Management of internal dryness of Imperial mandarin

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FINAL REPORT

CT04002

Management of internal dryness of Imperial Mandarin

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CT04002 Management of internal dryness of Imperial Mandarin
Final report 31 December 2010

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Media summary

Granulated or ‘dry’ fruit is a major quality issue for Imperial mandarins. Fruit that are granulated taste dry, look white or colourless compared to normal mandarins, and are relatively tasteless. As Imperials are the premier mandarin in the Australian domestic market, with an estimated annual wholesale value of $80 million, this poses a significant problem to the industry. Fruit from all growing regions in Australia can be affected.

Research conducted by AgriScience Queensland in collaboration with growers in the central Burnett region over the past three years has provided insights that will be of use to growers and benefit consumers. It is now clear that granulation is triggered early in fruit development, so growers need to be watching seasonal weather conditions and pay particular attention to nutrition and irrigation at this time. Granulation appears to be highest in years with warm winters, above average rainfall in spring when young fruit are developing or above average rainfall in late summer. This suggests that granulation is linked to the competition for resources between flowers, developing fruit and expanding flush, and possibly also to moisture availability in spring and late summer.

In research trials under commercial conditions, the application of extra nitrogen fertilizers during the early stage of fruit development reduced the proportion of granulated fruit in the range of 5% to 35%. Granulation is highest on trees with low crop loads, so management to reduce biennial bearing is important, as is choice of rootstock and maintenance of a balanced nutrition program.

On-farm trials that reduced irrigation frequency were mostly unsuccessful but a trial under controlled nursery conditions suggests this may be an important area for further research, because of the link between sugar accumulation in fruit, mild water deficits and reduced granulation.

Technical summary

Granulation of Imperial mandarins (*Citrus reticulata* cv. Imperial) is a significant problem for the Australian domestic mandarin market. Incidence varies with season and because affected fruit cannot reliably be detected by appearance or density, a significant proportion of granulated fruit reaches the market in some years. Trials into management practices in the Central Burnett region of Queensland, Australia, suggest that the problem is triggered in early fruit development, affects fruit on the inside of the canopy more than the outside and is associated with low crop loads. As the problem can affect both larger and smaller fruit, incidence appears to be linked to the time of fruit inception rather than to rates of growth. This implies that the seasonal conditions prevailing at the time of flowering and flush are critical in setting the path to granulation. On the basis of four years of survey data, the seasonal conditions that may pose a risk are warmer winters that advance flowering, and heat periods or rainfall during flush events that exacerbate competition between flowers, flush and young fruit.

Trials into management practices to reduce granulation were established following the hypothesis that granulation is linked to higher water potential in juice cells, that is, cells with lower total soluble solids and higher turgor pressure. Nutrition strategies, plant growth regulator applications and thinning strategies were trialled to increase levels of soluble solids, and reduced irrigation frequency strategies were trialled to reduce cell turgor pressure.

Application of additional nitrogen during Stage I of fruit development was the most successful of the management practices tested. Broadcast applications had more effect than foliar applications. Reduced irrigation frequency after fruit set did not reduce granulation in field trials, regardless of the stage of fruit development during treatments. However, reduced frequency with reduced volume was effective under controlled conditions in potted trees, both when imposed over the whole growth season and when imposed only in Stage I of fruit development. Manual thinning in Stage II of fruit development and application of auxins and giberellic acid (GA^3) at the time of transition from Stage I to Stage II of fruit development did not reduce granulation.

Testing of non-invasive technologies for use in the packing line to detect granulated fruit was largely unsuccessful. Technologies tested included interactance and transmittance optical geometries, nuclear magnetic resonance, computed tomography – X ray; on-line transmission X ray, firmness measurements by accelerometer, acoustic frequency and acoustic velocity. These results were attributed to the lack of chemical differences, density difference and the interference of the skin and flavedo.

1 Introduction

1.1 The problem

Market research in Australia indicates that granulation or 'dryness' of mandarins is the main quality concern of consumers (McKinna 2003). Fruit that are 'granulated' taste dry, look white or colourless compared to normal mandarins, and are relatively tasteless. Imperial mandarins are particularly prone to granulation. As Imperials are the premier mandarin in the Australian domestic market, with an estimated annual wholesale value of $80 million, this poses a significant problem to the industry. Fruit from all growing regions in Australia can be affected.

It is not possible to reliably identify affected fruit from its external appearance so agronomic solutions are needed to help growers reduce the incidence of granulation. A non-invasive method of detecting affected fruit, that is, one that does not require the fruit to be cut, would also be of great benefit to the industry.

1.2 What is granulation?

The juice sacs of granulated citrus are hardened, gelled or granular and an opaque white in colour. Extractable juice levels are low. Soluble solids (sugars and acids) are at lower concentrations than normal fruit and granulated fruit as a consequence are 'tasteless'.

The opaque white tissue in granulated vesicles appears to be due to thicker epidermises (Goto and Araki 1983) and/or to thickened juice cell walls (Bartholomew *et al.* 1941; Burns 1990; Burns and Achor 1989; Goto and Araki 1983; Mahmoud 1954; Shomer *et al.* 1988; Zhang *et al.* 1999). Work done for this project by Central Queensland University on characterising granulation found that in granulated vesicles there was a proliferation of small, outer juice cells (Appendix 2). A decrease in carotin or carotenoids also contributes to the whitened effect (Bartholomew *et al.* 1941; El-Zeftawi 1978; Goto and Araki 1983).

Granulated fruit have a lower extractable juice percentage (Awasthi and Nauriyal 1972a; Chakrawar and Singh 1977; Daulta and Arora 1990; Kaur *et al.* 1991; Kotsias 2004; Sharma and Saxena 2004; Singh and Singh 1980a; Zong *et al.* 1979). However, several studies indicate that the total moisture content is in actuality higher than in non-granulated fruit (Awasthi and Nauriyal 1972b; Chakrawar and Singh 1977; Singh and Singh 1980a; Zong *et al.* 1979) although Ding et al. (2009) found reduced moisture content in late stages of fruit development. It is likely that the extractable juice levels are lower because moisture is either bound to cell walls (Bartholomew *et al.* 1941), or bound in gels created by pectins. The gelation of cell wall contents may be a side effect of the process of cell wall thickening in that some of the pectins manufactured may remain 'free' pectins, that is, they are not incorporated into the cell wall, thus leading to the gelatinization of the juice (Goto 1989).

Published studies indicate that the main changes to the components of juice in granulated fruits are decreased soluble solids, including decreased sugars and decreased acids (Awasthi and Nauriyal 1972b; Ding *et al.* 2009; El-Zeftawi 1978; Gilfillan and Stevenson 1977; Kotsias 2004; Sandhu and Singh 1989; Sharma and Saxena 2004; Sharma *et al.* 2006; Shomer *et al.* 1988; Sinclair and Jolliffe 1961; Singh and Singh 1980a; Sinha *et al.* 1962).

1.3 What causes granulation?

1.3.1 Existing hypotheses

Since the 1930s, there has been extensive research into granulation in the main citrus growing regions of the world which has found a wide range of factors associated with the disorder but has not elicited a clear explanation of the cause. These factors include: variety, rootstock, fruit maturity, crop load, soil type, tree age, tree vigour, tree health, fruit size and position and climate and a range of farming practices including irrigation frequency, use of oil sprays, use of various PGRs and nutrient applications.

The main theories that have emerged are that granulation is due to:

- sink competition between plant organs, (Agusti et al. 2001; Chakrawar and Singh 1978; 2004; Kaur et al. 1990; Kaur et al. 1991);
- a normal maturation/senescence process, (Chen *et al.* 2005; Singh and Singh 1979);
- temperature and/or moisture stress (Burns and Achor 1989);
- inefficient transport of carbohydrates, water or minerals into the fruit (Chakrawar and Singh 1978; Chakrawar et al. 1980); or
- too rapid growth (Cassin et al. 1969).

Of these, the 'sink competition' theory appears to have most support in the research to date. 'Sink competition' refers to the partitioning of available carbohydrates between plant organs including fruits, roots and leaves. Organs are considered to have a greater 'sink strength' if they receive a greater allocation of carbohydrates or appear to have 'priority' over other organs when carbohydrates are limited.

There is considerable evidence to support this hypothesis, for example, the lower levels of soluble solids in granulated fruit, and the prevalence of granulation on young trees with vigorous vegetative growth. Nevertheless, it leaves much unexplained, for example, why sink competition might lead to cell wall thickening and the gelation of vesicles, why some vesicles in a fruit will granulate and others not, and why climate and seasonal conditions have a large influence on incidence of granulation.

1.3.2 A new hypothesis

A review and reinterpretation of the literature has led to the development of a new theory on the cause of granulation, which incorporates all the factors covered in the literature and suggests a fundamental or underlying cause for granulation. This is that granulation is caused by higher water potential in juice cells.

Juice cells in citrus are not directly connected to the vascular system. Water and sucrose have to travel long distances either between cells or through cells. Several studies suggest that juice cells rely on osmoregulation to attract and retain water in competition with other

plant tissues, that is, by increased levels of solutes in juice, particularly sugars (Barry *et al.* 2004; Yakushiji *et al.* 1996). As sugars accumulate in juice cells, acids must be broken down to provide an energy source to drive the accumulation process against an increasing sugar gradient, which is why acids decline and sugars increase during ripening.

The new hypothesis suggests that not only sugar concentrations are important but that all the components of water potential are significant in triggering granulation. Water potential describes the 'free energy' of water. Water will move—where barriers are permeable—from cells of high water potential to cells of low water potential. The main components of water potential are gravity, pressure and solute concentration. Cells that are under high pressure, that is, that are turgid, and/or low in solutes (acids, sugars) have high water potential and are at risk of losing water to cells with low water potential, that is, cells that have high solute concentrations and/or low turgor. (Gravity is unlikely to be significantly different between adjacent cells.)

It is possible that the cell wall thickening in granulation is a protection against moisture loss by cells that are high in water potential. The thickening may also be a simple process of growth triggered by turgor pressure. The gelation of granulated cells may be a by-product of the development of structural carbohydrates such as pectins used in cell wall thickening. Burns (1990) and Wang (2005) provide evidence that the thickening of cell walls occurs at the expense of soluble sugars and acids. There seems to be the possibility of an exacerbating loop, where the process of cell wall building not only uses up acids and sugars, thus increasing water potential further, but also provides a greater barrier to influx of sucrose into the cell, increasing water potential even further (Figure 1). The hypothesis and its genesis are outlined in detail in Hofman and Smith (2008).

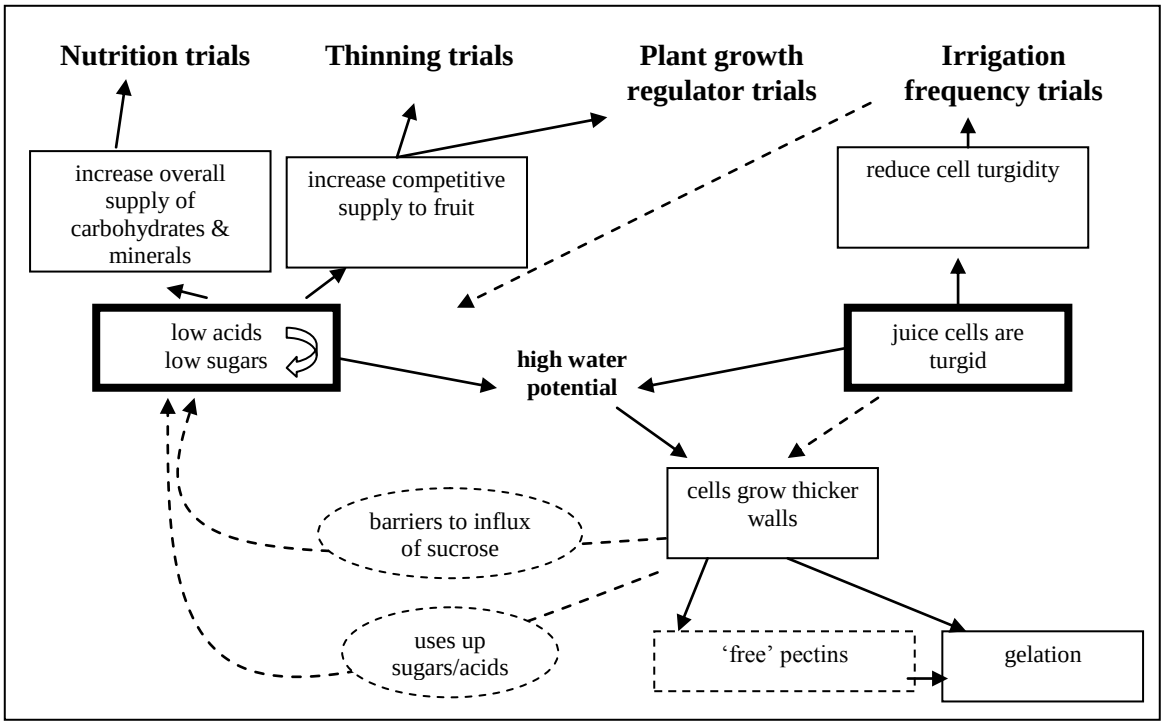

Figure 1 The water potential hypothesis and its relationship to the research program

This hypothesis suggests that ameliorating granulation requires a dual focus: a focus on factors that affect the supply of sucrose to the developing fruit and the production of acids

within the cells, and a focus on factors that affect availability of moisture to the developing fruit (Figure 1).

1.4 Research program

The research program for this project focused on on-farm trials over three years to investigate options for growers to reduce the incidence of granulation. Trials in the initial year (2007-08) covered four main areas (thinning, plant growth regulators, nutrition and irrigation frequency strategies) that had been reported in previous research to have some success, where these were compatible with the research hypothesis. Thinning, nutrition and growth regulator strategies might improve soluble solid levels by increasing the supply of nutrients and photoassimilates from the leaves into fruit, particularly in competition with other plant organs. On the other side of the equation, reduced irrigation frequency should reduce cell turgor and cause increased soluble solids as the cells osmoregulate to attract and retain moisture.

The trial program evolved over three years in response to the success or failure of the various management strategies and to availability of trial sites. Thinning and plant growth regulator strategies had no success in the first year of trials, so these were abandoned: the research program in 2008/09 and 09/10 focussed instead on those nutrition and irrigation frequency strategies which showed most promise. In 2009-10, trials to investigate in more detail aspects of nitrogen application strategies were also included. The trial program is outlined in Table 1 .

In order to better understand the factors affecting granulation, around 40 blocks of Imperials in the Central Burnett and Bundaberg region were sampled each harvest over four years (2007 to 2010). In addition, at selected trial sites, individual fruit were tagged to assess the effect on granulation of growth patterns and position in the canopy.

The project also included investigation by Central Queensland University of technologies suitable for use in packing sheds for the non-invasive assessment of granulated fruit over three years from 2005/06 to 2007/08. The University's final report is included as Appendix Two to this report.

Table 1 Trial program 2007/08 to 2009/10

Year	Thinning trials	Plant growth regulator trials	Irrigation frequency (+ nutrition) trials	Nutrition trials
2007/08	**Trial E** **Trial I**	**Trial F** **Trial G** **Trial H**	**Trial C**: reduced frequency in Stages I, II and III (including foliar nutrients)	**Trial A**: identifying key nutrients **Trial B**: identifying key nutrients
2008/09			**Trial D**: reduced frequency (including +N and +N+Zn in Stage I) **Trial J:** reduced frequency (including +N in Stage I)	**Trial A**: continued with revised treatments **Trial K:** + N, K and Zn in Stage I
2009/10			**Trial D** continued **Trial J** continued **Trial N**: reduced frequency and volume nursery trial	**Trial A** continued **Trial P**: timing of winter N **Trial M**: comparing foliar nutrients and broadcast N in Stage I

2 Materials and methods

A range of materials and methods were common to all or several trials and these are first outlined below. This is followed by details specific to each trial. Survey methodology is then explained.

2.1 Location of trial sites

All trials, with the except of Trial N which was undertaken at Bundaberg Research Station, were conducted on Imperial trees on commercial orchards in the Central Burnett region, around Mundubbera and Gayndah. In order to protect confidentiality, these sites and the sites used in the annual survey are not identified but details of physical attributes and soil type are noted where relevant.

Selection of trial sites with a reasonable probability of granulation was problematic due to the seasonal variation in granulation. Blocks with a known history of granulation tended to have older, declining trees. The level of granulation on declining trees can be highly variable, i.e. trees that look similarly compromised can have very little granulation or heavy incidence (see Section 3.1.5). This makes it difficult to allow for tree health in statistical analysis, as correlation coefficients are very low. Leaving such trees out of trial designs made it difficult to use fully randomised complete block designs. Therefore, differing methods were used to account for tree health at different trial sites, including:

- selecting plots that had the healthiest trees at the sacrifice of a complete block design (Trial J, K);
- using tree health as a covariate (Trials B,D, E, F, H, K and P); or
- removing sick trees from the plot average (Trial A and M).

The process used is mentioned for each trial in the outlines below.

At all trials, normal management practices, where these were not the subject of treatments, were conducted by the grower. This included application of macro- and micro-nutrients, pest management, irrigation, thinning and pruning practices. In most trials, growers harvested fruit after the initial sampling and before the second sample one month later.

2.2 General methodology

2.2.1 Fruit characteristics

Estimating granulation of mature fruit

The effects of treatments at trials were assessed twice during each harvest season, the first just before the first commercial picking by the grower, and the second one month later.

Samples of 12 fruit were picked randomly at approximately shoulder height from the north east corner of each tree (the latter to reduce variability within the sample). At Trials F, G, H, I and E, 20 fruit were picked at random. At Trials I and E (thinning trials) fruit were picked from around the tree. At all trials, samples included fruit from both outside and inside the canopy, to a maximum of arm's length within the canopy. Sunburnt fruit and fruit less than 50mm in diameter were not sampled.

Fruit were cut in half in the field along the equator and individually rated on an 11 point scale from 0 (no visible granulation) in 0.5 steps up to 5 (completely opaque and white). These visual ratings were subsequently converted to an approximate percentage granulation. This calibration was based on a sample of 480 fruit from a range of orchards collected in 2010. The estimated % granulation is based on:

- the whiteness of the flesh (its L* value as measure by a chromometer) and
- the percentage juice by estimated volume of the pulp.

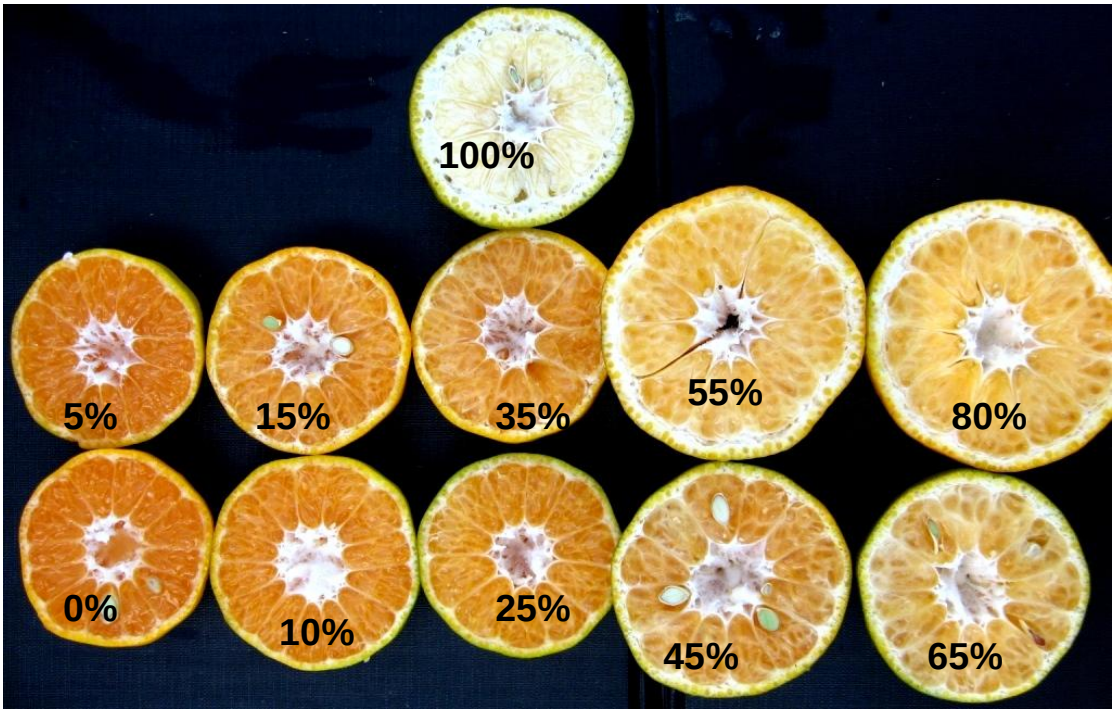

Figure 2 Estimated granulation ratings of fruit

A figure of 100% granulation is fruit that is very white with minimal extractable juice. A figure of 0% is fruit with no visible evidence of granulation, that is, vesicles have thin translucent walls, colour is deep orange and extractable juice percentage is high (Figure 2). Details of this estimation are provided in Appendix One.

Percentage granulation figures for individual fruit were then combined to calculate two parameters:

- Mean granulation percentage. This incorporates both the severity and the incidence of the disorder in the sample.
- Granulation incidence. This was defined as the proportion of fruit in the sample rated at 25% granulation or above. At 25% fruit are noticeably paler, vesicle walls are strongly evident and fruit is quite 'chewy' or 'crunchy'. Percentage juice by volume

if peel and vacant core are excluded is 57% (s=11%). Percentage juice by weight at this level is 36% (s=6%). The current maturity standard for juice content of Imperials promulgated by Citrus Australia is 33%.

Granulation of immature fruit

Fruit thinned by the growers in January of each year were assessed for granulation and compared to final granulation means to see if granulation could reliably be detected before harvest, and whether treatment effects could be detected at this stage of fruit development. If so, and if late intervention strategies could be found, assessing thinned fruit would provide useful information to growers two months ahead of harvest.

Thirty fruit were sampled from the ground around the middle tree of three tree plots, or between the two central trees of four tree plots. These were assessed visually for granulation using the same 11 point scale used for mature fruit.

Fruit size

Fruit size of harvested samples was measured across the diameter of the cut sample with a ruler to the nearest millimetre. Fruit size of tagged fruit before harvest was measured with electronic callipers across the equatorial diameter to the nearest tenth of a millimetre.

Juice samples

Juice samples were taken from a majority of survey blocks in 2007 and 2010 using the same 12 fruit sample used to measure granulation. At relevant trial sites in 2009 and 2010, juice samples were taken from one tree in each replicate plot for key nitrogen and potassium treatments.

Juice was extracted by simple squeezing by hand or by use of a domestic juicer. Five millilitres of juice were extracted from each fruit and combined into one sample per tree.

Juice samples were assessed for Brix level, acidity and Brix:acid ratio following standard procedures. Brix^{o} measurements are corrected for temperature and acidity (Stevens and Baier 1939).

External colour

In order to assess the effects of nitrogen and potassium treatments on fruit colour in relevant trials, selected treatments at Trials A, J, K, M and P were assessed both before and after placement in a ripening room in mesh bags with 10 ppm ethylene at 24^{o} C for 2.5 days. Five additional fruit per replicate were picked at the first sample date from the same tree used for juice samples. Colour was measured using a chromometer (Minolta Chroma Meter CR 200). Output was converted to a Citrus Colour Index using the formula 1000.a/L.b (Jiminez-Cuesta 1981). The 'L' value is a measure of the luminosity or brightness of the light reflected from the fruit, the 'a' value measures the difference between light in the red and green zones. Negative values of 'a' indicate green colours, while positive values indicate red colours. The 'b' value measures the difference in the yellow and blue zones. Negative 'b' values indicate blue colours while positive values indicate yellow colours.

2.2.2 Tree characteristics

Tree health

Tree health was visually rated on a nine point scale of 1, 1.5, 2, 2.5 up to 5 where 1 represented a very healthy tree (no twig dieback, dark green and dense canopy), 2 = healthy, dark green canopy, a few dead twigs, 3 = healthy green leaves but thin canopy cover, 4= not healthy, often yellow leaves, invariably very thin canopy cover, and 5= very unhealthy, yellow leaves, close to collapse.

Canopy cover

Trees at survey sites were rated for density of canopy on a scale of 1 to 5 where 1 represents a very open canopy and 5 a very dense canopy.

Crop load

Crop loads were measured as the number of fruit in a cubic quadrat 50x50x50cm, that is, $0.125m^3$, in the north east quadrant of the canopy at chest height. Crop loads were visually estimated in categories of 0-5, 6-10, 11-15, 16-20, 21-25, 26-30 per quadrat before the first harvest at trials, or at time of sampling at survey sites. For a smaller number of trials, crop loads were also measured in December or early January, prior to thinning.

Flowering intensity and advancement and vegetative flush

The relative load of flowers and of flush on trees were rated at selected trials as 1=low, 2=medium and 3=heavy. Similarly, the relative advancement of flowering was rated as 1=advanced, few flowers, lots of fruit; 2 = more fruit than flowers, 3 = medium, mixture of buds, flowers and fruit; and 4 = slow, lots of buds and flowers, few fruit.

Size of rootstock and scion

Trunk circumferences were measured with a tape measure at 10 cm below and above the graft union.

2.2.3 Other characteristics

Sprinkler output

Comparative sprinkler output was measured using five cups placed at 30 cm intervals along four 1.5m axes at 90° angles around the sprinkler (total 20 cups) and left for 30 mins. Sprinklers were generally located 30-100cm from the trunk of the tree.

Leaf analyses

Equal numbers of spring flush leaves were picked from around the tree using the method outlined in Vock *et al.* (1997) and submitted for nutrient analyses to Incitec Pivot Nutrient Advantage Laboratory Services at Werribee, Victoria. Sample details are outlined in Table 2
.

Table 2 Dates, sites and purpose of leaf sampling

Date of sampling	Sample sites	Purposes
27 November 2007	Trials A and B: all treatments	Check efficacy of nutrient applications
	Trial C: control and boron treatments	Check boron levels to ensure not approaching toxicity
19, 22 February 2008	Trials A and B: all treatments	Check efficacy of nutrient applications
	Trial C: all nutrient treatments with sample each from 'control' and 'low frequency' irrigation treatments	Test link between nutrient levels and granulation at subsequent harvest (2008)
	Trials F, G, H and I : control treatments	
11 December 2008	Trials A and K: all treatments	Check efficacy of nutrient applications
	Trials J and D: all nutrient treatments, with sample each from 'control' and 'low frequency' irrigation treatments	Test link between nutrient levels and granulation at subsequent harvest (2009)
3 July 2009	Trials A, D, J and K: single sample from whole trial	Test link between nutrient levels and granulation at previous harvest (2009)
	8 survey sites with histories of high or low granulation (same trees as surveyed)	
20 February 2010	Trials A , P and M: all treatments	Check efficacy of nutrient applications
	Trials J and D: all nutrient treatments with sample each from 'control' and 'low frequency' irrigation treatments	Test link between nutrient levels and granulation at subsequent harvest (2010)
	8 survey sites with histories of high or low granulation (same trees as surveyed)	

Soil nitrogen

Soil samples were collected by extracting three cores per plot to a depth of 60cm. Cores were drilled manually with a 7cm auger at the dripline of the tree, but at different positions on different sampling dates to avoid contamination from previous drilling. Cores were mixed to make one sample per plot.

Sixty millilitres of 0.01M CaCl were added to 30 g soil and the solution tumbled for one hour. There were two subsamples from each soil sample. After settling, nitrate levels of the solution were assessed using colorimetric analysis using a Palintest® photometer (Kingsway, Team Valley, Gateshead, Tyne and Wear, England).

2.3 Tagging of individual fruit

2.3.1 Tagging to assess effects of fruit growth rates

Fruit were tagged at Trials A, J and K in 2008/09 and Trials A, J, P and M in 2009/10 to ascertain if there was a relationship between granulation severity and rates of growth overall or at particular times in fruit development and/or position in the tree and granulation severity. Fruit were tagged when 5-10mm in diameter. If tagged fruit were lost in the natural fruit drop process, replacement fruit were tagged. Fruit were tagged in the north west quadrant of the canopy so as not to affect harvest sampling in the north east quadrant. Diameters were measured at approximately fortnightly intervals using electronic callipers. Horizontal distance to the trunk and total width of the canopy were measured with a rigid tape measure. Fruit were harvested, cut and assessed for granulation at the first harvest date at each trial site.

In 2009/10 fruit were tagged in pairs of a large and a small fruit in the same position in the canopy (where possible on the same twig) in order to compare the relative effects of size/growth and position in the canopy. The same number of paired fruit were tagged on the outside of the canopy and on the inside.

Photosynthetically active radiation (PAR) around each tagged fruit was measured in 09/10 using a Li-cor® LI-250A light meter with a single LI-190SA quantum sensor, holding the sensor above the leaves closest to the tagged fruit. The sensor records were an average over 15 seconds. Readings were taken during a clear day at mid morning, noon (overhead sun) and mid afternoon and expressed as a percentage of readings in the interrow in direct sunlight. Trial J was measured on two days, giving two average daily readings per tagged fruit; other trials were measured only on one date.

2.3.2 Tagging for assessment of sample date differences

In order to understand changes in granulation between sample dates, pairs of fruit were tagged at Trials A, J, K, M and P in 2008/09 and 2009/10. Pairs of fruit in the same position (within 10cm of each other) and of equal diameter (± 5 mm) were tagged on four or five trees at each trial. One of the pair was harvested and rated on the first sample date and the second on the second sample date, one month later.

2.4 Survey methodology

The main objectives of the survey were to improve understanding of the key factors associated with granulation and to identify seasonal trends.

Thirty-five blocks of commercially grown Imperials around Gayndah, Mundubbera, Paradise Dam and Bundaberg were surveyed in 2007 and 40 in 2008, 2009 and 2010.

In each block, 12 ripe fruit from each of nine trees were sampled using the same methodology as used in trials (see Section 2.2 above). Each block was sampled once during the season. Trees were selected as the 5^{th}, 10^{th} and 15^{th} tree (or closest healthy tree) in three alternate rows from an accessible point on the block. The same trees were sampled each year, except where trees had been removed. In these cases an alternative adjacent tree was sampled.

Methods used to estimate granulation, crop load (after thinning), tree health and extent of flush at time of harvest and to measure fruit diameter and circumference of rootstock and scion were the same as that used in trials (see Section 2.2 above). Soil type, tree age and rootstock were also recorded.

2.5 Data on seasonal conditions

Trends in key seasonal parameters as published by the Bureau of Meteorology for Gayndah Post Office/Airport (Station 039039) were examined against mean granulation data from the survey from 06/07 to 09/10. These included:

- average daily minimum temperatures,
- average daily maximum temperatures ,
- number of 'warm' days (>5ºC in winter, >30ºC in stages I and III, >35ºC in Stage II),
- total rainfall,

- number of days with rainfall,
- mean relative daily humidity at maximum temperature (%), and
- mean relative daily humidity at minimum temperature (%).

Data were totalled or averaged for each month as well as for physiological stages of fruit development defined by Bain (1958)and estimated to be as follows:

- Winter—May to August inclusive,
- Stage I of fruit development (cell division and multiplication) —September to November inclusive,
- Stage II of fruit development (cell expansion) —December to February inclusive, and
- Stage III of fruit development (ripening) —March and April inclusive.

Rough estimates of granulation for 1997-98, 2000-01 and 2003/04 were also included in this analysis, using a small survey done in 2000-01 and from trial work in 2003/04 (Fullelove *et al.* 2004). The year 1997/98 was anecdotally a very 'dry' year but there is no estimate available for the incidence and severity of granulation in that year. The mean granulation in 09/10 was thus substituted for illustrative purposes in Figure 23.

2.6 Data analyses

Data were analysed in Genstat 11 (Lawes Agricultural Trust, Rothamsted, UK).

Correlation coefficients (r) were calculated using Pearson product-moment correlation and the significance of correlation coefficients were tested using two sided tests of correlations different from zero. The *p* values quoted are the probability of achieving a correlation coefficient the same as calculated, if the null hypothesis (that there is zero correlation between the two variables) were true. In this report, strong correlations are rare, so correlation coefficients ≥ 0.3000 or ≤ -0.3000 and with a *p* value < 0.0500 are considered to be of substance.

Treatment means were tested for significant differences using analysis of variance or REML (restricted maximum likelihood) procedures, as noted for each trial in the outlines below. Means are considered significantly different at the 95% level of confidence, and estimates of the least significant differences (lsd) between means were calculated at the same level. Covariates of crop load and/or tree health were included in some trial analyses to improve the comparison of treatment means.

2.7 Thinning trials (I and E)

2.7.1 Overview

Granulation is associated with low crop load (El-Zeftawi 1973; 1978; Ritenour *et al.* 2004) and large fruit (Awasthi and Nauriyal 1972c; Bartholomew *et al.* 1941; Burns and Albrigo 1997; 1998; Hearn 1987; Lloyd 1961; Sandhu and Singh 1989; Sharma and Saxena 2004; Sharma *et al.* 2006; Sinclair and Jolliffe 1961; Van Noort 1969). The reason for this is thought to be preferential carbohydrate partitioning into vegetative (leaf and root) growth in 'off years'. Carbohydrate storage after a heavy crop or 'on year' is also much depleted (Goldschmidt and Golomb 1982; Monselise *et al.* 1981). In 'off years', it is possible that granulation may be due to the resulting low sucrose availability to the fruit.

