Horticulture Innovation Australia

Final Report

Reducing mango industry losses from resin canal discolouration

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Summary

Discolouration of mango fruit resin canals causes economic loss to growers. Detection of this quality defect has increased during the past two Australian mango seasons, particularly among the early-season fruit produced in Northern Territory. In 2013, a one-year scoping project, MG12018, started to define the features and potential causes of resin canal discolouration (RCD). The current follow-on project was undertaken to confirm preliminary leads and identify potential factors that contribute to RCD expression. The project focused on 'Kensington Pride' fruit produced near Darwin in the Northern Territory.
As a first step, we surveyed growers and packers of mango fruit about their experience with RCD. Twenty-one out of the 26 surveyed growers and packers from the Darwin area were impacted by RCD in 2013. Nine growers considered RCD was an economic issue for their business. Four growers and two packers estimated that RCD accounted for 10-30% lost production and 5-25% lost packout. The survey highlighted considerable variation in production and postharvest practices employed by each business.

As a second step, we developed a standard procedure for accurately diagnosing and quantifying RCD. In 'Kensington Pride' mangoes, the first signs of RCD developed during early stages of fruit ripening and reached a maximum at the eating ripe stage. These findings highlight the importance of assessing fruit for RCD at the eating ripe stage to ensure the full extent of the defect is consistently measured. The use of a standardised protocol, where fruit are evaluated for internal symptoms of RCD at eating ripe, should enable industry personnel to accurately and consistently diagnose and rate the severity of this defect.

Thirdly, we monitored fruit from tree to market to identify production and postharvest practices causing RCD. Incidence and severity of RCD that developed in 'Kensington Pride' mangoes varied markedly among fruit sourced from nine orchards in the Northern Territory during the 2014 season. In six out of the nine orchards, RCD was significantly higher in commercially transported and ripened fruit relative to fruit ripened with no commercial handling. We also observed that fruit affected by RCD exhibited significantly lower total soluble solids content at eating ripe than fruit without symptoms. This provides a potential lead to follow in developing a better understanding of the underlying causes of RCD.

As a fourth step, we studied the involvement of bacteria in RCD development. The bacterial organisms, *Pantoea agglomerans* and an *Enterobacter* species, were consistently recovered from Australian mango fruit exhibiting RCD symptoms. No such bacteria were isolated from asymptomatic resin canals. *Pantoea agglomerans* was also detected in mango wash and water dump solutions at several Northern Territory mango orchards and pack sheds. 'Kensington Pride' mangoes exposed to solutions containing bacteria tended to develop more RCD than fruit processed in bacteria-free solutions. Foliar applications of copper hydroxide and postharvest hot water treatment of inoculated fruit reduced the level of RCD. These data provide circumstantial evidence for a possible role of bacteria in RCD development.

Taken overall, this study highlighted that RCD is a serious economic issue for several mango businesses in the Northern Territory. While several factors have been identified as potential contributors to RCD, the underlying cause is still largely unknown. Further research is needed to confirm these preliminary leads, identify precise causes, and to develop reliable control measures.

Keywords

Bacteria; Discolouration; ‘Kensington Pride’; Mango; Northern Territory; Postharvest; Production; Resin Canals; Total Soluble Solids

Introduction

Resin canals are a distinctive feature of members of the Anacardiaceae plant family, which includes cashew, pistachio and mango (Venning, 1948). These canals store resinous secretions under pressure within leaves, flowers and fruit (Joel, 1981). Given the caustic nature of the stored resin or sap (Loveys et al., 1992), it has been proposed that the canal system functions to defend against herbivores (Joel, 1980). The resin has also been hypothesised to help modulate plant water status based on observations that plants with resin canals are relatively drought tolerant (Downton, 1981; Kallarackal et al., 1990), and that the canals are spatially associated with vascular tissue (Venning, 1948).

In mango fruit, the resin canals form a complex network that extends from just under the skin and into the flesh (Juliano and Cuevas, 1932). The canals are typically flesh-coloured in appearance. However, discoloured (brown-black) resin canals have occasionally been observed in freshly cut ripe fruit (Moore, 2012). These internal symptoms are usually accompanied by dark lines of the discoloured canals on the fruit skin. Because this quality defect is often only observed in ripe fruit after the point of sale, there are concerns about its impact on consumer purchasing behaviour.

There has been increased detection of mango fruit with the resin canal discolouration (RCD) at wholesale markets in Melbourne, Sydney and Brisbane over the past two seasons (T. Rudge, C. Cope, T. Campbell, pers. comm., 2013). While the incidence of RCD is highest for fruit produced by some growers in the Darwin area, the defect has also been detected in fruit from north Queensland. RCD has been more commonly observed in 'Kensington Pride' fruit but other Australian and Asian varieties are also susceptible to developing this defect (Moore, 2012). To-date, there has been limited research into RCD. Preliminary work by Macnish et al. (2014a) during the HIA Ltd project MG12018 suggests that:

- RCD is occasionally found in green fruit with severe injury or infection.
- RCD incidence can vary markedly between different orchards and for different harvest dates.
- RCD increases in severity over time as fruit develop from firm ripe to overripe.
- RCD is higher in fruit exposed to commercial handling as compared to fruit ripened off the tree.
- RCD can be present in the flesh without symptoms being exhibited on the skin.
- RCD can be higher in immature fruit as compared to fruit of more advanced harvest maturity.
- RCD can be higher in fruit harvested within 12 hours of a rain event relative to 60 hours after rain.
- RCD could possibly be associated with bacteria with a likeness to *Pantoea* and *Enterobacter* species, since these organisms were isolated from discoloured resin canals.

The purpose of this follow-on project was to pursue these promising leads with a view to identifying the precise causes of RCD. This 1-year project focused on the following four activities:

1. Survey growers and packers of mango fruit about their experience with RCD.
2. Develop a standard procedure for accurately diagnosing and quantifying this defect.
3. Monitor fruit from tree to market to identify production and postharvest practices causing RCD.
4. Confirm the identity and possible involvement of bacteria in the development of RCD.

Methodology

Activity 1. Survey of growers and packers about RCD

The overall aim of this work was to survey growers and packers of mango fruit for key records of fruit history and handling with a view towards identifying potential factors that contribute to mango RCD. We developed a confidential questionnaire that was used to survey growers and packers of mango fruit from the Darwin area about their experience with RCD (see Appendix 1 for survey questions). The questionnaire was reviewed and modified by the Chair of the Charles Darwin University Human Research Ethics Committee to the satisfaction of acceptable ethical practices as set out in the National Statement on Ethical Conduct in Human Research. The questionnaire was translated into Vietnamese and Khmer.

Twenty-six mango growers and packers from the Darwin area were surveyed about their experience with RCD during July and August 2014. The businesses surveyed had a history of either low or high incidence of RCD. The survey documented key production (e.g. chemical, irrigation regimes) and/or postharvest (e.g. chemical use, pack line procedures, transport conditions) practices employed by each business. It also established the historical level of RCD that has been typically observed among fruit at each business. The results of the survey were also used to identify potential industry collaborators and guide the general direction of activity 3 (see below).

Activity 2. Development of a standard RCD assessment procedure

The purpose of this activity was to develop a standardised procedure that could be used by multiple operators to consistently diagnose and accurately rate the severity of RCD. Based on observations from project MG12018, external and internal symptoms of RCD usually reach a maximum in ripe fruit. In the present activity, 'Kensington Pride' fruit were harvested from an orchard in the Darwin production area with a history of RCD. The fruit were picked from the same block of trees in early September and again in late October 2014 following commercial procedures. Fruit were graded for uniform appearance, quality and size, treated with a postharvest fungicide and insecticide, and then packed into cardboard trays. Twenty-four trays each containing 16 fruit were randomly sampled and air-freighted from Darwin to the DAFQ laboratory in Nambour, Queensland within 24 hours.

Fruit from the first harvest were treated with 10 parts per million (ppm) of ethylene at 20°C for 2 days to trigger ripening as per commercial protocols. Fruit from the second harvest that were of a more advanced maturity were not exposed to ethylene. All fruit were maintained at 20°C to simulate wholesale and retail handling and display. Sub-samples of one to three trays of fruit were assessed every 1-2 days until all fruit reached eating ripe. The fruit were assessed for external symptoms of RCD and then for internal symptoms after removal of the fruit skin using a vegetable peeler. The severity of the defect was expressed as a percentage of the fruit skin and flesh surface area affected. Fruit skin and flesh colour, fruit firmness, total soluble solids content, titratable acidity, starch content and weight loss

were also determined at each assessment time to identify possible relationships with RCD. Photographs of typical external and internal RCD symptoms were also captured to assist personnel in assessing RCD.

Activity 3. Sequential sampling of fruit from tree to market

Preliminary data from project MG12018 indicated that the incidence of RCD was 2- to 9-fold higher in 'Kensington Pride' mangoes that were exposed to commercial handling and distribution as compared to fruit ripened directly off the tree. The current activity aimed to establish potential supply chain handling factors that may exacerbate the expression of RCD. 'Kensington Pride' fruit from nine orchards in the Darwin and Katherine production area of the Northern Territory were studied given the high probability of these fruit to develop RCD. The orchards were selected in consultation with local mango industry representatives based on findings from project MG12018 and the survey activity described above. The nine orchards had a history of low to high RCD incidence and different production and management characteristics (e.g. early flowering induction, chemical inputs) suspected of contributing to the defect.

Fruit were harvested on the commercial harvest date during the 2014 season from trees considered to be representative of each orchard. The fruit were de-stemmed in a commercial mango de-sapping solution used at each orchard. The fruit were then transported in bins to local pack sheds and processed (i.e. washed, treated, graded, packed) on a commercial pack line. The packed fruit were transported in refrigerated truck trailers from the sheds to wholesalers in Melbourne, Sydney or Brisbane within 4-5 days. These fruit were gassed with 10-100 ppm of ethylene at 20°C for 2-3 days as per commercial practice. In order to identify potential postharvest handling steps or stresses that exacerbate RCD, three replicate trays each containing 16 fruit were sequentially sampled at five steps (i.e. off-tree, from bin, end of pack line, after truck transport, after commercial ripening) along the commercial handling and distribution chain. Additional fruit sampled at the end of the pack line were maintained under simulated transport and ripening conditions at the NT DPI&F Coastal Plains facility near Darwin. All fruit were then held at 20°C and evaluated for RCD using the standard assessment protocol developed in activity 2.

Activity 4. Possible role of bacteria in RCD development

In project MG12018, bacteria with a likeness to *Pantoea* and *Enterobacter* species were isolated from mango fruit with discoloured resin canals. In the current activity, a series of additional tests were completed to determine if these bacteria were directly associated with RCD development. As a first step, mango fruit expressing RCD symptoms were randomly sampled from growers and wholesalers during the 2014 season and tested for the presence of bacteria. Samples of harvest aid and shed dump water from farms and pack sheds in the Northern Territory with RCD were also collected at random and tested for bacteria. Fruit processed in these solutions were tracked to market and assessed for RCD.

Field and laboratory experiments were also completed. Firstly, a field inoculation experiment was established at the South Edge Research Station near Mareeba, Queensland. 'Kensington Pride' flowers and fruit at different development stages were inoculated with the *Pantoea* bacteria previously isolated from mangoes with RCD. The inoculated fruit were harvested at commercial maturity and evaluated for RCD using the standard protocol developed in activity 2. Secondly, an anti-bacterial spray experiment was set up at two orchards near Darwin. 'Kensington Pride' trees were sprayed with and without ManKocide®, a copper-based fungicide, at the recommended rate every 2 weeks from flowering until the

commercial harvest date. The fruit were harvested, packed and transported to wholesalers in Sydney and Melbourne. The efficacy of the copper treatment to reduce RCD was assessed. Thirdly, a postharvest hot water dip experiment was completed at the DAFQ laboratory in Mareeba. 'Kensington Pride' fruit from an orchard near Mareeba were harvested at commercial maturity and dipped in 30 or 52°C water for 5 minutes. The level of RCD that developed on treated fruit was assessed.

Outputs

- Recommendations on a standard assessment protocol to aid industry and research workers to accurately diagnose and quantify the extent of RCD in mango fruit.
- Recommendations on the selection of orchards with a relatively high risk of producing fruit with RCD to guide future research into identifying factors that contribute to RCD.
- An oral presentation to the "Australian Mango Industry Association (AMIA) Field Day" in Walkamin, Queensland on 30 July 2014 by Andrew Macnish entitled "Resin canal discolouration: What we know and where to from here". The presentation provided an overview of the MG14004 project and proposed research activities and was attended by 40 growers.
- An article entitled "Resin canal discolouration – what the research is telling us" contributed by Andrew Macnish and Cameron McConchie was published in the September 2014 issue of Mango Matters on pages 30-31. This magazine is distributed to Australian mango growers and supply chain operators that are members of the peak body AMIA.
- An oral presentation to the "Small Groups Meeting" in Berry Springs, Northern Territory on 26 March 2015 by Cameron McConchie entitled "Reducing mango industry losses from resin canal discolouration – an interim report". The presentation provided an update on the MG14004 project activities and findings and was attended by 25 growers and partners.
- An oral paper presentation to the 10th Australian Mango Industry Conference in Darwin, Northern Territory on 28th May 2015 by Andrew Macnish entitled "Resin canal discolouration: Cause, effect, management, future research". The presentation provided an update on research progress and was attended by 60 mango growers, packers, wholesalers, retailers and researchers.
- An interview to ABC Northern Territory Country Hour radio was aired in Darwin on 15 June 2015 in which Andrew Macnish described research activities towards understanding potential causes of RCD in mango fruit. The interview was posted on the ABC Rural website.
- A video presentation was produced on 25th June 2015 by Trevor Dunmall (AMIA) and Andrew Macnish entitled "Resin canal discolouration – Outcomes of research by the Australia mango industry". The presentation provided an update on RCD research efforts towards understanding and managing the defect. The video was posted on the AMIA website.
- An oral paper presentation to the XI International Mango Symposium in Darwin, Northern Territory on 30th September 2015 by Andrew Macnish entitled "Towards identifying factors that contribute to mango resin canal discolouration". The presentation provided a summary of key RCD research findings and was attended by 80 scientists and mango industry representatives and growers.

Outcomes

Activity 1. Survey of growers and packers about RCD

Twenty-one out of the 26 surveyed mango growers and packers from the Darwin area were impacted to some degree by RCD in 2013. Twelve of these businesses were also impacted by RCD in the 2012 mango season. Nine growers considered RCD was an economic issue for their business. Four growers and two packers estimated that RCD accounted for 10-30% lost production and 5-25% lost packout.

The survey highlighted considerable variation in practice among the different growers. For example, the practice of applying chemical treatments to mango trees to initiate flowering varied between each business. The survey also documented differences in irrigation practices and approaches used to determine when to resume fruit picking after a rain event. Six proprietary brands of mango wash were used by the different growers to de-sap fruit either on harvest aids or in tubs. It was also evident from the survey that fruit could remain in harvest bins in the field for varying durations prior to packing.

There was substantial variation in practice at each of the surveyed pack sheds. The procedures for processing mango fruit on the pack line varied markedly among the businesses. Of the eight sheds that were impacted by RCD in 2013, five packers washed the fruit in a water dump while a sixth packer used a hot water treatment instead. In contrast, of the six sheds that had no RCD, five packers did not wash fruit in dump water but two did treat fruit in a hot water dip. Only two sheds included a chlorine sanitiser in the water dump solution. All eight sheds with a history of RCD applied an insecticide to the fruit while seven out of the eight packers treated the fruit with a fungicide. Conversely, of the six sheds that had not experienced RCD, four packers did not apply an insecticide or a fungicide to the fruit.

Activity 2. Development of a standard RCD assessment procedure

RCD was not present in green-mature 'Kensington Pride' fruit at harvest. The first signs of RCD developed as fruit started to ripen. RCD symptoms were first observed in partially ripe fruit once the flesh had softened to sprung/firm soft and the fruit skin had developed 50% yellow colour. The appearance of RCD symptoms also corresponded to a ripening-related increase in flesh colour and total soluble solids content and a decrease in starch and acids content. The incidence and severity of RCD steadily increased as fruit continued to ripen. RCD expression reached a maximum at 1-3 days prior to the eating ripe stage. Of interest, RCD symptoms were only visible through the fruit skin in about one-third of fruit that exhibited internal symptoms. A series of photographs of typical symptoms were captured and together with these findings were used to develop a standard RCD assessment protocol.

The current study also determined if the presence or absence of RCD in 'Kensington Pride' fruit was related to specific ripening parameters. There were no significant differences in skin and flesh colour, fruit firmness, weight loss, starch and acids content of fruit both with and without RCD symptoms.

However, fruit affected by RCD exhibited significantly lower total soluble solids content at three out of the five assessment times as compared to fruit without symptoms of RCD.

Activity 3. Sequential sampling of fruit from tree to market

The incidence and severity of RCD that developed in 'Kensington Pride' mangoes varied markedly among fruit sourced from nine mango orchards in the Northern Territory during the 2014 season. For fruit harvested and ripened directly off the tree (no commercial handling), the incidence of internal symptoms of RCD ranged from 0 to 45% depending upon the orchard. Likewise, for the same fruit that were commercially picked, packed, transported and ripened, the RCD incidence varied from 4 to 75%. This variable pattern was not related to differences in fruit dry matter content at harvest nor the harvest date. In general, a greater proportion of fruit exhibited RCD symptoms in the flesh without obvious signs visible through the skin.

