

Identifying factors that contribute to mango resin canal discolouration

Dr Andrew Macnish
The Department of Agriculture, Fisheries and
Forestry, Qld

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Andrew Macnish et al

Department of Agriculture, Fisheries and Forestry
(Queensland)

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31 May 2014



W.E Pack



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1. Media summary

Resin canal discolouration (RCD) is a quality defect that reduces the market value of ripe mango fruit. Market reports of RCD have increased during the past two mango seasons, particularly for early-season 'Kensington Pride' fruit produced near Darwin in the Northern Territory. A 1-year project was undertaken during the 2013 mango season to start identifying possible factors that contribute to RCD. The research focused on 'Kensington Pride' fruit produced in the Darwin production area. The research team monitored fruit from orchard to market, conducted specific trials, and completed detailed scientific evaluation of affected fruit. Preliminary findings suggest that RCD:

- Can also occasionally be found in green fruit on the tree or at grading but only when the fruit or stems have severe physical injury or pathogen infection.
- Occurrence can vary considerably between different orchards and for different harvest dates.
- Increases in severity as fruit develop from firm ripe to overripe.
- Incidence is higher in fruit that are exposed to commercial handling procedures than those ripened directly off the tree.
- Can be present in the flesh without symptoms being exhibited on the skin.
- Incidence can be higher in early-season fruit than those picked later.
- Incidence can be higher in fruit harvested soon after a rain event.
- Symptoms may be associated with bacterial infection.

The observations to-date suggest that RCD is likely to occur when field conditions result in the production of 'sensitive' fruit that, in turn, express the defect when exposed to the common harvest and postharvest stresses. Follow-on R&D will aim to confirm the causes of RCD and to develop reliable control measures.

2. Technical summary

Resin canals are a distinctive feature of mango (*Mangifera indica*). These canals store resinous secretions under pressure within the plant tissues. In fruit, the canals are typically flesh-coloured in appearance but brown-black canals have occasionally been observed in ripe mangoes. These internal symptoms are usually accompanied by dark outlines of the discoloured canals on the fruit skin. Resin canal discolouration (RCD) is a disorder that reduces the aesthetic quality and marketability of ripe mango fruit. There has been increased detection of RCD at Australian wholesale markets over the past two mango seasons, particularly for early-season 'Kensington Pride' fruit produced near Darwin in the Northern Territory.

A pre-season survey of mango growers, packers and wholesalers highlighted that rain around harvest, early fruit harvest maturity, and/or over-use of chemicals were among the most commonly perceived factors that could be associated with this defect. A 1-year scoping project was undertaken during the 2013 season to start identifying possible factors that contribute to RCD. The research focused on 'Kensington Pride' fruit produced in the Darwin production area. Three activities were completed:

1. Monitoring with traceability to determine how fruit affected by RCD were handled.
2. Specific trials to test the survey ideas above to identify RCD contributory factors.
3. Scientific evaluation of RCD symptoms to better understand the likely causes.

Our preliminary findings suggested that RCD:

- Is also occasionally found in green fruit on the tree or at grading but only when the fruit and/or stems have severe physical injury or pathogen infection.
- Incidence can vary markedly between different orchards in a close geographic area and for different harvest dates from the same orchard.
- Increases in severity over time as fruit develop from firm ripe to overripe. Accordingly, surveys at the wholesaler stage may under-estimate RCD, because fruit at the retail stage will be riper and likely to display more severe symptoms.
- Incidence is relatively higher in fruit that are exposed to commercial handling and distribution procedures as compared to fruit ripened directly off the tree.
- Can be present in the flesh without symptoms being exhibited on the skin. Thus, the incidence of the defect is potentially higher than can be estimated by supply chain surveys based on external appearance only
- Incidence and severity can be higher in early-season fruit as compared to late-season fruit of more advanced harvest maturity.
- Incidence can be higher in fruit harvested within 12 hours of a rain event relative to fruit picked 60 hours after rainfall.
- Could possibly be associated with bacterial organisms with a likeness to *Pantoea* and *Enterobacter* species, since these were isolated from discoloured resin canals.

Taken overall, our observations to-date suggest that RCD is likely to occur when field conditions result in the production of 'sensitive' fruit that, in turn, express the defect when exposed to common harvest and postharvest stresses. Further research is required to confirm our preliminary findings. A follow-on R&D project is currently being developed with a view to better understanding factors contributing to mango

RCD and developing reliable strategies to minimise this quality defect.

3. Introduction

Resin canals or ducts 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, stems, flowers and fruit (Joel, 1981). In mango fruit, the canals form a complex network that runs from just under the skin and along the seed through the flesh (Juliano and Cuevas, 1932). Given the caustic nature of the stored resin or sap (Loveys et al., 1992), it has been proposed that the canal system functions as a chemical defence barrier against herbivores (Joel, 1980). Water-containing resin canals may also help maintain plant water status during drought (Downton, 1981; Kallarackal et al., 1990).

Mango fruit resin canals are typically flesh-coloured in appearance, although discoloured (brown-black) canals have been observed in ripe fruit (Moore, 2012). This internal discolouration is usually visible through the fruit skin as darkened outlines of the canals. Market reports of Resin Canal Discolouration (RCD) have increased during the past two mango seasons, particularly for early-season 'Kensington Pride' fruit produced near Darwin in the Northern Territory (T. Rudge, C. Cope, T. Campbell, pers. comm., 2013). Because RCD can develop during ripening of fruit after packing, there are concerns about its adverse impact on consumer purchasing behaviour and the ongoing reputation of the early season fruit.

To-date, there has been very limited research directed at understanding the underlying biology of and/or factors that contribute to the manifestation of RCD, nor the development of commercial control measures. This disorder is complex in that there appears to be sets of interacting pre- and postharvest factors rather than a single cause. Moreover, the inherent susceptibility of fruit reportedly varies with cultivar, site, region and season. A review by Moore (2012) outlined some of the basic physical features of RCD and highlighted the history of its occurrence in Northern Territory mango fruit. The Australian Mango Industry Association (AMIA) helped facilitate a survey of several growers, packers and wholesalers prior to the 2013 season. The findings suggested that rain around harvest, early fruit harvest maturity, and/or over-use of pre- and postharvest chemicals were commonly perceived as factors associated with this defect.

Based on the preliminary survey, a 1-year scoping study was commissioned to start identifying factors that contribute to mango RCD. The project focussed on the following three activities:

1. Fruit monitoring with traceability to determine how fruit affected by RCD were handled from orchard to market.
2. Specific trials to start testing some of the above ideas, and involving sampling along the supply chain with a view to identifying RCD contributory factors.
3. Detailed scientific evaluation of affected fruit to better understand the symptoms of RCD and the likely nature of contributing factors.

4. Materials and Methods

4.1. Fruit monitoring

The overall aim of this activity was to help establish a monitoring program for product history, handling conditions and outturn assessment with a view towards identifying factors that contribute to mango RCD.

We sought and received the commitment of a key mango pack house (W.E. Pack Pty Ltd) near Berry Springs (12°42'S, 131°00'E), Northern Territory to participate in the study. W.E. Pack is a central shed that treats, grades, packs and distributes mango fruit from up to 50 growers in the Northern Territory. In consultation with the pack house, we identified 14 commercial mango orchards to be involved in the fruit monitoring activity. The selected orchards produced fruit exhibiting varying degrees of RCD during the 2012 mango season.

Green mature 'Kensington Pride' fruit were randomly sampled from 34 consignments at the Berry Springs shed during the 2013 mango season. The fruit were sourced from the 14 orchards described above. Briefly, the fruit were harvested, de-stemmed and treated with a commercial mango de-sapping solution as per standard practice at each orchard. The fruit were transported by flatbed trucks to the Berry Springs shed within 24 hours of harvest. Upon arrival at the shed, the fruit were loaded onto a commercial mango pack line and washed in a chlorinated water solution for 30 seconds. They were then treated with a postharvest fungicide (0.6 mL/L Scholar[®]; Syngenta Australia Pty Ltd) for 30 seconds via overhead sprayers and brushed with rotating soft brushes for 1 minute to improve fungicide coverage. A random selection of 10-72 fruit from each consignment was sampled after grading. The sample fruit were transported by car to the Department of Primary Industry and Fisheries, Berrimah Farm laboratory near Darwin (12°27'S, 130°50'E), Northern Territory within 1 hour. The fruit were maintained at 20°C until eating ripe. Fruit were then cut with a knife and assessed for RCD symptoms. The incidence of RCD was expressed as the proportion (%) of fruit within each sample that exhibited symptoms.