Another possible factor may be involved—water status in the tree. Crop load affects sap flow in branches: a heavy crop load has a lower sap flow, and a heavy crop load also increases water stress (Yonemoto *et al.* 2004). Water supply to the fruit in an 'on year' may thus be relatively more restricted.

The effect of artificial reduction of crop load through thinning has not been researched to any extent to date. Chanana and Nijjar (1985) found no significant differences under thinning regimes of Hamlin orange: this is the only published study on the effects of thinning on granulation. In terms of other characteristics that may be linked to granulation it is noteworthy that Bevington *et al.* (1998) reported that thinning had no effect on juice content, Brix or acidity of Imperial mandarins and that it increased the average fruit size on the tree by removing the smaller fruit, not by increasing growth rates of remaining fruit. Jones *et al.* (1974) also found no increase in fruit size of Valencia oranges after thinning in the first year.

However, if granulation is due to uneven partitioning of carbohydrates to fruit, then reducing the number of fruit might reduce granulation by increasing their availability to remaining fruit. This is the hypothesis tested in the thinning trials in this project.

Supporting this hypothesis is the fact that position of fruit on the tree effects granulation: fruit inside the canopy tend to granulate more (Awasthi and Nauriyal 1972c; Bartholomew *et al.* 1941; Lloyd 1961). This may be due to lower light availability to leaves, reducing the supply of photosynthates to nearby fruit, or to the lower density of leaves inside the canopy. Outside canopy fruit have been found to have higher °Brix and lower acid than inside positions (Syvertsen and Albrigo 1980). It is not clear to what extent carbohydrates move around the tree, although Kriedemann (1969) suggests that fruit are fed mainly from nearby leaves.

Therefore, treatments in the two trials also included the stripping of half the tree or of some limbs selectively in order to identify whether this reduces granulation by reallocation of carbohydrates produced by leaves on stripped limbs to remaining fruit on other parts of the tree.

2.7.2 Trial E Thinning

Objective

Assess the effects on granulation of manual thinning treatments.

Plant materials

Imperials on Troyer rootstock, planted in 1989, on sandy loam soil, not mounded. Row spacing was 7m and tree spacing varied from 3.2 to 4.7m with an average of 3.9m.

Experimental design

This trial had a randomised blocked design with single tree plots and five replicates of each treatment. At least one guard tree was left between all plots.

Treatments

Treatments were applied on 16 and 17 January 2008 when fruit were on average 44.1mm in diameter. Treatments were as follows:

- Control: Trees were thinned as per normal commercial practice (10-15 fruit per 50cm cubic quadrat) by the grower, also in mid January
- Heavy thinning of the whole tree (leaving 5-7 fruit per quadrat)
- Stripping of half the tree (north west and north east quadrants)
- Stripping of alternate limbs (approx half of the tree in total)
- Heavy thinning of alternate limbs (approx half of the tree in total) – fruit were sampled from both untouched limbs and thinned limbs.

Measurement and analysis

Sampling was done on only one date (18/3/08) rather than two at this trial as too few fruit were left on the tree for a second sample after harvesting by the grower.

Data for granulation at harvest, crop load before thinning (16 January 2007) and diameter of thinned fruit were analysed using analysis of variance, with tree health as recorded on 6 March 2008 as a covariate.

2.7.3 Trial I Thinning

Objective

As for trial E.

Plant materials

Imperials on Troyer rootstock planted in 1990, on a sandy loam soil, not mounded. Row spacing was 6.7m and tree spacing was 3.1m.

Experimental design

As crop load at this site was very variable, a stratified approach was taken to treatment allocation. On 23 January 2008, crop load was assessed on four sides of each tree with a $0.125m^3$ quadrat. Forty-eight trees (16 in each of three rows) were stratified into trees with 'high' (mean of > 8 fruit per quadrat), 'medium' (3-8 fruit) and 'low' crop loads (<3 fruit). Note that >8 fruit is by industry standards a comparatively low crop load. Trees with naturally uneven bearing patterns (*i.e.* heavy loads on some limbs, light loads on other limbs) were also identified. Six young fruit were also sampled randomly: one from each quadrant of the outside canopy and two from inside the canopy. Fruit were cut equatorially and assessed visually for granulation. From this assessment, 12 trees with 'high' crop load and average granulation above 1.5 were randomly selected and allocated randomly to the three treatments, and four 'naturally low' crop load and four 'naturally uneven' loads were selected randomly. Trees thus selected were spatially well-scattered through the trial site. A guard row was left between all treatment rows.

Treatments

Stratification, in effect, provided three 'control' treatments:

- Naturally 'high' crop load

- Naturally 'low' crop load
- Naturally variable bearing pattern

These were compared to the following treatments:

- Heavy thinning of naturally high crop load trees (half or slightly over half of fruit removed from trees. The number of fruit removed ranged from 142 to 273 per tree.
- Stripping of alternate limbs of naturally high crop load trees.

Thinning treatments were undertaken on 29 January 2008. These were in addition to thinning by the grower earlier that week. Fruit at that date was on average 43.4mm in diameter.

Measurement and analysis

Granulation of immature fruit was assessed on a sample of six fruit per tree on 23/1/08 (prior to stratification). At harvest, samples of 20 fruit were taken from around the tree or from relevant limbs on each tree on 18/03/2008 and 16/04/2008. Analysis of variance (no blocking, no covariates) was used to assess difference between means.

2.8 Plant growth regulator trials (F, G and H)

2.8.1 Overview

Plant growth regulators (PGRs) were included in the research program as they play a role in regulating source-sink relationships, increasing 'sink strength' or photosynthate partitioning to developing fruits. Most studies of auxin and gibberellic acid (usually GA_3) application report favourable results in reducing granulation (Chakrawar and Singh 1978; El-Zeftawi 1973; Erickson 1968; Erickson and Richards 1955; Fullelove *et al.* 2004; Hield and Erickson 1962; Kaur *et al.* 1991; Powell and Krezdorn 1977; Singh and Chohan 1984; Singh and Singh 1981a; Stewart 1949), while all reports of GA application report favourable results. However, some studies report increased granulation with auxin application e.g. Miller *et al.* (1999), Hirose *et al.*(1972), Pan *et al.* (1998) and Gallasch *et al.* (1998).

Auxins are used in the citrus industry to manage crop load and/or increase fruit size. Auxins applied before the end of the natural fruit drop period have been shown to increase fruit drop, probably through the induction of ethylene synthesis. Auxins applied early in Stage II of fruit development, after the natural fruit drop period in November, have been shown to increase fruit size (Agusti *et al.* 1994; Agusti *et al.* 1995; Agusti *et al.* 2002; El-Otmani *et al.* 1993; Guardiola and Garcia-Luis 1997; Guardiola and Lazaro 1987). These trials tested the hypothesis that the mechanism that increases fruit size may also reduce granulation, by increasing the sink strength of fruit. If this is so, it will need to be reconciled with the fact that large fruit size is generally associated with increased granulation.

In general, studies reporting reduced granulation suggest that spraying later in fruit development (that is, late Stage II and/or through Stage III) is more effective than spraying early in fruit development. Monthly sprays appear to be more effective than single sprays (Chakrawar and Singh 1978; Kaur *et al.* 1991; Singh and Chohan 1984; Singh and Singh 1981a). This approach, therefore, was also taken in the three trials outlined below, with the exception of Trial F where a single spray was also included as a treatment.

2.8.2 Trials F, G and H Plant growth regulators

Objectives

Evaluate the effectiveness of GA_3 or auxins in reducing granulation when applied at the end of Stage I of fruit development.

Plant material

PGRs were tested at three trial sites as follows:

- Trial F: Troyer rootstock, planted in 1989 in sandy loam soil, no mounding. Row spacing was 7m and tree spacing varied from 3.2m to 4.7m with an average of 3.9m.
- Trial G: Troyer rootstock, planted in 1997 on sandy (granite) soil, no mounding. Row spacing was 6.8m and tree spacing was 3.6m. Trees were mulched by the grower with sawdust.
- Trial H: Troyer rootstock, planted in 1990 on sandy loam soil on mounds. Row spacing was 7.4m and tree spacing was 3.75m.

Experimental design

- Trial F: Randomized blocked design, with five replicates and single tree plots. Guard trees were left between plots.
- Trials G and H: Randomized complete block designs, with five replicates and single tree plots. Guard trees were left between plots.

Treatments

Treatments included a control and applications of:

- 356-TPA (triclopyr) as 'Tops' at 20 ppm ai (2 tablets) per 100L water
- 2,4-DP as Corasil™ , a 25 g a.i./L Dichlorprop-p (present as the 2-ethylhexyl ester) emulsifiable concentrate product manufactured by Nufarm, at 200ml per 100L water
- GA_3 as Ralex® (40g/L GA) at 50 ml /100L water;
- Kelpak, a 100% liquid seaweed concentrate that includes auxins (11.0 mg/L); and cytokinins (0.031 mg/L) at 200ml/100L water.

Treatments were applied monthly for three months beginning at the end November, approximately the time of transition to Stage II of fruit development. Spray dates were:

- Trial F: 20/11/07 (when average fruit size was 22.1 mm, s=4.0), 18/12/07 and 17/1/08
- Trial G: 21/11/07 (when average fruit size was 25.3 mm, s=4.8), 19/12/07 and 18/1/08
- Trial H: 30/11/07 (when average fruit size 26.2 mm, s=3.7), 8/1/08, and 8/2/08. Note that Corasil was applied by the grower to all trees at 200ml/100L at 700 L/ha on 1/11/2007 in order to increase fruit drop.

Trial F included an additional four treatments consisting of a single application of each product on the first date (20/11/07).

Application volume was six litres per tree. Wetter was added to Kelpak, Tops and Corasil sprays and pH of mix adjusted where necessary with hydrochloric acid.

Measurement and analysis

In PGR trials, 20 fruit were harvested from the north east quadrant of the tree.

Twelve fruit in each treatment in three replicates were tagged and measured on each spray date and at harvest.

Data for granulation at harvest and diameter of tagged fruit were analysed using analysis of variance, with tree health and crop load as recorded on 6/3/08 as a covariate for Trial F and as recorded on 19/3/08 for Trial H. Trees in Trial G were similar in tree health and crop load so no covariates were used in analyses.

Trial F and G were sampled at harvest on 18/03/2008 and 16/04/2008; Trial H on 19/03/08 and 17/04/08.

2.9 Irrigation frequency trials (C, D, J and N)

2.9.1 Overview

Reducing frequency

Several studies have shown that granulation is reduced where irrigation water is reduced in volume, or the frequency of irrigation is reduced (Bartholomew *et al.* 1941; Benton 1940; Bevington *et al.* 1998; Goldhamer and O'Connell 2005; Lloyd 1961; Malik *et al.* 1981; Raina and Lakhanpal 1997; Scuderi 1970; Singh and Singh 1980b; Sites *et al.* 1951).

It is hypothesised that the effect of reduced irrigation frequency is through a wetting and drying cycle which imposes a slight drought stress on the tree. Fruit from citrus trees under mild water deficits appear to osmoregulate and thus have higher total soluble solids (TSS) and acid concentrations than unstressed trees (González-Altozano and Castel 1999; Hutton *et al.* 2007; Moon and Mizutani 2002; Mostert and van Zyl 2000; Peng and Rabe 1998; Spiegel-Roy and Goldschmidt 1996; Treeby *et al.* 2007; Verreynne *et al.* 2001). Frequent and regular irrigation may be a contributing factor to the lower levels of TSS found in granulated fruit. It may also increase juice cell turgor. Lower sugar levels and/or increased turgor may increase cell water potential, the hypothesised underlying cause of granulation.

The critical factor appears to be frequency rather than volume *per se*, in that it is the wetting and drying cycle that drives changes in osmotic gradients. Irrigation treatments thus were designed to provide the same volume of water but approximately half as often.

Critical stage of fruit development

Treatments were also designed to elicit information on the effects on granulation of reducing irrigation frequencies in different fruit development stages: I, II or III.

There are varying results reported in the literature for effects of reduced irrigation at different stages, not only for granulation *per se* but also for TSS and acid levels. Mostert and van Zyl (2000) found that quality factors were influenced by dry regimes during Stage I (flowering and cell division) for grapefruit and navels. Peng and Rabe (1998) found that deficit irrigation also has an influence on TSS in Stage II, but no effect on acidity or juice content. Sites *et al.* (1951) found that both Stage I and Stage II deficits increased TSS and acids. Reduced irrigation during ripening (Stage III) appears to have a particularly large effect on TSS levels (Mostert and van Zyl 2000). González-Altozano and Castel (1999)

found that deficits in Stage III increased TSS and acids but there were no differences from deficits in earlier stages.

Goldhamer (2005) tested varying the times of water deficits to early, middle and late season (roughly Stages I, II and III) and found that granulation was lower than the well-irrigated control for **all** times. González-Altozano and Castel (1999) also found that deficits all season increased TSS and acids but fruit size and yield decreased.

Methodology for all field irrigation trials

At all irrigation trials, treatments were to reduce frequency in Stage I, Stage II, Stage III, all three stages and in Stages II and III. In control treatments, normal frequency was maintained. Stage I treatments commenced after fruit set as deficit irrigation during flowering and fruit set is generally noted as detrimental in that it increases fruit drop (González-Altozano and Castel 1999; Mostert and van Zyl 2000). Dates of the treatment periods for irrigation trials are shown in Table 3

Table 3 Trials C, D, J and N: Irrigation trials treatment dates

	Stage I	Stage II	Stage III
Trial C			
2007-08	4/9/07-26/11/07	27/11/07-26/2/08	27/2/08-10/4/08
Trial D			
2008-09	2/10/08-3/12/08	4/12/08-4/3/09	5/3/09-28/4/09
2009-10	24/9/09-17/11/09	18/11/09-24/2/10	25/2/10-22/4/10
Trial J			
2008-09	6/10/08-9/12/08	10/12/08-3/3/09	4/3/09-30/3/09
2009-10	22/9/09-18/11/09	19/11/09-23/2/10	24/2/10-14/4/10
Trial N			
2009-10	28/9/09-15/11/09	16/11/09-11/2/10	12/2/10-6/4/10

Low frequency treatments were applied by the simple expedient of installing a second irrigation line with a tap that was turned off at alternate irrigation events. In this second line (the low frequency line), two microsprinklers were installed per tree so that trees received the same amount of water as control trees but half as often. Enviroscan™ units were installed in two 'control' and two 'low frequency all stages' plots at each trial. These were used to monitor soil moisture levels but were not used for decisions on irrigation scheduling. Growers decided on irrigation scheduling based on experience and seasonal conditions and turned taps on and off.

Nutrition factors

To date, there are few published studies on the interaction of nutrient applications and irrigation in citrus. Romero *et al.* (2006) found that deficit irrigation of Clemenules mandarins reduced N and Mg leaf mineral nutrition but not other nutrients in two years of trials. Because of promising results in reducing granulation from both irrigation frequency and nutrition trials in 2007/08, it was considered important to assess their impact in combination. Consequently, at Trials C, D and J, nutrition treatments were factored into irrigation trials to assess interactions. At Trial C this consisted of foliar sprays of N, B, Zn and P. The nutrition treatments tested in Trials D and J from 2009 onwards were those that were most effective in the previous year's trials, that is, the application of broadcast nitrogen in Stage I and the application of additional foliar zinc sprays in Stage I.

2.9.2 Trial C Irrigation frequency and foliar nutrients

Objectives

Test the effects on granulation of:

- reduced frequency of irrigation in key fruit development stages, and
- foliar applications of key nutrients (N, P, B, and Zn).

Plant materials

Imperials on Troyer rootstock, planted in 1994 on sandy loam, mounded. Row spacing was 7.3m and tree spacing was 3.6m.

Experimental design

This trial had a randomised split plot design with near-neighbour balance for irrigation treatments. There were four replicates of irrigation treatments in plots of 12-13 trees. One replicate each of the 'control' and 'LF all stages' treatments were on Cleopatra rootstock: these were excluded from the analysis. Four nutrient treatments were applied to random single trees in each of the irrigation plots. All nutrient plots were separated by one or more guard trees

Treatments

The six irrigation treatments were to reduce frequency in Stage I, Stage II, Stage III, all three stages and in Stages II and III (see Table 3 for dates of the three stages). In control treatments, normal frequency was maintained. Actual irrigation and rainfall dates are shown in Figure 3. Trees were irrigated using 70L/hr microsprinklers. The four foliar nutrient treatments are shown in Table 4 .

Table 4 Trial C Foliar nutrient treatments:

Treatment	Application dates	Application rates
Nitrogen	25/10/07, 27/11/27, 14/1/08, 15/2/08	1% w/v low biuret urea
Boron	24/10/07, 14/1/08	50g/100L Solubor
Zinc	24/10/07, 27/11/07, 14/1/08, 15/2/08	100g/100L zinc sulfate on 24/10/07 and 30ml Zinc100/100L water on 27/11/2007
Phosphorus	25/10/07, 27/11/07, 14/1/08, 15/2/08	0.3% w/v MAP

All applications five litres per tree. Low biuret urea at 0.2% w/v added to Zn and B sprays. Wetter added to all sprays (10ml/150L).

All trees were fertigated with calcium nitrate on 9/10/07 and 21/11/07 and with potassium nitrate on 21/12/07 and 24/1/08. Foliar nutrient sprays on all trees before nutrient treatments commenced included K and Zn on 16/7/07, calcium nitrate on 18/8/07, B on 9/9/07 and N (low biuret urea) on 17/9/07.

Measurement and analysis

Samples for granulation assessment were taken on 12/03/2008 and15/04/2008. The differences between treatment means were analysed using restricted maximum likelihood (REML), excluding trees on Cleopatra rootstock, with no covariates.

2.9.3 Trial D Irrigation frequency and additional N and Zn in Stage I

Objectives

Evaluate the effects on granulation of:

- reduced frequency of irrigation in key fruit development stages, and
- applications of additional N and zinc foliars in Stage I within irrigation frequency treatments.

Plant materials

Imperials on Troyer rootstock and sweet orange interstock planted in 1990. The soil was a sandy loam, mounded, overlaying a heavy clay loam at approximately 1m depth. Row spacing was 8m and tree spacing was 4m.

Experimental design

This trial had a randomised complete block design of three tree plots in four blocks. There was a single guard tree between plots.

Treatments

The six irrigation treatments were to reduce frequency in Stage I, Stage II, Stage III, all three stages and in Stages II and III (see Table 3 for dates of the three stages). In control treatments, normal frequency was maintained. Actual irrigation and rainfall dates are shown in Figure 4 (08/09) and Figure 5 (09/10). Trees were irrigated using 50L/hr microsprinklers. Note that in 2010, frequent rain meant there were no irrigation events at all in Stage III i.e. no low frequency treatment could be applied. Table 5 shows the three nutrient treatments.

Table 5 Trial D Nutrient treatments

Treatment	2008/09	2009/10
Control	Grower applied 1 kg/tree urea on 20/6/2008 and 2 zinc foliars (50ml/100L at 3000L/ha on 4/7/08 and 33 ml/100L at 3000L/ha on 28/8/08.	Grower applied 0.5 kg/tree urea on 28.6.10 and 0.5 kg/tree urea on 21/9/10; and two zinc foliars (50ml/100L at 3000L/ha in July 09 and 33 ml/100L in August 09.
Additional N in Stage I.	As for control + broadcast 0.6 kg/tree urea on 20/10/08.	As for control + broadcast 0.35 kg/tree urea on 3/9/2010.
Additional N in Stage I plus foliar Zinc in Stage I	As for control + broadcast 0.6 kg/tree urea on 20/10/08, and zinc as a foliar 40ml/100L of Zinc100 (with 0.2% low biuret urea) on 17/10/08 and on 20/11/2008.	As for control + broadcast 0.35 kg/tree urea on 3/9/2010 and zinc as a foliar 100g/100L zinc sulfate on 30/9/09 (with 0.2% low biuret urea) and 50ml/100L Zinc100 on 23/10/09 and 12/11/09.
Foliar sprays all 6L/tree		

Measurement and analysis

Samples for granulation assessment were taken on 2/04 and 28-29/4 in 2009, and on 26/3 and 22/04 in 2010. Differences between treatment means were analysed using analysis of variance. All three trees in each plot were used in data analyses. Crop loads estimated before thinning each year (18/12/08 and 6/1/10) and tree health ratings (at 30/9/08 and at 6/1/10) were used as covariates.

2.9.4 Trial J Irrigation frequency plus extra N in Stage I

Objectives

Test the effects on granulation of:

- reduced frequency of irrigation in all key fruit development stages; and
- applications of additional N in Stage I within irrigation frequency treatments.

Plant materials

Imperials on Troyer rootstock, planted approximately 1990 on mounds on a sandy loam with a clay layer at depths from 0.4 to 1.2 m. Row spacing was 7.5m and tree spacing was 3.8m, with young trees interplanted progressively in 2009 and 2010 between mature trees. Trees were mulched in 2008 (but not in 2009) with hay.

Experimental design

This trial had a randomized, blocked design with three tree plots with at least one guard tree between plots. The large number of sick or missing trees meant that a complete block design could not be used. There were 10 replicates of each irrigation treatment, divided into two nutrient treatments. After removal by the grower of several trees in October 2009, trial design was adjusted in 09/10 to leave eight replicates of each irrigation treatment, divided into two nutrient treatments. Blocking was not used for analysis of 2009/10 data.

Treatments

The six irrigation treatments were to reduce frequency in Stage I, Stage II, Stage III, all three stages and in Stages II and III (see Table 3 for dates of the three stages). In control treatments, normal frequency was maintained. Actual irrigation and rainfall dates are shown in Figure 6 (08/09) and Figure 7 (09/10). Note that in 2010, frequent rain in Stage III meant there were no irrigation events at all during this period, that is, no low frequency treatment could be applied. Trees were irrigated using 70L/hr microsprinklers.

The two nutrient treatments were a control (no additional N applications) and the application of additional N in Stage 1, that is, broadcast of broadcast 0.6 kg/tree urea. Dates of application were 23/10/08 in the first year and 2/9/09 in the second year

Grower applications before treatments commenced in 08/09 included fertigation with calcium nitrate on 13/8/08, 3/9/08 and 23/10/08. In the 09/10 growing season, the grower applied 1kg/tree of granulated ammonium sulphate (20.2% N) in July.

Measurement and analysis

Samples for granulation assessment were taken on 30/3 and 24/04 in 2009, and on 17/03 and 14/04 in 2010. Analysis of variance was used to assess differences between treatment means. As noted above, blocking was used in 08/09 analysis but not in 09/10.

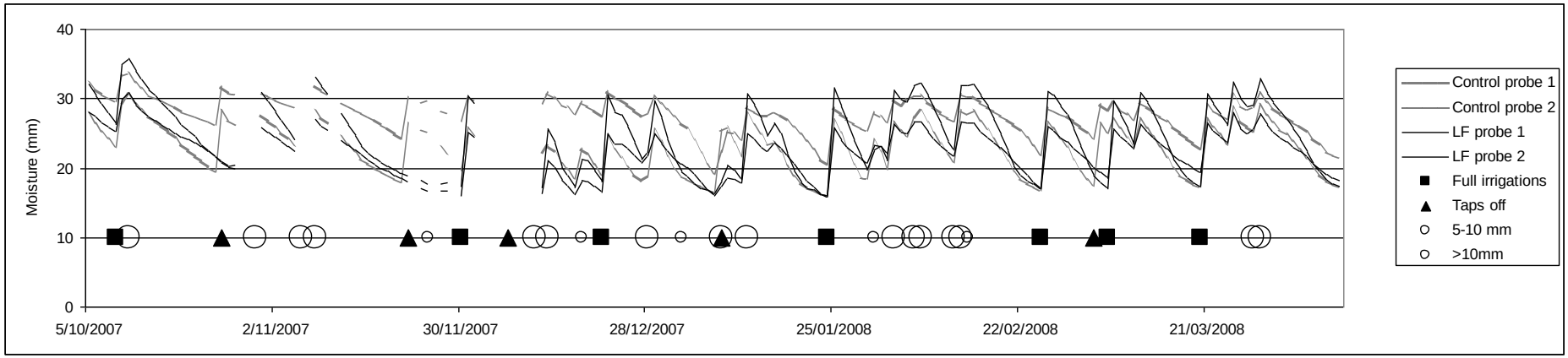

Figure 3 Trial C 07/08 Soil moisture at probes in two 'control' plots and two 'low frequency all Stages' plots at 10cm depth, and dates of irrigations and rainfall events.

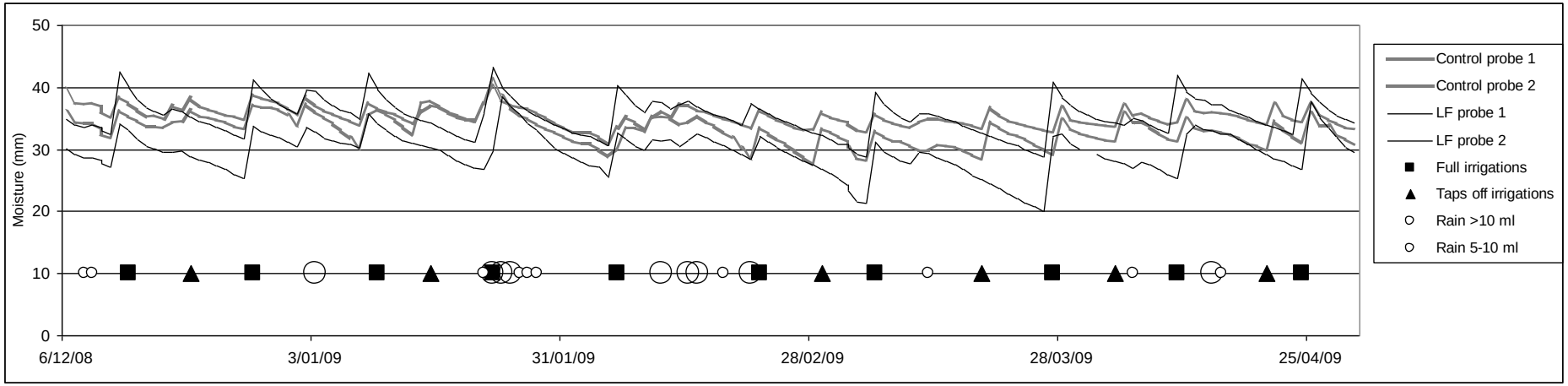

Figure 4 Trial D 08/09 Soil moisture at probes in two 'control' plots and two 'low frequency all Stages' plots at 10cm depth, and dates of irrigations and rainfall events.

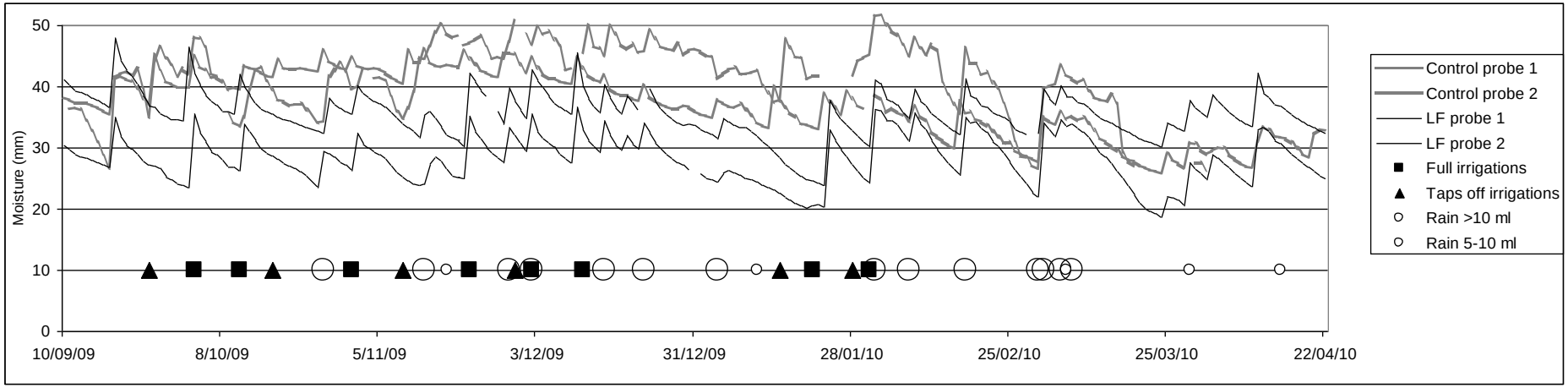

Figure 5 Trial D 09/10 Soil moisture at probes in two 'control' plots and two 'low frequency all Stages' plots at 10cm depth, and dates of irrigations and rainfall events.

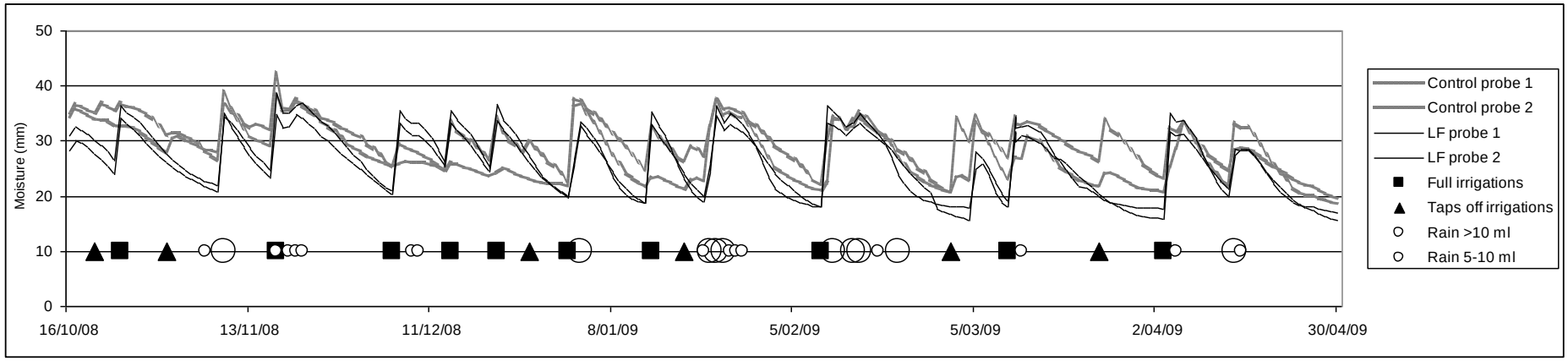

Figure 6 Trial J 08/09 Soil moisture at probes in two 'control' plots and two 'low frequency all Stages' plots at 10cm depth, and dates of irrigations and rainfall events.

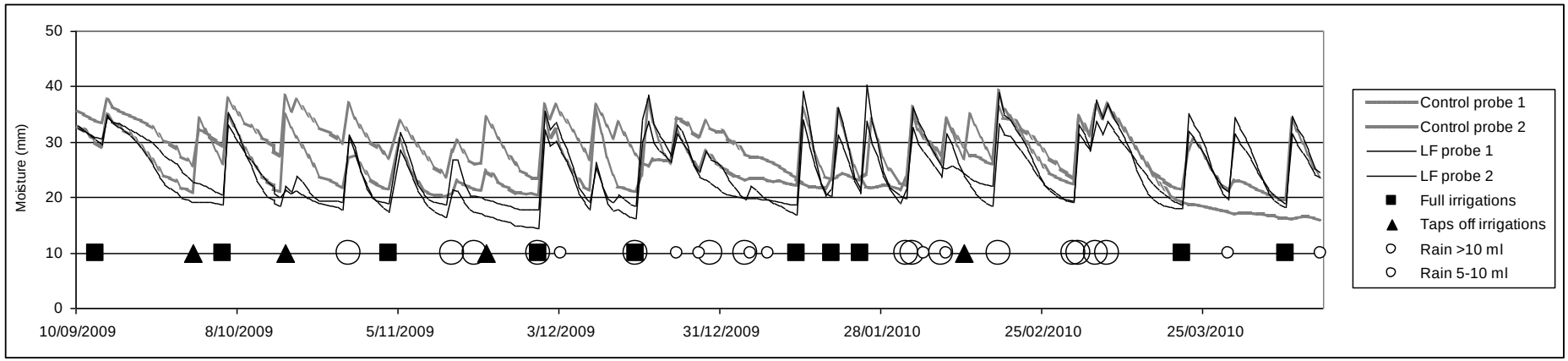

Figure 7 Trial J 09/10 Soil moisture at probes in two 'control' plots and two 'low frequency all Stages' plots at 10cm depth, and dates of irrigations and rainfall events

2.9.5 Trial N Irrigation volume and frequency trial in pots

Objective

Test the effect of reduced frequency and volume of irrigation in all key fruit development stages on granulation.