In six out of the nine orchards, the RCD incidence and severity was significantly higher in commercially transported and ripened fruit relative to fruit ripened directly off the tree. A similar trend was observed for fruit that were subjected to simulated transport and ripened in the Northern Territory. There was no consistent effect of ethylene gassing on the levels of RCD that developed in fruit exposed to commercial and simulated transport. When the data from all nine orchards were combined, a significant incremental increase in RCD incidence and severity occurred at each distinct step along the commercial harvesting, handling and distribution continuum. With the exception of fruit from one orchard, the total soluble solids content in ripe fruit displaying RCD symptoms was significantly lower (0.5 to 1.2 °Brix) than in matching ripe symptomless fruit. There was, however, no consistent association between the time taken for fruit to reach eating ripe and the incidence and severity of RCD that developed.

Activity 4. Possible role of bacteria in RCD development

Pantoea agglomerans bacteria were isolated from resin canals in 10% of 'Calypso™', 33% of 'Keitt' and 80% of 'Kensington Pride' mango fruit exhibiting RCD symptoms. An *Enterobacter* species was also often isolated from fruit affected by RCD. No bacteria were isolated from healthy asymptomatic resin canals. Samples of water used to process 'Kensington Pride' fruit at seven orchards and three packing sheds in the Northern Territory were also tested. There was considerable variation in the clarity of the rinse and mango wash solutions. With the exception of the mango wash at one orchard and the dump water at two sheds, all other solutions were colonised by bacteria. Where bacteria were detected, *Pantoea agglomerans* was also present except in the dump water used at one shed.

'Kensington Pride' fruit from all seven surveyed orchards were exposed to solutions containing *Pantoea agglomerans* bacteria either in the pre-wash rinse, mango wash and/or the shed dump. The incidence of RCD that developed in fruit that were exposed to these solutions during commercial picking and packing varied greatly between orchards. The lowest incidence of RCD was recorded in fruit that were exposed to bacteria in the mango wash but processed in relatively bacteria-free solution at the shed. The highest incidence of RCD was found in fruit that were exposed to bacteria in solutions at harvest and at the shed.

Artificial inoculation of green 'Kensington Pride' mango fruit on trees with *Pantoea agglomerans* bacteria

was generally associated with a slight increase in RCD in fruit. In particular, fruit inoculated during later stages of development tended to show a higher RCD incidence. Foliar applications of copper hydroxide, an anti-bacterial agent, reduced the incidence and severity of RCD in inoculated fruit. Postharvest dipping of inoculated fruit into hot (i.e. 52°C) water for 5 minutes also reduced RCD levels relative to fruit dipped in 30°C water. Applying copper hydroxide sprays to non-inoculated 'Kensington Pride' mango trees during fruit development did not consistently reduce the incidence and severity of RCD.

Evaluation and Discussion

Activity 1. Survey of growers and packers about RCD

Twenty-one out of 26 surveyed mango growers and packers from the Darwin area were impacted by RCD in 2013. Six businesses estimated that RCD accounted for up to 30% lost production and packout. These survey data represent the first attempt to quantify the true extent of RCD in the Darwin mango crop. The data also highlight that RCD is a serious economic issue for several mango businesses in the Northern Territory. The survey also catalogued considerable variation in key production (e.g. chemical, irrigation regimes), harvesting (e.g. wash solutions, time fruit remain in bins) and packing (e.g. chemical use, pack line procedures, transport conditions) practices employed by the different businesses. While it was not possible to infer from the survey responses alone if particular practices were directly associated with RCD, the survey did highlight potential leads to guide the general direction of research activities 2, 3 and 4 (see below). The survey also served to identify potential industry collaborators who were willing to assist with these activities and any future research into RCD.

Activity 2. Development of a standard RCD assessment procedure

External and internal symptoms of RCD were seldom present in green-mature 'Kensington Pride' fruit. The first signs of RCD developed during early stages of fruit ripening and reached a maximum at the eating ripe stage. These findings both confirm and extend the observations by Macnish et al. (2014b). The findings highlight the importance of assessing fruit for RCD when they are at the eating ripe stage to ensure the full extent of the defect is consistently measured. We also observed that the internal symptoms of RCD were not always visible through the fruit skin. Thus, there is a risk that fruit with RCD but with no obvious external symptoms could be overlooked during re-packing/culling procedures at wholesale and retail. The use of a standardised protocol, where fruit are evaluated for internal symptoms of RCD at eating ripe, would enable operators to consistently and accurately diagnose and rate the severity of this defect. In future, there may be scope to test non-invasive methods (e.g. near-infrared spectroscopy) that accurately determine the presence of discoloured canals within fruit. These methods could also potentially be used to predict which fruit will develop symptoms during ripening.

The relationship between specific ripening attributes and the development of RCD in 'Kensington Pride' fruit was also studied. While there were no differences in skin and flesh colour, fruit firmness, starch and acids content of fruit both with and without RCD symptoms, fruit afflicted by the defect generally exhibited a significantly lower total soluble solids content. While these observations were based on a relatively small dataset, it suggests a potential relationship exists between low total soluble solids and the presence of RCD. Further research will be required to confirm this relationship and its exact nature. For example, it is possible that fruit with low soluble solids content, such as those that are harvested immature, are more prone to developing RCD. It is also possible that the typical ripening-related increase in total soluble solids is disrupted in fruit that are affected by RCD.

Activity 3. Sequential sampling of fruit from tree to market

'Kensington Pride' mango fruit sampled from nine orchards in the Northern Territory displayed varying levels of RCD. This variation was consistent with reports by Macnish et al. (2014b) from the 2013 mango season. This activity helped to identify orchards where the incidence of RCD in fruit was relatively high. We recommend that future research should focus on fruit from these orchards given their higher propensity to developing RCD. In general, the RCD incidence and severity was significantly higher in commercially transported and ripened fruit relative to fruit ripened off the tree. These findings confirm observations by Macnish et al. (2014b) during the 2013 season. Higher levels of RCD in commercially handled fruit resulted from incremental increases in expression of the defect at distinct handling steps. While no single step was a sole contributing factor, these data presumably reflect an accumulation of handling stress that elevates expression of RCD. Further research is needed to identify specific harvest and postharvest handling practices or stresses that appear to exacerbate RCD symptom development.

Fruit affected by RCD exhibited significantly lower total soluble solids content at the eating ripe stage than fruit without symptoms. Mango fruit harvest maturity, as determined by dry matter content, is related to fruit eating quality, as measured by total soluble solids (Hofman et al., 2011). Thus, it is possible that RCD-affected fruit with low total soluble solids content may have been harvested with low dry matter content. Macnish et al. (2014a) reported that early-season 'Kensington Pride' fruit harvested with a dry matter content of 13% developed 30-35% more RCD than mid- and late-season fruit picked at 15 and 17% dry matter, respectively. As noted above, it is also possible that RCD development disrupts fruit ripening processes such as carbohydrate metabolism that otherwise contributes to the typical ripening-related increase in soluble solids. Additional research is warranted to confirm the relationship between soluble solids and RCD development in mango fruit. Use of non-destructive methods such as near-infrared spectroscopy to determine the fruit dry matter and total soluble solids content at harvest through to ripe could help test for relationships with RCD symptom development.

Activity 4. Possible role of bacteria in RCD development

Bacterial organisms including *Pantoea agglomerans* and an *Enterobacter* species were consistently recovered from Australian mango fruit exhibiting RCD symptoms. No such bacteria were isolated from asymptomatic resin canals which suggest that they were not endophytic. *Pantoea agglomerans* was also detected in mango wash and water dump solutions at several Northern Territory mango orchards and pack sheds. These observations may relate to variable practice in refreshing and sanitising solutions. It is well established that wash water contaminated with microorganisms can enter and colonise fresh produce through damaged tissues such as stem scars (Solomon and Sharma, 2009). 'Kensington Pride' mangoes exposed to bacteria in solutions tended to develop more RCD than fruit processed in bacteria-free solutions. However, the incidence of RCD was also often high in fruit not exposed to solutions.

Inoculation of developing 'Kensington Pride' mango fruit with *Pantoea agglomerans* resulted in a slight increase in RCD. The defect was, however, also observed in non-inoculated control fruit. This may be attributed to cross contamination from the inoculum spray or that factors other than bacteria are responsible for the naturally high level of RCD. Foliar applications of copper hydroxide, an anti-bacterial

agent, and postharvest hot water treatment of inoculated fruit reduced the incidence and severity of RCD. However, the copper treatment did not reduce RCD that developed on non-inoculated fruit. Taken overall, these data provide circumstantial evidence for a role of bacteria in RCD development in mango fruit. Further detailed research is required to confirm this possible association.

Recommendations

This 1-year project confirmed and extended the initial findings from project MG12018 to further characterise the nature and extent of RCD in Australian mango fruit. The research highlighted several factors that potentially contribute to RCD development. It has also identified orchards in the Northern Territory with a higher risk of RCD developing in fruit. Additional follow-on R&D will be crucial to pursue existing leads with a view to developing strategies for implementing improved practices for reducing RCD. The following are preliminary recommendations to aid future efforts to reduce the occurrence and commercial impact of RCD in mangoes. While the recommendations have a specific focus on 'Kensington Pride' fruit produced in the Northern Territory, resolving RCD issues will have broader benefits and enhance the reputation of the whole Australian mango industry to reliably produce high quality fruit.

- Follow best mango production and handling practices that limit stress on trees and fruit to maximise fruit quality outcomes. RCD likely develops as a result of interacting sets of factors rather than single causative factors.

- Complete further R&D to compare the influence of best vs existing management practices on the occurrence of RCD in fruit. The research should focus on fruit produced at orchards with a history of relatively high RCD to accelerate progress into identifying the cause and control of RCD.

- Practice good orchard and shed hygiene to reduce the risk of microorganisms contaminating fruit. Observations from the current project provide circumstantial evidence that bacteria play a role in RCD development. Random sampling of mango processing solutions used by growers and packers highlighted that bacteria were often present.

- Complete additional detailed research to confirm the potential role of bacteria in the development of RCD. A series of structured, rigorous experiments employing approaches such as Koch's postulates should be undertaken to establish a causative relationship between bacteria and expression of RCD.

- Undertake additional R&D to confirm the possible association between fruit total soluble solids content and the presence of RCD. Preliminary data from the current project suggest that mangoes with RCD have low soluble solids in fruit at ripe. The research should investigate whether this response is a cause or effect.

- Identify the specific commercial harvesting, handling and/or distributing steps or stresses that exacerbate RCD in mango fruit. Observations from the current project indicate that RCD incidence is relatively higher in fruit that are exposed to commercial harvest, pack house and distribution procedures as compared to fruit ripened directly off the tree.

- Quantify the true extent of RCD in retail markets to better understand the commercial impact of this defect. Train produce section managers and independent inspectors to evaluate fruit for RCD using the standard assessment procedure developed as part of the current project.

Scientific Refereed Publications

None to report.

Intellectual Property/Commercialisation

No commercial IP generated.

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Appendices

Appendix 1: Survey of mango growers and packers about resin canal discolouration

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Appendix 4: The potential role of bacteria in mango resin canal discolouration

Appendix 1

Survey of mango growers and packers about resin canal discolouration

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Introduction

Resin canal discolouration (RCD) is a quality defect that reduces the marketability of mango fruit (Moore, 2012). There has been increased detection of mango fruit with RCD at Australian wholesale markets over the past two years (T. Rudge, pers. comm., 2014). The incidence of RCD appears to be highest for 'Kensington Pride' fruit produced by some growers near Darwin in the Northern Territory. While symptoms of RCD are very occasionally seen in green fruit at the pack shed, the defect is most commonly found in ripening fruit at wholesale and retail (Macnish et al., 2014).

To-date, there has been limited research into RCD. Preliminary work by Macnish et al. (2014) highlighted several factors such as harvest maturity, rain at harvest, and commercial handling and distribution procedures that may contribute to RCD. The aim of the current study was to survey growers and packers of mango fruit from the Darwin area about their experience with RCD. Key records of fruit history, production and handling were documented with a view towards guiding future research activities.

Materials and Methods

Questionnaire design

A three page questionnaire was developed the NT DPI&F and DAFQ project team to survey growers and packers of mango fruit about their experience with RCD (see below). The questionnaire was reviewed and modified by the Chair of the Charles Darwin University Human Research Ethics Committee to the satisfaction of acceptable ethical practices as set out in the National Statement on Ethical Conduct in Human Research. The questionnaire consisted of open- and closed-ended questions. The questions were presented in English, Vietnamese and Khmer.

Since a primary objective of the survey was to determine the number of growers and packers who had a history of producing or packing fruit with RCD, a series of questions asked whether fruit yields in 2013 or prior years had been affected by RCD. The survey respondents were asked to estimate the

percentage of fruit that had been afflicted by RCD and whether this was considered to be economically significant. The respondents were asked if RCD varied across the harvest season and between orchard blocks or harvest bins and batches. They were also asked about how and when they were notified that fruit consignments were affected by RCD, given that the defect typically develops in ripening fruit at the wholesale and retail level.

In a series of additional questions, growers were asked about their farm characteristics such as the number of trees and cultivars grown. They were also asked about their production (e.g. floral induction treatments, pesticide use, irrigation schedules) and harvesting (e.g. timing of harvest, type of mango wash, water quality) practices. Packers were also asked additional questions about their pack shed (e.g. time fruit remain in bins, dump water quality, fungicide use, pre-cooling temperature) and distribution (transport duration and temperature, use of ethylene gas) process. Both the growers and packers were asked to comment about factors that possibly contribute to RCD such as flowering flush, fruit maturity, rain event, tree nutrition/health, and tree age.

Survey interviews

The survey was undertaken in the Darwin production area of the Northern Territory given that fruit from this district have previously recorded a relatively high incidence of RCD. It consisted of a 30 minute face-to-face interviews between 26 individual growers or packers and a single common interviewer. The survey was completed in August 2014 just prior to the mango harvest season. The questionnaire responses were anonymous and resulting data was handled confidentially.

Results and Discussion

Grower responses

Twenty-six growers were surveyed. Fourteen of these growers also operated pack sheds and packed their own fruit. Some of their responses are also captured in the separate packer survey results below. Twenty-one out of the 26 surveyed growers either detected or were alerted to the presence of RCD in their fruit during the 2013 mango season. Twelve of these growers were also impacted by RCD in the 2012 season. Nine growers considered RCD was a serious economic issue for their business. Four of these growers estimated that RCD accounted for 10-30% of lost production in 2013. These businesses were relatively small to medium size with 800-9,000 trees under cultivation. The other 17 growers that were impacted by RCD in 2013 estimated that the defect affected <1-5% of their crop. Of the 21 growers who had RCD-affected fruit, 23% had detected the defect at harvest, 62% were notified of its presence by their packers, 62% were alerted to the issue by wholesalers, and 5% were advised of the problem by retailers.

In keeping with general best practice and advice, 24 of the 26 surveyed growers indicated that they applied chemical treatments to their trees to initiate flowering. The other two growers either followed organic production practices or were a non-commercial scale operation. Out of the 24 growers who applied floral induction treatments, three used Cultar® alone, one used potassium nitrate (KNO_3) alone, and 20 used both Cultar® and KNO_3. Nine growers incorporated cincturing in addition to Cultar® and KNO_3. There was, however, considerable variation in the practice of applying these compounds. This

difference in practice likely contributed to the flowering time varying from April to August and fruit harvests spanning from June to November.

The survey showed that 24 of the 26 surveyed growers used an array of registered insecticides and fungicides during fruit production. Among the more commonly used insecticides were dimethoate, Lorsban®, Bugmaster® and Bulldock®. The fungicides Amistar®, Mancozeb and Octave® were favoured by most of the surveyed growers. Fourteen of the growers indicated that they also used a copper-based fungicide (e.g. ManKocide®). The survey did not collect data on the frequency and dose of the applied insecticides and fungicides. The survey highlighted variation in irrigation practice. Growers indicated that they either increased, maintained or decreased rates of irrigation to trees just prior to harvest. Depending upon the grower, the irrigation volume varied from 100-1,890 L/tree/week. There was no universal approach used by growers to how they resumed fruit picking after a rain event. Twelve growers indicated that they delayed harvesting after rain until the fruit appeared dry. The other 12 growers in the survey usually waited for a specific time (e.g. 1-5 hours after light rain, 1-3 days after heavy rain) before re-commencing harvesting.

Nineteen growers indicated that they de-sapped fruit into mango wash solution using harvest aids. Two growers de-sapped fruit into solutions in tubs on the back of a truck or trailer, while two de-sapped fruit in the pack shed. Two growers picked and de-sapped fruit by hand without mango wash. Six proprietary brands of mango wash were used by the different growers. Most growers indicated that they used mango wash at the recommended label rates. While 15 growers estimated that their fruit were treated in mango wash for 1-5 minutes, the other nine surveyed growers indicated that the treatment varied from 15 seconds to 15 minutes. For the growers who used harvest aids, only four sprayed the solution to waste. The remaining growers changed the harvest aid solution after 2 to 10 bins of fruit had been processed or when the water clarity had deteriorated. Like the mango wash treatment, the duration that fruit remained in harvest bins in the field varied considerably. Fourteen growers claimed that they collected the bins within 60 minutes while the other 12 growers typically left fruit in bins for 1-12 hours. Picking fruit after rain, floral induction treatments, older trees, tree health/nutrition and handling practices of wholesalers were some of the suggestions by growers as possible drivers of RCD.