The remaining fruit in each consignment was packed into single layer cardboard trays, palletised, pre-cooled and transported by refrigerated trucks to wholesalers in Melbourne (37°48'S, 144°57'E), Victoria within 5 days. Produce inspectors contributing to the market survey component of project MG13015 "Improving Mango Quality Through Accurate Harvest Maturity" were engaged to assess fruit consignments for the presence of RCD. Briefly, the inspectors removed two to four trays (32-64 fruit) of mangoes from random consignments from our study plus those from other shipments soon after fruit arrived in Melbourne. The inspectors assessed fruit for RCD symptoms as visualised through the skin. The proportion (%) of the sample trays that contained at least one fruit with RCD was determined.

We also developed a template to start cataloguing key records of fruit production and handling practices that may assist in identifying factors associated with RCD. The template was designed as a questionnaire and is attached as Appendix 1. We are now preparing to commence surveying Northern Territory growers and packers about their practices and experiences during the 2013 mango season. The aim of this work is to build up a database on orchards and sheds that had relatively low and high

incidence of RCD. The questionnaire responses will help us trace back to potential factors contributing to RCD. It will also assist with planning trials and seeking collaborators for the follow-on project (MG14004) set to commence in July 2014.

4.2. Specific trials

The general aim of this activity was to start testing some commonly perceived factors associated with RCD that were highlighted by growers during a pre-season survey.

4.2.1. Plant material

'Kensington Pride' mango (*Mangifera indica*) fruit were harvested from two commercial orchards near Lambells Lagoon (12°35'S, 131°11'E) and Berry Springs in the Northern Territory. The orchards were chosen based on observations that their fruit exhibited a relatively high incidence of RCD during the 2012 mango season (T. Elliott, pers. comm., 2013). Fruit were harvested with pedicels attached and taken to nearby sheds for processing.

4.2.2. General processing

Upon arrival at the shed, each fruit was inverted and their pedicel was detached by hand at the abscission zone. The de-stemmed fruit were immediately placed into a plastic bin containing a 50 L commercial de-sapping solution (2.5 g/L mango wash powder; Harvey Distributors) for 2 minutes. The solution was not replaced during processing of all fruit in the particular trial. Half of the fruit from each tree were transported by car at ambient temperature (ca. 35°C) to the Berrimah Farm laboratory near Darwin, Northern Territory within 1 hour and served as library tray controls. These fruit were maintained at 20°C until eating ripe. The remaining half of the fruit were transported by car to a commercial pack shed near Berry Springs, Northern Territory within 1 hour.

4.2.3. Commercial packing and shipment

Fruit were loaded onto a commercial mango pack line and treated as described above (section 4.1.). The treated fruit were packed into standard single layer cardboard mango trays lined with plastic moulded cup inserts. A datalogger (HOBO U12-013; Onset Computers Corp.) that recorded temperature and relative humidity was inserted into a randomly selected tray. The trays were built into the top layer of a randomly selected pallet. The palletised fruit were pre-cooled to 22°C overnight.

The pallet containing sample fruit was randomly loaded into a solid-walled refrigerated truck trailer equipped with air suspension. An additional 21 pallets were loaded into the trailer to ensure a full load. The pallets were transported from the pack shed in Berry Springs to a wholesaler in Melbourne, Victoria within 5 days. Upon arrival, the pallets were removed from the trailer and sample trays were retrieved. The sample fruit were exposed to 10 µL/L (parts per million) ethylene gas at 20°C for 2 days to initiate uniform ripening as per standard commercial practice (Ledger et al., 2012). The experimental fruit were then held at 20°C for 2 days prior to being air-freighted to the Department of Agriculture, Fisheries and Forestry, Maroochy Research Facility near Nambour (26°37'S, 152°57'E), Queensland within 1 day. These fruit were maintained at 20°C until eating ripe.

4.2.4. Trial 1: Fruit maturity

One row of 35 'Kensington Pride' trees considered to be representative of the Lambells Lagoon orchard was marked for use in the trial. These trees were subjected to standard commercial production practices. Thirty-seven fruit were harvested at 0800-1000 from each of eight randomly selected trees within the marked row at 2 weeks before, at, and 2 weeks after the predicted commercial harvest date to capture early-, mid-, and late-season stages of maturity, respectively. Individual fruit were labelled and processed as described above.

4.2.5. Trial 2: Rain event

'Kensington Pride' fruit were harvested at commercial maturity ($14.4 \pm 0.1\%$ dry matter content; DMC) from the orchard near Lambells Lagoon that was used in trial 1. The same single row of 35 trees was used to source fruit. Thirty-seven fruit were harvested at 0800-1000 from each of eight randomly selected trees within the marked row at 12 hours after a significant rain event (14 mm) and again after 2 days of sustained dry weather. The trees continued to receive daily supplementary irrigation as per standard commercial practice during the trial sampling period. The fruit were labeled and processed as described above.

4.2.6. Trial 3: Production practices

One row of 20 'Kensington Pride' trees that were well maintained (i.e. exposed to standard production practices) and another cluster of 10 trees that had been neglected (i.e. no irrigation or chemical applications) at the Berry Springs orchard were marked for use in the trial. Thirty-seven fruit were harvested at 1100-1300 from each of five randomly selected trees from both the marked row of well maintained trees and cluster of neglected trees. Individual fruit were labelled and processed at the nearby pack shed as outlined above.

4.2.7. Trial 4: Fruit wounding

In the first experiment, 32 replicate 'Kensington Pride' fruit from eight randomly selected trees used in trial 1 and 2 at the Lambells Lagoon orchard were labelled for wounding treatment. A hypodermic needle was inserted through each fruit pedicel four times either above or below the abscission zone to simulate insect damage at 0900-1100 at 2 weeks before the predicted commercial harvest date (Figure 1). At the same time, a knife was used to make four horizontal (4-5 cm-long \times 3 mm-deep) cuts to one cheek of additional fruit to simulate damage caused by magpie geese (Figure 1). An additional 32 control fruit were not wounded. After 2 weeks, the fruit were picked at 0900-1000 and processed as described above.

In a second experiment, 20 replicate 'Kensington Pride' fruit were randomly selected from three trees at the Lambells Lagoon orchard. These trees were chosen because they bore the occasional green fruit with RCD symptoms, possibly indicating that the remaining fruit may be sensitive to developing the defect. As described above, four horizontal cuts were made to one cheek per fruit at 0900-1100 at 2 weeks before the predicted harvest date. An additional 20 control fruit were not wounded. After 2 weeks, the fruit were picked at 0900-1000 and processed as outlined above.

