UNIVERSITY *of* TASMANIA

# RESIN CANAL DISCOLOURATION IN MANGO (*Mangifera indica* L.) FRUIT

**by**

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# Statements and Declarations

## Declaration of Originality

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## Statement of Co-Authorship

The following people and institutions contributed to the publication of work undertaken as part of this thesis:

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  Umar Muhammad contributed 60% (conducted all experimental work, analysed data, wrote the initial manuscript). Cameron McConchie, Lucy Tran-Nguyen, Constancio Asis, Alieta Eyles, Roger Stanley, Alistair Gracie (40%) guided experimental design, advised on statistical analysis, revised and edited the manuscript.

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**C. Industry and media communications**

- Advances in what we know about resin canal discolouration. https://www.industry.mangoes.net.au/resource-collection/rcd2019

- Mango industry discovers highly infectious bacteria as cause of ugly veins of resin canal discolouration. https://www.treecrop.com.au/news/mango-industry-discovers-highly-infectious-bacteria-cause-ugly-veins-resin-canal-discolouration/

- Step closer to understanding mystery mango disorder. https://www.goodfruitandvegetables.com.au/story/5981543/step-closer-to-understanding-mystery-mango-disorder/
- Clues about mysterious mango disorder discovered. https://www.scimex.org/newsfeed/clues-about-mysterious-mango-disorder-discovered
- Fixing ugly fruit https://www.utas.edu.au/tia/news-events/news-items/fixing-ugly-fruit https://www.freshplaza.com/article/9104657/australian-researchers-trying-to-fix-ugly-fruit/
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We, the undersigned, endorse the above stated contribution of work undertaken for the above peer-reviewed and submitted manuscripts contributing to this thesis:

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1. Alistair Gracie (Thesis Principal Advisor; UTAS Associate Professor): conception and design of the project; advising on individual experiments; critically revising the draft of the work so as to contribute to interpretation.
2. Roger Stanley (Thesis Advisor; UTAS Professor): advising on experiments relating to market survey and consumer studies (Chapter 3); critically revising the draft of the work so as to contribute to interpretation.
3. Alieta Eyles (Thesis Advisor; UTAS Research Fellow): advising on each experiment; critically revising the draft of the work
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5. Cameron McConchie (Thesis Advisor; DPIR, NT. Research leader): advising on experiments relating to postharvest and supply chain studies (Chapter 4 and 6); critically revising the draft of the work so as to contribute to interpretation.
6. Constancio (Tony) Asis (DPIR NT. Senior Research Agronomist): contributing to certain experiments in Chapter 4 and 6. Advising on experimental design and data analysis.
7. John Bowman (UTAS Professor of Microbiology): Advising on data analysis and critically reviewing Chapter 5.

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

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# Abstract

Resin canal discolouration (RCD) in mango fruit is a quality defect and a major concern to the Australian mango industry. RCD appears as red-brown resin canals that form networks through the flesh and irregular brown mottling across the peel. It occurs sporadically and has been observed at all stages along the supply chain from field harvest to retail display with symptoms becoming more obvious during fruit ripening. The propensity for RCD appears complex as genetic, agronomic and environmental factors have been posited as possible causes. This thesis aimed to elucidate factors associated with RCD expression including its possible causes in order to identify potential pre- and post-harvest interventions to manage RCD.

The impact of RCD on consumer perception of mango quality and their specific decision to purchase and consume mangoes with RCD was investigated in a consumer survey of three supermarkets in Sydney. The survey of 135 mango purchasers revealed that the majority of consumers (89%) were regular buyers and preferred to buy Kensington Pride (KP) (36%) and Calypso$^{TM}$ (32%) cultivars because of their taste (50%), aroma (19%) and fruit colour (18%). Moreover, the majority of the respondents (87%) were not willing to buy RCD-affected fruit. This study suggests that RCD can significantly affect consumers' perception on fruit quality and influence their purchasing behaviour.

To characterize the progression of RCD symptoms during fruit ripening, the incidence (%) and severity (rating score) of RCD on fruit peel, flesh and seed along with other physio-chemical attributes (peel colour, flesh colour, firmness, dry matter content and total soluble solids) were explored at eating ripe stage. Fruit with RCD had significantly lower total soluble solids (TSS) compared with non-affected fruit. There were no other associations between these fruit attributes and the presence of RCD.

To date, progress on RCD-related research has been stymied by an inability to consistently induce RCD expression in mangoes. In this thesis, evaluation of two inoculation methods (needle and spray) found it was possible to reliably induce RCD at different stages of development (harvest green to eating-ripe) by inoculating with the extracted flesh from RCD -infected fruit as an inoculum source. In particular, while all inoculated fruit developed RCD, regardless of the stage of fruit development, fruit inoculated at 'harvest green' had the highest RCD severity by the eating-ripe stage. A

second study monitored RCD expression and severity by sequential sampling at 0, 3, 6, 9, 12 and 15 days after inoculation (DAI) at harvest green stage. Symptoms of RCD was first observed at 6 DAI on the seed testa, then in the flesh at 9 DAI and finally in the peel at 15 DAI.

The bacteria present in RCD fruit were identified using 16S rRNA sequencing technique and next generation sequencing (NGS) techniques were used to assess overall microbial diversity and their abundance in both RCD-infected and control fruit. Significant difference in microbial communities were observed between RCD-infected and control fruit, suggesting a bacterial association with RCD expression. The two dominant genera identified in RCD-infected mangoes were *Pantoea* and *Tatumella*.

Novel approaches to manage RCD expression were explored. The efficacy of a commercial postharvest sanitiser, Nylate®, to manage RCD was investigated. This postharvest treatment applied in varying concentrations, durations and timing were ineffective at controlling RCD expression.

The susceptibility of mango cultivars B74 (Calypso™) and National Mango Breeding Program cultivars (1243, 1201 and 4069) to RCD was evaluated using the two artificial inoculation tests. Symptoms of RCD were observed in all cultivars, however the level of RCD incidence and severity differed among cultivar demonstrating a genetic difference in susceptibility to RCD.

Overall, this study highlights the role of bacteria associated with RCD expression. It also documents the increase in RCD incidence with maturity along the supply chain. Despite the lack of postharvest control of RCD using Nylate®, alternative sanitisers and application at earlier stages such as pre-harvest and at harvest should be explored. Results from the cultivar screening offers new research possibilities to the longer-term reduction of RCD through genetic selection of less susceptible cultivars. A better understanding of the pre- and post-harvest factors affecting RCD will assist the mango industry in the reduction of RCD incidence and the associated economic losses.

Keywords: Resin canal, mango, consumers perception, pathogens, pathogenicity, post-harvest, sanitisers.

# Explanatory Note on Thesis Structure

This thesis has been written in the form of individual manuscripts for each chapter. As a result, some elements of repetition between chapters may occur. Chapter 1 includes an introduction to the research work and the thesis aims. Chapter 2 summarises the available knowledge on RCD and the research gaps. Chapters 3, 4, 5 and 6 are experimental chapters. Chapter 7 is a general discussion.

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# Chapter 1: General Introduction

## 1.1 Research background

Mango (*Mangifera indica* L.) is a tropical, edible fruit that is popular globally due to its distinct sensory properties and nutritional content (Mahato et al. 2016; Ntsoane et al. 2019). The demand for mango fruit continues to rise as evidenced by a worldwide production volume increase from 35.3 million tonnes in 2007 to 50.6 million tonnes per annum in 2017 (Faostat 2019). Australia produces approximately 60,000 tonnes of mango annually for the domestic and international market (AMIA, 2019). Kensington Pride (KP), is the dominant cultivar grown in Australia and represents approximately 80% of Australia's total production (Bally 2006b; Hofman et al. 2009). Honey Gold, B74 (Calypso$^{TM}$) and R2E2 are account for the majority of the remaining 20% of Australia's production (Dillon et al. 2013).

Mango is a climacteric fruit (Litz 2009). The fruit can ripen fully once harvested at the mature green stage of development (referred to as "harvest mature") (Hofman et al. 2009). Stage of maturity at time of harvest should be optimised to ensure fruit is of premium quality at retail (Bally 2006a). Typically, fruit maturity of mangoes is tested by the evaluation of the dry matter content before harvest. For KP mango, 15% dry matter is considered the best commercial harvest stage (AMIA 2016). To avoid mechanical damage, harvested fruits are handled carefully at packhouse where they are subjected to postharvest washing, brushing, drying, grading and packing before further transport and distribution (Taiti et al. 2016).

In Australia, about 85-90% of the total mango production that is destined for domestic markets is first transported to commercial ripeners/distribution facilities (Innovation 2018). Here, the fruit is ripened to the desired stage of softness by exposure to ethylene concentration between 10-100 ppm (Ledger et al. 2010). The duration and concentration of ethylene exposure depend on the stage of fruit maturity and the desired stage of fruit softness after ripening treatment. Subsequently, ripe fruit is either marketed directly to supermarket retail chains or through wholesalers in open markets.

Supermarkets account for 82% of total retail sales of fresh mangoes, the remaining 18% are sold for food services (Innovation 2018).

A current challenge facing the Australian mango industry is the sporadic expression of a discolouration that appears throughout the flesh and on the peel surface. This cosmetic discolouration reduces the marketability of fruit, and is estimated to cause economic losses between AUD$ 5 million to AUD$10 million per annum in the domestic market. A detailed description of the disorder has been provided by Macnish et al. (2014) and reported as resin canal discolouration (RCD). Resin canals are a distinguishing feature of healthy mango trees as similarly observed in other members of the *Anacardiaceous* family (Venning 1948). These resin canals form an important network system in both the stem and fruit (Juliano & Cuevas 1932), and are filled with a viscous, corrosive sap resin (Joel 1981) comprising primarily of lipids and terpenoids and it is acidic in nature (Hodges et al. 1979; Strom et al. 2002). These resin networks have been suggested to contribute the tree's chemical defence against insects and has antiseptic, antifungal and antibacterial properties (Joel 1980; Loveys et al. 1992; Franceschi et al. 2005; Hood et al. 2015).

Resin canal discolouration in mango fruit (Figure 1.1) appears as black markings on the fruit surface due to red or dark brown veining of resin canals during fruit ripening (Asis et al. 2017). Symptoms can occasionally be seen through the peel as a black coloured network of sub cuticle resin canals (Holmes et al. 2009). RCD is rarely visible at the harvest mature or green stage but becomes evident as the fruit transitions to the eating ripe stage. RCD has been reported to occur in Australian mango cultivars KP, B74 (Calypso$^{TM}$), Keitt and in some Asian cultivars grown in Australia (Moore 2012; Hoffman et al. 2013a; San et al. 2015; Macnish 2016). It is unclear from the published and 'grey' literature if RCD has been reported in mangoes growing outside of Australia. There are suggestions that KP has a higher propensity for expression of RCD (Macnish et al. 2015). This disorder has shown to be responsible for almost 32% of fruit loss of this variety (Macnish et al. 2015; Asis et al. 2017). However, it is still unclear if the high level of

incidence in KP represents genetic propensity or if it is due to its dominance in the marketplace.

A B C

Figure 1.1: Photographs of Kensington Pride fruit with resin canal discolouration on fruit peel at the mature harvest green stage (A) and at eating ripe stage (B), and the flesh at eating ripe stage (C).

The underlying cause of the expression of RCD is unknown. Previous studies have examined a range of both pre- and postharvest factors that may be linked with RCD expression including plant nutrition, fruit maturity at harvest, rainfall before harvest, physical damage to fruit, fruit processing and pathogens (Moore 2012; Hoffman et al. 2013a; Macnish et al. 2015; Asis et al. 2017). Evidence to support for the potential role of bacteria was first reported by Macnish et al. (2014) whereby bacteria from RCD infected mango fruits was identified as *Pantoea agglomerans* and *Enterobacter cowanii*. There continues to be a lack of clarity if these bacteria have a causal role in the expression of RCD. However, understanding the fundamental causes of the expression of RCD is paramount in developing effective control measures for the mango industry.

## 1.2 Research approach and aims

Most of the research on RCD has been conducted in the last two decades and has highlighted different causal factors but has not identified a single cause behind this defect (e.g. rainfall at harvest, plant nutrition or pathogens) (Moore 2012; Hoffman et al. 2013a; Macnish et al. 2015; Asis et al. 2017). Furthermore, consumer purchasing decisions regarding RCD fruit have not been documented, and there are no known control measures. Therefore, many aspects of RCD research warrant further investigation to improve our understanding of the factors associated with RCD expression and propensity. This new knowledge will permit more targeted strategies to reduce RCD incidence.

Resin canal discolouration is a fruit quality defect of the commercially important KP mango. The core aim of this study was to better understand the underlying mechanisms leading to RCD in mango. In elucidating the cause of RCD, a whole supply chain perspective was taken that included consumer perception, post-harvest operations and agronomic practices.

The specific aims of this study were:

1. To understand the impact of RCD on consumer perceptions, level of satisfaction and repeat purchase intentions of RCD-affected fruit (Chapter 3)
2. To determine whether RCD is due to pathogens (transmissible agents) in cv. KP mango fruit and to develop a robust method to artificially induce RCD expression (Chapter 4)
3. Identification of bacterial species associated with RCD fruits and to examine the role of microbes in RCD expression by determination of microbial diversity (bacterial and fungal) communities from RCD and control mango fruits (Chapter 5)
4. To evaluate the efficacy of a commercial sanitiser as a postharvest control measure of RCD and to examine the response of related mango cultivars to exposure to RCD (Chapter 6)

# Chapter 2: Mango Resin Canal Discolouration: A Review of Contributing Factors and Management Challenges

## 2.1 Introduction

Mango (*Mangifera indica,* L.) is a popular fruit due to its unique flavour and texture and its dietary and medicinal benefits (Litz 2009). It is grown commercially in tropical and subtropical regions and, by volume, is the fifth most abundant fruit cultivated globally (Rajan & Hudedamani 2019; Li et al. 2020). Consumers generally select fruit based on previous eating experience and external appearance. It is therefore, important that fruit are free from defects, such as skin blemishes that reduce the aesthetic appeal of fruit and affect the marketability of mangoes (Hofman et al. 2009). One of the major external defects of mango fruit is resin canal discolouration (RCD). It causes economic losses of 10% to 30% and is mostly observed in mango from Northern Territory (NT) Australia (Macnish et al. 2014; Asis et al. 2017).

The objectives of this review are to: (i) describe mango fruit quality with a focus on physiological changes during postharvest ripening; (ii) synthesis current knowledge of internal and external disorders and blemishes affecting mango; (iii) highlight the incidence and expression of RCD, in the context of Australian production systems; (iv) identify knowledge gaps of the underlying factors that potentially contribute to RCD expression and suggest research priorities for management practices to minimise or even prevent the occurrence of RCD.

## 2.2 Mango fruit physiology and quality attributes

### 2.2.1 Fruit growth and maturity

Maturity at harvest plays a key role in determining the eating quality and shelf life of mango (Sivakumar et al. 2011). Mango fruit growth follows a sigmoidal pattern in which the seed develop first followed by an increase in fleshy mesocarp (Mukherjee & Litz 2009). The fruit takes approximately 90 days to complete its development and attain physiological maturity. This duration varies with cultivar, management practices and growing conditions (Lizada 1993). Commercially, mangoes are harvested at physiologically fully developed but mature 'hard green' stage (Kader 2002) and then ripened to achieve the desired taste and texture (Singh et al. 2013). Fruit harvested prior to this stage result in lower quality fruit with poor colour and taste development, and is generally considered unacceptable to consumers (Sivakumar et al. 2011).

Assessment of fruit maturity at harvest is based on fruit size, shape, visual and biochemical parameters referred to as fruit maturity/harvest indices (Lizada 1989). These visual/external indices usually consider changes in fruit peel and flesh colour, fruit shoulder growth and softness. Total soluble sugars (TSS) and dry matter content (DMC) are considered among the key biochemical maturity indices. For mangoes, a TSS of around 8 °Brix is typical of mature fruit but can vary depending on cultivar (Jha et al. 2006).

DMC is an aggregate of starch and sugars and other (relatively constant) cell components like cell walls, fibres and protein of the fruit. DMC is considered an important criterion of fruit maturity because during ripening, starch is converted to sugar (Jha, SN et al. 2010). DMC $\geq$ 15% before harvest is considered ideal for Kensington Pride (KP) and B74 whereas values $\geq$ 14% are recommended for Honey Gold (AMIA, 2016). On the other hand, fruit harvested above the recommended DMC values suffer

increased softness and susceptibility to bruising and peel browning discolouration, as well as poor flavour and reduced postharvest storage life (Vazquez-Salinas & Lakshminarayana 1985; Sivakumar et al. 2011).

### 2.2.2 Mango fruit ripening

Mango is highly perishable when stored at ambient temperature therefore, management of the fruit ripening process is very important for maintaining and delivering high quality fruit to consumers. Natural fruit ripening typically takes 9-12 days when stored at 25 ± 1 °C and 60-65 % relative humidity (RH) (Ledger et al. 2010). While ripening, fruit undergo various irreversible changes in both flesh and peel colour, texture softening and alterations in TSS, acidity and aromatic compounds (Ntsoane et al. 2019). However, the rate of these changes differ among cultivars (Vazquez-Salinas & Lakshminarayana 1985).

As a climacteric fruit, ripening in mangoes is initiated by an increase in both respiration rate and ethylene biosynthesis (Lalel et al. 2003b; Singh et al. 2013). The spike in respiration vary with cultivar occurring 4 and 5 days after harvest for KP and Alphonso, respectively, while for Kent and Haden, 9 and 11 days after harvest (Lalel et al. 2003b; Singh et al. 2013). Ethylene plays an important role in triggering the series of physiological and biochemical changes that occur during the ripening process (Pristijono et al. 2019; Rosman et al. 2019). The production of ethylene similarly varies with cultivar ranging from very low (0.02 - 0.18 ppm) to high (5 - 8 ppm) (Cua & Lizada 1989; Zaharah & Singh 2011) and further modified by storage period, temperature and fruit maturity stage.

The shelf life of mangoes can be extended by slowing the ripening process using a range of postharvest technologies as described in a recent review by Ntsoane et al. (2019). For example, storage at low temperatures such as 12 °C can decrease ethylene production and respiration rate allowing fruit to be stored for up 3-4 weeks (Mitra 1997). However, storage at below 12 °C may cause chilling injury in some cultivars. Apart from low

temperature storage, the ripening process can be delayed by exogenous application of the ethylene antagonist, 1-methylcyclopropane (1-MCP), which is effective in inhibiting ethylene action and is active at extremely low concentrations, readily available for commercial use, and nontoxic (Singh et al. 2008)

## 2.3 Physio-chemical characteristics of mango fruit

### 2.3.1 Fruit colour

Mango peel colour is an essential attribute of quality and plays a vital role in consumer acceptability and likely purchase. Fruit peel and flesh colour is assessed visually by comparing with colour charts (Holmes et al. 2009), as well as with handheld colourimeter, which is a robust and more accurate in measurement (Ayala-Silva et al. 2005; Leon et al. 2006). Fruit with inadequate or uneven peel colour development typically sell at reduced prices (Sivakumar et al. 2011).

During ripening, the colour of the fruit peel typically turns from light green to light yellow, yellow, reddish, orange-yellow or violet yellow (Sabuz et al. 2019). These colour changes occur as a result chlorophyll degradation and accumulation of anthocyanins and carotenoids (Medlicott et al. 1986). A range of carotenoids have been identified including all-*trans*-*β*-*carotene*, all-*trans*-violaxanthin and 9-*cis*-*violaxanthin* (Mercadante et al. 1997). However, the rate of chlorophyll degradation and accumulation of colour pigments varies among cultivars. Tommy Atkins and Keitt generally develop more reddish blush skin colour compared to other commercial cultivars (Mitcham & McDonald 1993; Li et al. 2009). Development of red colour is linked with the accumulation of anthocyanins, while carotenoids and xanthophylls dominance is related to yellow (Medlicott et al. 1986). A study on KP found fruit colour can managed by avoiding high nitrogen application before harvest (Ledger 1989), by picking the fruit at optimum maturity stage and by ripening the fruit at 18-22 $^{\circ}$C with 10 ppm ethylene for two to three days (Ledger et al. 2010).

### 2.3.2 Fruit softening

Fruit softening is the most essential textural parameter linked with quality and postharvest storage life of mangoes (Jha et al. 2007). Softening is defined as the process of decreasing the fruit tissue cohesion. Fruit softening is due the activity of hydrolysing enzymes that depolymerize the cell wall and pectin substances in the middle lamella (Singh & Janes 2000; Brummell & Harpster 2001). The prominent enzymes involved include exo and endo *polygalacturonases* (PGs), *pectate lyases* (PLs), *pectin methylesterase* (PMEs), *β-galactosidases* (β -Gals) and *α-arabinofuranosidases* (Lazan et al. 1986; Prasanna et al. 2003). However, fruit softening can be regulated by application of calcium chloride which inhibits the enzymatic activity and softening in Amrapali and Dashehari mangoes, while exogenous application of ethylene rapidly increases the softening process (Reddy & Srivastava 1999).

### 2.3.3 Sugars and total soluble solids

As mentioned, mango ripening is accompanied by the conversion of starch into sugars. Total sugars are the sum of non-reducing (sucrose) and reducing (glucose and fructose) sugars (Suwonsichon et al. 2012). Several enzymes have been shown to increase the activities during the hydrolysis of starch into sugars while fruit ripening (Lalel et al. 2003a; Lavanya et al. 2019). The most important enzymes include *amylase*, *sucrose synthase* and *invertase* rise in *α*- and *β-amylases* enzymes activities (Litz 2009). An increase in total sugars concentration from 2.69% to 11.16% during ripening was reported for an Indian mango cultivar (Doreyappa Gowda & Huddar 2001). Yashoda et al. (2006) found a rise in total sugars from 7% to 20% from unripe to the ripe stage in 'Alphonso.' In contrast, total sugars increased from 6.2% to 14% in KP (O'Hare 1995).

### 2.3.4 Organic acids

Ripening is accompanied by the reduction of organic acids (Aubert et al. 2019; Quirós-Sauceda et al. 2019). The major organic acids identified in mangoes include citric acid, malic acids, tartaric acids, ascorbic acids, α-ketoglutaric acids and oxalic acids (Sung et al. 2019). During fruit ripening, reduction of citric acid concentration resulted in a decrease in fruit acidity (Medlicott & Thompson 1985). Gowda and Huddar (2001)

recorded a decline from 2.71 to 0.04% of acidity in various cultivars during ripening. Ascorbic acid decreased from 40.83 to 11.08 mg/100 g during ripening (Gowda & Huddar 2001). A similar trend was also reported for Tommy Atkins, Keitt and Kent (Manthey & Perkins-Veazie 2009).

### 2.3.5 Fruit flavour (taste, aroma)

Flavour is a unique attribute which is a balance between fruit taste, mouthfeel and aromatic properties of the fruit (Medlicott & Thompson 1985). Mango fruit aroma is a complex combination of numerous volatile mainly, alcohols, aldehydes and esters (MacLeod & de Troconis 1982). In KP cultivar, a total of 61 volatile compounds were identified in the fruit (Lalel et al. 2003a). Pino et al. (2005) identified a total of 372 volatile compounds from 20 Cuban mango cultivars, the prominent being monoterpenes and sesquiterpenes. Engel and Tressl (1983) reported more than 300 volatile compounds in Keitt, Kent and Tommy Atkins mango cultivars.

## 2.4 Postharvest diseases and disorders of mango

### 2.4.1 Diseases

Postharvest diseases reduce fruit quality causing heavy economic losses by downgrading fruit marketability. The most serious postharvest disease of mango fruit is mango anthracnose, caused by *Colletotrichum gloeosporioides* (Dembele et al. 2020). Infected fruit develop brown to black spots which become more obvious as the fruit ripens. Management of anthracnose at postharvest stage is achieved by hot water dip and chemical application or a combination of both approaches (Pavitra Kumari & Singh 2017; Manasa et al. 2018; Sharma et al. 2019). Hot water dip at between 50 to 55 °C for 3 to 15 minutes has been shown to provide moderate control of mango anthracnose with no residual effect and is considered a viable option for organic management (Falade et al. 2017). Mango anthracnose can also be managed postharvest by use of several fungicides such as thiabendazole (1,000 to 2,000 ppm), prochloraz (1,000 ppm) and benomyl (9500 ppm) (Arauz 2000; Nelson 2008). However, the combination of hot water

dip and fungicides has proved the most effective control measure – postharvest dip in 250 ppm prochloraz at 53 °C for 3 minutes had more then 98% efficacy (Arauz 2000; Nelson 2008; Alvindia & Acda 2015).

Stem end rot (SER) is also an important postharvest problem and have been reported to cause significant fruit loss, particularly where mangoes are grown in dry areas. SER named because of its symptoms as grey brown area starts appearing at the stem end (pedicel) of mango fruit during ripening and storage. This disease is linked with various fungal pathogens, mainly those belonging to the *Botryosphaeriaceae* family (Galsurker et al. 2020). Management approaches of SER are aimed to preventing or delaying symptoms during ripening. Among the chemical treatments, preharvest fungicide spray with difolatan, copper hydroxide, baycor, aliette, benlate or copper oxychloride is considered to be most efficient in reducing SER (Darvas 1981; Muirhead et al. 1982; Galsurker et al. 2020). For postharvest management of SER, the industry have historically relied on dipping, spraying or coating with various fungicides such as prochloraz, imidazole, benomyl and fludioxonil as recently reviewed by Galsurker et al. (2020). Biological control with various microbial antagonists such as bacteria, fungi and yeast has also been shown to be effective for controlling SER (Korsten et al. 1997; Sharma et al. 2009). Alternative approaches for managing SER include hot water dip, irradiation and low temperature storage of mango fruit (Farkas 2006; Alvindia & Acda 2015; Galsurker et al. 2020).

### 2.4.2 Disorders

Physiological disorders alter the visual appearance of mango fruit (Hofman et al. 2009). Prominent internal disorders in mangoes are soft nose, jelly seed and spongy tissue (Seshadri et al. 2019). These physiological disorders can damage fruit internally (flesh and seed) and externally (peel) and occur as a result of several metabolic changes within the fruit (Sivakumar et al. 2011). Disorders may be caused by a range of biotic and abiotic factors along with postharvest handling practices (Shivashankar 2014).

Among the plant nutrients, Ca is considered the most important nutrient in relation with postharvest disease and disorders of mangoes (Conway et al. 1994; Sabir & Sabir 2017). Low Ca level in mango fruit has been linked with reduced storage life and physiological disorders (Raymond et al. 1998). For example, Torres et al. (2008) associated internal breakdown in mango that had higher mesocarp nitrogen content, and lower mesocarp calcium content. Similarly, Bitange et al. (2020) found a deficiency in Ca caused spongy tissue in mango fruit. In Kent, soft nose symptoms were associated with fruit that had lower Ca concentration (5.85 mg/100 g) than healthy fruit (8.02mg/100 g) (Burdon et al. 1990).

Improper postharvest storage conditions such as storage at low temperature (< 10 °C) can cause chilling injury (CI) in mango and other tropical fruit (Biswas et al. 2016; Deltsidis et al. 2016; Ikeda et al. 2016). This storage disorder result in discolouration of the peel, greyish scald and uneven ripening (Han et al. 2006). CI is responsible for cellular leakage of metabolites such as amino acids, sugars and minerals (Joseph & Aworh 1991; Sharma et al. 1999). At the onset of CI, only the fruit peel is affected however, in the later stage of CI, both peel and fruit pulp are damaged, which leads to exposure of the mesocarp rich in sugar for subsequent colonisation by microorganisms (Singh et al. 2012).

#### 2.4.2.1 Lenticel discolouration

Lenticels are microscopic opening present on the peel of mango fruit and functions as a medium for gaseous exchange (Bally et al. 1999). However, lenticels and the surrounding tissues discolour as light purple or black spots resulting in lenticel discolouration (LD) or lenticel spotting (Bezuidenhout et al. 2005). LD does not reduce the internal quality of the fruit, but it can downgrade the cosmetic look and marketability of the fruit. LD is due to the accumulation of phenolic compounds in the vacuole of sub lenticellular cells (Du Plooy et al. 2006). The cause of LD remains unknown however, factors that increase susceptibility include contamination of mango peel with sap during mango desapping, rainfall event and heavily wind before fruit harvest and hot water treatment (O'Hare & Prasad 1991; Rymbai et al. 2012).

#### 2.4.2.2 Under-skin browning

Under-skin browning (USB) is a fruit quality issue, predominantly affecting Honey Gold fruit. It appears as a skin injury under the epidermis, causing no damage to the flesh, with symptoms developing during ripening (Hofman et al. 2009; Holmes et al. 2009). The mechanism and causes of USB are still unknown, however, Hofman et al. (2009) suggested that USB can be minimised by slowing the cooling process before and after the packing of fruits. Fruit produced in hotter areas are relatively more susceptible to USB, which may be due to the large temperature gradient in the field and post-harvest operation (Hofman et al. 2009).

## 2.5 Resin canal discolouration

Resin Canal Discolouration (RCD) has become an emerging issue for the industry (Macnish et al. 2014). RCD is considered to be a fruit physiological disorder, which causes the discolouration of the peel and flesh, appearing as browning of the cells with a cluster of discrete spots gradually becoming dark red or brown streaks along the resin with the ripening of the fruit. RCD first appears at near ripe to ripe stage as distinctive internal red “veining” discoloration of the fruit and later, as surface blemishes.

This defect has been reported in KP, Keitt, B74 (Calypso $^{TM}$) and Carabao cultivars (Moore 2012; San et al. 2015; Macnish 2016). In Australia, RCD is commonly reported in NT predominantly affecting KP mango, which is the most cultivated and popular cultivar of Australia. RCD is responsible for about 32% of KP fruit loss in NT (Macnish et al. 2014). This cosmetic defect is a serious constraint to maintaining the supply of high quality mango for the domestic market (Macnish et al. 2014; San et al. 2015).

Mango belongs to the family *Anacardiaceae*, which are characterized by the presence of a complex system of secretory cells called resin canals (Venning 1948). The anatomy, ultrastructure and development of the resin canal system in mango fruit has been well

studied (Joel 1980). These secretory cells are known to produce sap or resinous substance (Venning 1948). The resin is composed of viscous lipid-soluble mixture of volatile and non-volatile terpenoid and phenolic compounds (Strom et al. 2002). The physiological role of resin is not well studied. However, it has antibacterial and antifungal properties and act as a natural defence against insects and herbivores (Joel 1980; Loveys et al. 1992; Hassan et al. 2011). In some plants it is thought to have an role in healing of the wounds and infections (Lombardero et al. 2000; Franceschi et al. 2005; Hood et al. 2015).

A major concern with RCD is the difficulty in detecting this disorder at an early stage. RCD symptoms might be seen at the time of harvest and supply chain but become more visible during fruit ripening which leads to fruit being unsaleable at the supermarket and negatively affects the whole industry (Hoffman et al. 2013b). The late stage appearance however, means affected fruit cannot be effectively screened out at the packhouse and currently no accurate records are kept on the incidence at retail. Growers may therefore be unaware of the true incidence of RCD for their fruit.

## 2.6 Factors associated with RCD

Macnish et al. (2014) conducted a number of preliminary studies on identifying the causes of RCD in KP in Australia. This work identified a range of potential factors as contributing to the expression RDC including nutrition, cultivar, fruit maturity, environment and post-harvest processing (Table 2.1). However, as discussed below, there is still limited understanding on the factors and underlying causes responsible for RCD incidence.

### 2.6.1 Genotype

Genotype can play a significant role in tolerance to RCD. For instance, KP, which is indigenous to Australia, is considered to be more susceptible to RCD than other cultivars such as B74 (Calypso$^{TM}$) however, this has not been directly assessed (Macnish et al.

2015). There is evidence from studies of other mango disorders that cultivars may have differing tolerance to discolouration disorders. For example, Oosthuyse (1999) found that Tommy Atkins and Keitt were more prone to LD as compared with Kent. Similarly, San et al. (2015) identified Honey Gold to be more susceptible to USB then KP fruit. Further research may assist with the identification of genotypes showing enhanced tolerance to RCD. It is unclear if the high level of incidence in KP represents a genetic susceptibility to RCD or if it is due to its dominance in the marketplace

Table 2. 1. A summary of potential factors linked with RCD along the mango supply chain and research gaps in literature.

| | Factor | Farm | Packhouse | Transit | Retail |
|---|---|---|---|---|---|
| Available literature | Mango season | ✔ | | | |
| | Rainfall event before harvest | ✔ | | | |
| | Crop nutrition | ✔ | | | |
| | Fruit maturity | ✔ | | | |
| | Preharvest bacterial inoculation | ✔ | | | |
| | Survey of growers/packers | | ✔ | ✔ | |
| | Fruit wounding | ✔ | | | |
| | Commercial processing | | ✔ | | |
| | Microbial association | ✔ | ✔ | | |
| Research gaps | Fruit marketability | | | | ? |
| | Fruit internal quality | | ? | | ? |
| | Genotype (tolerance) | ? | | | |
| | Role of pathogen/s | ? | ? | | |
| | Progression of RCD symptoms | | ? | ? | |
| | Control measures | ? | ? | ? | |

✔ = Preliminary studies only, **?**= No literature available

### 2.6.2 Preharvest conditions

Preharvest elements may be unique to specific Australian conditions and production systems. The incidence of RCD has been shown to be more commonly observed in fruits produced in Northern Territory as compared to Queensland and Western Australia (Macnish et al. 2014). Therefore, orchard location, management and environmental conditions could influence the propensity for mangoes to develop RCD as discussed below (Johnson et al. 1992).

#### 2.6.2.1 Nutrition

A preliminary study by Asis et al. (2017) found variation in nutrient ratios in orchards with RCD and without RCD fruits. Moreover, RCD-affected fruit had higher N, P, K, Mg, S, Cu, Fe and Zn than control fruit (Asis et al. 2017). However, there is still a need to investigate the possible relationship between nutritional status and RCD propensity across the mango growing regions of Australia.

#### 2.6.2.2 Fruit harvest maturity

Macnish et al. (2014) identified fruit maturity as having an important role in RCD incidence with immature fruit being more susceptible to RCD. In particular, KP fruit harvested early with DMC of < 13% had the highest RCD incidence (64%) than fruit harvested later with 15% and 17% DMC in a commercial orchard in Darwin, NT. Thus, this single study suggests that fruit should be harvested at the optimal maturity stage to minimize RCD incidence.

#### 2.6.2.3 Rainfall

A rainfall event prior to harvest has been reported to exacerbate symptoms of RCD. Macnish et al. (2014) harvested 12 and 60 hours immediately after a 14 mm rainfall event and found RCD symptoms to be 45% higher in fruits harvested 12 hours after the rainfall event. Exposure of rainfall just prior to harvest has similarly been reported to enhance susceptibility to mango stem end cavity disorder (Mead & Winston 1989)

lenticels damage and skin browning (Jacobi et al. 2001) and chilling injury (Hofman et al. 1997). In terms of orchard management of plant water status, lenticel spotting in KP has shown to be reduced by withholding irrigation for 12 weeks after flowering and from seven weeks before harvest (Lechaudel et al. 2002).