Plant materials

Two-year old Imperial trees in 40cm diameter pots on Trifoliata rootstock under polyethylene at the Bundaberg Research Facility. Plants were potted in the citrus potting mix used at the Facility (Smith *et al.* 2008). Nutrient applications to all trees ceased in May 2009 in order to increase the likelihood of granulation.

Experimental design

There were four replicates of six treatments arranged in a randomized design across two benches.

Treatments

The six irrigation treatments were to reduce volume and frequency in Stage I, Stage II, Stage III, all three stages and in Stages II and III (see Table 3 for dates of the three stages). In control treatments, normal frequency was maintained. Treatment trees were irrigated when soil moisture as measured by tensiometers in two pots fell below -55kPa. Non-treatment trees received watering every second day until 2/11/09 when frequency was changed to daily. Trees received 3.5-4 L of water per irrigation.

Measurement and analysis

Fruit were harvested progressively when ripe as assessed by colour change, between 1/2/2010 and 6/4/2010. The difference between treatment means was analysed using analysis of variance.

2.10 Nutrition trials (A, B, K, M and P)

2.10.1 Overview

These trials tested whether or not decreased or increased levels of nutrient applications influence the prevalence of granulation, hypothetically through effects on soluble solid concentrations. In the first year of trials the main objective was to test the effects of key nutrients identified in the literature (N, K, P, B and Zn) to establish effects on granulation under local conditions.

High N is reported to contribute to granulation by stimulating vegetative and/or root growth, directing photosynthates away from fruit development (Bartholomew *et al.* 1941; Matsumoto 1964). In the Central Burnett, N is generally applied in late winter to support development of healthy vegetative growth to support fruiting wood and applications at other times are discouraged to avoid stimulating vegetative flushes or increasing acid levels (Bevington 1988; Chapman 1986). However, it may be possible to use N fertilisation to increase photosynthates supplies to fruit, thus increasing sugar levels and reducing granulation.

Foliar sprays of nitrogen have been found to increase fruit size and early maturity (El-Otmani *et al.* 2002; El-Otmani *et al.* 2004b).

Potassium (K), which is 40% of the total mineral content of citrus fruit, is a strong determinant of citrus fruit size and acidity (Spiegel-Roy and Goldschmidt 1996). Imperial mandarins are heavy users of K. Most characterisation studies find high levels of K in granulated fruit (Bartholomew *et al.* 1941; Sinclair and Jolliffe 1961); but other studies suggest that deficiencies of K can exacerbate granulation and that applications of K reduced granulation (Kotsias 2004; Singh and Chohan 1982; Singh and Singh 1981b). Over-application of K is generally found to produce large, coarse fruit with thick peels (Bevington 1988; Davies and Albrigo 1994), reduced juice content, increased acidity and regreening (Bevington 1988; Chapman 1968; Chapman 1982; El-Otmani *et al.* 2004a). Central Burnett growers generally apply broadcast fertilisers of K in winter and early summer, the latter application depending on crop load.

Boron (B) could conceivably be associated with granulation because it is known to facilitate translocation of photosynthates. Applications of boric acid have been found to significantly reduce incidence of granulation (Kaur *et al.* 1990; Kotsias 2004; Singh and Singh 1981b). In the central Burnett growers tend to apply annual, low dosage sprays in winter to promote flowering and fruit set.

Zinc (Zn) could be associated with granulation because of its importance in chlorophyll biosynthesis. Zn deficiencies can also result in loss of capacity to produce sufficient endogenous auxins. It is suggested by some authors that decreased auxin may increase oxidisation of sugars (faster respiration) thus decreasing sugar levels (Singh and Chohan 1982; Singh and Singh 1981a).

Citrus are considered to have a low requirement for phosphorous (P) (Davies and Albrigo 1994). Growers rarely apply P in the central Burnett although some are reportedly doing so with good results. Phosphorus could be indirectly associated with granulation because excess P produces low acid, low sugar fruit (Chapman 1968). Some studies have found higher levels of P in granulated fruit or leaves of granulating trees (Bartholomew *et al.* 1941; Munshi *et al.* 1978; Sinclair and Jolliffe 1961); some have found lower levels (Awasthi and Nauriyal 1972d; Gilfillan and Stevenson 1977; Singh and Singh 1980c) and others no relationship (Chanana and Nijjar 1984).

Trials A and B in 07/08 therefore included additional N applications in Stage I and in Stage II of fruit development to see if additional N applications could be used to induce granulation for future trials and to improve understanding of the critical period for onset of granulation. Treatments that applied additional P, K, B and Zn in Stage I were included, as well as treatments that reduced levels of K, B and Zn.

In the second year of trials (08-09), treatments at Trial A were adjusted to focus on those which showed more promise. This included dropping 'low' nutrient treatments (with the exception of 'low K'). As there was a very low of incidence of granulation at Trial B in 07/08, this trial was dropped altogether. Trial K was established to further test N, P and K additions. In addition, N and Zn treatments were factored into irrigation frequency trials D and J.

In the third year of the project (09-10), variable timing of N winter applications were tested at Trial P, and foliar and broadcast N applications were compared at Trial M. Foliar applications could be preferable in situations where leaching of nutrient into waterways is an issue, where soils were unfavourable to efficient urea application, or where nitrogen could be added to other foliar sprays to save on costs.

2.10.2 Trial A Identifying key nutrients

Objectives

The objectives of this trial were to:

- test key nutrients identified in the literature or through local experience (N, K, P, B, Zn) to establish effects on granulation incidence/severity;
- test timing of additional N applications to ascertain if additional N applications can be used to reliably induce granulation for future trials; and.
- test differential timing of additional N applications (Stage I and Stage II) to see if this indicates the critical period for onset of granulation.

Plant materials

Imperials on Troyer rootstock planted in 1994 on mounds on a heavy loam soil/light clay. Treatment trees were interplanted with young Valencias on Troyer around 1m high. Row spacing was 7m, trees were planted at 4.4m intervals (not including interplants).

Experimental design

This trial had a randomised complete block design with near-neighbour balance, with four tree plots and six replicates of each treatment. Treatments were applied to all four trees in each plot.

Treatments

Treatments are shown in Table 6 . Note that plots which had received 'low B' treatments in 07/08 were subjected to '+N foliar' treatment in 08/09 and 09/10; and those which had received low Zn treatments in 07/08 were subjected to '+ zinc soil' treatment in the following years.

Measurement and analysis

Samples for assessment of granulation assessment in 2008 were taken on 25/3 and 15/4; in 2009 on 1/4 and 27/4; and in 2010 on 25/3 and 21/4. The three healthiest trees in the plot were used in analysis (the same three trees for all three years of data) except at the sample on 17/4/08 when only the two central trees of each four-tree plot were sampled. Means were separated using analysis of variance. Crop loads estimated at harvest each year were used as covariates.

Table 6 Trial A and B nutrient treatments

	Trial B	Trial A		
Treatment	2007-08	2007-08	2008-09	2009-10
Control	1.2 kg/tree urea on 2/8/07 0.9 kg/tree SoP on 29/8/07 and 0.6 kg/tree SoP on 12/12/07 foliar Zn as $ZnSO_4 7H_2O$ 100g/100L on 19/9/07	Foliar Zn as $ZnSO_4 7H_2O$ 150g/100L on 3/7/07 0.648 kg/tree urea on 26/6/07 0.9 kg/tree SoP on 29/8/07 and 0.6 kg/tree SoP on 12/12/07 Solubor at 50g/100L foliar spray on 10/9/07 Foliar Zn as $ZnSO_4 7H_2O$ at 100g/100L on 25/9/07	0.65 kg/tree urea on 26/6/08 and 0.31 kg/tree urea on 18/9/08 0.9 kg/tree SoP on 5/9/08 and 0.9 kg/tree SoP on 13/11/08 Foliar Zn as ZinTrac at 30ml/100L on 25/11/08	0.65 kg urea/tree on 28/6/09 and 0.31 kg/urea per tree on 5/8/09 0.73 kg/tree SoP on 29/9/09 and 1.15 kg/tree SoP on 10/12/09 Solubor at 50g/100L foliar spray on 10/9/07 Foliar Zn as $ZnSO_4 7H_2O$ at 100g/100L on 6/7/09 and (tops only0 on 3/8/09; and as ZinTrac at 60ml/100L on 28/8/09. Boron as Redibor-Plus at 50g/100L on 28/8/09
Low N	No application of broadcast N on 2/8/07	na	na	na
+ N Stage I	As for control +1.2 kg/tree urea on 5/10/07	As for control + 0.6 kg/tree urea on 5/10/07	As for control + 0.6 kg/tree urea on 8/10/09	As for control + 0.35 kg urea/tree on 17/9/09
+ N Stage II	As for control +1.2 kg/tree urea on 12/12/07	As for control + 0.6 kg/tree urea on 12/12/07	As for control + 0.8 kg/tree urea on 3/12/09	As for control+ 0.35 kg urea/tree on 26/11/09
High K	As for control + extra 0.6 kg/tree SoP on 5/10/07+extra 1.2 kg/tree SoP on 12/12/07	As for control +0.6 kg/tree SoP on 5/10/07+1.2 kg/tree SoP on 12/12/07	As for control + 0.9 kg/tree SoP on 5/9/09 + 0.9 kg/tree SoP on 3/12/09	As for control + 0.9 kg/tree SoP on 17/9/09 + 0.9 kg/tree SoP on 26/11/09
Low K	As for control but without application of SoP on 12/12/07	As for control but without application of SoP on 12/12/07	As for control but without application of SoP on 13/11/08	As for control but without application of SoP on 26/11/09
High B	na	As for control + foliar sprays of Solubor at 50g/100L on 19/10 and 12/12/07	As for control + foliar sprays of Solubor at 50g/100L on 16/10/08 and 13/11/08	As for control + 5 foliar sprays of Solubor at 50g/100L fortnightly from 9/9/09-5/11/09
Low B	na	As for control but without foliar spray on 10/9/07	na	na
High Zn	As for control + foliar sprays of Zn as $ZnSO_4 7H_2O$ at 100g/100L on 19/10/07 and Zinc100 at 40 ml/100L on 12/12/07	As for control + foliar sprays of Zn as $ZnSO_4 7H_2O$ at 100g/100L on 19/10/07 and Zinc100 at 40ml/100L on 12/12/07	As for control +foliar sprays of Zinc100 at 40 ml/100L on 16/10/08 and 13/11/08	As for control + foliar sprays of $ZnSO_4 7H_2O$ at 100g/100L on 9/9/09, 24/9/09 and 8/10/09 and 50 ml/100L of Zinc100 on 22/10/09 and 5/11/09

	Trial B		Trial A	
Treatment	2007-08	2007-08	2008-09	2009-10
Low Zn	As for control but without zinc foliar spray on 19/9/07	As for control but without zinc foliar spray on 25/9/07	na	na
Extra P	As for control + 1.8 kg SuperMo/tree on 5/10/07 + 1.8 kg Supermo/tree on 12/12/07	As for control + 1.7 kg SuperMo/tree on 5/10/07 + 0.9 kg Supermo/tree on 12/12/07 (banded on drip line)	As for control + 0.75 L/tree Polyphos in 15L water, banded along dripline on 5/10/08 plus foliar sprays of MAP on 16/10/08 and 13/11/08	As for control + 0.75 L/tree Polyphos in 15L water, banded along dripline on 16/9/09
N foliars	na	na	As for control + foliar sprays of LoBi urea at 1.5% wv on 16/10/08 and 13/11/08	As for control + 5 foliar sprays of LoBi urea at 1.5% wv fortnightly from 9/9/10-5/11/10
10. + Zn soil	na	na	As for control +0.5kg/tree $ZnSO_47H_2O$ in 7L water, banded along dripline on 24/9/08	As for control +0.5kg/tree $ZnSO_47H_2O$ in 7L water, banded along dripline on 16/9/09
Notes:	Urea = 46% N SoP= sulphate of potash = 41.5% K $ZnSO_47H_2O$ = 22% Zn Zinc100 = 100% Zn Polyphos^{TM} =20%P, 13%N Solubor = Disodium octaborate tetrahydrate $Na_2B_8O_{13}4H_2O$ = 20.5% B; Zintrac^{TM}=40%Zn Foliars were sprayed at the rate of 6 litres per tree. Low biuret urea at 0.2% was added to all foliar sprays. Wetter added to all foliar sprays except Zinc100 sprays and pH adjusted where necessary with hydrochloric acid.			

2.10.3 Trial B Identifying key nutrients

Objectives

As for Trial A.

Plant materials

Imperials on Troyer rootstock, planted 1995, in rows 8m apart, with 4m between trees, on a clay loam soil (not mounded).

Experimental design

This trial had a randomised complete block design with near-neighbour balance, with three tree plots and eight replicates of each treatment, with no guards. Treatments were applied to all trees in each plot.

Treatments

A similar range of nutrient treatments to Trial A was included at Trial B, with the addition of a 'low N' treatment. Details are shown in Table 6 .

Measurement and analysis

Samples for granulation assessment were taken on 19/3/08 and 17/4/08. On the second sample date, only the central tree of each plot was sampled. Means were separated using analysis of variance. Means were adjusted for covariates of tree health and crop load measured at harvest (19/3/08).

2.10.4 Trial K Additional N, P and K

Objectives

Test repeatability of response to extra N in Stage I that was found in Trials A and B in 07-08, and, in comparison, to see:

- if doubling the amount of urea at this stage further reduces granulation;
- if the response could be improved by providing a 'package' of N-P-K rather than 'straight' N; and
- if N foliars would provide similar results to application of broadcast urea.

Plant materials

Imperials on Troyer rootstock planted in 1989 on a sandy loam soil, not mounded. Row spacing was 7m and tree spacing varied from 3.2 to 4.7m with an average of 3.9m.

Experimental design

The frequency of sick trees in this block meant that plots of healthy trees could be randomised but not blocked. There were seven replicates of three tree plots, with at least one guard tree between plots. Broadcast and soil treatments were applied to guard trees, or to half the guard tree where there was only one tree between plots.

Treatments

All treatments were timed for Stage I of fruit development. The treatments were:

- Control—no additional applications.
- Zinc foliars—sprays of Zinc100 at 40ml/100L on 17/10/08 and 12/11/08 (7L/tree).
- Zinc soil application—0.5 kg/tree of $ZnSO_4 7H_2O$ in 7L water, banded along dripline on 24/9/08.
- Extra N broadcast—0.6kg/tree of urea on 9/10/08. This is equivalent to 35% of the recommended annual requirement of N (Vock et al. 1997).
- Double extra N broadcast—1.2kg/tree of urea applied on 9/10/08.
- Nitrogen foliars—sprays of LoBi urea at 1.5% wv on 17/10/08 and 12/11/08 (7L/tree).
- Extra K— 1 kg/tree sulphate of potash (41.5%K) broadcast on 24/9/09 and 1kg/tree on 11/12/08.
- Extra NPK—broadcast application on 9/10/08 of 1.5kg/tree of 'Nitropshoska blue' (125.214.1) plus 0.22kg/tree of urea. Together this provides approximately 35% of the annual requirement of both N and K, and 100% of P (Vock et al. 1997).

Low biuret urea at 0.2% was added to zinc foliar sprays. Wetter was added to foliar sprays. The pH of sprays was adjusted where necessary with hydrochloric acid.

Applications by the grower to all trees before commencement of treatment applications included 1kg/tree urea and 1.5kg/tree sulphate of potash and KNO_3 and zinc foliar sprays.

Measurement and analysis

Samples for granulation assessment were taken on 31/03/09 and 28/04/09. Means were separated using analysis of variance. Means were adjusted for covariates of tree health measured on 27/11/08 and crop load measured at harvest (18/3/08).

2.10.5 Trial M Foliar applications of nutrients

Objectives

To test whether foliar applications of:

- nitrogen or a nitrogen and zinc combination in Stage I provide similar results to that achieved for broadcast urea; and
- calcium nitrate are effective in reducing granulation.

Plant materials

Imperials on Troyer rootstock planted in 1997 on sandy soil (granite based), not mounded. Row spacing was 6.8m and tree spacing was3.6 m.

Trial design

This trial had a fully randomized complete block design, with four blocks and five-tree plots. Guard row between blocks, but no guard trees between plots.

Treatments

All foliar treatments consisted of five sprays of seven litres per tree at approximately fortnightly intervals in Stage I of fruit development. Dates of application for all foliar treatments were 9/9/09, 23/9/09, 7/10/09, 21/10/09 and 4/11/09. Treatments were as follows:

- Control—no additional applications.
- Broadcast N— additional 0.6 kg/tree urea on 9/9/09.
- Foliar zinc and nitrogen— $ZnSO_4 7H_2O$ at 100g/100L plus 1% wv low biuret urea on 9/9, 23/9 and 7/10. Zinc100 at 50ml/100L plus 1% wv low biuret urea on 21/10/09 and 4/11/09.
- Foliar nitrogen— 1.5% wv LoBi.
- Foliar calcium— $Ca(NO_3)_2$ at 1% wv.

Low biuret urea at 0.2% was added to calcium foliar sprays. Wetter was added to all foliar sprays except Zinc100 sprays. The pH of sprays was adjusted where necessary with hydrochloric acid.

Grower applications prior to the trial commencing on all trees included 1kg/tree urea on 24/7/09; foliar spray incorporating 1% wv KNO_3, 0.5% wv low biuret urea and 150g/100L of $ZnSO_4 7H_2O$ on 8/7/09; and a foliar spray incorporating 0.5% low biuret urea and 0.5% wv $Ca(NO_3)_2$ on 19/8/09.

Measurement and analysis

Samples for assessment of granulation were taken on 18/03/10 and 14/04/10. The three healthiest trees in each plot were used in analyses of treatment means. All trees were used for correlation analysis (Table 8).

2.10.6 Trial P Timing of winter application of nitrogen

Objective

Assess whether timing and quantity of winter urea application affects granulation levels.

Plant materials

Imperials on Troyer rootstock, planted 1995, in rows 8m apart, with 4m between trees, on a clay loam soil (not mounded).

Experimental design

This trial had a randomised complete block design, with three-tree plots and four replicate plots per treatment, with no guards.

Treatments

Treatments were as follows:

- Control (normal N in July)—application of 1.2 kg/tree urea on 8/7/09 (=176 kg/ha N).
- Low N in July—application of 0.6 kg/tree urea on 8/7/09 (=88 kg/ha N).
- Normal N in August—application of 1.2 kg/tree urea on 6/8/09 (=176 kg/ha N).
- Split application of normal N—application of 0.6 kg/tree urea on 8/7/09 and 0.6 kg/tree urea on 6/8/09 (=total of 176 kg/ha N).
- Zero N—no application of urea at any date.

Measurement and analysis

Only one sample was taken at this trial (7/4/2010). Means were analysed using analysis of variance. Tree health assessed on 8/7/09 was used as a covariate in analyses.

3 Results and discussion

Data collected over the life of the project has significantly improved our understanding of the physiological aspects of granulation. This knowledge is outlined below, as it informs the interpretation of the results of management trials which follows.

3.1 Improved understanding of the causes of granulation

A range of relationships with fruit, tree and block characteristics are evident from survey data collected from 2007 to 2010, and from assessment of those same characteristics within trial blocks.

The clearest associations are with vigorous rootstock types, larger fruit diameter, lower crop load, acid content of juice, tree health and tree age, as discussed below. There is relatively less evidence for links to lighter soil types, Brix°, tree health, canopy density and the degree of rootstock benching (as measured by difference in the cross-sectional area of rootstock and scion).

The large number of factors involved and the variability of those factors indicates the complexity of the issue and suggests either multiple causes or supports the concept of a unifying underlying cause (such as juice cell water potential) that itself has multiple causes.

3.1.1 Rootstocks

Survey blocks planted on Troyer citrange rootstocks had higher mean granulation rates in all years than blocks on Cleopatra or Swingle (Figure 8). DPI&F trials of rootstocks for Imperial mandarins indicate that fruit harvested over the 2001 to 2006 seasons were, on average, highly granulated on Volkameriana rootstocks, followed by Troyer, Carizzo, Sweet Orange and C32. Granulation was consistently lower on Cleopatra, Benton and, particularly, Swingle rootstocks (Smith 2006).

These findings broadly support research that suggests that vigorous, early fruiting rootstocks are more inclined to granulate (Awasthi and Nauriyal 1972c; Bain 1949; Jawanda *et al.* 1978). The link may be through total soluble solids in fruit (sugars and acids) and/or through plant water relations. While it is the foliage that supplies sucrose to the plant, the rootstock appears to have the strongest influence on the amount (Gardner 1969). Rootstocks also have significant effects on plant water relations (Albrigo 1978; Castle and Warrington 1995). Vigour may be due to higher hydraulic conductivity (ease of movement of water through the plant) (Syvertsen and Graham 1985) and extent of root systems (Syvertsen 1981). The more vigorous rootstocks tend to have higher stem and leaf water potential (Albrigo 1978; Barry *et al.* 2004; Camacho-B *et al.* 1974; Crocker *et al.* 1974). It seems that hydraulic conductivity and TSS are inversely related (Barry *et al.* 2004; Yonemoto *et al.* 2004). Overall, there appears to be a 'tradeoff' between vigour and fruit quality.

Table 7 Survey data correlations between mean granulation per tree and tree-based measurments 2007 to 2010.

Parameter	Survey year	Correlation coefficient (r)	*P*	N (no of trees)
Mean fruit diameter	2007	0.2661	0.0000	287
	2008	0.2535	0.0000	277
	2009	**0.4514**	**0.0000**	332
	2010	**0.4168**	**0.0000**	333
Crop load	2007	0.0646	0.2753	287
	2008	-0.2304	0.0001	277
	2009	-0.1494	0.0064	332
	2010	-0.2981	0.0000	333
Flush at harvest (m)	2007	0.1881	0.0014	287
	2008	-0.1374	0.0222	277
	2009	0.1837	0.0008	332
	2010	-0.2646	0.0000	333
Tree health rating	2007	0.0151	0.7987	287
	2008	0.0909	0.1312	277
	2009	0.0724	0.1880	332
	2010	0.2175	0.0001	333
Brix^{o}	2007	-0.0984	0.1192	252
	2010	-0.2535	0.0000	297
% acid	2007	**-0.3714**	**0.0000**	252
	2010	**-0.3884**	**0.0000**	297
Brix: acid ratio	2007	**0.4146**	**0.0000**	252
	2010	0.2944	0.0000	297
Canopy cover rating	2008	-0.0666	0.2690	277
	2009	-0.1204	0.0280	332
	2010	-0.0457	0.4059	333
Scion circumference	2007	**-0.3514**	**0.0000**	287
	2008	-0.2141	0.0003	277
	2009	-0.2071	0.0001	332
	2010	-0.2034	0.0002	333
Rootstock circumference	2007	-0.2979	0.0000	287
	2008	-0.2034	0.0007	277
	2009	-0.1866	0.0006	332
	2010	-0.1309	0.0168	333
Scion/rootstock difference in cross sectional area	2007	-0.1522	0.0098	287
	2008	-0.1378	0.0218	277
	2009	-0.114	0.0379	332
	2010	0.0189	0.7313	333

Bold text = correlations where values of r ≥0.3 and $p<0.05$; p= two sided test of correlations different from zero

Table 8 Trial data correlations between mean granulation per tree and tree-based measurements.

			First sample			Second sample		
	Year	Trial	r	*p*	n	r	*p*	n
Mean fruit diameter at harvest								
	2008	G	-0.0912	0.6647	25	0.1466	0.4843	25
	2009	A	0.2805	0.0000	240	0.1690	0.0087	240
		D	**0.4083**	**0.0000**	215	**0.4259**	**0.0000**	215
		J	**0.6131**	**0.0000**	180	**0.3175**	**0.0000**	180
		K	**0.4944**	**0.0000**	168	**0.3097**	**0.0000**	168
	2010	A	0.2374	0.0002	240	-0.1811	0.0049	240
		D	**0.3754**	**0.0000**	216	-0.0318	0.6418	216
		J	**0.5927**	**0.0000**	145	-0.1961	0.0181	145
		M	0.1550	0.1235	100	0.2882	0.0036	100
		P	**0.6592**	**0.0000**	51	na	na	na
Crop load pre thinning								
17 12 08	2009	A	-0.0819	0.2063	240	-0.0898	0.1655	240
18 12 08		D	-0.1196	0.0803	215	0.0548	0.4237	215
17 12 08		J	-0.0480	0.5221	180	-0.1070	0.1527	180
7 1 09		K	-0.1435	0.0635	168	-0.0056	0.9421	168
7 1 10	2010	A	0.0519	0.4232	240	-0.0548	0.3981	240
6 1 10		D	-0.2515	0.0002	216	-0.2037	0.0026	216
13 1 10		J	-0.1671	0.0476	141	-0.1695	0.0445	141
6 1 10		M	-0.0589	0.5608	100	-0.0344	0.7343	100
14 1 10		P	**-0.5014**	**0.0002**	51	na	na	na
Crop load at harvest								
	2008	A	-0.1277	0.0482	240	-0.2446	0.0052	129
		B	-0.2875	0.0000	240	**-0.3899**	**0.0000**	104
		C	**0.3417**	**0.0000**	245	**0.3108**	**0.0000**	245
		F	**-0.5791**	**0.0000**	45	-0.2622	0.0818	45
		G	0.1068	0.6113	25	0.3197	0.1193	25
		H	-0.2484	0.1857	30	**-0.5282**	**0.0027**	30
	2009	A	-0.0625	0.3347	240	-0.0446	0.4917	240
		D	-0.0014	0.9841	215	0.0156	0.8206	215
		J	0.0617	0.4106	180	-0.0185	0.8048	180
		K	0.1055	0.1734	168	0.1403	0.0698	168
	2010	A	-0.0639	0.3241	240	-0.0889	0.1698	240
		D	-0.1240	0.0690	216	-0.2094	0.0020	216
		P	**-0.4455**	**0.0011**	51	na	na	na
Tree health rating								
14 3 08	2008	A	0.0222	0.7319	240	-0.0029	0.9737	129
14 3 08		B	**0.3776**	**0.0000**	240	**0.3687**	**0.0001**	104
6 3 08		C	0.0308	0.6314	245	-0.0213	0.7404	245
6 3 08		F	-0.0247	0.8718	45	-0.1223	0.4236	45
18 03 08		G	-0.2075	0.3197	25	0.1406	0.5026	25
19 3 08		H	**-0.5463**	**0.0018**	30	**-0.5353**	**0.0023**	30
3 12 08	2009	A	0.1021	0.1148	240	0.1524	0.0181	240
4 12 08		D	-0.1203	0.0784	215	-0.2452	0.0003	215
4 12 08		J	-0.0368	0.6239	180	-0.0283	0.7057	180
27 11 08		K	0.1627	0.0351	168	0.0484	0.5336	168
7 1 10	2010	A	0.1607	0.0127	240	0.2042	0.0015	240
6 1 10		D	0.0791	0.2470	216	0.0856	0.2103	216
13 1 10		J	**0.3240**	**0.0001**	145	**0.3354**	**0.0000**	145
6 1 10		M	0.0568	0.5747	100	-0.1516	0.1322	100

			First sample			Second sample		
	Year	Trial	r	*p*	n	r	*p*	n
14 1 10		P	**0.3751**	**0.0067**	51	na	na	na
Brix^{o}								
	2009	A	0.0646	0.7082	36	-0.2281	0.1809	36
		D	**-0.4307**	**0.0356**	24	-0.3587	0.0852	24
		J	-0.2500	0.2860	20	-0.1422	0.5498	20
		K	**-0.5416**	**0.0002**	42	**-0.4397**	**0.0036**	42
	2010	A	0.0846	0.5943	42	0.1560	0.3238	42
		D	0.0366	0.8322	36	0.1171	0.4964	36
		J	0.3136	0.0805	32	-0.3229	0.0714	32
		M	**-0.5764**	**0.0078**	20	**-0.7234**	**0.0003**	20
		P	-0.2806	0.1742	25	na	na	na
% acid								
	2009	A	**-0.5184**	**0.0012**	36	**-0.5515**	**0.0005**	36
		D	**-0.5202**	**0.0092**	24	-0.2327	0.2739	24
		J	**-0.7368**	**0.0002**	20	**-0.6229**	**0.0033**	20
		K	**-0.6623**	**0.0000**	42	**-0.3932**	**0.0100**	42
	2010	A	**-0.3675**	**0.0166**	42	-0.0381	0.8105	42
		D	**-0.4345**	**0.0081**	36	-0.2509	0.1400	36
		J	**-0.6576**	**0.0000**	32	**-0.5220**	**0.0022**	32
		M	**-0.8101**	**0.0000**	20	**-0.6077**	**0.0045**	20
		P	**-0.4164**	**0.0384**	25	na	na	na
Brix:acid ratio								
	2009	A	**0.5524**	**0.0005**	36	**0.4996**	**0.0019**	36
		D	0.2927	0.1651	24	-0.0024	0.9912	24
		J	**0.6513**	**0.0019**	20	**0.6396**	**0.0024**	20
		K	**0.5280**	**0.0003**	42	0.0988	0.5336	42
	2010	A	**0.5619**	**0.0001**	42	0.1823	0.2478	42
		D	**0.5347**	**0.0008**	36	**0.4496**	**0.0059**	36
		J	**0.7037**	**0.0000**	32	**0.4317**	**0.0136**	32
		M	**0.5720**	**0.0084**	20	0.2351	0.3185	20
		P	0.3627	0.0747	25	na	na	na
Canopy cover rating								
7 1 10	2010	A	-0.1408	0.0292	240	-0.1533	0.0175	240
6 1 10		D	-0.0177	0.7956	216	-0.0714	0.2965	216
13 1 10		J	-0.2622	0.0017	141	**-0.3696**	**0.0000**	141
6 1 10		M	-0.0667	0.5097	100	0.0851	0.3996	100
14 1 10		P	-0.1021	0.4757	51	na	na	na
Scion circumference								
	2008	A	0.0492	0.4479	240	0.1627	0.0654	129
	2009	A	-0.0741	0.2531	240	-0.0109	0.8669	240
		J	0.1480	0.0474	180	0.1389	0.0629	180
		K	0.0669	0.3888	168	0.0944	0.2237	168
	2010	A	0.2292	0.0003	240	0.2254	0.0004	240
		M	-0.0365	0.7182	100	-0.0457	0.6518	100
		P	-0.0304	0.8322	51	na	na	na
Rootstock circumference								
	2008	A	0.0423	0.5146	240	0.0645	0.4680	129
		A	-0.0021	0.9747	240	-0.0055	0.9320	240
		D	0.0126	0.8546	215	-0.1884	0.0056	215
		J	0.1380	0.0647	180	0.1216	0.1040	180

	Year	Trial	First sample			Second sample		
			r	*p*	n	r	*p*	n
		K	0.0495	0.5238	168	0.1038	0.1804	168
	2010	A	0.2502	0.0001	240	0.1976	0.0021	240
		D	-0.0181	0.7912	216	-0.1447	0.0336	216
		M	-0.0112	0.9119	100	0.0034	0.9734	100
		P	-0.0279	0.8457	51	na	na	na
Rootstock - scion – difference in cross sectional area as % of rootstock area								
	2008	A	-0.0641	0.3228	240	-0.1771	0.0447	129
	2009	J	-0.0185	0.8051	180	-0.0460	0.5393	180
	2010	A	-0.1158	0.0734	240	-0.1364	0.0347	240
		M	0.0646	0.5228	100	0.0896	0.3753	100
		P	0.0549	0.7021	51	na	na	na
Flowering load rating								
24 9 08	2009	A	0.0505	0.4359	240	-0.0467	0.4715	240
24 9 08		D	0.1295	0.0580	215	-0.0441	0.5200	215
5 10 08		J	-0.2025	0.0064	180	-0.1835	0.0137	180
24 9 08		K	-0.0532	0.4935	168	0.0066	0.9320	168
26 8 09	2010	J	-0.1568	0.0644	140	-0.1133	0.1827	140
Advancement of flowering rating								
24 9 08	2009	A	-0.1391	0.0313	240	-0.0251	0.6986	240
24 9 08		D	0.0243	0.7227	215	-0.0744	0.2773	215
5 10 08		J	-0.2425	0.0010	180	-0.2003	0.0070	180
26 8 09	2010	J	-0.0723	0.3960	140	-0.0322	0.7061	140
Extent of flush rating								
24 9 08	2009	A	-0.0475	0.4642	240	-0.0884	0.1721	240
26 11 08		A	0.1335	0.0388	240	0.0482	0.4576	240
4 12 08		D	0.2021	0.0029	215	0.0648	0.3446	215
4 12 08		J	0.0967	0.1965	180	0.0387	0.6061	180
5 10 08		J	0.0037	0.9610	180	-0.0307	0.6823	180
24 9 08		K	0.0332	0.6694	168	-0.0013	0.9864	168
27 11 08		K	0.1327	0.0863	168	0.1572	0.0418	168
26 8 09	2010	J	-0.0384	0.6520	140	-0.1030	0.2260	140
7 10 09		P	**0.4122**	**0.0027**	51	na	na	na
Sprinkler output								
	2008	P	**0.6719**	**0.0002**	26	na	na	na
	2009	K	-0.2773	0.1453	29	-0.2745	0.1496	29
		J	0.0189	0.8749	72	-0.1862	0.1172	72
		M	-0.0619	0.6564	54	-0.0738	0.5959	54
		P	0.0961	0.5349	44	na	na	na

Bold text = correlations where values of r ≥0.3 and p<0.05; *p*= two sided test of correlations different from zero

Survey data for 2010 demonstrates that even rootstocks such as Cleopatra which are less prone to granulation are not immune in a 'dry' year. The poorer results in 2010 for Cleopatra are partly linked to crop load: 36% of trees on Cleopatra rootstocks had significantly lower crop loads (>10 fruit per quadrat less) in 2010 than in 2009, compared to 25% of trees on Troyer rootstocks.