Shed responses

Fourteen mango packers were surveyed. Eight out of the 14 sheds were impacted by RCD during the 2012 and 2013 mango seasons. Two of these packers indicated that RCD was a serious economic issue for their business and affected 5 and 25% of their fruit packout in 2013. The six other packers with RCD experience estimated that the defect impacted <1% of their packout. Of the eight packers with a history of dealing with RCD-affected fruit, 50% had identified the defect in fruit in the field, 75% had seen symptoms in fruit at grading, 88% were notified of an issue by wholesalers, while 25% had heard directly from retailers. Typically, the packers were notified by wholesalers and retailers if fruit consignments had RCD within 7-21 days of dispatch from the shed.

The survey also highlighted considerable variation in practice among the different sheds. For example, eight of the surveyed packers indicated that they routinely pack fruit within 12 hours of arrival at the shed, while the other six sheds usually leave fruit in bins for 12-24 hours prior to packing. The fruit are held at ambient temperature (24-38°C) as they wait for processing. Following packing and depending upon the particular shed, the fruit are typically cooled to 12-19°C and held for 4-72 hours prior to

dispatch. The surveyed packers also indicated that temperatures in trucks can vary from 13°C to >18°C for the 2-5-day shipment to southern markets.

Of the eight sheds that were impacted by RCD in 2013, five packers washed the fruit in a water dump while a sixth packer used a hot water treatment instead. In contrast, of the six sheds that had no RCD, five packers did not wash fruit in dump water but two did treat fruit in a hot water dip. Only two sheds included a chlorine sanitiser in the water dump solution. Of those using water dumps, the frequency at which the water was changed varied from 5 to 15 bins or 2 to 4 times a day depending upon when solution became discoloured. All eight sheds with a history of RCD applied an insecticide to the fruit while seven out of the eight packers treated the fruit with a fungicide. Conversely, of the six sheds that had not experienced RCD, four packers did not apply an insecticide or a fungicide to the fruit. Based on their experience, packers still perceived that production factors such as rain before harvest, poor tree nutrition, and older trees were likely responsible for RCD development.

Conclusions

Twenty-one out of 26 surveyed mango growers and packers from the Darwin area were impacted to some degree by RCD in 2013. Four growers and two packers estimated that RCD accounted for 10-30% lost production and 5-25% of the fruit packout, respectively. These survey data highlight that RCD is a serious economic issue for several mango businesses in the Northern Territory. The survey also documented considerable variation in key production (e.g. chemical, irrigation regimes), harvesting (e.g. wash solutions, time fruit remain in bins) and packing (e.g. chemical use, pack line procedures, transport conditions) practices employed by the different businesses. While it is not possible to infer from the survey responses alone that these variable procedures are associated with RCD, it does provide guidance for the general direction of future research into RCD. The survey also served to identify potential industry collaborators who could assist with this research.

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Mango grower survey questionnaire: resin canal disorder (RCD)

All information is confidential and will not be shared outside of the research project team.

1. What age demographic does your business management fall in? 18 – 35yrs; 36 – 55yrs; 56 – 75yrs.

2. Which varieties do you grow?
_____

3. How many trees in your orchard? <500; 500 – 1500; 1500 – 3000; 3000 – 5000; 5000 – 10000; 10000 – 15000; >15000.

4. Was RCD observed in your fruit in either:
   (a) 2012
   (b) 2013
   (c) both seasons

5. Have your fruit had RCD in previous seasons?
   (a) Yes
   (b) No

6. Which varieties have been affected?_____
   ____

7. Who notified you that your fruit had RCD?
_____

8. What period of time after harvest was the notification given?
_____

9. By your estimation, what percentage of your fruit was showed signs of RCD?
_____

10. Does RCD occurrence vary much across the season? Yes ____ No ____ How much?
_____

11. In your experience, does RCD incidence vary within particular blocks in the orchard?
    (a) Yes
    (b) No
    (c) Don't know.

12. Did you observe any incidence of RCD around the following:
    (a) Flowering flush
    (b) Fruit maturity
    (c) Rain event
    (d) Soil type
    (e) Tree nutrition
    (f) Tree vigour/health
    (g) Tree age
    (h) Other
    _____

13. Do you use any of the following induction treatments?
    (a) Cultar®
    (b) Potassium nitrate
    (c) Thiourea
    (d) Ethrel®
    (e) Cincturing
    (f) Other
    _____
    _____

14. Last season, when did your trees flower?
    _____

15. What was your harvest period in 2013?
    _____

16. Do you use any of the following pesticides in field?
    (a) Amistar®
    (b) Octave®

(c) Mancozeb®
(d) Fenthion®
(e) Dimethoate
(f) Endosulfan
(g) Other
_____

17. What is your typical irrigation schedule leading up to harvest?
_____

18. Do you normally pick fruit in the:
(a) AM
(b) PM
(c) Both AM and PM

19. How do you de-sap fruit?
(a) Harvest aid
(b) In shed

20. What type of mango wash do you typically use and at what concentration?
_____

21. How long are fruit kept in mango wash? _____

22. How often do you change the solution? _____

23. What water source do you use for the mango wash?
(a) Bore
(b) Dam
(c) Tank
(d) Treated (chlorination)

24. How long do fruit typically stay in bins in the field?
(a) Immediately transported to shed.
(b) <30 min
(c) 30-60 min
(d) > 1 hour

25. Do you pack your own fruit or use a commercial shed?
_____

26. Would you be willing and interested to collaborate in an industry project to research the incidence and causes of RCD?
    (a) Yes
    (b) No
27. Any other comments or insights you would like to share around RCD?

_____
_____

Mango packer survey questionnaire: resin canal disorder (RCD)

All information is confidential and will not be shared outside of the research project team.

1. What age demographic does your business management fall in? 18 – 35yrs; 36 – 55yrs; 56 – 75yrs.

2. Was any fruit you packed reported as expressing RCD last season?
   (a) Yes
   (b) No

3. When was RCD detected?
   (a) Grading
   (b) Wholesaler/market
   (c) At retail.
   (d) All of the above

3. If RCD was detected in your consignments after dispatch, who notified you that the fruit had RCD?_____
4. How soon after dispatch was RCD detected?
   _____

5. By your estimation, what percentage of your total pack-out was affected by RCD?
   _____

6. Was RCD observed in your fruit in either:
   (a) 2012
   (b) 2013
   (c) both seasons

7. Have your fruit had RCD in previous seasons?
   (c) Yes
   (d) No

8. Since which year has RCD been an economic issue for your business?
   _____

9. Did RCD incidence fluctuate across the last season?
   (a) No
   (b) Yes - how much?
   _____

10. In your experience, does RCD vary between bins/batches?
   (a) No
   (b) Not sure
   (c) Yes - any ideas why?
   _____
   _____

11. Based on your observations, did RCD incidence vary with any of the following?
   (a) Flowering flush
   (b) Fruit maturity
   (c) Rain event
   (d) Tree nutrition/health
   (e) Tree age
   (f) Other
   _____
   _____

12. On average, how long are fruit held in bins before packing?
   (a) <30 min
   (b) 30-60 min
   (c) 1-2 hours
   (d) 2-3 hours

13. At what temperature are bins held in the shed prior to packing?_____

14. Describe your basic pack-line process? (circle which apply to your business)
   (a) Water dump
   (b) Hot water dip

(c) Insecticide treatment
(d) Fungicide treatment
(e) Brushes

14. How long are fruit in the water dump?
_____

15. What additives do you add to the water?
_____

15. How often is the dump water changed?
_____

16. How do you clean the dump?
_____
_

17. Do you treat fruit with:
(a) Scholar
(b) Sportak

18. What temperature do you cool fruit to? ___ °C

19. How long do you normally store fruit prior to dispatch?
_____

20. What is the typical transport set temperature for your fruit? ____ °C

21. What is the typical duration of transport?
_____

22. Are your fruit usually ripened with ethylene?:
(a) Yes
(b) No

23. Would you be willing and interested to collaborate in an industry project to research the incidence and causes of RCD?
(c) Yes

(d) No

Any other comments or insights you would like to share around RCD?
_____
_____

Appendix 2

Developing a standard assessment protocol for mango resin canal discolouration

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Introduction

Resin canals are a distinctive feature of mango fruit (Venning, 1948). They form a network that runs under the skin and along the seed (Juliano and Cuevas, 1932). While canals are normally flesh coloured, brown-black resin canals have occasionally been found in Australian mango fruit during the past two seasons. Symptoms of resin canal discolouration (RCD) typically develop as fruit ripen (Macnish et al., 2014). The discoloured canals are usually also visible through the fruit skin as dark lines. Unlike superficial skin browning disorders, fruit afflicted by RCD are unmarketable and must be graded out once visible symptoms are evident.

Given that the external symptoms of RCD share a general likeness with other skin browning disorders, there is a risk that this relatively new quality defect could be misdiagnosed. Accordingly, the purpose of the current study was to develop a protocol that can be used by multiple operators to consistently and accurately diagnose RCD. We also aimed to identify the optimal time during fruit ripening to quantify the severity of this quality defect. Fruit ripening parameters (e.g. fruit firmness, skin colour, soluble solids content) were also determined to identify possible relationships with RCD.

Materials and Methods

Plant material

'Kensington Pride' fruit were sourced from an orchard near Lambells Lagoon in the Northern Territory with a history of RCD. Commercial crews picked fruit from the same block of trees in early September 2014 and again in late October 2014 when the fruit dry matter content was 13.1 $\pm$ 0.6% and 17.7 $\pm$ 0.2%, respectively. At harvest, the fruit were de-stemmed in a commercial mango wash solution to prevent sapburn injury. The de-sapped fruit were placed into harvest bins. The bins were loaded onto a flatbed truck and transported to a pack shed in Berry Springs, about 40 km away.

General processing

The fruit were processed on a commercial pack line at the shed. Briefly, the fruit were washed in

chlorinated water, treated with a fungicide and insecticide, and graded for uniform appearance quality and size. The fruit were packed into single layer cardboard mango trays lined with plastic moulded cup inserts. Twenty-four trays each containing 16 fruit were sampled from the end of the pack line. An additional 10 fruit were collected for the determination of fruit dry matter content. The sample trays were transported by a car to the airport in Darwin, Northern Territory within 1 hour. They were then air-freighted to the Sunshine Coast airport in Queensland within 24 hours. The trays were collected and transported by a car to the nearby DAFQ laboratory in Nambour.

Fruit ripening

Upon arrival at the laboratory, the fruit were removed from trays, labelled, and packed back into trays in a completely randomised design. The fruit were cooled to 20°C. Fruit from the first harvest date were treated with 10 parts per million (ppm) of ethylene at 20°C for 2 days to trigger ripening as per commercial protocols (Ledger et al., 2010). Fruit from the second harvest that were of a more advanced maturity were not exposed to ethylene. Thereafter, all fruit were maintained in a cold room at 20°C and 70-90% relative humidity to simulate retail handling until they reached the eating ripe stage whereby the skin colour was 70-100% yellow and the flesh was soft (Ledger et al., 2010).

Fruit quality assessment

Sub-samples of one to three trays of fruit (i.e. 16-48 fruit) from both harvest dates were assessed every 1-2 days of shelf life. Fruit from the first harvest date were assessed for firmness by hand pressure using the scale: 0 = hard, 1 = rubbery, 2 = sprung, 3 = firm soft, 4 = soft (Holmes et al., 2009). Fruit firmness is a good predictor of ripening in 'Kensington Pride' mango. Each fruit was then assessed for external symptoms of RCD and for internal symptoms after removal of the fruit skin using a vegetable peeler. The severity of the defect was expressed as a percentage of the skin and flesh surface area affected. Photographs of external and internal RCD symptoms were also captured.

Fruit from the second harvest date were weighed individually upon arrival in the laboratory and again at each assessment time to calculate weight loss. The fruit skin colour was rated using the scale: 1 = 0-10%, 2 = 10-30%, 3 = 30-50%, 4 = 50-70%, 5 = 70-90%, 6 = 90-100% yellow (Holmes et al., 2009). Fruit firmness was determined as described above. The presence of skin defects (e.g. sapburn, wounds) and disease was also recorded. Each fruit was then assessed for RCD symptoms as described above. The flesh colour was measured with a Minolta Chromameter using the L*, a* and b* coordinates. Ten fruit from each sub-sample with and/or without RCD symptoms were assessed for total soluble solids content, titratable acidity and starch content. Briefly, cheek flesh tissues were cut from fruit and juiced using a garlic press. The juice total soluble solids content was measured using an Atago digital refractometer. The juice titratable acidity, expressed as citric acid equivalents, was determined by titration with 0.1 N NaOH to pH 8.2 with a Mettler Toledo titrator. The fruit starch content was determined by dipping the cut fruit surface into a 10 g/L potassium iodide plus 2.5 g/L iodine solution for 30 seconds (Beattie and Wild, 1973). The starch-staining patterns were scored as the percentage of the flesh surface area.

Results and Discussion

RCD was not present in hard green-mature fruit at harvest for either the early or late picking date. The first visible signs of RCD developed as fruit started to ripen. For fruit harvested early in the season with a dry matter content of 13.1%, RCD symptoms were evident by day 6 of their 17-day shelf life in association with slight flesh softening (Figure 1). For fruit harvested later in the season with a dry matter content of 17.7%, symptoms of RCD were visible by day 4 of their 8-day shelf life once the fruit skin was 50% yellow colour and the flesh was sprung to firm soft (Figure 2). The appearance of RCD symptoms in the late-season fruit also corresponded to a ripening-related increase in flesh colour and total soluble solids content and a decrease in starch and acids content (Figure 2). The incidence and severity of RCD steadily increased as fruit from both harvest dates continued to ripen. RCD expression reached a maximum at 1-3 days prior to the eating ripe stage on day 14 and day 7 at 64% and 89% incidence for early- and late-season fruit, respectively (Figures 1, 2). There was no consistent association of skin defects or disease with RCD (data not shown).

Taken overall, these findings highlight the importance of assessing fruit for RCD when they are at the eating ripe stage to ensure the full extent of the defect is consistently measured. Otherwise, fruit quality surveyors could under-estimate RCD if assessing partially ripe fruit. In the present study, we also observed that RCD symptoms were only visible through the fruit skin in about one-third of fruit that exhibited internal symptoms (Figure 3). This finding highlights the risk that fruit with RCD in the flesh but with no obvious external symptoms could be overlooked during re-packing/culling procedures at wholesale and retail. There may be scope to develop and test non-invasive methods (e.g. near-infrared spectroscopy) that more accurately determine the presence of discoloured canals within fruit. These methods could also potentially be used to predict which fruit will likely develop symptoms during ripening. Photographs of fruit displaying different levels of RCD were captured to aid industry and research workers to diagnose and rate the severity of RCD (Figures 4, 5).

The current study also aimed to determine if the presence or absence of RCD in fruit was related to specific ripening parameters. We found that there were no significant differences in skin and flesh colour, fruit firmness, weight loss, starch and acids content of fruit both with and without RCD symptoms (data not shown). However, fruit affected by RCD exhibited significantly lower total soluble solids content at three out of the five assessment times (Figure 6). While these observations were based on a relatively small dataset, it suggests a potential relationship exists between low total soluble solids and the presence of RCD. Further research will be required to confirm this relationship and its exact nature. For example, it is possible that fruit with low soluble solids content, such as those that are harvested immature, are more prone to developing RCD. It is also possible that the typical ripening-related increase in total soluble solids is disrupted in fruit that are affected by RCD.

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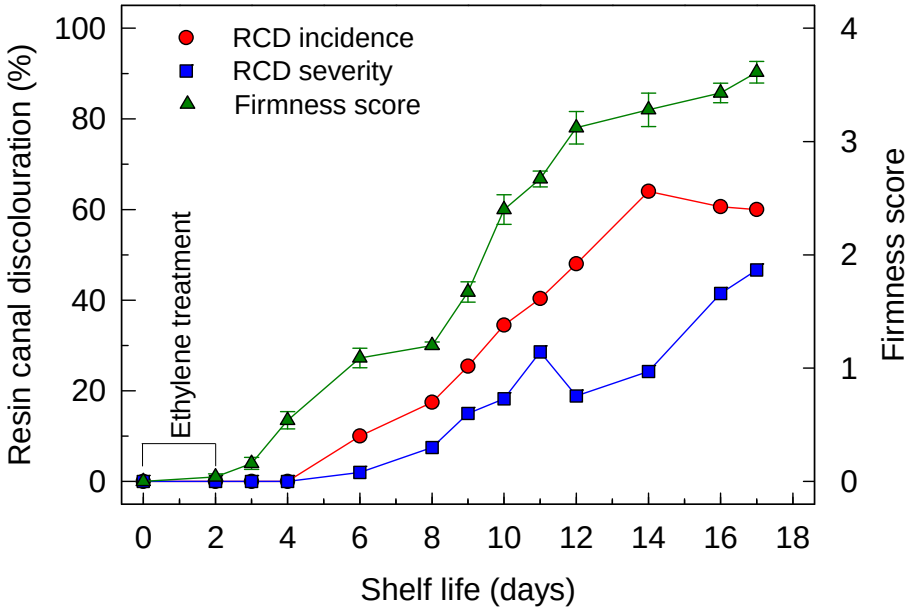

Figure 1. Changes in 'Kensington Pride' mango fruit firmness and RCD incidence and severity during shelf life at 20°C. Green-mature (13.1% dry matter content) fruit were harvested in September 2014. They were treated with 10 ppm of ethylene for 2 days at 20°C and then allowed to ripen at 20°C. Different sets of fruit were assessed every 1-2 days until all fruit were eating ripe (firmness score of 4). Firmness scores: 0 = hard green, 1 = rubbery, 2 = sprung, 3 = firm soft, 4 = soft. RCD data represent internal symptoms.