### 2.6.3 Postharvest handling and management

RCD has been linked to poor fruit handling during harvest (Lu et al. 2013; Macnish et al. 2014). For example, a RCD was found to occur between two and nine times higher in KP mangoes exposed to commercial handling and distribution than unprocessed fruit, indicating that industry practices used in processing may contribute to the occurrence of this disorder (Macnish et al. 2014). A survey of growers about their experience with RCD attributed 10-30% fruit loss in production to RCD while a further 5-25% fruit loss occurred at the packhouse (Hoffman et al. 2013b; Macnish et al. 2015).

Such results suggest that RCD could potentially be managed by application of various post-harvest approaches such as hot water treatment, chemicals, cold and modified atmosphere storage. These techniques have been shown effective against other mango postharvest diseases and disorders as detailed in Table 2.2 (Arauz 2000; Akem et al. 2010; Diedhiou et al. 2014; Naqvi et al. 2014).

Table 2.2. Selected postharvest treatments for the management of disorders and discolouration of mango.

| Treatments | Benefits | References |
|---|---|---|
| Heat treatment | Reduce chilling injury, delay ripening, reduce microbial contamination | (McCollum et al. 1993) |
| Antimicrobial and anti-browning agents | Improve fruit quality, reduce microbial load | (Baskaran et al. 2013; Lopez-Galvez et al. 2013) |
| Nitric oxide | Slow ripening process, improve fruit quality | (Singh et al. 2009; Zaharah & Singh 2011; Manjunatha et al. 2012) |
| Sulphur dioxide | Prevent postharvest decay | (Palou et al. 2010; Sivakumar et al. 2010; Cantín et al. 2012; Rivera et al. 2013) |
| Ozone | Easily incorporated into existing cold storage, washing system, better efficacy than chlorine | (Huyskens-Keil et al. 2012; Hassenberg et al. 2013; Ali et al. 2014) |
| CA* | Retard senescence, associated biochemical and physiological changes, reduction in decay severity | (Hoehn et al. 2009; Prange et al. 2012; Chong et al. 2013) |
| MAP* | Delay senescence, and slow down rate of respiration and deterioration | (Linke & Geyer 2013; Sousa-Gallagher & Mahajan 2013) |

*MAP=modified atmosphere storage, CA= controlled atmosphere storage

### 2.6.4 Microbial associations

Postharvest pathogens cause significant losses in mango at post-harvest, distribution and consumption stage (Aguirre-Güitrón et al. 2019; Shao et al. 2019). There is preliminary evidence to indicate multiple bacterial species are among the possible causes of RCD. Two species of bacteria, *Pantoea agglomerans,* and *Enterobacter cowanni*, were identified from fruit with RCD symptoms by using Biolog tests (Macnish et al. 2014). However, these same two bacteria were also isolated from intact symptomless fruit and in wash water used to de-sap fruit. Therefore, there remains uncertainty on the role of pathogenic bacteria in causing RCD. *Pantoea agglomerans* and *Enterobacter* sp. have been reported as endophytes in other tropical crops such as citrus (Soto-Muñoz et al. 2015), pome (Buron-Moles et al. 2012; Torres et al. 2014) and stone fruit (Buron-Moles et al. 2012; Soto-Muñoz et al. 2014). Further study is required for the confirmation of bacteria in causing RCD.

#### 2.6.4.1 Pathogen identification

Bacterial detection and verification have been reported with PCR based techniques in various fruits. Patel (2001) found 16S rRNA sequencing techniques as most effective in bacterial identification. This method utilises the selective binding of universal primer pairs to highly conserved sequences within the hypervariable regions of the 16S rRNA gene that contains sufficient phylogenetic information for taxonomic classification. The 16S rRNA gene sequencing method is the most utilized method in the field of microbiome research and provides detailed information on specific bacteria present as compared to conventional enumeration methods. However, this method is expensive, and the analysis is complicated and time consuming, and it does not differentiate between live and dead bacteria (Hamady and Knight, 2009, Gorni et al., 2015,). Further, this technique has limitations providing 90% accurate identification at the genus level but only 65-83% accuracy at the species level (Mignard & Flandrois 2006).

Alternatively, a comparison of microbial communities of RCD and asymptomatic fruits could be useful alternative approach for investigation of potential pathogens. Next-generation sequencing (NGS) has been widely applied for the investigation of microbial community structures in diseases of different fruit such as mango, grapes, apple and

olives (Abdelfattah et al. 2018; Gao et al. 2019; Hall & Wilcox 2019, apple (Angeli, 2019; Singh et al. 2019). Diskin et al. (2017) used this method to study stem end rot of mango fruit and found significantly differences in microbial community between resistant and susceptible mango fruit. This method captures the entire metagenome of organisms instead of focusing on fragments of 16S rRNA genes (Fricker et al. 2019). This approach allows to characterize unique bacterial communities and bacteria that are unable to grow in laboratory conditions. However, the major disadvantages of metagenomics include the cost and risk of missing previously uncharacterized species or strains of bacteria (Emily et al., 2019). For higher resolution of plant pathogen species, Multilocus sequence analysis (MLSA) is a more reliable approach than 16S rRNA for differentiating and identifying species . However, this is a more expensive and laborious approach. Housekeeping genes such as *rpo*B and *hsp*60 have successfully been used for species identification of *P. agglomerans* and *Enterobacter cowanii* (Brady et al. 2009; Delétoile et al. 2009).

#### 2.6.4.2 Postharvest control of bacteria

If there is a microbial association with RCD, post-harvest sanitisers could potentially be used to manage RCD (Kakani et al. 2018). The mango industry currently manages microbial contamination in the wash water by a combination of hot water dips and chlorine at 50-200 ppm (Li et al. 2001; Shen et al. 2013). Chlorine application offer a number of advantages including good oxidising effect, affordable cost, relatively simple operation and widely commercially available (Zhang et al. 2019). Other sanitisers such as sodium hypochlorite, calcium hypochlorite, chlorine bromide and chlorine gas have also been reported in the mango industry (Zhang et al. 2019).

Chlorine-based sanitisers may pose environmental and health and safety issues and may be expensive to use (Issa-Zacharia et al. 2010). These concerns have led to the development of alternative sanitisers that are eco-friendly such bromo-chloro-dimethyl-hydantoin (BCDMH) (Nylate®). Nylate® is a chlorine-based disinfectant currently in use in Australia (Premier 2013). Previous research has shown that this sanitiser is effective against microbial pathogens in fruits and vegetables. Nylate® application (5 ppm) for 45

s was effective in reducing microbes in baby leafy salad vegetables (Bliss 1996; Premier 2013). Similarly, Nylate® (10-12 ppm) was effective in controlling microbial growth and significantly enhanced shelf life of cut flowers (Jones et al. 1993; Jones & Hill 1993). However, Dixon et al. (2004) reported that 15-20 ppm Nylate® could not prevent fruit rot in avocado. To date, no study has yet investigated the efficacy of using postharvest sanitisers such as Nylate® in managing RCD.

## 2.7 Conclusion

RCD is a substantial mango fruit quality defect, however, little is known of its aetiology as highlighted in Table 2.1 (Macnish et al. 2015). Macnish et al. (2014) reported possible factors contributing to the expression of RCD including abiotic factors such as fruit injury, fruit maturity, rainfall before harvest, and nutrition as well as biotic factors such as bacterial infection. The literature review indicates that there is an urgent need to determine the role of bacteria in causing RCD. The use of NGS molecular techniques offers a new approach for characterising differences in microbial communities (both bacteria and fungi) between RCD and control fruit. Other aspects of RCD research, which require attention include an understanding of the impact of RCD on consumer's acceptability and fruit marketability. Further, developing robust inoculation methods that allow consistent induction of RCD expression will greatly assist in addressing basic knowledge gaps such as gaining an understanding of progression of expression of RCD symptoms.

# Chapter 3: Perception of Australian consumers on resin canal discoloration (RCD) in mango

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# Chapter 4: Transmission of RCD causative agents by artificial inoculation and evaluation of fruit quality

## 4.1 Abstract

Resin canal discolouration (RCD) is a major concern of the Australian mango industry. Progress in this field of research has been stymied by an inability to consistently induce RCD expression in mangoes. Two inoculation techniques were developed to try and demonstrate transmission of infective agent in Kensington Pride 'KP' cultivar. One method involved puncturing the mango skin with syringe needles while the other relied on passive transmittance by spraying the skin with inoculum in apparently healthy fruit. RCD incidence (%) and severity (rating score) along with physico-chemical attributes (skin colour, firmness, dry matter content, and total soluble solids) were recorded following inoculation treatment in fully ripened fruit. Both needle and spray methods were found to reliably induce RCD at three different stages of fruit ripening (harvest green, after commercial ripening and eating-ripe stage) confirming that RCD can be expressed at any stage of ripeness. A separate study monitoring RCD expression over time found symptoms to be first observed on the surface of the seed on day six after inoculation. Further research is recommended to develop validated and verifiable hygienic practices of harvesting, packing, cooling, ripening and handling in the supply chain to minimise contamination with an infective agent.

Key words: Inoculation, Kensington Pride, mango, resin canal discolouration.

## 4.2 Introduction

In Australia, quality and quantity of mango production can be reduced by a range of diseases and physiological disorders (Bally et al. 1999). Skin physiological disorders are among the major concerns (San et al. 2015). Despite growing production and consumption of cv. KP fruit the major local cultivar, delivering high quality fruit to consumers has remained a challenge for the Australian mango industry (Hofman et al. 2009; Marques et al. 2016). One emerging challenge is resin canal discolouration (RCD) in cv. KP is one of the most important fruit defects potentially experienced by domestic consumers (Macnish et al. 2014).

RCD is linked with discolouration of the cells of the well branched network of sub-cuticle resin canals (Macnish et al. 2014). A visible black streak can be seen within the skin of affected fruit. Sometimes it is associated with browning of resin canals in the outer layers of the pulp (San et al. 2015). Factors considered to contribute to the development of RCD in cv. KP mangoes include stress sensitised fruit, physical injury, fruit production location, commercial processing and pathogens (Macnish et al. 2014). This previous investigation found that RCD severity increased as fruit developed from firm-ripe to overripe. The symptom generally appeared on the peel and flesh but sometimes were not externally visible.

In a previous study Macnish et al. (2014) attempted to induce artificially RCD symptoms in cv. KP mango fruit. However, these experiments were conducted on developing fruit on mangoes trees using pure bacterial culture of *Pantoea agglomerans*. The farm used had a history of RCD fruit and they observed up to 40% RCD symptoms in control of untreated fruits. Furthermore, they sprayed the inoculum on fruits after wounding with sterilized sandpaper and accessed the RCD visually at eating ripe stage. The results from these inoculations were inconsistent and a number of causes, in addition to infection, were concluded to contribute to RCD.

Fruit quality of RCD fruits is a major concern of the Australian mango industry. RCD result in reduced economic profits by affecting fruit quality and marketability. However, it has been found that fruit having less than 12% dry matter content (DMC) at harvest exhibit more RCD symptoms and RCD infected fruit had low total soluble solids (TSS) (Macnish et al. 2014). Fruit peel and flesh colour, firmness, starch and acid content was non-

significant difference between RCD affected fruit and fruit without RCD symptoms (healthy fruits) (Hoffman et al. 2013b; Macnish et al. 2014). However, RCD occurs sporadically during the growing season, and remains unpredictable. Preliminary surveys of thousands of mangoes under different orchard conditions also confirm that it is difficult to detect RCD in green mangos, with symptoms more easily visible in ripe fruit. Therefore, there is a need to confirm the relationship of fruit quality with RCD fruit and to develop a consistent method to produce RCD symptoms for research purposes.

The aims of this study were to determine whether RCD is due to pathogens (transmissible agents) in cv. KP mango fruit. Development of a reliable artificial inoculation method and timing of susceptibility to transmission obtained from the different inoculation trials can then suggest the ways to prevent RCD. Moreover, the ability to reliably infect fruit with RCD allows better characterisation of the impact of RCD on fruit quality.

## 4.3 Materials and methods

Three experiments using cv. KP were conducted at the Berrimah Farm Northern Territory Department of Primary Industry and Resources (DPIR), Darwin, Australia as follows: 1) Method development to artificially inoculate mangoes with crude extracts of RCD infected fruit, 2) Characterisation of expression of RCD symptoms during ripening following artificial inoculation, and 3) Characterisation of RCD expression at three stages of fruit ripening [i. mature green at farm level, ii. after commercial ripening and iii. eating ripe at retail stage] as a means to determine the likely points in the supply chain when fruit could be infected along the supply chain.

### 4.3.1 General

#### 4.3.1.1 Plant material

Hard green mango fruit with 4-5 cm attached peduncle were harvested between 9 to 11 am from mature trees (>10 years old) grown at three locations in the Northern Territory (Australia) (Experiment 1: 12°26’17”S 130°50’28”E; Experiment 2: 14°28’0”S 132°16’0”E; Experiment 3:$12^0$ 35’S $131^0$18’E). The selected fruits were free from blemishes, defects and disease. Weather conditions (i.e. rainfall and temperature) supplementary data

Figure 4.8 & 4.9) prior to, and during harvest were typical of previous seasons. The mangoes were directly transported to the Berrimah Farm Laboratory in fruit bins within two hours after harvest. Fruits were not processed commercially, nor were they treated with Mango Wash® or commercial fungicides as per commercial practice but were kept to avoid cross-contamination by packing in separate boxes. However, they were physically desapped as per protocol described by (Holmes et al. 1992). Briefly, the fruit was positioned upside down on a metal mesh to allow the sap to drain away following removal of the stem.

#### 4.3.1.2 Inoculum preparation

The RCD inoculum was sourced from infected fruits which had visible RCD symptoms and been commercially shipped from Darwin to a wholesaler in Sydney (Figure 4.1). The RCD infected fruit were transported to Berrimah Farm on 19 September 2018 via courier within two days. Infected RCD fruit was cut into 2 cm x 2 cm segments using sterilised scalpel and blended into a homogenous slurry with sterile distilled water in a 3:1 ratio.

### 4.3.2 Method development for artificial inoculation

Desapped fruit (*n* = 90) harvested on 20 Sept 2018 were packed into nine boxes (10 fruits per box). The experiment was arranged as a complete randomised design, with each box representing a replicate.

On the day of harvest, three boxes were each subjected to one of the following three treatments: 1) uninoculated control (Control), 2) spray treatment whereby inoculum was sprayed thrice on each side of the fruit cheek using a sterile hand sprayer on one occasion (Inoculated spray) (Fig 4.1) ; 3) needle puncture treatment whereby inoculum was applied with a sterile injector comprising of six syringe injectors into stem end side of the fruit cheek penetrating 1-2mm in depth (Inoculated needle)

The mangoes were stored at 25±1 °C and 60-65% relative humidity (RH) and upon reaching eating ripe stage (after ten days of storage), the external surface of the mangoes was visually assessed for RCD symptoms (see Section 4.3.5.1). Latex gloves were changed to minimise cross contamination between each treatment. After inoculation, fruits were allowed to dry completely prior to packing in cardboard boxes. Boxes were enclosed with a polythene bag at 25±1 °C to maintain high humidity

conditions for bacterial growth. After 12 hours, bags were removed, and fruits were stored at 25±1 °C and 60-65% RH until ripe.

A B C

Figure 4.1: Artifical inoculation methods examined include A) source of Inoculum B) spray application and C) needle puncture application.

### 4.3.3 Characterisation of expression of RCD symptoms during ripening following artificial inoculation.

A total of 108 mangoes were harvested on 2 October 2018. The design was a completely randomised design with fruit (*n* = 9) assigned to one of two treatments: uninoculated control (Control) or spray application as explained in Experiment 1 (Inoculated spray).

After treatment, fruits were stored at 25±1 °C and 60-65% RH and on days 0 (before treatment) and 3, 6, 9, 12 and 15 day of the storage period, three fruit from each treatment/one replication were assessed for RCD rating on seed, flesh and peel and total soluble solids (TSS), firmness and flesh colour (see sections 4.6).

### 4.3.4 Characterisation of RCD expression at three stages of fruit ripening along the supply chain

A total of 360 mangoes (10 fruits per box) were harvested on 4 September 2018. The experimental design was a factorial split-plot randomised complete block design with three replicates (one box assigned as a replicate). The main factor was four inoculation treatments, and the subfactor fruit ripeness. The treatments included 1) Control spray (as experiment 1). 2), needle puncture control where sterile water was injected into the fruits using the same technique as explained for inoculated needle (Control needle), 3)

Inoculated needle (as experiment 1). There were three sub factor levels based on where fruit was sourced i.e. 1) packhouse to represent a mature green stage (R1), 2) post-ethylene treatment following commercial protocol to represent near ripe stage (R2), and 3) retail store to represent ready-to-eat stage (R3) (Figure 4.2). Harvested fruits were stored in ripening rooms (20 $^{o}$C and 90% RH) for three and six days prior to treatment for R2 and R3 treatments, respectively. During this storage period, fruits in these treatments were exposed to 10 ppm continuous ethylene for three days according to the commercial ripening protocol (Nguyen et al. 2000; Ho et al. 2016). All inoculation treatments were performed on a single day and subsequently, fruits were stored at 25±1 °C and 60-65% RH and allowed to ripen for assessment.

Ripeness stage R1 Ripeness stage R2 Ripeness stage R3

Figure 4.2: Different ripening stages of mango at harvest (R 1), 3 days ethylene treatment (R 2), 3 days ethylene treatment + 3 days storage (R 3)

After ten days of storage (inoculation), the fruit was cut open with a sharp knife and assessed for RCD rating in the seed, flesh and peel along with DMC, TSS and fruit peel colour (see section 4.5.3.1).

### 4.3.5 Fruit quality assessments

#### 4.3.5.1 RCD assessment

Fruits were visually assessed for RCD according to a rating scale developed by Macnish et al. (2015) where RCD score of 1 = 0-15%, 2= 15-30%, 3= 30-45%, 4= 45-70%, 5= 70-85%, 6= 85-100% RCD on the fruit surface (peel). The same scale was used for destructive assessments of RCD on fruit flesh and fruit seed. The frequency of RCD was

determined by calculating the percentage of RCD-infected fruit out of the total number of fruit for each treatment.

#### 4.3.5.2 Fruit peel colour visual assessment

Fruit peel colour is based on changes in percentage of yellow, which typically becomes higher as it ripens. The fruit peel colour was determined by a visual rating score based on % yellow where 1 = 0-10%, 2 = 10-30%, 3 = 30-50%, 4 = 50-70%, 5 = 70-90%, 6 = 90-100% (Holmes et al. 2009).

#### 4.3.5.3 Flesh colour

Flesh colour (three times on each side of the fruit) was measured with a Minolta Chroma Meter, model CR-400 (Minolta Corp, Ramsay, NJ USA), which provided L*(ranging from 0 for black to 100 for white), a* (degree of redness a+ or greenness a−), and b* (degree of yellowness b+ or blueness b−) values (setting D for daylight 6500$^{o}$C, using the CIE L*, a*, b* (Ayala-Silva et al. 2005; Leon et al. 2006).

#### 4.3.5.4 Fruit dry matter content and firmness

Dry matter content (DMC) was measured by using a handheld F-750 (Felix Instruments, Camas, WA) with wavelength range of 285 to 1200 nm. Fruit at room temperature were placed on the instrument lens at the setup mode to measure the spectrum parameters of fruit samples. The data was sorted and analysed using the F-750 data viewer software (v1.1.0.51) (Anderson et al. 2017). Flesh firmness was measured by TA.XT plus texture analyser (Stable Micro Systems, UK) with conical head probe of diameter 2 mm in compression mode. The start of penetration test was the contact of the probe and mango surface and the finish when the probe penetrated the tissue to depth of 5 mm with pre-test, test and post-test speed of 1.5, 1.5 and 10 mm/s, respectively. The 1 cm flesh slices, without peel, were cut with a cylinder and flesh firmness was measured as the maximum force in compression (Newton) recorded in a force–time curve obtained from a texture analyser during the compression of mango by the conical probe (Jha, SK et al. 2010).

#### 4.3.5.5 Fruit total soluble solids

A Digital Refractometer (Atago-PAL-1, Tokyo, Japan) was used for the measurement of total soluble solids (TSS). It was calibrated with demineralized water before actual

measurement. A drop of homogeneous juice was placed on the prism of refractometer and TSS (°Brix) was recorded directly from the digital screen of refractometer at room temperature (Jin et al. 2018).

## 4.4 Statistical analysis

Method development for the artificial inoculation and for the characterisation of expression of RCD symptoms during ripening following artificial inoculation were conducted according to a completely randomised design. The method for characterisation of RCD expression at three stages of fruit ripening along the supply chain was a factorial split-plot randomised complete block design. Data were subjected to analysis of variance (ANOVA) using Statistical Tools for Agricultural Research version 2.0.1 (http://bbi.irri.org). To normalise distribution, data were subjected to square root transformation to meet the assumption of ANOVA (Osborne 2010). Fisher's protected least significant difference post hoc tests were used to determine significant differences among treatment means. Analysed data average values were back transformed for interpretation.

## 4.5 Results

### 4.5.1 Method development for artificial inoculation

Resin canal discolouration (RCD) expression and severity in hard green mango cv. KP fruit was significantly higher ($P \leq 0.05$) in inoculated fruits, as compared to uninoculated fruit of control treatment at eating ripe stage (ten days after inoculation). However, the needle puncture and a surface spray inoculum treatment were not statistically different from each other, RCD severity was found to be slightly higher in spray inoculated fruits (Figure 4.3 B). In terms of a number of fruits (frequency) exhibited the any symptom of RCD was 100% RCD symptoms in both treatments. Moreover, 6.6% of RCD fruits were found in the control treatment (Figure 4.3 A).

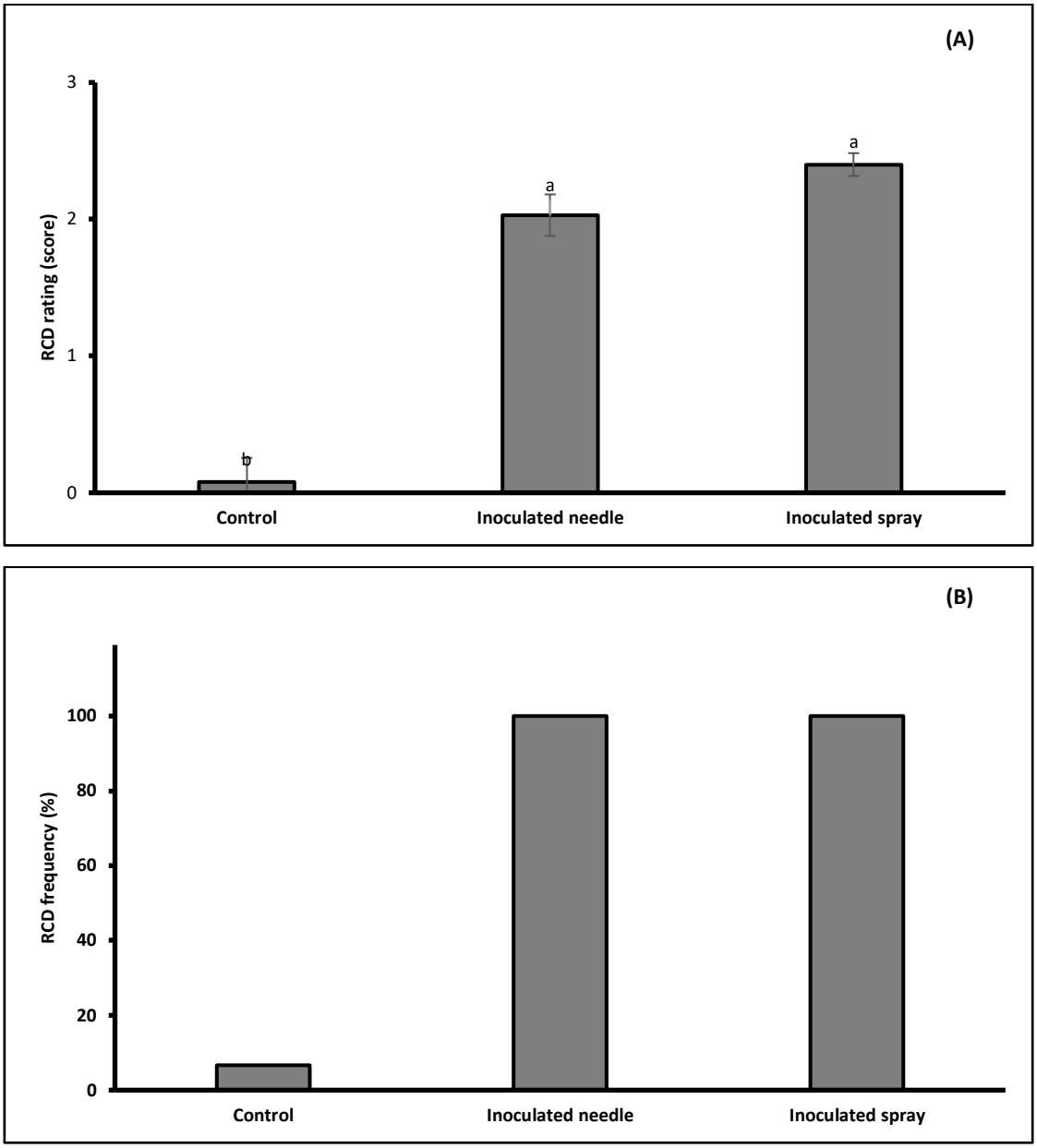



Figure 4.3: Effect of inoculation treatments on the incidence and severity of RCD in cv. KP mango (B), percentage of RCD fruits (A).

Vertical bars represent ± SE of means. n = 30 (10 fruit × 3 replications). Letters on the data points represent the difference between the treatments. Treatments not sharing letters differ significantly from each other (P≤0.05). Fruits were assessed at eating ripe stage (ten days after treatment).

### 4.5.2 Characterisation of expression of RCD symptoms during ripening following artificial inoculation

During fruit ripening, RCD expression and severity showed significantly increased ($P \leq 0.05$) in fruit subjected to inoculation treatments. However, the first visible RCD symptoms were recorded on the seed surface in fruits after six days of treatment, whereas in the flesh and peel it was observed from day 9 to 15. TSS and DMC were found to be significantly lower only at day six, and a non-significant difference was found in fruit firmness (Figures 4.5 A, B and C).

A rapid increase in RCD symptoms were recorded in fruit peel between day 12 and 15 over the assessment period. (Figures 4.4 A, B and C). Furthermore, 100% RCD symptoms was found in seed at day 6, also started increasing in mesocarp and peel along the ripening period (Figures 4.4 D, E and F). A non-significant relation was observed in fruit flesh colour, however, L*, a* and b* values were lower at day 15 (Figures 4.5 D, E, F).

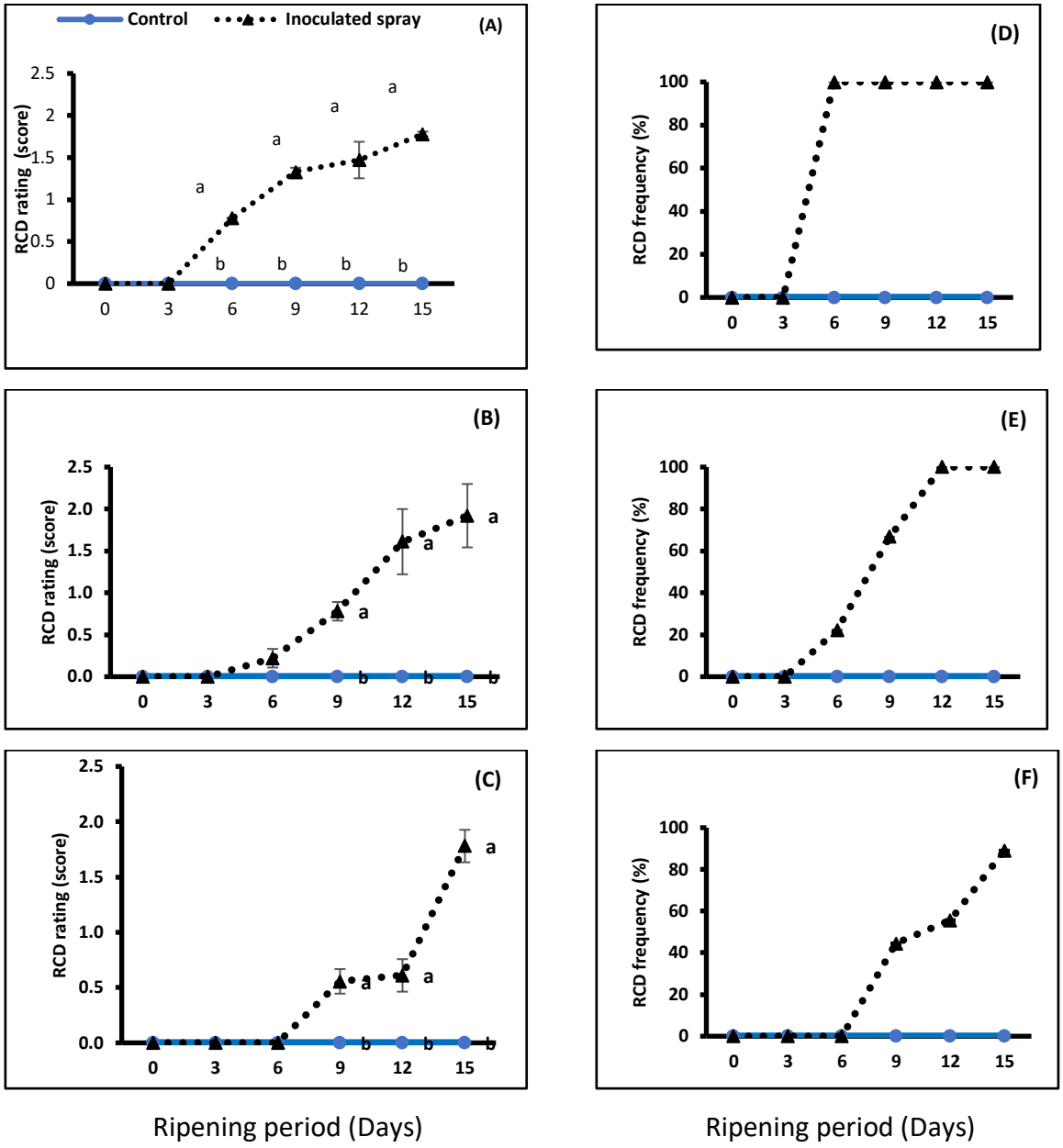



Figure 4.4: Incidence of RCD on seed (A), flesh (B) and peel (C) and percentage of RCD fruits on seed (D) and flesh (E) and peel (F) KP mango during ripening (Days).

Vertical bars denote SE of means. n=3. Letters on the data points represent the difference between the treatments. Treatments not sharing letters differ significantly from each other.

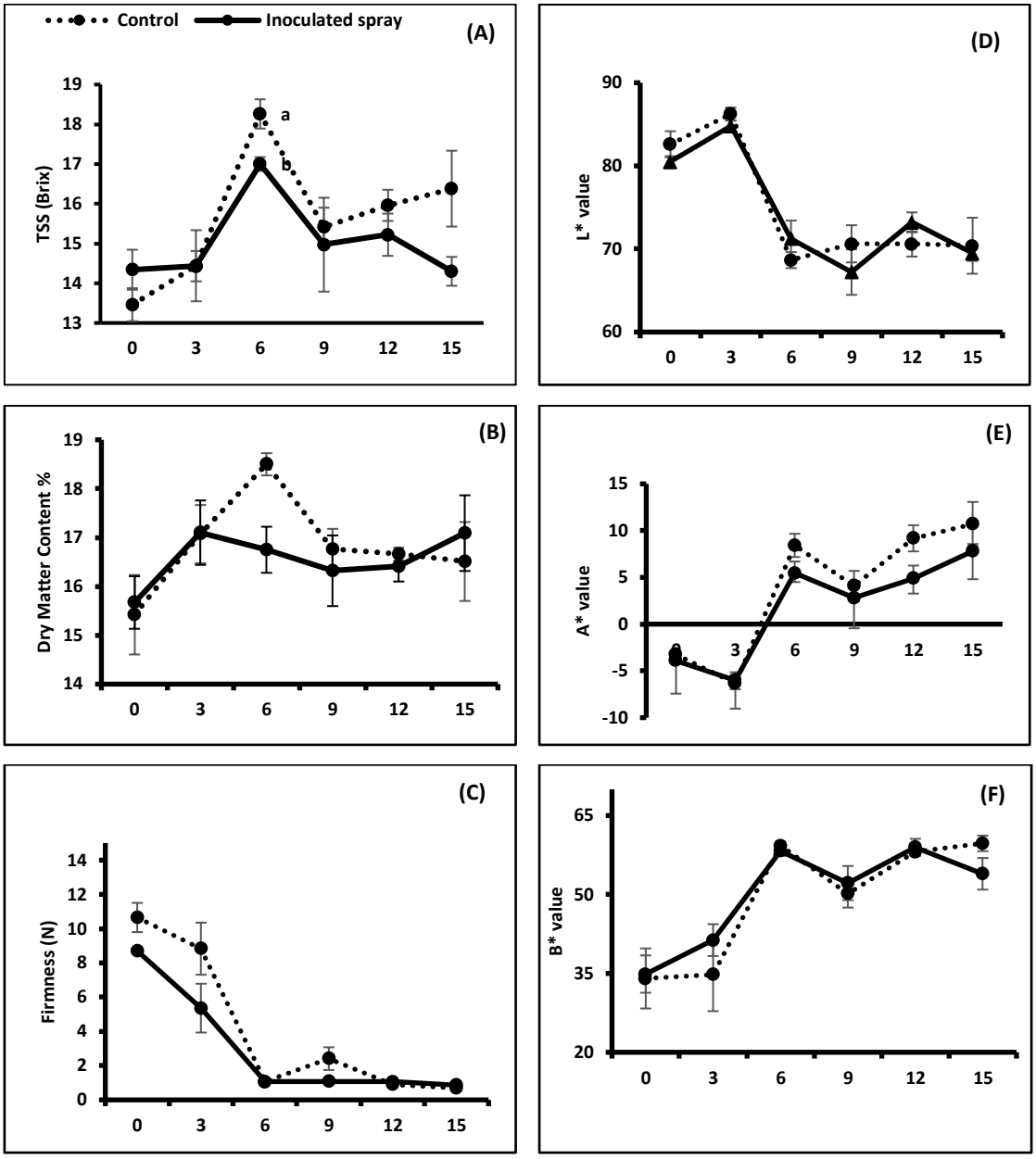



Figure 4.5: Changes of TSS (A), DMC (B), Firmness (C), Flesh colour L* (brightness) value (D), A* (green to red) value (E) and B* (blue to yellow) value (F) on RCD affected and control fruits KP mango during ripening.

Vertical bars denote SE of means. n=3, Letters on the data points represent the difference between the treatments. Treatments not sharing letters differ significantly from each other.