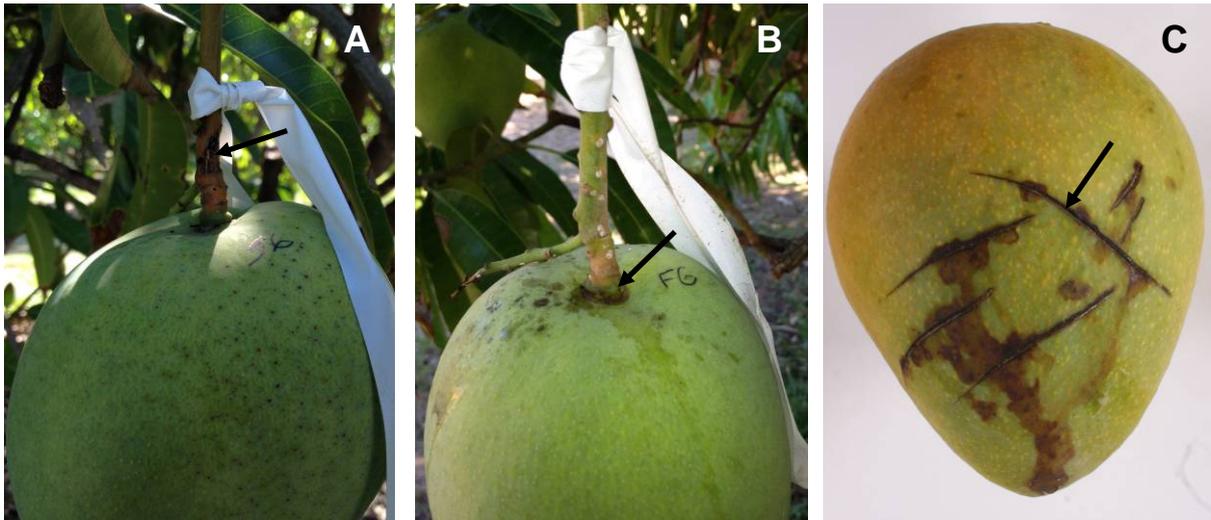


Figure 1. Photographs showing artificial wounding to ‘Kensington Pride’ mango pedicels and fruit at a commercial orchard near Lambells Lagoon, Northern Territory. (A) Wounds above the pedicel abscission zone, (B) Wounds below the pedicel abscission zone, (C) Wounds to the fruit cheek. Arrows point to the wound sites.

4.2.8. Fruit evaluation

Fruit DMC was determined for five randomly selected fruit from each sample tree. Briefly, a wedge of tissue was removed from both cheeks of each fruit within 1 day of harvest and dried in an oven at 70°C to a constant weight. The DMC was expressed as the proportion (%) of dry weight relative to the initial fresh weight of the sample.

The incidence and severity of RCD was determined for fruit at eating ripe both before and after removing the fruit skin with a vegetable peeler. The incidence of RCD was determined as the proportion (%) of fruit exhibiting darkened canals relative to the total number of fruit in a sample. The severity of RCD was determined as the proportion (%) of the surface area of individual fruit displaying visible symptoms.

The incidence of disease was also determined for fruit at eating ripe. Disease incidence was expressed as the proportion (%) of fruit displaying visible signs of disease relative to the initial number of fruit in each treatment.

4.2.9. Experiment design and data analysis

Fruit were arranged in a randomised complete block design within the 20°C evaluation rooms. Data are generally presented as means \pm standard errors. Depending upon the experiment, data were analysed as one-way ANOVAs using the generalised linear model procedure of GenStat (version 14, VSN International). Where significant ($P \leq 0.05$) treatment effects were determined by ANOVA, data means were separated by the least significant difference test at $P = 0.05$.

4.3. Detailed examination of affected fruit

The aim of this activity was to undertake detailed examination of fruit affected by RCD to better understand the symptoms and the likely contributing factors.

4.3.1. RCD anatomy

Five ripe 'Kensington Pride' fruit from the maturity trial that exhibited symptoms of RCD were selected for anatomical studies. Segments comprising 3 mm square blocks of fruit skin and flesh tissues showing visible signs of RCD were excised from each fruit. These explants were chemically-fixed in formaldehyde acetic acid solution (ethyl alcohol, glacial acetic acid, formaldehyde, distilled water) for 3 days at 20°C. Fixed tissues were dehydrated in a graded series of 50, 70, 90, 95 and 100% ethanol for 2 hours at each step. The dehydrated explants were then gradually infiltrated with 100% xylene. The xylene solution was progressively replaced with paraffin wax (Paraplast Plus; Sigma) over 2 days at 20°C. Infiltrated tissues were embedded into fresh molten paraffin wax. Transverse 5-7 µm-thick tissue sections through the fruit skin and flesh were cut using a stainless steel knife on a microtome. The sections were transferred to glass slides and stained with toluidine blue O for 5 seconds. Stained sections were washed with distilled water and viewed under bright field illumination with a compound light microscope (Eclipse TS100; Nikon Instruments Inc.). The sections were photographed using a digital camera (EOS 40D; Canon Inc.). About 20 sections through resin canals were examined.

4.3.2. Fruit pathology

Isolation procedures

Symptoms of RCD were observed on green 'Kensington Pride' fruit from two mango orchards near Berry Springs, Northern Territory. Three replicate fruit from each orchard with and without visible RCD symptoms were selected and air-freighted to Brisbane (27°28'S, 153°01'E), Queensland within 24 hours. The fruit were surface sterilised with 70% ethanol and allowed to dry. Small portions of skin were removed from both symptomatic (i.e. discoloured) and asymptomatic tissue, and from resin canal tissue underneath the skin. The extracts were plated onto peptone yeast extract agar (PYEA) agar, and the plates incubated at 24°C for 24 hours. Bacteria growing from tissues were sub-cultured by streaking onto fresh PYEA, and single colony isolates established and maintained. Bacteria were also isolated from 'Kensington Pride' fruit from south east Queensland displaying RCD symptoms.

In a parallel study, symptoms of RCD were also observed on ripe 'Kensington Pride' fruit harvested from trees near Mareeba (16°59'S, 145°25'E), Queensland. Three replicate fruit with and without visible RCD symptoms were collected from one tree. Symptomatic and asymptomatic resin canals from each fruit were excised, plated onto nutrient agar and incubated as described above.

Preliminary identification

The bacterial isolates from the Berry Springs fruit were collected and identified via DNA sequencing. Briefly, genomic DNA from bacterial colonies was extracted using the Promega Wizard® Genomic DNA Purification kit according to the manufacturer's instructions. A 16S rRNA gene fragment was then amplified using a polymerase chain reaction (PCR) master mix with the primers R16 (CTTGTACACACCGCCCGTCA) and R23 (GGTACTTAGATGTTTCAGTTC). The amplified PCR products were separated by electrophoresis on an agarose gel and sent for purification and sequencing at the Macrogen sequencing facility in Seoul, Korea. The sequences were then compared against existing GenBank bacterial

database entries using the BLAST program (National Center for Biotechnology Information).

As a complementary approach, the bacterial isolates from the Mareeba fruit were collected and identified using a biochemical test. Briefly, the carbon source utilisation profiles of the isolates were characterised using the Biolog Gram Negative MicroPlate™ (Biolog Inc.) technique according to the manufacturer's instructions. The resulting metabolic profiles were then analysed with the Micro Station GEN III ID system to tentatively identify the bacterial isolates.

Pathogenicity tests

Isolates of the opportunistic bacteria *Pantoea agglomerans* and *Enterobacter cowanii* were incubated on nutrient agar at 30°C for 48 hours. The cultures were removed from the agar and bacterial suspensions were made in sterile water. The number of bacteria in the water suspensions were enumerated and adjusted to a concentration of 1×10^7 colony forming units/mL.

'Kensington Pride' fruit were harvested at commercial maturity from trees maintained at the South Edge Research Station near Mareeba, Queensland. The fruit were transported to the Department of Agriculture, Fisheries and Forestry laboratory in Mareeba, Queensland within 30 minutes. Upon arrival at the laboratory, the fruit pedicels were detached by hand at the abscission zone and each fruit was immediately inverted to allow sap to drain for 30 minutes. The pedicels were then re-cut with a sharp knife.

A 40 µL drop of the *P. agglomerans* and *E. cowanii* suspensions were applied either individually or in combination to the cut pedicel surface of seven replicate fruit. Additional fruit that were treated with or without a 40 µL drop of sterile water served as controls. The inoculated fruit were then placed into closed plastic containers lined with moist paper towel for 2 days at room temperature (ca. 27-30°C). The fruit were then removed from containers and maintained at room temperature until ripe. The fruit were evaluated after 2 weeks for the presence or absence of visible symptoms of RCD. Bacteria were re-isolated from diseased tissue adjacent to the inoculated stem ends and identified as described above.

4.3.3. Fruit mineral analysis

Ripe 'Kensington Pride' fruit from the third harvest of the maturity trial at the Lambells Lagoon orchard were selected during evaluation in the laboratory near Nambour. The skin and underlying flesh was excised from five replicate fruit with and without symptoms from a single tree. Skin and flesh samples were carefully separated using a knife and dried to a constant weight at 70°C for 3 days. The dried samples were sent to the CSBP Ltd soil and plant analysis laboratory in Perth, Western Australia for determination of mineral content.