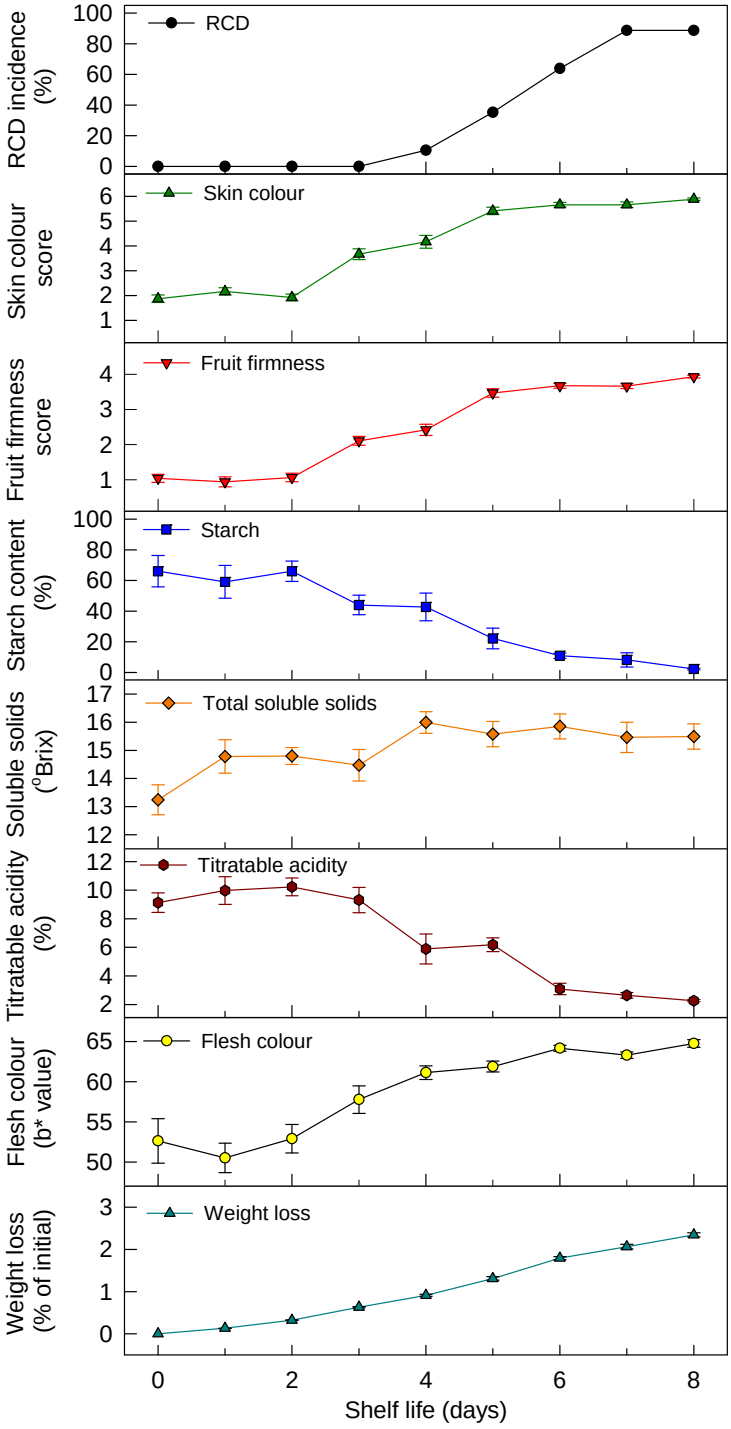

Figure 2. Changes in 'Kensington Pride' mango fruit RCD incidence and ripening attributes during shelf life at 20°C. Green-mature (17.7% dry matter content) fruit were harvested in October 2014. They were allowed to ripen at 20°C. Different sets of fruit were assessed daily until all fruit were eating ripe (firmness score of 4). Firmness scores: 0 = hard green, 1 = rubbery, 2 = sprung, 3 = firm soft, 4 =

soft. Colour scores: 1 = 0-10%, 2 = 10-30%, 3 = 30-50%, 4 = 50-70%, 5 = 70-90%, 6 = 90-100% yellow skin. RCD data represent internal symptoms.

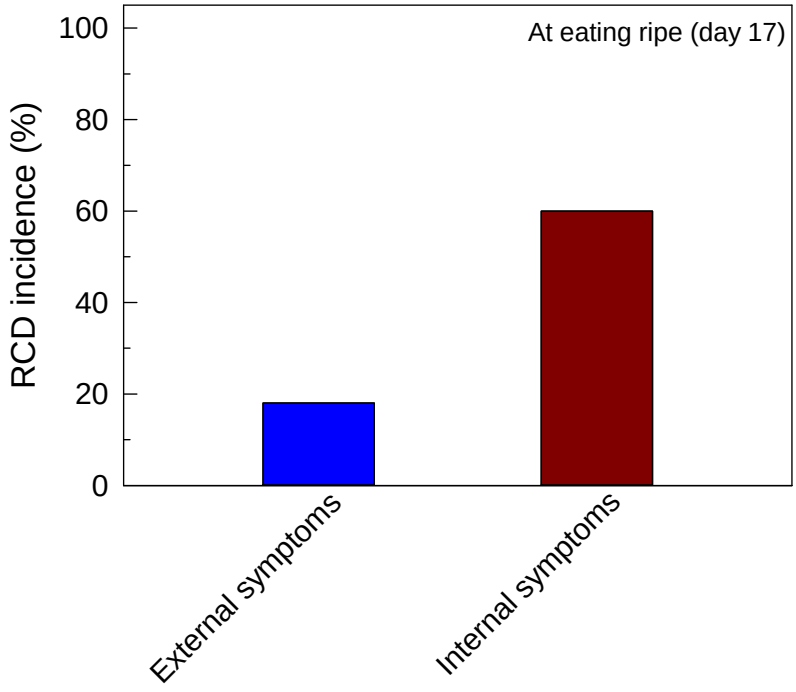

Figure 3. The proportion (%) of 'Kensington Pride' mango fruit in which symptoms of RCD were visible through the skin and in the flesh at eating ripe. Green-mature (13.1% dry matter content) fruit were harvested in September 2014. They were treated with 10 ppm of ethylene for 2 days at 20°C and then allowed to ripen at 20°C for 17 days.

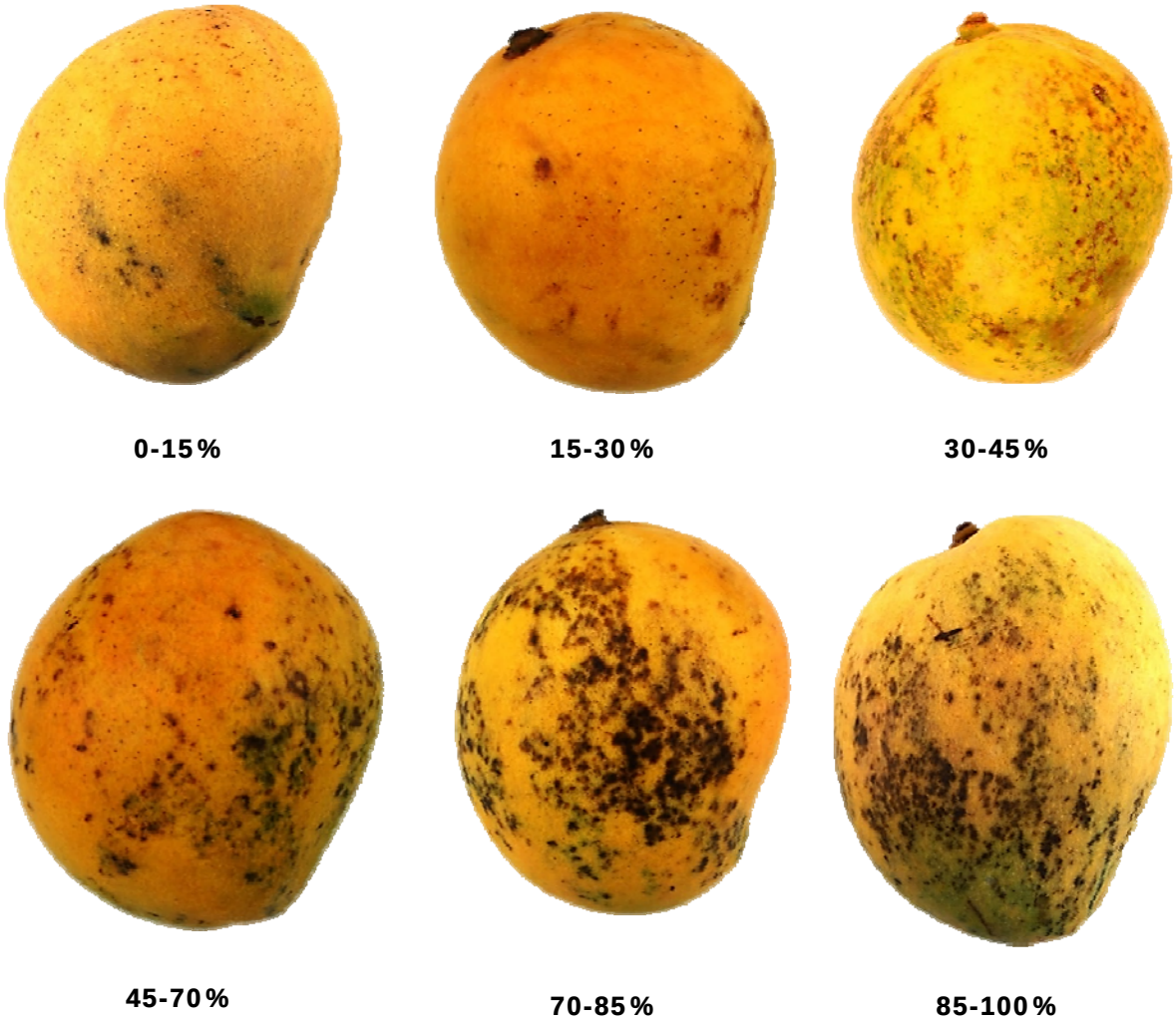

Figure 4. Photographs showing varying severity of external symptoms of RCD on 'Kensington Pride' mango fruit. RCD severity was expressed as the proportion (%) of the fruit skin surface with obvious RCD symptoms.

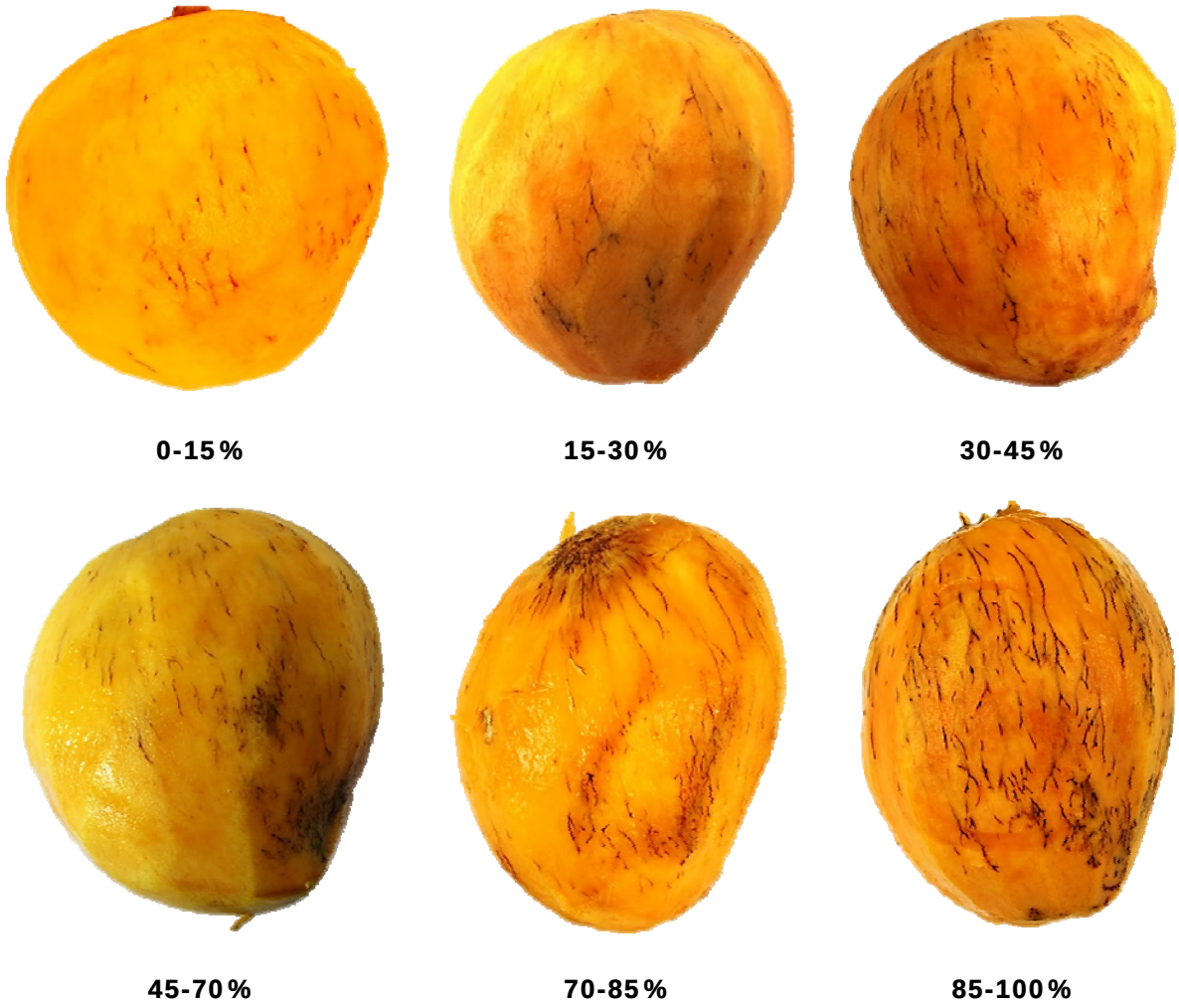

Figure 5. Photographs showing varying severity of internal symptoms of RCD on 'Kensington Pride' mango fruit. RCD severity was expressed as the proportion (%) of the fruit flesh surface with obvious RCD symptoms.

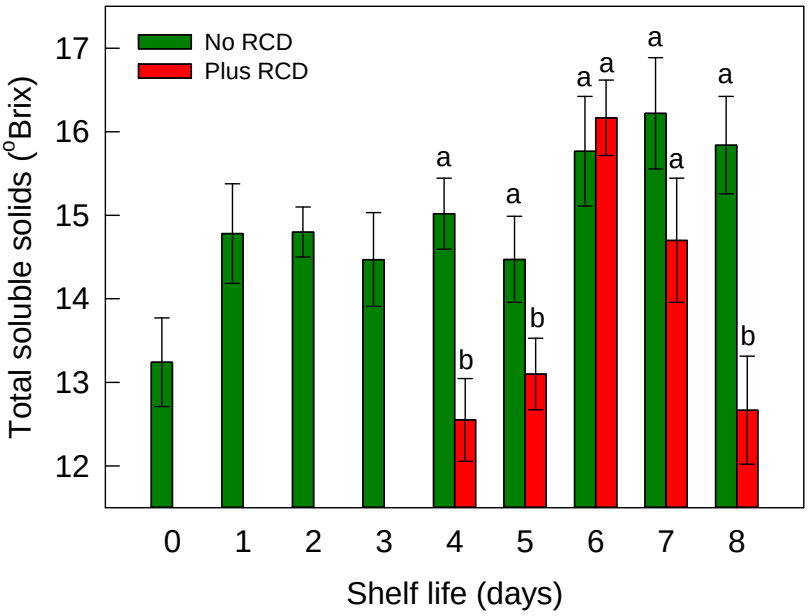

Figure 6. Changes in the total soluble solids content of 'Kensington Pride' mango fruit that developed RCD symptoms during shelf life at 20°C. Green-mature (17.7% dry matter content) fruit were harvested in October 2014. They were allowed to ripen at 20°C. RCD symptoms were evident by day 4 of shelf life.