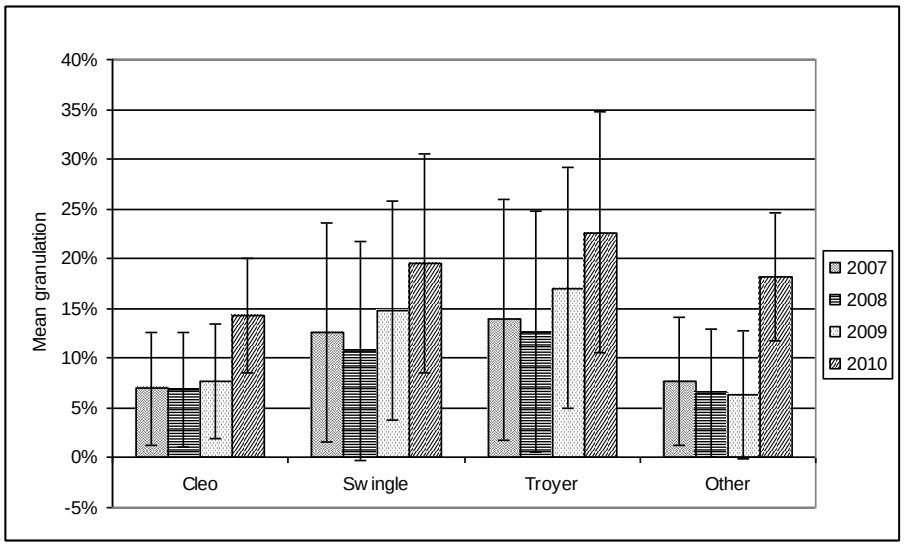

Figure 8 Mean granulation by rootstock in surveys from 2007 to 2010. Bars represent standard deviations. Number of blocks on Cleopatra = 9, Swingle = 5, Troyer citrange = 20, other = 3 (Emperor, Sweet orange and Trifoliata).

3.1.2 Soil types

Earlier observations suggested that granulation in the Central Burnett is associated with lighter soils (Fullelove 2001; Fullelove *et al.* 2004). Of the surveyed blocks, the one planted on sand had higher mean granulation rates than those on heavier soils in 2007 and 2009, but not in 2008 or 2010 (Figure 9). The other soil types show no clear trend, in fact, the clay loam blocks (n=5) show on average the highest granulation in 2010.

Some growers suggest sandy soils are linked to granulation due to their inability to hold water, thus causing water stress. However, the research hypothesis suggests that any such link may be due to the lower CEC and nutrient-holding capacity or to the higher plant-availability of water in sandy soils. It is possible that in 2010 more frequent rainfall in Stage I and/or Stage III meant increased plant-available water in the clay loam soils, thus increasing granulation on these soils in that year.

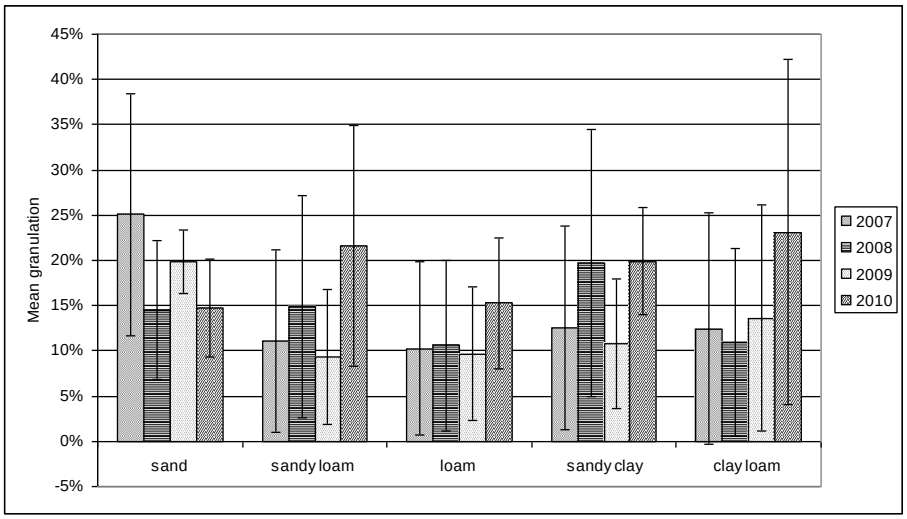

Figure 9 Mean granulation by soil type in surveys from 2007 to 2010. Bars represent standard deviations. No. of blocks in 2010 = 1 on sand, 19 on sandy loam, 11 on loam, 2 on sandy clay, and 5 on clay loam.

3.1.3 Crop load

Several studies demonstrate that granulation is more prevalent on trees with low crop loads (El-Zeftawi 1973; 1978; Gravina *et al.* 2004; Ritenour *et al.* 2004). Survey data supports this general trend (Figure 10), although correlations are not strong (Table 7 Table 8). At very low crop loads (below 10 per 0.125m^3 quadrat), fruit is likely to be granulated, but beyond 15 fruit per quadrat there is a less distinct difference in most years. On trial blocks, correlations vary widely, with *r* values from +0.3417 to -0.5791 (Trial C and Trial F 2008 respectively). Correlations were stronger in 2008 and 2010 than in 2009, that is, in those years tending towards 'off years', where a greater range of crop loads were observed.

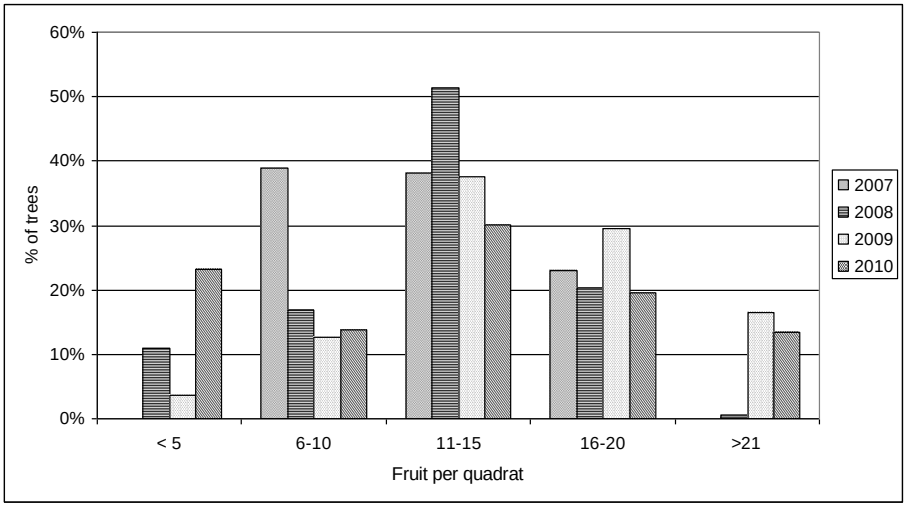

Figure 10 Mean granulation by crop load in surveys from 2007 to 2010. Bars represent standard deviations.

Lower crop loads seem to be one of the reasons why granulation was high in 2010 (Figure 11). Crop loads on 32% of survey trees in 2010 were reduced by ≥ 10 fruit per quadrat compared to the previous year, whereas in 2009 only 16.9% of trees had lower

crop loads than in the previous year (Table 9). In addition, average fruit size in 2010 was higher than previous years (Figure 14). This would also help to account for increased granulation in that year, as discussed in Section 3.1.6 below.

Correlations between granulation and crop load measured before thinning on trial blocks tend to be slightly stronger and more consistently negative than crop load measured at harvest (Table 8). This could reflect the importance of the natural crop load in determining granulation, or could be because thinning reduces the extreme values at the higher end of crop load estimates.

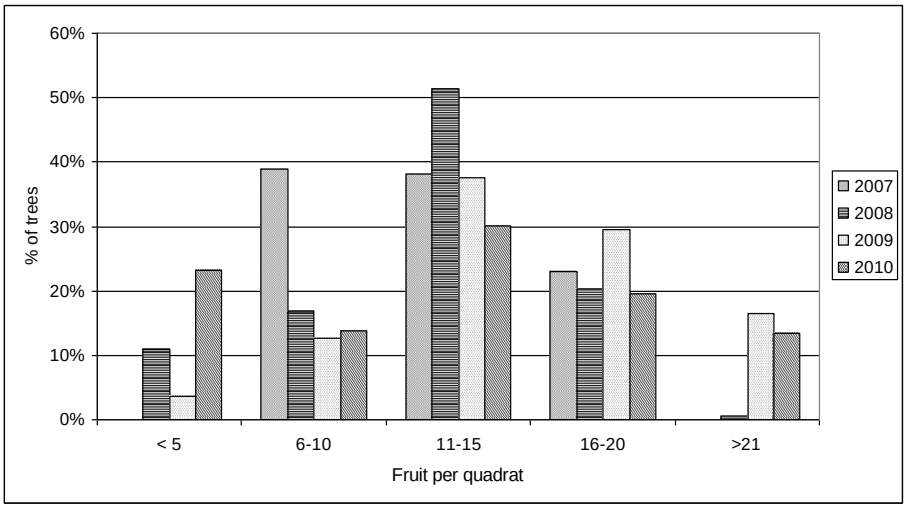

Figure 11 Distribution of trees by crop load in surveys from 2007 to 2010.

Table 9 Effect of annual change in crop load on granulation by tree in surveys from 2007 to 2010

Change in crop load between:	*2007 and 2008*		*2008 and 2009*		*2009 and 2010*	
Change in no of fruit per 0.125m^3 quadrat	% of trees (n=265)	Mean change in granulation	% of trees (n=326)	Mean change in granulation	% of trees (n=303)	Mean change in granulation
-25					2.0%	33.0%
-20	0.8%	-1.7%			5.6%	24.6%
-15	4.5%	18.8%	1.2%	13.0%	11.2%	14.5%
-10	4.9%	10.3%	2.2%	3.9%	12.9%	11.7%
-5	21.5%	1.1%	13.2%	1.8%	14.2%	13.6%
0	36.2%	1.5%	26.4%	-1.4%	22.8%	7.4%
5	24.9%	-1.9%	25.2%	-3.4%	17.5%	4.7%
10	6.4%	-5.6%	14.4%	-7.5%	9.9%	1.9%
15	0.8%	-19.6%	10.4%	-9.8%	3.0%	-0.9%
20			6.1%	-0.4%	1.0%	-7.2%
25			0.6%	-1.9%		
30			0.3%	-29.2%		
% of trees with crop load decreases	31.7%		16.6%		45.9%	
% of trees with crop load increases	32.1%		57.1%		31.4%	

A common explanation for the association between granulated fruit and low crop load is that in an ‘off year’ the tree directs photoassimilates into vegetative growth (Bartholomew *et al.* 1941; Benton 1940; El-Zeftawi 1978; 2004). However, survey

observations do not support this explanation because increases in crop load occurred at the same time as canopy density increases, and decreased crop load is not associated with corresponding increases in canopy density (Figure 12). Other possible explanations are that carbohydrate reserves are low in early fruit development (due to depletion in the previous year), or that any photoassimilates produced over the season are preferentially directed into storage reserves. A study by Monselise *et al.* (1981) of two Wilking trees – a strongly biennnial cultivar – shows that the effect of an 'on year' is to deplete starch from roots, while after an 'off year' reserves accumulate in roots in very large amounts (the ratio of starch in roots of the 'off year' to the 'on year' was 17.2:1).

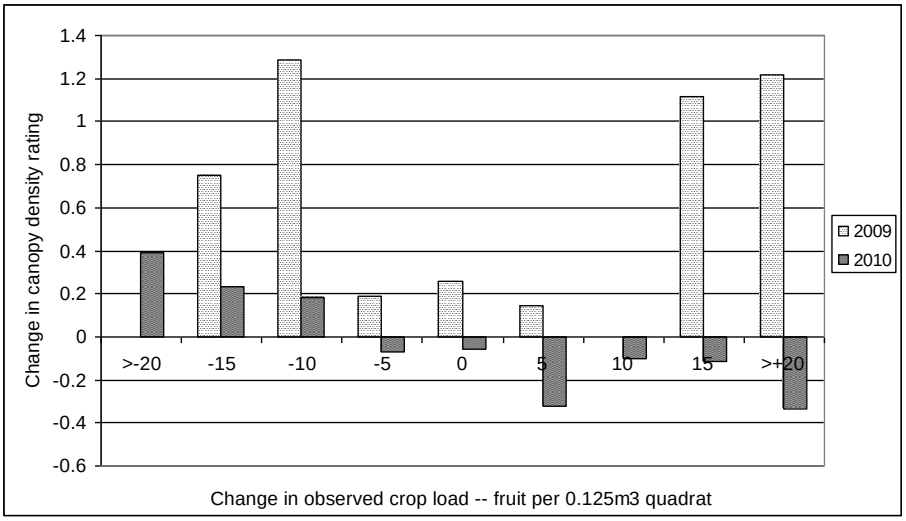

Figure 12 Mean change in canopy density rating by change in observed crop load in surveys 2008 to 2009 and 2009 to 2010. Positive changes in canopy density rating equate to increases in canopy density.

There is also evidence that rates of photosynthesis slow down in 'off years' (Lenz 1980). Similarly, there is evidence that in an 'off year' sap flow is higher than when there is a heavy crop load (Yonemoto *et al.* 2004). Jones *et al.* (1975) record a reduction in feeder roots in 'on years', leading to reduced root activity and thus increased water stress. Thus a reduced water supply to individual fruits in an 'on year' may help prevent granulation.

The link of increased granulation to low crop load suggests that reducing biennial bearing is an important aspect in the management of granulation.

3.1.4 Brix and acid levels

A decrease in total soluble solids (TSS) in granulated vesicles is well established in most research (Awasthi and Nauriyal 1972b; Ding *et al.* 2009; El-Zeftawi 1978; Gilfillan and Stevenson 1977; Kotsias 2004; Sandhu and Singh 1989; Sharma and Saxena 2004; Sharma *et al.* 2006; Shomer *et al.* 1988; Sinclair and Jolliffe 1961; Singh and Singh 1980a; Sinha *et al.* 1962).

Survey data show a negative correlation between the acid levels of the juice sample from a tree (combined sample of 12 fruit) and the granulation of that fruit ($r = -0.37$ in 2007 and -0.39 in 2010). This relationship might be even stronger if not for the difficulty of extracting 5mls of juice from fruit rated above 55%, and for the propensity of less-

granulated vesicles in a partially granulated fruit to easily release juice when squeezed in the field in comparison to more-granulated vesicles in the same fruit.

Correlations between acid level and granulation in trial trees are consistently negative and are overall the strongest of the variables assessed (Table 8). Values of 'r' range from -0.3675 to -0.8101 at the first sample date. Correlation coefficients are generally lower for the second sample. As the trees sampled in trials were limited to N, K and control treatments, this suggests a link to increased nutrition, as discussed in Section 3.2.4 below. Fruit diameter from treatments with high acid levels tend to be slightly smaller.

Correlations between Brix° and granulation are much less consistent than those with acid, varying widely for both survey and trial data (Table 7 Table 8). While differences in maturity on the range of sampling dates and sites would explain much of the variability for survey data, it would be expected that this would also apply to acid levels, and moreover would not be relevant at trial sites. Correlation coefficients for trial data range from -0.5746 (Trial M, 2010, p= 0.0078) to +0.3136 (Trial J, 2010, p=0.0805). If the water potential hypothesis is valid, more consistently negative correlations of granulation with Brix would be expected, particularly on the second sample date.

3.1.5 Tree health

There is no consistent linear relationship between observed tree health and granulation (Table 7 Table 8). Although unhealthy trees (rated 3.5 and over) produce on average more granulated fruit, some produce perfect fruit, as shown in very high standard deviations of mean granulation (vertical bars in Figure 13). The reason for this dichotomy remains unclear, but is possibly due to whether or not there are sufficient stored levels of carbohydrates to support fruit development.

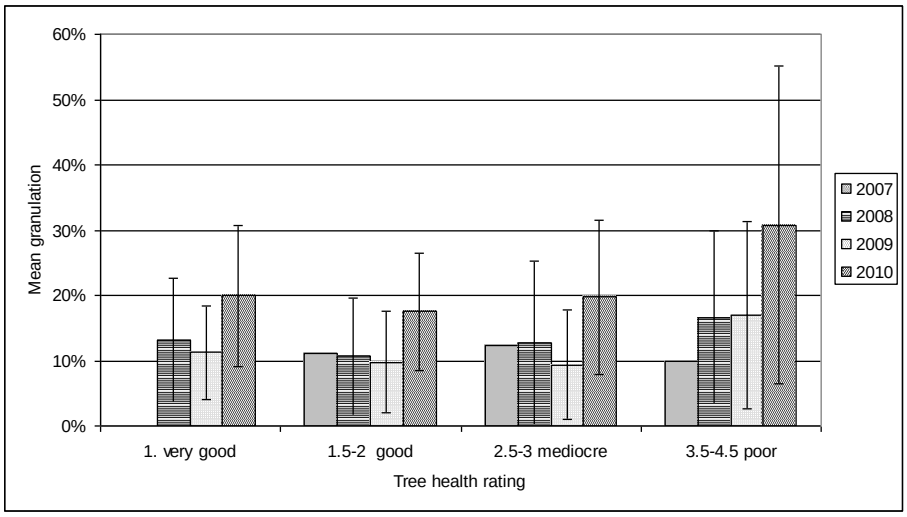

Figure 13 Mean granulation by tree health rating in surveys from 2007 to 2010. Bars represent standard deviations.

3.1.6 Fruit size

The correlation between larger fruit and increased granulation is evident at all trials and in the survey data in all years (Table 7 , Table 8). This is in line with most reported research (Awasthi and Nauriyal 1972c; Bartholomew *et al.* 1941; Burns and Albrigo 1997; 1998; Gravina *et al.* 2004; Hearn 1987; Lloyd 1961; Sandhu and Singh 1989; Sharma and Saxena 2004; Sharma *et al.* 2006; Sinclair and Jolliffe 1961; Van Noort 1969).

However, severely granulated fruit can be both large and small, as is shown by the lower mean and high standard deviations in Figure 14. Tagged fruit from trials show this dichotomy was particularly evident in 2010. This dichotomy is not as evident in survey data because survey fruit less than 50mm in diameter were not sampled. Only one other published study has illustrated this trend of granulation in both extremes of fruit size: Sandhu and Singh (1989) found very small (<59mm) Jaffa sweet oranges as well as larger fruits (>80mm) had the highest levels of granulation.

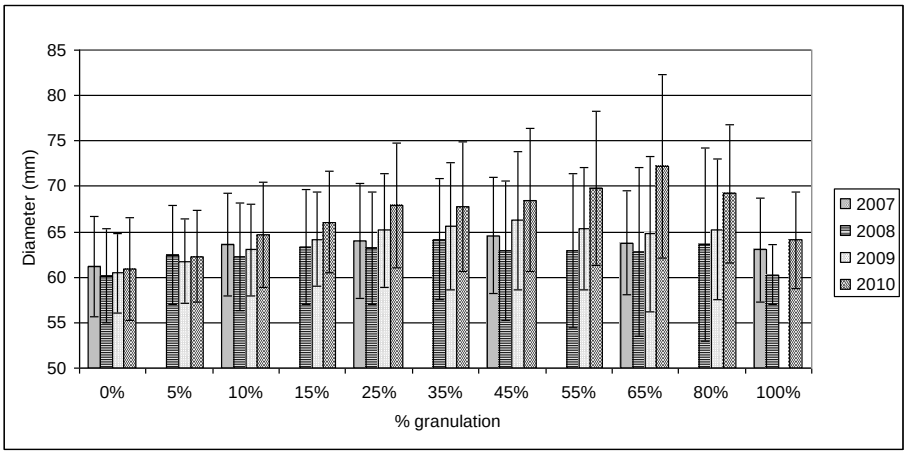

Figure 14 Fruit size by granulation rating in surveys from 2007 to 2010. Bars represent standard deviations.

A possible reason for the link between large fruit and granulation is that large fruit generally have lower sugar levels and lower acidity but a higher sugar: acid ratio (Bevington *et al.* 1998). This is generally supported by survey and trial data (Table 8).

Fruit were tagged at trials to see if the link between final diameter and granulation was due to a faster (or slower) growth rate. Data from Trials A and J in 08/09 and in 09/10 show that, on average, growth rates for tagged fruit were remarkably consistent and linear from the date of tagging over the whole growing season, whether fruit are on the inside or the outside of the canopy, or are small or large when first tagged, and regardless of granulation rating at harvest (Figure 15, Figure 16, Figure 18, Figure 19). Final fruit size appears to be determined mostly by the date fruit begin to grow rather than to variable rates of growth, that is, large fruit start to grow earlier than smaller fruit.

Table 10 Correlations between mean diameter and Brix and acid in surveys in 2007 and 2010 and in trials in 2009 and 2010.

Sample (n = no of trees)	% acid		Brix		Brix acid	
	r	*p*	r	*p*	r	*p*
Survey data 2007 (n= 252)	-0.1652	0.0086	-0.1164	0.0650	0.1649	0.0087
Survey data 2010 (n=297)	**-0.5084**	**0**	-0.1365	0.0186	**0.3798**	**0**
Trial A 2009 (n=36)	**-0.4984**	**0.0020**	0.2329	0.1716	**0.6013**	**0.0001**
Trial D 2009 (n=24)	**-0.5795**	**0.0030**	0.0115	0.9576	**0.5663**	**0.0039**
Trial J 2009 (n=20)	-0.3544	0.1252	0.0145	0.9517	0.3741	0.1042
Trial K 2009 (n=42)	**-0.6400**	**0**	-0.2661	0.0886	**0.6655**	**0**
Trial A 2010 (n=42)	**-0.4452**	**0.0031**	-0.3038	0.0504	**0.3877**	**0.0112**
Trial D 2010 (n=36)	-0.2024	0.2364	-0.1645	0.3376	0.1196	0.4872
Trial P 2010 (n=25)	**-0.5791**	**0.0024**	-0.3350	0.1016	**0.4668**	**0.0187**
Trial J 2010 (n=32)	**-0.4686**	**0.0068**	**0.4150**	**0.0182**	**0.5487**	**0.0011**
Trial M 2010 (n=20)	-0.1088	0.648	-0.1814	0.4441	0.0061	0.9797

Bold text = correlations where values of r ≥0.3 and p<0.05; *p*= two sided test of correlations different from zero

Although fruit growth is generally considered to be sigmoidal rather than linear (Bain 1958; Storey and Treeby 1999), a simple extrapolation of the mean growth line as a straight line to find the date that fruit diameter equals 0 mm could be indicative of the likely differences between the dates that fruit start to grow. Such an extrapolation in 08/09 suggests an average inception date of around 6/9/08 for large fruit and 20/9/08 for small fruit at Trial A, and 7/9/08 and 21/9/08 for Trial J. In 2009/10 extrapolating the mean growth line suggests an inception date on average of 30/8/09 for large fruit and 10/9/09 for small fruit at Trial A, and 29/8/09 and 12/9/09 for Trial J. This is in accord with observations on the extent of flowering in these years, with the exception of Trial J in 2008 where observation was that flowering was 7-10 days later than these dates suggest.

Similarly, in 08/09 the strength of the correlation between size and granulation at both trials remained fairly consistent over time, suggesting that there is no particular phenological stage of fruit growth particularly associated with granulation, unless it is the growth before the time fruit was first tagged and measured (early November) (Table 11).

This finding on the consistent rate of growth supports the work by Bevington *et al.* (1998) who reported that hand thinning increases the average fruit size on the tree by removing the smaller fruit, not by any effect on increasing growth rates on remaining (larger) fruit. Fruit diameter in February was strongly linearly related to fruit size at harvest.

In short, tagged fruit data suggests that granulation is linked not so much to faster or slower growth rates but to when growth starts. This date is apparently as early as the petals unfolding (Bartholomew *et al.* 1941). This brings seasonal conditions at the time of flowering and fruit inception under the spotlight, as discussed below.

Table 11 Correlation at Trials A and J between diameters of tagged individual fruit and granulation; and granulation and distance from the trunk, pedicel diameter and mean PAR reading as a % of PAR reading in the interrow.

	Trial A				Trial J			
	2008-09		2009-10		2008-09		2009-10	
	r	n	r	n	r	n	r	n
% of canopy width	**-0.4472**	226	-0.0759	248	**-0.3083**	219	-0.1656	284
Diameter								
early Oct			0.1494	186			0.0316	149
late Oct			0.0672	248			-0.0866	250
early Nov	**0.6074**	180	0.0595	248	**0.4972**	184	-0.1000	284
late Nov	**0.5528**	216	0.0249	248	**0.4911**	217	-0.0897	284
early Dec	**0.5493**	226	0.0245	248	**0.4806**	219	-0.0949	284
late Dec	**0.5356**	226	0.0234	248	**0.4713**	219	-0.0818	284
early Jan	**0.5664**	226	0.0446	248	**0.4572**	219	-0.0847	284
late Jan	**0.5764**	226	0.0764	248	**0.4477**	219	-0.0649	284
early Feb	**0.5938**	226	0.0761	248	**0.4424**	219	-0.0395	284
late Feb	**0.5775**	226	0.0983	248	**0.4539**	219	-0.0470	284
early Mar	**0.5815**	226	0.1094	248	**0.4396**	219	-0.0153	284
late Mar	**0.5585**	226	0.1389	248	**0.4379**	219	-0.0194	284
at harvest	**0.5614**	226	0.0912	248	**0.4272**	219	-0.0194	284
Pedicel diameter:								
November 2008	**0.5098**	216	na		**0.4052**	219	na	na
at harvest 2009	**0.4692**	226	na		**0.3946**	219	na	na
Feb 2010	na	na	0.1757	248	na	na	0.1118	284
Mean PAR readings as a % of interrow:								
Trial A 9/12/09	na		-0.0897	248	na		na	
Trial J 23/12/09	na		na		na		-0.1489	284
Trial J 27/1/2010	na		na		na		-0.1120	284

Bold text= correlations where values of r ≥

3.1.7 Fruit position

Tagged fruit data at Trials A and J show that there was a difference in mean granulation depending on the position of fruit in the tree (Figure 17, Figure 20). Fruit inside the canopy tend to granulate more than fruit on the outside whether large or small and even though mean size and growth rates are similar. This applies even though trees in these trials were pruned to be relatively open.

In 2008/09 larger fruit at both Trial A and J were more granulated on average than smaller fruit both on the inside as well as the outside of the canopy (Figure 17). In 2009/10, however, smaller fruit were, on average, more granulated both on the inside as well as the outside of the canopy (Figure 20). This reflects the strong dichotomy in granulation of very small and very large fruit in 2010 discussed above in Section 3.1.6.

The correlation coefficient (r) for the position of fruit (distance from the trunk as a percentage of the total canopy width) and granulation of the fruit was -0.4384 at Trial A and -0.3083 at Trial J in 08/09, and -0.739 and -0.166 respectively in 2010. Bartholomew et al. (1941) also found that position was more important than fruit size.

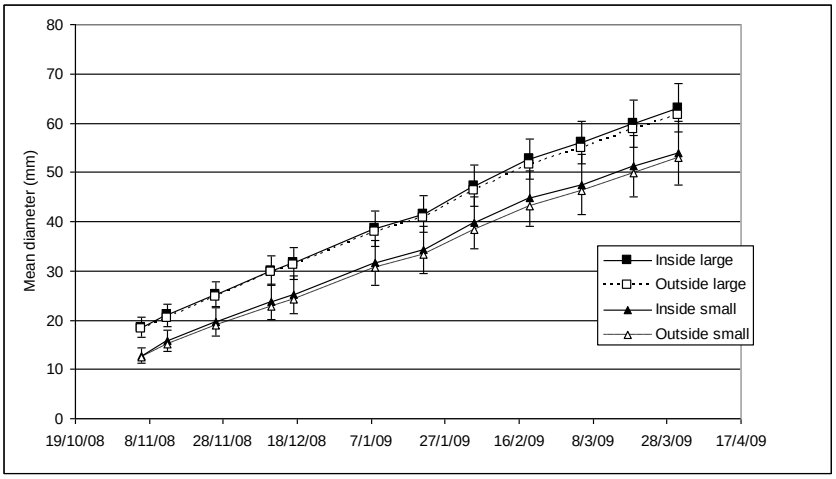

Figure 15 Trial A 2008/09 Mean size of tagged fruit measured at ~ fortnightly intervals. Bars represent standard deviations for fruit inside the canopy. Fruit were not matched into pairs.

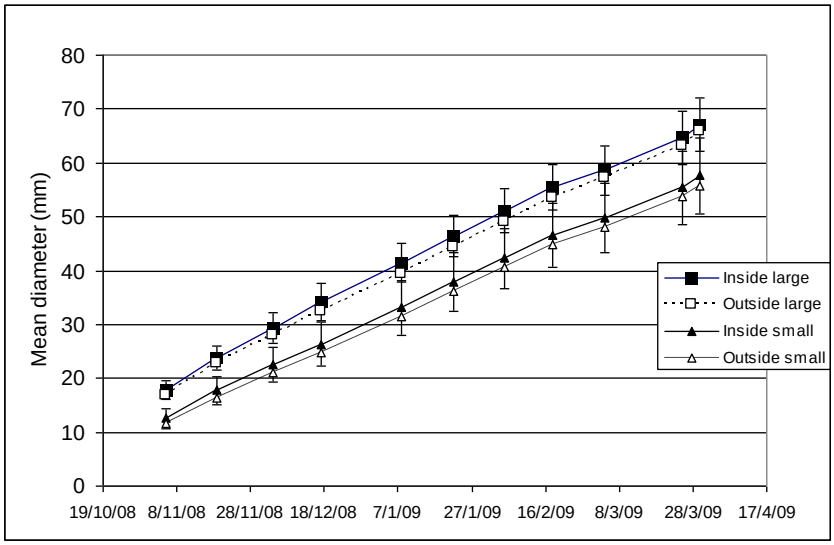

Figure 16 Trial J 2008/09 Mean size of tagged fruit measured at ~ fortnightly intervals. Bars represent standard deviations for fruit inside the canopy. Fruit were not matched into pairs.

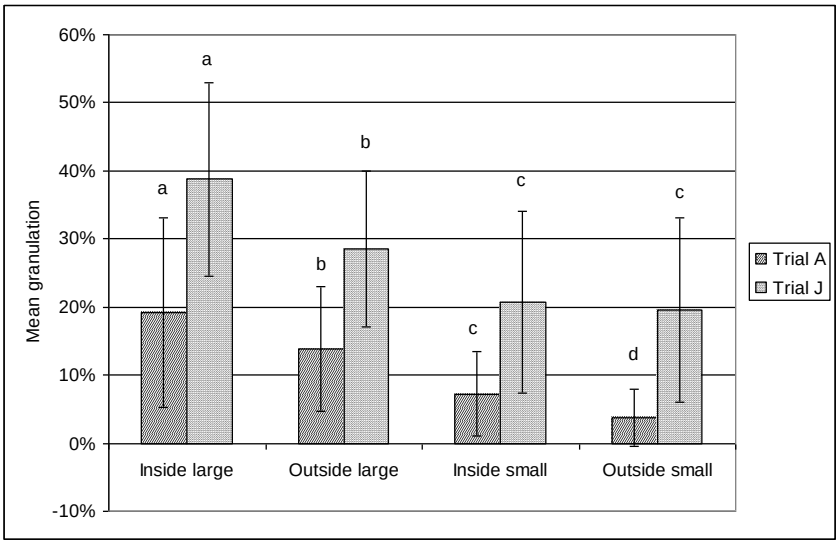

Figure 17 Trials A and J 2008/09 Mean granulation of tagged fruit by size and position. Vertical bars represent standard deviations. Fruit were not matched into pairs and sample numbers vary. Difference between treatments means assessed by two tailed t-tests assuming equal variances. Means in each trial with the same subscript are not significantly different at the 95% confidence level.