### 4.5.3 Characterisation of RCD expression at three stages of fruit ripening along the supply chain

Inoculation techniques and ripening stages significantly influenced RCD expression (Table 4.1). Inoculation treatments, surface spray and needle puncture, showed a significant effect on RCD severity and expression on the surface of mango with respect to different ripening stages ($P \leq 0.05$). However, 76% and 66% infected fruits found in surface spray and needle puncture treatments, respectively, whereas 21% and 4% RCD fruits were recorded in control fruits of needle puncture and surface spray fruits (Figure 4.6 A and C). The presence of RCD symptoms in uninoculated control fruit could be due to cross contamination. RCD severity and expression in mango flesh in treated fruits were also found to be significantly higher ($P \leq 0.05$) as compared with control fruits (Figure 4.6 B). About 20% higher number of fruits with surface spray treatment had RCD symptoms on mango flesh as compared with needle injected fruits (Fig 4.6 D).

RCD severity was significant high in the fruit peel (48%) in ripe stage R1 (Figures 4.7 B & D), whereas it was (52%) higher in the flesh of the ripe stage R1 (i.e. eating ripe stage) (Figures 4.7 A & C). There was a significant ($P \leq 0.05$) interaction between inoculation treatments and ripening stages for RCD severity on mango peel. In particular, at ripeness stage, R1, RCD severity was higher in the surface spray than in the inoculated needle, however, this response was the opposite at R3 (Table 4.3).

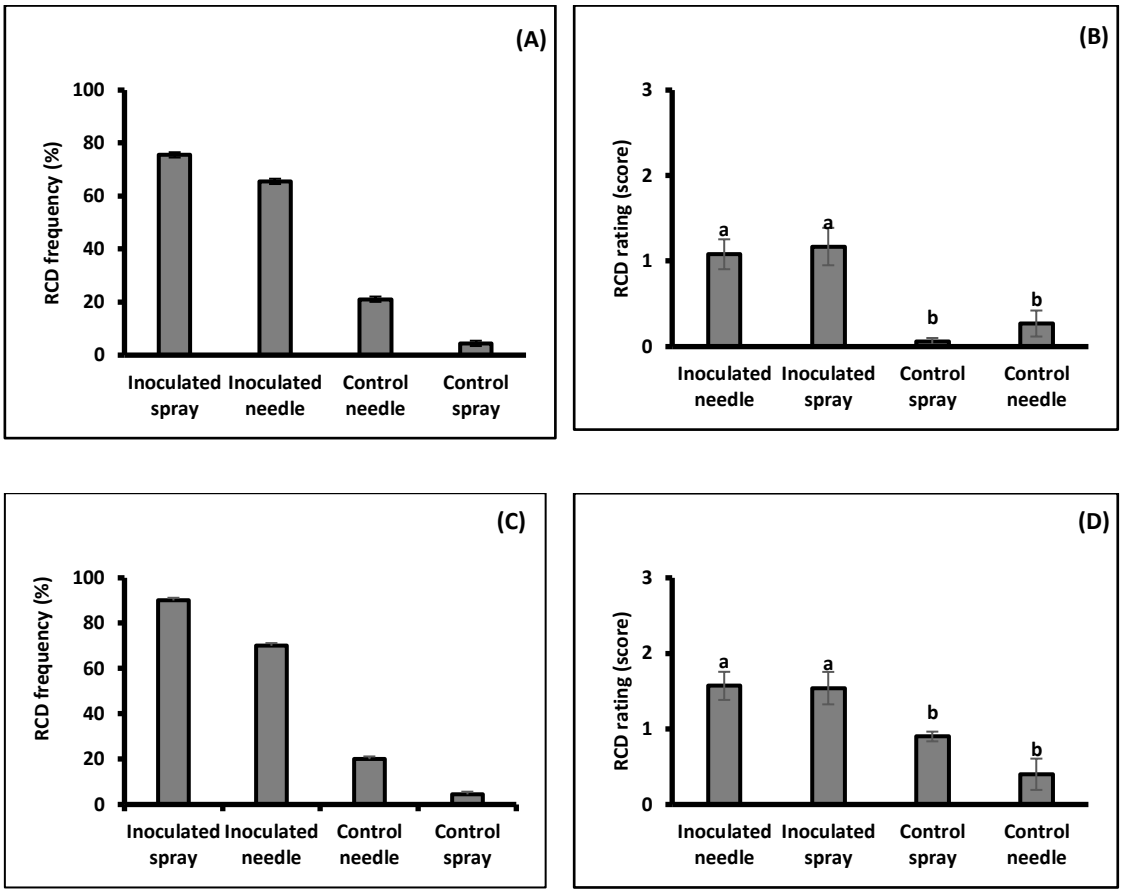



Figure 4.6: Effect of artificially inoculation treatments on the incidence and severity of RCD in cv. KP mango peel (B), flesh (D), percentage of RCD fruits having RCD on the peel (A), flesh (C).

Vertical bars represent ± SE of means. n = 30 (10 fruit × 3 replications). Letters on the data points represent the difference between the treatments. Treatments not sharing letters differ significantly from each other.

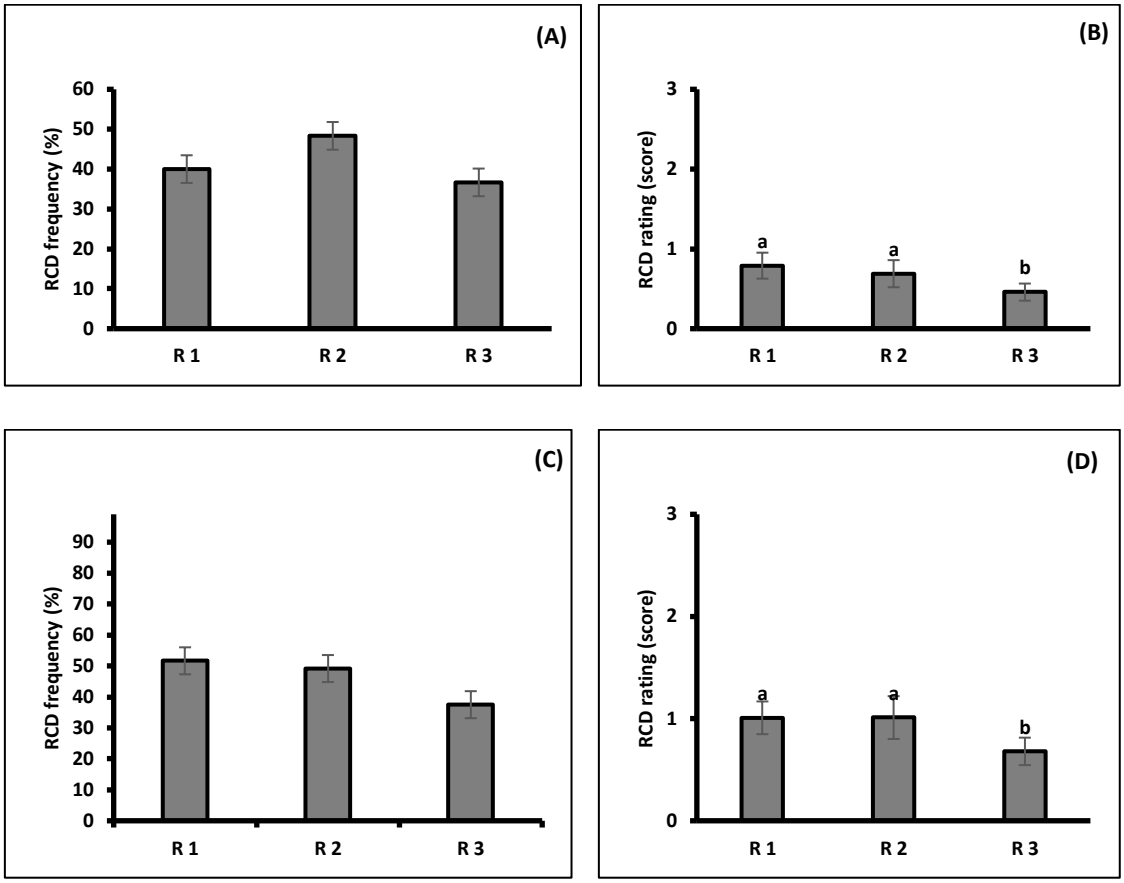



Figure 4.7: Effect of artificially inoculation treatments with respect to ripening stages on the incidence and severity of RCD in cv. KP mango peel (B), flesh (D), percentage of RCD fruits having RCD on the peel (A), flesh (C).

Vertical bars represent ± SE of means. n = 30 (10 fruit × 3 replications). Letters on the data points represent the difference between the treatments. Treatments not sharing letters differ significantly from each other.

#### 4.5.3.1 Physio-chemical changes in RCD fruits with respect to different ripening stages

Fruits with both inoculation treatments, surface spray and needle puncture had significantly ($P \leq 0.05$) lower TSS as compared to untreated fruits with respect to different mango ripening stages (Table 4.2). Whereas, a non-significant impact was recorded on other physio-chemical properties, (peel colour), dry matter content (DMC) and fruit firmness (Table 4.2). A significantly ($P \leq 0.05$) higher mean values of DMC and TSS was observed in ripe stage 1. however, peel colour and fruit firmness remained unaffected (Table 4.3).

Table 4.1: Two-way ANNOVA test of different fruit quality parameters measured after ten days of storage at eating ripe stage based on factors of inoculation and ripeness stage.

| **Parameters** | **Inoculation (I)** | **Ripeness (R)** | **I X R** |
|---|---|---|---|
| RCD severity on fruit peel | *** | * | ** |
| RCD severity on fruit flesh | *** | ns | ns |
| Mango peel colour | ns | ns | ns |
| DMC | ns | *** | ns |
| TSS | * | *** | ns |
| Fruit firmness | ns | ns | ns |

*, ns are significant and non-significant where, *= *P<0.05*, **= *P* <0.01, ***= *P* <0.001. I = Inoculation technique, R = ripening stage ; (R 1), 3 days ethylene treatment (R 2), 3 days ethylene treatment + 3 days storage (R 3). TSS = total soluble solids. DMC = dry matter content. All the parameters were measured at eating ripe stage after ten day of storage.

Table 4.2: Mean values of fruit peel colour, DMC and TSS mango fruit influenced by inoculation treatments

| Treatments | Mango Peel colour | DMC | TSS |
|---|---|---|---|
| | (score) | (%) | (°Brix) |
| Inoculated spray | 5.34 | 15.85 | 15.52bc |
| Inoculated needle | 5.46 | 15.96 | 15.11c |
| Control spray | 5.15 | 16.3 | 16ab |
| Control needle | 5.44 | 16.58 | 16.17a |

Mean values not sharing the same letter differ significantly from each other by Tukey's LSD test at P = 0.05. DMC = dry matter content. TSS = total soluble solids.

Table 4.3: Mean values of RCD score in fruit peel and interaction between inoculation methods and fruit ripening stages.

| Treatments | RCD severity on mango peel | | |
|---|---|---|---|
| | Ripeness stages | | |
| | R1 | R 2 | R 3 |
| Inoculated spray | 1.53a | 1.32a | 0.96b |
| Inoculated needle | 1.25b | 1.22a | 1.28a |
| Control spray | 0.79c | 0.74c | 0.71c |
| Control needle | 0.77c | 0.97b | 0.87bc |

Mean values not sharing the same letter differ significantly from each other by Tukey's LSD test at P = 0.05. R1 = ripeness stage 1. R2 ripeness stage 2. R3 = ripeness stage 3. (R 1), 3 days ethylene treatment (R 2), 3 days ethylene treatment + 3 days storage (R 3).

## 4.6 Discussion

Inoculation studies supported a primary role of transmissible agents (pathogens) in RCD expression. Homogenates from RCD infected fruit applied via surface spray or needle inoculation elicited characteristic RCD symptoms. To the best our knowledge this is the first study to reliably induce RCD. An inoculation method tested by Macnish et al. (2014) sprayed inoculum onto fruit after wounding with sterilised sandpaper recorded 40% RCD symptoms in control fruits. Macnish et al. (2014) inoculated fruit prior to harvest in a commercial mango orchard that had a history of RCD. In this study RCD severity was higher in surface spray fruit. The reasons for this response remain unclear but may be linked to the amount of inoculum applied which may be lower with the needle application. The development of reliable inoculation methods in this study permitted further investigations of RCD expression including progression of RCD development and the effect of fruit maturity on susceptibility to RCD expression.

Incidence and severity of RCD expression on the fruit peel was influenced by ripening stage. The higher incidence of RCD severity and expression in ripening stages 1 and 2 (at mature green and after commercial ethylene ripening) as compared to ripening stage 3 (eating ripe stage). This was a surprising result as we predicted a higher susceptibility and therefore incidence of RCD at ripening stage 3. Ripened fruit are characterised by a reduction in acidity and an increase in sugar content. These physiochemical changes usually promote bacterial growth (Rooban et al. 2016). In this case, the lower RCD severity on the peel at ripening stage 3 may be due to the short incubation time. Fruit at eating ripe stage was assessed 2 to 3 days after inoculation whereas the earlier stages were assessed 6 to 10 days after inoculation.

RCD symptoms first appeared around the seed 6 days after artificial spray inoculation. The symptoms then progressed to the flesh and peel from days 9 to 12 (Figure 4.4). The detection of symptoms on the seed is an unexpected result as no physical injury was applied by the inoculation method. It is therefore difficult to explain how the transmissible agent migrated/transferred the flesh surrounding the seed. The homogenate may have triggered a physiological response that led to RCD symptoms around the seed rather than being due to a direct transfer of the causal agent to the inner flesh. Further studies are needed to elucidate what induced this response.

The time study revealed that changes in physiological parameters of mango fruit were not directly associated with RCD symptom progression. While both DMC and TSS were significantly higher in RCD fruit on day 6 this was not sustained for the remainder of the ripening period when RCD symptoms increased. Previous studies have found much lower TSS in fruit with physiological disorders than in healthy fruit (Assis et al. 2004). Similarly, Katrodia (1985) observed the hydrolytic enzymatic activities in damaged fruit were three times lower than healthy tissues during ripening. Furthermore, Lima et al. (2001) monitored amylase activity, starch and reducing and non-reducing sugars contents and found much lower amylase activity and reducing and non-reducing sugars in spongy tissue affected fruits during ripening.

## 4.7 Conclusion

This study has confirmed the potential role of transmissible agents in RCD expression. Two artificial inoculation methods (surface spray and inoculated needle) were developed using RCD homogenate to reliably induce RCD symptoms. However, further studies are required to compare symptom progression between artificially induced and naturally occurring RCD. To our knowledge, this is the first study to confirm that RCD can be induced at all post-harvest ripening stages. These findings have important implications to the mango industry. In particular, management of RCD will require mitigation strategies that reduce contamination of the transmissible agent along the entire supply chain.

## 4.8 Supplementary Data

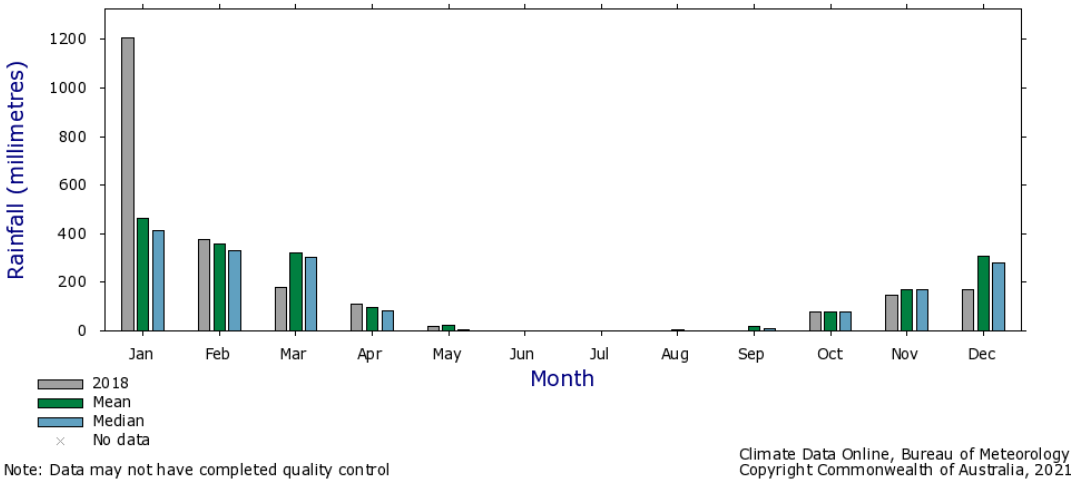



Figure 4.8 Average rainfall (millimeters) during fruit harvest in 2018

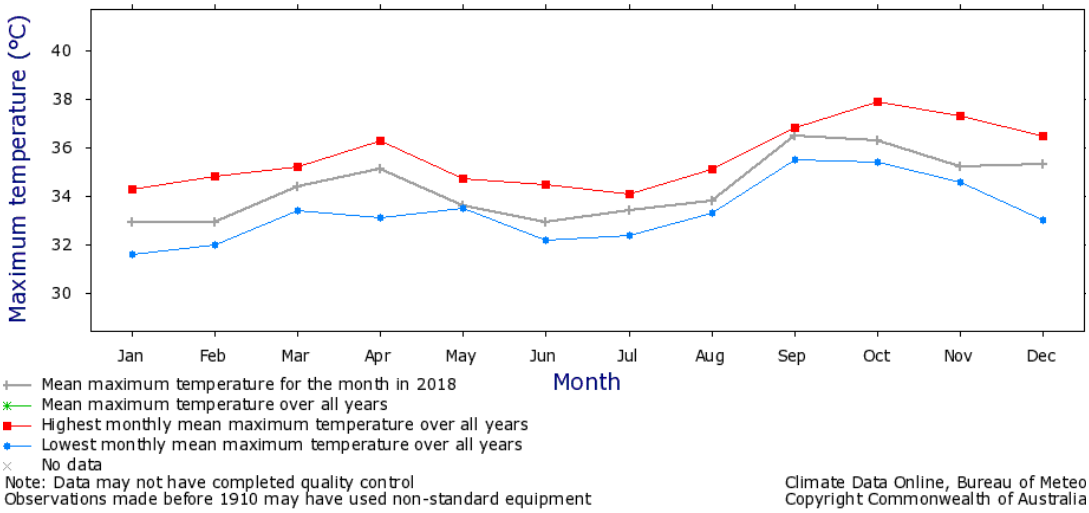



Figure 4.9 Maximum and minimum temperature during fruit harvest in 2018

# Chapter 5: Identification of bacteria and microbial communities associated with resin canal discolouration in mango fruit

## 5.1 Abstract

Resin canal discolouration (RCD) severely impacts the fruit quality of mango however, the role of bacterial pathogens as a causal agent remains unclear. Two approaches were utilised to elucidate the identity of bacteria associated with RCD. In the first study, multilocus sequence analyses of three genes, *rpo*B, *hsp*60 and 16S rRNA showed that the dominant genus present in RCD-infected fruit grown in Darwin, Australia was *Pantoea*. In a subsequent study, next-generation sequencing techniques (NGS) compared the overall microbial community composition of RCD-infected and control (visually healthy mango) fruit. Significant differences in bacterial and fungal communities of RCD affected and control fruit were observed. RCD-infected fruit was dominated by the bacterial genus *Tatumella* while the fungal community of RCD fruit was largely dominated by yeasts of the genera *Meyerozyma* (family *Debaryomycetaceae*) and *Naganishia* (family *Tremellaceae*). Overall, these results provide circumstantial evidence for a role of bacterial pathogens, potentially of more than one genus, in RCD development.

Keywords: Mango, resin canal discolouration, fruit microbiome, microbial communities

## 5.2 Introduction

Mango crop production can be limited by fungal and bacterial diseases. The most prominent fungal disease of mango fruit is anthracnose caused by *Colletotrichum gloeosporioides* followed by stem end rot (SER) mainly caused by members of the family *Botryosphaeriaceae*, such as *Neofusicoccum* sp., and *Lasiodiplodia theobromae* (Aguirre-Güitrón et al. 2019; Diskin et al. 2019; Ekanayake et al. 2019; Montecalvo et al. 2019; Shao et al. 2019). The most serious bacterial disease of mango fruit is bacterial black spot caused by *Xanthomonas campestris* pv. *mangiferaeindicae*. However, other bacterial diseases that affect the mango tree rather than the fruit include apical necrosis caused by *Pseudomonas syringae* pv. *syringae* and more recently by *Pantoea agglomerans* as identified in mango trees in Canary Island orchards (McMillan & Wang-Wong 1992; Lee & Tzeng 2006; Gutiérrez-Barranquero et al. 2019).

Bacterial pathogens are considered among the possible causes of RCD (Macnish et al. 2015). Macnish et al. (2014) isolated and identified strains of *Pantoea agglomerans* and *Enterobacter cowanii* from infected-RCD fruit based on 16S rRNA sequences. However, these species were also isolated from both intact symptomless fruit and in wash water used to de-sap fruit (Macnish et al. 2014). Moreover, identification on the basis of 16S rRNA gene analysis has recognised limitations at resolving phylogenetic relationships at the species level within a genus (Delétoile et al. 2009; Srinivasan et al. 2015). Therefore, there remains uncertainty on the role of pathogenic bacteria in causing RCD.

Multilocus sequence analysis (MLSA) has proven to be a more reliable approach for characterisation of bacterial species than 16S rRNA sequence technique (Brady et al. 2008). MLSA defines an isolate by comparison of sequences obtained from internal fragments of several housekeeping genes (Delétoile et al. 2009). Housekeeping genes such as *rpo*B and *hsp*60 have successfully been used for species identification of *P. agglomerans* and *Enterobacter cowanii* (Mollet et al. 1997; Hoffmann & Roggenkamp 2003; Kämpfer et al. 2005; Brady et al. 2008; Brady et al. 2009; Delétoile et al. 2009).

Given that RCD could potentially be caused by more than one or a combination of pathogens, a complementary approach for exploring this microbial association is by high throughput next-generation sequencing (NGS). NGS has been used for profiling microbial community structures for a range of fruit diseases of grapes, apples and olives (Liu et al. 2018; Gao et al. 2019; Hall & Wilcox 2019; Singh et al. 2019).

To the best of our knowledge, the microbial communities of mango fruit have only been reported in two previous studies. Diskin et al. (2017) characterised microbial communities colonising the stem end of green-unripe and fully ripe mango fruit stored at 5 or 12 °C. This study found SER to be correlated with increases in relative abundance of pathogenic fungal families *Pleosporaceae* and *Botryosphaeriaceae* and members of the bacterial family *Chitinophagaceae* which are known to be effective degraders of polysaccharide (Diskin et al. 2017). More recently, Galsurker et al. (2020) compared the microbial communities of mango fruit with or without a short stem by using internal transcribed spacer (ITS) amplification (~0.5 cm), and examined the incidence of SER and the associated microorganisms during cold storage (12 °C for 3 weeks) and during shelf life (20 °C for 7 days). Galsurker et al. (2020) found that SER was significantly lower in fruit harvested with a short stem than without one and this response was correlated with an increase in the abundance of the fungal pathogen families *Dothioraceae* and *Pleosporaceae*, and also members of the bacterial family *Sphingomonadaceae*.

The objectives of the research presented here included (i) isolation and identification of bacteria from RCD-infected fruit, and (ii) comparison of microbial communities of RCD-infected and control fruit. From this a causative agent may be revealed that could could eventually lead to the development of methods that may prevent or control RCD incidence.

## 5.3 Materials and methods

Experiments were conducted at the Berrimah Farm Science Precinct (hereafter referred to as Berrimah Farm), Northern Territory Department of Primary Industry and Resources (NT DPIR), Darwin (12°26'17"S 130°50'28"E), Australia.

### 5.3.1 Bacteria isolation and identification using mulitilocus analysis

In the 2018-2019 season, eighteen mango fruit Kensington Pride (KP) and R2E2 showing RCD symptoms were collected from a retail store, open market and commercial mango orchards in Darwin (Table 5.5). Fruit were transported to Berrimah Farm for analysis. Sections of fruit showing the symptoms of RCD (10 mm long) were sampled on fruit skin and flesh using a sterile surgical blade. All samples were immediately stored at -80 °C.

Following sample collection, the potential pathogens were isolated using a general tissue isolation method (Daub 1986). Infected fruit flesh (5 mm) were surface sterilized with 75% (v/v) ethanol solution for 3 min. After rinsing three times with sterile distilled water, the tissue was placed onto nutrient agar (NA) media in 90 mm petri plates (King et al. 1954). Plates were then incubated at 26-28 °C in an incubator for 48 hours. For each of the resultant cultures, single bacterial colonies were then re-streaked to new NA media plates for further growth with the objective of obtaining pure individual colony cultures for each sample. However, further studies only proceeded with three pure cultures labelled as Mu1, Mu2 and Mu3 (three replications each) as there were contamination issues with the remaining cultures.

Bacterial identification at the genus level was conducted by using universal bacterial 16S rRNA primers *Fd*1/*Rp*1 (Table 5.1). Briefly, single bacterial colonies were sub-cultured from the primary isolation culture, each individual colony were randomly picked by using a sterile disposable inoculating loop and agitated in 100 μL SDW in a 2 mL tube, and then 1 μL from the tube was transferred into microtubes and used directly for PCR assays. Each 50 μL PCR reaction contained 25 μL of MyFi 2×PCR master mix (Bioline,

Taunton, MA), 2 μM of forward and 2 μM of reverse primers, 22 μL SDW and 1-2 μL of template DNA 16S rRNA PCR assay parameters were initial denaturation at 95 °C for 1 min, followed by 35 cycles of denaturation at 95 °C for 45 s, annealing at 42 °C for 30 s, extension at 72 °C for 1 min, and final extension was at 72 °C for 5 min (Weisburg et al. 1991).

For species-level identification of cultures, Mu1, Mu2, Mu3, two housekeeping genes, beta subunit of RNA polymerase (*rpo*B) and heat shock protein 60 (*hsp*60) were selected – both bacterial markers have shown high specificity and sensitivity to allow for finer taxonomic resolution (Hoffman and Rogenkamp 2003) (Table 5.1). PCR conditions were as follows; for *rpo*B, initial denaturation was at 95 °C for 1 min, followed by 30 cycles of denaturation at 95 °C for 45 s, annealing at 57 °C for 30 s, extension at 72 °C for 1 min and final extension was at 72 °C for 5 min. Whereas for *hsp*60, similar conditions with *rpo*B PCR parameters except a different annealing temperature (59 °C) was used. Amplification products were run on a 1.5% (w/v) agarose gel electrophoresis along with 5 μL SYBR™ red dye (Invitrogen, Thermo Fisher Scientific, US), in 1 x TAE buffer at 100 V for 30 minutes and visualised and photographed under UV light. A 1.0 kb plus DNA Ladder (Invitrogen, Thermo Fisher Scientific, US) was run alongside the PCR products. PCR products were then purified using the PCR purification kit (Bioline ISOLATE II PCR and Gel Kit) according to the manufacturer's protocol. PCR products were sent for sequencing at the Australian Genome Research Facility using the same primers used for amplification.

The assigning of an isolate to a particular species was based on the percentage of sequence similarity with the *rpo*B and *hsp*60 genes from NCBI GenBank (https://www.ncbi.nlm.nih.gov/).

Table 5.1 List of primers used for characterisation of pure cultures, bacterial and fungal microbiome studies.

| Gene | Primer | Primer sequences | PCR product size (bp) | Reference |
|---|---|---|---|---|
| 16S rRNA | *Fd*1 | 5'-CCG AAT TCG TCG ACA ACA GAG TTT GAT CCT GGC TCA G-3' | 1200 | (Weisburg et al. 1991) |
| | *Rp*1 | 5'-CCC GGG ATC CAA GCT TAC GGT TAC CTT GTT ACG ACT T-3' | | |
| *rpo*B | *rpo*B-F | 5'-AAA AAC GTA TTC GTA AGG ATT TTG GTA A-3' | 1000 | (Hoffmann & Roggenkamp 2003) |
| | *rpo*B-R | 5'-CCA GCA GAT CCA GGC TCA GCT CCA TGT T-3' | | |
| *hsp*60 | *hsp*60-F | 5'-GGT AGA AGA AGG CGT GGT TGC-3' | 340-400 | |
| | *hsp*60-R | 5'-ATG CAT TCG GTG GTG ATC ATC AG-3' | | |
| 16S rRNA | *27F* | 5'-AGA GTT TGA TCM TGG CTCA G-3' | 300* | (Caporaso et al. 2010) |
| | *519R* | 5'-GWA TTA CCG CGG CKG CTG-3' | | |
| ITS 1 | *F* | 5'-CTT GGT CAT TAG AGG AAG TAA-3' | | |
| ITS 2 | *R* | 5'-GCT GCG TTC TTC ATC GAT GC-3' | | |

*= Sequence read length base pair (bp)

### 5.3.2 Microbiome analysis

For total genomic DNA extraction, samples were collected during the 2018-19 mango season. A total of 20 fruit (ten RCD and ten control fruit) were used for DNA extractions (Table 5.5). For DNA extraction, about 100-150 mg of previously frozen mango fruit sample were finely ground in a pre-chilled mortar and pestle to obtain a homogenized mixture. Genomic DNA was extracted using a Bioline Isolate II plant DNA kit following the manufacturer's protocol. DNA concentration was quantified using a Qubit® 3.0 fluorometer (Thermo Fisher Scientific, US) (Simbolo et al. 2013) . The DNA concentration was adjusted ( to minimum concentration 10 ng/μL) as required for the analysis.

Subsequent PCR amplification and sequencing using the V1 and V3 16S rRNA primers were performed by the Australian Genome Research Facility.

## 5.4 Bioinformatical and statistical analysis

In order to assess the diversity profile of bacterial and fungal communities, primers targeting the 16S rRNA gene and ITS regions were used (Table 5.1). Subsequent analysis of the sequence data was performed using the SEED pipeline (Větrovský et al. 2018). FastQ files were unarchived using 7zip v 19 (I. Pavlov,www.7-zip.org) and then joined with Fast1-join (Aronesty 2013) in SEED2 (Větrovský et al. 2018). Joined files were then filtered by PHRED score (minimum 8) and then clustered using U-PARSE/USEARCH v. 8.1.1861 (Edgar 2013), assuming an OTU radius of 97% similarity. FASTA files from this process were retained. Data were compiled into a single non-redundant sequence file using the aforementioned process and then classified using the Silva non-redundant 16S rRNA gene list (release 132, https://www.arb-silva.de/). The identified sequences were then converted into a local database using makeblastdp v. 2.6 (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The local database was then used to classify the retained individual FASTA files to create an OTU read abundance table for further analysis. The top two most abundant bacterial-sourced reads (ignoring plant-derived sequences) were placed into a phylogenetic tree using ngphylogeny.fr (Lemoine et al. 2019), selecting default parameters with the outgroup sequence from *Legionella pneumophila*. The tree was displayed in ITOL (Letunic & Bork 2019).

To assess bacterial and fungal community compositions, PRIMER6 and PERMANOVA+ (version 6.1.12 and version 1.0.2; Primer-E, Quest Research Ltd, Auckland, New Zealand) were used to conduct permutation multivariate analysis of variance (PERMANOVA) (Anderson et al. 2005). This analysis was performed for the 2018-2019 samples only as there were insufficient data for the 2017-18 samples. For the bacterial analysis chloroplast and mitochondrial reads were removed and sequence read data was organised at the lowest taxonomic level possible and was normalised for sampling depth, square root transformed, and a resemblance matrix created by calculation of Bray-Curtis coefficients. PERMANOVA was conducted using default settings with 9999 permutations; The PERMANOVA derived significance values were considered significant

when P < 0.01, while 0.01 < to P < 0.05 were considered only marginally significant. For ITS data the analysis was done identically.

## 5.5 Results

### 5.5.1 Identification of bacteria associated with RCD fruit

From the 16S rRNA, *rpo*B and *hsp60* sequence data, the three isolates (Mu1, Mu2, Mu3) obtained were identified as belonging to the genus *Pantoea* (Table 5.2, 5.3 and 5.4). This allows for sequence error and incompleteness. Strain Mu3 speciation in *Pantoea* could not be pinpointed exactly but was most close to uncultured and/or unspeciated *Pantoea* species. Isolates Mu1 and Mu2 were most similar overall to *Pantoea dispersa*.

A neighbour-joining phylogenetic tree was constructed using the data from isolate Mu2 (due to this strain having the best quality sequences) based on the housekeeping gene *rpoB* along with other related members of family *Enterobacteriaceae.* The tree revealed that the isolate formed a close cluster with representatives of 19 strains of *Pantoea dispersa* that have been genome sequenced and additional strains that have *rpoB* gene sequences (Figure 5.1). It must be noted that the tree only shows different sequences, Strains with identical sequences are included for the singe sequence homologs shown (e.g. WP_145886289.1, WP_021508205). These sequences include the *rpoB* from the genome sequenced *P. disp*ersa type strain CCUG 25232$^T$ (under accession code GCA_008692915.1) and also include sequences from bacterial symbionts of the brown-winged green bug *Plautia stali*. Interestingly, another sequence derived from *P. dispersa* LMG 2603$^T$ was more similar to *P. agglomerans* LMG 1286$^T$, which may suggest an issue in the disposition of the LMG 2603$^T$ strain since all other *P. dispersa* strains group closely together. The CCUG 25232$^T$ strain was derived from the LMG culture collection and as a result thus further clarification is required through sequencing of the original type strain culture deposited as ATCC 14589$^T$. Despite the aberrant position of type strains in the tree, it is fairly conclusive that isolate Mu2 clusters with the species *P. dispersa* (Figure 5.1). Further analysis is required to further confirm the speciation of isolates Mu1 and Mu3.