5. Results and Discussion

5.1. Fruit monitoring

The incidence of RCD in ‘Kensington Pride’ fruit varied considerably among 14 orchards from the Darwin production area during the 2013 mango season (Figure 2). For example, the incidence of RCD within samples of individual fruit consignments varied from 0% at orchards 1, 2, 3, 5 to 75% at orchard 6. These data highlight the possibility that different production practices may contribute to differing fruit ‘sensitivity’. The occurrence of RCD also varied substantially for fruit collected from the same orchard at different harvest dates across the season. For instance, fruit from orchard 8 packed on 24 September 2013 had no RCD while those packed on 4 October 2013 developed 40% RCD. Despite this variation, there was no consistent pattern in RCD incidence over time for any orchard. Of interest, RCD was occasionally found in fruit on the tree or grading table, but only when the fruit and/or stems had severe and recent physical injury or pathogen infection (Figure 3A).

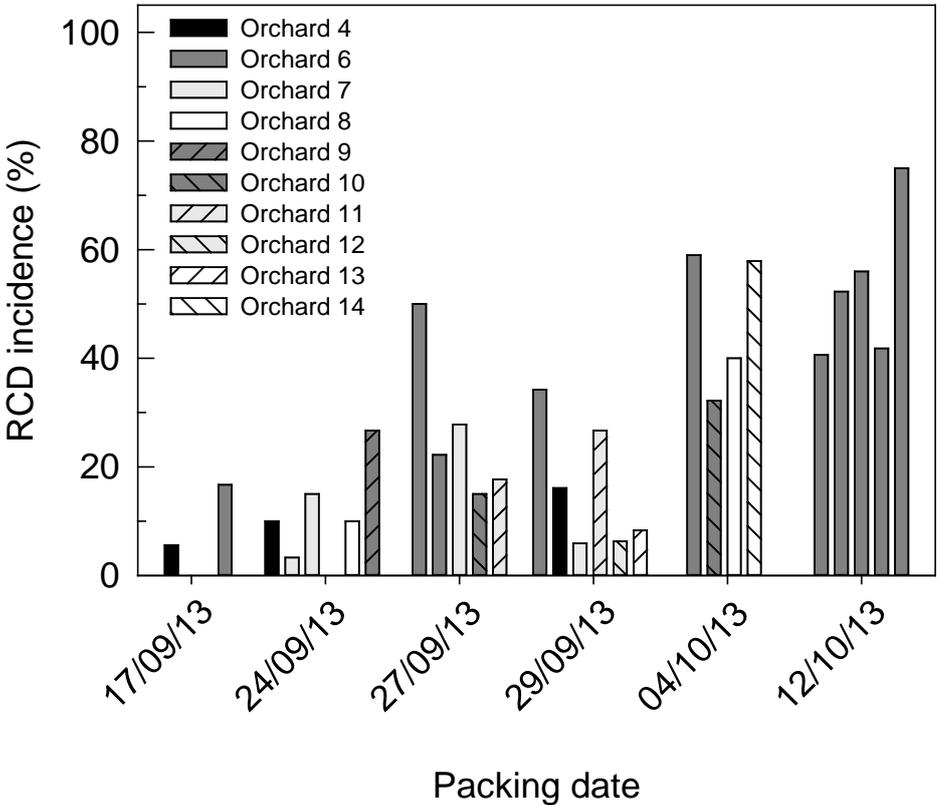


Figure 2. Incidence of RCD in ‘Kensington Pride’ fruit sourced from 14 orchards in the Darwin, Northern Territory production area during the 2013 mango season. Fruit were sampled at random during grading at a pack house near Berry Springs, Northern Territory. Fruit were maintained at 20°C until eating ripe and then assessed for internal symptoms of RCD. Data represent the mean of 10-72 replicate fruit, depending upon the orchard. Orchards 1, 2, 3 and 5 recorded no RCD.



Figure 3. Photographs of 'Kensington Pride' mango fruit showing symptoms of RCD. (A) A green fruit displaying superficial symptoms of RCD through the skin and several sites of physical injury. (B) A ripe fruit with the skin removed showing the underlying symptoms of RCD in the flesh.

The extent of RCD in parallel consignments that were transported to wholesalers in Melbourne also varied markedly between orchards and across harvest/packing dates. This data is presented in the final report of project MG13015. While the incidence of RCD appeared to be highest for fruit produced by some growers in the Darwin area, the defect was also detected at relatively low levels in fruit from north Queensland during the 2013 mango season (T. Dunmall, pers. comm., 2014). RCD was most commonly observed in 'Kensington Pride' fruit but other Australian and Asian mango varieties can also develop this defect (Moore, 2012).

5.2. Specific trials

5.2.1. Trial 1: Fruit maturity

The incidence and severity of RCD in commercially handled 'Kensington Pride' mangoes was significantly higher for fruit picked early in the season (<13% DMC) than those harvested 2-4 weeks later with a DMC of 15-17% (Table 1). RCD was present in the flesh of 13-19% of fruit without obvious symptoms on the skin. Thus, the incidence of the defect is potentially higher than can be estimated by supply chain surveys based on external appearance only. RCD incidence was less in the immature library tray fruit (not commercially harvested, packed or transported). The reasons for the difference between the commercially handled and the library tray treatments is unclear, and requires further investigation.

Visible signs of disease accompanied RCD symptoms in 43, 41 and 82% of early, mid and late-season fruit, respectively. We also observed that RCD increased in severity as fruit developed from firm ripe to overripe (data not shown; Figure 3B). Accordingly, surveys at the wholesale level may under-estimate the extent of RCD

reaching consumers, because fruit at retail will be riper and likely to display more severe symptoms. The incidence of RCD was 1.3-9.2-fold higher in fruit that were exposed to commercial handling as compared to library tray control fruit that were ripened directly off the tree (Table 1). This observation points to the possibility that RCD is likely to occur when 'sensitive' fruit are exposed to specific harvest and postharvest stresses.

5.2.2. Trial 2: Rain event

Harvesting mature (14.4% DMC) 'Kensington Pride' fruit within 12 hours of a 14 mm rain event resulted in significantly higher RCD incidence and severity following commercial handling than fruit picked 60 hours after rain (Table 2). In contrast, there was no effect of rain on RCD levels that developed in library tray fruit. Nevertheless, the incidence of RCD was higher in fruit that were commercially handled than those maintained as library trays, as per our findings from trial 1. RCD was evident in the flesh of 15-21% of fruit that displayed no symptoms on the skin. There was no association of disease with RCD for fruit picked at either harvest time.

Table 1. The incidence and severity of RCD in 'Kensington Pride' mango fruit as visualised through the skin and in the flesh. Fruit were harvested every 2 weeks to capture three stages of maturity as indicated by dry matter content. The fruit were then commercially packed and transported from Berry Springs, Northern Territory to Nambour, Queensland via a wholesaler in Melbourne, Victoria. Additional fruit were ripened off the trees (no commercial harvesting or handling) and maintained as library trays in Darwin, Northern Territory. Fruit were held at 20°C and assessed for RCD upon reaching eating ripe. Incidence and severity data represent the mean of 128 fruit sourced from eight trees. Data followed by different letters are significantly different at $P = 0.05$.

Harvest date	Dry matter content (%) ¹	Commercially packed and shipped trays				Library trays
		Skin		Flesh		Flesh
		Incidence (%) ²	Severity (%) ³	Incidence (%)	Severity (%)	Incidence (%)
3/10/13	12.7 ± 0.1	48.8 a	52.0 a	66.4 a	56.6 a	7.2 c
17/10/13	14.6 ± 0.2	30.3 b	36.4 b	43.4 b	38.5 b	21.6 b
31/10/13	16.9 ± 0.2	32.4 b	16.8 c	46.7 b	18.2 c	36.6 a

¹Data represents the mean ± standard error of 40 fruit.

²The proportion (%) of fruit that displayed RCD symptoms.

³The proportion (%) of the fruit surface that exhibited RCD symptoms.