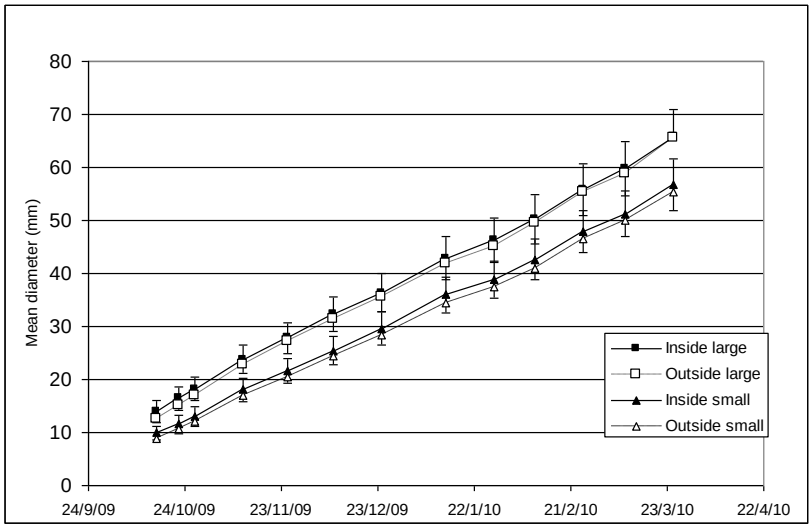

Figure 18 Trial A 2009/10 Mean size of tagged fruit measured at ~ fortnightly intervals. Bars represent standard deviations for fruit inside the canopy. Fruit were matched pairs of large and small (n=62 pairs inside, 62 pairs outside).

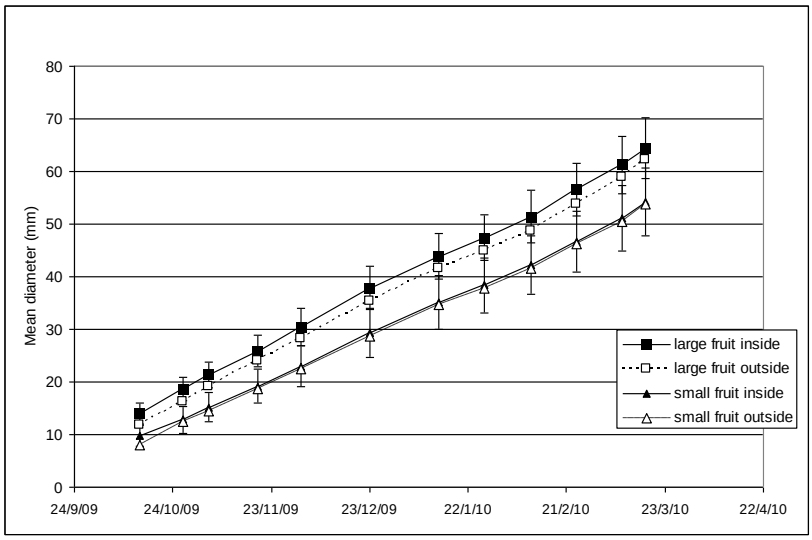

Figure 19 Trial J 2009/10 Mean size of tagged fruit measured at ~ fortnightly intervals. Bars represent standard deviations for fruit inside the canopy. Fruit were matched pairs of large and small at each location (n=71 pairs inside, 71 pairs outside).

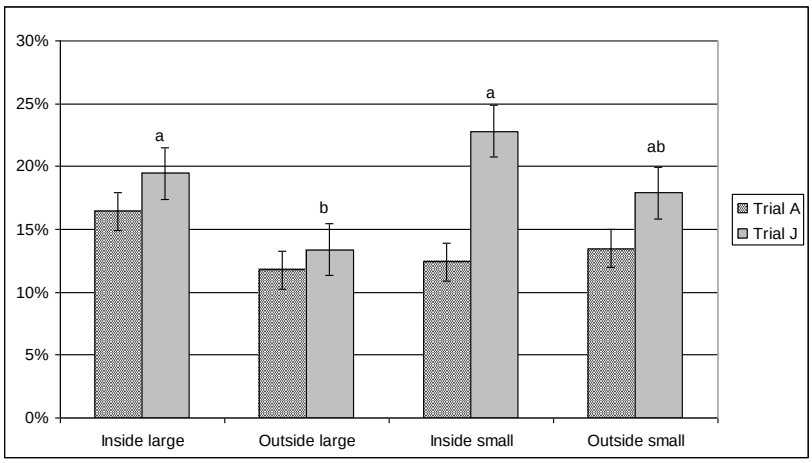

Figure 20 Trials A and J 2009/10 Mean granulation of tagged fruit by size and position. *P* (trial A=0.131); *p* (Trial J)= 0.013. Means with the same or no subscript at each trial are not significantly different at the 95% confidence level. Vertical bars represent standard errors of the means. Difference between treatments means assessed by analysis of variance.

The reason why inside fruit granulate more may be because nearby leaves appear to play a significant role in sucrose supply. Leaves export their assimilates primarily to nearby fruits (Kriedemann 1969; 1970). As there are fewer leaves on the inside of the tree, inside fruit are lower in soluble solids. There may also be less light for photosynthesis inside the tree. The pressure aspect of the water potential hypothesis may also be relevant here, that is, higher turgor due to less competition for water from transpiration in the shade of the canopy and/or increased water availability closer to the trunk may be contributing to increased granulation.

Measurements of photosynthetically active radiation (PAR) in 09/10 showed no correlations at either Trial A or J. This may be due to the difficulties of measuring light accurately or may be another instance of the lack of correlation between all measured parameters and granulation in that year (Table 11).

The significance of position in the tree in determining final granulation suggests that supply of water and assimilates from nearby leaves to the fruit is also important.

3.1.8 Harvest date

Trial data often show higher mean granulation at the first sample date than at the second (one month later)(see for example Figure 28, Figure 31, Figure 36, Figure 38, Figure 42, Figure 43, Figure 44). The data discussed above suggests that this is not due to the fruit 'getting better' over the ensuing month, but that the early large fruit (those prone to granulation) are picked by the grower before the second sample.

Tagging of sample pairs of the same size at the first harvest date and harvesting them at different dates removes the effect of commercial harvesting practice between sample dates. However, there are no clear trends in the results of this tagging, with some samples showing significant increases, others decreases and others no significant difference (Table 12). Over all trials in 2009, the second fruit in 45% of pairs had lower granulation and in 36% had higher granulation; but in 2010 only 39% had lower granulation and 42% higher granulation (Figure 21).

Table 12 Mean ratings and correlations of pairs harvested at different sample dates

Trial and year	no of pairs	Mean granulation rating first sample date	Mean granulation rating second sample date	*p*	Conclusion
A 2009	47	12.2%	6.1%	0.0008	2nd mean lower
J 2009	77	19.7%	28.1%	0.0009	2nd mean higher
K 2009	74	22.7%	13.5%	0.0008	2nd mean lower
A 2010	76	16.9%	12.3%	0.0028	2nd mean lower
J 2010	34	18.2%	19.3%	0.6561	means not different
M 2010	33	11.8%	16.2%	0.0685	means not different
P 2010	27	25.4%	29.4%	0.1753	means not different

p =t-test for paired two sample for means (two tail)

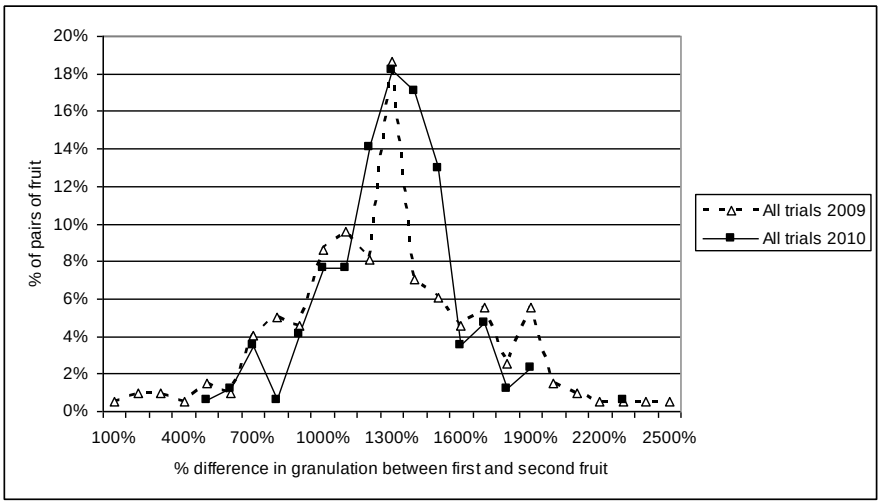

Figure 21 Distribution of fruit pairs by difference in granulation rating for all trials combined 2009 and 2010.

Across all trials, differences in ratings of the pairs does not correlate with the growth of the second fruit (r=0.03981 in 2009 and 0.1597 in 2010), which confirms the lack of relationship between growth and granulation of tagged fruit as discussed in Section 3.1.6 above. However, in 2009, at Trial J and K, but not Trial A, the size of the fruit when tagged appears weakly related to the difference in rating (r =0.3629, 0.2964, and -0.0898 respectively), that is, larger fruit 'got worse'. In 2010, this was the case for tagged fruit at Trials J and P, but not at Trials A and M (r= 0.3324, 0.2432, 0.0007 and -0.1919 respectively). This suggests that although granulation appears to be triggered early, and is more evident in larger fruit that begin to develop first, once in train it may exacerbate as time goes by.

In short, data is insufficient to support any conclusions about whether fruit improve or decline during Stage III but the overall impression is that there is no real change. On the other hand, the variable results could be interpreted to mean there are opportunities for late intervention to limit the severity of granulation. While *limiting* severity may be a possibility, the physiological nature of granulation as a process of cell and vesicle wall thickening and gelation, suggests that it is unlikely, once triggered, to be reversed.

3.1.9 Seasonal conditions

Survey data indicates that of the four years covered, mean granulation across all blocks was the highest in 2010. Mean granulation was 11.6% in 2007, 13.4% in 2008, 10.3% in 2009 and 19.8% in 2010 (Figure 23). In 2010, 33% of fruit were rated at 25% granulated or higher, compared to 23%, 27% and 16% in 2007, 2008 and 2009 respectively. In 2010, the proportion of partly granulated fruit (rated at 10% granulated through to 35% granulated) increased significantly, rather than the very granulated fruit (rated 65-100%)(Figure 22).

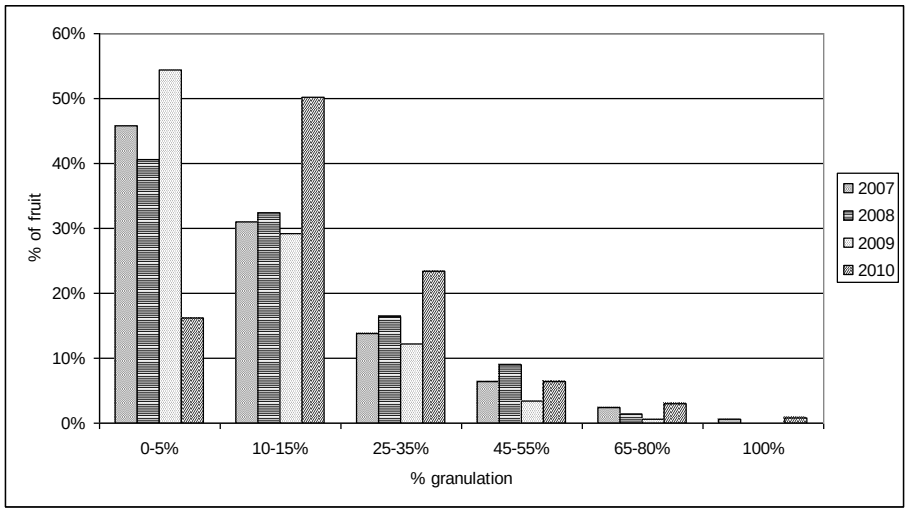

Figure 22 Proportion of fruit by granulation rating in surveys from 2007 to 2010.

The examination of data on seasonal conditions in Gayndah indicated several factors that followed a roughly similar trend to mean granulation of survey blocks shown in Figure 23.

Possible trends include:

- higher rainfall in early Stage I, particularly September (Figure 24);
- higher rainfall in February (late Stage II). Note that this is very late in fruit development which may suggest that early fruit development is not the only period of importance in determining final granulation severity (Figure 25);
- warmer winter temperatures, particularly August (Figure 26). This pattern fits closely to the pattern of observed granulation in the last three years, but overall is less close than the rainfall patterns. However, averaging temperature over a month would blunt peaks of shorter periods, such as those in winter/spring of 2010 (Figure 27).

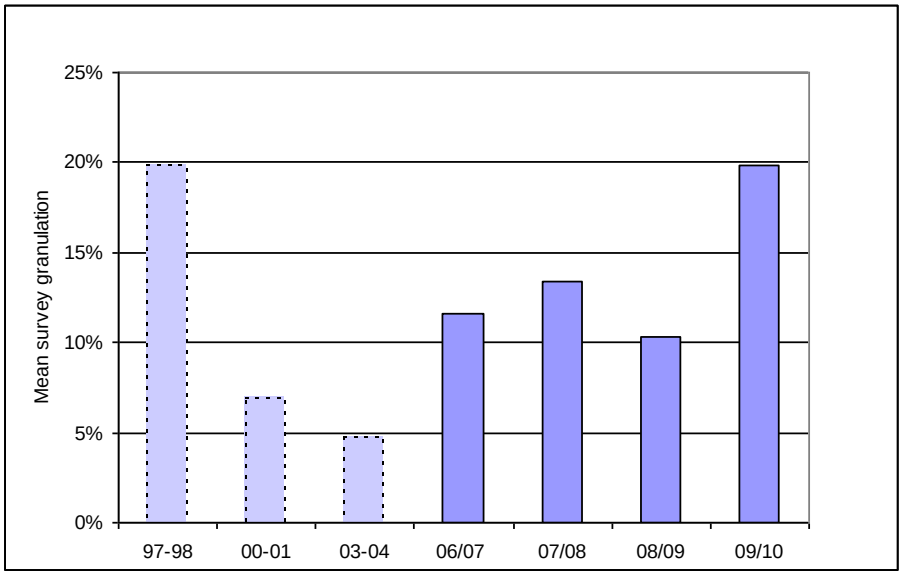

Figure 23 Mean granulation of all surveyed blocks 06/07 to 09/10 and estimates for 97/98,00/01 and 03/04.

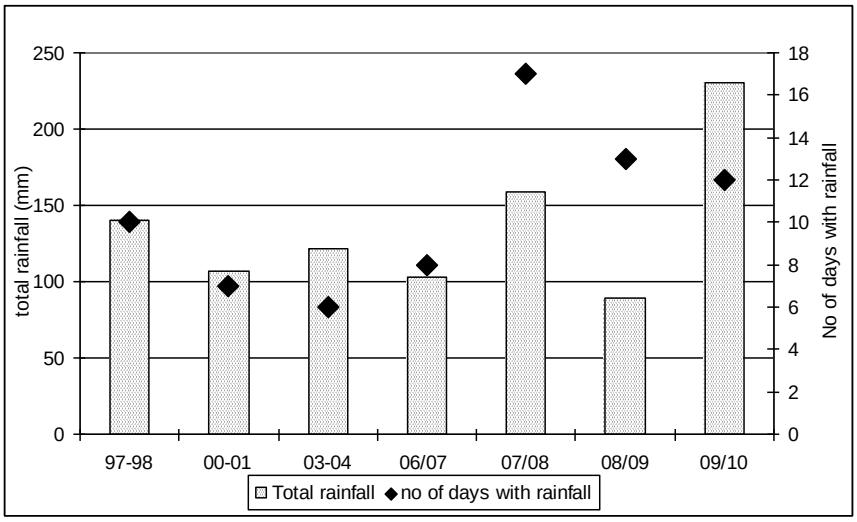

Figure 24 September rainfall and number of days with rain at Gayndah in selected years.

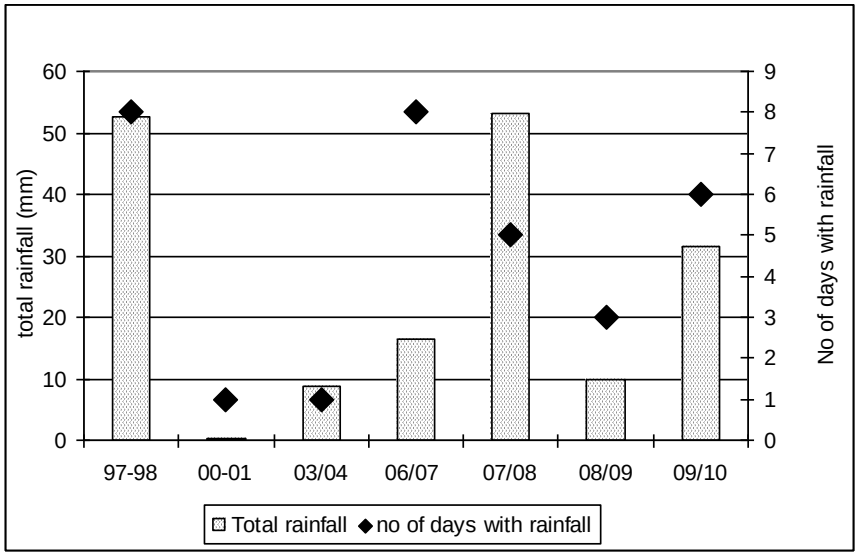

Figure 25 February rainfall and number of days with rain at Gayndah in selected years.

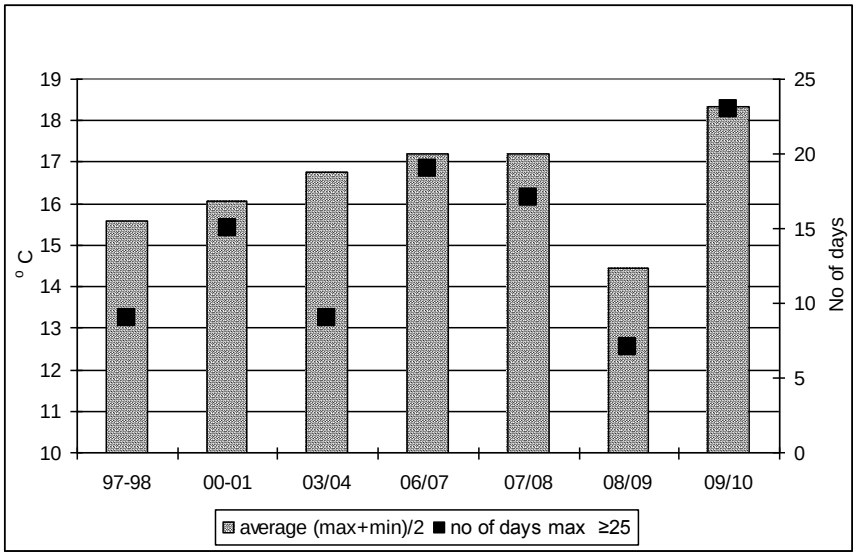

Figure 26 August temperatures (average of daily maximums and minimums) and number of days where the daily maximum was ≥25ºC at Gayndah in selected years.

None of these possible trends match the trends in granulation precisely but an exact match would be unlikely as weather data is for a single point (Gayndah PO/airport) which would not reflect varying conditions at all survey sites in Gayndah, Bundaberg and Mundubbera. Crop load can also have a strong seasonal impact, for example, this was an important influence in the 09/10 year as discussed in Section 3.1.3 above. Similarly, an exact match could not be expected if the seasonal trend is one of an 'either/or' scenario. The exceptions to a trend may indicate that not all factors need to occur for the year to be 'dry'. For example, in 97/98 winter was not particularly warm, nor was it wet in February. However, it was very wet in Stage I.

These suggested patterns fit with two published reports. Van Noort (1969) reported increased granulation of navels in South Africa when winter or early spring is warmer than average, followed by heavy rain in late summer or early autumn. Ritenour *et al.* (2004) suggest that the severe granulation in Florida's 2003 navel oranges crop was possibly due to higher than average daily temperatures in February and March, (i.e. August and September in the southern hemisphere), higher maximum (but not average) temperatures in October and November (i.e. April and May), a compressed bloom period around 10 March (September), low fruit set, late summer and early autumn rains in August to September) (i.e. February – March).

Van Noort (1969) suggests the reason for observed patterns is that early heat causes accelerated development of fruit so it is more advanced at harvest. This assumes that granulation is a product of maturity which is not supported by the growth patterns observed in tagged fruit data nor by the detectable levels of granulation in thinned fruit examined in January and February.

As discussed in Section 3.1.6, tagged fruit data suggests seasonal conditions at the time fruit start growing is likely to be the key issue. This implicates the extent of competition from flowers and expanding flush. This is a time of heavy carbohydrate demand on the tree to support flower, fruit and flush development simultaneously. One estimate is that 27% of the tree's total production of photoassimilates for the year are used in the processes of flowering and early abscission of fruitlets (Bustan and Goldschmidt 1998). Moreover, a new vegetative growth flush has a stronger demand on assimilates than fruit before the flush becomes a 'source' rather than a 'sink'(Kriedemann 1969). Thus seasonal conditions that advance flowering, accelerate or decelerate the bloom, and/or promote flush development may exacerbate competition for carbohydrates.

This explains the three identified trends in seasonal conditions. Warm winters may cause earlier flowering, so reserves built up over winter may be inadequate to meet demand. If late winter/early spring heat compresses the bloom period, the effect may be to exacerbate competition from flowers and/or rapidly expanding flush. Rainfall in early spring (September) may stimulate flush and/or increase cell turgor in young fruit. Rainfall at this time may also leach nutrients from the soil. Similarly, late Stage II rainfall (February) may again stimulate flush/root growth and/or sustain cell turgor.

In most years it is the larger fruit that are most affected, as they begin to develop at a time when there is the greatest competition from flowers and expanding flush. Shorter temperature or rainfall peaks during late bloom and early fruit development may explain the years, such as 2010, in which smaller (later) fruit have a tendency to granulate as well as the

larger (earlier) fruit. There were two peaks in daily maximum temperatures in late winter of 2010 (Figure 27). If the second peak caused increased flush development and expansion (particularly where accompanied by rainfall), this might explain the prevalence of granulated smaller fruit in that year.

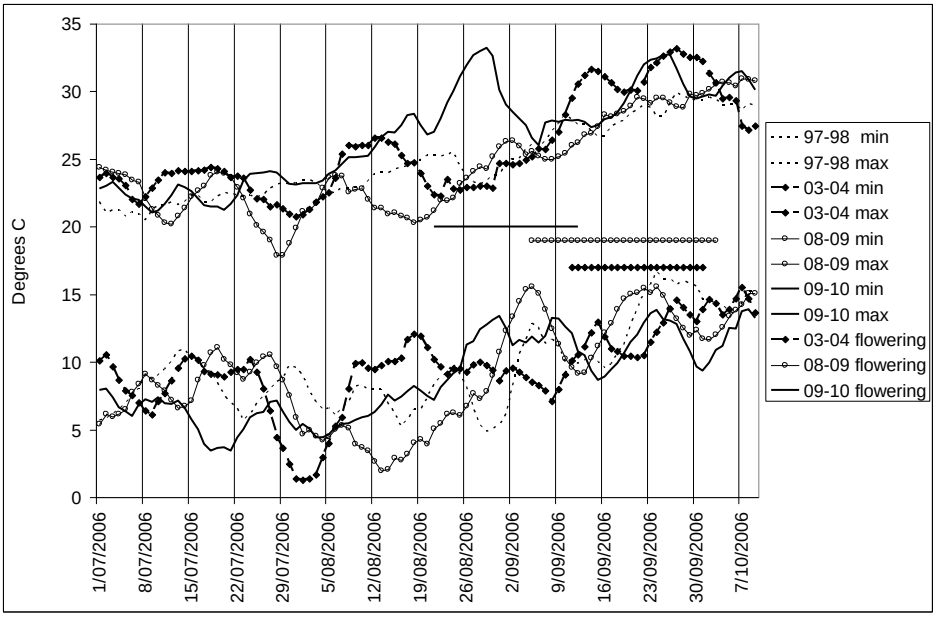

Figure 27 Smoothed (seven day average) daily maximum and minimum temperatures at Gayndah PO/airport for 97/98, 03/04, 08/09 and 09/10 and approximate timing of bloom in 03/04, 08/09 and 09/10. Flowering dates in 97/98 uncertain.

In summary, higher than average temperatures or rainfall in late winter and early Stage I appear to be the key seasonal risk factors. The importance of early fruit development suggests that growers need to pay attention to the extent and timing of flowering and flush and whether these are likely to exacerbate the already high competition for carbohydrates between flowers, fruit and flush.

3.1.10 Rootstock and scion size and 'benching'

Survey data shows consistently weak negative correlations between scion and rootstock circumferences and granulation, that is, larger and older trees are less likely to granulate (Table 7). This correlation is not evident in trial data where trees are more consistent in size (Table 8).

The degree of benching at rootstock and scion unions, particularly on Troyer rootstock, has been suggested as the reason for the prevalence of granulation in this rootstock. However, neither survey nor trial data show correlations between the size of the 'bench' at the join or scion and rootstock (Table 7 , Table 8).

3.1.11 Early estimation of crop granulation

Mean granulation ratings of fruit thinned from trees in late January and early February correlate well with final granulation ratings from the tree (Table 13).

Although results suggest that granulation is triggered early in fruit development, assessing granulation at this stage may still be of some practical use to the grower. It could prompt

decisions on whether or not to reduce irrigation frequency (see discussion in Section 3.2.3), indicate the need to assess some areas or blocks to see whether fruit will be so badly granulated that they should not be harvested, or, where levels of granulation are not too severe, to pick earlier rather than later once fruit is mature.

Table 13 Correlation of thinned fruit ratings with granulation ratings at harvest

Trial	Date thinned	Harvest sample	n	r	*p*
Trial A [a]	28/01/2010	1	57	**0.6172**	**0**
		2	57	**0.4990**	**0.0001**
Trial D [b]	10/02/2010	1	32	**0.4277**	**0.0146**
		2	32	0.3246	0.0699
Trial M [c]	3/02/2010	1	20	**0.5288**	**0.0165**
		2	20	**0.7332**	**0.0002**
Trial J [b]	11/02/2010	1	32	**0.6631**	**0**
		2	32	**0.6214**	**0.0001**

a Four-tree plots: thinned fruit lying between two central trees compared to means of those two trees at harvest
b Three-tree plots: thinned fruit around single central tree compared to plot means for harvested fruit
c Three-tree plots: average of thinned fruit around all trees compared to plot means for harvested fruit
Bold text = correlations where values of r ≥0.3 and p<0.05; *p*= two sided test of correlations different from zero

3.1.12 Conclusions and implications for management

It is now clear that granulation is triggered in early fruit development; therefore management needs to focus on supporting flowering and early fruit development. A key area would be focusing on nutrition to support the expansion of flush and the development of young fruit. Canopy management is also likely to be an area where improvements could be made; opening up the canopy to allow light inside may help reduce granulation of fruit on the inside of the canopy. Boosting carbohydrate storage over autumn/winter through appropriate nutrition may also help.

Observation of climate variables in winter and early spring may indicate those years where the risk of granulation is highest. Analysis to date suggests that warmer winter temperatures, earlier flowering time and rainfall or heat spikes that stimulate flush could indicate risk years.

Management practices that prevent biennial bearing will also reduce the risk of 'dry' years. Growers should be wary of leaving fruit on the tree late into the season and should consider appropriate thinning and pruning strategies to smooth bearing patterns.

3.2 Results of management trials

3.2.1 Thinning trials

Results at Trial I suggest that granulation is not reduced by thinning heavily or stripping alternate branches treatments late in Stage II of fruit development (Figure 28). There was no

significant difference between mean granulation ratings for trees with naturally high crop loads, or the two thinning treatments. The low 'natural' crop load is more strongly associated with granulation than the thinning treatments (p=0.005, second sample date).

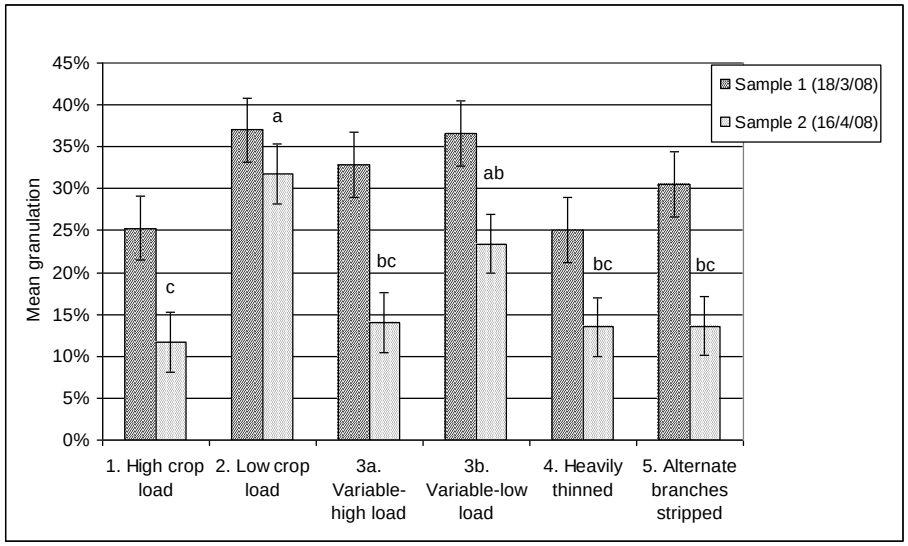

Figure 28 Trial I 2007-08 Mean granulation by thinning treatment. P= 0.155 (sample 1) and p=0.005 (sample 2): means with the same subscript are not significantly different at the 95% confidence level. Vertical bars represent standard error of the means.

The association of low crop load with granulation appears to apply on a limb for limb basis, as well as for the tree as a whole. This, and the lack of effectiveness of the stripping of alternative branches treatment, suggests that there is either no significant reallocation of carbohydrates/water around the tree or, if there is, it has little effect on granulated fruit at this later stage of fruit development (mid-Stage II).

Table 14 Trial I 2007-08 Percentage of fruit rated ≥25% granulated by treatment and mean crop load before thinning

Treatment	1. High crop load	2. Low crop load	3a. Variable-high crop load limbs	3b. Variable-limbs with low crop load	4. Heavily thinned trees	5. Alternate limbs stripped	*p/ lsd*
Sample 1	63%	86%	85%	91%	70%	78%	p=0.077
Sample 2	18%	69%	31%	53%	30%	25%	p=0.002
	c	a	bc	ab	bc	c	lsd= 24%
Crop load/tree before thinning[1]	9.4	1.8	6.0		10.1	10.2	p=0.001
	a	c	b		a	a	lsd =2.4

Notes: Means with the same or no subscript on each sample date are not significantly different at the 95% confidence level

1. Mean no of fruit in 0.125m^3 quadrat

At Trial E, mean granulation for the heavy thinning and stripping of alternative limbs was significantly higher than for the other treatments (p=0.049) (Figure 29). However, the mean crop loads before thinning of the 'heavy thinning' and ' stripping of alternate limbs' treatments (8 and 10 per 0.125m^3 quadrat respectively) were significantly lower (p=0.023) than the other treatments (13-14 per quadrat). The granulation means are most likely due to the 'natural' crop load rather than thinning treatments.

Table 15 Trial E 2007-08 Percentage of fruit rated ≥25% granulated by treatment

Treatment	1. Control	2. Heavily thinned trees	3. Half tree stripped	4. Alternate limbs stripped	5a. Alternate limbs thinned– untouched limbs	5b Alternate limbs thinned – thinned limbs	*p* value
Sample 1	76%	89%	71%	92%	65%	66%	p=0.177

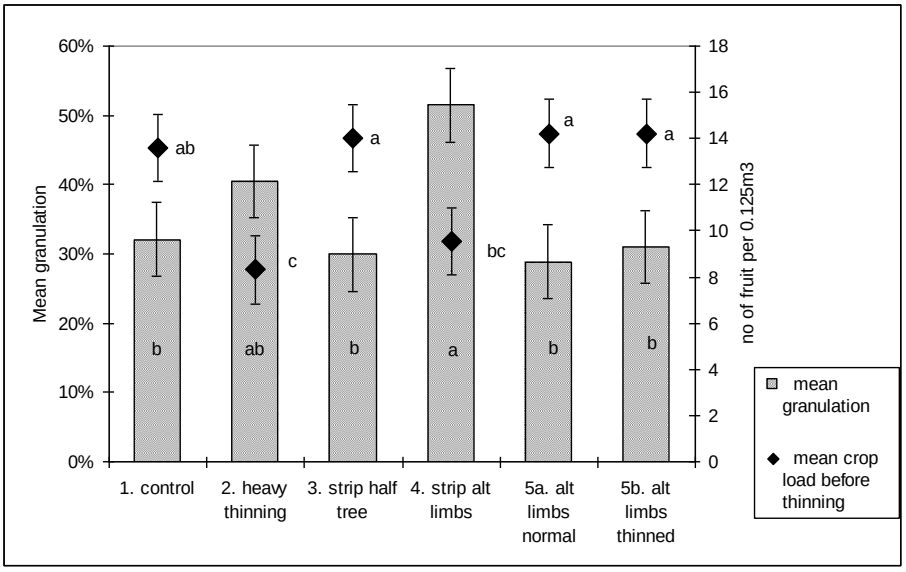

Figure 29 Trial E 2007-08 Mean granulation by thinning treatment and crop load before thinning: *p*(granulation means)= 0.049; *p* (crop load means)=0.023. Means with the same subscript are not significantly different at the 95% confidence level. Vertical bars represent standard error of the means. Means adjusted for covariate of tree health rating on 6/3/08.