Appendix 3

Sequential sampling and monitoring of mango fruit for resin canal discolouration from tree to market

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Introduction

Discolouration of mango fruit resin canals causes economic loss to growers (Moore, 2012). Detection of this quality defect has increased during the past two Australian mango seasons, particularly among the early-season 'Kensington Pride' fruit produced in the Northern Territory. The symptoms of resin canal discolouration (RCD) are typically absent in green fruit and develop as the fruit ripen (Macnish et al., 2014b). Preliminary data from the HIA Ltd project, MG12018, suggested that the incidence of RCD was 2- to 9-fold higher in fruit that were exposed to commercial handling and distribution as compared to fruit ripened directly off the tree (Macnish et al., 2014a).

The aim of the current study was to establish potential harvest and postharvest handling factors that may exacerbate the expression of RCD. 'Kensington Pride' fruit from orchards in the Northern Territory with a history of low to high RCD incidence were tracked from tree to market. Fruit were sequentially sampled at different steps along the commercial harvest and distribution chain in order to identify the steps or stresses that potentially contribute to RCD symptom expression.

Materials and Methods

Plant material

'Kensington Pride' fruit were sampled from nine orchards in the Darwin (Darwin River, Lambells Lagoon, Noonamah, Berry Springs) and Katherine region of the Northern Territory (Table 1). These orchards were selected on the basis of findings from project MG12018 and the survey activity described in Appendix 1. The nine orchards had a history of low to high RCD incidence and different production and management characteristics (e.g. early flowering induction, chemical inputs) suspected of contributing to the defect. Fruit were harvested from a selected row of 20-30 trees considered to be representative of each orchard on the commercial harvest date. Tree, row and orchard block characteristics were documented at each harvest time.

Experimental

In order to identify potential harvest and postharvest handling steps or stresses that exacerbate RCD, three replicate trays each containing 16 fruit were randomly sampled at five sequential steps (off-tree, from harvest bin, end of pack line, after commercial transport, after commercial ripening) along the commercial handling and distribution chain (Figure 1). Additional fruit sampled at the end of the pack line were maintained under simulated transport and ripening conditions at the NT DPI&F Coastal Plains facility near Middle Point in the Northern Territory (Figure 1).

Sampling off the tree

Fruit that were sampled directly off the trees were harvested with stems attached. They were transported by car to the Coastal Plains facility within 1 hour. The fruit were de-stemmed and inverted to prevent sap exuding onto the fruit surface. These fruit were packed into cardboard mango trays and treated with 10 ppm of ethylene for 2 days at 20°C to trigger ripening (Ledger et al., 2010). They were then transported by car to the NT DPI&F laboratory at Berrimah within 1 hour and maintained at 20°C until they reached eating ripe.

Sampling from harvest bins

Other than fruit harvested and ripened directly off the tree, fruit were harvested by commercial crews. The fruit were de-stemmed and treated with a commercial mango wash solution as per the standard practice at each orchard. At orchards 1 and 2, the fruit were rinsed and de-stemmed in water prior to immersion in a mango wash solution. At orchards 3-9, the fruit were de-sapped directly in mango wash on harvest aids. The fruit were then placed into harvest bins adjacent to the trees. Except where small volumes of fruit were picked, fruit were generally sampled from the third and fourth bin that had been processed in the mango wash solution since it was prepared. The sampled fruit were packed into mango trays and transported by car to the Coastal Plains facility within 1 hour. They were treated with ethylene and transported to the laboratory in Berrimah as described above.

Sampling from the pack line

The harvest bins from which fruit were sampled were labelled and tracked to commercial pack sheds. Fruit in bins from orchards 3, 4, 5, 6, 8 and 9 were collected from the field on the same day of harvest and transported by a flatbed truck or utility truck to pack sheds in Noonamah, Berry Springs or Katherine within 5-45 minutes. These fruit were either packed within 2-3 hours or maintained in the shed at ambient temperature (ca. 24-38°C) until being packed on the next day (Table 1). Fruit in bins from orchards 1, 2 and 7 were held in sheds at each orchard at ambient temperature until the next day. They were then transported by a flatbed truck or utility truck to a pack shed in Berry Springs within 15-45 minutes. These fruit were generally packed within 1-2 hours (Table 1). Fruit in the labelled bins were tracked on the pack lines. At each pack shed the fruit were dumped into a water bath to rinse off field dust, treated with a fungicide and insecticide, brushed to ensure good coverage of the pesticides, and graded for uniform quality. The fruit were packed into single layer cardboard mango trays lined with plastic moulded cup inserts. Trays that each held 16 fruit were sampled at random from the end of the pack line. The sampled fruit were transported by car to the Coastal Plains facility within 30-60 minutes. They were treated with ethylene and maintained in the laboratory in Berrimah as described above.

Sampling after simulated transport and ripening

Additional trays each containing 16 fruit from the sample bins were collected at random from the end of the pack lines. They were transported by car to the Coastal Plains facility within 30-60 minutes. Fruit in trays were maintained in cold rooms operating at 13 or 19°C for 5 days to simulate interstate truck transport. The fruit were then treated with or without ethylene as described above. They were maintained in the laboratory in Berrimah as described above. In order to simulate transport and handling of fruit that are processed for juicing, a further set of packed fruit in trays were maintained at ambient temperature (ca. 24-38°C) at the Coastal Plains facility until they reached eating ripe.

Sampling after commercial transport and ripening

Additional trays each containing 16 fruit from the sample bins were collected from the pack lines. The trays were built into pallets and held at 13, 15 or 16°C depending upon the shed. The palletised fruit were loaded into refrigerated truck trailers and transported from the pack sheds to fresh produce wholesalers in Melbourne, Sydney or Brisbane within 3-4 days (Table 1). A datalogger that recorded the air temperature within the trailer was included in most shipments. The wholesalers removed the sample trays from pallets and treated the fruit with or without ethylene at 18-20°C for 2-3 days as per recommended commercial practice (Ledger et al., 2010). The sample fruit held by wholesalers in Melbourne and Sydney were air-freighted to the Sunshine Coast airport in Queensland within 8 hours. They were then transported by car to the nearby DAFQ laboratory in Nambour. The sample fruit held by wholesalers in Brisbane were transported by car directly to laboratory within 2 hours. All fruit were maintained in the laboratory at 20°C and 70-90% relative humidity until they reached eating ripe.

Fruit assessment

Six to ten fruit were sampled off the selected row of trees for determination of dry matter content at harvest. Fruit from each sequential sub-sample were evaluated for RCD at eating ripe using the standard assessment protocol developed in Appendix 2. Individual fruit were also assessed at eating ripe for total soluble solids content, disease and other skin defects.

Results and Discussion

The incidence of RCD varied considerably among fruit from the nine selected orchards (Table 2). For fruit harvested and ripened directly off the tree, internal symptoms of RCD varied from 0 to 45% depending upon the orchard. Likewise, for the same fruit that were commercially picked, packed, transported and ripened, the RCD incidence varied from 4 to 75%. This variable pattern did not appear to be related to differences in fruit dry matter content at harvest nor the harvest date (Tables 1, 2). In general, a greater proportion of fruit exhibited RCD symptoms in the flesh without obvious signs on the skin (data not presented). Fruit from orchards 1 and 2 developed relatively high levels of RCD in the flesh. These fruit were harvested on the day after 10-25 mm of rain (Table 1). Harvesting fruit soon after rain has previously been associated with elevated RCD expression (Macnish et al., 2014b). Orchards 1 and 2 also had a history of RCD during the 2013 mango season. Fruit from orchard 4, a 'neglected' block of trees that received no chemical (e.g. floral initiation, pesticide sprays) inputs during

production, also developed a moderately high occurrence of RCD. Preliminary data reported by Macnish et al. (2014a) suggested that fruit produced from trees with no chemical inputs may develop higher levels of RCD. Although orchards 6 and 8 were managed by the same grower, the incidence of RCD in these fruit was markedly different. This study represents the first report of RCD in fruit from orchard 9.

In six out of the nine orchards, the RCD incidence was significantly higher in commercially transported and ripened fruit relative to fruit ripened directly off the tree (Table 2). A similar trend was observed for fruit that were subjected to simulated transport and ripened in the Northern Territory. These findings confirm similar observations by Macnish et al. (2014b) during the 2013 mango season. There was no consistent effect of ethylene gassing on the levels of RCD that developed in commercially transported fruit. Similarly, ethylene treatment generally did not affect RCD expression in fruit exposed to simulated transport at 19°C. There was also no consistent effect of simulated transport at 13°C vs 19°C on RCD occurrence in eating ripe fruit. However, allowing fruit to ripen at ambient (ca. 35°C) temperature as opposed to at 19-20°C was associated with significantly less RCD in fruit from five out of the nine orchards. Fruit sampled from orchards 5, 8 and 9 after packing developed more RCD than those ripened directly off the tree or from the field bins. Sampling fruit from orchard 3 out of field bins or the end of the pack line was also associated with a higher occurrence of RCD relative to fruit ripened off trees.

As reported for the incidence data, the severity of RCD also varied substantially for fruit from the different orchards (Table 3). RCD severity, expressed as the proportion (%) of the fruit flesh area affected by symptoms, varied from 0 to 20% for fruit harvested and ripened directly off the tree at the different orchards. RCD severity was highest in fruit that were commercially transported in six of the nine tested orchards. The severity of the defect was significantly greater in commercially transported fruit than those exposed to simulated transport in the Northern Territory. RCD symptoms that developed in commercially transported fruit were also significantly more severe than in fruit ripened off the tree or sampled from field bins and the pack lines. There was no consistent association between the time taken for fruit to reach eating ripe and the incidence and severity of RCD that developed (data not presented). The time to reach eating ripe was considerably less for fruit harvested at a more advanced stage of maturity. The time to eating ripe was generally reduced when fruit were maintained at an elevated storage temperature or exposed to ethylene. There was also no association between RCD symptom development in fruit and the presence of skin defects or disease (data not presented).

When the data from all nine orchards were combined, a significant incremental increase in RCD incidence was observed to occur with progressive steps along the commercial handling and distribution chain (Figure 2). A similar cumulative increase in the severity of RCD symptoms was also observed for commercially handled fruit (data not presented). While no single handling step could be identified as a sole contributing factor, these data may reflect an accumulation of handling stress from multiple steps that triggers elevated expression of RCD in sensitive fruit. Further research is needed to identify and better understand the particular handling stresses that potentially contribute to an increase in RCD symptoms in fruit in the marketplace.

With the exception of fruit from orchard 3, the total soluble solids content in ripe fruit displaying RCD symptoms was consistently and significantly lower (0.5 to 1.2 °Brix) than in matching ripe symptomless fruit (Table 3). Mango fruit harvest maturity, as determined by dry matter content, is closely related to fruit eating quality, as measured by total soluble solids (Hofman et al., 2011). Thus, our observations

that RCD-affected fruit often have low total soluble solids content at ripe potentially suggests that these individual fruit may have been harvested with a relatively low dry matter content. Preliminary data reported by Macnish et al. (2014a) indicated that early-season 'Kensington Pride' mango fruit harvested with a dry matter content of 13% developed 30-35% more RCD than mid- and late-season fruit picked at 15 and 17% dry matter, respectively. Alternatively, it is also possible that RCD symptom development in mango fruit simply disrupts normal internal ripening processes such as carbohydrate (e.g. sugar) metabolism that otherwise contributes to the typical ripening-related increase in total soluble solids.

Taken together, our findings highlight the need for further research to identify specific commercial harvest and postharvest handling practices that may exacerbate RCD development. Additional research is also warranted to confirm the potential relationship between low total soluble solids and RCD development in mango fruit. It may be feasible to utilise use non-destructive methods such as near-infrared spectroscopy to determine the fruit dry matter and total soluble solids content at harvest through to ripe to help test and establish relationships with RCD symptom development. Our current research has identified several orchards where RCD was relatively high. We recommend that any future research should focus on fruit from these orchards given their higher propensity to developing RCD.

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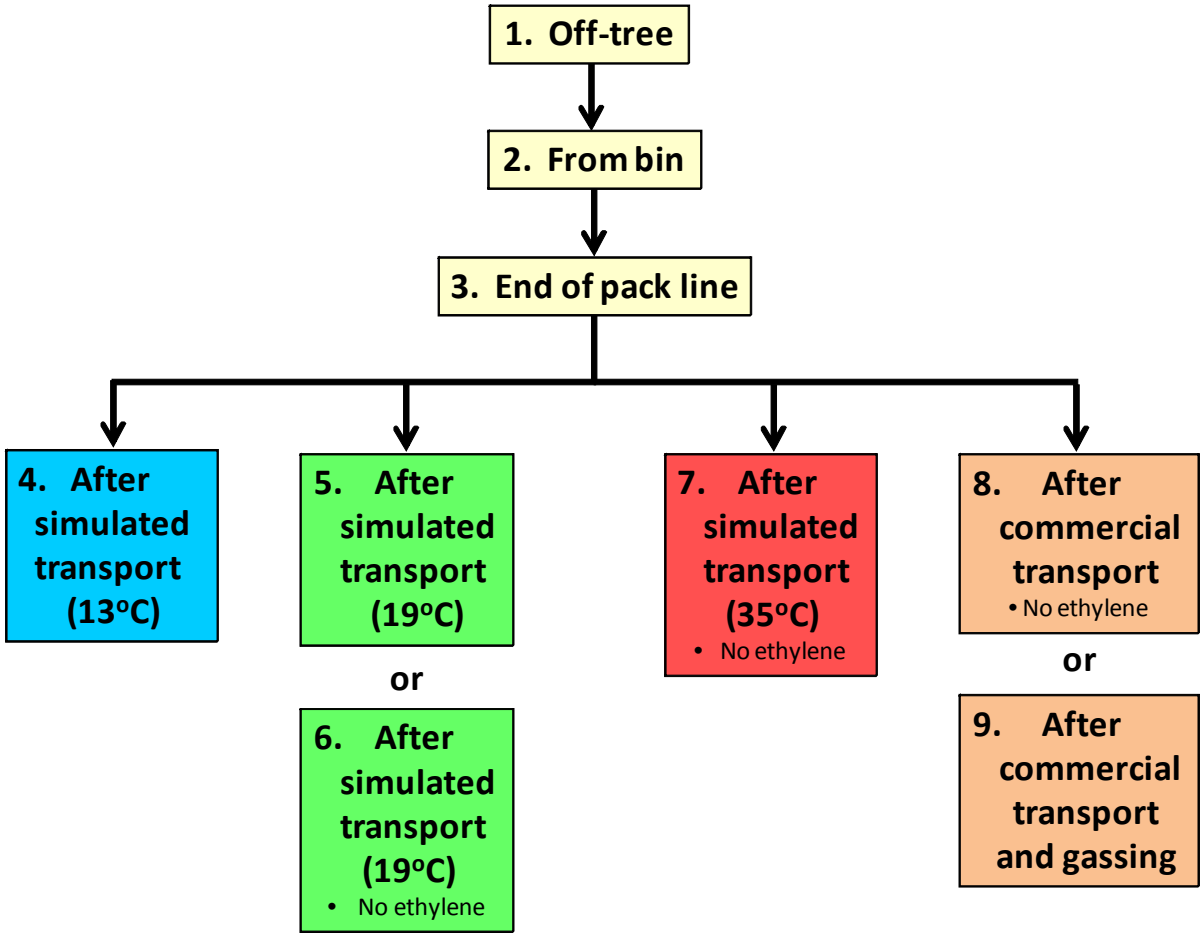

Figure 1. Flow diagram showing steps along a commercial and simulated supply chain where 'Kensington Pride' mango fruit were sequentially sampled. Three trays each containing 16 fruit were removed at every step. Unless specified otherwise, all fruit were treated with ethylene gas after sampling to initiate ripening. Fruit were assessed for RCD once they reached eating ripe. The fruit were sourced from nine orchards in the Northern Territory with a history of RCD.

Table 1. Commercial harvesting, packing and shipping dates for 'Kensington Pride' mango fruit that were sampled from nine orchards in the Northern Territory as part of a sequential sampling trial. NA indicates that data is not available.

Orchard	Orchard location	Harvest date	Harvest time	Pack shed	Pack date	Pack time	Dispatch date	Shipment temperature range and average	Arrive date	Wholesaler location
1	Darwin River	15/10	3 pm	1	16/10	3 pm	17/10	NA	21/10	Sydney
2	Lambells Lagoon	15/10	5 pm	1	16/10	2 pm	17/10	11-19°C, average 16°C	21/10	Melbourne
3	Noonamah	16/10	10 am	2	17/10	8 am	18/10	NA	22/10	Melbourne
4	Noonamah	16/10	1 pm	2	17/10	9 am	18/10	NA	22/10	Melbourne
5	Berry Springs	28/10	9 am	2	29/10	11 am	30/10	13-20°C, average 17°C	2/11	Melbourne
6	Berry Springs	29/10	9 am	3	29/10	12 pm	30/10	16-19°C, average 17°C	2/11	Brisbane
7	Lambells Lagoon	29/10	11 am	1	30/10	10 am	31/10	13-15°C, average 14°C	3/11	Sydney
8	Lambells Lagoon	13/11	9 am	3	13/11	1 pm	14/11	NA	18/11	Brisbane
9	Katherine	11/11	9 am	4	11/11	12 pm	13/11	NA	16/11	Brisbane

Table 2. The incidence (%) of RCD that developed in ripe 'Kensington Pride' mango fruit sourced from nine orchards in the Northern Territory in 2014. The fruit were harvested at the commercial harvest date and sequentially sampled at nine different stages of commercial or simulated handling and distribution. Data represent the average of three trays (48 fruit). Refer to Figure 1 for sampling and treatment details. NA indicates not available. Data for individual orchards followed by different letter are significantly different. ^{NS} indicates not significant. Data in bold font reflect the maximum incidence at each orchard.