Table 2. The incidence and severity of RCD in ‘Kensington Pride’ mango fruit as visualised through the skin and in the flesh. Fruit were harvested at commercial maturity at 12 and 60 hours after a rain event. The fruit were commercially packed and transported from Berry Springs, Northern Territory to Nambour, Queensland via a wholesaler in Melbourne, Victoria. Additional fruit were ripened off the trees (no commercial harvesting or handling) and maintained as library trays in Darwin, Northern Territory. Fruit were held at 20°C and assessed for RCD upon reaching eating ripe. Incidence and severity data represent the mean of 128 fruit sourced from eight trees. Data followed by different letters are significantly at $P = 0.05$.

Harvest time	Commercially packed and shipped trays				Library trays
	Skin		Flesh		Flesh
	Incidence (%) ¹	Severity (%) ²	Incidence (%)	Severity (%)	Incidence (%)
12 h after rain	46.4 ^{NS}	56.7 a	67.6 a	58.8 a	35.9 ^{NS}
60 h after rain	31.6	35.3 b	46.6 b	38.0 b	36.7

¹The proportion (%) of fruit that displayed RCD symptoms.

²The proportion (%) of the fruit surface that exhibited RCD symptoms.

^{NS} indicates no significant difference between data.

5.2.3. Trial 3: Production practices

The incidence and severity of RCD that developed in ‘Kensington Pride’ mangoes after commercial handling was substantially higher in fruit picked from neglected trees than those exposed to standard production practices (Table 3). These differences were not observed in the library tray fruit. This further suggests that fruit may be exposed to stress during distribution along the supply chain that increases expression of RCD. It is important to note that the fruit harvested from neglected trees had lower DMC (13%) than those picked from well maintained trees (17% DMC). The fruit harvested from neglected trees also displayed a 7-fold higher incidence of decay (49%) than fruit from well-maintained trees (7%) at ripe. Almost half (i.e. 43-47%) of the fruit affected by RCD also displayed visible signs of disease when ripe. The incidence of RCD in fruit from neglected trees that were commercially handled was higher than fruit maintained as library trays.

Table 3. The incidence and severity of RCD in ‘Kensington Pride’ mango fruit as visualised through the skin and in the flesh. Fruit were harvested from trees that had been exposed to standard or no chemical applications. The fruit were commercially packed and transported from Berry Springs, Northern Territory to Nambour, Queensland via a wholesaler in Melbourne, Victoria. Additional fruit were ripened off the trees and maintained as library trays in Darwin, Northern Territory. Fruit were held at 20°C and assessed for RCD upon reaching eating ripe. Incidence and severity data represent the mean of 80 fruit sampled from five trees. Data followed by different letters are significantly different at $P = 0.05$.

Treatment	Dry matter content (%) ¹	Commercially packed and shipped trays				Library trays
		Skin		Flesh		Flesh
		Incidence (%) ²	Severity (%) ³	Incidence (%)	Severity (%)	Incidence (%)
Neglected	13.2 ± 0.4	56.6 a	47.9 a	64.5 a	50.0 a	31.8 ^{NS}
Well maintained	17.4 ± 0.6	10.5 b	31.7 b	16.3 b	26.9 b	31.9

¹Data represents the mean ± standard error of 40 fruit.

²The proportion (%) of fruit that displayed RCD symptoms.

³The proportion (%) of the fruit surface that exhibited RCD symptoms.

^{NS} indicates no significant difference between data.

5.2.4. Trial 4: Fruit wounding

Wounding the cheeks of green ‘Kensington Pride’ fruit at 2 weeks before harvest resulted in an increased number of ripe fruit exhibiting RCD symptoms through the skin relative to non-wounded control fruit (Table 4). However, pre-harvest wounding of the fruit did not increase the incidence or severity of RCD that developed in the flesh. Wounding fruit pedicels either above or below the abscission zone at 2 weeks before harvest did not increase RCD incidence or severity in ripe fruit.

In a second experiment, pre-harvest wounding of the cheeks of green fruit on ‘Kensington Pride’ trees that appeared to be sensitive to RCD resulted in significantly higher incidence of the defect in both the skin and flesh at eating ripe than non-wounded control fruit (Table 5). Thus, it is possible that more RCD occurs when field conditions result in the production of ‘sensitive’ fruit that are then damaged by e.g. passing tractors, severe wind rub or insect/pest activity, or maybe damaged during harvest, then exposed to postharvest stresses. There was no significant difference, however, in the severity of RCD that developed in fruit from either treatment, and significant RCD occurs on fruit with no obvious damage to the skin.

Table 4. The incidence and severity of RCD in ‘Kensington Pride’ mango fruit as visualised through the skin and in the flesh. Fruit were harvested at commercial maturity from trees at an orchard near Lambells Lagoon, Northern Territory. The fruit were wounded with a hypodermic needle above and below the pedicel abscission zone (AZ) at 2 weeks before harvest. The cheek of additional fruit was wounded with a knife. Non-wounded fruit acted as controls. All fruit were commercially packed and transported from Berry Springs, Northern Territory to Nambour, Queensland via a wholesaler in Melbourne, Victoria. Fruit were held at 20°C and assessed for RCD at eating ripe. Incidence and severity data represent the mean of 32 fruit sourced from eight trees. Data followed by different letters are significantly different at $P = 0.05$.

Treatment	Commercially packed and shipped trays			
	Skin		Flesh	
	Incidence (%) ¹	Severity (%) ²	Incidence (%)	Severity (%)
No wounding	32.4 b	16.8 ^{NS}	46.7 ab	18.2 ^{NS}
Plus wounding above AZ	22.6 b	17.1	22.6 c	17.1
Plus wounding below AZ	27.6 b	19.0	27.6 bc	19.7
Plus wounding on fruit	55.6 a	40.0	55.6 a	40.0

¹The proportion (%) of fruit that displayed RCD symptoms.

²The proportion (%) of the fruit surface that exhibited RCD symptoms.

^{NS} indicates no significant difference between data.

Table 5. The incidence and severity of RCD in ‘Kensington Pride’ mango fruit as visualised through the skin and in the flesh. Fruit were harvested at commercial maturity from trees that appeared sensitive to RCD at an orchard near Lambells Lagoon, Northern Territory. The cheek of fruit was wounded with a knife at 2 weeks before harvest. Non-wounded fruit acted as controls. All fruit were commercially packed and transported from Berry Springs, Northern Territory to Nambour, Queensland via a wholesaler in Melbourne, Victoria. Fruit were held at 20°C and assessed for RCD at eating ripe. Incidence and severity data represent the mean of 20 fruit sourced from three trees. Data followed by different letters are significantly different at $P = 0.05$.

Treatment	Commercially packed and shipped trays			
	Skin		Flesh	
	Incidence (%) ¹	Severity (%) ²	Incidence (%)	Severity (%)
No wounding	15.0 b	25.0 ^{NS}	25.0 b	28.8 ^{NS}
Plus wounding on fruit	65.0 a	7.5	75.0 a	10.5

¹The proportion (%) of fruit that displayed RCD symptoms.

²The proportion (%) of the fruit surface that exhibited RCD symptoms.

^{NS} indicates no significant difference between data.

5.3. Detailed examination of affected fruit

5.3.1. RCD anatomy

Discoloured resin canals were visible in cross section as dark circular microscopic structures in freshly cut mango skin and flesh tissues (Figure 4A). The canals were located within 0.2 mm of the fruit surface and extended into the flesh. In chemically-fixed transverse tissue sections, resin canals were hollow and devoid of their resinous secretions (Figure 4B). The innermost cell layer of discoloured canals stained a reddish purple when treated with toluidine blue dye (Figure 5). In non-discoloured canals, this cell layer did not colour when exposed to toluidine blue.