The results of these trials support the conclusion that granulation is triggered early in fruit development and that late intervention strategies will have lesser effect than early intervention. They also support the need for reducing biennial bearing patterns. Thinning does not seem to be a strategy that can reliably be used to reduce the granulation of harvested fruit. Fruit tagging showed that both small and large fruit can granulate in some years, and that final fruit size is determined mostly by inception date. However, thinning may be an important tool in reducing biennial bearing.

3.2.2 Plant growth regulator trials

Overall, the spraying of plant growth regulators late in Stage I was not effective in reducing granulation: none of the treatments at the three trials in 2007/08 reduced granulation compared to the control.

At both Trial F and Trial G there was no significant difference between mean granulation or incidence of granulation on either sample date (Figure 30, Figure 31, Table 16). At Trial H, the 356-TPA ('Tops') treatment increased granulation in comparison to the control, natural auxins ('Kelpak') and 2-4DP ('Corasil') treatments (p=0.032 and 0.031 on the first and second sample dates respectively).

Table 16 Trials F, G, H 2007-08 Mean percentage of fruit rated ≥25% granulated by treatment

			Product in treatment					
Trial	Sample date	No of sprays in treatment	Control	24 D-P 'Corasil'	Natural auxins 'Kelpak'	GA3 'Ralex'	356-TPA 'Tops'	*p* value/lsd
F	1(18/3/08)	1 spray	72%	73%	74%	70%	89%	*p*=0.471
		3 sprays		77%	67%	86%	84%	
	2 (16/4/08)	1 spray	53%	53%	64%	68%	66%	*p*=0.845
		3 sprays		79%	67%	69%	70%	
G	1(18/3/08)	3 sprays	39%	47%	42%	45%	37%	*p*=0.888
	2 (16/4/08)	3 sprays	13%	36%	20%	29%	28%	*p*=0.109
H	1 (19/3/08)	3 sprays	14%	19%	14%	27%	53%	*p*=0.054
	2 (17/4/08)	3 sprays	17%	13%	10%	30%	42%	*p*=0.012
			bc	bc	c	ab	a	lsd=0.1889

Trial F and H: means adjusted for covariates of tree health and crop load recorded on 6/3/08

This result at Trial H may be related to fruit growth since the mean size at harvest of fruit under the Tops treatment was significantly greater than the control (64.6 and 57.7 mm respectively)(*p*=0.032) (Figure 35). At Trial F, growth patterns were similar and mean diameters at harvest were not significantly different between treatments (Figure 33). At Trial G, growth rates were consistent between treatments (Figure 34) and mean diameters were only statistically different for the first two measurement dates (*p*=0.028 and 0.010) with fruit in the Corasil treatment being slightly larger.

At Trial H and F, granulation is better correlated with diameters measured at the later dates than with earlier diameters (Table 17). This is different from the patterns observed in the tagged fruit discussed in Section 3.1.6 and suggests that artificially exacerbated growth rates may be a factor. There may be a 'trade off' between boosting fruit size and fruit quality.

These trial results indicate that plant growth regulators applied late in Stage I are not able to solve the granulation problem and in some instances may exacerbate it.

Table 17 Trials F, G and H Correlations of granulation with mean diameter on four dates(all treatments)

Trial G n=167			Trial H n=201			Trial F n=299		
Date	r	*p*	Date	r	*p*	Date	r	*p*
21/11/07	-0.1869	0.0156	30/11/07	0.2209	0.0016	19/11/07	-0.0704	0.2248
19/12/07	-0.1105	0.1551	8/1/08	0.3465	0	18/12/07	0.0364	0.5306
18/1/08	-0.1229	0.1136	8/2/08	0.4020	0	16/01/08	0.1938	0.0008
18/3/ 08	-0.0822	0.2911	19/3/08	0.3720	0	18/3/08	0.3512	0

p= Two-sided test of correlations different from zero

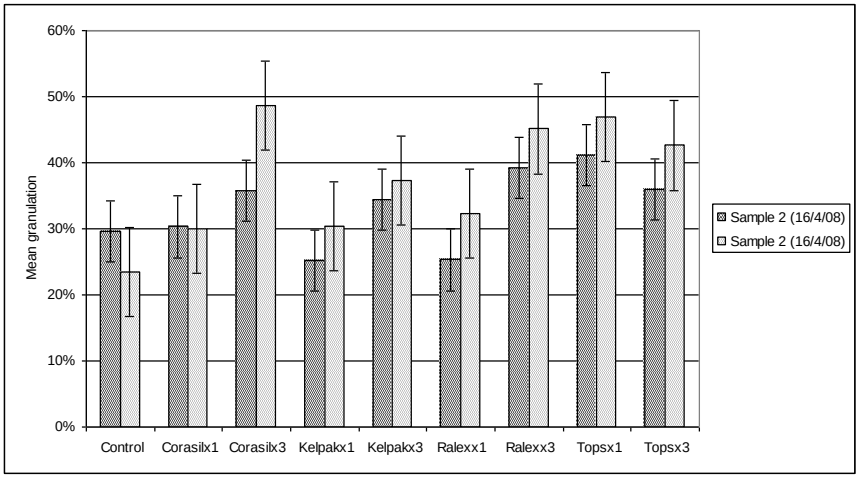

Figure 30 Trial F 2007-08 Mean granulation by PGR treatment: *p*(first sample)= 0.210; *p*(second sample)= 0.162. Vertical bars represent standard error of the means. Means adjusted for covariates of tree health and crop load on 6/3/08.

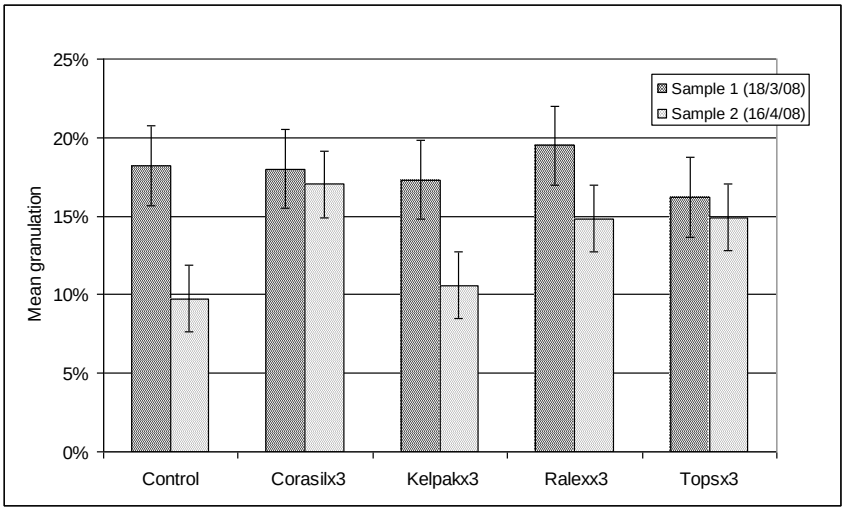

Figure 31 Trial G 2007-08 Mean granulation by PGR treatment: *p* (first sample)= 0.915; *p* (second sample)= 0.120. Vertical bars represent standard error of the means.

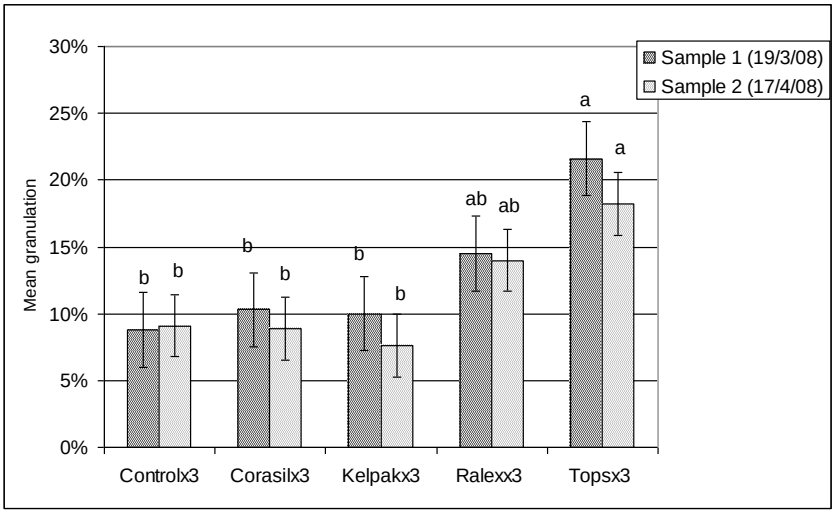

Figure 32 Trial H 2007-08 Mean granulation by PGR treatment: *p* (first sample)= 0.032; *p* (second sample)= 0.031 Means in each sample date with the same subscript are not significantly different at the 95% confidence level. Vertical bars represent standard error of the means. Means adjusted for covariates of tree health and crop load on 18/3/08.

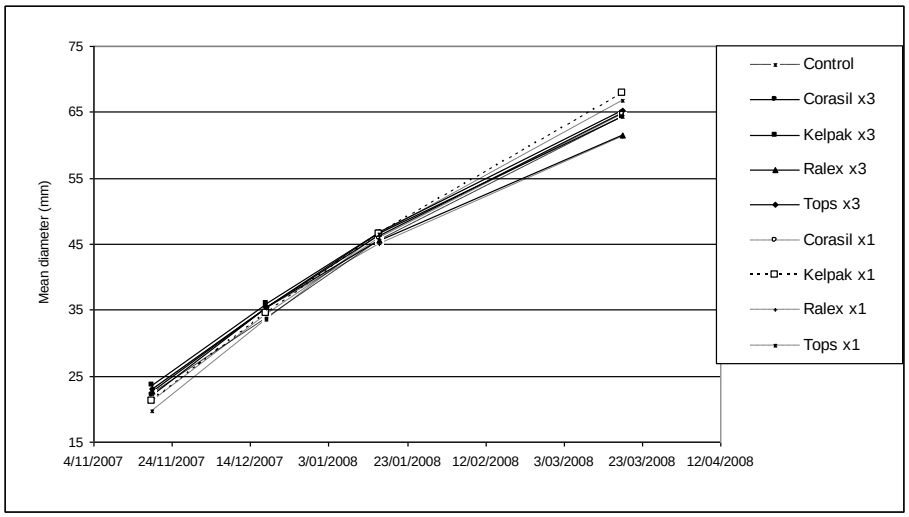

Figure 33 Trial F 2007-08 Mean fruit diameter by treatment on four dates.

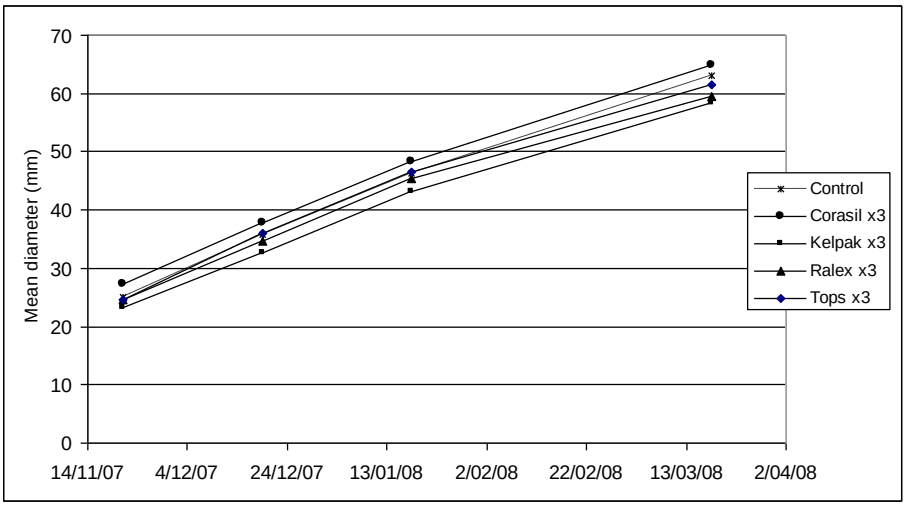

Figure 34 Trial G 2007-08 Mean fruit diameter by treatment on four dates.

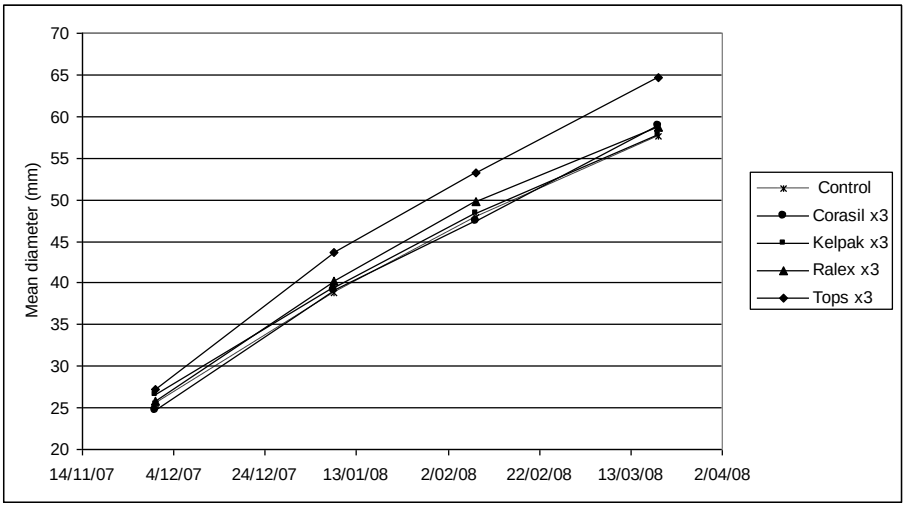

Figure 35 Trial H 2007-08 Mean fruit diameter by treatment on four dates.

3.2.3 Irrigation frequency trials

Overall, field trials of reduced irrigation frequency were not effective in reducing granulation. However, very promising results from a trial in pots in the nursery suggest some trial design factors need to be addressed in future research before ruling this out as a potential management option.

In the first year of trials (07-08), the reduced irrigation frequency treatments at Trial C showed encouraging results for the effectiveness of reducing irrigation frequency all year, although means were not statistically different (p=0.201 and 0.086 on the first and second sample dates respectively). Mean granulation was lowest for the 'low frequency all stages' treatment, at 29.6%% compared to the control of 36.2% on the first sample date and 21.3% compared to 26.7% on the second sample date (Figure 36). The proportion of fruit rated >25% was reduced from 93% to 73% on the first sample date (p=0.075) (Table 18).

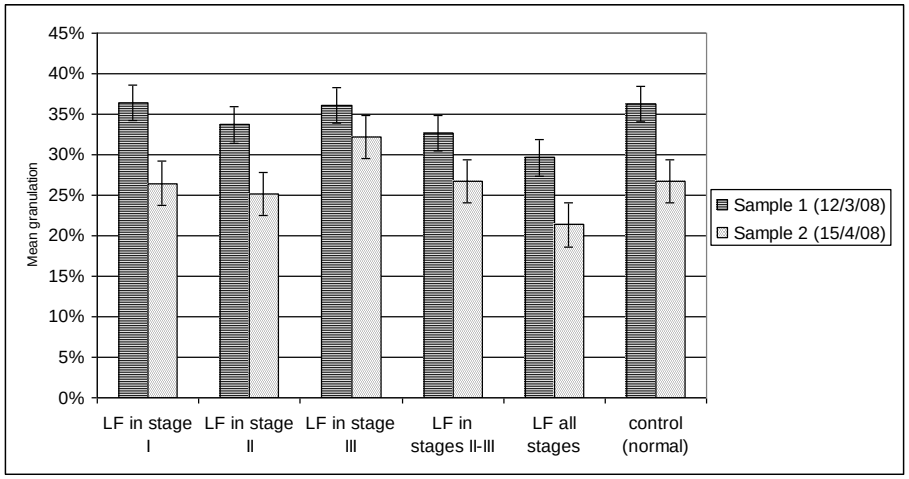

Figure 36 Trial C 2007-08 Mean granulation by irrigation frequency treatment: p (first sample)= 0.201, p(second sample)= 0.086. LF= low frequency. Vertical bars represent average standard errors of the mean. Means adjusted for spatial variation.

However, low frequency irrigation treatments at both Trial J and Trial D in the following two years were not able to replicate these findings (Figure 37, Figure 38, Figure 39, Figure 40). There was no benefit in reducing irrigation frequency at any stage of fruit development in either trial in 2008-09 or 2009-10 and some treatments showed an increase in granulation compared to the control. (Note that in 09/10 frequent rainfall in Stage III meant there were no irrigations at either trial and no treatment effects could be expected.)

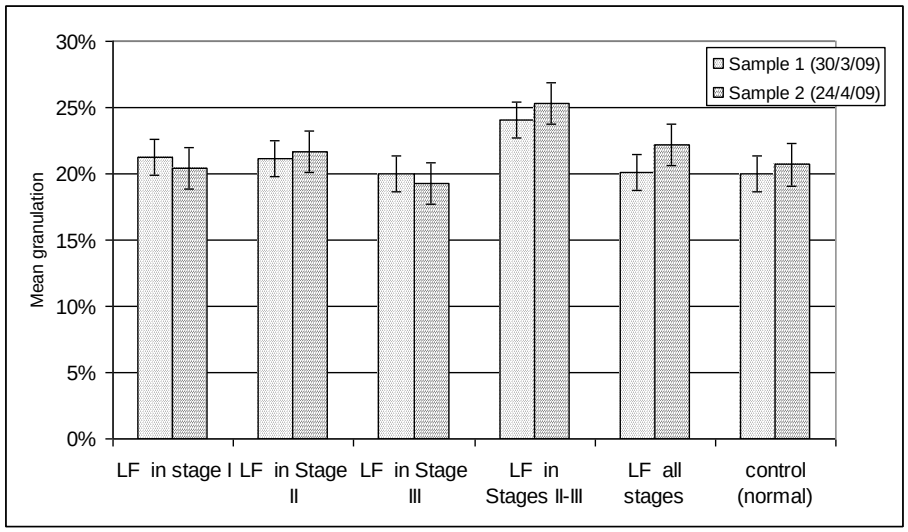

Figure 37 Trial J 08-09 Mean granulation by irrigation frequency treatment: *p*(first sample)=0.359, *p(*second sample) =0.268. LF= low frequency. Vertical bars represent standard errors of the means.

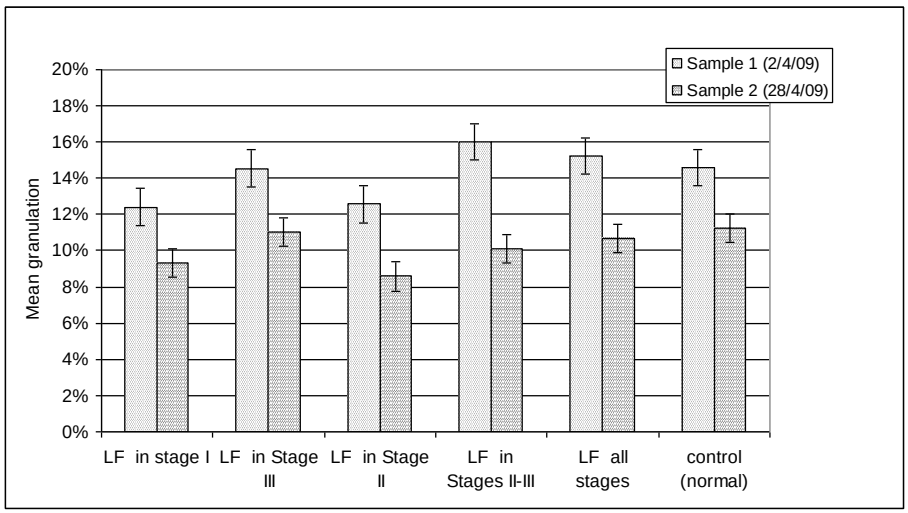

Figure 38 Trial D 08-09 Mean granulation by irrigation treatments: *p*(first sample)= 0.081, *p*(second sample) =0.124. LF= low frequency. Vertical bars represent standard errors of the means. Means adjusted for covariates of crop load 18/12/08 and tree health 30/9/08.

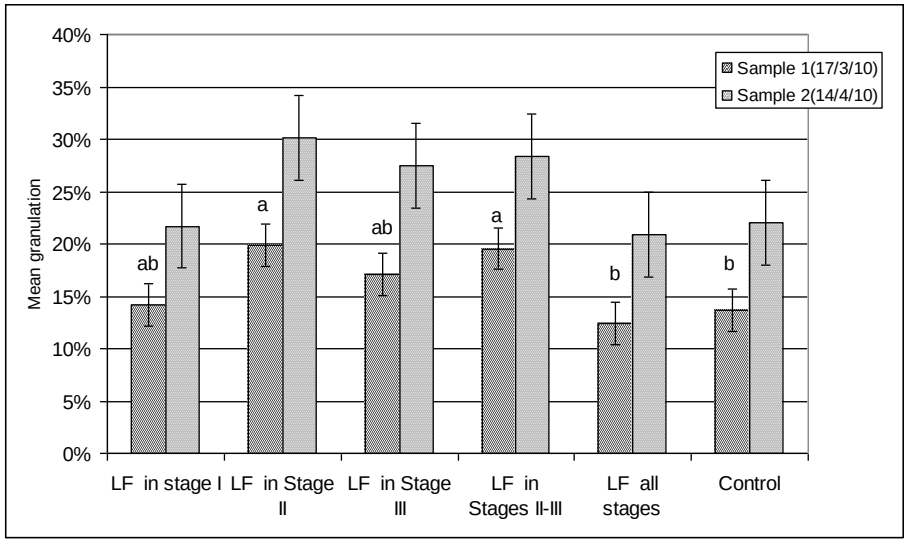

Figure 39 Trial J 09-10 Mean granulation by irrigation treatments: *p*(first sample)=0.048; *p*(second sample) =0.438. Means in each sample date with the same or no subscript are not significantly different at the 95% confidence level. LF= low frequency. Vertical bars represent standard errors of the means.

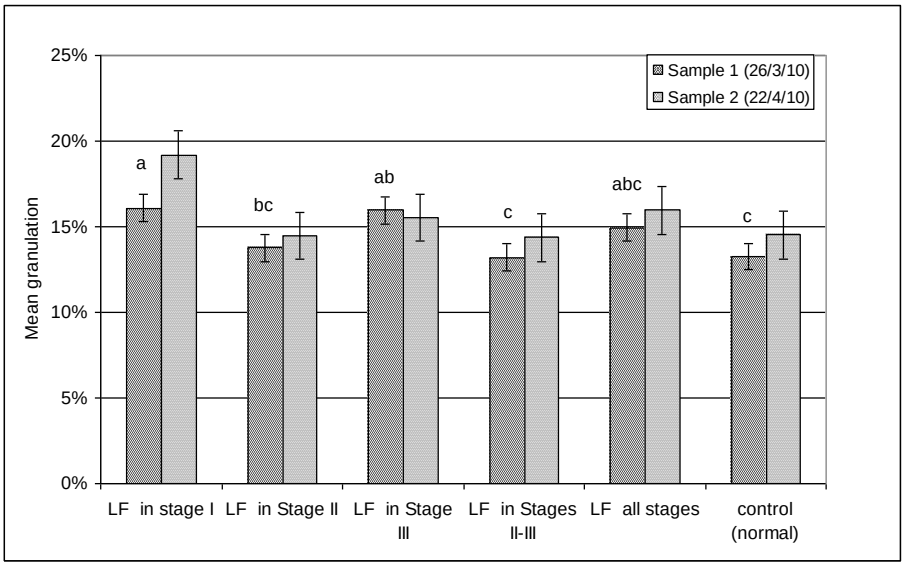

Figure 40 Trial D 09-10 Mean granulation by irrigation treatments: *p* (first sample)= 0.023; *p*(second sample) =0.120. Means in each sample date with the same or no subscript are not significantly different at the 95% confidence level. LF= low frequency. Vertical bars represent standard errors of the means. Means adjusted for covariates of crop load and tree health 6/1/10.

Table 18 Trials C, D, J and N Proportion of fruit ≥ 25% granulated by irrigation treatment

Trial and year	Sample	Lf in Stage I	LF in Stage II	Lf in Stage III	LF in Stages II & III	LF in all stages	Control [1]	p value / lsd
Trial C 07/08	1 12/3/08	92%	84%	89%	84%	73%	93%	p=0.075
	2 15/4/08	66%	59%	81%	69%	53%	66%	p=0.228
Trial D 08/09	1 2/4/09	19%	27%	21%	33%	29%	27%	p=0.100
	2 28/4/09	6%	8%	3%	6%	9%	12%	p=0.107
Trial D 09/10	1 26/3/10	29% a	17% bc	26% ab	15% c	22% abc	14% c	p=0.016 lsd=10%
	2 22/4/10	36%	20%	24%	23%	24%	18%	p=0.044
Trial J 08/09	1 30/3/09	52%	54%	49%	67%	52%	46%	p= 0.09
	2 24/4/09	42%	47%	41%	59%	49%	43%	p= 0.145
Trial J 09/10	1 17/3/10	23%	40%	34%	39%	16%	20%	p=0.060
	2 14/4/10	35%	51%	44%	49%	34%	38%	p=0.559
Trial N 09/10	Various dates	52% bc	89% a	92% a	79% ab	38% c	106% a	p=0.002 lsd=31%

Notes: 1 For Trial N, control = 'daily watering' treatment LF= low frequency.

Trial D means adjusted for covariate of tree health and crop load pre thinning (18/12/08 and 6/1/10); Trial N means adjusted for covariate of crop load

Means in each sample date with the same or no subscript are not significantly different at the 95% confidence level.

There was a positive response in both 08/09 and 09/10 to nutrient treatments in both Trials J and D (except in 09-10 at Trial D) (see Section 3.2.4), but there was no interaction between irrigation treatments and nutrient treatments in any year of either trial.

In the trial of trees in pots at the Bundaberg Research Facility (Trial N), mean granulation for trees watered daily was signficantly higher than trees under the lower frequency regimes (p<0.001). Treatments with the least granulation were the 'low frequency in all stages' treatment and the 'low frequency in Stage I' treatment, with mean granulation of 17.6% and 23.4% respectively, compared to 77.8% for the 'daily watering' treatment.

These treatments did not reduce crop load on average (p=0.465) but may have affected fruit size, although means were not quite significantly different (p=0.072, lsd=7.6mm)(Figure 41). The mean diameter of fruit when harvested (varying dates) was 60.2 mm for the 'low frequency in all stages' treatment, 67.3mm for the 'low frequency in Stage I treatment', and 68.5 mm for the 'daily watering' treatment.

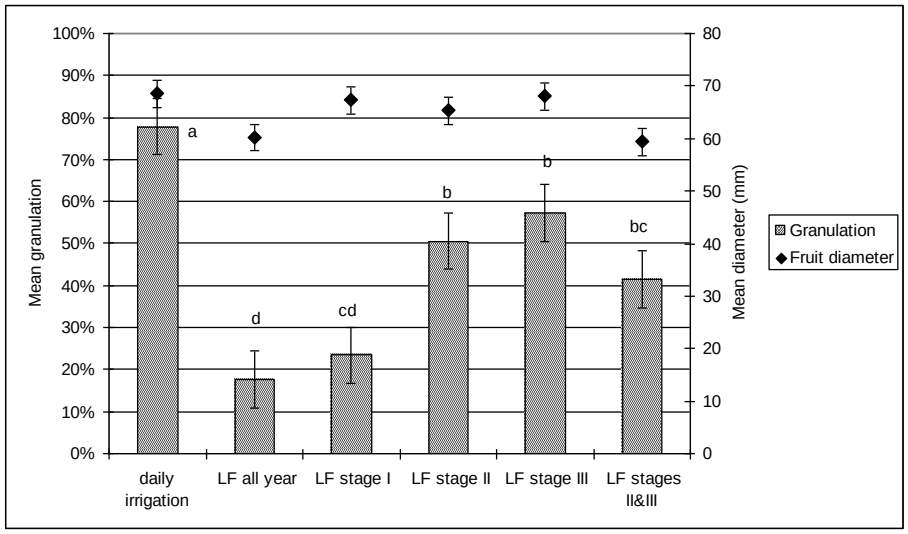

Figure 41 Trial N 2009-10 Mean granulation and diameter by treatment: p(granulation) <0.001. Means with the same subscript are not significantly different at the 95% confidence level. Means for fruit diameter were not significantly different (p =0.072). LF= low frequency. Vertical bars represent standard errors of the means.

While the results at Trials J and D may reflect the ineffectiveness of reducing irrigation frequency as a management practice in the field, it is also possible that trial design and implementation were at fault.

The number of 'taps off' irrigation events, where only control treatments were watered, were in practice much lower than envisaged when the trials were designed, and may not have been sufficient to stimulate treatment effects.

- At trial C in 07/08, the number of 'taps off' irrigations was five, compared to six full irrigations, and there were 10 major rainfall events (groups of days with greater than 10 ml rainfall on each day)(Figure 3).
- At both Trials D and J in 08/09, the total number of 'taps off' irrigations was six, compared to nine full irrigations, and there were six major rainfall events (Figure 4 and Figure 6).
- At Trial D in 09/10 there were six 'taps off' irrigations compared to eight full irrigations, and 12 major rainfall events (Figure 5).
- At Trial J in 09/10, there were only four 'taps off' irrigations compared to seven full irrigations, and 11 major rainfall events (Figure 7).

The disparity in numbers of 'taps off' and 'full' irrigations at Trial J was partly due to the grower's unwillingness to turn the taps off during the hottest months (December 2008 and January 2009 particularly). It means that 'low frequency' trees, by virtue of having two sprinklers rather than one, received more volume overall than control trees over this period. The grower at Trial D, however, consistently turned the taps off at every second irrigation, so this lapse alone could not explain the lack of results for Stage II treatments at both trials. Similarly, in 2009-10 rainfall was slight during Stage I and irrigations met the designed pattern but this did not have any significant effect on reduction of granulation in the Stage I LF treatment.

Rainfall totals or the number of rainy days do not provide an explanation. Rainfall at Gayndah from September to April inclusive was similar in 07/08 (626mm, 70 days with rainfall) to 09/10 (672mm, 67 days with rainfall), but somewhat lower in 08/09 (481mm, 71 days with rainfall), although rainfall in Stage I was highest in 07/08. More consistent patterns between the two trials would also be expected if rainfall events were the main reason.

In summary, the difference in results between the three field trials and the pot trial remains inexplicable. Trials in which both frequency and volume can be better controlled are needed to ascertain whether or not this management strategy has potential.

Sprinkler output

In 2009 at Trial B there was a strong correlation between the output of sprinklers and the granulation of individual trees in the year before treatments were applied (r (df=24) =0.6719, p=0.002). This correlation supported the contention that increasing irrigation increases granulation. However, at Trials P, K, J and M in 2010 there were no significant correlations (Table 8). The range of measurements was similar for both years at Trial P (in 2009 the range was 147-705 mm, s= 167mm, and in 2010 the range was 74-760 mm, s=127), so the reason for the lack of correlation in the second year is unclear. The effects of sprinkler output may have been overridden by treatment effects or by more regular rainfall, but data is insufficient to draw any conclusions.

3.2.4 Nutrition trials

Overview: nutrient treatments compared

In general, the effects of increased or decreased nutrient applications in Trials A, B and K were incremental reductions or increases in mean granulation. In most years, treatment means were not significantly different from the control or each other. None of the trials suggested that specific deficiencies or toxicities *per se* (except in N) are the main issue behind granulation.

Of all the nutrient treatments tested, the greatest and most consistent reduction in granulation over the range of trials and years was from the application of nitrogen in early Stage 1. Treatments where nitrogen was withheld or drastically reduced (Trial B, Trial P) showed dramatic increases in the rate of granulation.

There were no clear benefits from increased phosphorus applications, or from increased or decreased potassium levels. At both trial A and Trial B, a low granulation mean (not significantly different) for extra applications of Zn foliars also seemed promising and this treatments was pursued in subsequent trials. However, results in the following years were not consistent.

Granulation was highest for treatments with low levels of nitrogen, boron and zinc in the first year of trials. These latter two treatments were subsequently discontinued in the following two years at Trial A and replaced with a soil based zinc treatment and a foliar N application. However, soil application of zinc resulted in higher granulation in both years.

Results are explored in more detail below.

Timing of N applications

In Trial A, differences between treatments were not significant for all three years of treatments except for the first sample in 2008/09. However, over the three years, the best results were for 'extra N in Stage 1' in the form of broadcast urea, and also, less consistently, 'extra N in Stage II' and 'high zinc' (Figure 42, Figure 43, Figure 44 and Table 19).