Sampling step	**Orchard**								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Fruit dry matter content (%)	12.7	14.5	13.2	13.7	16.2	16.7	15.7	17.7	NA
1. Off tree, plus ethylene	15.1 c	45.1 ^{NS}	13.3 c	28.3 b	4.2 cd	0.0 d	2.0 c	0.0 d	0.0 d
2. From bin, plus ethylene	22.9 bc	48.9	**51.9 a**	17.4 bc	2.1 d	2.0 cd	10.0 b	0.0 d	0.0 d
3. End of pack line, plus ethylene	20.0 c	38.6	46.3 ab	22.9 bc	14.8 b	7.1 bcd	2.1 c	51.6 b	9.5 c
8. After commercial shipment, no ethylene	35.3 ab	55.8	37.9 b	24.1 bc	18.8 b	3.4 cd	4.7 bc	78.6 a	9.5 c
9. After commercial ethylene gassing	**45.2 a**	**74.6**	24.1 c	**44.0 a**	**38.8 a**	3.8 cd	**19.0 a**	76.9 a	11.9 bc
4. After simulated shipment 13^{o}C, plus ethylene	27.1 bc	53.7	24.1 c	40.8 a	2.0 d	11.9 ab	6.3 bc	**85.2 a**	17.1 ab
5. After simulated shipment 19^{o}C, plus ethylene	40.8 a	50.0	19.6 c	27.3 b	14.3 bc	14.6 ab	5.8 bc	37.5 c	21.4 ab
6. After simulated shipment 19^{o}C, no ethylene	16.9 c	57.1	24.6 c	28.4 b	15.7 b	9.3 bc	2.1 c	83.3 a	**26.2 a**
7. After storage at 35^{o}C, no ethylene	22.2 bc	56.3	14.6 c	13.4 c	3.9 d	**17.1 a**	0.0 c	61.0 b	7.0 cd

Table 3. The severity (% of fruit area) of RCD that developed in ripe 'Kensington Pride' mango fruit sourced from nine orchards in the Northern Territory in 2014. The fruit were harvested at the commercial harvest date and sequentially sampled at nine different stages of commercial or simulated handling and distribution. Data represent the average of three trays (48 fruit). Refer to Figure 1 for sampling and treatment details. NA indicates not available. Data for each orchard followed by different letter are significantly different. ^{NS} indicates not significant. Data in bold reflect the maximum severity at each orchard.

Sampling step	**Orchard**								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Fruit dry matter content (%)	12.7	14.5	13.2	13.7	16.2	16.7	15.7	17.7	NA
1. Off tree, plus ethylene	8.6 cd	20.2 bcd	20.3 b	10.5 ^{NS}	6.1 cde	0.0 e	8.0 ^{NS}	0.0 e	0.0 ^{NS}
2. From bin, plus ethylene	9.5 cd	15.9 def	3.8 d	6.4	12.3 b	2.0 e	5.4	0.0 e	0.0
3. End of pack line, plus ethylene	5.7 d	11.1 ef	17.7 b	4.5	3.5 de	4.7 de	1.0	11.0 d	7.3
8. After commercial shipment, no ethylene	19.3 b	**48.8 a**	**31.4 a**	16.6	18.1 a	45.0 b	**14.0**	33.7 b	14.0
9. After commercial gassing, plus ethylene	**26.2 a**	27.0 b	18.6 b	**16.9**	**22.3 a**	**60.0 a**	9.3	**49.7 a**	6.2
4. After simulated shipment 13^{o}C, plus ethylene	10.8 c	17.7 cde	10.4 c	9.3	8.8 bc	12.8 c	2.3	11.3 d	4.4
5. After simulated shipment 19^{o}C, plus ethylene	6.4 d	24.0 bc	5.6 cd	10.4	3.7 de	18.7 c	1.0	9.3 d	2.9
6. After simulated shipment 19^{o}C, no ethylene	8.5 cd	12.2 ef	6.2 cd	9.0	2.4 e	1.3 e	1.0	22.8 c	**15.2**
7. After storage at 35^{o}C, no ethylene	15.2 b	10.9 f	7.9 cd	7.8	7.0 cd	2.7 e	0.0	3.5 e	10.7

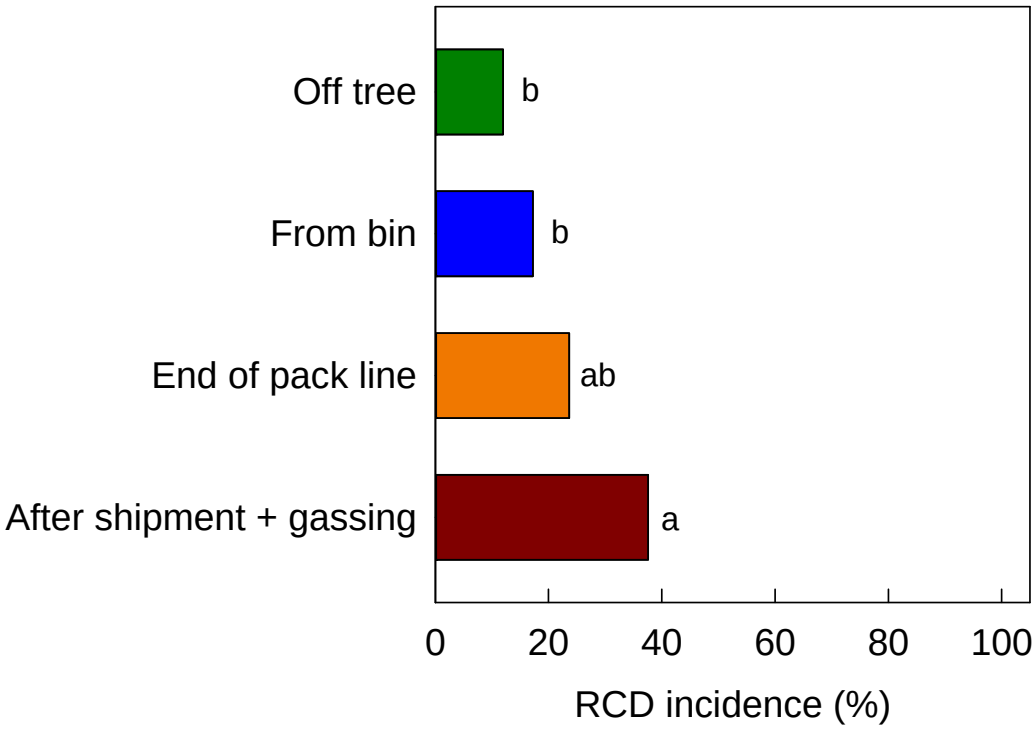

Figure 2. The incidence (%) of RCD that developed in ripe 'Kensington Pride' mango fruit when averaged across nine orchards in the Northern Territory in 2014. The fruit were harvested at the commercial harvest date and sequentially sampled at different stages of commercial harvesting, handling and distribution. Refer to Figure 1 for sampling and treatment details. Data followed by different letter are significantly different.

Table 3. The average total soluble solids content in ripe 'Kensington Pride' mango fruit with and without symptoms of RCD. The fruit were picked at the commercial harvest date from nine orchards in the Northern Territory in 2014. They were exposed to simulated handling and distribution treatments (see Figure 1, steps 1-7). Data from sampling steps 1-7 were pooled for presentation. Data from individual orchards followed by different letters are significantly different. ^{NS} indicates not significant.

Orchard	Total soluble solids content (°Brix)	
	No RCD	Plus RCD
1	11.6 a	10.9 b
2	13.1 a	12.3 b
3	12.3 ^{NS}	11.4
4	10.9 a	10.5 b
5	15.1 a	14.6 b
6	16.0 a	14.8 b
7	15.0 a	14.4 b
8	17.1 b	16.3 a
9	16.2 a	15.7 b

Appendix 4

The potential role of bacteria in resin canal discolouration

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Introduction

Resin canals in mango fruit are typically flesh-coloured in appearance. Brown to black discolouration of resin canals has, however, been occasionally observed in Australian mango fruit (Moore, 2012). Market reports of resin canal discolouration (RCD) have increased since 2012, particularly for early-season 'Kensington Pride' fruit. Preliminary observations from the HIA Ltd project, MG12018, indicated that *Pantoea agglomerans* and *Enterobacter cowanii* bacteria were isolated from fruit with discoloured canals (Macnish et al., 2014). No bacteria were isolated from asymptomatic resin canals. Postharvest inoculation of 'Kensington Pride' mangoes with *Pantoea agglomerans* and *Enterobacter cowanii* induced symptoms of RCD in 57% and 85% of fruit, respectively (Macnish et al., 2014). Taken together, these observations suggest that bacteria may potentially play a role in RCD.

In the current study, a series of additional tests were completed with a view to confirming the possible association of bacterial colonisation and the development of RCD in mango fruit. We tested fruit and mango wash solutions from farms and packing sheds for the presence of bacteria. We also inoculated mango fruit on trees at different stages of development with the isolated bacteria. In addition, the effects of applying anti-bacterial copper-based treatments to fruit in the field and hot water dip treatments to fruit during postharvest handling were also evaluated.

Materials and Methods

Trial 1: Bacterial isolation from fruit and wash water

Seventy-seven 'Kensington Pride', 'Calypso' and 'Keitt' mango fruit exhibiting visible symptoms of RCD were randomly collected during the 2014/15 mango season. The fruit were sourced directly from growers in the Northern Territory and north Queensland or from wholesalers in the markets once symptoms were evident. The fruit were air-freighted to the DAFQ laboratory in Mareeba, Queensland within 24-48 hours of collection. The fruit were maintained at room temperature (ca. 24°C) until they were ripe. The ripe fruit were cut open and portions of both symptomatic (i.e. discoloured) and asymptomatic (i.e. healthy) resin canal tissue was removed. The extracts were plated onto nutrient agar media and incubated at 30°C for 4 days. Bacteria growing from tissues were identified to the species level using physiological and biochemical (i.e. Biolog) tests.

Samples of mango wash water were randomly collected from harvest aids at seven orchards near Darwin in the Northern Territory during commercial harvesting in October 2014. Samples of the dump water used to rinse the fruit prior to packing were also collected from three nearby pack

sheds. The samples were held at 4°C for 12-24 hours. They were then packed into an insulated foam container with ice packs and delivered to the laboratory in Mareeba within 72 hours of collection. Serial dilutions of each sample were made in sterile distilled water. A 50 µl aliquot of each dilution was plated onto nutrient agar medium. The inoculated plates were incubated and assessed for the presence of microorganisms as described above. 'Kensington Pride' fruit that were processed in the mango wash and dump water were also tracked from the sheds to wholesalers in Melbourne, Victoria and Sydney, New South Wales. These fruit were then air-freighted to the DAFQ laboratory in Nambour, Queensland within 24 hours and assessed at eating ripe for RCD as described in Appendix 2. The incidence of RCD on fruit was compared to the bacterial population present in water samples.

Trial 2: Field inoculation studies

Based on the results from the bacterial isolation work in which *Pantoea agglomerans* was predominantly found in fruit showing RCD symptoms, a field inoculation trial was established. This experiment was set up in August 2014 at the South Edge Research Station near Mareeba. *Pantoea agglomerans* is a common epiphyte. It inhabits numerous environments, and has been reported as an emerging pathogen (Teresa et al., 2009). The epidemiology of *Pantoea* is largely unknown. Therefore, an attempt was made to inoculate 'Kensington Pride' mango flowers and fruit on trees at different growth stages given that the physical environment surrounding the phyllosphere experiences daily cycles in temperature, radiation, relative humidity, wind velocity, and leaf wetness.

A bacterial culture of *Pantoea agglomerans* was mass multiplied using nutrient broth. Cultures that were 48 hours old were used for inoculations. Prior to inoculation, the culture concentration was adjusted to 10^7 (10 million) colony forming units per mL (cfu/mL). Flowering panicles and both wounded (rubbed with sterilised sand paper) and non-wounded tissue sites on fruit at different development stages were sprayed with the bacterial suspension using an atomiser until the point of run-off (Figure 1). After inoculation, the flowers and fruit were covered with polyethylene bags for 48 hours to maintain optimum relative humidity levels for bacterial growth. To half of the trees in which the panicles were inoculated, ManKocide®, a copper-based fungicide, was sprayed onto panicles and developing fruit at the label rate on seven occasions every 14 days after inoculation until 2 weeks prior to the harvest date. In total, ten treatments were tested as described below:

1. Inoculation of panicles at the flowering stage.
2. Inoculation of fruit at the pea stage.
3. Inoculation of fruit at the marble stage.
4. Inoculation of fruit at golf ball stage.
5. Inoculation of fruit at the mature green stage.
6. Non-inoculated controls with no copper sprays.
7. Inoculation of panicles + golf ball stages.
8. Inoculation of pea + mature green stages.
9. Inoculation of golf ball + mature green stages.
10. Inoculation of panicles followed by seven sprays with copper (ManKocide®) every 14 days.

Each treatment consisted of four replicated trees and ten wounded and ten non-wounded fruit per replication. The inoculated fruit were examined every 2 weeks throughout the growing cycle for external symptoms of RCD, tissue necrosis, dieback, water soaked spots, chlorotic lesions or discolouration. Some inoculated fruit were observed to abscise from trees during the growing cycle.

The trial fruit were harvested at the mature green stage and inspected for external symptoms of RCD. Fruit with stems attached were transported by car to the nearby laboratory in Mareeba. The

fruit were de-stemmed in a mango wash solution as per commercial procedures. The wash water was changed for fruit from the different field treatments. Fruit were air-dried, packed in cartons and stored at room temperature (ca. 24°C). The final assessment for RCD was made at the eating ripe stage as recommended in Appendix 2. The fruit were cut open and the severity of RCD at the stem end and within the fruit was recorded and rated using the following 6-point scale: 0 = no symptoms, 1 = 1-5%, 2 = 6-15%, 3 = 16-30%, 4 = 31-50%, 5 = >50% of the fruit surface affected by RCD.

Trial 3: Effect of copper treatments in field

Two mango orchards with a history of RCD were identified near Berry Springs and Darwin River in the Northern Territory. Two representative blocks of 'Kensington Pride' trees were selected at each orchard in consultation with the growers. Developing fruitlets on one block of trees were sprayed four times with ManKocide®, as described above in trial 2, from mid-August to late September. The other block of trees was not sprayed with ManKocide® and served as controls. Trees from both treatments received all other insecticide and fungicide treatments in line with commercial practice.

Fruit at both orchards were harvested in mid- and late-October 2014 to capture two fruit maturity stages. Fruit with stems attached were transported by a car to the NT DPI&F Coastal Plains facility near Middle Point, Northern Territory within 1 hour. The fruit were de-stemmed in a commercial mango wash solution, rinsed in water, and treated with Scholar®, a fungicide, as per label instructions. The solutions were changed for fruit from the different treatments. The fruit were packed into single layer cardboard mango trays and transported to a commercial pack shed in Berry Springs. The sample trays were built into pallets and cooled to 16°C within 12-16 hours. Palletised fruit were transported by refrigerated trucks to wholesalers in either Sydney or Melbourne within 4-5 days. Upon arrival, the sample trays were removed from pallets and air-freighted to the Sunshine Coast airport in Queensland within 24 hours. The trays were then transported by a car to the nearby DAFQ laboratory in Nambour. Fruit was assessed at eating ripe for RCD as described in Appendix 2.

Trial 4: Effect of postharvest hot water treatments

Commercially picked and packed green mature 'Kensington Pride' mango fruit were procured from a grower near Mareeba with a history of RCD. The fruit were harvested from trees that had been sprayed with and without copper hydroxide (provided as ManKocide®) at the label rate every 2 weeks between flowering and harvest. Clean, blemish-free fruit were transported by a car to the DAFQ laboratory in Mareeba. In the laboratory, a 10^8 cfu/ml *Pantoea agglomerans* bacteria suspension was prepared as per trial 2. The fruit were randomly selected for treatment as follows:

1. Dip non-copper sprayed fruit in 30°C tap water for 5 minutes.
2. Dip non-copper sprayed fruit in 30°C suspension of *Pantoea agglomerans* for 5 minutes.
3. Dip non-copper sprayed fruit in 52°C tap water for 5 minutes.
4. Dip non-copper sprayed fruit in 52°C bacterial suspension (as above) for 5 minutes.
5. Dip copper sprayed fruit in 30°C tap water for 5 minutes.

Each treatment was comprised of 15 replicate fruit. All treated fruit were stored at room temperature (ca. 24°C). They were assessed for RCD incidence and severity at the eating ripe stage as per the protocol developed in Appendix 2. Fruit disease severity was calculated using the formula:

Disease severity = ∑ of disease ratings / Total number of ratings x maximum disease grade] x 100.