Table 5.2 Sequence similarities (%) of 16S rRNA sequence based on NCBI search of RCD mango fruit samples collected in Darwin during 2018-2019.

| Isolate | # | Blast Hits from GenBank | Similarities % |
|---|---|---|---|
| | 1 | MH767047 uncultured *Enterobacter* sp. | 95.4 |
| | 2 | KR029327 *Pantoea* sp. | 95.4 |
| Mu1 | 3 | KY882117 *Pantoea dispersa* | 95.3 |
| | 4 | KJ184895 *Erwinia* sp. | 95.4 |
| | 5 | AB273743 *Pantoea* sp. | 94.4 |
| | 1 | KX212185 uncultured bacterium | 99.7 |
| | 2 | EU931561 *Pantoea agglomerans* strain ZFJ-6 | 95.0 |
| Mu2 | 3 | KX179628 *Pantoea* sp. | 95.0 |
| | 4 | CP002433 *Pantoea* sp. | 93.6 |
| | 5 | KU867653 bacterium strain | 93.5 |
| | 1 | MK459433 *Pantoea* sp. | 98.6 |
| | 2 | MH767079 uncultured *Enterobacter* sp. | 98.0 |
| Mu3 | 3 | MK500861 *Pantoea dispersa* | 98.0 |
| | 4 | LC007797 gammaproteobacterium | 95.4 |
| | 5 | JN391535 *Pantoea* sp. | 95.4 |

Table 5.3 Sequence similarities (%) of *rpo*B gene sequences based on NCBI search of RCD mango fruit samples collected in Darwin during 2018-2019.

| Isolate | # | Blast Hits from GenBank | Similarities % |
|---|---|---|---|
| Mu1 | 1 | MH015165 *Pantoea brenneri* | 91 |
| | 2 | CP025799 *Dickeya zeae* | 90.9 |
| | 3 | CP017581 *Pantoea stewartii* subsp. *stewartii* DC283 | 90.6 |
| | 4 | CP001836 *Dickeya zeae* | 90.6 |
| | 5 | CP005991 *Enterobacter* sp. | 90.4 |
| | 6 | LMG 2603$^T$ *Pantoea dispersa* | 0.0 |
| Mu2 | 1 | MH015168 *Pantoea dispersa* | 99.4 |
| | 2 | MH015167 *Pantoea dispersa* | 99.4 |
| | 3 | CP009880 *Pantoea* sp. | 99.3 |
| | 4 | CP002433 *Pantoea* sp. | 93.6 |
| | 5 | CP011427 *Pantoea vagans* | 93.5 |
| | 6 | LMG 2603$^T$ *Pantoea dispersa* | 0.0 |
| Mu3 | | Found error in sequencing results | No data |

Table 5.4 Sequence similarity (%) of *hsp*60 sequences based on NCBI search of RCD mango fruit isolates collected in Darwin during 2018-2019.

| Isolate | # | Blast Hits from GenBank | Similarities % |
|---|---|---|---|
| Mu1 | 1 | LC007537 gammaproteobacterium | 99.3 |
| | 2 | MH015031 *Pantoea dispersa* | 98.7 |
| | 3 | LC007583 gammaproteobacterium | 98.7 |
| | 4 | LC007455.1 *Pantoea dispersa* | 98.3 |
| | 5 | CP045216.1 *Pantoea dispersa* | 98.0 |
| | 6 | LMG 2603$^{T}$ *Pantoea dispersa* | 0.0 |
| Mu2 | 1 | LC007537 gammaproteobacterium | 99.3 |
| | 2 | MH015031 *Pantoea dispersa* | 98.7 |
| | 3 | LC007583 gammaproteobacterium | 98.7 |
| | 4 | LC007554 gammaproteobacterium | 98.7 |
| | 5 | MH781991.1 *Pantoea* sp. | 97.0 |
| | 6 | LMG 2603$^{T}$ *Pantoea dispersa* | 0.0 |
| Mu3 | | Found error in sequencing results | No data |

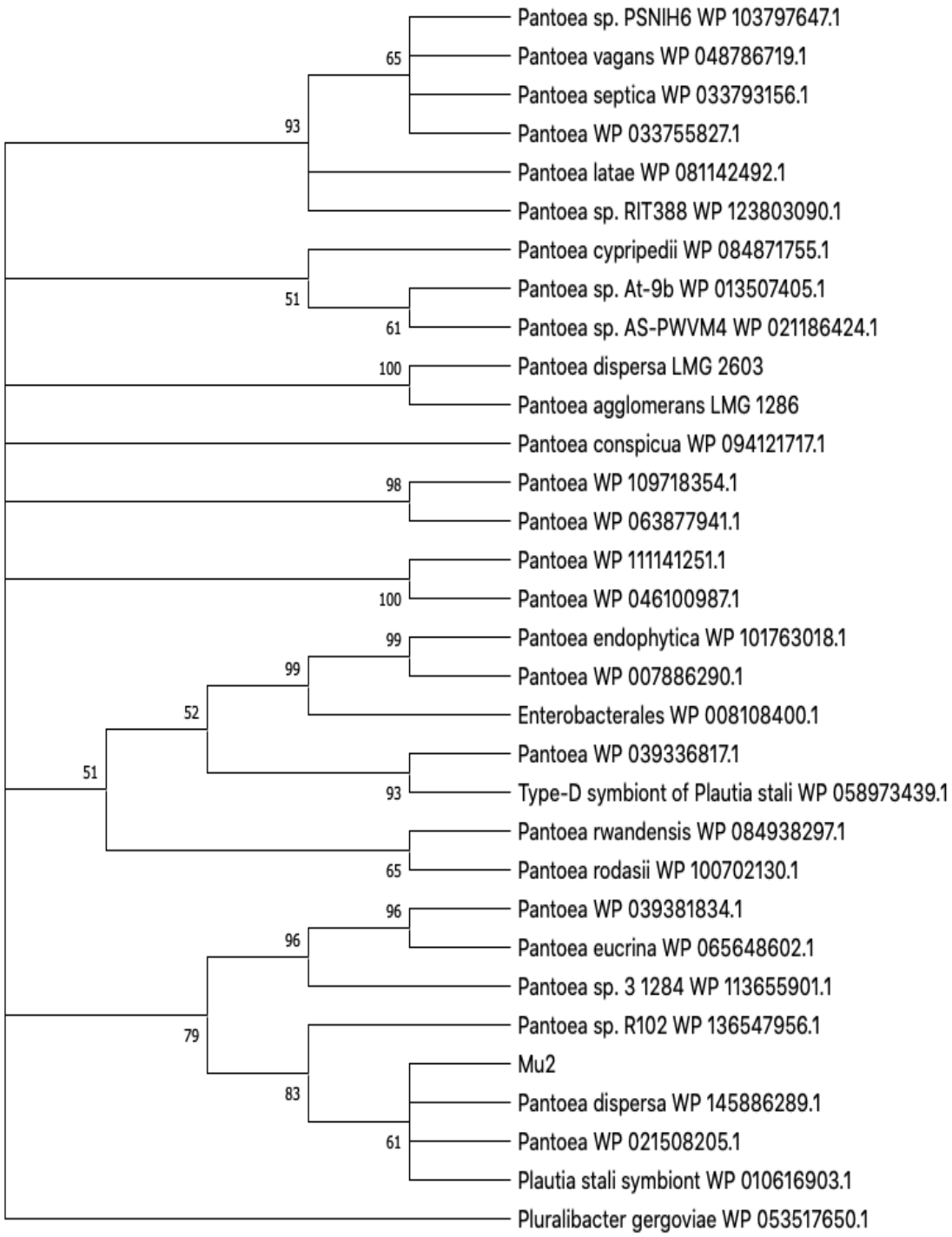



Figure 5.1 Neighbour joining tree based on the nucleotide sequences of *rpo*B of Mu2 and 27 other *rpo*B sequences. Percentage bootstrap values are based on 1000 replicates. *P. dispersa* (LMG 2603$^{T}$) and *P. agglomerans* (LMG 1286$^{T}$) are the type strains. *P. dispersa* CCUG 25232T is under sequence WP_021508205.1. *Pluralibacter gergoviae* WP 053517650.1 was used as the outgroup for the tree.

### 5.5.2 Determination of bacterial community make-up from RCD and control fruit

After removal of mango chloroplast and mitochondrial reads a total of 193811 16S rRNA sequence reads were obtained from RCD and control fruit combined. For control fruit, chloroplast and mitochondrial reads vastly dominated (99.83-99.98%) and includes only 416 bacterial reads (11 to 136 reads per sample). For RCD fruit, the average number of bacterial reads per sample was 19339 (4250 to 36929 reads per sample) representing 5 to 42% of the total sequences from these samples. This major difference is strongly suggestive that the bacterial load was much higher in the RCD fruit while the plant background DNA swamped the bacterial 16S rRNA gene contributions in control fruit. To compare the data, it was necessary to normalise and transform the data with the caveat that this may be distorting in downstream analyses.

The average number of OTUs was higher in the RCD fruit with an average of 18 ± 4 OTUs (range 14 to 23 across sample) compared to the control fruit which had an average of 9 ± 4 OTUs (5 to 12 OTUs across samples). There were 59 bacterial OTUs collectively. These OTUs were classified mainly only to the genus level due to lack of resolution with the primer set, especially amongst members of the order *Enterobacterales*.

### 5.5.3 1.5.3. Determination of relationships between RCD and control fruit

PERMANOVA and PCoA analyses both indicated that the bacteria community of RCD-infected fruit was highly significantly different to control fruit (PERMANOVA test, P=0.0001 F=20.518; Figure 5.2A). To allow for bacterial read number discrepancy through sampling normalisation two analyses were obtained. The first included all the data while the second analysis included a rarefaction based normalisation, performed by removing the least abundant OTUs (bottom 50% in terms of relative read abundance) from the RCD samples. In conclusion these data provided very similar PERMANOVA outcomes (data not shown) and thus the full data analysis is only shown here. In the PCoA plot of the bacterial community, most of the samples collected from control fruit

were clustered in the upper left side of the graph. For RCD fruit the microbial composition was different located on the opposite side of the graph (Figure 5.2A). This difference is mainly defined by axis PCO1 in which the majority (55.8%) of the variation is explained.

Similarly, the fungal community of RCD-infected fruit differed from control fruit, however one RCD sample overlapped with the control samples resulting in a marginally significant result (PERMANOVA test, P=0.0409 F=2.2986; Figure 5.2B).

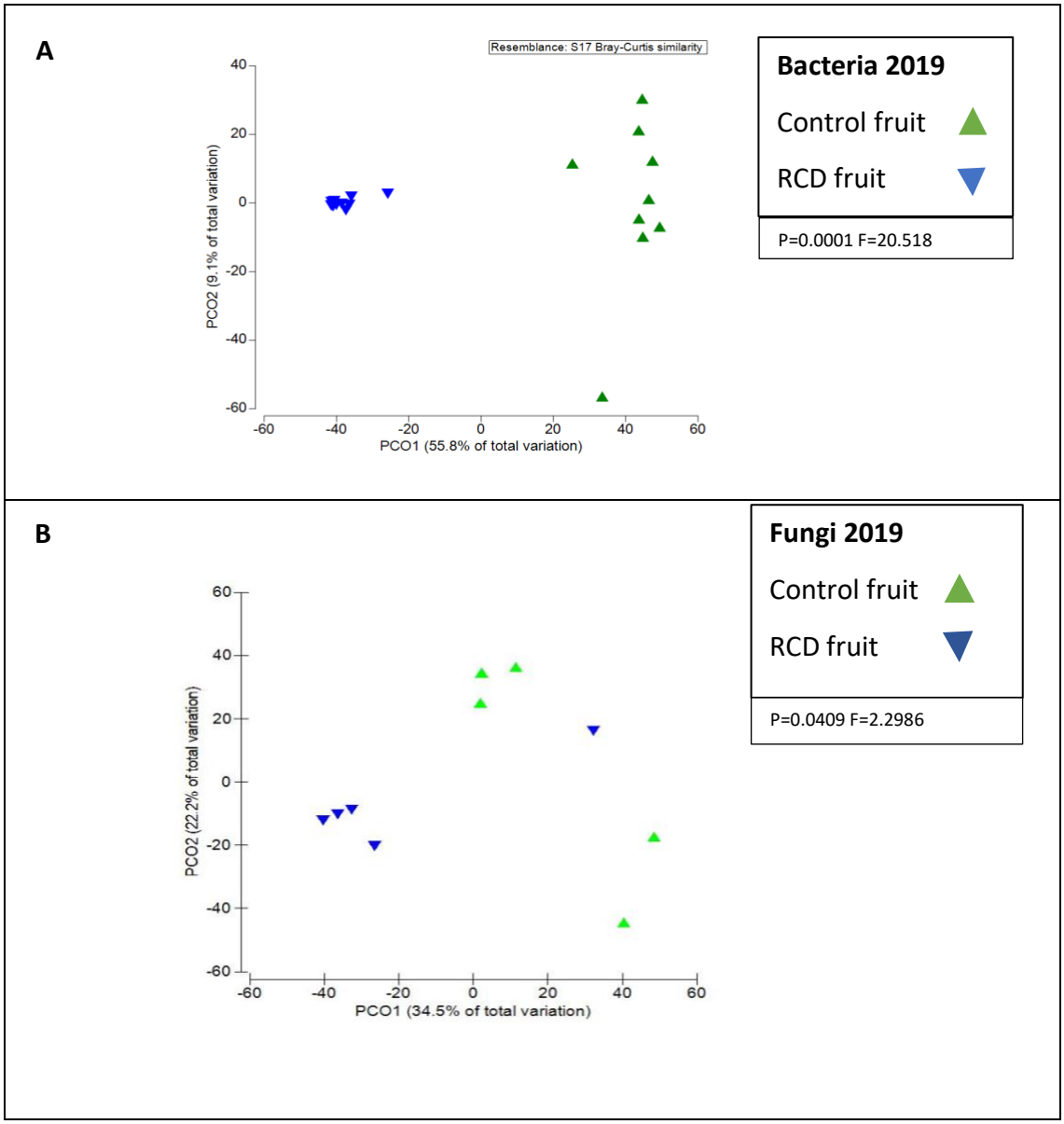



Figure 5.2 Principle component analysis of the bacterial community structure determined from taxa classifications derived from 16S rRNA (A) and ITS sequence analysis data (B). Comparisons are shown between control and RCD fruit for 2018- 2019 samples (n= 10). The classification of replicate data treatment for each of the community components was assessed using PERMANOVA.

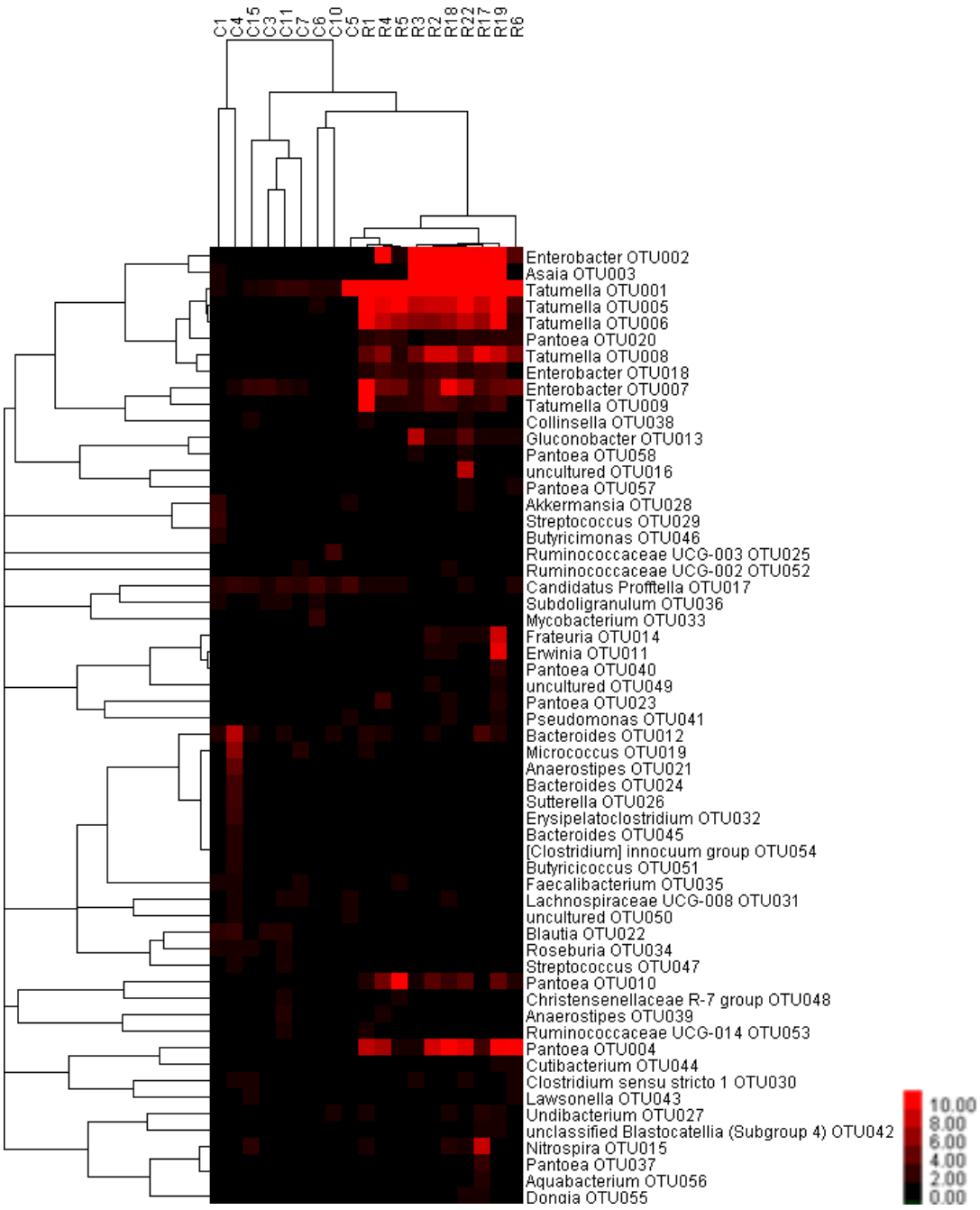



Figure 5.3 Heat map of the relative abundance of bacteria taxa of control (C) and RCD (R) fruit (n = 10) drawn using the program Java TreeView (Saldanha 2004). The colour bar in the bottom right side of the heat map indicates the percentage of given taxa present in the samples. The cluster analysis on the left is based on unsupervised complete linkage and un-centred correlations using Cluster 3 (De Hoon et al. 2004).

The sequencing results revealed that the most relatively abundant OTU from the RCD fruit samples (n=10) was *Tatumella* OTU001, which was found in all RCD samples at high proportions (median 91.5%, range 76.2-98.5% of reads). Most control fruits also had

detectable levels of *Tatumella* OTU001 but it was proportionally less abundant with a median abundance of 16.7% (range 0 to 93.5%). A penalised *t*-test of 16S rRNA gene relative abundance (Boorsma et al. 2005) indicated that *Tatumella* OTU001 was much more predominant in the RCD fruit compared to the control fruit (p=0.0005). Another 14 OTUs (*Enterobacter* OTU002, OTU007, OTU018; *Asaia* OTU003; *Pantoea* OTU004, OTU010, OTU020; *Tatumella* OTU005, OTU006, OTU008, OTU009; *Bacteroides* OTU012; *Gluconobacter* OTU013; *Candidatus* "Profttella" OTU017) were present in the majority of RCD samples (5 or more out of 10) (Figure 5.3 and Table S5.2) with abundances varying between samples. Only *Tatumella* OTU005 showed significant differences as found for OTU001 (p=0.003) though was much less abundant (median 0.2% in RCD fruit, not detected in the controls). Given the discrepancy in sampling, only the OTU001 results seem credible. Besides OTU001, the control fruit showed a relatively higher abundance of OTU017 compared to RCD fruit though this was at the margins of significance (p=0.04) OTU017 sequences are similar to that the citrus aphid symbiont *Candidatus* "Profttella". This OTU was present in all control samples but only sporadically detected in RCD samples.

The neighbour-joining phylogenetic tree showed that the OTU001 grouped with the species *Tatumella terrea*. The other *Tatumella* OTUs (OTU005, OTU006, OTU008, OTU009) also grouped with this species as well (data not shown). By comparison, the next most common OTU in RCD fruit, *Pantoea* OTU002, seems more novel and most closely grouped with various *Pantoea* species and not *Enterobacter.* The classification of bacteria in the *Enterobacter-Pantoea-Erwinia* group is problematic due to a confusing and dynamic taxonomy. Furthermore, the sequences obtained are partial therefore, classifications should be taken with some caution (Figure 5.4). The analysis of RCD fruit from a microbiological point of view requires further work that is not in the scope of this study, such as genome sequence data and/or metagenomics.

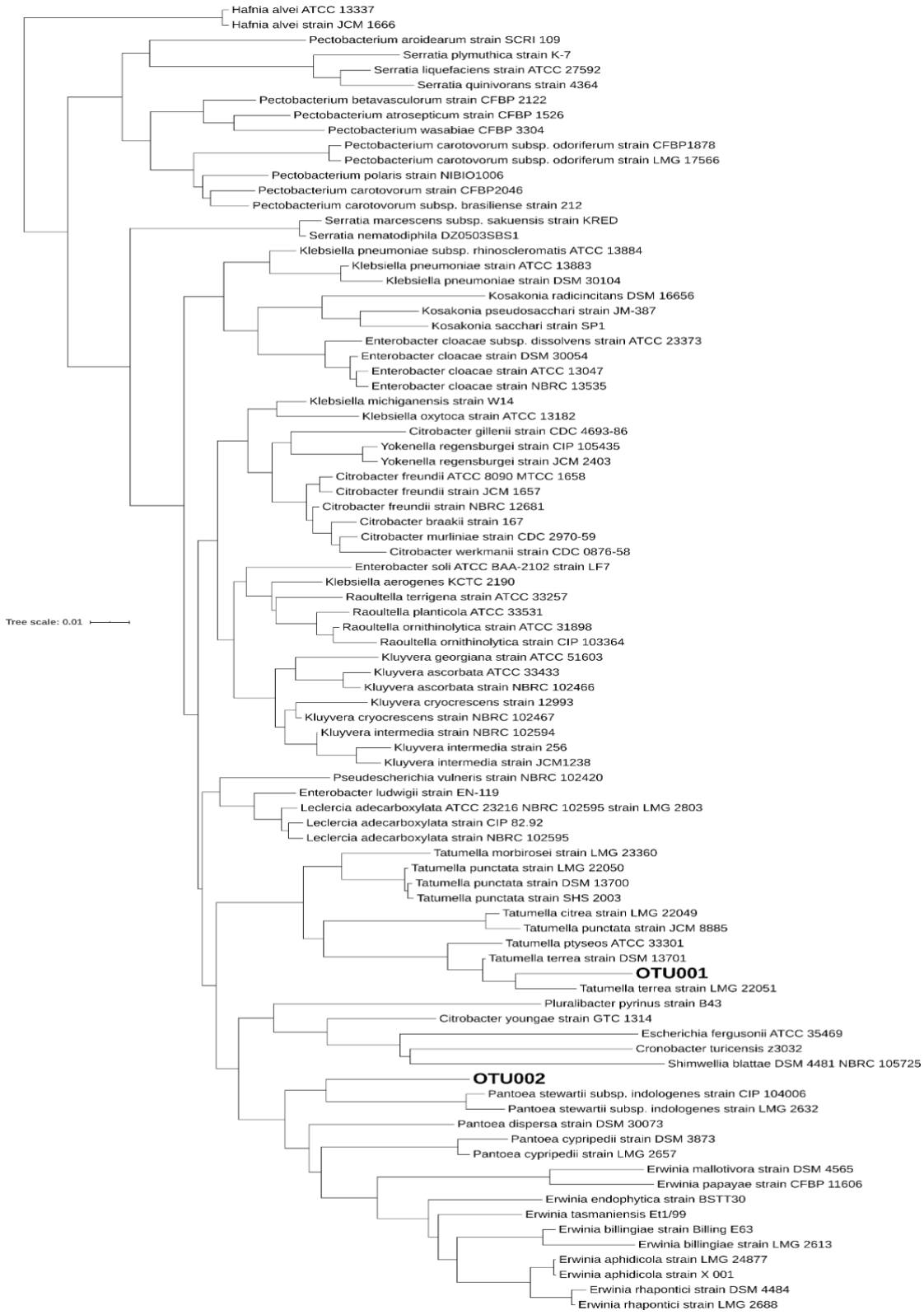



Figure 5.4 Phylogenetic tree derived from 16S rRNA gene sequence data showing the positions of the OUT1 within the genus *Tatumella* and OUT2 found within the *Pantoea* genus groups.

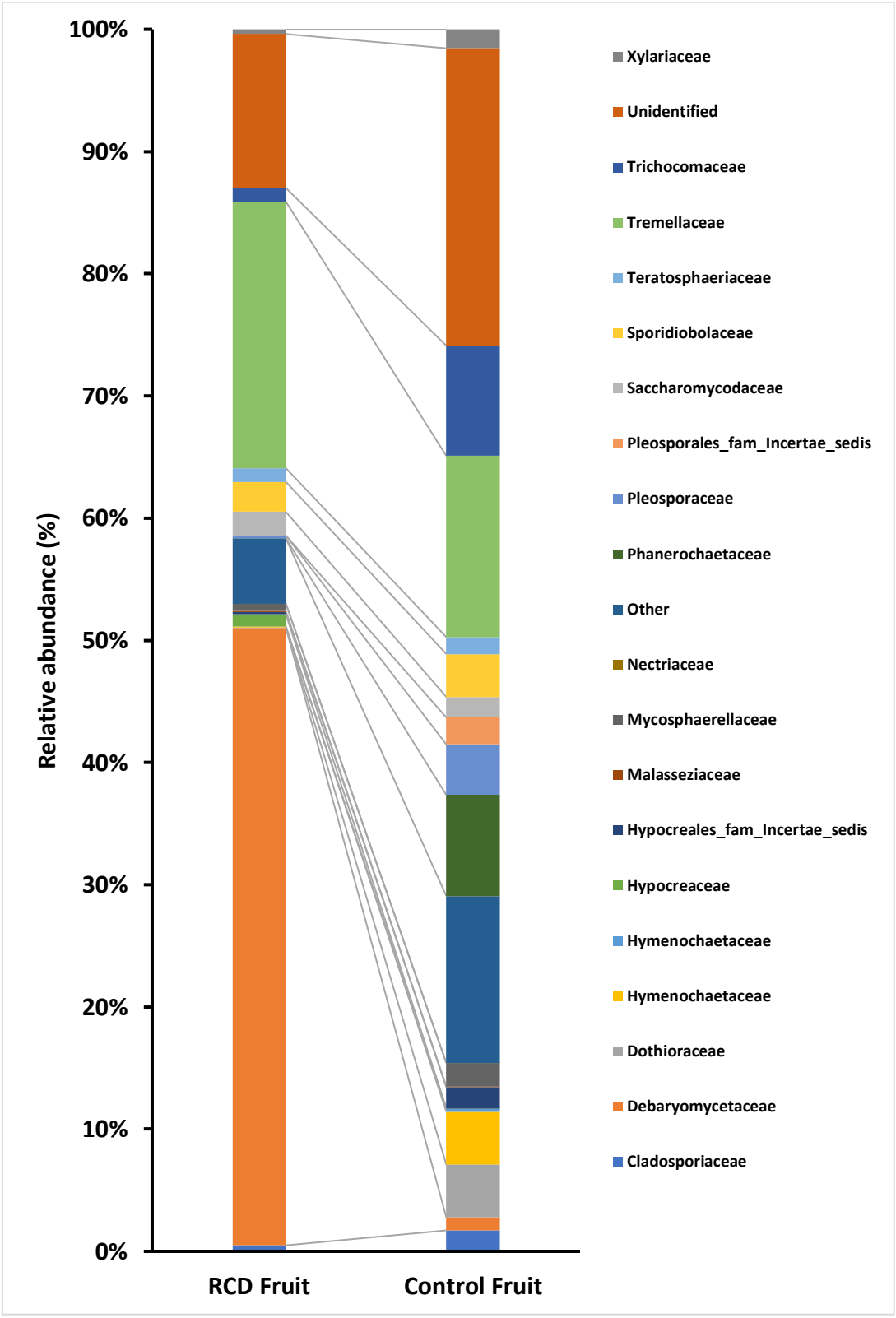



Figure 5.5 Relative abundance of common fungal families in control and RCD infected fruit sampled during 2018-2019, n=10. The uncommon fungal families are mentioned as “other” and unclassified data is mentioned as “unidentified”.

For the 2018-2019 samples, taxonomic assignment of ITS sequences indicated that, for four of the five RCD fruit samples analysed yeasts of the genera *Meyerozyma* (family *Debaryomycetaceae*, range 2.1-77.6% of ITS reads) and *Naganishia* (family *Tremellaceae*, <0.1-37.1%) largely dominated the fungal community present. *Meyerozyma* was mainly found at very low levels in the control fruit except for one sample where *Meyerozyma* reads made up 6.3% of the total. *Naganishia*, formerly of the *Cryptococcus albidus* clade (Liu et al. 2015), was found in high proportions in 3 out of the 6 control fruit samples analysed. In most RCD fruit *Naganishia* and *Meyerozyma* when combined represented most reads (72.2-99.9% of the total). One RCD sample had a more diverse range of predominating fungi including both ascomycetes and basidiomycetes. Amongst the ascomycetes these included *Catenulostroma* (*Teratosphaeriaceae*), *Aspergillus* (*Trichocomaceae*), *Debaromycetaceae* and unclassified *Helotiales* and *Pleosporales*. Basidiomycetes present included yeast-like genera *Rhodotorula* and *Rhodosporidiobolus* (*Sporidiobolaceae*, *Sporidiobolales*). By comparison, control samples had quite diverse fungal patterns with *Aspergillus*, *Aureosporidium* (*Dothioraceae*), *Phanerochaete* (*Phanerochaetaceae*), *Naganishia*, and unclassified *Pleosporales* found in 3 or 4 of the samples, but no individual group was present in all samples. Members of family *Botryosphaeriaceae* were detected at only low levels (1.2% of reads) in one RCD fruit, at trace levels (<0.01% of reads) in a second RCD fruit, and at trace levels in one control fruit sample.

## 5.6 Discussion

During a growing season when RCD incidence was low, the discovery of mango fruit showing clear symptoms of RCD provided a valuable opportunity to commence studies on the identification of microorganisms present in RCD fruit. Multilocus analysis using specific genes *rpo*B and *hsp*60 identified *Pantoea* as the most frequently identified bacterial genus amongst the three isolates. However, Blast searches within NCBI search, showed *P. dispersa* did not match with the type stains *P. dispersa* (LMG 2603$^{T}$) (Table 5.3). Previously, *P. agglomerans* and *Enterobacter cowannii* was reported in RCD fruit (Macnish et al. 2015). However, the sequences of the bacterial isolates from (Macnish et al. 2015) was not available in the NCBI GenBank (https://www.ncbi.nlm.nih.gov/) and could not be included in our phylogenetic studies. *Pantoea* spp. have been previously

reported to cause diseases in different fruit such as citrus (Soto-Muñoz et al. 2015), pome (Torres et al. 2011; Soto-Muñoz et al. 2014) and stone fruit (Torres et al. 2014), melon (Bruton et al. 1991) and pear (Lindow et al. 1998). More recently *P. agglomerans* that was isolated from mango tree (leaves, stems and immature fruit) showing necrotic symptoms was confirmed to be the causal agent of bacterial necrotic disease via pathogenicity tests (Gutiérrez-Barranquero et al. 2019). The phylogenetic analysis based on the housekeeping gene, *rpo*B revealed that the *Pantoea* isolate shared less than 40% sequence similarity with type strains *P. dispersa* (LMG 2603$^{T}$), however, this type strain was almost similar to *P. agglomerans* (LMG 1286) (Figure 5.1). Further studies are recommended for verification of identified species as potential causal organisms.

The microbiome analysis showed that the average number of bacterial reads in the control fruit was low with only 416 reads per samples compared to 19339 reads per sample in RCD fruit. These results suggest the bacterial load in the control fruit was very low, which appears reasonable given that the fruit was intact and thus protected from microbial contamination. However, future work should also assess the total viable count of control fruit using a standard culture technique to unequivocally confirm this. Further, control fruit was swamped with mango DNA, which can occur following milling and physico-chemical lysis of plant material (Lucaciu et al. 2019). This co-amplification may have contributed to this low number of bacterial reads and future studies should aim to minimise contributions of plant DNA by optimising primer choice or by blocking plant genes (Powell et al. 2012).

The PERMANOVA and PCoA analyses both indicated that the bacteria community of RCD-infected fruit was highly significantly different to control fruit. The most abundant genus was *Tatumella* (median 91%) in RCD fruit. *Tatumella* has been previously reported from one sample out of 77 RCD-infected mango fruit collected from Northern Territory (Macnish et al. (2015). Furthermore, *Tatumella ptyseos* as well as *Pantoea citrea* have been reported as a causative agent of pink disease in pineapple (Marín-Cevada et al. 2010). Other bacteria genera found in RCD fruit were *Enterobacter* and *Pantoea*.

The dominant presence of *Tatumella* in all RCD fruit samples suggest that this bacterial genus is potentially linked with the incidence of RCD. However, this result is unexpected and contrasts with the finding from the current multilocus sequence study and from

previous studies that identified *Pantoea* as the potential causal agent of RCD. *Tatumella* is a close relative of *Pantoea* (Marín-Cevada et al. 2010) and some members of *Pantoea* including *Pantoea citrea* were transferred to the genus *Tatumella* in 2010 (Brady et al. 2010). These results suggest that RCD may be associated with more than one bacterial genus that, nonetheless, have similar phenotypic and genetic characteristics. However further studies are required to explore this further.

The fungal community of RCD-infected fruit differed from control fruit. A total 19 fungal families were found common in both RCD and control fruit. However, control fruit had a more diverse fungal community as compared with RCD fruit. The fungal community of RCD fruit was largely dominated by yeasts of the genera *Meyerozyma* (family *Debaryomycetaceae*) and *Naganishia* (family *Tremellaceae*). *Meyerozyma* is a skin dwelling ascomycetous yeast, the anamorphs of which are classified as *Candida* species. *Naganishia*, formerly of the *Cryptococcus albidus* clade has been previously identified in other fruit (Piombo et al. 2020).

Diskin et al. (2017) studied the microbiome diversity of mango fruit with stem end rot (SER) and identified *Pleosporaceae, Dothioraceae, Davidiellaceae*, and *Botryosphaeriaceae* as the four prominent fungal families associated with mango SER. Members of these families have been identified as endophytes which can switch from endophytic colonization to necrotrophic stage to cause disease (Litz 2009; Galsurker et al. 2020). This study detected only trace or very low levels of members of *Botryosphaeriaceae*, *Dothioraceae* and *Pleosporaceae*, indicating that these potential fungal pathogens are not linked with RCD expression.

The important fungal families observed in the control fruit includes, *Trichocomaceae*, *Teratosphaeriaceae*, *Sporidiobolaceae*, *Xylariaceae, Pleosporales*, *Phanerochaetaceae*, *Cladosporiaceae*, *Pleosporaceae*, *Dothioraceae Mycosphaerellaceae* and *Hypocreales*. The *Trichocomaceae* is an indicator of increased decay processes and contains most familiar pathogenic fungi such as *Penicillium* and *Aspergillus*. *Teratosphaeriaceae, Sporidiobolaceae* and *Xylariaceae* recognised as endophytes and reported in plant and human pathogens (Crous & Groenewald 2011; Adams et al. 2013; Kemler et al. 2013; Schaeffer et al. 2017; Urbina & Aime 2018). *Pleosporales* and *Phanerochaetaceae* are reported as causal organism of important agricultural crops such as leaf blight on maize,

glume blotch on wheat and stem canker in cabbage (Kruys et al. 2006; Zhang et al. 2009; Justo et al. 2017). Whereas *Cladosporiaceae* family causes the tomato leaf mould disease (Deshmukh & Rai 2005). The *Mycosphaerellaceae* and *Hypocreales* are opportunistic pathogens and pathogenic to plant and animals (Lombard et al. 2016; Yuping et al. 2016). The presence of a diverse community in fruit is correlated with resistance to diseases and disorders (Diskin et al. 2017).