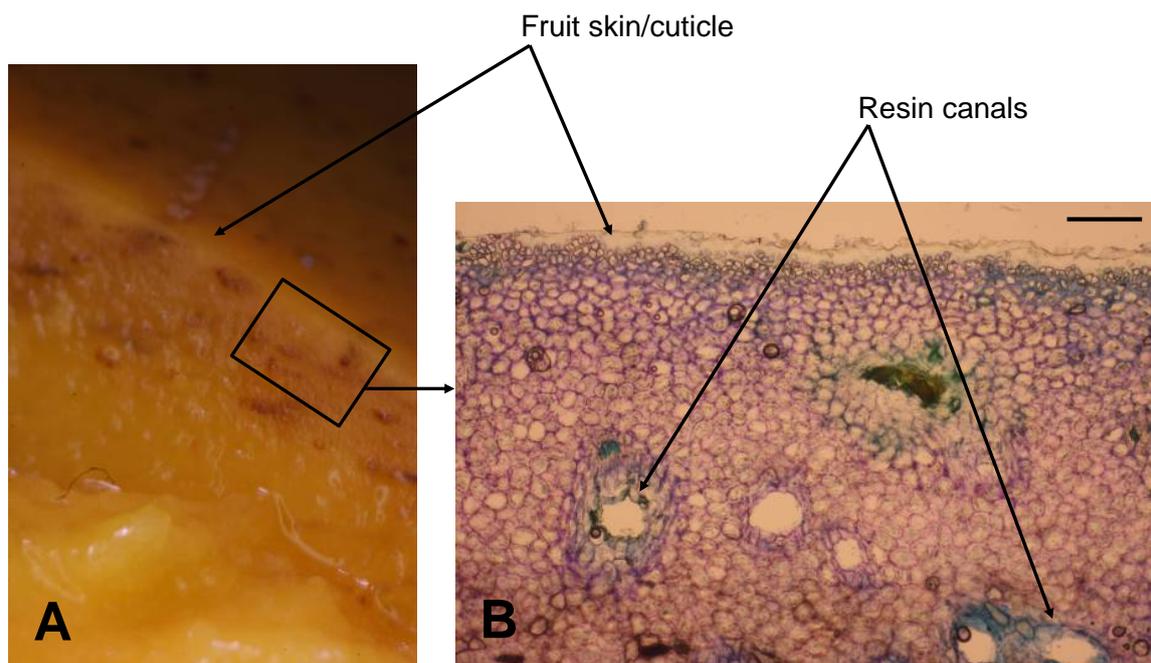


Figure 4. Photographs of ‘Kensington Pride’ mango fruit skin and flesh tissues. (A) Side view of excised fruit skin and flesh showing dark circular resin canals. (B) Transverse tissue section of formaldehyde acetic acid-fixed fruit skin and flesh stained with toluidine blue O dye showing the cuticle and numerous resin canals. The two resin canals indicated by the arrows have significant staining from the toluidine blue, suggesting accumulation of polyphenols that are commonly associated with brown discoloration in plant tissues. The other canals are not stained and likely would not contribute the RCD. The scale bar represents 0.2 mm.

Toluidine blue is a well-known histochemical stain for demonstrating the presence of polyphenols. Thus, these preliminary observations suggest that the cell layer surrounding discoloured canals may be rich in polyphenolic compounds. Previous research has established that the cell layer lining canals actively secretes the resin into the canals (Joel and Fahn, 1980). At harvest, resin freely exudes from ruptured canals at the cut surface. This can result in brown-black ‘sapburn’ damage to the fruit skin (Brown et al., 1986). The resin exudate appears to disrupt the skin cell structure enabling enzymes such as polyphenoloxidase to mix with its phenolic substrates and catalyse the browning reaction (Saby John et al., 2002) common in plant tissues, e.g. the brown colour in bruised apple flesh.

Our observations that RCD is associated with commercial handling and/or physical wounding of fruit suggests it may be a type of stress-related disorder. Discolouration of plant tissues is a recognised problematic response to physical wounding stress. Accordingly, it is possible that RCD results from injury or dysfunction of the specialised cells lining the resin canals. This in turn, may trigger enzymatic oxidation of polyphenolics deposited in these specialised cells and account for the brown discolouration of affected resin canals. Further research is clearly required to characterise the underlying biology and symptoms of RCD.

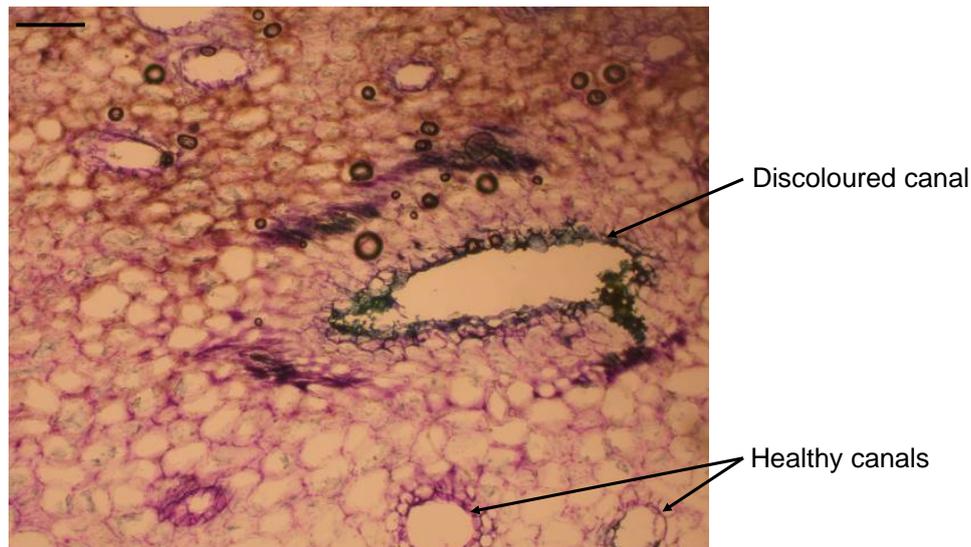


Figure 5. Photograph of a transverse tissue section through formaldehyde acetic acid-fixed ‘Kensington Pride’ mango fruit flesh tissues stained with toluidine blue O dye showing the cuticle and discoloured (symptomatic) and healthy (asymptomatic) resin canals. The scale bar represents 0.1 mm.

5.3.2. Fruit pathology

Two bacterial species were consistently recovered from discoloured resin canals in fruit sourced near Mareeba, Queensland. The metabolic profiles of the bacteria on Biolog microplates were similar to the database profile of *Pantoea* and *Enterobacter* species. No bacterial organisms were isolated from asymptomatic resin canals which suggest that the organisms were not endophytic. Bacteria were also isolated from discoloured resin canals in fruit sampled near Berry Springs, Northern Territory. A BLAST search with the isolated 16S rRNA bacterial gene fragments supported the Biolog results that the isolates probably belong to a species of *Pantoea*. The highest homologies to the 16S rRNA were *P. stewartii* subsp. *stewartii* with 99% matching identity. The DNA sequence data did not show a strong match for *Enterobacter* in the samples from the Berry Springs orchard.

Bacteria from the genus *Pantoea* and *Enterobacter* are generally associated with plants, either as epiphytes, endophytes or pathogens (Rosenblueth and Martinez-Romero, 2006). These bacteria have a wide plant host range including maize, rice, sweet potato, wheat and grasses. They appear to enter plants through intercellular spaces and cracks in roots and have also been reported to colonise vascular tissues

(Ruppel et al., 1992; McCully, 2001). For example, *Pantoea stewartii* subsp. *stewartii* is a gram-negative xylem-dwelling bacterium that causes Stewart's bacterial wilt of sweet corn (Pepper, 1967). Our data from the current study of mango RCD is still very preliminary and further research with a greater number of fruit will be required to confirm the present findings.

In the pathogenicity tests, postharvest inoculation of mature green 'Kensington Pride' mangoes from Mareeba, Queensland with the opportunistic bacteria *P. agglomerans* and *E. cowanii* induced symptoms of RCD in 57% and 85% of ripe fruit, respectively (Figure 6). Co-inoculation with both bacterial organisms induced RCD symptoms in 85% of fruit at eating ripe. The severity of RCD was greatest for fruit inoculated with the mixed culture (data not shown). While no RCD developed in negative control fruit inoculated with sterile water, 57% of non-inoculated fruit also developed RCD. RCD in the non-inoculated fruit were often accompanied by infection with fungal pathogens such as *Colletotrichum* and damage from fruit fly larvae (data not shown).