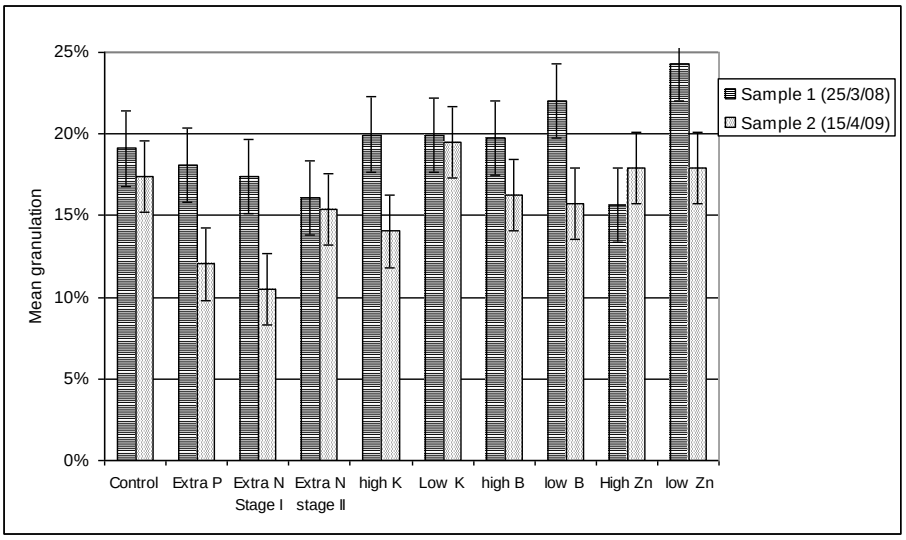

Figure 42 Trial A 07/08 Mean granulation by nutrient treatment: *p*(first sample)= 0.260, *p*(second sample) =0.133. Vertical bars represent standard errors of the means. Means adjusted for covariate of crop load at harvest.

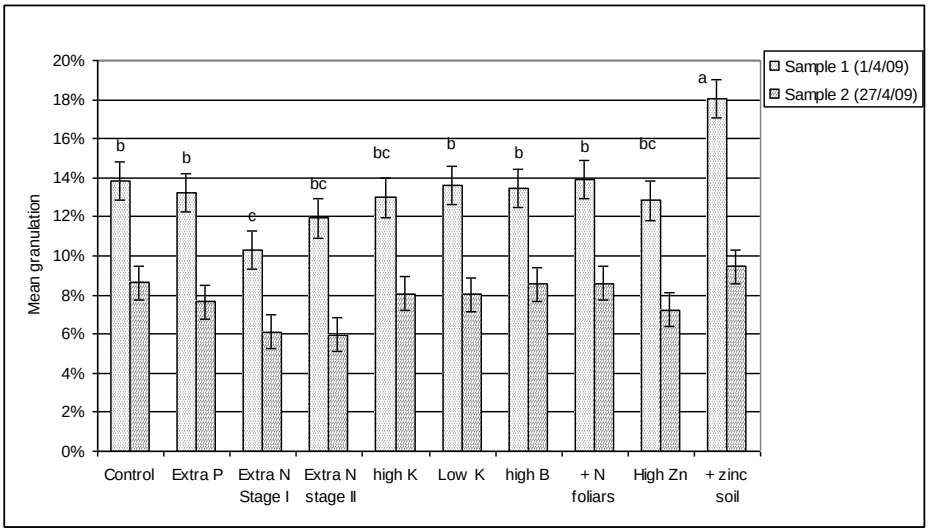

Figure 43 Trial A 08/09 Mean granulation by nutrient treatment: *p*(first sample)= 0.001, *p*(second sample) = 0.121: means in each sample date with the same or no subscript are not significantly different at the 95% confidence level. Vertical bars represent standard errors of the means. Means adjusted for covariate of crop load at harvest.

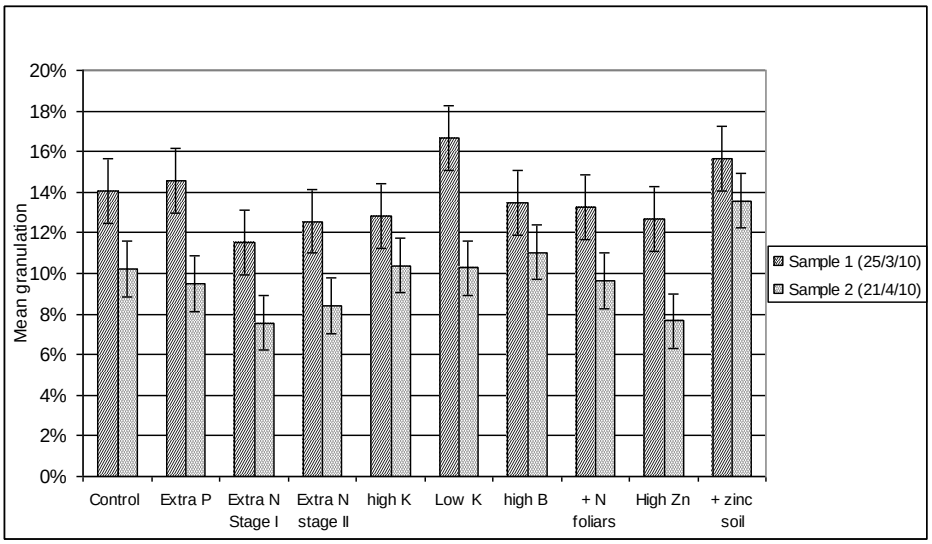

Figure 44 Trial A 09/10 Mean granulation by nutrient treatment: #(first sample)= 0.498, *p*(second sample) =0.114. Vertical bars represent standard errors of the means. Means adjusted for covariate of crop load at harvest.

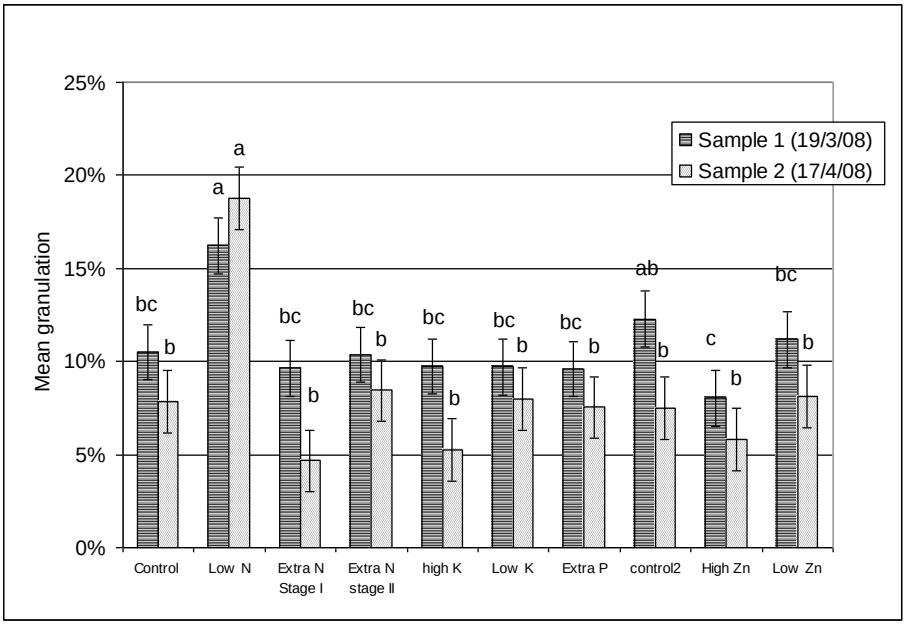

Figure 45 Trial B 07/08 Mean granulation by nutrient treatment: *p*(first sample)= 0.042, *p*(second sample) <0.001; means in each sample date with the same subscript are not significantly different at the 95% confidence level. Vertical bars represent standard errors of the means. Means adjusted for covariates of tree health and crop load at harvest.

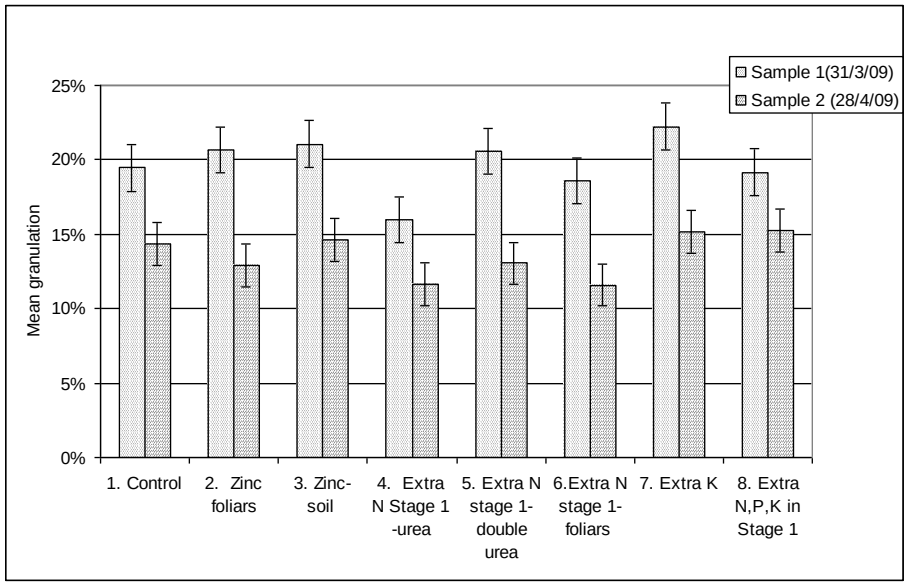

Figure 46 Trial K 08/09 Mean granulation by nutrient treatment: *p*(first sample)= 0.167, *p*(second sample) =0.374. Vertical bars represent standard errors of the means. Means adjusted for covariates of crop load and tree health.

At Trial K (2008/09), the mean granulation for the additional Stage I treatment (broadcast urea) was the lowest on the first sample date (*p*=0.167)(Table 20 Figure 46).

Table 19 Trial A and B Proportion of fruit ≥ 25% granulated by nutrient treatment

Trial and year	Sample	Control	Low N	Extra P	Extra n Stage I	Extra n Stage II	High K	Low K	High B	Low B	+ N foliars	High Zn	Low Zn	Soil Zn	*p value*/ lsd
A 07/08	1 (25/3/08)	50%	--	47%	44%	41%	53%	53%	46%	56%		39%	61%	--	*p*=0.513
A 07/08	2 (15/4/08)	36%	--	18%	20%	33%	25%	46%	34%	36%		42%	38%	--	*p*=0.121
A 08/09	1 (1/4/09)	22% ab	--	19% bc	9% c	12% bc	20% bc	18% bc	21% b	--	22% ab	14% bc	--	33% a	*p*=0.011 lsd= 11%
A 08/09	2 (27/4/09)	8%	--	5%	2%	1%	5%	7%	8%	--	10%	5%	--	9%	*p*=0.176
A 09/10	1 (25/3/10)	20%	--	25%	12%	17%	16%	34%	23%	--	21%	16%	--	27%	*p*=0.414
A 09/10	2 (21/4/10)	10 % abc	--	10% abc	9% c	2% bc	4% abc	9% abc	9% ab	--	13% ab	2% c	--	16% a	*p*=0.037 lsd=9%
B 07/08	1 (19/3/08)	24% bc	40% a	19% bc	20% bc	18% bc	20% bc	20% bc	--	--		11% c	20% bc	--	*p*=0.013 lsd= 13%
B 07/08	2 (17/4/08)	10% a	40% b	11% a	0% a	12% a	3% a	8% a	--	--		4% a	8% a	--	*p*<0.001 lsd=13%

Notes: Trial A means adjusted for covariate of crop load
Means in each sample date with the same or no subscript are not significantly different at the 95% confidence level.

Table 20 Trial K Proportion of fruit ≥ 25% granulated by nutrient treatment

Sample	Control	Zinc foliars	Zinc- soil application	Extra N broadcast	Double N broadcast	N foliars	Extra K	Extra N,P,K	*p value*/ lsd
1 (31/3/09)	50%	50%	53%	35%	55%	43%	60%	46%	*p* = 0.097
2 (28/4/09)	26%	24%	26%	21%	20%	18%	31%	28%	*p* = 0.397

Notes: Means adjusted for covariates of tree health and crop load
Means in each sample date with the same or no subscript are not significantly different at the 95% confidence level.

Additional N also performed well in the irrigation trials, with far more effect and consistency than the irrigation treatments. At Trial J in 2008/09 decreases in granulation with the application of N were not significant, but in 09/10 decreases were strongly significant (Figure 47). The proportion of fruit rated ≥25% granulated was reduced from 42% to 16% on the first sample date and, on the second sample date, from 61% to 23% (Table 21). At Trial D in 2009 but not 2010, granulation was reduced by applications of N although the only significantly different mean was for the 'N plus zinc' treatment at the first sample date in 2009 (Figure 48). There was no significant interaction with irrigation treatments at either trial in either year.

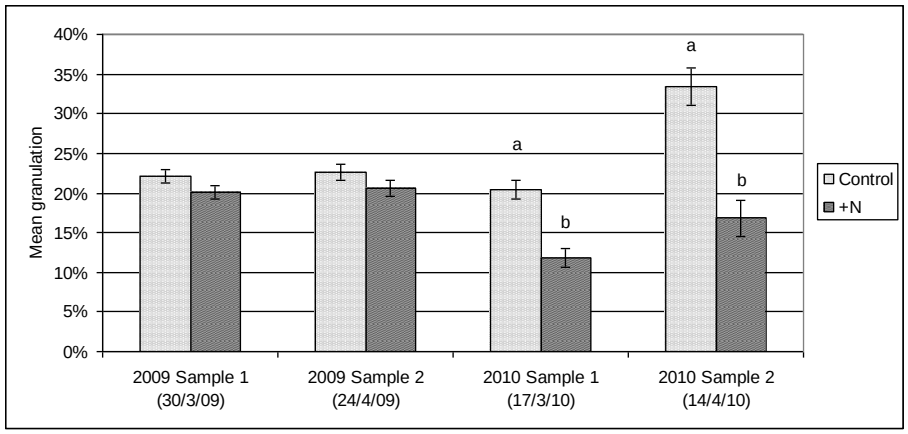

Figure 47 Trial J 08/09 and 09/10 mean granulation by nutrient treatment: *p*(2009 first sample) =0.118, *p*(2009 second sample) =0.194, *p*(2010 first sample) <0.001, *p*(2010 second sample) <0.001. Means on each sample date with the same or no subscript are not significantly different at the 95% confidence level. Vertical bars represent standard errors of the means.

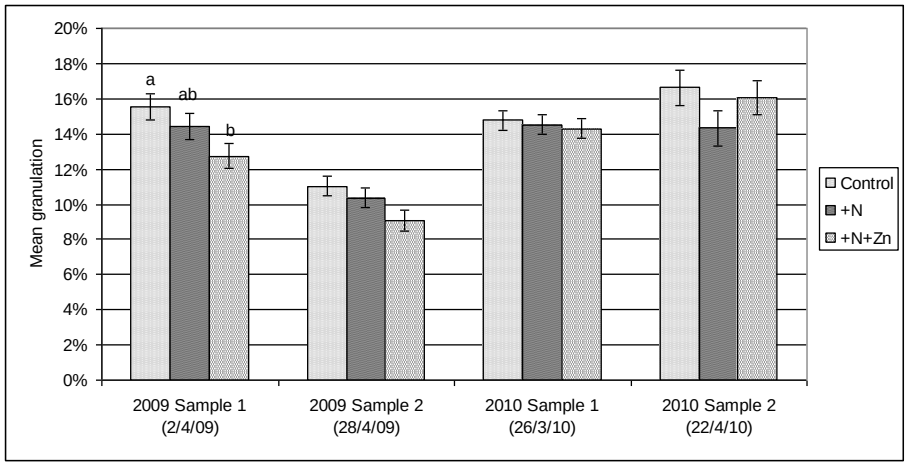

Figure 48 Trial D 08/09 and 09/10 mean granulation by nutrient treatment: *p*(2009 first sample) = 0.031, *p*(2009 second sample)= 0.067, *p*(2010 first sample) =0.855, *p*(2010 second sample) =0.229. Means on each sample date with the same or no subscript are not significantly different at the 95% confidence level. Vertical bars represent standard errors of the means.

Table 21 Trials D and J 08/09 and 09/10 Proportion of fruit rated ≥25% granulated by nutrient treatment.

	Control	+N	+N+Zn	ese	p value/lsd
Trial D					
2009 Sample 1 (2/4/09)	31% a	26% ab	21% b	3%	*p*=0.024 lsd = 7.3%
2009 Sample 2 (28/4/09)	8%	9%	5%	2%	*p*=0.224
2010 Sample 1 (26/3/10)	22%	20%	20%	3%	*p*=0.809
2010 Sample 2 (22/4/10)	27%	21%	24%	3%	*p*=0.399
Trial J					
2009 Sample 1 (30/3/09)	57%	50%	na	3%	*p*=0.106
2009 Sample 2 (24/4/09)	48%	46%	na	3%	*p*=0.565
2010 Sample 1 (17/3/10)	42% a	16% b	na	4%	*p*<.001 lsd= 11.1%
2010 Sample 2 (14/4/10)	61% a	23%b	na	5%	*p*<.001 lsd=13.7%

Means in each sample date with the same or no subscript are not significantly different at the 95% confidence level.

In Trial A, the extra N in Stage II treatments (application in early December) were less effective in reducing granulation than the Stage I treatments, although the difference in means was not significant in any years of the trial. The same amount of N was applied in Stage I and Stage II treatments. Analysis of soil N levels shows that soil N levels under Stage II treatments remained higher over subsequent months, suggesting that less of the Stage II application is used, even over the whole growing season, than that applied in Stage I (Figure 50). The earlier time is apparently more conducive to N uptake but as accumulated N stocks are not taken up in the spring following Stage II application, this may suggest that uptake may also be determined by heightened (even excess) availability in soil solution at the time of most demand.

In view of the hypothesis presented in Section 3.1.9, it is suggested that the effectiveness of the Stage I timing is due to supporting the growth and expansion of the spring flush, so that this flush moves more quickly from being a sink to a source.

The provision of N in winter also appears to be important, to be stored for mobilisation during the subsequent spring flush. Nitrogen during the spring flush is mostly sourced from storage organs: only 10% to 30% is supplied from soil (Martinez *et al.* 2002; Mooney and Richardson 1992; Sweet *et al.* 2009). Trial P tested three timings of winter N – early July and early August and a split application between these two dates – but there were no statistically significant different effects (Figure 49, Table 22). However, further research may be warranted into timing options with a greater differential than one month and how these could be best combined with Stage I applications.

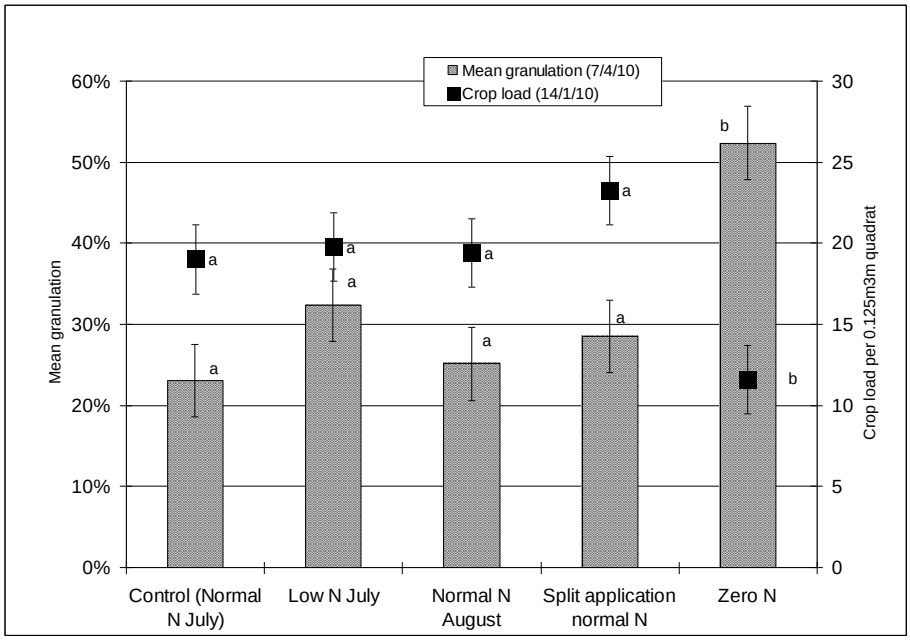

Figure 49 Trial P 09/10 Mean granulation and crop load by nutrition treatment: *p* (mean granulation)=0.001, *p*(mean crop load) = 0.025. Means with the same or no subscript are not significantly different at the 95% confidence level. Vertical bars represent standard error of the means. Means adjusted for covariate of tree health assessed on 8/7/09.

Table 22 Trial P Proportion of fruit rated ≥25% granulated by nutrient treatment

Sample	Control (Normal N July)	Low N July	Normal N August	Split application-normal N	Zero N	*p value*
1 (7/4/10)	45% b	63% ab	45% b	46% b	94% a	*p* = 0.049 lsd= 37%

Means adjusted for covariate of tree health assessed on 8/7/09. Means with the same subscript are not significantly different at the 95% confidence level.

It is hypothesised that aspects of the growers' practices in winter applications of N at trial sites may explain some trial results, for example,

- The low level of granulation overall at Trial B in 07/08 was unexpected, as the block had a history of granulation: mean granulation when surveyed in 2006/07 was 34.5%, in the trial year it was 10.9%. In hindsight it appears that the reduced granulation of this block may have been partly due to the comparatively high amount of urea applied in winter (1.2 kg/tree to all trees except the 'low N' treatment) compared to other trials and to the grower's applications in previous years.
- In Trial A, after the first year, the grower applied a second urea broadcast (0.31kg/tree) to all trees in the trial in September in 2008 and August in 2009. This possibly accounts for the relatively low levels of granulation in 2009 (13.7%) and 2010 (13.9%) compared to 2008 (19.0%).
- At Trial J, there were large reductions in granulation in response to extra N in Stage I in 09-10, particularly at the second sample date. At this trial, the winter application was only 1 kg of Granam applied by the grower in winter, that is, 202 g N, a relatively low amount.

- In Trial D in 2008-09, the combination of additional urea plus two extra zinc sprays reduced mean granulation by a small but significant amount, from 15.5% to 12.7% (p=0.031), or from 31% to 21% of fruit rated above 25% (p=0.100). In 2010, however, there was no difference between nutrient treatment means. This lack of result in 09/10 may be because in that year the grower applied N in a split dose in July and September, rather than a single dose in July. In addition, in response to grower concerns about the effect of extra N on the ability of fruit to degreen the fruit, treatment amounts were reduced from 0.6 kg urea per tree in 08/09 to 0.35 kg/tree in 09/10. The smaller amount applied in treatments and/or the subsequent grower application may have nullified treatment effects.

Measurement of soil N levels support the view that the tendency of some growers in the central Burnett in recent years to reduce N applications in order to encourage earlier maturity may be increasing granulation, at least in sandy and sandy loam soils. Additional broadcast N applications in Stage I at Trials J, M, P and K appear to be all used (or lost) by the time of soil sampling in late Stage II (Figure 51, Figure 52, Figure 53, Figure 54). In contrast, at Trial A, on a clay-loam soil, there was a build up of soil N over the three years of trials (Figure 50).

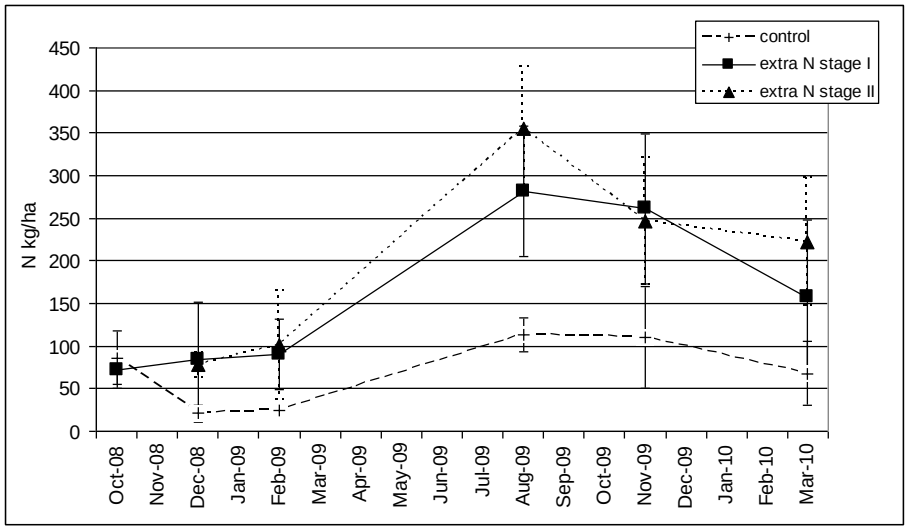

Figure 50 Trial A 08/09 to 09/10 Soil N levels by treatment. Vertical bars show standard deviations, n= 3 plots per treatment.

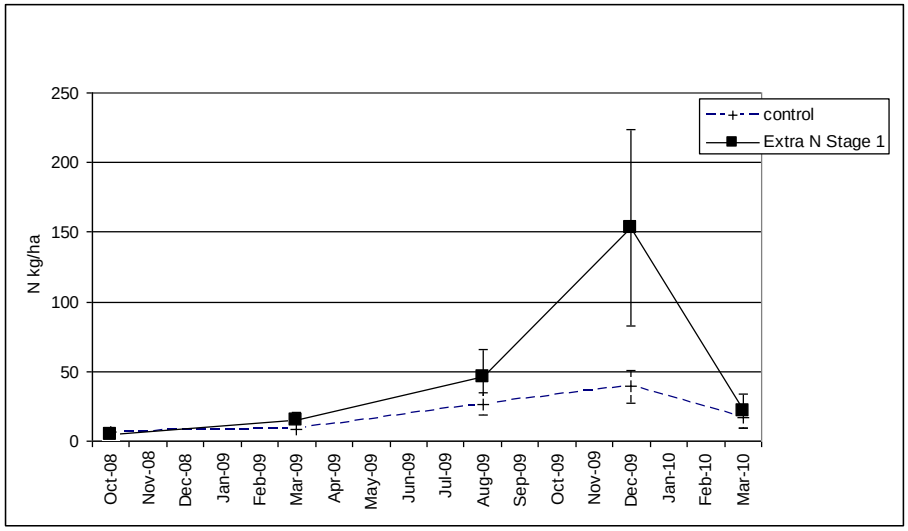

Figure 51 Trial J 08/09-09/10 Soil N levels by treatment. Vertical bars show standard deviations, n= 12 plots per treatment sin 08/09 and 6 plots in 09/10

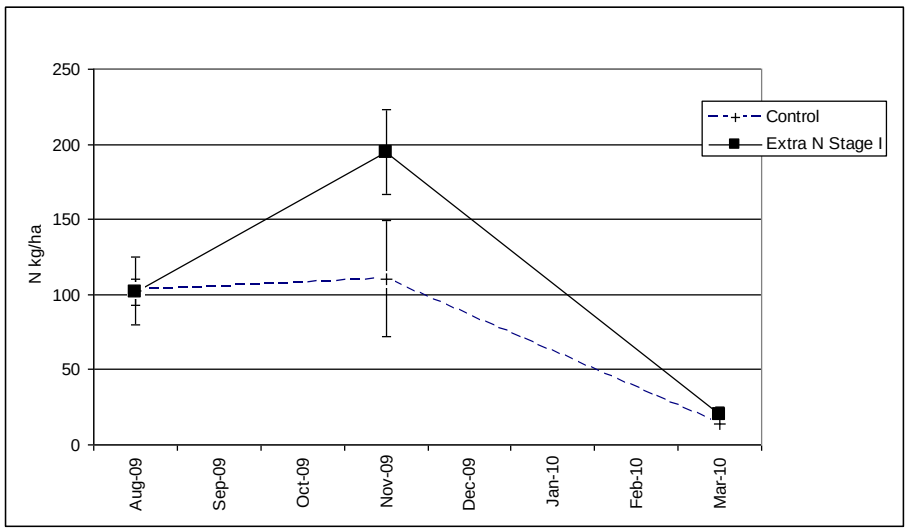

Figure 52 Trial M 09/10 Soil N levels by treatment. Vertical bars show standard deviations, n= 4 plots per treatment.

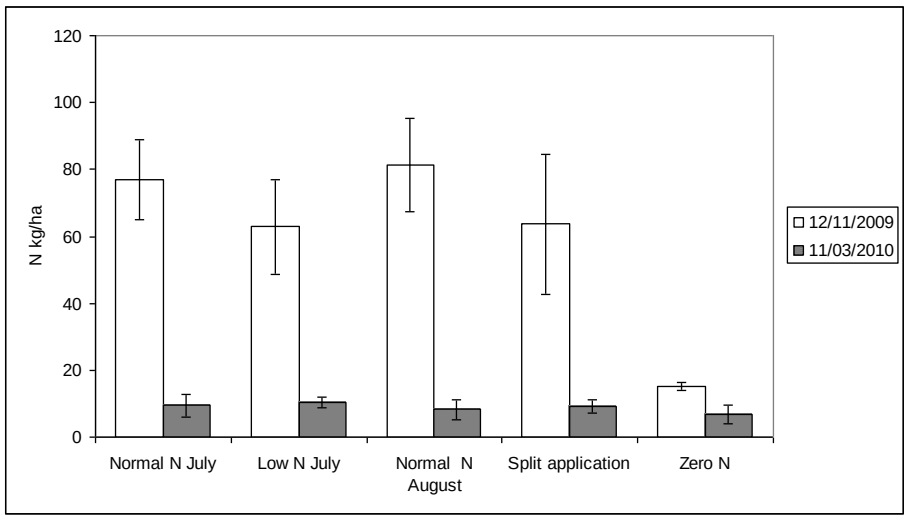

Figure 53 Trial P 09/10 Soil N levels by treatment. Vertical bars show range, n= 2 plots per treatment.

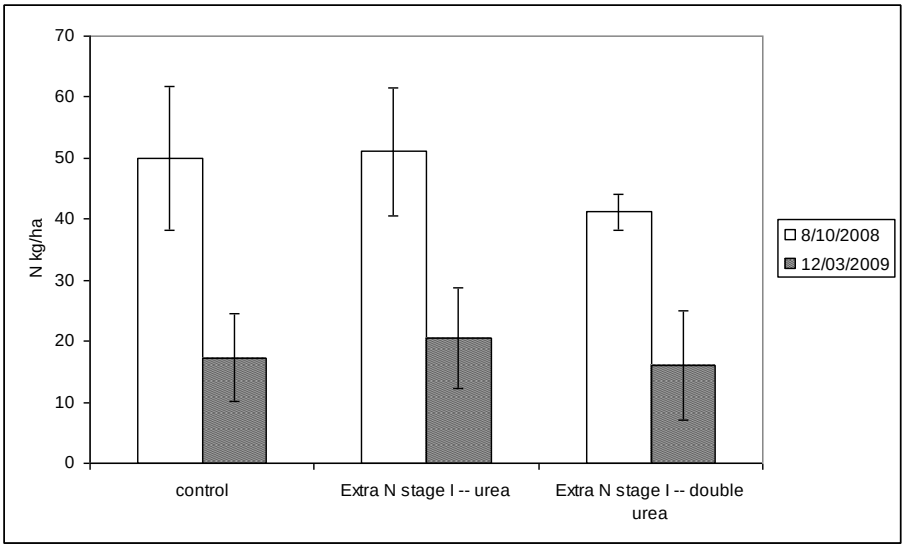

Figure 54 Trial K 08/09 Soil N levels by treatment. Vertical bars show standard deviations, n= 3 plots per treatment.

Rate of nitrogen application

Trial K tested two rates of applications in Stage 1: the 'extra N broadcast' application was 0.6 kg/tree urea and the 'double extra N broadcast' application was 1.2 kg/tree urea. While treatment differences were not significant at this trial (p=0.167 and 0.374 for the first and second samples respectively), means on the first sample date show an average decrease against the control for 'extra N broadcast' but not for the 'double extra N broadcast' (Figure 46). It is possible that this high rate of urea caused increased pH to the extent of inhibiting nitrification for a critical period. This may have been only temporary, as by the time of soil sampling in March, soil N levels were similar to the other treatments (Figure 54). No increase in vegetative growth was observed on these trees.

In terms of winter N applications, Trial B illustrates the importance of applying sufficient amounts. The only treatment mean that was significantly different from other means was the 'low N' treatment, which served to almost double the incidence of granulation (Figure 45). At Trial P, the zero N applications also resulted in significantly increased granulation above all other treatments, with double the granulation of the 'normal N in July' treatment (p=0.001) (Figure 49). The 'low N in July' treatment resulted in 9.3% higher granulation than 'normal N in July', although means were not significantly different (lsd= 12.4%). These results suggest that quantity is more important than timing for winter applications.

Foliar nitrogen

Overall, trial results suggest foliar N treatments can help reduce granulation to some extent, but gains are not as large nor as consistent as for broadcast N treatments.

Foliar N application reduced granulation at Trial C (Table 23), although gains were smaller than for the 'low frequency all stages' treatment' (Table 18).

Table 23 Trial C Mean granulation and proportion of fruit rated at **≥25% by foliar nutrition treatment.**

	Zinc	Boron	Phosphorus	Nitrogen	Control	p value
Mean granulation:						
Sample 1 (12/3/08)	33.0%	34.1%	36.4%	31.7%	35.4%	p=0.099
Sample 2 (15/4/08)	25.4%	26.7%	28.6%	23.7%	27.6%	p =0.105
% of fruit rated at ≥25%:						
Sample 1 (12/3/08)	82% a	85% ab	93% b	80% a	89% b	p=0.01
Sample 2 (15/4/08)	61%	63%	73%	61%	70%	p=0.084

Means on each sample date with the same or no subscript are not significantly different at the 95% confidence level.