## 5.7 Conclusion

Multilocus sequence analysis of three isolates obtained from RCD-infected fruit identified them as belonging to the genus *Pantoea*. In a first, this research characterised the bacterial and fungal communities of RCD-infected fruit using a metagenomics approach. Our data show that both the bacterial and fungal community of control fruits, without RCD symptoms, was significantly different to that of RCD-infected fruit. From a bacterial perspective, RCD-infected fruit was largely dominated by the genus *Tatumella* while prominent fungal families found in RCD fruit were non-pathogenic. Overall, these results provide circumstantial evidence for a role of bacterial pathogens in RCD development. By underlining critical advances in microbial studies, the present work is paving the way for future studies addressing the role of pathogens in the propensity of RCD. However, further studies are still warranted to confirm the pathogen species and the potential role of mango microbiota. Moreover, this work will facilitate the development of novel approaches to postharvest control of RCD.

## 5.8 Supplementary Data

Table 5.5 RCD samples collection detail and location

| Sample # | Sample Code | Cultivar | Symptom site on fruit | Sample | Location |
|---|---|---|---|---|---|
| 1 | Mu3 | KP | Peduncle | RCD | NT, Orchard 1 |
| 2 | Mu18 | KP | Peduncle | Control | NT, Orchard 1 |
| 3 | Mu10 | KP | Peduncle | RCD | NT, Orchard 1 |
| 4 | Mu6 | KP | Peduncle | RCD | NT, Orchard 3 |
| 5 | Mu9 | KP | Peduncle | Control | NT, Orchard 3 |
| 6 | Mu1 | KP | Peduncle | RCD | Commercial store |
| 7 | Mu4 | KP | Nose end | Control | Commercial store |
| 8 | Mu13 | KP | Nose end | RCD | NT, Orchard 2 |
| 9 | Mu12 | KP | Nose end | RCD | NT, Orchard 2 |
| 10 | Mu15 | KP | Nose end | Control | NT, Orchard 2 |
| 11 | Mu5 | KP | Nose end | RCD | NT, Orchard 2 |

| | | | | | |
|---|---|---|---|---|---|
| 12 | Mu8 | KP | Nose end | RCD | NT, Orchard 2 |
| 13 | Mu2 | R2E2 | Peduncle | RCD | NT, Orchard 3 |
| 14 | Mu7 | R2E2 | Peduncle | RCD | NT, Orchard 3 |
| 15 | Mu11 | R2E2 | Peduncle | RCD | NT, Orchard 3 |
| 16 | Mu14 | R2E2 | Nose end | RCD | NT, Orchard 3 |
| 17 | Mu16 | R2E2 | Nose end | Control | NT, Orchard 3 |
| 18 | Mu17 | R2E2 | Nose end | RCD | NT, Orchard 3 |

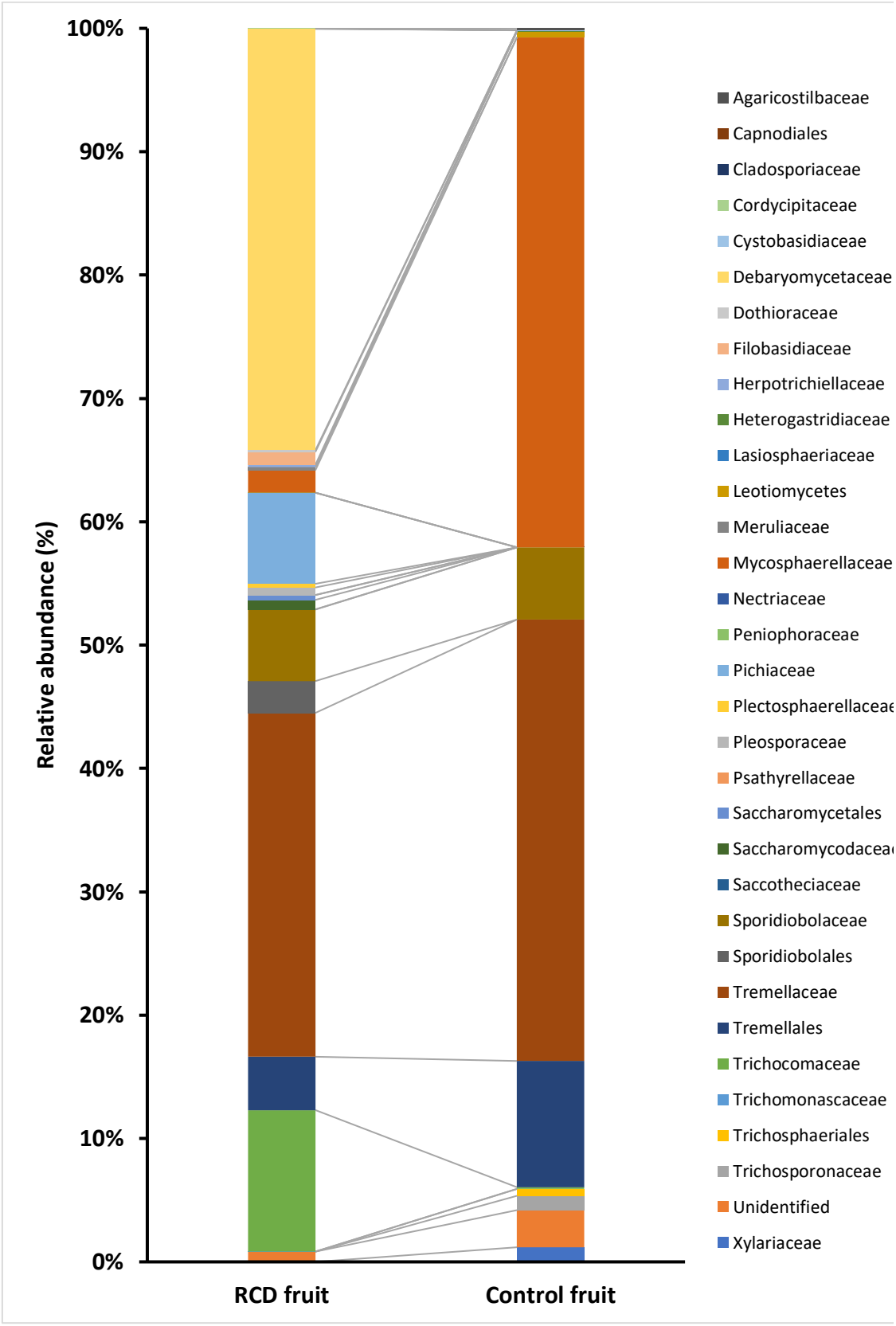



Figure S5.1: Percentage of fungal families in RCD infected and control fruit from the preliminary study of 2017-18, n=2. Unclassified data is mentioned as “unidentified”.

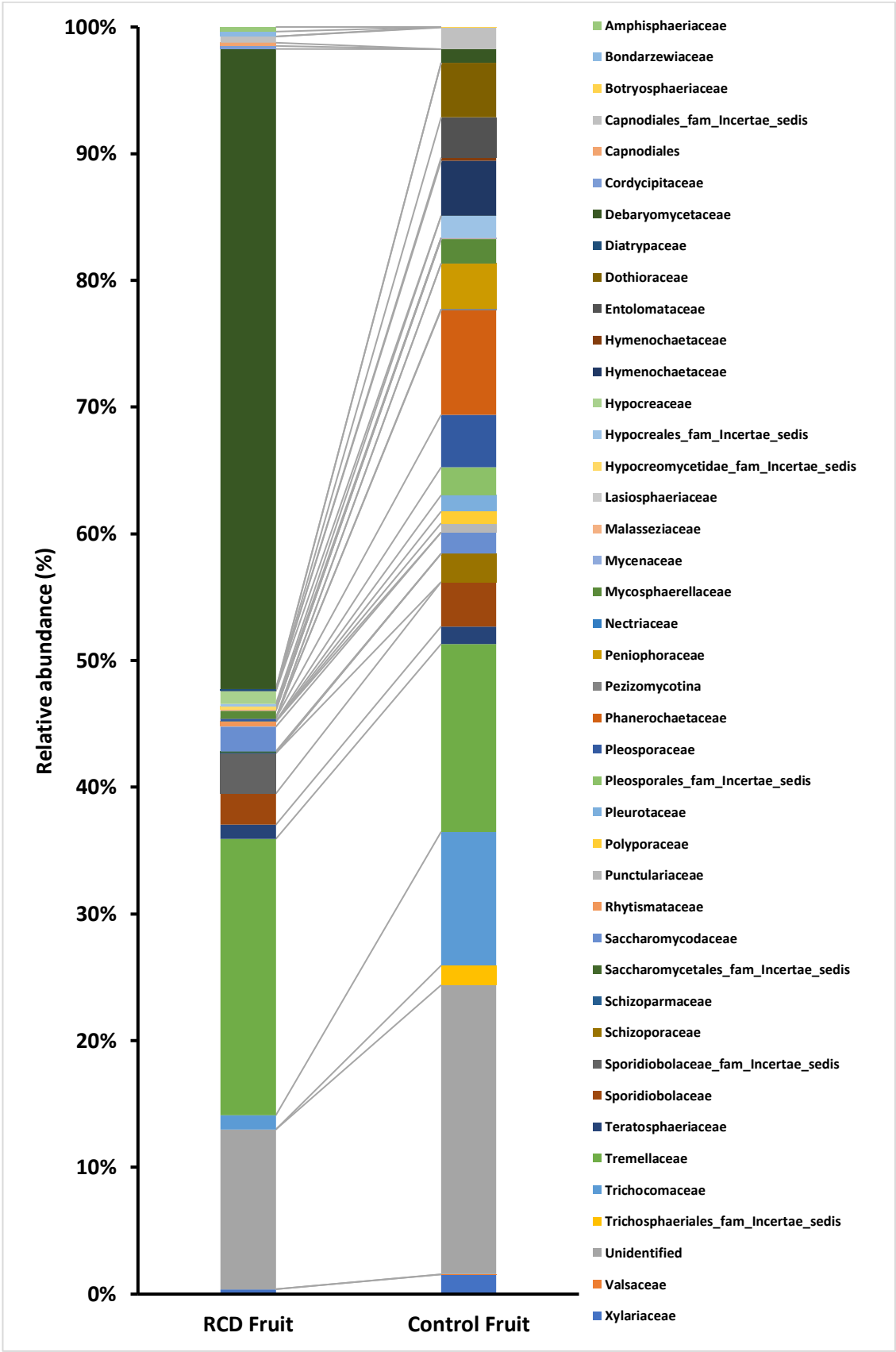



Figure S5.2: Relative abundance of all fungal families identified in RCD infected and control fruit from the 2019, n=10. Unclassified data is mentioned as “unidentified”.

# Chapter 6: Evaluation of resin canal discolouration susceptibility and postharvest control measures.

## 6.1 Abstract

Resin canal discolouration (RCD) is a critical postharvest problem for the Australian mango industry, however, management options for this quality defect remains limited. In this study, two options were investigated: 1) Evaluation of a commercial postharvest sanitiser (Nylate®) dip to minimise RCD incidence and 2) Screening for host resistance to RCD of four cultivars (National Mango Breeding Program 1243, 1201, 4069 and B74) using artificial inoculation with RCD infected homogenate. Results showed that a range of 10 postharvest dip treatments using Nylate® solution did not prevent the expression of RCD. The incidence of RCD severity (rating score) recorded for seed, flesh and peel at the eating ripe stage confirmed that RCD severity varied amongst the four cultivars with 1201 cultivar exhibiting the most severe symptoms. However, variation in RCD severity suggests there exists a potential of using genetic resistance as future means of managing RCD.

Key words: Inoculation, Kensington Pride, mango, postharvest sanitizer, resin canal discolouration.

## 6.2 Introduction

Initial studies on resin canal discolouration (RCD) by Macnish (2016) suggested a range of potential management strategies including postharvest application of sanitisers and identification of genotypes showing tolerance to RCD. Given that microbial contamination in mango fruit can occur at any stage throughout production and supply chain process (Mathew et al. 2018), the inclusion of an effective postharvest sanitisation step may offer a potential strategy for managing RCD.

In Australia, a wide range of postharvest sanitisers are used to treat against pathogens (bacterial and fungal) in fruits and vegetables (Bliss 1996; Premier 2013). For mango, the most widely used postharvest sanitiser is chlorinated water at concentrations ranging from 50 ppm to 200 ppm (Ayhan et al. 1998; Mathew et al. 2018). Chlorine application offer a number of advantages including good oxidising effect, affordable cost, relatively simple operation and widely commercially available (Zhang et al. 2019). Although chlorine-based sanitisers are effective antimicrobial agents, their efficacy can be quickly compromised in the presence of organic matter (Keskinen et al. 2009). Further, if not used correctly, chlorine may pose health and safety issues and may be expensive to use (Issa-Zacharia et al. 2010). These concerns have led to the development of alternative sanitisers that are eco-friendly such as peroxyacetic acid (PAA) and bromo-chloro-dimethyl-hydantoin (BCDMH) (Nylate®).

Recently, Nylate® was registered as a postharvest sanitiser in Australia in 2018 (APVMA 2018). Bromo-chloro-dimethyl-hydantoin (BCDMH) (Nylate®) reacts slowly with water to release hypochlorous and hypobromous acidy (Dixon et al. 2004). It has been shown to be extremely effective against fungal and bacterial pathogens, however the literature on this sanitiser is scarce. The major advantage of Nylate® is that it is a stable compound, effective across a broad pH range and at much lower concentrations than chlorine. Whilst chlorine needs to be used at concentrations > 50 ppm, Nylate® can be used at 5 to 10 ppm (Bliss 1996; Premier 2013). However, Nylate®is more expensive than chlorine which limits its use initially, but its other advantages make the overall cost of applied BCDMH as a disinfectant financially competitive to chlorine treatment (Soracco et al.

1985). To our knowledge, no study has yet investigated the efficacy of using postharvest sanitisers such as Nylate® in managing RCD. At this stage, it is unclear the transmission mechanisms of RCD. However, being a surface sterilising agent, Nylate may have limited efficacy for managing pathogens in the flesh.

A second possible control measure for RCD is the identification of genotypes that show enhanced tolerance to RCD. Mango KP and B74 (Calypso™) cultivars are popular commercially grown cultivars in Australia with their market share at 47% and 23% respectively (Dillon et al. 2013). RCD symptoms have been observed in mango cultivars KP, B74, Keitt and in some Asian cultivars (Moore 2012; Macnish 2016). However, the susceptibility of these cultivars to RCD has not yet been formally examined due to an inability to reliably infect fruit with RCD.

In this study, the efficacy of a commercial sanitiser (Nylate®) as a postharvest control measure of RCD was evaluated. Secondly, this study was to provide an understanding about the response of specific cultivars to infection using the artificial inoculation protocols as developed in chapter 4. To this end, an evaluation of RCD propensity was conducted for one commercial cultivar (B74) and three new cultivars (1243, 1201 and 4069) developed by the National Mango Breeding Program (NMBP) that are not yet grown commercially.

## 6.3 Materials and Methods

Fruit for the first experiment was sourced from a mango orchard in the Katherine Research Station (14°28’0”S 132°16’0”E), whereas fruit for the second experiment were sourced from different areas of Darwin ($12^0$ 35’S $131^0$18’E). All fruit were harvested at the hard green stage and transported to Berrimah Farm Science Precinct Postharvest Laboratory, Northern Territory Department of Primary Industry and Resources (NT DPIR).

### 6.3.1 Postharvest sanitiser dip for minimizing RCD incidence

Thirty fruit (10 fruit per box) were assigned to one of ten treatments using a completely randomised design (CRD). Treatments details are given in Table 6.1. (N=300)

Fruit were artificially inoculated using the spray protocol developed in Chapter 4 describes briefly about the inoculation protocol. For all sanitiser treatments, fruit were dipped collectively into the sanitiser treatment for a given time and then left to drain freely for 10 minutes to remove sanitiser solution (Fig 6.1). Fruit were packed into polyethylene bags (56L black pack) and sealed with tape in order to provide the favourable conditions/humidity for microbial growth. The inoculation prior to sanitation treatments were selected to mimic the different scenarios where fruits are exposed to bacterial infection before they are transported into the packing house.

Figure 6.1: Postharvest sanitiser Nylate® dip application of KP mango fruit.

Table 6.1: Details of postharvest sanitiser dip treatments.

| Treatment | Description | Treatment | Time in bag after treatment | Sanitation | Sanitation time |
|---|---|---|---|---|---|
| 1 | Water control | Water* | 24h | No | 0 |
| 2 | Uninfected pulp control | Uninfected pulp | 24h | No | 0 |
| 3 | Infected pulp control | Infected pulp | 24h | No | 0 |
| 4 | Sanitiser prior to inoculation | Infected pulp | 24h | Yes prior to inoculation | 90 sec |
| 5 | Inoculation 5 min short sanitation | Infected pulp | 5 min | Yes, after inoculation | 90 sec |
| 6 | Inoculation 6h short sanitation | Infected pulp | 6h | Yes, after inoculation | 90 sec |
| 7 | Inoculation 6h medium sanitation | Infected pulp | 6h | Yes, after inoculation | 300 sec |
| 8 | Inoculation | Infected pulp | 6h | Yes, after inoculation | 600 sec |

| | | | | | |
|---|---|---|---|---|---|
| | 6 h long sanitation | | | | |
| 9 | Inoculation<br>12 h short sanitation | Infected pulp | 12 h | Yes, after inoculation | 90 |
| 10 | Inoculation<br>24h short sanitation | Infected pulp | 24 h | Yes, after inoculation | 90 |

* Water from a Milli-RO®4 plant

Fresh Nylate® solution was prepared at recommended commercial rate of 30 mg/L, pH 8.0 on the same day of treatment. Briefly, the solution was prepared at room temperature using reverse osmosis water from a Milli-RO®4 plant.

All treatments were stored at room temperature (25±1 °C; 60-65% RH) for ten days until fruit had reached the eating-ripe stage (Lalel et al. 2003a, 2004), when they were assessed for RCD severity and frequency of infection symptoms of the seed, flesh and peel using protocols as explained in Chapter 4.

### 6.3.2 RCD susceptibility in four mango cultivars

A total of 90 fruit (n = 90) (10 fruits per box) of each cultivar (NMBP 1243, 1201, 4069 and B74) were used in this study. Cultivars NMBP 1243 and 4069 were harvested on 20 Oct 2018 and 25 Oct 2018, respectively, while cultivars NMBP 1201 and B74 were both harvested on 25 Oct 2018. Inoculation treatments of individual cultivars was conducted separately. Fruits were subjected to either inoculated control, uninoculated control (Control spray), spraying of RCD inoculum onto the fruit surface (Inoculation spray) and puncture RCD inoculum into the fruits (Inoculated needle) on the day of harvest. Mango fruit which had been inoculated with RCD homogenate in previous experiments (Chapter 4) and stored in at low temperature (12±1 °C, 80-85% RH) and were used as a

source of inoculum for this trial. The same inoculation protocol was followed as explained in Chapter 4.

After treatment, fruits were stored at 25±1 °C and 60-65% RH, upon reaching eating ripe stage (after ten days of storage), they were assessed visually for RCD symptoms on the fruit surface (peel), flesh and seed. The experiment was arranged as a complete randomised design, with each box representing a replicate.

### 6.3.3 RCD severity and fruit quality parameters

As previously described in Chapter 4, RCD incidence was assessed by using a rating scale where 1 = 0-15% RCD, 2= 15-30% RCD, 3= 30-45% RCD, 4= 45-70% RCD, 5= 70-85% RCD, 6= 85-100 % RCD on the fruit surface (Macnish et al. 2015). A digital refractometer (Atago-PAL-1, Tokyo, Japan) was used for the measurement of total soluble solids (TSS). A drop of juice was placed on the prism of refractometer and TSS (°Brix) was recorded directly from the digital screen of refractometer at room temperature (Jin et al. 2018).

## 6.4 Statistical analysis

Both experiments were conducted according to a completely randomised design. Data gathered from each experiment were subjected to analysis of variance (ANOVA) with means separation at 5% LSD using Statistical Tools for Agricultural Research version 2.0.1 (http://bbi.irri.org). To normalise the data distribution required for statistical analysis, data were subjected to square root transformation to meet the assumption of ANOVA (Osborne 2010). Analysed data average values were back-transformed for interpretation.

## 6.5 Results

### 6.5.1 Postharvest sanitiser dip for minimizing the RCD incidence

RCD severity (rating) were estimated on the peel, flesh, and peel at the ripe eating stage (ten days after storage) for all treatments exposed to postharvest Nylate® sanitizer dip. Statistical analysis revealed no significant difference in RCD rating on fruit peel between all treatments (Table 6.2). However, RCD symptoms on peel exhibited very low levels

only in treatment 4. A non-significant effect of sanitiser treatments were observed in RCD severity at fruit flesh; however, there was comparatively higher RCD symptoms were recorded in treated fruits compared to controls. Moreover, highest RCD severity were found on fruit flesh in fruits of treatment 3 (Infected pulp control).

At seed level, the severity of RCD symptoms was significant (P≤0.05) control untreated fruits exhibited with no RCD symptoms (Table 6.2). RCD symptoms were higher in fruits of treatment nine, which was postharvest dip after 12h inoculation. The lowest RCD seed level in seed was found in treatment eight which longest the time duration of postharvest dip 10min dip after 6h of inoculation. Moreover, all treated fruits showed RCD symptoms on fruit seed at eating ripe stage.

Thus, it can be concluded that postharvest Nylate® sanitizer dip could not stop the infection after surface inoculation. There was a non-significant difference observed in total soluble solids (TSS) among all treatments (Fig 6.2).

Table 6.2: RCD rating (score) of mango cv KP fruit after ten days of storage at 25±1 °C; 60-65%RH

| Treatment number | Treatment description | RCD assessment (score) | | |
|---|---|---|---|---|
| | | Fruit Peel | Fruit* Flesh | Fruit Seed |
| 1 | Water control | 0.00 | 0.00 | 0.00c |
| 2 | Uninfected pulp control | 0.00 | 0.00 | 0.00c |
| 3 | Infected pulp control | 0.00 | 0.32 | 0.84ab |
| 4 | Sanitiser prior to inoculation | 0.00 | 0.10 | 0.74ab |
| 5 | Inoculation 5 min short sanitation | 0.04 | 0.20 | 0.60ab |
| 6 | Inoculation 6h short sanitation | 0.00 | 0.24 | 0.57b |
| 7 | Inoculation 6h medium sanitation | 0.00 | 0.27 | 0.57b |
| 8 | Inoculation 6h long sanitation | 0.00 | 0.04 | 0.56b |
| 9 | Inoculation 12h short sanitation | 0.00 | 0.07 | 0.90a |
| 10 | Inoculation 24h short sanitation | 0.00 | 0.30 | 0.67ab |

*Means not sharing a letter are significantly different from each other (P≤0.05)*

*There was no significant treatment effect for fruit flesh.

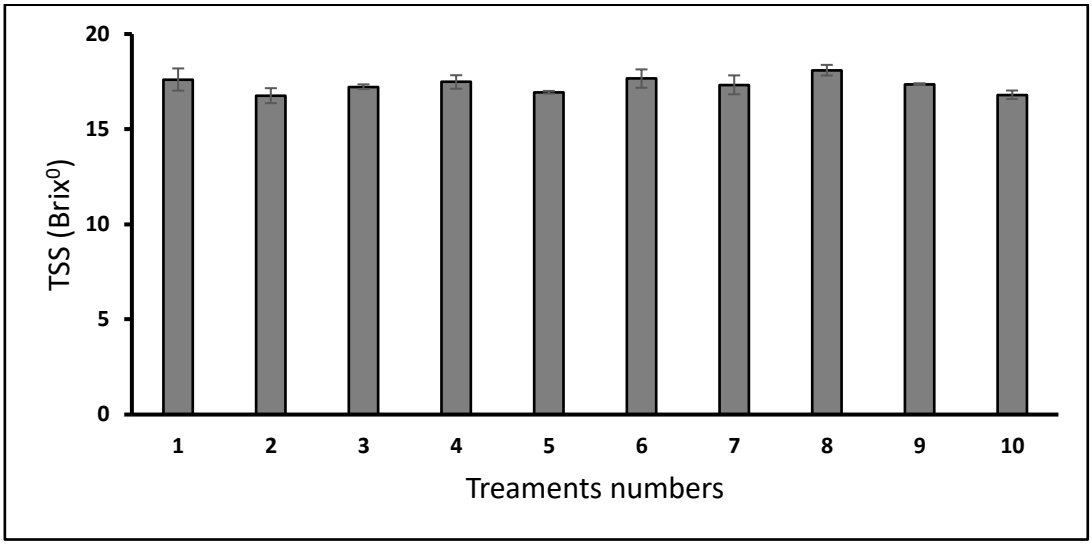



Figure 6.2: Effect of postharvest sanitizer treatments on total soluble solids (TSS) of KP mango. Vertical bars denote SE of means. n = 3. Letters on the data points represent the difference between the treatments.

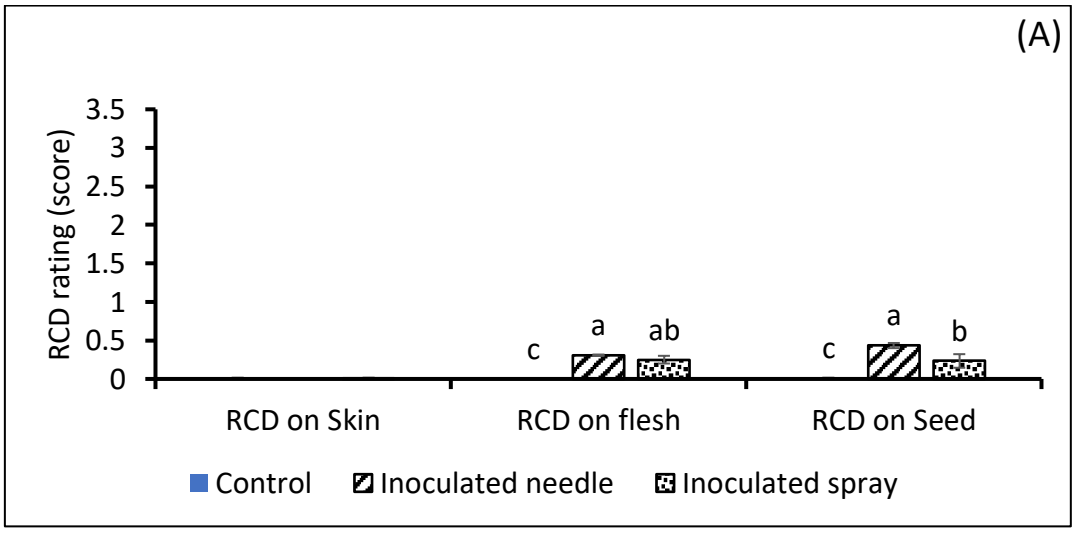
### 6.5.2 Susceptibility of four mango cultivars

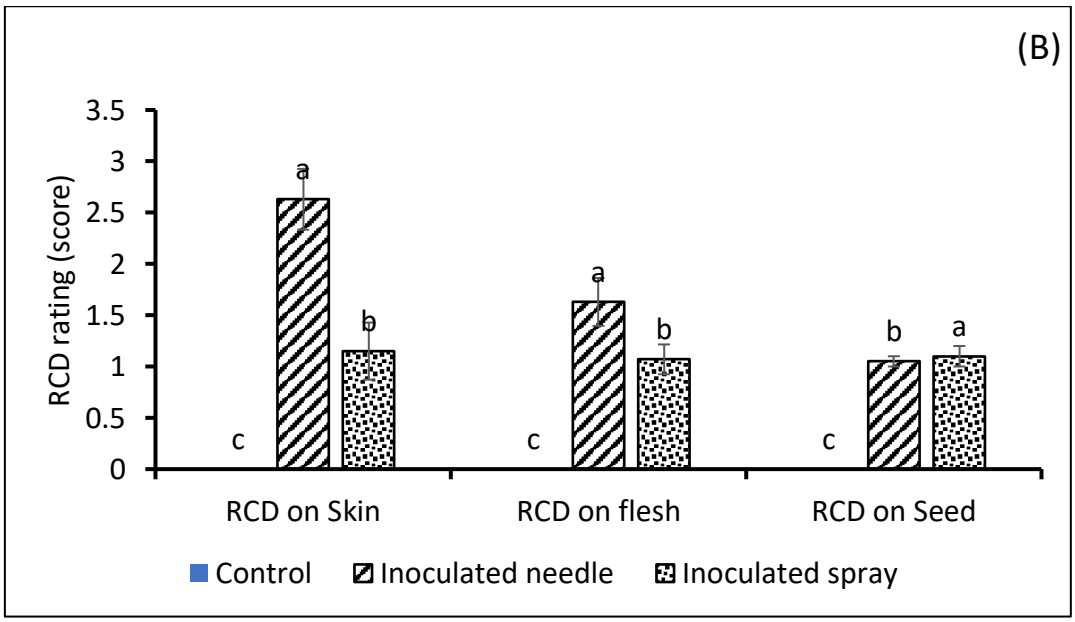
The effect of inoculation treatments using the surface spray and needle puncture on RCD severity (score) on mango peel, mango flesh and mango seed were recorded for four cultivars (NMBP 1243, 1201, 4069 and B74) after ten days post-inoculation treatments (Fig 6.3). Cultivar NMBP 1201 had the highest RCD severity on fruit peel with a severity score of (2.63) (Fig 6.3 B) while the lowest RCD severity was recorded in B74 cultivars (Fig 6.3 C). Moreover, cultivars NMBP 1243 and 4069 did not exhibit symptoms on peel. Needle inoculated fruits had most severe RCD rating (score) on fruit peel and flesh than spray inoculated fruits in the cultivar NMBP 1201 (Fig 6.3 B).

A similar trend was observed in mango flesh were fruits treated with needle inoculation had significantly higher symptoms. However, more severe symptoms were observed in cultivar NMBP 1201 as compared with controls (Fig 6.3 B). However, RCD severity on mango seed revealed that all four cultivars showed signs of RCD. Still, they were higher in inoculated spray fruits where NMBP 1201 was most susceptible to RCD on mango seed followed by B74, 1243 and significantly lower RCD symptoms were recorded in NMBP 4069 cultivar (Fig 6.3 A, B, C and D).

Treated fruits of 1201 and 4069 had significantly lower (P≤0.05) TSS than uninoculated control fruits (Fig 6.4 A and C). Moreover, there was no difference in TSS of B74 fruits at eating ripe stage (Fig 6.4).

Overall, results of this study revealed that all four cultivars are susceptible to RCD after inoculation. However, the intensity of symptoms varied amongst cultivars. Mango cultivar NMBP 4069 appeared the most resistant to RCD incidence as compared to other cultivars (Fig 6.3 B).



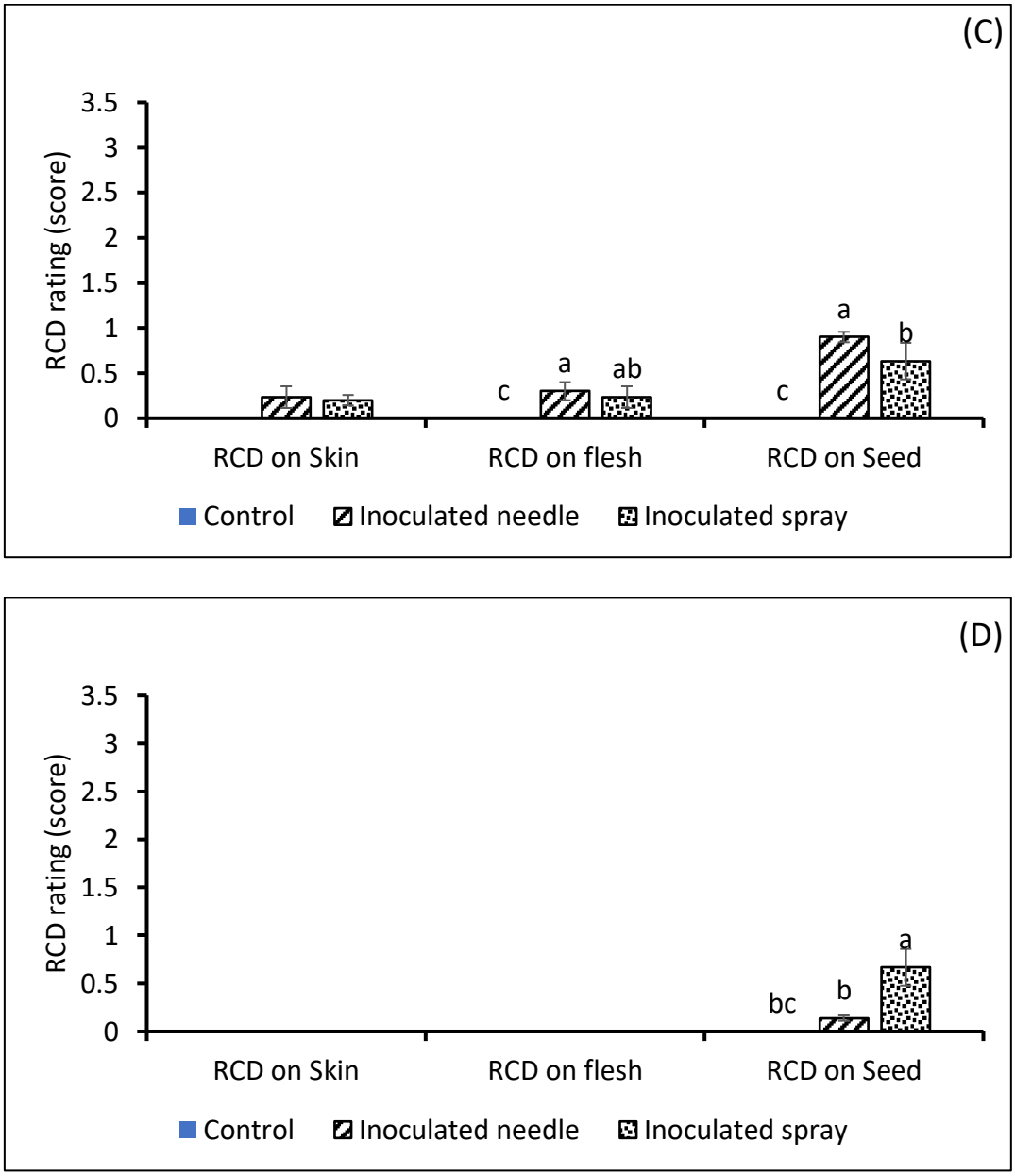
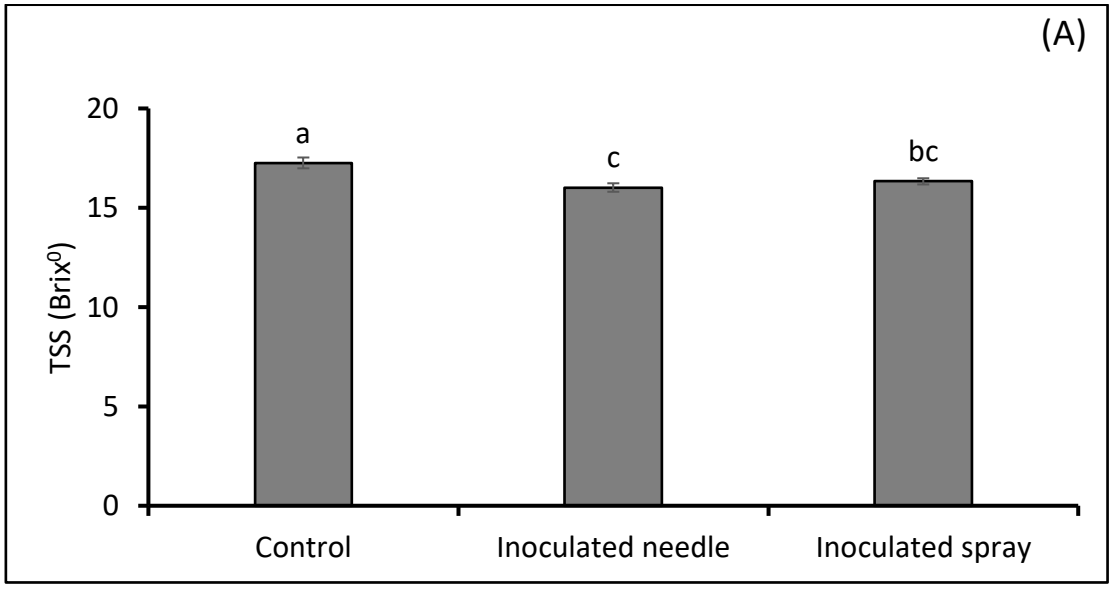



Figure 6.3: Incidence of RCD for four mango cultivars; Graph A (NMBP 1243); Graph B (NMBP 1201); Graph C (B74); Graph D NMBP 4069. Vertical bars denote SE of means. N = 3. Letters on the data points represent the difference between the treatments. Treatments not sharing letters differ significantly from each other.