Trial 5: Effect of storage temperature

Commercially picked and packed green mature 'Kensington Pride' mango fruit were collected from the same Mareeba grower described in trial 4. Fruit with no external symptoms of RCD were inoculated with either *Pantoea agglomerans* or a mixture of *Pantoea agglomearnas and Enterobacter cowanii* by applying a 40 µl bacterial suspension (10^7 cfu/ml) to the cut stem end of fruit. The fruit were incubated for 4 days at room temperature (ca. 27°C) before transferring an equal number of fruit to 13°C and 25°C for 15 days. The fruit were then cut open and data on RCD incidence and severity was recorded. Tissue from discoloured resin canals was isolated for bacterial testing.

Overall experiment design and data analysis

A randomised block design was used for the field experiment while postharvest studies employed completely randomised designs. Data were analysed using a generalised linear model assuming a binomial distribution and log link. The results showed the predicted proportions on the log scale, the associated standard error, the p-value, back-transformed means and the 95% least significant difference (LSD). The predicted proportions were the mean proportions of fruit with the given level of RCD. Where significant effects were found, 95% pairwise LSDs were used to make comparisons.

Results and Discussion

Trial 1: Bacterial isolation from fruit and wash water

Isolations were made from 77 mango fruit with discoloured and healthy resin canals. *Pantoea agglomerans* was isolated from resin canals in 10% of 'Calypso', 33% of 'Keitt' and 80% of 'Kensington Pride' fruit exhibiting RCD symptoms (Table 1, Figure 2). The maximum incidence of 100% was found in a particular sample of Northern Territory fruit received from a wholesaler at the Brisbane markets. An *Enterobacter* sp. was also often isolated from fruit affected by RCD while *Tatumella ptyseos* was found in one sample of Northern Territory fruit. Similarly, Leff and Fiere (2013) reported that fruits and vegetables harboured diverse bacterial communities, and the communities on each produce type were significantly distinct from one another. These differences were often attributable to distinctions in the relative abundance of Enterobacteriaceae taxa. A few members of this family like *Tatumella ptyseos* have been described as the causative agent of pink disease in pineapple (Vianey et al., 2009) and *Pantoea stewartii* as a cause of bronzing (i.e. discolouration of pulp) in jackfruit fruit (Ruben et al., 2015). No bacterial organisms were isolated from healthy asymptomatic resin canals which suggest that these organisms were not endophytic.

Samples of water used to process 'Kensington Pride' fruit at seven farms and three packing sheds in the Northern Territory were also tested. At two farms, the fruit were rinsed and de-stemmed in water prior to immersion in a mango wash solution. At all other farms, the fruit were de-sapped directly in mango wash on harvest aids. There was considerable variation in the clarity of the rinse and mango wash solutions (Figure 3). On average, 51% of the solutions were colonised by *Pantoea agglomerans* (Table 1). *Micobacterium* sp. (27%), *Enterobacter* spp. (16%) and *Pseudomonas* spp. (6%) were also present in solutions. With the exception of the mango wash at farm 1 and the dump water at sheds A and C, all other solutions contained detectable levels of bacteria (Table 2). Where bacteria were detected, *Pantoea agglomerans* was also present except in the dump water at shed A. Bacteria were present at 10^6 to 10^7 (1-10 million) cfu/ml, consistent with the inoculum levels typically applied to fruit to induce RCD symptoms. These findings highlight the wide distribution of *Pantoea agglomerans* in the environment and its presence across all the tested farms.

Fruit from all seven surveyed farms were exposed to solutions containing *Pantoea agglomerans* bacteria either in the pre-wash rinse, mango wash and/or the shed dump. The incidence of RCD that developed in fruit that were exposed to these solutions as part of commercial picking and packing operations also varied greatly between farms (Table 2). The highest incidence of RCD was found in fruit from farm 2. These fruit were exposed to solutions containing bacteria at harvest and at the shed. The lowest incidence of RCD was recorded in fruit from farms 6 and 7 that were exposed to bacteria in the mango wash but processed in bacteria-free solution at the shed. While these data may point to a loose association between exposure to bacteria and RCD development, it is important to consider that the RCD incidence was also often high in the same fruit not exposed to commercial solutions (i.e. off-tree samples). Further research is needed to confirm if a relationship exists between exposure to bacteria and RCD, including the importance of the bacteria concentration, handling step where inoculation occurs, and environmental conditions following inoculation.

Solomon and Sharma (2009) reported that wash water contaminated with microorganisms, including pathogens, can infiltrate the intercellular spaces of fresh produce through damaged tissues, lenticels and stem end scars. *Pantoea agglomerans* were shown to form an aggregate colony or biofilm on cilantro leaves and these aggregates may limit the effectiveness of sanitising treatments on produce. This study does not rule out the possibility of other bacterial organisms/closely-related strains associated with RCD that might co-exist in plants, irrigation water, soil, or even insect vectors. They may vary in their distribution among different environments and production practices across farms. In addition, their variation may be due to differences in quality produce (less fruit damage) and the result of better pre- and postharvest management strategies.

Trial 2: Field inoculation trial

No visual RCD symptoms were observed on inoculated and non-inoculated fruit throughout the growing cycle. Several inoculated fruit abscised from trees at early stages. RCD became evident in some of the inoculated fruit at the eating ripe stage. The symptoms included red-brown discoloured canals that ran from the skin through the flesh. The majority of the RCD symptoms were found at the stem end and extended towards the seed and flesh. From a total of 400 inoculated fruits, only 258 fruits could be harvested at the green mature stage because of fruit drop. Of the harvested fruits, 33% on average showed RCD. The incidence of RCD varied from 19 to 51% for fruit inoculated at the different development stages (Figure 4). The highest incidence of RCD was observed in fruit inoculated at the mature green stage late in season (51%). RCD symptoms were, however, also observed in non-inoculated control fruit. This may be attributed to cross contamination from spraying the inoculum on adjacent trees or that factors other than bacteria are responsible for the naturally high level of RCD.

The severity of RCD that developed at the fruit stem end and within the flesh of the inoculated fruit was also assessed. There were generally no significant effects of the inoculation treatments on the severity of RCD that developed in fruit (Figure 5A). However, a significantly higher number of fruits that were inoculated at the marble stage or at flowering followed by fortnightly copper sprays ended up developing the maximum RCD severity of category 5 (>50% RCD) (Table 3). Repeating the analysis for RCD that developed within the fruit flesh showed that significant treatment effects were present for the proportion of fruit in RCD category 3 (16-30%) and category 5 (>50%) (Figure 5B). Pairwise comparisons showed that inoculation of fruits at the mature green stage and golf ball or pea size + green mature stage resulted in a significantly higher proportion of fruit with RCD categories of 3 and 5 as compared to inoculation at all other development stages (Table 4).

Trial 3: Effects of copper treatments in the field

Applying copper via ManKocide® sprays to 'Kensington Pride' mango trees during fruit development did not consistently reduce the incidence and severity of RCD that developed in fruit at eating ripe (Tables 5, 6). There was also no significant effect of harvest maturity (e.g. 13% vs 15% dry matter content) on the level of RCD that developed in these copper-treated and non-treated fruit. In the current study, copper was applied four times during mid to late stages of fruit development. It is typically reccommended that multiple copper treatments commence just after flowering in mango. Thus, it is possible that this delay in applying copper to mango trees dimished the potential treatment benefits.

Trial 4: Effects of hot water treatment

Overall, postharvest treatment in 52°C hot water for 5 minutes and pre-harvest copper sprays reduced the incidence and severity of RCD in inoculated 'Kensington Pride' fruit (Figure 6). The level of RCD incidence and severity was relatively high in non-inoculated fruit and this may be related to latent infection from the field or contamination during the fruit washing process. These infections potentially become active following harvest and during storage at relatively high transport and ripening temperatures. For example, delayed pathological decay was associated with an outbreak of illness due to Salmonella serotype Newport from consumption of mango (Sommer et al. 1992). The hot and cold water treatment for fruit fly disinfestation were the most likely source of this contamination, where entry of bacteria into the stem end segments (83%) was significantly higher than into middle side (19%) or blossom end (9%) segment (Pentado et al., 2004).

Trial 5: Effect of storage temperature

Inoculated and non-inoculated 'Kensington Pride' fruit that were stored at 13°C for 10 days developed less RCD as compared to fruit stored at 25°C (Figures 7, 8). This may be directly attributed to reduced rates of bacteria growth at the lower temperature. It may also simply reflect that the fruit stored at 13°C did not ripen completely in 10 days as those held at 25°C. Our previous research has shown that RCD symptoms typically reach a maximum in eating ripe fruit. *Pantoea* species require a higher temperature and less acidic pH for optimal growth. As fruit ripen, sugar levels increase, skin softens, and the natural defence barriers weaken.

The epidemiology of plant diseases caused by *Pantoea* spp. is relatively unknown. Environmental factors influence the severity of disease at high humidity and moderate temperature conditions (between 20-25°C in maize; 28-35°C in onion bulbs). The organism has also been reported to produce indole-3-acetic acid (IAA; plant growth promotor), which could play a role in pathogenesis. Very low concentrations of IAA affect plants and promote cell wall loosening during cell elongation. New reports of *Pantoea* spp. on previously unreported hosts have highlighted the re-emergence of this bacterium as a potentially economically important plant pathogen (Teresa et al., 2009).

In this study, only one bacterial organism/strain was used for field inoculations, to avoid any cross contamination. However, given that the artificial inoculation with a mixture of bacteria resulted in higher disease severity and RCD incidence (Figure 7), we cannot not rule out the possibility that other closely-related bacterial pathogens (*Enterobacter sp.* and *Tatumella sp.*) could also be associated with RCD and co-exist in plants, irrigation water, soil, and insect vectors. These bacterium may vary in distribution among different environments and production practices across farms.

Summary

Bacterial species including *Pantoea agglomerans* were consistently recovered from Australian mango fruit with RCD symptoms. Artificial inoculation of 'Kensington Pride' mango fruit with isolated *Pantoea agglomerans* induced RCD symptoms. Fruit inoculated during later stages of development on the tree also tended to show a higher RCD incidence. Foliar applications of copper sprays and postharvest hot water treatment of artificially inoculated fruit could reduce the incidence and severity of RCD. Copper treatments did not consistently reduce RCD that developed on non-inoculated commercial mango fruit. Taken together, these data provide circumstantial evidence for a role of bacteria in RCD development in mango fruit. Further research is required to confirm this possible association. This research should address the following questions:

- RCD in mango may be caused by common epiphytic/environmental bacteria under certain environmental conditions (high relative humidity and high temperature). Can these bacteria gain entry into the fruit through lenticels, the stem end and via areas of skin damage caused by insect-pest, diseases and in harvesting and packaging process?
- RCD may originate in the form of field infection that remains latent until the fruit ripens so can copper sprays reduce the inoculum load and RCD in mango?
- Relatively high bacterial populations were found in mango wash water, so can using the correct concentration of disinfectants (fungicide and chlorine) help reduce the potential for contamination of fruit with bacteria and RCD?
- Can RCD be reduced by timely application of chemicals to reduce insect pest pressure in the field given that high population of flatids and plant hoppers can transmit bacteria such as *Pantoea* (Nault and Rodriguez, 1985)?

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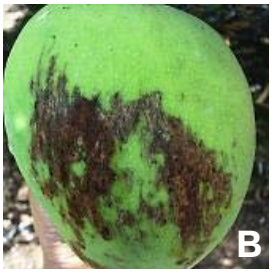

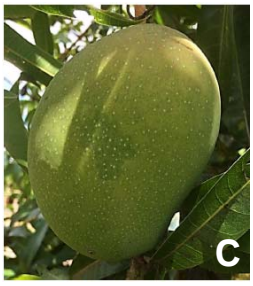

Figure 1. A. Spray inoculation treatment of 'Kensington Pride' mango trees at flowering and different fruiting stages with bacterial suspensions; B. Spray inoculated wounded fruit; C. Spray inoculated non-wounded fruit.

Table 1. The occurrence (%) of different species of bacteria in mango fruit exhibiting symptoms of RCD and in mango wash and dump water samples.

Date	Variety	Location	Isolations from RCD fruit	*Occurrence of bacteria (% of fruit tested)*					
				Pantoea agglomerans	***Enterobacter sp. (E.cowanii (E.gergoviae)***	***Tatumella ptyseos***	***Pectbacterium cyperipedi***	***Microbacterium barkeri***	***Pseudomonas sp (P.stutzeri and P.medonica)***
Fruit									
Jan-2014	'Kensington Pride'	Mareeba	1	100	100	0	0	0	0
July-2014	'Calypso'	Northern Territory	10	10	0	0	0	0	0
Sept-2014	'Kensington Pride'	Northern Territory	14	85	43	100	6	0	0
Nov-2014	'Kensington Pride'	Brisbane market	16	94	50	0	0	0	0
Jan-2015	'Kensington Pride'	Mareeba	30	61	19	0	0	0	0
Apr-2015	'Keitt'	Mareeba	6	33	0	0	0	0	0
		Total	**77**	**75**	**27**	**18**	**1**	**0**	**0**
Wash water									
Dec-2015	Kensington Pride	Northern Territory	33	51	16	0	0	27	6

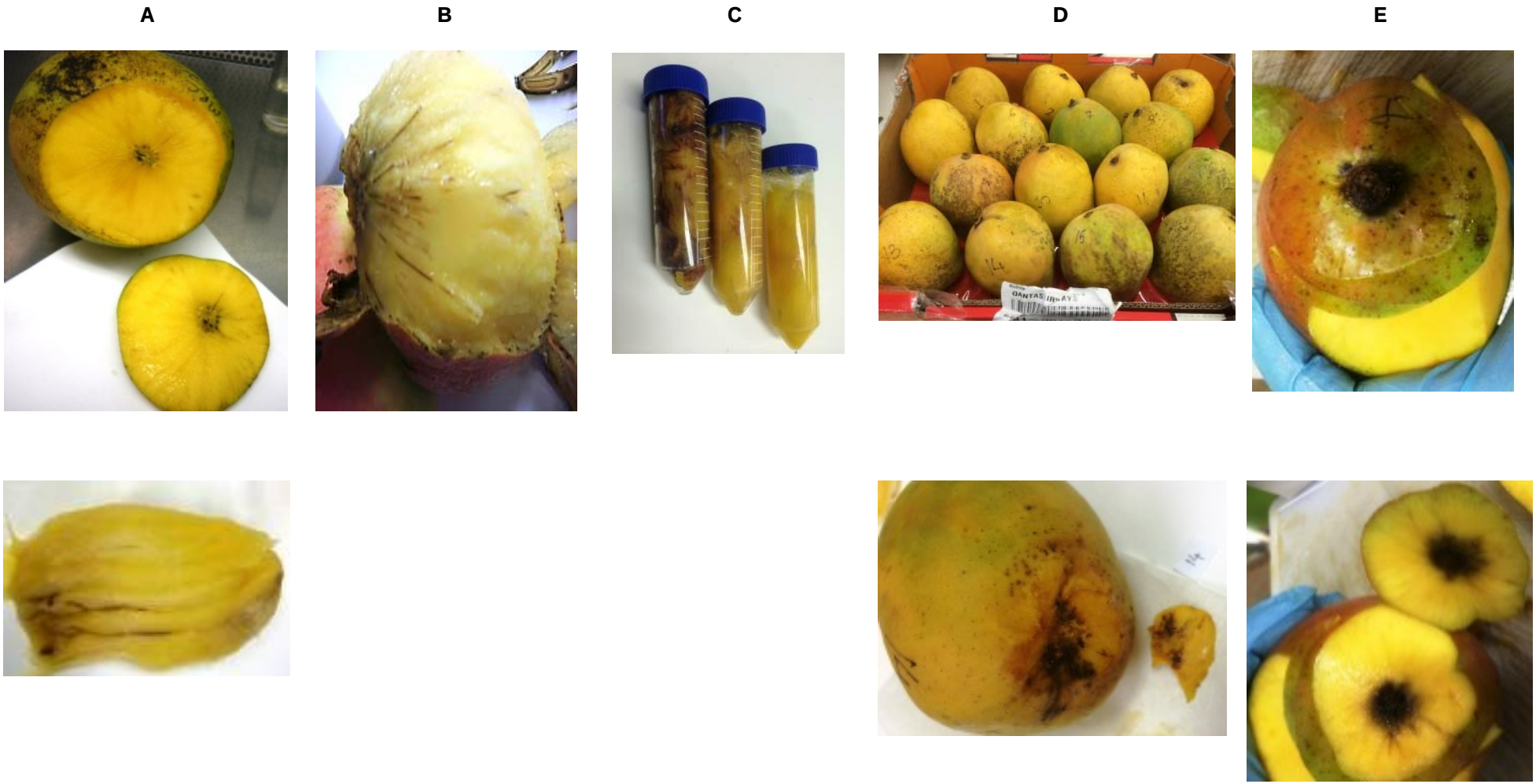

Figure 2. Photographs of the symptoms of RCD on fruit from different locations; A. Mareeba ('Kensington Pride'), B. Northern Territory ('Calypso'), C. Northern Territory ('Kensington Pride'), D Brisbane Market ('Kensington Pride') E. Mareeba ('Kensington Pride').