While these findings are promising and provide some circumstantial evidence for bacterial involvement in RCD, these preliminary data are based on a very limited number of fruit. Because these bacteria are opportunistic and may function as secondary pathogens, further research is required to confirm their full identity, and the relationship between these bacteria and the development of RCD symptoms.

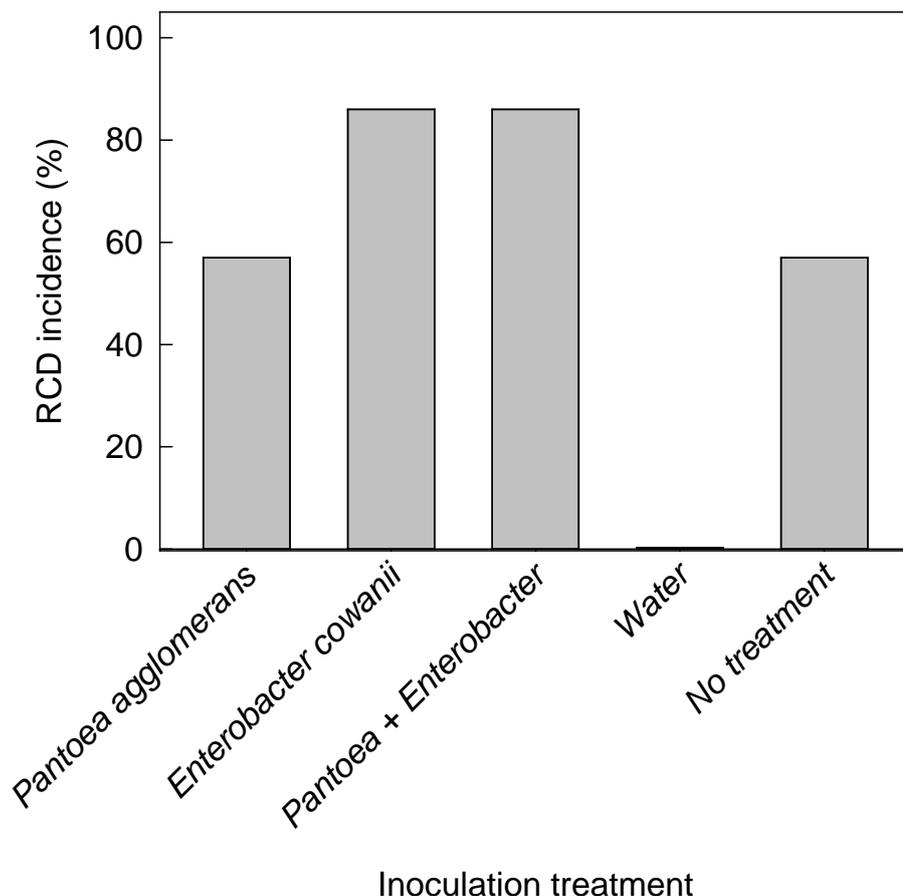


Figure 6. The incidence of RCD in ripe 'Kensington Pride' mango fruit following inoculation with 1×10^7 *Pantoea agglomerans* and *Enterobacter cowanii* bacteria

either alone or in combination. Green mature fruit were harvested from trees near Mareeba, Queensland and inoculated through the cut stem end. Fruit inoculated with sterile water acted as a control. Fruit were incubated at 20°C until reaching eating ripe. Data represent the mean of 7 replicate fruit.

5.3.3. Fruit mineral analysis

There were no consistent or significant differences in the mineral content of ripe 'Kensington Pride' fruit skin and flesh with RCD symptoms as compared to control fruit without the defect (Table 6). However, given that just five fruit were used in this study, we cannot rule out the possibility that fruit nutrition may play a role in RCD development. Further research involving a greater number of fruit is needed to resolve this issue.

Table 6. The mineral content of ripe 'Kensington Pride' mango skin and flesh with and without visible symptoms of RCD. Green mature fruit were harvested from an orchard near Lambells Lagoon in the Northern Territory. The fruit were commercially packed and transported from Berry Springs, Northern Territory to Nambour, Queensland via a wholesaler in Melbourne, Victoria. The fruit were maintained at 20°C until reaching eating ripe. Data represent the mean \pm standard error of five fruit from the same tree.

Nutrient	Skin ^{NS}		Flesh ^{NS}	
	No RCD	Plus RCD	No RCD	Plus RCD
Boron (mg/kg B)	12.3 \pm 2.5	11.5 \pm 1.0	8.5 \pm 2.4	6.2 \pm 1.3
Calcium (% Ca)	0.11 \pm 0.03	0.13 \pm 0.03	0.09 \pm 0.06	0.04 \pm 0.01
Chloride (% Cl)	0.11 \pm 0.01	0.11 \pm 0.01	0.12 \pm 0.02	0.11 \pm 0.01
Copper (mg/kg Cu)	6.6 \pm 1.0	9.6 \pm 3.5	10.9 \pm 3.9	7.4 \pm 0.8
Iron (mg/kg Fe)	37.2 \pm 6.7	40.1 \pm 0.7	23.4 \pm 2.4	21.2 \pm 2.6
Magnesium (% Mg)	0.11 \pm 0.02	0.12 \pm 0.01	0.09 \pm 0.03	0.05 \pm 0.01
Manganese (mg/kg Mn)	38.5 \pm 18.9	39.1 \pm 29.0	14.3 \pm 4.4	13.2 \pm 5.4
Nitrate (mg/kg NO ₃)	<40.0	<40.0	<40.0	<40.0
Phosphorus (% P)	0.08 \pm 0.00	0.08 \pm 0.00	0.10 \pm 0.02	0.09 \pm 0.01
Potassium (% K)	0.73 \pm 0.03	0.76 \pm 0.05	0.87 \pm 0.10	0.98 \pm 0.10
Sodium (% Na)	< 0.01	< 0.01	< 0.01	< 0.01
Sulphur (% S)	0.05 \pm 0.00	0.05 \pm 0.01	0.05 \pm 0.01	0.04 \pm 0.00
Nitrogen (% N)	0.46 \pm 0.09	0.47 \pm 0.09	0.48 \pm 0.07	0.32 \pm 0.06
Zinc (mg/kg Zn)	11.3 \pm 1.1	7.4 \pm 1.3	7.0 \pm 0.7	8.4 \pm 0.5

^{NS} indicates no significant difference between data.

6. Technology transfer

6.1. Pre- and post-season meetings

The following series of regular meetings were held to discuss project planning, logistics, key findings and commercial impacts of the R&D.

- A pre-season meeting was held in Tinbeerwah, Queensland on 14 June 2013 between Andrew Macnish (DAFF), Trevor Dunmall (AMIA) and Tim Elliott (W.E. Pack Pty Ltd). The meeting discussed project scope, timing and access to fruit. W.E. Pack committed to work closely with DAFF to plan trials, source fruit and help monitor fruit in market.
- A meeting that outlined our progress towards identifying factors contributing to RCD was held in Berry Springs, Northern Territory on 30 October 2013. In attendance was DAFF (Andrew Macnish, Peter Hofman), DPI&F (Cameron McConchie, Bob Williams), W.E Pack Pty Ltd (Tim Elliott), Northern Territory Farmers Association Inc. (Grant Fenton) and several mango growers from the Darwin area. The meeting served as an opportunity to receive feedback different segments of the mango industry.
- A post-season meeting was held in Nambour, Queensland on 17 January 2014 between Andrew Macnish and Peter Hofman (DAFF), Trevor Dunmall (AMIA) and Tim Elliott (W.E. Pack Pty Ltd). The meeting discussed project progress and outlined some of the preliminary findings.
- A project update was presented to the Northern Territory mango advisory panel in Darwin, Northern Territory on 21 May 2014. In attendance were: Andrew Macnish (DAFF), Bob Williams, Cameron McConchie, Warren Hunt, Lucy Tran-Nguyen (DPI&F), Trevor Dunmall (AMIA), Ross Maxwell (Jabiru Tropical Orchards), Tim Elliott (W.E. Pack Pty Ltd) and Trevor Lake (Olloo Farms).