(A)
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10
5
0
TSS (Brix$^0$)
a
c
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Control
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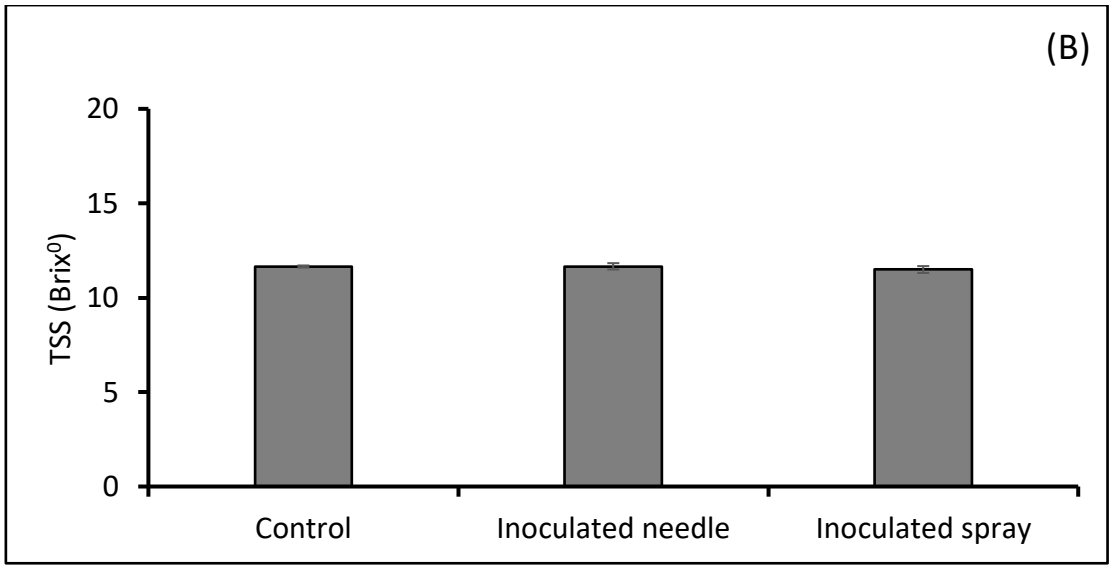
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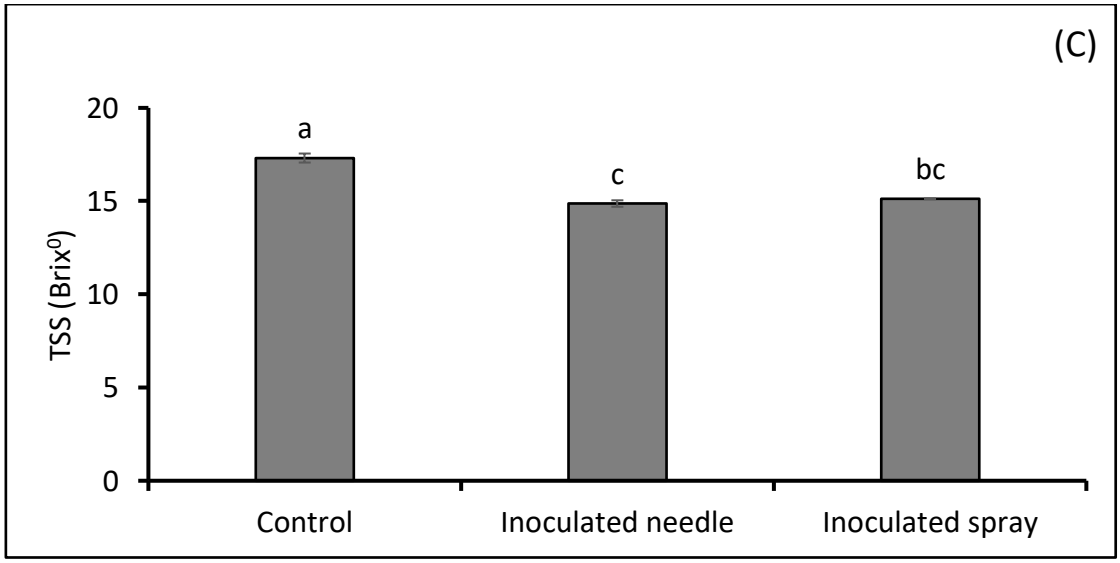
(C)
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TSS (Brix$^0$)
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c
bc
Control
Inoculated needle
Inoculated spray

Figure 6.4: Changes in total soluble solids (TSS) of different mango cultivars (A, 1201. B, B74. C 4069*). Vertical bars denote SE of means. N = 3. Letters on the data points represent the difference between the treatments. Treatments not sharing letters differ significantly from each other.

* TSS of cv 1243 was not included in this study.

## 6.6 Discussion

### 6.6.1 Postharvest control measures

Previously Nylate® has been reported to control pathogens associated with fresh fruits and vegetables (Premier 2013). However, the result of this study indicated that Nylate® was unable to control RCD in KP mangoes, at least using the concentration as recommended by the product manufacturer. However, it is possible that concentration of Nylate® was insufficient to perform good antimicrobial activity. A pervious study showed that dipping avocado in a Nylate® solution of 15-20 ppm was not effective for managing avocado ripe rots (Dixon et al. 2004). Another possible reason for the observed lack sanitiser effect may be related to the overall low levels of RCD symptoms induced by the artificial inoculation protocol in this trial. In particular, RCD scores expressed in the inoculated fruit were much lower (0.84) than that observed for inoculated fruit in chapter 4. The low level of symptoms could be due to differences in fruit locations and time of harvest. It has been reported that RCD incidence varies from location and time of harvest (early or late) for the same cultivar (Macnish et al. 2015). For example, a higher incidence was observed in fruit harvested early in the season compared to those harvested later in the season. Furthermore, the inoculum source used in this trial had been stored in the cold temperature 12±1 °C and 80-85% RH for two weeks prior to use in this experiment, which may have reduced the viability of the inoculum as even the control treatment showed less symptoms than that induced in previous experiments (Chapter 4)

In this study, novel observations were made regarding the effect of Nylate® on the management of RCD. However, further studies are needed to investigate factors that may affect the efficacy of Nylate® as a sanitiser of mango. However, different

concentration of Nylate® could be evaluated further. For example, 5 mg/L Nylate® dip for 45 sec was shown to reduce 91.3% of microbial total plate count (CFU) per gram of spinach baby leaf (Premier 2013). Nylate®at concentrations of 10 mg/L and 12 mg/L effectively controlled the microbial growth and significantly enhanced the vase life of cut flowers (Jones et al. 1993; Jones & Hill 1993). A reduction in pathogen may be achieved by applying different fruit dip duration in Nylate® solution. Nylate® dip for 30 minutes significantly reduced the pathogens in broccoli (Behrsing et al. 2000). Keeping in view the findings of this study, further trials using higher concentration and dipping the fruits for longer time Nylate® solution or applying alternative sanitisers may result in effective RCD control measure. However, care should be taken when using higher concentrations of sanitisers to ensure it does not damage the fruit and reduce their shelf life (Nogales-Delgado et al. 2013) or the fruit do not become hazardous to consume.

### 6.6.2 RCD susceptibility of different mango cultivars

All four cultivars displayed RCD symptoms on the seed using both the inoculation surface spray and needle puncture methods. However, only two of the four cultivar displayed RCD symptoms on the peel (Fig 6.3). Overall, cultivar 4069 showed the least effect in term of RCD severity. These findings highlighted that RCD is not specific to a particular cultivar, however its propensity varied from cultivar to cultivar. The observed variation in symptoms amongst the cultivars indicate that there is genetic variation to RCD expression. Macnish (2016) observed the incidence in KP, B74 (Calypso™) and Keitt cultivars. However, he found higher rate of RCD expression in the KP fruit that can be concluded due to its genetic or its market dominance as KP contributes about the 80% of total mango produce in Australia (AMIA, 2019). Moreover, KP fruit had higher fibre flesh then B74 and Keitt cultivars that could be a contributing factor towards RCD incidence (Jabeen 2016). Moreover, NMBP cultivars 1243, 1201 and 4069 have been reported with very low fibre content (Bally 2008; Anonymous 2019). A comparative genetic study along with fibre content in the flesh of newly developed NMBP cultivars could be used as a potential screening tool for identifying differences in RCD propensity among the cultivars.

## 6.7 Conclusion

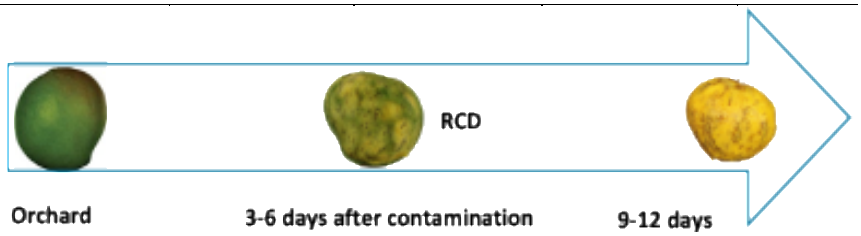
RCD in mango cv. KP fruit is a postharvest defect and related to the presence of pathogens. In this study, RCD could not be minimised by the application of Nylate® at the postharvest stage. Once infected, RCD could not be controlled by commercially relevant exposure time to sanitiser. Similarly, treatment with a sanitiser before artificial inoculation did not prevent infection. This study has answered some critical questions related to its relationship with a specific cultivar of symptoms and severity. Some fruit had no commercial sign of RCD when inoculated on the surface. These results suggest that RCD expression differs from cultivar to cultivar.

# Chapter 7: General Discussion

The research presented in this thesis has advanced our understanding of resin canal discoloration (RCD) of mango fruit by elucidating fundamental pathological mechanisms underpinning expression (Table 1). Until the time of this study, very little information was available about RCD. This study has elucidated the impact of RCD on Australian consumers' perceptions of mango fruit quality and willingness to buy RCD-affected fruit (Chapter 3), understanding the role of pathogens through artificially inoculation and monitoring the progression of RCD symptoms during fruit ripeness (Chapter 4), identification of bacterial species associated with RCD and determination of microbial diversity (bacterial and fungal) communities from RCD and control mango fruits (Chapter 5), the efficacy of a commercial sanitiser as a postharvest control measure of RCD and to examine the response of related mango cultivars sourced from different orchards in the Northern Territory (NT), Australia to exposure to RCD (Chapter 6)

Table 7.2. A summary of key factors studied and their association with RCD expression.

| No | Factor | Preharvest | Harvest | Packhouse | Transit | Retail |
|---|---|---|---|---|---|---|
| 1 | Rainfall before harvest | ? | | | | |
| 2 | Crop nutrition | ✗ | | | | |
| 3 | Genotype | ✓ | | | | |
| 4 | Fruit handling | | | ? | ? | |
| 5 | Pathogen/s | ✓ | ✓ | ✓ | ✓ | ✓ |
| 6 | Control measures* | ? | ? | ? | ? | |




* Postharvest sanitisers (Nylate®) used in this research project could not control the symptoms of RCD

✓ = Associated with RCD symptoms, **?** no relationship found with RCD

## 7.1 Research findings

### 7.1.1 The effect of RCD on consumer satisfaction and intention to make a repeat purchase

The visual appeal of mango is a crucial factor that determines the final quality of the fruit at the consumer level. A consumer survey (n = 135) at three retail stores in Sydney, during the 2017 mango season confirmed that RCD was affecting the consumers' intentions to repeat the purchase of mangoes (Chapter 3), in agreement with earlier studies that visual fruit quality has a negative impact on fruit marketability (Ledger et al. 2003; Hofman et al. 2009; Sivakumar et al. 2010). In this study, 87% of consumers were unwilling to buy RCD-infected fruit that showed symptoms in the store. Moreover, a

survey of the retail stores managers revealed that RCD contributed around 5% of fruit loss at the retail store level. This study showed that RCD significantly affected consumers perception of fruit quality and decreased the intention to repeat purchase. However, the study was conducted in only three retail stores in Sydney with limited participants. This study provides insight in estimating the contribution of RCD in terms of loss to the Australian mango industry at the endpoint of the value chain and consumption.

### 7.1.2 Causative factors of RCD

A primary aim of this study was to answer the fundamental questions about the cause of RCD in mango fruit. Previous studies (Macnish 2016) have been preliminary and the specific causal organisms' identification had not been achieved or conclusively demonstrated. To answer this question, identification of bacterial species from RCD fruits was carried out on KP mango (Chapter 5).

From the 16S rRNA, *rpo*B and *hsp*60 sequence data of the three isolates (Mu1, Mu2, Mu3) obtained we identified these as belonging to the genus *Pantoea*. Isolates Mu1 and Mu2 were most similar overall to *Pantoea dispersa*. This limited data supports previous studies that this species causes postharvest diseases in several horticultural crops such as citrus (Soto-Muñoz et al. 2015), pome (Torres et al. 2011; Soto-Muñoz et al. 2014) and stone fruits (Torres et al. 2014), melon (Bruton et al. 1991), pear (Lindow et al. 1998). However, in this study there was difficulty in obtaining quality DNA for sequencing due to a lack of sufficient RCD-infected samples, particularly in 2018. Further, contamination issues with the pure cultures also impeded optimisation of DNA analysis. D. Future studies should investigate isolates sampled from RCDinfected fruit for a range of cultivars, locations and production systems.

### 7.1.3 Study of microbiome communities of RCD fruits

Our research shows that there is a difference in bacterial and fungal populations in mango cv. KP fruit with RCD symptoms and control fruit (Chapter 5). The sequencing result revealed that the most relatively abundant OTU samples from the RCD fruit samples was *Tatumella* OTU001 that was found in all RCD samples at high proportions

(median 91.5%, range 76.2-98.5% of reads). By comparison, the next most common OTU in RCD fruit was *Pantoea* OTU002. The results from the microbiome study do not support the isolate study. This may be due to a range of factors such as timing of fruit sampling during disease progression. Further, the classification of bacteria in the *Enterobacter-Pantoea-Erwinia* group is problematic due to a confusing and dynamic taxonomy.

The genus *Tatumella* and *Pantoea* belong to family *Enterobacteriaceae*, which is known pathogen in mango, such as bacterial apical necrosis of mango trees (Gutiérrez-Barranquero et al. 2019). Previously, Macnish et al. (2015) found the same species from one sample out of 77 RCD fruits collected from Northern Territory. Furthermore, members of *Tatumella* genus have been reported as causative agents of pink disease in pineapple (Marín-Cevada et al. 2010).

Fungal families found prominent in RCD fruits were either non-pathogenic or secondary pathogens. Despite the presence of secondary pathogens, which can exist as endophytes (Diskin et al. (2017)) this present study suggests that fungi are not responsible for RCD expression. Instead, these results provide circumstantial evidence for a role of bacterial pathogens in RCD development. Further studies are required to unequivocally confirm the role of bacteria in RCD. We recommend isolation from mature healthy and RCD infected fruits representing all fruit ripening stages.

### 7.1.4 Progression of expression of RCD symptoms

Previous studies have reported that RCD symptoms manifest at eating ripe stage (Macnish et al. 2015). However, in this study the first symptom of RCD was observed on the seed six days after artificial inoculation (Figure 7.1). Subsequently, symptoms were then noted in the flesh and peel, from day 9 to 15 (Chapter 4). It is unclear why the symptoms were first evident adjacent to the seed given the bacteria was applied to the surface of the fruit. In another study, we inoculated fruit at three stages of ripening (at harvest stage, after commercial ripening stage and fruit at retail store stage) and RCD

symptoms were induced at all stages, however a higher severity was observed when inoculated at the earlier ripening stages.

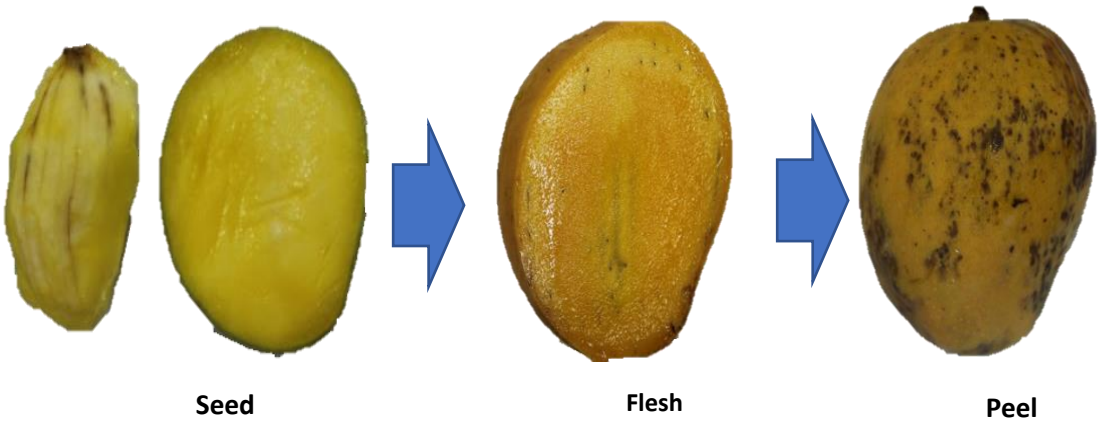



Figure 7.1: First symptom of RCD expression during fruit ripening occurs next to the seed between days 3 to 6, then in the flesh from days 6 to 9 and lastly in the peel at days 9 to 12 days after inoculation treatment.

### 7.1.5 Post-harvest management of RCD

In our study, novel observations were made regarding the post-harvest management of RCD. Firstly, we evaluated the efficacy of a Nylate® post-harvest sanitiser dip for the management of RCD (Chapter 6). However, the results of this study indicated the non-significant effect of Nylate® in controlling RCD in KP mangoes once infected. Keeping in view the findings of this study, there is a need for developing post-harvest management protocol for RCD by using a range of different available post-harvest sanitisers to disinfest fruit at the post-harvest stage. Secondly, we inoculated the different mango cultivars for evaluation of RCD susceptibility. We found that cultivar 4069 appeared more resistant towards RCD severity. This may be due to the genetic variability of cultivar having less resin canals in the flesh as compared with KP mango (Jabeen 2016). This information could be helpful in breeding purposes for screening and developing an RCD resistant cultivar.

## 7.2 Future research and Industry recommendations

This project identified factors that contribute to RCD incidence in mango fruit. Our findings support the hypothesis that RCD is caused by bacterial pathogens. However, we provide evidence that fruit may differ in susceptibility to infection based on genotype (e.g. National mango breeding program cultivars 1243, 1201, 4069 and B74), production practises and environment (e.g. Darwin and Katherine). Further, evaluation of RCD susceptibility of other commercial cultivars, like Honey Gold, R2E2, Nam doc mai and of fruit from different growing locations could be explore.

Our data showed that fruit exposed to pathogens regardless of the stage of ripeness express RCD symptoms. Infection therefore may occur during production, harvesting, packhouse operations, transportation and at commercial retail outlets. Primary points of exposure are likely to be during harvesting, which involves mango picking tools to sever the fruit from a tree and wash water to remove the sap that excludes from the cut stem, and from combining healthy and disease fruit. These are key points of transfer of bacteria from one fruit to another. Despite our improved understanding of RCD expression, knowledge gaps still remain and future work could consider the following research areas:

- The visual discoloration associated with RCD reduced consumers' willingness to purchase fruit. However, RCD arguably does not affect taste therefore industry could conduct consumer awareness campaigns to educate them on this knowledge as a way to reduce unnecessary loss.
- RCD is difficult to detect in the early stages of expression. Early detection and sorting of the RCD would be of benefit to industry. Such approaches could include non-destructive methods based on near-infrared (NIR) technologies which have been successful used to detect other visual disorders in fruit (Mogollón et al. 2020).
- The contribution of pre-harvest harvest factors to the prevalence of RCD requires further investigation. These could include the role of rainfall, production location and agronomic practices.

- The artificial inoculation studies showed that RCD first appeared around the seed then flesh and peel as fruit attained the eating ripe stage. However, this progression of symptoms is not typical of natural infections where symptoms are generally observed in the flesh and peel and only occasionally around the seed. Further examination of the anatomy and biochemistry of resin canals during RCD development may explain for this observed discrepancy.
- The study on mode of entry and infection of pathogens could be explored, such as application of apoplastic dye in the inoculation solution followed by sectioning of fruit would reveal how far bacteria might travel into the fruit through lenticles and intercellular spaces.
- We provide evidence that RCD is linked to bacterial infection. However, a detailed study is needed to determine whether a single or combination of bacterial species can trigger the expression of RCD. Following identification of the causal agents could enable their detection in the supply chain. For example, quantitative real-time PCR techniques could be developed to detect possible points of contamination along the supply chain. Industry could then use this knowledge to manage RCD by strategic application of sanitisers to reduce inoculum load

# References

AMIA, 2019. Mango production https://www.industry.mangoes.net.au/mango-production (accessed on 25.09.2019).

AMIA Australian Mango Industry Association. 2017. http://www.industry.mangoes.net.au/ . [Online]. (accessed on 15.06.2019).

AMIA Australian Mango Industry Association. 2016. http://www.industry.mangoes.net.au/ . [Online]. (accessed on 15.09.2019).

Abdelfattah, A, Ruano-Rosa, D, Cacciola, SO, Nicosia, MGLD & Schena, L 2018, 'Impact of Bactrocera oleae on the fungal microbiota of ripe olive drupes', *PloS one*, vol. 13, no. 11, p. e0199403.

Adams, RI, Amend, AS, Taylor, JW & Bruns, TD 2013, 'A unique signal distorts the perception of species richness and composition in high-throughput sequencing surveys of microbial communities: a case study of fungi in indoor dust', *Microbial ecology*, vol. 66, no. 4, pp. 735-741.

Aguirre-Güitrón, L, Calderón-Santoyo, M, Bautista-Rosales, PU & Ragazzo-Sánchez, JA 2019, 'Application of powder formulation of Meyerozyma caribbica for postharvest control of Colletotrichum gloeosporioides in mango (Mangifera indica L.)', *LWT*, p. 108271.

Akem, C, MacManus, G, Lakhesar, D, Boccalatte, P & Stockdale, K 2010, 'Comparison of fungicide dips efficacies for the control of mango postharvest diseases', in *IX International Mango Symposium 992*, pp. 385-392.

Ali, A, Ong, MK & Forney, CF 2014, 'Effect of ozone pre-conditioning on quality and antioxidant capacity of papaya fruit during ambient storage', *Food chemistry*, vol. 142, pp. 19-26.

Alvindia, DG & Acda, MA 2015, 'Revisiting the efficacy of hot water treatment in managing anthracnose and stem-end rot diseases of mango cv.‘Carabao’', *Crop Protection*, vol. 67, pp. 96-101.

Anderson, T, N, Subedi, P, P, Walsh & B, K 2017, 'Manipulation of mango fruit dry matter content to improve eating quality', *Scientia horticulturae*, vol. 226, pp. 316-321.

Anderson, M, Gorley, R & Clarke, R 2005, 'Permanova', *Permutational multivariate analysis of variance, a computer program. Department of Statistics, University of Auckland*, vol. 24.

Anonymous 2019, 'Australian National Mango Breeding Program', *Department of Primary Industries and Regional Development. Government of Western Australia. https://www.agric.wa.gov.au/mangoes/australian-national-mango-breeding-program*.

APVMA 2018, 'Agricultural and veterinary chemicals ', *Australian Pesticides and Veterinary Medicines Authority*, pp. 5-6.

Arauz, LF 2000, 'Mango anthracnose: Economic impact and current options for integrated managaement', *Plant Disease*, vol. 84, no. 6, pp. 600-611.

Aronesty, E 2013, 'Comparison of sequencing utility programs', *The open bioinformatics journal*, vol. 7, no. 1.

Asis, C, Meschiari, L & McConchie, C 2017, 'Ionome balance analysis of mango fruit from orchard with and without resin canal discolouration', in *XII International Mango Symposium 1244*, pp. 221-228.

Assis, de, JS, Silva, José, D, Moraes & de, PLD 2004, 'Nutritional balance and physiological disorders in mango'Tommy Atkins'', *Revista Brasileira de Fruticultura*, vol. 26, no. 2, pp. 326-329.

Aubert, C, Chalot, G, Lurol, S, Ronjon, A & Cottet, V 2019, 'Relationship between fruit density and quality parameters, levels of sugars, organic acids, bioactive compounds and volatiles of two nectarine cultivars, at harvest and after ripening', *Food chemistry*, p. 124954.

Ayala-Silva, T, Schnell, RJ, Meerow, AW, Winterstein, M, Cervantes, C & Brown, JS 2005, 'Determination of color and fruit traits of half-sib families of mango (Mangifera indica L.)', in *Proc. Fla. State Hort. Soc*, vol. 118, pp. 253-257.

Ayhan, Z, Chism, G & Richter, E 1998, 'The shelf-life of minimally processed fresh cut melons', *Journal of food quality*, vol. 21, no. 1, pp. 29-40.

Bally, I 2008, 'New mango hybrids from Australia', in *IV International Symposium on Tropical and Subtropical Fruits 975*, pp. 55-61.

Bally, I, Harris, M, Kulkarni, V, Hamilton, D, Johnson, P, Robinson, D & Leonardi, J 1999, 'The Australian national mango breeding project', in *VI International Symposium on Mango 509*, pp. 225-232.

Bally, IS 2006a, 'Mangifera indica (mango)', *Traditional trees of pacific islands. Holualoa, Hawaii: Permanent Agriculture Resources. http://www. traditionaltree. org*.

Bally, IS 2006b, 'Mangifera indica (mango)', *Species profiles for pacific island agroforestry*, pp. 1-25.

Bartley, J & Schwede, A 1987, 'Volatile flavor components in the headspace of the Australian or “Bowen” mango', *Journal of Food Science*, vol. 52, no. 2, pp. 353-355.

Baskaran, SA, Upadhyay, A, Kollanoor-Johny, A, Upadhyaya, I, Mooyottu, S, Roshni Amalaradjou, MA, Schreiber, D & Venkitanarayanan, K 2013, 'Efficacy of Plant-Derived

Antimicrobials as Antimicrobial Wash Treatments for Reducing Enterohemorrhagic Escherichia Coli O157: H7 on Apples', *Journal of food science*, vol. 78, no. 9, pp. M1399-M1404.

Behrsing, J, Winkler, S, Franz, P & Premier, R 2000, 'Efficacy of chlorine for inactivation of Escherichia coli on vegetables', *Postharvest biology and technology*, vol. 19, no. 2, pp. 187-192.

Bezuidenhout, J, Robbertse, P & Kaiser, C 2005, 'Anatomical investigation of lenticel development and subsequent discolouration of 'Tommy Atkins' and 'Keitt'mango (Mangifera indica L.) fruit', *The Journal of Horticultural Science and Biotechnology*, vol. 80, no. 1, pp. 18-22.

Biswas, P, East, A, Hewett, E & Heyes, J 2016, 'Intermittent Warming in Alleviating Chilling Injury—a Potential Technique with Commercial Constraint', *Food and Bioprocess Technology*, vol. 9, no. 1, pp. 1-15.

Bitange, NM, Chemining'wa, GN, Ambuko, J & Owino, WO 2020, 'Can calcium sprays alleviate jelly seed in mango fruits?', *Journal of Agriculture and Rural Development in the Tropics and Subtropics (JARTS)*, vol. 121, no. 1, pp. 35-42.

Bliss, W 1996, 'Control of water born pathogens in hydroponics using Nylate', in *Proceedings of the 9th International Congress on Soilless Culture, ISOSC, St Helier, Jersey*, pp. 61-73.

Boorsma, A, Foat, BC, Vis, D, Klis, F & Bussemaker, HJ 2005, 'T-profiler: scoring the activity of predefined groups of genes using gene expression data', *Nucleic Acids Res*, vol. 33, no. Web Server issue, pp. W592-595.

Brady, C, Cleenwerck, I, Venter, S, Vancanneyt, M, Swings, J & Coutinho, T 2008, 'Phylogeny and identification of Pantoea species associated with plants, humans and the natural environment based on multilocus sequence analysis (MLSA)', *Systematic and applied microbiology*, vol. 31, no. 6-8, pp. 447-460.

Brady, C, Venter, S, Cleenwerck, I, Engelbeen, K, De Vos, P, Wingfield, M, Telechea, N & Coutinho, T 2009, 'Isolation of Enterobacter cowanii from Eucalyptus showing symptoms of bacterial blight and dieback in Uruguay', *Letters in applied microbiology*, vol. 49, no. 4, pp. 461-465.

Brady, CL, Venter, SN, Cleenwerck, I, Vandemeulebroecke, K, De Vos, P & Coutinho, TA 2010, 'Transfer of Pantoea citrea, Pantoea punctata and Pantoea terrea to the genus Tatumella emend. as Tatumella citrea comb. nov., Tatumella punctata comb. nov and Tatumella terrea comb. nov and description of Tatumella morbirosei sp nov', *International Journal of Systematic and Evolutionary Microbiology*, vol. 60, no. 3, pp. 484-494.

Brummell, DA & Harpster, MH 2001, 'Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants', in *Plant cell walls*, Springer, pp. 311-340.

Bruton, B, Wells, J, Lester, G & Patterson, C 1991, 'Pathogenicity and characterization of Erwinia ananas causing a postharvest disease of cantaloup fruit', *Plant Disease*, vol. 75, no. 2, pp. 180-183.

Burdon, J, Moore, K & Wainwright, H 1990, 'A preliminary examination of the physiological disorder'soft-nose'in mango fruit', *Tropical Fruits, XXIII IHC 296*, pp. 15-22.

Buron-Moles, G, López-Pérez, M, González-Candelas, L, Viñas, I, Teixidó, N, Usall, J & Torres, R 2012, 'Use of GFP-tagged strains of Penicillium digitatum and Penicillium expansum to study host-pathogen interactions in oranges and apples', *International Journal of Food Microbiology*, vol. 160, no. 2, pp. 162-170.

Cantín, CM, Minas, IS, Goulas, V, Jiménez, M, Manganaris, GA, Michailides, TJ & Crisosto, CH 2012, 'Sulfur dioxide fumigation alone or in combination with CO 2-enriched atmosphere extends the market life of highbush blueberry fruit', *Postharvest Biology and Technology*, vol. 67, pp. 84-91.

Caporaso, JG, Kuczynski, J, Stombaugh, J, Bittinger, K, Bushman, FD, Costello, EK, Fierer, N, Pena, AG, Goodrich, JK & Gordon, JI 2010, 'QIIME allows analysis of high-throughput community sequencing data', *Nature methods*, vol. 7, no. 5, p. 335.

Chong, KL, Peng, N, Yin, H, Lipscomb, GG & Chung, T-S 2013, 'Food sustainability by designing and modelling a membrane controlled atmosphere storage system', *Journal of Food Engineering*, vol. 114, no. 3, pp. 361-374.

Conway, WS, Sams, CE & Kelman, A 1994, 'Enhancing the natural resistance of plant tissues to postharvest diseases through calcium applications', *Annu. Rev. Phytopathol*, vol. 18, pp. 361-387.

Cracknell, T, A,, Cid Ballarin, M, Socorro Monzon, A, Fernandez Galvan, D & Rosell Garcia, P 2004, 'Incidence of internal fruit breakdown in various mango (Mangifera indica L.) cultivars', *Acta horticulturae*, pp. 315-318.

Crous, P & Groenewald, J 2011, 'Why everlastings don't last', *Persoonia: Molecular Phylogeny and Evolution of Fungi*, vol. 26, p. 70.

Cua, A & Lizada, M 1989, 'Ethylene production in the 'Carabao'mango (Mangifera indica L.) fruit during maturation and ripening', in *Symposium on Tropical Fruit in International Trade 269*, pp. 169-180.

Daddiego, L., Bianco, L., Capodicasa, C., Carbone, F., Dalmastri, C., Daroda, L., Delfiore, A., DE Rossi, P., DI Carli, M., Donini, M., Lopez, L., Mengoni, A., Paganin, P., Perrotta, G. & Bevivino, A 2018, 'Omics approaches on fresh-cut lettuce reveal global molecular responses to sodium hypochlorite and peracetic acid treatment'. *Journal of the Science of Food and Agriculture,* 98, pp 737-750.

Darvas, J 1981, 'Pre-harvest chemical control of post-harvest avocado diseases', *South African Avocado Growers' Association Yearbook*, vol. 7, pp. 57-59.

Daub, ME 1986, 'Tissue culture and the selection of resistance to pathogens', *Annual Review of Phytopathology*, vol. 24, no. 1, pp. 159-186.

De Hoon, MJ, Imoto, S, Nolan, J & Miyano, S 2004, 'Open source clustering software', *Bioinformatics*, vol. 20, no. 9, pp. 1453-1454.

Delétoile, A, Decré, D, Courant, S, Passet, V, Audo, J, Grimont, P, Arlet, G & Brisse, S 2009, 'Phylogeny and identification of Pantoea species and typing of Pantoea agglomerans strains by multilocus gene sequencing', *Journal of clinical microbiology*, vol. 47, no. 2, pp. 300-310.

Deltsidis, A, Brecht, J, Bai, J & Baldwin, E 2016, 'Tomato chilling injury threshold defined by the volatile profiles of pink harvested tomato fruit', in *Acta Horticulturae*, vol. 1120, pp. 143-149.

Dembele, DD, Kamara, A, Grechi, I, Silué, N, N'goran, N, Yéo, Y, Rey, J-Y & Koné, D 2020, 'Morphological characteristics and distribution of Colletotrichum isolates morphotypes infecting mango (Mangifera indica L.) in the north of Côte d'Ivoire', *African Journal of Food, Agriculture, Nutrition and Development*, vol. 20, no. 3, pp. 15837-15856.