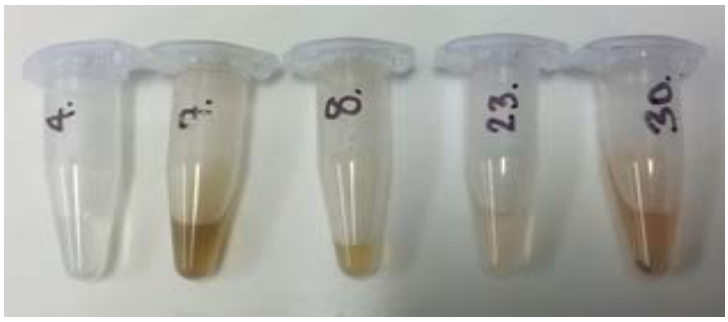

Figure 3. Photograph showing an example of the variation in clarity of mango wash water samples collected from Northern Territory mango farms in 2014.

Table 2. Total bacterial populations in 1 mL samples of mango wash and dump water solutions collected from seven mango farms and three packing sheds in the Northern Territory during the 2014 season. The incidence of RCD that developed on fruit processed in these solutions after commercial transport and ripening was also measured.

Farm	Pack shed	Farm harvest aid		Shed pack line	RCD incidence (%)	
		Water rinse	Mango wash	Dump water	Plus commercial handling	No commercial handling
1	A	2.0×10^9*	0	0	47	15
2	A	4.3×10^9*	1.9×10^9*	2.2×10^6	71	45
3	B	N/A	3.5×10^9*	5.6×10^6*	24	21
4	B	N/A	3.2×10^9*	4.9×10^6*	44	28
5	B	N/A	4.9×10^9*	1.0×10^9*	39	2
6	C	N/A	3.6×10^9*	0	4	0
7	A	N/A	9.0×10^9*	0	19	2

*Denotes presence of *Pantoea agglomerans.*

N/A denotes where the water rinse was not used prior to mango wash.

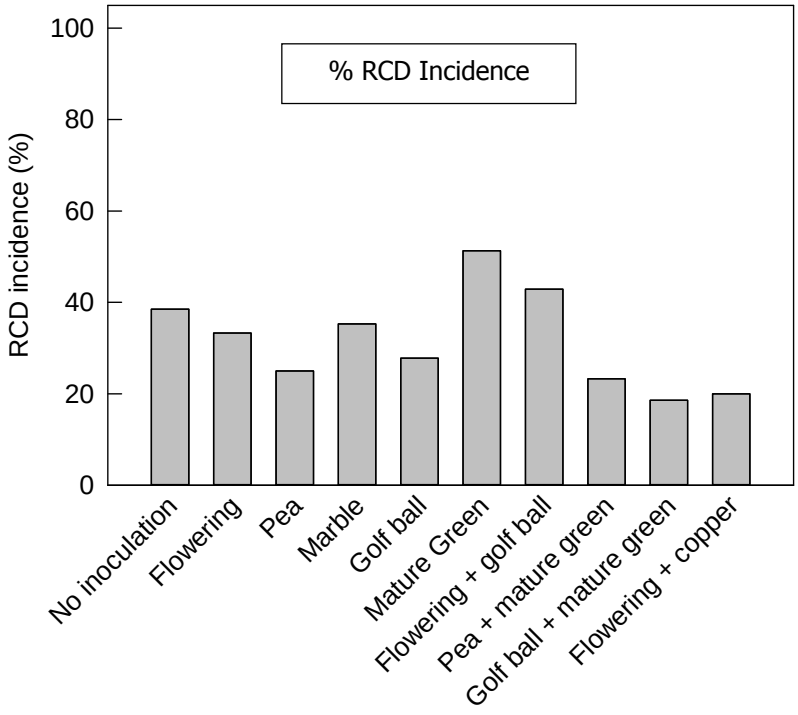

Figure 4. The incidence of RCD that developed in ripe 'Kensington Pride' fruit in response to artificial inoculation with *Pantoea agglomerans* at different flower and fruit development stages.

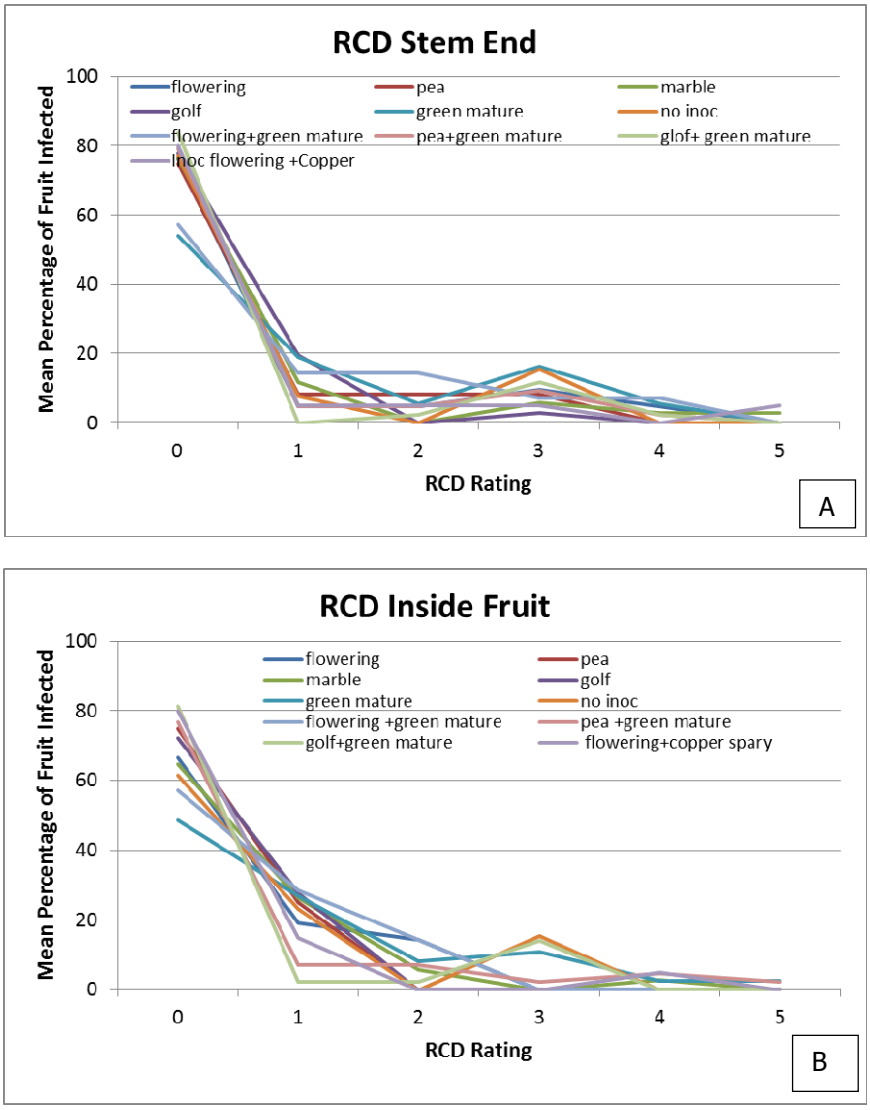

Figure 5. The severity of RCD that developed at the stem end and within the flesh of 'Kensington Pride' mango fruit after inoculation at different development stages with *Pantoea agglomerans*. Standard error bars were omitted due to a clarity issue. Details are provided in Table 3 and 4. RCD rating score: 0 = no symptoms; 1 = 1-5%; 2 = 6-15%; 3 = 16-30%; 4= 31-50%; and 5 = >50% of fruit had RCD.

Table 3. RCD severity at the stem end of artificially inoculated 'Kensington Pride' fruit.

Stem End	RCD = 0		RCD = 1		RCD = 2		RCD = 3		RCD = 4		RCD = 5	
Treatment	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se
1. Flowering stage	0.762	0.109	0.048	0.052	0.048	0.039	0.095	0.066	0.048	0.035	0.000 a	0
2. Pea stage	0.75	0.146	0.083	0.09	0.083	0.067	0.083	0.082	0	0	0.000 a	0
3. Marble stage	0.765	0.085	0.118	0.062	0	0	0.059	0.042	0.029	0.022	**0.029 b**	0.013
4. Golf stage	0.778	0.081	0.194	0.074	0	0	0.028	0.028	0	0	0.000 a	0
5. Maturity green fruit stage	0.541	0.096	0.189	0.073	0.054	0.031	0.162	0.063	0.054	0.028	0.000 a	0
6. Control no inoculation, no cu spray	0.769	0.136	0.077	0.083	0	0	0.154	0.103	0	0	0.000 a	0
7. Flowering + golf	0.571	0.154	0.143	0.106	0.143	0.079	0.071	0.071	0.071	0.051	0.000 a	0
8. Pea stage + Maturity at green stage	0.791	0.072	0.047	0.036	0.047	0.027	0.093	0.046	0.023	0.017	0.000 a	0
9. Golf + green fruit stage	0.837	0.066	0	0	0.023	0.019	0.116	0.051	0.023	0.017	0.000 a	0
10. Inoculation at flowering stage only + 7 Cu sprays	0.8	0.104	0.05	0.055	0.05	0.041	0.05	0.05	0	0	**0.050 b**	0.022
p-value	0.451		0.168		0.17		0.759		0.303		0.003	
Average 95% LSD	0.3102		0.1951		0.1038		0.1798		0.0594		0.0138	

Data represent the mean and standard error of 258 fruits.

Mean values followed by the same letter within the same column were not significantly different in LSD (Least significant difference) at P=0.05.

Table 4. RCD severity inside fruit in artificially inoculated 'Kensington Pride' fruit.

Inside fruit	RCD = 0		RCD = 1		RCD = 2		RCD = 3		RCD = 4		RCD = 5	
Treatment	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se
1. Flowering stage	0.667	0.122	0.191	0.107	0.143	0.066	0.000 a	0	0	0	0.000 a	0
2. Pea stage	0.75	0.149	0.25	0.156	0	0	0.000 a	0	0	0	0.000 a	0
3. Marble stage	0.647	0.098	0.265	0.094	0.059	0.035	0.000 a	0	0.029	0.018	0.000 a	0
4. Golf stage	0.722	0.089	0.278	0.093	0	0	0.000 a	0	0	0	0.000 a	0
5. Green mature stage	0.487	0.098	0.27	0.091	0.081	0.039	**0.108 c**	0.036	0.027	0.017	**0.027 b**	0.011
6. Control, no inoculation	0.615	0.161	0.231	0.146	0	0	**0.154 bc**	0.071	0	0	0.000 a	0
7. Flowering +Golf	0.571	0.157	0.286	0.151	0.143	0.081	0.000 a	0	0	0	0.000 a	0
8. Pea stage +maturity at green stage	0.767	0.077	0.07	0.048	0.07	0.034	**0.023 ab**	0.016	0.047	0.02	**0.023 b**	0.009
9. Golf+green mature stage	0.814	0.071	0.023	0.029	0.023	0.02	**0.140 c**	0.037	0	0	0.000 a	0
10.Inoculation at flowering + 7 copper sprays	0.8	0.106	0.15	0.1	0	0	0.000 a	0	0.05	0.03	0.000 a	0
p-value	0.375		0.171		0.061		<0.001		0.058		0.008	
Average 95% LSD	0.3329		0.3068		0.097		0.0603		0.0318		0.0082	

Data represent the mean and standard error of 258 fruits. Mean values followed by the same letter within the same column were not significantly different in LSD (Least significant difference) at P=0.05.

Table 5. Effect of applying copper hydroxide (provided as ManKocide®) to 'Kensington Pride' mango trees on the incidence and severity of RCD that developed in fruit at eating ripe. The copper treatments were applied to trees at an orchard near Berry Springs in the Northern Territory. The treatments were applied every 2 weeks between fruit set and the first fruit harvest date. Fruit were harvested at two maturity stages. Data followed by different letters are significantly different.

Harvest	Dry matter content (%)	Treatment	Incidence	Severity [NS]
13 October	12.8 ± 0.4	No copper	7.8 b	17.5
	13.3 ± 0.6	Plus copper	28.3 a	8.6
28 October	14.7 ± 0.3	No copper	27.0 a	26.4
	15.4 ± 0.1	Plus copper	19.4 a	24.5

[NS] indicates no significant difference among data.

Table 6. Effect of applying copper hydroxide (provided as ManKocide®) to 'Kensington Pride' mango trees on the incidence and severity of RCD that developed in fruit at eating ripe. The copper treatments were applied to trees at an orchard near Darwin River in the Northern Territory. The treatments were applied every 2 weeks between fruit set and the first fruit harvest date. Fruit were harvested at two maturity stages. Data followed by different letters are significantly different.

Harvest	Dry matter content (%)	Treatment	Incidence	Severity
13 October	13.3 ± 0.2	No copper	11.5 b	11.2 a
	14.1 ± 0.2	Plus copper	32.1 a	12.7 a
28 October	14.3 ± 0.2	No copper	21.1 a	40.5 a
	15.6 ± 0.2	Plus copper	21.2 a	17.5 b

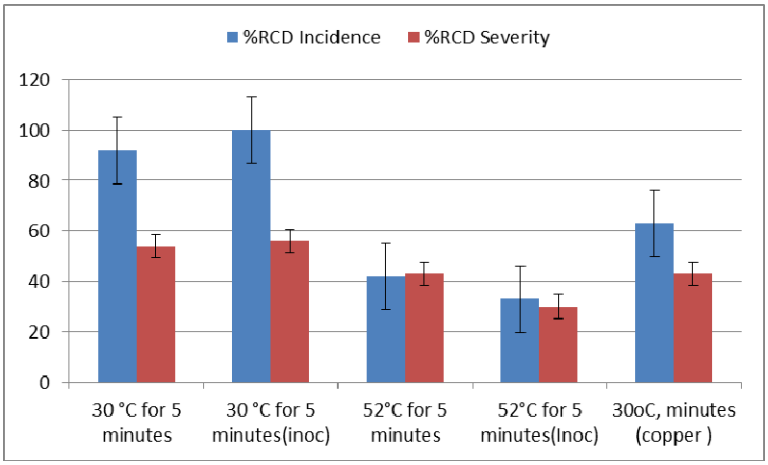

Figure 6. RCD incidence and severity in 'Kensington Pride' in ripe mango fruit. The fruit were exposed to postharvest water dips at different temperatures. Additional fruit were treated with copper sprays prior to harvest. 75 fruit were assessed at the eating ripe stage.

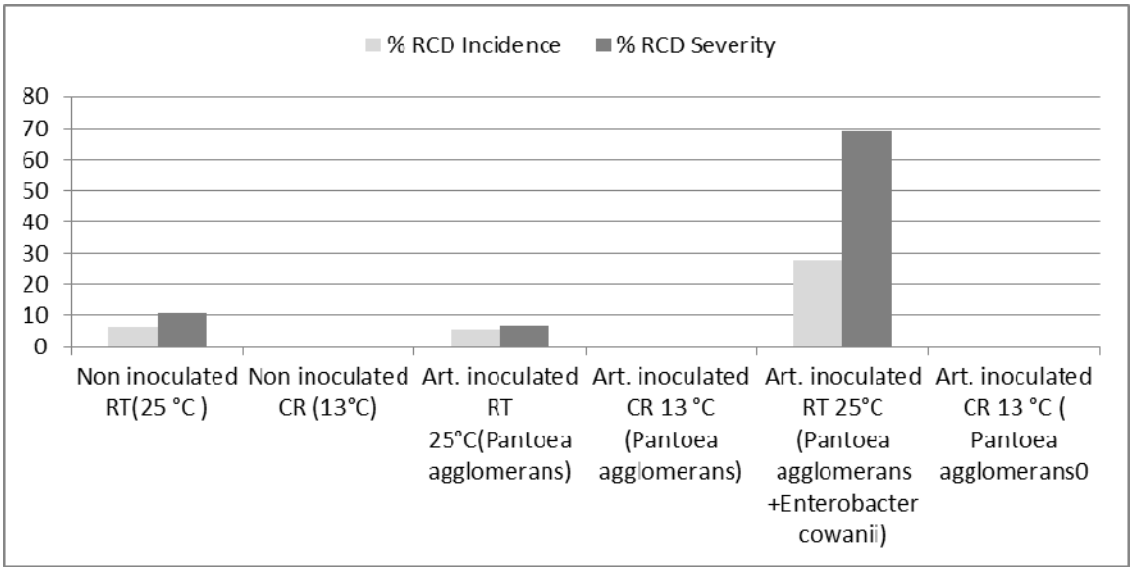

Figure 7. RCD incidence and severity in ripening 'Kensington Pride' mango fruit after 10 days of storage at 13 and 25°C. Green mature fruit were inoculated and non-inoculated with *Pantoea agglomerans* and/or *Enterobacter cowanii* bacteria prior to storage.

Figure 8. Photographs of RCD symptoms on 'Kensington Pride' mango fruit after 10 days of storage at 25°C (A) and 13°C (B). Green mature fruit were inoculated and non-inoculated with *Pantoea agglomerans* and/or *Enterobacter cowanii* bacteria prior to storage.