6.2. Publications and conference presentations

6.2.1. Popular articles

Hofman, P., Macnish, A., Joyce, D., Campbell, T., McConchie, C., Moore, C. (2013). Progress on the investigation into resin canal disorder in mangoes. The Plant Industries NT Newsletter, Northern Territory Government. November 2013. pp. 3-5.

Macnish, A., McConchie, C., Hofman, P., Joyce, D., Campbell, T. (2014). Towards identifying factors that contribute to mango resin canal discolouration. *Mango Matters* 14(1), 9-12.

6.2.2. Conference presentations

Macnish, A., Hofman, P. (2013). Resin canal – What we know and where to from here? 9th Australian Mango Conference, Cairns, Qld, 14-17 May.

Macnish, A., Hofman, P., Campbell, T., McConchie, C., Hunt, W. (2014). Identifying factors that contribute to mango resin canal discolouration. 2014 Northern Territory Mango R&D Forum, Darwin, NT, 20-21 March.

6.3. RCD workshop

A workshop that outlined the R&D progress towards identifying factors contributing to mango RCD was held on 24 May 2014 at a pack house near Berry Springs, Northern Territory. The event was advertised and facilitated by the AMIA and attracted 39 growers and packers of mango fruit from the Darwin production area. The presenters were Tim Elliott (W.E. Pack Pty Ltd), Trevor Dunmall (AMIA), Andrew Macnish (DAFF) and Bob Williams (DPI&F). Topics covered were 1) The extent of RCD in the market, 2) Update on R&D activities completed during the 2013 season, 3) Proposed ideas and activities for future R&D on RCD. The presenters fielded numerous questions from interested attendees and took onboard several useful suggestions for future R&D activities. The workshop also served to stimulate commitments from growers for closer collaboration in future R&D into RCD.

7. Recommendations

This 1-year scoping project has started to highlight several potential factors that contribute to mango RCD. Additional follow-on R&D will now be necessary to pursue existing leads in more detail and to confirm current findings across multiple seasons. Strategies for implementing improved practices for reducing RCD could then be developed through close negotiation and discussion with mango growers, packers and supply chains.

The following are preliminary recommendations to aid future efforts to reduce the commercial impact of RCD. While the recommendations have a specific focus on 'Kensington Pride' mangoes produced in the Northern Territory, resolving RCD will have wider benefits and enhance the reputation of the whole mango industry.

- 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. Thus, future research into RCD should include sequential sampling of fruit during key stages of commercial handling to identify contributing factors.
- Preliminary results suggest a potential involvement of bacteria in RCD. This needs to be confirmed with more extensive and rigorous testing on fruit from several production areas. If confirmed, then effort should be channelled towards understanding the epidemiology (the causal organisms, how it spreads and how it can be controlled) to provide commercial control measures to minimise RCD.
- RCD increases in severity over time as fruit develop from firm ripe to overripe. Accordingly, surveys at the wholesale level may under-estimate the extent of RCD reaching consumers, because fruit at retail will be riper and likely to display more severe symptoms. Where an appreciation of the full extent of RCD is required, we recommend fruit are held until ripe and then assessed.
- RCD can be present in the flesh of fruit but without obvious symptoms being exhibited through the skin. Thus, the incidence of the defect is potentially higher than can be estimated by supply chain surveys based on external appearance only. To determine the full extent of RCD, we suggest the fruit skin should be removed when ripe prior to assessing internal symptoms.
- RCD can be misdiagnosed as other mango skin browning disorders, when evaluated based on external features only. To ensure accurate diagnosis and quantification of RCD in the market and R&D experiments, a standard assessment procedure should be developed and widely shared with industry and research personnel.

Taken overall, our observations to-date suggest that RCD is likely to occur when field conditions result in the production of 'sensitive' fruit that, in turn, express the defect when exposed to specific harvest and postharvest stresses. Close attention should be paid to following best practices, including maintaining orchard hygiene, harvesting fruit at optimal maturity and minimising harvest and postharvest stress.

8. References

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9. Appendix I

Farmer's survey questionnaire: resin canal discolouration (RCD)

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

1. Business name: _____
2. What age demographic does your business management fall in?
18 – 35; 36 – 55; 56 – 75yrs.
3. Which varieties do you grow? _____
4. How many trees in your orchard?
<500; 500–1500; 1500–3000; 3000–5000; 5000–10000; 10000–15000; >15000.
5. Was RCD observed in your fruit in either:
(a) 2012 (b) 2013 (c) both seasons
6. Have your fruit had RCD in previous seasons?
(a) Yes (b) No
6. Since which year has RCD been an economic issue for your business? _____
7. Which varieties have been affected? _____
8. Who notified you that your fruit had RCD? _____
9. What period of time after harvest was the notification given? _____
10. By your estimation, what percentage of your fruit was showed signs of RCD? _____
11. Does RCD occurrence vary much across the season? Yes ____ No ____
How much? _____
12. In your experience, does RCD incidence vary within particular blocks in the orchard?
(a) Yes (b) No (c) Don't know.
13. 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 _____
14. Do you use any of the following induction treatments?
(a) Cultar®
(b) Potassium nitrate
(c) Ethrel®
(d) Cincturing
(e) Other _____

15. If so at what rates? _____
16. Last season, when did your trees flower? _____
17. What was your harvest period in 2013? _____
18. Do you use any of the following pesticides in field?
(a) Amistar®
(b) Octave®
(c) Mancozeb®
(d) Fenthion®
(e) Dimethoate
(f) Other _____
19. What is your typical irrigation schedule leading up to harvest? _____
20. Do you normally pick fruit in the:
(a) AM
(b) PM
(c) Both AM and PM
(d) Night hours
21. How do you de-sap fruit?
(a) Harvest aid (b) In shed
22. What type of mango wash do you typically use and at what concentration?

23. How long are fruit kept in mango wash _____
24. How often do you change the solution? _____
25. What water source do you use for the mango wash?
(a) Bore
(b) Dam
(c) Tank
(d) Treated (chlorination)
26. 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
27. Do you pack your own fruit or use a commercial shed? _____
28. Would you be willing and interested to collaborate in an industry project to research the incidence and causes of RCD?
(a) Yes (b) No
29. Any other comments or insights you would like to share around RCD?

Packing shed's survey questionnaire: resin canal discolouration (RCD)

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

1. Business name: _____
2. What age demographic does your business management fall in?
18 – 35yrs; 36 – 55yrs; 56 – 75yrs.
3. Was any fruit you packed reported as expressing RCD last season?
(a) Yes (b) No
4. When was RCD detected?
(a) Grading
(b) Wholesaler/market
(c) At retail.
(d) All of the above
5. If RCD was detected in your consignments after dispatch, who notified you that the fruit had RCD? _____
6. How soon after dispatch was RCD detected? _____
7. By your estimation, what percentage of your total pack-out was affected by RCD? ____
8. Was RCD observed in your fruit in either:
(a) 2012 (b) 2013 (c) both seasons
9. Have your fruit had RCD in previous seasons?
(a) Yes (b) No
10. Since which year has RCD been an economic issue for your business? _____
11. Did RCD incidence fluctuate across the last season?
(a) No
(b) Yes - how much? _____
12. In your experience, does RCD vary between bins/batches?
(a) No (b) Not sure (c) Yes - any ideas why?

13. 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 _____
14. 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
15. At what temperature are bins held in the shed prior to packing? _____

16. 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
 - (f) Hot water sprays
17. How long are fruit in the water dump? _____
18. What additives do you add to the water? _____
19. How often is the dump water changed? _____
20. How do you clean the dump? _____
21. Do you treat fruit with:
- (a) Scholar
 - (b) Sportak
22. What temperature do you cool fruit to? _____ °C
23. At what temperature is your cool room set? _____ °C
24. How long do you normally store fruit prior to dispatch? _____
25. What is the typical transport set temperature for your fruit? ____ °C
26. What is the typical duration of transport? _____
27. Are your fruit usually ripened with ethylene?:
- (a) Yes
 - (b) No
28. Would you be willing and interested to collaborate in an industry project to research the incidence and causes of RCD?
- (a) Yes
 - (b) No
29. Any other comments or insights you would like to share around RCD?
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