Deshmukh, S & Rai, M 2005, *Biodiversity of fungi*, Enfield: Science Publishers, Inc.

Diedhiou, PM, Diallo, Y, Faye, R, Mbengue, AA & Sene, A 2014, 'Efficacy of different fungicides against mango anthracnose in Senegalese Soudanian agroclimate', *American Journal of Plant Sciences*, vol. 5, no. 15, p. 2224.

Dillon, NL, Bally, IS, Wright, CL, Hucks, L, Innes, DJ & Dietzgen, RG 2013, 'Genetic diversity of the Australian national mango genebank', *Scientia horticulturae*, vol. 150, pp. 213-226.

Diskin, S, Feygenberg, O, Maurer, D, Droby, S, Prusky, D & Alkan, N 2017, 'Microbiome alterations are correlated with occurrence of postharvest stem-end rot in mango fruit', *Phytobiomes*, vol. 1, no. 3, pp. 117-127.

Diskin, S, Sharir, T, Feygenberg, O, Maurer, D & Alkan, N 2019, 'Fludioxonil–A potential alternative for postharvest disease control in mango fruit', *Crop Protection*, p. 104855.

Dixon, J, Elmsly, T & Smith, D 2004, 'Alternatives to Sportax ', *New Zealand Avocado Growers' Association Annual Research Report*, vol. 4, pp. 32-35.

Doreyappa Gowda, I & Huddar, A 2001, 'Studies on ripening changes in mango (Mangifera indica L.) fruits', *Journal of Food Science and Technology*, vol. 38, no. 2, pp. 135-137.

Du Plooy, W, Combrinck, S, Botha, B, Van der Merwe, C & Regnier, T 2006, 'Development of discolouration in mango lenticels', in *VIII International Mango Symposium 820*, pp. 665-672.

Edgar, RC 2013, 'UPARSE: highly accurate OTU sequences from microbial amplicon reads', *Nature methods*, vol. 10, no. 10, p. 996.

Ekanayake, G, Abeywickrama, K, Daranagama, A & Kannangara, S 2019, 'Morphological Characterization and Molecular Identification of Stem-end Rot Associated Fungal Species Isolated from 'Karutha Colomban'Mango Fruits in Sri Lanka', *Journal of Agricultural Sciences–Sri Lanka*, vol. 14, no. 2.

Emily C, Gotschlich, R & Colbert, T 2019 'Past, present, and future,Best Practice & Research Clinical Rheumatology', *Methods in Microbiome Research*, vol 33, pp. 101498.

Engel, KH & Tressl, R 1983, 'Studies on the volatile components of two mango varieties', *Journal of agricultural and food chemistry*, vol. 31, no. 4, pp. 796-801.

Falade, M, Enikuomehin, O & Borisade, O 2017, 'Sequence of Application Benomyl and Plant Extracts in the Control of Cowpea Anthracnose Caused by Colletotrichum lindemuthianum Sensu Lato', *Asian Journal of Advances in Agricultural Research*, pp. 1-9.

Faostat, 2016, 'Agriculture Organization of the United Nations Statistics Division (2014)', *Production Available in: http://faostat3. fao. org/browse/Q/QC/S [Review date: April 2015]*.

Faostat, 2019, 'Agriculture Organization of the United Nations Statistics Division (2014)', *Production Available in: http://faostat3. fao. org/browse/Q/QC/S [Review date: April 2015]*.

Farkas, J 2006, 'Irradiation for better foods', *Trends in Food Science & Technology*, vol. 17, no. 4, pp. 148-152.

Forohling, A., Rademacher, A., Rumpold, B., Klocke, M. & Schluter, O 2018, 'Screening of microbial communities associated with endive lettuce during postharvest processing on industrial scale. *Heliyon*, 4, e00671.

Franceschi, VR, Krokene, P, Christiansen, E & Krekling, T 2005, 'Anatomical and chemical defenses of conifer bark against bark beetles and other pests', *New Phytologist*, vol. 167, no. 2, pp. 353-376.

Fricker AM, Podlesny D, Fricke WF 2019, 'What is new and relevant for sequencing-based microbiome research? A mini-review. *J Adv Res*, vol.19, pp. 105e12.

Galsurker, O, Diskin, S, Duanis-Assaf, D, Doron-Faigenboim, A, Maurer, D, Feygenberg, O & Alkan, N 2020, 'Harvesting Mango Fruit with a Short Stem-End Altered Endophytic Microbiome and Reduce Stem-End Rot', *Microorganisms*, vol. 8, no. 4, p. 558.

Gao, H, Yin, X, Jiang, X, Shi, H, Yang, Y, Dai, X, Chen, Y & Wu, X 2019, *Diversity and pathogenicity of microbial communities causing grape sour rot in eastern coastal areas of China*, 2167-9843, PeerJ Preprints.

Gowda, I & Huddar, A 2001, 'Studies on ripening changes in mango (Mangifera indica L.) fruits', *Journal of food science and technology*, vol. 38, no. 2, pp. 135-137.

Gorni, C, Allemand, D, Rossi, D & Mariani, P 2015, 'Microbiome profiling in fresh-cut products' *Trends in Food Science & Technology*, vol 46. Pp 295-301.

Gutiérrez-Barranquero, JA, Cazorla, FM, Torés, JA & de Vicente, A 2019, 'Pantoea agglomerans as a new etiological agent of a bacterial necrotic disease of mango trees', *Phytopathology*, vol. 109, no. 1, pp. 17-26.

Hall, ME & Wilcox, WF 2019, 'Identification and frequencies of endophytic microbes within healthy grape berries', *American Journal of Enology and Viticulture*, vol. 70, no. 2, pp. 212-219.

Hamady, M. & Knight, R 2009, 'Microbial community profiling for human microbiome projects: Tools, techniques, and challenges' *Genome Research*, 19, pp. 1141-1152.

Han, J, Tian, SP, Meng, XH & Ding, ZS 2006, 'Response of physiologic metabolism and cell structures in mango fruit to exogenous methyl salicylate under low-temperature stress', *Physiologia Plantarum*, vol. 128, no. 1, pp. 125-133.

Hassan, M, Dann, E, Coates, L, Hofman, P & Irving, D 2011, 'Retention of the fruit peduncle at harvest retains sap and contributes to resistance against post-harvest anthracnose disease in 'Kensington Pride', but not in 'R2E2'mango', *The Journal of Horticultural Science and Biotechnology*, vol. 86, no. 3, pp. 261-266.

Hassenberg, K, Huyskens-Keil, S & Herppich, W 2013, 'Impact of postharvest UV-C and ozone treatments on microbiological properties of white asparagus (Asparagus officinalis L.)', *Journal of Applied Botany and Food Quality*, vol. 85, no. 2, p. 174.

Ho, BT, Hofman, PJ, Joyce, DC & Bhandari, BR 2016, 'Uses of an innovative ethylene-α-cyclodextrin inclusion complex powder for ripening of mango fruit', *Postharvest biology and technology*, vol. 113, pp. 77-86.

Hodges, JD, Elam, WW, Watson, WF & Nebeker, TE 1979, 'Oleoresin characteristics and susceptibility of four southern pines to southern pine beetle (Coleoptera: Scolytidae) attacks', *The Canadian Entomologist*, vol. 111, no. 08, pp. 889-896.

Hoehn, E, Prange, RK & Vigneault, C 2009, 'Storage technology and applications', *Modified and controlled atmospheres for the storage, transportation, and packaging of horticultural commodities. CRC Press, Taylor & Francis Group, Boca Raton*, pp. 17-50.

Hoffman, Peter, Andrew MacNish, Darryl Joyce, Terrence Campbell & Cameron McConchie, CM 2013a, 'Progress on the investigation into resin canal disorder in mangoes'. *The Plant Industries NT Newsletter*, vol. November 2013, no. Northern Territory Government. Darwin, pp. 3-5.

Hoffman, Peter, Andrew MacNish, Darryl Joyce, Terrence Campbell & Cameron McConchie, CM 2013b, 'Progress on the investigation into resin canal disorder in mangoes', *The Plant*

*Industries NT Newsletter*, vol. November 2013, no. Northern Territory Government. Darwin, pp. 3-5.

Hoffmann, H & Roggenkamp, A 2003, 'Population genetics of the nomenspecies Enterobacter cloacae', *Appl. Environ. Microbiol.*, vol. 69, no. 9, pp. 5306-5318.

Hofman, P, Marques, J, Taylor, L, Stubbings, B, Ledger, S & Jordan, R 2009, 'Skin damage to two new mango cultivars during irradiation and cold storage', in *VI International Postharvest Symposium 877*, pp. 475-481.

Hofman, PJ, Smith, LG, Joyce, DC, Johnson, GI & Meiburg, GF 1997, 'Bagging of mango (Mangifera indica cv.Keitt') fruit influences fruit quality and mineral composition', *Postharvest Biology and Technology*, vol. 12, no. 1, pp. 83-91.

Holmes, RJ, Ledger, SN, Macleod & WNB 1992, 'Handling systems to reduce mango sapburn', in *IV International Mango Symposium 341*, pp. 528-532.

Holmes, Rowland, Hofman, J, P, Barker & Leigh 2009, *Mango Quality Assessment Manual: A Guide to Assessing the Post-harvest Quality of Australian Mangoes*, Department of Employment, Economic Development and Innovation.

Hood, S, Sala, A, Heyerdahl, EK & Boutin, M 2015, 'Low-severity fire increases tree defense against bark beetle attacks', *Ecology*, vol. 96, no. 7, pp. 1846-1855.

Huyskens-Keil, S, Hassenberg, K & Herppich, W 2012, 'Impact of postharvest UV-C and ozone treatment on textural properties of white asparagus (Asparagus officinalis L.)', *Journal of Applied Botany and Food Quality*, vol. 84, no. 2, p. 229.

Ikeda, H, Shibuya, T, Nishiyama, M, Nakata, Y & Kanayama, Y 2016, 'Physiological Mechanisms Accounting for the Lower Incidence of Blossom-end Rot in Tomato Introgression Line IL8-3 Fruit', *The Horticulture Journal*, pp. OKD-015.

Innovation, H 2018, *Australian Horticulture Statistics Handbook, 2017/18*.

Issa-Zacharia, A, Kamitani, Y, Muhimbula, HS & Ndabikunze, BK 2010, 'A review of microbiological safety of fruits and vegetables and the introduction of electrolyzed water as an alternative to sodium hypochlorite solution'. African Journal of Food Science, vol. 4, no. 13, pp 778-789.

Jabeen, A 2016, 'Field and postharvest biology of dendritic spot and stem end rot of mango'. PhD thesis, University of Queensland, Australia.

Jacobi, KK, MacRae, EA & Hetherington, SE 2001, 'Postharvest heat disinfestation treatments of mango fruit', *Scientia horticulturae*, vol. 89, no. 3, pp. 171-193.

Jha, S, Chopra, S & Kingsly, A 2007, 'Modeling of color values for nondestructive evaluation of maturity of mango', *Journal of Food Engineering*, vol. 78, no. 1, pp. 22-26.

Jha, S, Kingsly, A & Chopra, S 2006, 'Physical and mechanical properties of mango during growth and storage for determination of maturity', *Journal of Food Engineering*, vol. 72, no. 1, pp. 73-76.

Jha, S, Narsaiah, K, Sharma, A, Singh, M, Bansal, S & Kumar, R 2010, 'Quality parameters of mango and potential of non-destructive techniques for their measurement—a review', *Journal of food science and technology*, vol. 47, no. 1, pp. 1-14.

Jha, S, Sethi, S, Srivastav, M, Dubey, A, Sharma, R, Samuel, D & Singh, A 2010, 'Firmness characteristics of mango hybrids under ambient storage', *Journal of Food Engineering*, vol. 97, no. 2, pp. 208-212.

Jin, X, Chen, W, Chen, H, Chen, W & Zhong, Q 2018, 'Comparative Evaluation of the Antioxidant Capacities and Organic Acid and Volatile Contents of Mango Slurries Fermented with Six Different Probiotic Microorganisms', *Journal of Food Science*, vol. 83, no. 12, pp. 3059-3068.

Joel, D 1980, 'Resin ducts in the mango fruit: a defence system', *Journal of Experimental Botany*, vol. 31, no. 6, pp. 1707-1718.

Joel, D 1981, 'The duct systems of the base and stalk of the mango fruit', *Botanical Gazette*, pp. 329-333.

Joel, D & Fahn, A 1980, 'Ultrastructure of the resin ducts of Mangifera indica L.(Anacardiaceae). 1. Differentiation and senescence of the shoot ducts', *Annals of Botany*, vol. 46, no. 2, pp. 225-233.

John, KS, Rao, LJM, Bhat, S & Rao, UP 1999, 'Characterization of aroma components of sap from different Indian mango varieties', *Phytochemistry*, vol. 52, no. 5, pp. 891-894.

Johnson, GI, Mead, AJ, Cooke, AW, Dean & JR 1992, 'Mango stem end rot pathogens-Fruit infection by endophytic colonisation of the inflorescence and pedicel', *Annals of Applied Biology*, vol. 120, no. 2, pp. 225-234.

Johnson, P & Parr, D 2000, 'Mango growing in Western Australia', Bulletin 4348.

Jones, R, Truett, J & Hill, M 1993, 'Postharvest handling of cut immature Eucalyptus foliage, *Australian Journal of Experimental Agriculture*, vol. 33, no. 5, pp. 663-667.

Jones, RB & Hill, M 1993, 'The effect of germicides on the longevity of cut flowers', *Journal of the American Society for Horticultural Science*, vol. 118, no. 3, pp. 350-354.

Joseph, K & Aworh, O 1991, 'Composition, sensory quality and respiration during ripening and storage of edible wild mango (Irvingia gabonensis)', *International journal of food science & technology*, vol. 26, no. 3, pp. 337-342.

Juliano, JB & Cuevas, NL 1932, 'Floral morphology of the mango (Mangifera indica L.) with special reference to the pico variety from the Philippines', *Philipp Agr*, vol. 21, pp. 15-24.

Justo, A, Miettinen, O, Floudas, D, Ortiz-Santana, B, Sjökvist, E, Lindner, D, Nakasone, K, Niemelä, T, Larsson, K-H & Ryvarden, L 2017, 'A revised family-level classification of the Polyporales (Basidiomycota)', *Fungal Biology*, vol. 121, no. 9, pp. 798-824.

Kader, AA 2002, *Postharvest technology of horticultural crops*, vol. 3311, UCANR Publications.

Kakani, G, Taylor, TM, Martínez-Gonzáles, NE, Rodriguez-García, MO, Martinez-Cardenas, C, Escalona-Buendia, HB & Castillo, A 2018, 'Using antimicrobials as a food safety measure during phytosanitary treatments in mangoes', *Postharvest biology and technology*, vol. 138, pp. 114-124.

Kämpfer, P, Ruppel, S & Remus, R 2005, 'Enterobacter radicincitans sp. nov., a plant growth promoting species of the family Enterobacteriaceae', *Systematic and applied microbiology*, vol. 28, no. 3, pp. 213-221.

Katrodia, J 1985, 'Spongy tissue in mango—causes and control measures', in *II International Symposium on Mango 231*, pp. 814-826.

Kemler, M, Garnas, J, Wingfield, MJ, Gryzenhout, M, Pillay, K-A & Slippers, B 2013, 'Ion Torrent PGM as tool for fungal community analysis: a case study of endophytes in Eucalyptus grandis reveals high taxonomic diversity', *Plos One*, vol. 8, no. 12, p. e81718.

Keskinen, LA, Burke, A & Annous, BA 2009, 'Efficacy of chlorine, acidic electrolyzed water and aqueous chlorine dioxide solutions to decontaminate Escherichia coli O157: H7 from lettuce leaves', *International Journal of Food Microbiology*, vol. 132, no. 2-3, pp. 134-140.

King, EO, Ward, MK & Raney, DE 1954, 'Two simple media for the demonstration of pyocyanin and fluorescin', *Translational Research*, vol. 44, no. 2, pp. 301-307.

Koch, AL 2007, 'Growth measurement', in *Methods for General and Molecular Microbiology, Third Edition*, American Society of Microbiology, pp. 172-199.

Korsten, L, De Villiers, E, Wehner, F & Kotzé, J 1997, 'Field sprays of Bacillus subtilis and fungicides for control of preharvest fruit diseases of avocado in South Africa', *Plant Disease*, vol. 81, no. 5, pp. 455-459.

Kruys, Å, Eriksson, OE & Wedin, M 2006, 'Phylogenetic relationships of coprophilous Pleosporales (Dothideomycetes, Ascomycota), and the classification of some bitunicate taxa of unknown position', *Mycological research*, vol. 110, no. 5, pp. 527-536.

Kulkarni, V, Bally, I, Brettell, R, Johnson, P & Hamilton, D 2000, 'The Australian national mango breeding program-In search of improved cultivars for the new millennium', in *International Symposium on Tropical and Subtropical Fruits 575*, pp. 287-293.

Lalel, HJD, Singh, Z & Tan, S 2003a, 'Distribution of aroma volatile compounds in different parts of mango fruit', *The Journal of Horticultural Science and Biotechnology*, vol. 78, no. 2, pp. 131-138.

Lalel, HJD, Singh, Z & Tan, S 2003b, 'The role of ethylene in mango fruit aroma volatiles biosynthesis', *The Journal of Horticultural Science and Biotechnology*, vol. 78, no. 4, pp. 485-496.

Lalel, HJD, Singh, Z & Tan, S 2004, 'Ripening temperatures in¯ uence biosynthesis of aroma volatile compounds inKensington Pride'mango fruit', *The Journal of Horticultural Science and Biotechnology*, vol. 79, no. 1, pp. 146-157.

Lavanya, EK, Rao, DB, Edukondalu, L, Lakshmypathy, R & Rao, VS 2019, 'Effect of Ethephon and Storage Temperature on Physico-chemical Changes during Ripening of Mango (Mangifera indica L.) Cv. Neelum', *Current Journal of Applied Science and Technology*, pp. 1-11.

Lazan, H, Ali, Z, Lee, K, Voon, J & Chaplin, G 1986, 'The potential role of polygalacturonase in pectin degradation and softening of mango fruit', *Asean Food J*, vol. 2, no. 3-4, pp. 93-98.

Lechaudel, M, Genard, M, Lescourret, F, Urban, L & Jannoyer, M 2002, 'Leaf-to-fruit ratio affects water and dry-matter content of mango fruit', *The Journal of Horticultural Science and Biotechnology*, vol. 77, no. 6, pp. 773-777.

Ledger, S 1989, 'Post-harvest handling of mangoes', in *III International Mango Symposium 291*, pp. 479-488.

Ledger, S, Campbell, T, Holmes, R, Kernot, I & Hofman, P 2003, 'Improving knowledge and practices in mango supply chains', In: Proceedings of the Australian Postharvest Horticulture Conference. Brisbane, Australia, 1-3 October, 2003, pp. 77-79

Ledger, SN, Barker, L, Hofman, PJ, Campbell, J, Jones, V, Holmes, R & Campbell, TP 2010, *Mango ripening manual*, Department of Employment, Economic Development and Innovation.

Lee, M & Tzeng, D 2006, 'Pantoea agglomerans MB-9 is a potential pathogen causing necrosis on mango leaf', *Plant Pathology Bulletin*, vol. 15, no. 1, pp. 63-68.

Lemoine, F, Correia, D, Lefort, V, Doppelt-Azeroual, O, Mareuil, F, Cohen-Boulakia, S & Gascuel, O 2019, 'NGPhylogeny. fr: new generation phylogenetic services for non-specialists', *Nucleic acids research*, vol. 47, no. W1, pp. W260-W265.

Leon, K, Mery, D, Pedreschi, F & Leon, J 2006, 'Color measurement in L∗ a∗ b∗ units from RGB digital images', *Food Research International*, vol. 39, no. 10, pp. 1084-1091.

Letunic, I & Bork, P 2019, 'Interactive Tree Of Life (iTOL) v4: recent updates and new developments', *Nucleic acids research*, vol. 47, no. W1, pp. W256-W259.

Li, W, Zhu, X-G, Zhang, Q-J, Li, K, Zhang, D, Shi, C & Gao, L-Z 2020, 'SMRT sequencing generates the chromosome-scale reference genome of tropical fruit mango, Mangifera indica', *BioRxiv*.

Li, Y, Brackett, RE, Chen, J & Beuchat, LR 2001, 'Survival and growth of Escherichia coli O157: H7 inoculated onto cut lettuce before or after heating in chlorinated water, followed by storage at 5 or 15 C', *Journal of food protection*, vol. 64, no. 3, pp. 305-309.

Li, Z, Wang, N, Raghavan, GV & Vigneault, C 2009, 'Ripeness and rot evaluation of 'Tommy Atkins' mango fruit through volatiles detection', *Journal of Food Engineering*, vol. 91, no. 2, pp. 319-324.

Lima, C, Adimilson, BC, Maria & F 2001, 'Changes in amylase activity starch and sugars contents in mango fruits pulp cv. Tommy Atkins with spongy tissue', *Brazilian Archives of Biology and technology*, vol. 44, no. 1, pp. 59-62.

Lindow, SE, Desurmont, C, Elkins, R, McGourty, G, Clark, E & Brandl, MT 1998, 'Occurrence of indole-3-acetic acid-producing bacteria on pear trees and their association with fruit russet', *Phytopathology*, vol. 88, no. 11, pp. 1149-1157.

Linke, M & Geyer, M 2013, 'Condensation dynamics in plastic film packaging of fruit and vegetables', *Journal of Food Engineering*, vol. 116, no. 1, pp. 144-154.

Litz, RE 2009, *The mango: botany, production and uses*, CABI. International, UK.

Liu, J, Abdelfattah, A, Norelli, J, Burchard, E, Schena, L, Droby, S & Wisniewski, M 2018, 'Apple endophytic microbiota of different rootstock/scion combinations suggests a genotype-specific influence', *Microbiome*, vol. 6, no. 1, p. 18.

Liu, X-Z, Wang, Q-M, Theelen, B, Groenewald, M, Bai, F-Y & Boekhout, T 2015, 'Phylogeny of tremellomycetous yeasts and related dimorphic and filamentous basidiomycetes reconstructed from multiple gene sequence analyses', *Studies in Mycology*, vol. 81, pp. 1-26.

Lizada, C 1993, 'Mango', in *Biochemistry of fruit ripening*, Springer, pp. 255-271.

Lizada, M 1989, 'Postharvest physiology of the mango-A review', in *III International Mango Symposium 291*, pp. 437-453.

Lombard, L, Houbraken, J, Decock, C, Samson, R, Meijer, M, Réblová, M, Groenewald, JZ & Crous, PW 2016, 'Generic hyper-diversity in Stachybotriaceae', *Persoonia: Molecular Phylogeny and Evolution of Fungi*, vol. 36, p. 156.

Lombardero, M, Ayres, MP, Lorio Jr, PL & Ruel, JJ 2000, 'Environmental effects on constitutive and inducible resin defences of Pinus taeda', *Ecology letters*, vol. 3, no. 4, pp. 329-339.

Lopez-Galvez, F, Ragaert, P, Palermo, LA, Eriksson, M & Devlieghere, F 2013, 'Effect of new sanitizing formulations on quality of fresh-cut iceberg lettuce', *Postharvest Biology and Technology*, vol. 85, pp. 102-108.

Loveys, B, Robinson, S, Brophy, J & Chacko, E 1992, 'Mango Sapburn: Components of Fruit Sap and Their Role in Causing Skin Damage', *Functional Plant Biology*, vol. 19, no. 5, pp. 449-457.

Lu, P, Chacko, EK, Bithell, SL, Schaper, H, Wiebel, J, Cole, S & Müller, WJ 2013, 'Productivity is negatively related to shoot growth across five mango cultivars in the seasonally wet-dry tropics of northern Australia', *Fruits*, vol. 68, no. 4, pp. 279-289.

Lucaciu, R, Pelikan, C, Gerner, S, Zioutis, C, Köstlbacher, S, Marx, H, Herbold, CW, Schmidt, H & Rattei, T 2019, 'A bioinformatics guide to plant microbiome analysis', *Frontiers in Plant Science*, vol. 10, p. 1313.

MacLeod, AJ & de Troconis, NG 1982, 'Volatile flavour components of mango fruit', *Phytochemistry*, vol. 21, no. 10, pp. 2523-2526.

MacLeod, AJ & Snyder, CH 1988, 'Volatile components of mango preserved by deep freezing', *Journal of agricultural and food chemistry*, vol. 36, no. 1, pp. 137-139.

Macnish 2016, 'Reducing mango industry losses from resin canal discolouration', *Horticulture Innovation Australia Limited, Sydney*, p. 53.

Macnish, A, Peter, H, Cameron, M & Terrence, C 2014, 'Identifying factors that contribute to mango resin canal discolouration', *Horticulture Innovation Australia Limited, Sydney*, pp. 1-33.

Macnish, A, McConchie, C, Hofman, P & Joyce, D 2015, 'Potential factors that contribute to resin canal discoloration in mango fruit', in *XI International Mango Symposium 1183*, pp. 311-318.

Mahato, AK, Sharma, N, Singh, A, Srivastav, M, Singh, SK, Singh, AK, Sharma, TR & Singh, NK 2016, 'Leaf Transcriptome Sequencing for Identifying Genic-SSR Markers and SNP Heterozygosity in Crossbred Mango Variety 'Amrapali'(Mangifera indica L.)', *Plos One*, vol. 11, no. 10, p. e0164325.

Manasa, B, Jagadeesh, S, Thammaiah, N, Jagadeesh, R & Nethravathi, PG 2018, 'Pre-harvest application of azoxystrobin minimised anthracnose of mango (cv.'Alphonso') both at field and postharvest level enhancing yield and quality of fruits', *Journal of Pharmacognosy and Phytochemistry*, vol. 7, no. 3, pp. 2962-2967.

Manjunatha, G, Gupta, KJ, Lokesh, V, Mur, LA & Neelwarne, B 2012, 'Nitric oxide counters ethylene effects on ripening fruits', *Plant signaling & behavior*, vol. 7, no. 4, pp. 476-483.

Manthey, JA & Perkins-Veazie, P 2009, 'Influences of harvest date and location on the levels of β-carotene, ascorbic acid, total phenols, the in vitro antioxidant capacity, and phenolic profiles of five commercial varieties of mango (Mangifera indica L.)', *Journal of agricultural and food chemistry*, vol. 57, no. 22, pp. 10825-10830.

Marín-Cevada, V, Caballero-Mellado, J, Bustillos-Cristales, R, Muñoz-Rojas, J, Mascarúa-Esparza, MA, Castañeda-Lucio, M, López-Reyes, L, Martínez-Aguilar, L & Fuentes-Ramírez, LE 2010, 'Tatumella ptyseos, an unrevealed causative agent of pink disease in pineapple', *Journal of Phytopathology*, vol. 158, no. 2, pp. 93-99.

Marques, EJN, de Freitas, ST, Pimentel, MF & Pasquini, C 2016, 'Rapid and non-destructive determination of quality parameters in the 'Tommy Atkins' mango using a novel handheld near infrared spectrometer', *Food chemistry*, vol. 197, pp. 1207-1214.

Mathew, EN, Muyyarikkandy, MS, Bedell, C & Amalaradjou, MA 2018, 'Efficacy of chlorine, chlorine dioxide, and peroxyacetic acid in reducing Salmonella contamination in wash water and on mangoes under simulated mango packinghouse washing operations', *Frontiers in Sustainable Food Systems*, vol. 2, p. 18.

McCollum, TG, D'Aquino, S & McDonald, RE 1993, 'Heat treatment inhibits mango chilling injury', *HortScience*, vol. 28, no. 3, pp. 197-198.

McMillan & Wang-Wong, A 1992, 'A new disease of mango in Costa Rica caused by Erwinia-like bacteria. Una nueva enfermedad del mango en Costa Rica causada por una bacteria parecida a Erwinia', in *Proceedings of the Annual Meeting of the Florida State Horticultural Society.*, vol. 105, pp. 288-289.

Mead, A & Winston, E 1989, 'Description of the disorder'stem-end cavity', possible causes and suggestions for reducing incidence in packing sheds', in *III International Mango Symposium 291*, pp. 265-271.

Medlicott, A, Bhogal, M & Reynolds, S 1986, 'Changes in peel pigmentation during ripening of mango fruit (Mangifera indica var. Tommy Atkins)', *Annals of Applied Biology*, vol. 109, no. 3, pp. 651-656.

Medlicott, AP & Thompson, AK 1985, 'Analysis of sugars and organic acids in ripening mango fruits (Mangifera indica L. var Keitt) by high performance liquid chromatography', *Journal of the Science of Food and Agriculture*, vol. 36, no. 7, pp. 561-566.

Mercadante, AZ, Rodriguez-Amaya, DB & Britton, G 1997, 'HPLC and mass spectrometric analysis of carotenoids from mango', *Journal of agricultural and food chemistry*, vol. 45, no. 1, pp. 120-123.

Mignard, S & Flandrois, J-P 2006, '16S rRNA sequencing in routine bacterial identification: a 30-month experiment', *Journal of microbiological methods*, vol. 67, no. 3, pp. 574-581.

Mitcham, EJ & McDonald, RE 1993, 'Respiration rate, internal atmosphere, and ethanol and acetaldehyde accumulation in heat-treated mango fruit', *Postharvest biology and technology*, vol. 3, no. 1, pp. 77-86.

Mitra, SK 1997, *Postharvest physiology and storage of tropical and subtropical fruits*, Cab International, UK.

Mogollón, R, Contreras, C, da Silva Neta, ML, Marques, EJN, Zoffoli, JP & de Freitas, ST 2020, 'Non-destructive prediction and detection of internal physiological disorders in'Keitt'mango using a hand-held Vis-NIR spectrometer', *Postharvest biology and technology*, vol. 167, p. 111251.

Mollet, C, Drancourt, M & Raoult, D 1997, 'rpoB sequence analysis as a novel basis for bacterial identification', *Molecular microbiology*, vol. 26, no. 5, pp. 1005-1011.

Montecalvo, MP, Opina, OS, Dalisay, TU & Esguerra, EB 2019, 'Efficacy of postharvest treatments in reducing stem end rot of'Carabao'mango (*Mangifera indica* L.) fruit', *Philippine Journal of Crop Science*, vol. 44, no. 1, pp. 36-43.

Moore, C 2012, 'Resin canal disorder of mangoes: An overview', *In The Plant Industries NT eNewsletter*, no. December 2012, W. Hunt, ed. Darwin, Australia: Northern Territory Government, pp. 3-4.

Muirhead, I, Fitzell, R, Davis, R & Peterson, R 1982, 'Post-harvest control of anthracnose and stem-end rots of Fuerte avocados with prochloraz and other fungicides', *Australian Journal of Experimental Agriculture*, vol. 22, no. 119, pp. 441-446.

Mukherjee, S & Litz, RE 2009, 'Introduction: botany and importance', *The mango: Botany, production and uses*, no. Ed. 2, pp. 1-18.

Naqvi, SAH, Perveen, R, Manzoor, SA & Abbas, Z 2014, 'Occurance of mango anthracnose on inflorescence in Multan (Punjab) and it's in vitro control through various fungicides', *International Journal of Biological science*, vol. 14, pp. 184-191.

Nelson, SC 2008, 'Mango anthracnose (Colletotrichum gloeosporiodes)'. *Plant Disease,* pp.1-9

Nguyen, H, McConchie, R, Hofman, P, Smith, L, Stubbings, B & Adkins, M 2000, 'Effect of ethylene and ripening temperatures on the skin colour and flesh characteristics of ripe'Kensington Pride'mango fruit', in *International Symposium on Tropical and Subtropical Fruits 575*, pp. 635-642.

Nogales-Delgado, S, Fernández-León, AM, Delgado-Adamez, J, Hernández-Méndez, MT & Bohoyo-Gil, D 2013, 'Effects of several sanitisers for improving quality attributes of

minimally processed Fragaria vesca strawberry', *Czech Journal of Food Sciences*, vol. 31, no. 1, pp. 49-54.

Ntsoane, ML, Zude-Sasse, M, Mahajan, P & Sivakumar, D 2019, 'Quality assesment and postharvest technology of mango: A review of its current status and future perspectives', *Scientia horticulturae*, vol. 249, pp. 77-85.

O'Hare, T 1995, 'Effect of ripening temperature on quality and compositional changes of mango (Mangifera indica L.) cv. Kensington', *Australian journal of experimental agriculture*, vol. 35, no. 2, pp. 259-263.

O'Hare, T & Prasad, A 1991, 'The alleviation of sap-induced mango skin injury by calcium hydroxide', *Frontier in Tropical Fruit Research 321*, pp. 372-381.

Oosthuyse, S 1998, 'Effect of Environmental Conditions at Harvest on the Incidence of Lenticel Damage in Mango'. *South African Mango Growers' Association Yearbook*, vol 18, pp 15-17

Oosthuyse, S 1999, 'Effects of each of the stages in the pack-line on the incidence of lenticel damage in 'Keitt'and 'Tommy Atkins' mango', *South African Mango Growers' Association Yearbook*, vol. 19, pp. 37-39.

Osborne, JW 2010, 'Improving your data transformations: Applying the Box-Cox transformation', *Practical Assessment, Research & Evaluation*, vol. 15, no. 12, pp. 1-9.

Palou, L, Serrano, M, Martínez-Romero, D & Valero, D 2010, 'New approaches for postharvest quality retention of table grapes', *Fresh Produce*, vol. 4, pp. 103-110.

Patel, JB 2001, '16S rRNA gene sequencing for bacterial pathogen identification in the clinical laboratory', *Molecular diagnosis*, vol. 6, no. 4, pp. 313-321.

Pavitra Kumari, R & Singh, R 2017, 'Anthracnose of mango incited by Colletotrichum gloeosporioides: A comprehensive review', *Int. J. Pure App. Biosci*, vol. 5, no. 1, pp. 48-56.

Pesis, E, Aharoni, D, Aharon, Z, Ben-Arie, R, Aharoni, N & Fuchs, Y 2000, 'Modified atmosphere and modified humidity packaging alleviates chilling injury symptoms in mango fruit', *Postharvest biology and technology*, vol. 19, no. 1, pp. 93-101.

Pino, JA, Mesa, J, MUNoz, Y, Martí, MP & Marbot, R 2005, 'Volatile components from mango (Mangifera indica L.) cultivars', *Journal of agricultural and food chemistry*, vol. 53, no. 6, pp. 2213-2223.

Piombo, E, Abdelfattah, A, Danino, Y, Salim, S, Feygenberg, O, Spadaro, D, Wisniewski, M & Droby, S 2020, 'Characterizing the Fungal Microbiome in Date (Phoenix dactylifera) Fruit Pulp and Peel from Early Development to Harvest', *Microorganisms*, vol. 8, no. 5, p. 641.

Prange, RK, Wright, AH, DeLong, JM & Zanella, A 2012, 'History, current situation and future prospects for dynamic controlled atmosphere (DCA) storage of fruits and vegetables, using chlorophyll fluorescence', in *VII International Postharvest Symposium 1012*, pp. 905-915.

Prasanna, V, Yashoda, H, Prabha, T & Tharanathan, R 2003, 'Pectic polysaccharides during ripening of mango (Mangifera indica L)', *Journal of the Science of Food and Agriculture*, vol. 83, no. 11, pp. 1182-1186.

Premier, R 2013, 'Evaluation of vegetable washing chemicals', *Australia: Horticulture Australia Ltd*. pp 1-57

Pristijono, P, Golding, J & Bowyer, M 2019, 'Postharvest UV-C Treatment, Followed by Storage in a Continuous Low-Level Ethylene Atmosphere, Maintains the Quality of 'Kensington Pride'Mango Fruit Stored at 20° C', *Horticulturae*, vol. 5, no. 1, p. 1.

Quirós-Sauceda, AE, Sañudo-Barajas, JA, Vélez-de la Rocha, R, Domínguez-Avila, JA, Ayala-Zavala, JF, Villegas-Ochoa, MA & González-Aguilar, GA 2019, 'Effects of ripening on the in vitro antioxidant capacity and bioaccessibility of mango cv.'Ataulfo'phenolics', *Journal of food science and technology*, vol. 56, no. 4, pp. 2073-2082.

Rajan, S & Hudedamani, U 2019, 'Genetic Resources of Mango: Status, Threats, and Future Prospects', in *Conservation and Utilization of Horticultural Genetic Resources*, Springer, pp. 217-249.

Ram, R, Rahim, M & Alam, M 2020, 'Diagnosis and management of nutrient constraints in mango', in *Fruit Crops*, Elsevier, pp. 629-650.

Raymond, Luc, Schaffer, Bruce, Brecht, K, J, Crane & H, J 1998, 'Internal breakdown in mango fruit: symptomology and histology of jelly seed, soft nose and stem-end cavity', *Postharvest biology and technology*, vol. 13, no. 1, pp. 59-70.

Reddy, Y & Srivastava, C 1999, 'Ethylene biosynthesis and respiration in mango fruit during ripening. Indian journal of Plant Physiology vol.4 pp, 32-35.

Rivera, SA, Zoffoli, JP & Latorre, BA 2013, 'Determination of optimal sulfur dioxide time and concentration product for postharvest control of gray mold of blueberry fruit', *Postharvest Biology and Technology*, vol. 83, pp. 40-46.

Rooban, R, Shanmugam, M, Venkatesan, T & Tamilmani, C 2016, 'Physiochemical changes during different stages of fruit ripening of climacteric fruit of mango (Mangifera indica L.) and non-climacteric of fruit cashew apple (Anacardium occidentale L.)', *Journal of Applied and Advanced Research*, vol. 1, no. 2, pp. 53-58.

Rosman, N, Asli, N, Abdullah, S & Rusop, M 2019, 'Methods for ripening mango to extend shelf life', in *AIP Conference Proceedings*, vol. 2151, p. 020018.

Rymbai, H, Srivastav, M, Sharma, R & Singh, S 2012, 'Lenticels on mango fruit: Origin, development, discoloration and prevention of their discoloration', *Scientia horticulturae*, vol. 135, pp. 164-170.

Sabir, FK & Sabir, A 2017, 'Extending postharvest quality attributes of grapes (V. vinifera L. cv.'Thompson Seedless') by preharvest calcium pulverizations', *Acta Scientiarum Polonorum. Hortorum Cultus*, vol. 16, no. 5.

Sabuz, A, Chowdhury, M, Molla, M, Khan, M & Miaruddin, M 2019, 'Effect of Ethephon on ripening and postharvest quality of mango', *Bangladesh Journal of Agricultural Research*, vol. 44, no. 3, pp. 453-467.

Saldanha, AJ 2004, 'Java Treeview—extensible visualization of microarray data', *Bioinformatics*, vol. 20, no. 17, pp. 3246-3248.

San, A, Webb, R, Joyce, D, Hofman, P, Macnish, A & Smyth, H 2015, 'Anatomy of skin disorders afflicting Australian mangoes', in *XI International Mango Symposium 1183*, pp. 331-336.

Sangeetha, C & Rawal, R 2008, 'Nutritional studies of Colletotrichum gloeosporioides (Penz.) Penz. and Sacc. the incitant of mango anthracnose', *World Journal of Agricultural Sciences*, vol. 4, no. 6, pp. 717-720.

Schaeffer, RN, Mei, YZ, Andicoechea, J, Manson, JS & Irwin, RE 2017, 'Consequences of a nectar yeast for pollinator preference and performance', *Functional Ecology*, vol. 31, no. 3, pp. 613-621.

Seshadri, S, Manoharan, S & Singh, HS 2019, 'Preventive Regulation of Jelly Seed Disorder in 'Amrapali'Mango (Mangifera indica) by Preharvest Spray', *Horticultural Plant Journal*, vol. 5, no. 2, pp. 70-78.

Shao, Y-z, ZENG, J-k, Hong, T, Yi, Z & Wen, L 2019, 'The chemical treatments combined with antagonistic yeast control anthracnose and maintain the quality of postharvest mango fruit', *Journal of Integrative Agriculture*, vol. 18, no. 5, pp. 1159-1169.

Sharma, A, Sharma, I & Sharma, M 2019, 'Efficacy and economics of fungicides for management of mango anthracnose', *Indian Phytopathology*, vol. 72, no. 2, pp. 361-366.

Sharma, R, Singh, D & Singh, R 2009, 'Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review', *Biological control*, vol. 50, no. 3, pp. 205-221.

Sharma, S, Rajat, K, Prasad, R & Vasudevan, P 1999, 'Biology and potential of Psidium guajava'. NISCAIR-CSIR, India, pp 414.421

Shen, C, Luo, Y, Nou, X, Wang, Q & Millner, P 2013, 'Dynamic effects of free chlorine concentration, organic load, and exposure time on the inactivation of Salmonella,

Escherichia coli O157: H7, and non-O157 Shiga toxin–producing E. coli', *Journal of food protection*, vol. 76, no. 3, pp. 386-393.

Shivashankar, S 2014, 'Physiological disorders of mango fruit', *Horticultural Reviews: Volume 42*, pp. 313-348.

Shivashankara, K & Mathai, C 1999, 'Relationship of leaf and fruit transpiration rates to the incidence of spongy tissue disorder in two mango (Mangifera indica L.) cultivars', *Scientia Horticulturae*, vol. 82, no. 3-4, pp. 317-323.

Simbolo, M, Gottardi, M, Corbo, V, Fassan, M, Mafficini, A, Malpeli, G, Lawlor, RT & Scarpa, A 2013, 'DNA qualification workflow for next generation sequencing of histopathological samples', *PloS one*, vol. 8, no. 6, p. e62692.

Singh, Rupinder, Dwivedi & N, U 2008, 'Effect of ethrel and 1-methylcyclopropene (1-MCP) on antioxidants in mango (Mangifera indica var. Dashehari) during fruit ripening', *Food chemistry*, vol. 111, no. 4, pp. 951-956.

Singh, Zora, Singh, K, R, Sane, A, V, Nath & Pravendra 2013, 'Mango-postharvest biology and biotechnology', *Critical Reviews in Plant Sciences*, vol. 32, no. 4, pp. 217-236.

Singh, J, Silva, KJP, Fuchs, M & Khan, A 2019, 'Potential role of weather, soil and plant microbial communities in rapid decline of apple trees', *PloS one*, vol. 14, no. 3, p. e0213293.

Singh, P, Singh, MK, Kumar, V, Kumar, M & Malik, S 2012, 'Effect of physico-chemical treatments on ripening behavior and post-harvest quality of Amrapali mango (Mangifera indica L.) during storage', *Journal of environmental biology*, vol. 33, no. 2, p. 227.

Singh, S, Singh, Z & Swinny, E 2009, 'Postharvest nitric oxide fumigation delays fruit ripening and alleviates chilling injury during cold storage of Japanese plums (Prunus salicina Lindell)', *Postharvest Biology and Technology*, vol. 53, no. 3, pp. 101-108.

Singh, Z & Janes, J 2000, 'Effects of postharvest application of ethephon on fruit ripening, quality and shelf life of mango under modified atmosphere packaging', in *IV International Conference on Postharvest Science 553*, pp. 599-602.

Sivakumar, D, Jiang, Y & Yahia, EM 2011, 'Maintaining mango (Mangifera indica L.) fruit quality during the export chain', *Food Research International*, vol. 44, no. 5, pp. 1254-1263.

Sivakumar, D, Terry, LA & Korsten, L 2010, 'An overview on litchi fruit quality and alternative postharvest treatments to replace sulfur dioxide fumigation', *Food Reviews International*, vol. 26, no. 2, pp. 162-188.

Soracco, RJ, Wilde, EW, Mayack, LA & Pope, DH 1985, 'Comparative effectiveness of antifouling treatment regimes using chlorine or a slow-releasing bromine biocide', *Water research*, vol. 19, no. 6, pp. 763-766.

Soto-Muñoz, Lourdes, Teixidó, Neus, Usall, Josep, Viñas, Inmaculada, Torres & Rosario 2014, 'Detection and quantification by PCR assay of the biocontrol agent Pantoea agglomerans CPA-2 on apples', *International Journal of Food Microbiology*, vol. 175, pp. 45-52.

Soto-Muñoz, Lourdes, Torres, Rosario, Usall, Josep, Viñas, Inmaculada, Solsona, Cristina, Teixidó & Neus 2015, 'DNA-based methodologies for the quantification of live and dead cells in formulated biocontrol products based on Pantoea agglomerans CPA-2', *International Journal of Food Microbiology*, vol. 210, pp. 79-83.

Soto-Muñoz, L, Teixidó, N, Usall, J, Viñas, I & Torres, R 2014, 'Detection and quantification by PCR assay of the biocontrol agent Pantoea agglomerans CPA-2 on apples', *International Journal of Food Microbiology*, vol. 175, pp. 45-52.

Soto-Muñoz, L, Torres, R, Usall, J, Viñas, I, Dashevskaya, S & Teixidó, N 2015, 'Environmental monitoring of the biocontrol agent Pantoea agglomerans CPA-2 applied to citrus fruit at preharvest', *Annals of Applied Biology*, vol. 167, no. 2, pp. 250-261.

Sousa-Gallagher, MJ & Mahajan, PV 2013, 'Integrative mathematical modelling for MAP design of fresh-produce: theoretical analysis and experimental validation', *Food Control*, vol. 29, no. 2, pp. 444-450.

Srinivasan, R, Karaoz, U, Volegova, M, MacKichan, J, Kato-Maeda, M, Miller, S, Nadarajan, R, Brodie, EL & Lynch, SV 2015, 'Use of 16S rRNA gene for identification of a broad range of clinically relevant bacterial pathogens', *Plos One*, vol. 10, no. 2.

Strom, B, Goyer, R, Ingram, L, Boyd, G & Lott, L 2002, 'Oleoresin characteristics of progeny of loblolly pines that escaped attack by the southern pine beetle', *Forest Ecology and Management*, vol. 158, no. 1, pp. 169-178.

Sung, J, Suh, JH, Chambers, AH, Crane, J & Wang, Y 2019, 'Relationship between Sensory Attributes and Chemical Composition of Different Mango Cultivars', *Journal of agricultural and food chemistry*, vol. 67, no. 18, pp. 5177-5188.

Suwonsichon, S, CHAMBERS IV, E, Kongpensook, V & Oupadissakoon, C 2012, 'Sensory lexicon for mango as affected by cultivars and stages of ripeness', *Journal of sensory studies*, vol. 27, no. 3, pp. 148-160.

Taiti, C, Marone, E, Bazihizina, N, Caparrotta, S, Azzarello, E, Petrucci, AW, Pandolfi, C & Giordani, E 2016, 'Sometimes a little mango goes a long way: A rapid approach to assess how different shipping systems affect fruit commercial quality', *Food analytical methods*, vol. 9, no. 3, pp. 691-698.

Torres, J, Castelló, M, Escriche, I & Chiralt, A 2008, 'Quality characteristics, respiration rates, and microbial stability of osmotically treated mango tissue (Mangifera indica L.) with or without calcium lactate', *Food Science and Technology International*, vol. 14, no. 4, pp. 355-365.

Torres, R, Solsona, C, Viñas, I, Usall, J, Plaza, P & Teixidó, N 2014, 'Optimization of packaging and storage conditions of a freeze-dried P antoea agglomerans formulation for controlling postharvest diseases in fruit', *Journal of applied microbiology*, vol. 117, no. 1, pp. 173-184.

Torres, R, Teixidó, N, Usall, J, Abadias, M, Mir, N, Larrigaudiere, C & Viñas, I 2011, 'Anti-oxidant activity of oranges after infection with the pathogen Penicillium digitatum or treatment with the biocontrol agent Pantoea agglomerans CPA-2', *Biological control*, vol. 57, no. 2, pp. 103-109.

Urbina, H & Aime, MC 2018, 'A closer look at Sporidiobolales: Ubiquitous microbial community members of plant and food biospheres', *Mycologia*, vol. 110, no. 1, pp. 79-92.

Vazquez-Salinas, C & Lakshminarayana, S 1985, 'Compositional changes in mango fruit during ripening at different storage temperatures', *Journal of food science*, vol. 50, no. 6, pp. 1646-1648.

Venning, FD 1948, 'The ontogeny of the laticiferous canals in the Anacardiaceae', *American Journal of Botany*, pp. 637-644.

Větrovský, T, Baldrian, P & Morais, D 2018, 'SEED 2: a user-friendly platform for amplicon high-throughput sequencing data analyses', *Bioinformatics*, vol. 34, no. 13, pp. 2292-2294.

Weisburg, WG, Barns, SM, Pelletier, DA & Lane, DJ 1991, '16S ribosomal DNA amplification for phylogenetic study', *Journal of bacteriology*, vol. 173, no. 2, pp. 697-703.

Yashoda, HM, Prabha, TN & Tharanathan, RN 2006, 'Mango ripening: changes in cell wall constituents in relation to textural softening', *Journal of the Science of Food and Agriculture*, vol. 86, no. 5, pp. 713-721.

Yuping, R, Kaiwen, Z, Wenying, H, Jinghong, H, Xiaowei, F, Shuang, C, Jiaoqing, T, Xiaoxi, X, Daoxian, K & Yao, L 2016, 'Observation of Fungi, Bacteria, and Parasites in Clinical Skin Samples Using Scanning Electron Microscopy', *Modern Electron Microscopy in Physical and Life Sciences*, p. 225.

Zaharah, S & Singh, Z 2011, 'Mode of action of nitric oxide in inhibiting ethylene biosynthesis and fruit softening during ripening and cool storage of ‘Kensington Pride’mango', *Postharvest biology and technology*, vol. 62, no. 3, pp. 258-266.

Zhang, Baodong, Huang, Chongxing, Zhang, Linyun, Wang, Jian, Huang, Xingqiang, Zhao, Yuan, Liu, Ying, Li & Cuicui 2019, 'Application of chlorine dioxide microcapsule sustained-release antibacterial films for preservation of mangos', *Journal of food science and technology*, vol. 56, no. 3, pp. 1095-1103.

Zhang, Y, Schoch, C, Fournier, J, Crous, P, De Gruyter, J, Woudenberg, J, Hirayama, K, Tanaka, K, Pointing, S & Spatafora, J 2009, 'Multi-locus phylogeny of Pleosporales: a taxonomic, ecological and evolutionary re-evaluation', *Studies in Mycology*, vol. 64, pp. 85-102.

# Appendix 1

## Pre-harvest pilot studies

The effect of pre-harvest factors on RCD expression remains unclear. Two pilot studies were conducted to explore the role of plant nutrition and rainfall on RCD expression.

## Pilot study 1: Role of leaf nutrients in RCD incidence

Background:

The onset of some postharvest diseases and disorders has been shown to be related to plant nutrition (Sangeetha & Rawal 2008). In mango for example, Ca is an important factor causing internal physiological disorders that reduce fruit quality, shelf life and disease resistance (Raymond et al. 1998). In particular, Ca deficiency in mango can cause internal breakdown of the fruit pulp resulting in soft nose development (Cracknell et al. 2004; Ram et al. 2020).

Primary objective**:** To relate the incidence of RCD with the concentration of Ca in the leaves of mango

Hypothesis: Low calcium level in the leaves results in higher incidence of RCD in the fruit.

Material and Methods:

Fully expanded mature leaves of mango KP trees (~6-8 m tall) from four orchards in Darwin (12°26'17"S 130°50'28"E), Australia, were collected. These orchards had a known history RCD in the previous mango season. At each orchard, the area was divided into three blocks and each block was considered as a replication. A total 20 leaves were randomly collected from 20 different trees within the block, and these leaves with pooled to formed one replication. Leaves were washed thoroughly with distilled water and oven-dried at 50 $^{o}$C until constant dry weight. The dried sample were finely ground with a with a 0.5 mm sieve in a Thomas Willy Mill (Thomas Scientific) and sent to soil and plant laboratory (CSBP) soil and plant analysis laboratory, Western Australia (https://csbp-fertilisers.com.au/agronomy/ lab) to be

analysed for phosphorus (P), potassium (K), calcium (ca), magnesium (Mg), sulphur (S), boron (B), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn), chlorine (Cl), nitrogen (N) and molybdenum (Mo).

Statistical analysis:

The concentration of each nutrient in the leaves was tested by one-way analysis of variance (ANOVA) with means separation at 5% LSD using Statistical Tools for Agricultural Research version 2.0.1 (http://bbi.irri.org).

Results and Discussion:

Eight out of 14 nutrients were significantly influenced by orchard locations (Table P1.1). Despite a known history of RCD affecting these orchards, there did not appear to be any similarities regarding plant nutrition. The design of the experiment could have included orchards without any previous history of RCD to help clarify the role of the plant nutrition in contributing to RCD.

Table P 1. 1 Nutrient content of mango tree leaves from orchards with history of RCD incidence

| Orchard | Nutrient element | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **N %** | **P %** | **K %** | **Ca %** | **Mg %** | **S %** | **Cl %** | **B %** | **Cu %** | **Fe mg/kg** | **Mn mg/kg** | **Zn mg/kg** | **Mo mg/kg** |
| **1** | 1.14bc | 0.11b | 1.16 | 1.34a | 0.32c | 0.15 | 0.14 | 141.08 | 66.99b | 46.18 | 246.06a | 38.06c | 0.41c |
| **2** | 1.20b | 0.13a | 1.03 | 0.71b | 0.46b | 0.16 | 0.15 | 126.44 | 12.30b | 27.68 | 119.64c | 58.98b | 0.87b |
| **3** | 1.11c | 0.14a | 1.04 | 1.17a | 0.56a | 0.16 | 0.14 | 111.93 | 329.08a | 37.64 | 200.40ab | 43.97c | 0.13d |
| **4** | 1.30a | 0.14a | 1.12 | 1.13a | 0.45b | 0.17 | 0.15 | 136.78 | 14.23b | 29.24 | 139.31bc | 71.21a | 1.15a |

## Pilot study 2: Effect of stimulated rainfall on RCD incidence

Background:

Little is known about the effect of a rainfall event occurring at or immediately before the time of harvest on RCD expression in mango fruit but has been observed to (Moore 2012; Macnish et al. 2014). It is considered that wet conditions, especially at or immediately before the time of fruit harvest has significantly increased the Lenticel Damage (LD) on Tommy Atkins mango fruit (Oosthuyse 1998). Similarly, low temperature along with wind before harvest may cause LD on mango fruit (Pesis et al. 2000).To date, no study has investigated the effect a rainfall event close to harvest on RCD incidence.

Primary objective: To evaluate the influence of a stimulated rainfall event prior to harvest on RCD incidence

Hypothesis: A rainfall event prior to harvest may increase expression of RCD

Material and methods:

This experiment was conducted on an orchard in Darwin, Australia, with a known history of RCD. Four treatments were applied on selected tree (Table P2.1). A total of three mango KP trees (6-8 m) were randomly selected for each treatment. Each single tree was considered as replicate and was bounded on both sides by a guard tree.

Table P 2. 1 Details of the four stimulation rainfall event treatments

| Treatments | Description |
|---|---|
| 1 | Stimulated rainfall that was allowed to reach the soil |
| 2 | Stimulated rainfall on tree canopy only |
| 3 | Stimulated rainfall applied below tree canopy only |
| 4 | No simulated rainfall application (control) |

A rainfall event was simulated applying 50 mm of irrigation water via a diesel run sprinkler, before rainfall event, tree normal drip irrigation was blocked with stopper and six sprinklers were installed, two at top, two on sides and two under the tree canopy. Total 500L of water through sprinklers applied in treatments 1 and 2 was based on the highest rainfall event (50 mm) recorded during the mango season. The amount of water applied per tree was calculated by a formula (L) = area of the canopy ($m^2$) x average rainfall (mm) as given in http://www.calctool.org/CALC/other/default/rainfall. Amount of rainfall 50 ml rainfall applied on each tree was simulated to highest average rainfall during mango season in Darwin http://www.bom.gov.au/jsp/ncc/cdio/weatherData/av?p_display_type=dataGraph&p_stn_num=014048&p_nccObsCode=139&p_month=10.

In treatment 1, the water was applied to top of the tree canopy and allowed to be absorbed by the soil. For treatment 2, the ground under each tree canopy was covered with plastic to collect excess run off and prevent the water reaching the roots (Fig. P2.2). For treatment 3, water equivalent to a 50 mm rainfall event was applied using the commercial drip watering system set up in the orchard. Each treatment, the stimulated rainfall event was carried out between 0900 and 1200 hours. Five fruits from each tree (15 fruit per treatment) were harvested at sampling times including just before the rainfall event, five minutes, 24 hrs, 48 hrs and 72 hrs after the stimulated rainfall event.

Harvested fruit were packed in cardboard boxes and transported to Berrimah Farm laboratory (DPIR) Darwin and the fruits were stored at 20 °C for ripening and after ten

days of storage, fruits were accessed for RCD severity, fruit peel colour assessment, fruit dry matter content (DMC) and total soluble solids (TSS), lenticel spotting, sap burn injury and stem end rot (SER) according to the protocol described in Chapter 5.

Figure P 2. 1 Rainfall treatments on tree canopy

Statistical analysis:

Data was subjected to one-way analysis of variance (ANOVA) using Statistical Tools for Agricultural Research version 2.0.1 (http://bbi.irri.org).

Results and Discussion:

There was no significant treatment effect on dry matter content (DMC), fruit colour, fruit firmness, total soluble solids (TSS), stem end rot (SER) (Table P2.3). Importantly, only lenticel spotting was significantly higher in treatment 2 (fruit harvested 5 minutes after rain) as compared with other treatments. Rainfall, especially at or immediately before the time of fruit harvest effect the mango fruit quality (Johnson et al. 1992). Lenticel spotting in KP fruit harvest in wet conditions already observed (Jacobi et al. 2001). RCD fruit was not observed in any treatments at eating ripe stage (Table P2.4).

Table P 2. 2 Assessment of fruit quality parameters of before and after rainfall event.

| Treatment | Dry Matter Content (DMC) | | | | | Colour | Firmness | TSS | SER | Lenticel spotting | RCD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Before* | 5 min** | 24 hr** | 48 hr** | 72 hr** | | | | | | |
| 1 | 15.42 | 16.49 | 15.36 | 15.35 | 15.37 | 5.27 | 3.2 | 16.51 | 1.17 | 1.00c | 0 |
| 2 | 14.82 | 15.05 | 14.2 | 14.71 | 15.22 | 5.57 | 3.03 | 16.73 | 0.67 | 1.92a | 0 |
| 3 | 13.84 | 14.64 | 15.05 | 14.28 | 14.88 | 5.83 | 2.93 | 14.91 | 0.67 | 1.50ab | 0 |
| 4 | 14.35 | 13.89 | 14.48 | 14.62 | 14.74 | 5.4 | 3 | 15.13 | 1.33 | 1.12bc | 0 |

* rainfall event, ** After rainfall event, TSS (Total soluble solids), SER (Stem end rot)

Figure P 2.2 KP mango at eating ripe stage different treatments of rainfall study (left to right treatments 1, 2, 3 and 4)

Conclusion:

Rainfall event applied once had no influence of RCD development in fruits harvested at different time period after the rainfall event. However, further research is needed to test the multiple rain events before harvest. Application of more than one rainfall event may increase wet conditions and humidity prior to harvest.

## Pilot study 3: Pathogenicity test of *Pantoea dispersa* associated with RCD incidence

### Background

Bacterial pathogens are considered among the possible causes of resin canal discolouration. Two bacterial species, *Pantoea agglomerans* and *Enterobacter cowanni* have been previously identified from infected RCD fruits by 16S RNA sequencing (Macnish et al. 2015). We identified *Pantoea dispersa* from mango fruit had RCD symptoms by 16S RNA sequencing study in chapter 5. A pathogenicity test using pure cultures of *Pantoea dispersa* was conducted to confirm its association with RCD.

Primary objective: A bacterial pathogen is associated with RCD

Hypothesis: *Pantoea dispersa* has the potential to cause RCD

Materials and Methods

A pure culture of *P. dispersa* (NTP-Dc 47912) was obtained from NT DPIR. This isolate had been isolated from mango leaf/twig tissue collected from a commercial mango orchard in Darwin. This study was conducted during the mango off season, so we used pure cultures from NT DPIR for artificial inoculation studies. The bacterium was grown in nutrient broth for 24 hr and the bacterial suspension was adjusted to $A_{600}$ = 0.1 ($\pm$ 0.004) using an Eppendorf Bio Photometer®. Serial dilutions of $10^{-4}$, $10^{-5}$ and $10^{-6}$ were prepared and 10 uL of each dilution was poured onto nutrient agar plates for colony counting (three plates/dilution) as described by (Koch 2007). The plates were incubated for 24 hr at 25 $^{0}$C in the dark, and then the number of colonies for each plate was counted using a semi-automatic colony counter (Gallenkemp (UK). It was estimated that the concentration of the *P. dispersa* bacterial suspension was $A_{600}$ = 0.1 was $2.70 \times 10^{8}$ cfu / ml (colony forming unit).

Experiment layout and fruit assessment

The experiment was conducted in a completely randomised design with the following treatments:

Table P 3. 1 Treatments name and description

| | |
|---|---|
| T1 | Injection with bacteria (inoculated needle) |
| T2 | Surface spray with bacteria (inoculated spray) |
| T3 | Injection with sterile water (control needle water) |
| T4 | Surface spray with sterile water (control spray water) |
| T5 | Control (untreated) |

Mango fruits were harvested with the 4-5 cm attached peduncle from a commercial KP orchard in Darwin (12°26'17"S 130°50'28"E) and transported to Berrimah Farm, NT DPIR. Fruits were de-sapped by removing the peduncle and placing stem end down on a metal mesh (Holmes et al. 1992).

A total of 150 fruit at the hard-green stage were used in this study in which 30 fruit (10 fruit per box) were assigned to each treatment with a box considered as one treatment replicate. Details of the artificial inoculation protocol are explained in chapter 4.

Fruit were handled in such a way to minimise the risk of cross-contamination; gloves were used, and hands were regularly disinfected with 70 % ethanol during fruit handling of each treatment. After inoculation, fruit were placed into new commercial cardboard mango trays which were then covered with a polythene bag for 12hr then the bag was removed, and fruit were retained in the boxes and allowed to ripen at ambient conditions (25 ± 1 °C and 60-65 % RH).

RCD incidence was assessed ten days post inoculation using a scale 1 = 0-15 %, 2 = 15-30 % 3 = 30-45 %; 4 = 45-70 %; 5 = 70-85 %; 6 = 85-100 % (Macnish et al. 2015).

Statistical analysis

The experiment was conducted using a completely randomised design, and the statistical analysis of variance (ANOVA) was performed with mean separation at 5% LSD using statistical tools for agricultural research version 2.0.1 (http://bbi.irri.org).

Results and discussion

Artificial inoculation with *Pantoea dispersa* (NTP-Dc 47912) successfully induced RCD symptoms ten days post inoculation. Percentage of RCD was higher with spray (77&) than needle inoculation (5%) method (Figs P3.2 A and B). Inoculation with sterile water spray (T3) and injection (T4) induced RCD symptoms though control T5 remained symptomless (Figs P3.2 A and B).

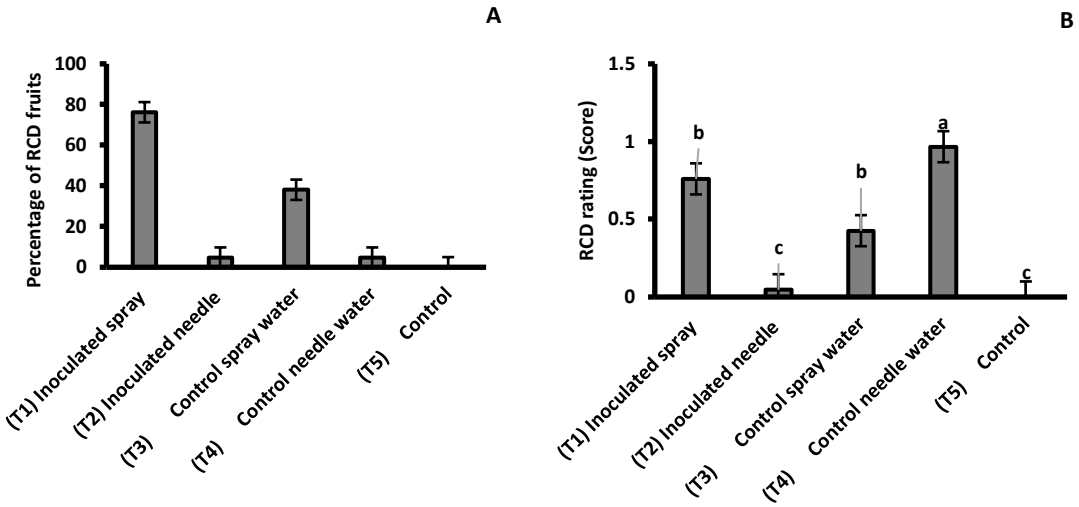



Figure P3.1: Effect of artificial inoculation treatments on (A) percentage, (B) severity of RCD fruits in Kensington Pride.

Vertical bars represent ± SE of means. n = 3 (1 replicate represents average of 10 fruit).. Treatments not sharing letters differ significantly from each other ($P \leq 0.05$).

Pathogenicity testing showed that *Pantoea dispersa* has potential to cause RCD (Fig. P3.2), however, the low severity rating suggested that there may be more than one pathogen responsible for RCD along with *P. dispersa.* Further, RCD symptoms were also observed in uninoculated control treatments, indicating that contamination had occurred. In a similar study, Macnish et al. (2015) inoculated mango at different preharvest stages of mango fruit development on mango tree (from flowering to mature green fruit) with of *P. agglomerans*. This study also observed RCD in 40 % of

uninoculated control fruit. However, in the present study, inoculation was done after harvesting the fruit. Due to limited project timeline of study, we could not conduct the further experiment to re isolate the *Pantoea dispersa* from inoculated fruits for the fulfilment of Koch's postulates. This observation suggests that scope for further research to segregate inoculation techniques and controlled environmental conditions for inoculation is required. Further studies are required to confirm the pathogen species through Koch's postulates and inoculation methods including inoculation at pre-harvest (e.g. hard green stage in the orchard) rather than post-harvest as was done in this study.

# Appendix 2

## Fact sheet and questionnaire used in the survey of perception of Australian consumers on resin canal discolouration in mango

ARC Training Centre for Innovative Horticultural Products

### Fact Sheet
### Resin Canal Disorder in Mango Fruit

**Muhammad Umar, Dr Alistair Gracie, Dr Alieta Eyles, Prof Roger Stanley**
ARC Training Centre for Innovative Horticultural Products
School of Land and Food, University of Tasmania

**What is Resin Canal Disorder RCD?**

- RCD is a visual disorder that affects the physical appearance of mango fruit.
- RCD does not affect the taste of mango fruit.
- Symptoms includes brown-black resin canals, which have been found both in hard green and ripe mango fruit.
- RCD is often visible through the fruit skin as dark canal outlines.
- RCD can be difficult to detect as it can be present in flesh but not visible in the skin.

**Visual assessment of RCD in mango using the rating scale**
**a) External with fruit peel.**

| RCD % | 0-15% | 15-30% | 30-45% | 45-70% | 70-85% | 85-100% |
|---|---|---|---|---|---|---|
| Score | 1 | 2 | 3 | 4 | 5 | 6 |

**b) Internal without fruit peel.**

| RCD % | 0-15% | 15-30% | 30-45% | 45-70% | 70-85% | 85-100% |
|---|---|---|---|---|---|---|
| Score | 1 | 2 | 3 | 4 | 5 | 6 |

tia Tasmanian Institute of Agriculture
University of Tasmania | Tasmanian Government
TIA is a joint venture of the University of Tasmania and the Tasmanian Government
Woolworths the fresh food people
Australian Government Australian Research Council
AUSTRALIAN MANGOES

Sr.#_____

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## *Consumer Questionnaire*

| | |
|---|---|
| **Store Name:** | |
| **Store Location:** | |
| **Date:** | |

Are you willing to participate in this survey? Yes/No

Gender: M/F

**Questions:**

1.a. Do you often buy mangoes during the mango season? Yes/No

1.b. How many mangoes do you usually buy per week? _____

2.a. Do you like to buy a specific variety of mango? Yes/No

If yes, which of the following:

☐ Kensington Pride (KP) ☐ Calypso ☐ R2E2 ☐ Honey Gold ☐ Other

2.b. Are you able to provide any reason why you prefer that variety/varieties?

_____

3. Are you generally satisfied with the fruit quality of mangoes sold in stores? Yes /No

4. Are you able to rank these fruit qualities from most important to least important?

☐ Colour ☐ Size ☐ Aroma ☐ Taste ☐ Texture

5.a. Have you seen this appearance in mango? Yes /No

5.b. If yes, when did you last see this appearance? This year/previous year

6.a. Would this appearance reduce your willingness to buy mangoes?

☐ Yes ☐ No ☐ maybe

7.a. Have you seen this appearance after purchasing it? Yes /No

7.b. if yes, what did you do with the affected mango – did you use it or discard it? _____

Sr.#_____

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## *Retail store manager Questionnaire*

| | |
|---|---|
| **Store Name:** | |
| **Store Location:** | |
| **Date:** | |

Gender: M/F

**Questions:**

1. How long have you been working in the fresh produce section?

☐ < 1 year ☐ 1-3 years ☐ > 3 years

2.a. Are you responsible for ordering mangoes? Yes /No

2.b. If yes, how many mangoes do you order each week/month? _____

3.a. Have consumers made any complaints about mango fruit quality this season? Yes /No

3.b. If yes, please specify, and what was the most common complaint?

_____

4.a. Have you seen Resin Canal Disorder (RCD) in mango? Yes /No

4.b. Have you seen RCD this year? Yes /No

4.c. If yes, when did (month/s) you last see RCD?

_____

4.d. Was it present in unripe or ripening fruit? unripe/ripe

4.e. What proportion of fruit had symptoms of RCD?

☐ <5 % ☐ 5-15% ☐ 15-25 % ☐ >25%

4.f. What do you do with RCD affected mangoes in store?

_____

_____