At Trial M, the 'N foliar' treatment in Stage I, but not the 'N+Zn foliar' treatment, significantly reduced granulation compared to the control on the first sample date, along with the 'broadcast N' treatment (p=0.017)(Figure 55 and Table 24). The difference between means was more dramatic on the second sample date, but statistical confidence is low (p= 0.157). However, in Trial A urea sprays in Stage I at 1.5% (two applications in 2007-08 and five applications in 09-10) did not appear to have any effect (Figure 43, Figure 44).

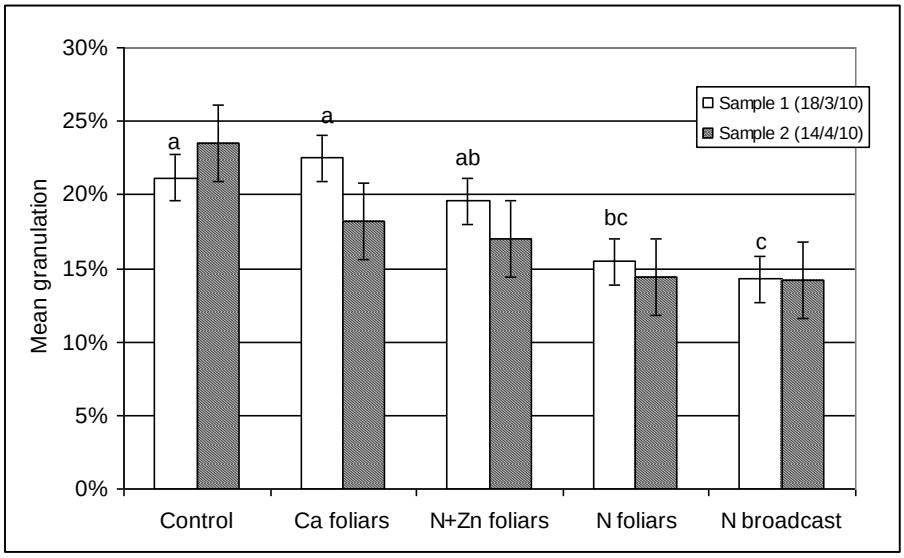

Figure 55 Trial M 2010 Mean granulation by nutrient treatment: p(first sample)=0.017, p(second sample) = 0.157. Means on each sample date with the same or no subscript are not significantly different at the 95% confidence level. Vertical bars represent standard errors of the means. Means adjusted for covariate of crop load on 6/1/10.

Table 24 Trial M Proportion of fruit rated ≥25% granulated by nutrient treatment.

Sample	Control	Ca foliars	N+Zn foliars	N foliars	N broadcast	*p value*/lsd
1 (18/3/10)	47%	56%	42%	19%	17%	p = 0.011
	a	a	a	b	b	lsd= 22%
2 (14/4/10)	43%	29%	29%	16%	19%	p=0.131

Means adjusted for covariate of crop load assessed on 6/1/10. Means in each sample date with the same or no subscript are not significantly different at the 95% confidence level.

At Trial M, the 'N foliars' and 'N+Zn foliars' treatments resulted in tip burn of the young flush, evident after the second spraying, with 11 of the 40 trees sprayed having 'medium' or 'severe' tip burn and the rest 'light' or 'very light' burn. All the N+Zn trees had 'light' or 'very light' tip burn. There was no tip burn at trials A, D and K where the same concentration was used. The burn at Trial M may have been due to the young age of the flush. No direct damage to the fruit was observed. Fruit load was reduced where tip burn was medium or severe (Table 25) with possible flow-on effects to increasing granulation.

Table 25 Trial M effects on crop load of tip burn from N foliars

Extent of tip burn	Mean crop load (no. of fruit per 0.125m^3 quadrat)	n (no. of trees)
no tip burn (all other treatments)	25.1	61
very light	25.0	15
light	21.8	14
medium	15.3	9
severe	11.3	2
Tip burn assessed 26/11/09; crop load measured on 6/1/2010		

Effects on Brix and acid levels of additional nitrogen in Stage I

Overall the negative correlation between granulation and acid levels noted in Section 3.1.4 appears to be reflected in higher acid levels for +N treatments (Table 26 and Table 27).

The application of N in Stage I and Stage II increased mean acid levels at Trial J in 2010 (at both sample dates, p <0.001 and p=0.006) and M in 2010 (at both sample dates, p=0.017, 0.028). At Trial A, increases in mean acid levels for Stage I and Stage II +N treatments in both years were not significant. At Trial D, increases in the mean acid levels for Stage I +N treatments were only evident in 2009 and were not significant. At Trial P, in contrast, the lower N treatments had higher acid levels (p=0.048). This may have been partly affected by a relatively late sampling date at this trial compared to the other trials: fruit may have been relatively more mature. There is a tendency for Stage II + N treatment acid levels to be higher than those of Stage I +N treatments. Fruit with higher acid levels tend to be smaller in diameter, though differences are not always significant.

The only trial for which Brix levels showed differences between treatments was at Trial M in which, at both sample dates, Brix was higher for the 'N broadcast' treatment than for any other treatment (Table 27). This echoes the puzzling general lack of correlation between Brix and granulation noted in Section 3.1.4.

Table 26 Mean Brix, acid , CCI, granulation and diameter by treatment 2009

		% acid		Brix		Brix: acid		CCI when picked	CCI after de-greening	Granulation		Diameter	
Sample date	n	1	2	1	2	1	2	1	1	1	2	1	2
Trial A													
Control	6	0.791 c	0.843	12.036	12.58	15.37 a	15.03	-9.2	1.396	15%	9%	60.53	61.81
+N Stage 1	6	0.857 ab	0.897	11.936	12.38	13.96 bc	13.82	-9.15	1.164	10%	6%	59.72	63.14
+N Stage 2	6	0.904 a	0.941	11.974	12.46	13.26 c	13.25	-8.28	1.383	12%	6%	60.32	62.62
High K	6	0.85 abc	0.877	12.142	12.65	14.4 abc	14.52	-7.02	1.564	13%	8%	59.5	63.44
Low K	6	0.805 bc	0.822	11.613	12.38	14.53 ab	15.14	-8.12	1.327	12%	8%	60.01	62.83
+N foliars	6	0.816 bc	0.861	11.765	12.29	14.44 abc	14.32	-8.69	1.321	15%	8%	61.83	62.94
ese		*0.022*	*0.027*	*0.151*	*0.207*	*0.420*	*0.469*	*0.756*	*0.109*	*1%*	*1%*	*0.806*	*0.655*
p value		*0.015*	*0.060*	*0.195*	*0.828*	*0.042*	*0.068*	*0.361*	*0.246*	*26%*	*28%*	*0.403*	*0.606*
lsd		*0.065*				*1.222*							
Trial D													
Control	8	0.728	0.753	10.516	10.35	14.47	13.79	-13.46	1.163	17%	14%	62.01	65.53
+N Stage 1	8	0.78	0.795	10.557	10.25	13.61	12.96	-13.98	0.968	12%	10%	60.62	65.33
+N+Zn Stage 1	8	0.764	0.753	10.687	10.56	14.05	14.11	-14.09	0.962	18%	11%	61.05	64.75
ese		*0.0166*	*0.020*	*0.141*	*0.214*	*0.302*	*0.372*	*0.783*	*0.132*	*2%*	*2%*	*0.627*	*0.637*
p value		*0.103*	*0.262*	*0.678*	*0.586*	*0.159*	*0.104*	*0.833*	*0.487*	*0.067*	*0.246*	*0.302*	*0.672*
Trial J													
Control	10	0.777	0.673	10.56	10.54	13.81	15.83 a	-7.97	1.094 a	0.197	0.23	60.3	66.42
+ N Stage 1	10	0.806	0.722	10.39	10.34	13.09	14.40 b	-9.39	0.798 b	0.222	0.235	59.82	65.08
ese		*0.0249*	*0.0189*	*0.219*	*0.273*	*0.313*	*0.281*	*0.551*	*0.0959*	*0.0212*	*0.0194*	*0.66*	*1.02*
p value		*0.434*	*0.087*	*0.602*	*0.602*	*0.129*	*0.003*	*0.09*	*0.047*	*0.426*	*0.858*	*0.613*	*0.365*
lsd							*0.8526*		*0.2909*				
Trial K													
Control	7	1.04	0.997	11.167	12.09	10.84	12.16	-6.35	1.172	17%	14%	61	62.98
+ N stage 1 - urea	7	1.129	1.074	11.76	12.75	10.48	12.05	-6.22	1.443	14%	10%	57.94	60.9
+ N stage 1 double urea	7	1.108	1.052	11.469	12.48	10.43	11.99	-6.19	1.281	22%	15%	60.19	61.58
+ N stage 1 foliars	7	1.044	1.008	11.299	12.42	10.93	12.41	-6.72	1.224	20%	12%	59.12	61.56
Extra K	7	1.059	1.061	11.56	12.5	10.99	11.83	-6.45	1.368	21%	18%	60.23	61.69

		% acid		Brix		Brix: acid		CCI when picked	CCI after de-greening	Granulation		Diameter	
Sample date	n	1	2	1	2	1	2	1	1	1	2	1	2
+N,P,K in Stage I	7	1.075	1.01	11.188	12.06	10.47	12.03	-7.64	1.213	18%	14%	58.71	61.96
ese		*0.045*	*0.046*	*0.197*	*0.363*	*0.352*	*0.464*	*0.780*	*0.099*	*2%*	*2%*	*0.948*	*1.035*
p value		*0.675*	*0.786*	*0.249*	*0.755*	*0.753*	*0.970*	*0.781*	*0.383*	*0.265*	*0.193*	*0.236*	*0.822*

Mean**S** in each trial with the same or no subscript are not significantly different at the 95% confidence level. CCI= Citrus Colour Index *ese*= standard error of the means; *lsd* = least significant difference

Table 27 Mean Brix, acid , CCI, granulation and diameter by treatment 2010

Treatment	n	% acid		Brix°		Brix: Acid		CCI when picked	CCI after de-greening	Granulation		Diameter (mm)	
Sample date		1	2	1	2	1	2	1	1	1	2	1	2
Trial A													
Control	6	0.81	0.70 b	11.32	12.08	14.10	17.28 ab	-4.87	1.22	17.4%	12.0%	67.31	65.5
+N Stage 1	6	0.82	0.69 b	10.90	11.51	13.36	16.67 ab	-5.96	0.89	9.7%	7.1%	66.36	64.4
+N Stage 2	6	0.86	0.76 ab	11.22	11.88	13.20	15.71 bc	-5.19	1.12	11.5%	7.2%	66.93	64.3
High K	6	0.89	0.86 a	11.50	12.64	13.04	14.97 c	-3.06	1.40	13.3%	10.4%	67.11	64.3
Low K	6	0.75	0.66 b	10.99	11.69	14.80	17.77 a	-4.19	1.13	15.6%	9.5%	67.75	66.7
+N foliars	6	0.84	0.71 b	11.29	11.92	13.47	16.67 ab	-5.66	0.96	16.0%	11.8%	64.35	63.4
+ zinc foliars	6	0.89	0.74 ab	11.20	11.63	12.91	15.89 bc	-7.00	0.88	13.2%	7.2%	65.49	65.3
ese		*0.05*	*0.04*	*0.30*	*0.35*	*0.55*	*0.56*	*0.94*	*0.15*	*2.1%*	*1.9%*	*0.791*	*1.2*
p value		*0.385*	*0.042*	*0.842*	*0.356*	*0.175*	*0.023*	*0.126*	*0.203*	*0.166*	*0.284*	*0.065*	
lsd (5%)			*0.12*				*1.628*						
Trial D													
Control	12	0.92	0.58	10.64	10.33	11.59 b	17.86	-5.93	1.54	17.1%	19.7%	70.03 a	67.8
+N Stage I	12	0.93	0.58	10.68	10.19	11.52 b	17.54	-6.56	1.53	14.3%	14.0%	69.14 a	68.3
+N+Zn Stage I	12	0.88	0.58	10.78	10.65	12.26 a	18.33	-6.00	1.44	16.0%	18.3%	66.58 b	67.7
ese		*0.019*	*0.01*	*0.13*	*0.21*	*0.21*	*0.33*	*0.52*	*0.09*	*1.4%*	*2.2%*	*0.706*	*0.7*
p value		*0.202*	*0.991*	*0.768*	*0.29*	*0.04*	*0.242*	*0.639*	*0.725*	*0.393*	*0.183*	*0.005*	
lsd (5%)						*0.6174*						*2.038*	
Trial J													
Control	16	0.87 a	0.51 a	10.20	9.61	11.80 a	18.90	-7.84	0.65	20.3% a	35.7% a	66.44 a	68.0
+ N Stage I	16	1.00 b	0.56 b	9.95	9.77	9.99 b	17.55	-8.97	0.63	10.8% b	14.3% b	63.2 b	67.8
ese		*0.0217*	*0.02*	*0.10*	*0.12*	*0.29*	*0.35*	*0.51*	*0.11*	*2.2%*	*3.6%*	*0.58*	*0.6*
p value		*<0.001*	*0.006*	*0.103*	*0.355*	*<.001*	*0.01*	*0.129*	*0.875*	*0.005*	*<0.001*	*<0.001*	
lsd (5%)		*0.062*	*0.03*			*0.838*	*1.01*			*6.37%*	*10.2%*	*1.676*	
Trial P													
Low N July	5	0.68 ab	0.72	10.07	10.57	15.09 abc	14.86	-0.61 a	2.52 a	26.9%	34.3%	66.33	63.5

Treatment	n	% acid		Brix°		Brix: Acid		CCI when picked	CCI after de-greening	Granulation		Diameter (mm)	
Sample date		1	2	1	2	1	2	1	1	1	2	1	2
Normal N July	5	0.60 b	0.77	9.93	11.49	16.70 a	15.29	-1.23 a	2.35 a	39.8%	44.3%	68.27	62.3
Normal N August	5	0.65 b	0.78	9.74	11.06	15.28 ab	14.40	-3.34 ab	2.08 ab	37.8%	36.1%	67.23	62.9
Split application normal N	5	0.68 ab	0.69	9.65	10.62	14.35 bc	15.38	-2.98 ab	2.08 ab	25.8%	31.9%	66.23	60.2
Zero N	5	0.75 a	0.78	9.72	10.29	13.08 c	13.35	-5.06 b	1.73 b	42.3%	48.0%	66.6	67.2
ese		*.03*	*0.06*	*0.15*	*0.36*	*0.701*	*0.70*	*0.97*	*0.15*	*7.8%*	*7.6%*	*0.896*	*2.4*
p value		*0.048*	*0.730*	*0.325*	*0.188*	*0.029*	*0.273*	*0.036*	*0.022*	*0.462*	*0.534*	*0.497*	
lsd (5%)		*0.09*				*2.103*		*2.909*	*0.46*				
Trial M													
Ca foliars	4	0.93	0.58	9.78 b	9.79 b	10.51	17.01	-6.91	0.87	22.5%	19.0%	67.33	68.3
Control	4	0.94	0.58	9.91 b	9.72 b	10.63	16.83	-5.05	1.07	22.6%	26.7%	67.19	66.0
+ N+Zn foliars	4	0.97	0.62	9.74 b	9.87 b	10.06	16.08	-7.72	0.78	20.9%	13.4%	66.94	66.5
+ N foliars	4	1.02	0.59	9.50 b	9.72 b	9.39	16.56	-7.56	0.82	20.0%	20.3%	64.98	69.3
+ N broadcast	4	1.16	0.74	10.78 a	11.15 a	9.33	15.22	-5.17	0.98	12.2%	11.2%	65.77	66.5
ese		*0.04*	*0.03*	*0.21*	*0.33*	*0.48*	*0.74*	*0.78*	*0.19*	*3.2%*	*4.7%*	*1.231*	*1.0*
p value		*0.017*	*0.028*	*0.009*	*0.04*	*0.236*	*0.476*	*0.08*	*0.812*	*0.194*	*0.221*	*0.612*	*0.195*
lsd (5%)		*0.13*	*0.11*	*0.64*	*1.02*	*1.48*	*2.29*						

Means in each trial with the same or no subscript are not significantly different at the 95% confidence level. CCI= Citrus Colour Index *ese*= standard error of the means; *lsd* = least significant difference

Effect of N and K on fruit colour

While there is a slight tendency for extra N treatments to be greener when picked, at most trials treatments did not affect colour after degreening (Figure 56 and Figure 57). There was no significant difference in treatment means for Citrus Colour Index values except at Trial P in 2010 (p=0.022) and Trial J in 2009 (p=0.05). At Trial J, fruit in the '+ N' treatments was slightly less coloured than control treatments after degreening in 2009. However, at Trial P, fruit from the 'zero N' treatment had poorer colour both before and after degreening.

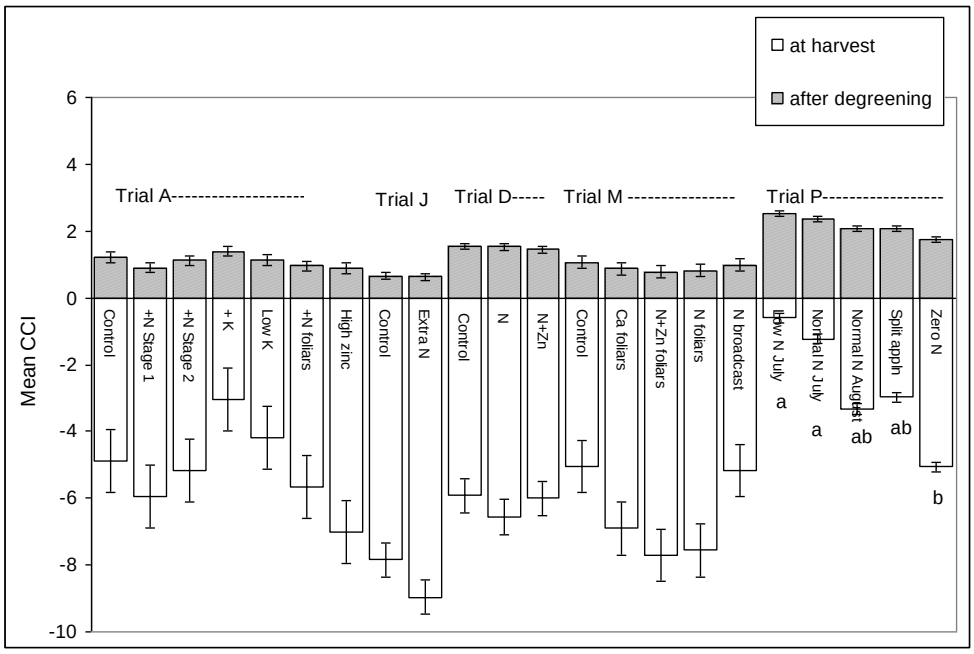

Figure 56 Mean CCI (Citrus Colour Index) values by treatment before and after degreening 2009. Within trials, treatment means were not significantly different except at Trial J (after degreening): p= 0.05.

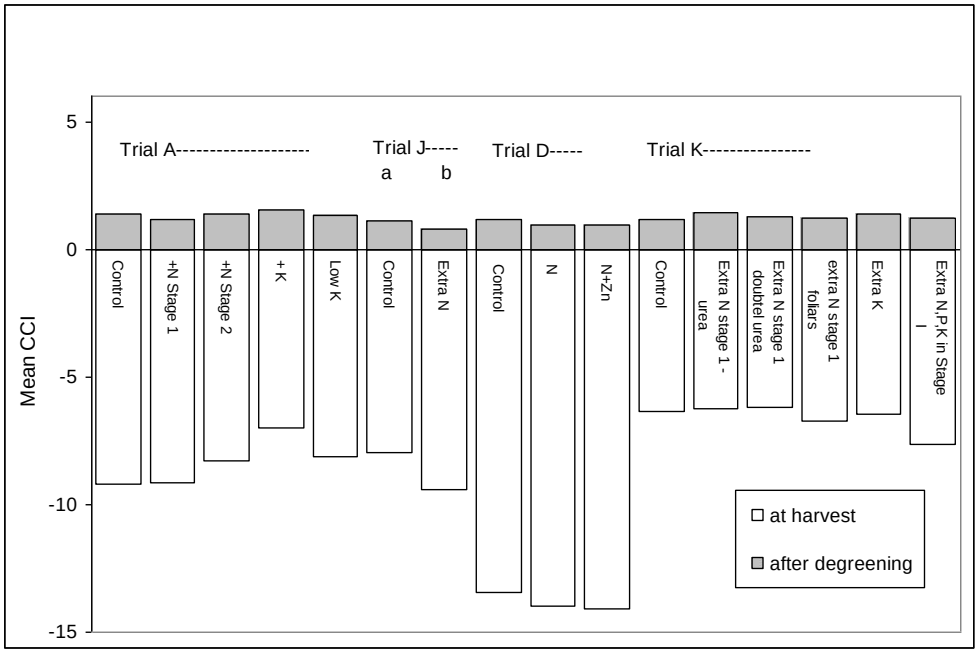

Figure 57 Mean CCI (Citrus Colour Index) values by treatment before and after degreening 2010. Within trials, treatment means were not significantly different except at Trial P (at harvest): p= 0.022.

Other macronutrients

Results at Trials A and B showed no clear benefit or detriment from extra phosphorus (P) (Figure 42, Figure 43, Figure 44, Figure 45, Table 19). Similarly, foliar sprays of P at Trial C slightly increased granulation (Table 23).

Results for potassium (K) were variable but overall do not suggest a significant role for K in comparison to N. Previous work by DPI&F indicated that excess potassium (K) may stimulate higher granulation (Fullelove *et al.* 2004). There were no clear trends in effects of 'high K' or 'low K' applications at Trial A in the three years of trials, or at Trial B in 07/08, although on the second sample date at Trial B, the reduction (non-significant) was similar to that of the 'extra N in Stage I' treatment. On the other hand, leaf levels of K are correlated with granulation, that is, high levels of leaf K mean higher levels of granulation.

Both acid and Brix levels were slightly higher for 'high K' treatments than for the control at Trial A in 08/09 and 09/10 and at Trial K in 08/09, but differences were generally not significant (Table 26 , Table 27). Similarly, 'low K' treatments at Trial A in 08/09 and 09/10 were lower in Brix and acid, but not significantly. The consistency of this trend, however, does not translate into trends in granulation means, despite the correlation between acid and granulation discussed above.

In Trial K, the mean proportion of fruit rated ≥25% for the 'extra K' treatment was 60% compared to 50% for the control (p=0.097). Interestingly, at the same trial, the 'extra N Stage 1-urea' treatment reduced the proportion of fruit rated ≥25% on the first sample date from 50% to 35%, while the 'extra N,P,K Stage I' treatment reduced it only to 46%. Both treatments contained the same amount of N (although in different forms) and were applied at the same time (Figure 46). This suggests a minimal role for additional P and K and/or no synergistic effect of the NPK combination.

Trial M tested foliar applications of calcium: there was no beneficial effect (Figure 55).

Micronutrients

Trials of extra zinc foliars at Trial A, B and C were promising, with reductions compared to the control in fruit rated ≥25% of 11% at Trial A (p=0.513), and 12% (p=0.013, lsd=13%) at Trial B and 7% at Trial C (p=0.01) at the first sample date. Treatments with low levels of boron and zinc at Trial A and B and 07/08 overall performed badly, and these treatments were dropped from the trial program in subsequent years.

Results in 08/09 and 09/10 did not convincingly fulfil zinc's early promise. While applications generally resulted in improvements, reductions in granulation tended to be smaller, were mostly non-significant and less consistent than the 'extra N in Stage I' treatments. In 2008/09, at Trial A at the first sample date there was a 7% reduction from the control (p=0.01, lsd=11%) but at Trial K at the first sample date, zero

difference from the control (p=0.097). In 2009/10 at Trial A at the first sample date there was a 5% decrease (p=0.414).

Trial D compared treatments of broadcast N with broadcast N in combination with with foliar Zn treatments within irrigation treatments. In 08/09, the N+Zn treatment had significantly less granulation than the control (21% of fruit rated ≥25% compared to 31%) but not significantly less than the N broadcast treatment (26%)(p=0.024 lsd=7%). In 09/10 there was no statistically significant difference between any of the three nutrient treatments *(p*=0.809), even though the number of zinc sprays was increased from two in 08/09 to three in 09/10. The pattern of N application, as discussed above in 'Timing of N applications' seemed to be more significant at this trial.

The zinc soil application treatments used in Trials A and K, on the other hand, appeared to be detrimental, as it resulted in an 11.1% increase in fruit rated 25% or higher at trial A in 2008-09 (p=0.011, lsd=11.3%) and 7% in 09/10 (p=0.414) (first sample dates). At Trial K, soil Zn application increased fruit rated at ≥25% by 3% (p=0.097). Leaf analysis undertaken in December 2008 and Feb 2010 suggest that there had been limited uptake by treatment trees of the soil-applied zinc (Table 28). Zn leaf levels are similar to the control so there must be an additional explanation for the increased granulation. It is possible that the Zn in soil interfered with uptake or metabolism of another nutrient such as N or iron (Fe) affecting photosynthesis, as has been demonstrated in corn and beets (Rosen *et al.* 1978; Sagardoy *et al.* 2009). This may have had a temporary effect at key stage in fruit development as low levels of other minerals were not evident by the time of sampling for leaf analysis (Table 28). Another possible explanation is that high Zn levels may have temporarily inhibited nitrification in the soil.

Table 28 Zinc, N and Fe plant tissue analysis for high Zn treatments Trials A and K.

	Trial and date of leaf sample	Control	Foliar zinc treatment	Soil zinc treatment
Zn	Trial A Dec 08 *	460	700	430
	Trial K Dec 08	67	160	69
	Trial A Feb 10	21	89	30
N% (Kjeldahl)	Trial A Dec 08	3.7	3.8	3.7
	Trial K Dec 08	3.2	3.5	3.2
	Trial A Feb 10	2.8	3.1	2.8
N (mg/kg)	Trial A Dec 08	210	330	300
	Trial K Dec 08	290	330	310
	Trial A Feb 10	<50	<50	<50
Fe (mg/kg)	Trial A Dec 08	74	78	79
	Trial K Dec 08	85	95	87
	Trial A Feb 10	56	57	57

* all treatments affected by foliar Zn application on 25/11/08 by grower

Leaf analysis

Leaf tissue analyses provide very little insight into causes of granulation. Correlation coefficients for nutrient levels and granulation vary widely and provide, at best, broad direction in terms of negative or positive associations (Table 29). There are significant negative correlations between leaf nitrogen levels and granulation in 07/08 and 08/09, but not 09-10, that is, low leaf N levels are associated with higher granulation. However, similarly strong negative correlations were found in one or more years for sulphur, magnesium, manganese, copper and sodium. As strong, but positive, correlations can be found for phosphorus, potassium and boron. Zinc and iron correlation coefficients vary from positive to negative.

An interesting point to note is that the average N levels in all leaf tests (regardless of level of granulation) in February 08 was 3.2%, with 50% of treatment values at trials falling between 3.0 and 3.3%. In February 2010, the average mean was 3.0%, with 50% of values at sites or survey blocks falling between 2.9 and 3.1%. This is well within and above the recommended range of 2.4-2.6% N (Vock *et al.* 1997) and suggests that recommendations for Imperials may need to be reviewed upwards.

Table 29 Correlation between mean granulation on first sample date with leaf nutrient levels.

Date of sampling	Nov 07		Feb 08		Dec 08		Jul 09		Feb 10	
Harvest year	2008		2008		2009		2009		2010	
	r	p	r	p	r	p	r	p	r	p
Nitrogen (Kjeldahl) %	-0.0031	0.9898	**-0.5532**	**0.0006**	**-0.5736**	**0.0014**	-0.5133	0.0879	-0.1440	0.3885
Nitrogen nitrate (mg/kg)	-0.3457	0.4475	**-0.6578**	**0.0030**	0.3438	0.0791	Insufficient data		-0.3819	0.1601
Sulfur	-0.1691	0.4759	-0.0204	0.9074	**-0.5010**	**0.0066**	-0.3522	0.2615	-0.1828	0.2719
Phosphorus	**0.5137**	**0.0205**	**0.5051**	**0.0020**	-0.0433	0.8269	0.1108	0.7318	**0.4811**	**0.0022**
Potassium	**0.5949**	**0.0057**	**0.5898**	**0.0002**	0.3560	0.0630	-0.0308	0.9244	0.3103	0.0579
Calcium	-0.2585	0.2712	**0.3525**	**0.0378**	**-0.4469**	**0.0171**	-0.1097	0.7343	-0.1619	0.3316
Magnesium	-0.3162	0.1743	**-0.6100**	**0.0001**	**-0.5463**	**0.0026**	-0.0420	0.8969	0.1676	0.3145
Sodium	**-0.4492**	**0.0469**	-0.2849	0.0971	-0.3498	0.0681	-0.0659	0.8387	**-0.3230**	**0.0480**
Chloride	-0.3985	0.0818	-0.2795	0.1039	-0.3425	0.0803	-0.3758	0.3590	-0.2539	0.1295
Copper	0.0836	0.7261	**-0.3499**	**0.0393**	**-0.5238**	**0.0042**	**-0.5888**	**0.0440**	-0.2052	0.2164
Zinc	**0.4684**	**0.0372**	0.2638	0.1257	**-0.6168**	**0.0005**	-0.3599	0.2505	0.0573	0.7327
Manganese	-0.1153	0.6282	**-0.4329**	**0.0094**	**-0.4550**	**0.0150**	-0.3032	0.3380	**-0.4877**	**0.0019**
Iron	**-0.6978**	**0.0006**	**-0.4862**	**0.0031**	**0.4427**	**0.0183**	-0.4630	0.1296	0.3097	0.0585
Boron	**0.5147**	**0.0202**	**0.8003**	**0.0000**	0.1974	0.3139	0.3368	0.2845	0.2114	0.2027
N:S Ratio	0.2770	0.2370	-0.1984	0.2533	0.1948	0.3207	0.0327	0.9196	-0.1592	0.3396
N: P Ratio	**-0.5522**	**0.0116**	**-0.5534**	**0.0006**	-0.0444	0.8226	-0.2142	0.5039	**-0.3590**	**0.0269**
N: K Ratio	**-0.4827**	**0.0311**	**-0.5796**	**0.0003**	**-0.5959**	**0.0008**	-0.1364	0.6725	**-0.3754**	**0.0202**

Nov 07 n for Nitrogen nitrate (mg/kg) =7, all other nutrients = 20
Feb 08 n for Nitrogen nitrate (mg/kg) =18, all other nutrients = 36
Dec 08 n for Nitrogen nitrate (mg/kg) =27, chloride = 27, all other nutrients = 28
Jul 09 n for chloride = 8, all other nutrients = 128
Feb 10 n for nitrate N =15, n for chloride =37, all other nutrients =38
Bold text = correlations where values of r ≥0.3 and $p<0.05$; p= two sided test of correlations different from zero probabilities

3.2.5 Spatial trends

Several trial sites show evidence of a spatial component to granulation, that is, certain parts of the block are more prone to granulation than others. At Trial A, this pattern is reasonably consistent over the three years of the trials. In Trial J, a pattern was evident in 08/09 but less so in 09/10.

These patterns are partly but not fully explained by tree health. They are linked to fruit size, as would be expected from the correlation of these two factors, but it is not clear why fruit size would vary spatially. Pollination differences seem unlikely given the location of the granulation-prone areas.

Variations in soil texture and structure would seem the most probable cause, but are difficult to characterize in sufficient detail. At Trial A, the soil profile to 1.2m depth seemed to be largely consistent across the block. Three Aquaspy™ moisture monitoring probes installed in January 2009 between pairs of trees of good health but with high, medium and low granulation as assessed at the 07/08 harvest showed that the average water use measured as the average difference from 'peaks' to 'troughs' in the top 10 cm was higher in the high granulation sites, and lower in the low granulation sites in both 09 and 10 (Table 30). This is consistent with the hypothesis that increased water availability and uptake is linked to increased granulation.

Table 30 Trial A Mean water use measured as soil moisture differences between highs (irrigation/rainfall) and lows, 10 cm probes

	Location of probe	Mean difference (units)	No of peaks
3/1/09 to 31/3/09	Low granulation zone	5.3	14
	Medium granulation zone	7.6	12
	High granulation zone	8.4	13
1/9/09 to 31/3/10	Low granulation zone	5.4	25
	Medium granulation zone	6.0	28
	High granulation zone	9.5	29

Probes installed in January 09. Units are on a scale of 0 to 100 and do not represent moisture %.

At Trial J, soil texture and structure varied significantly, ranging in colour from red to brown, and with a heavy clay layer underlying the sandy loam top soil to varying depths. The poorer end of the block tended to have shallower top soils, suggesting the hypothesis that water may be held in the upper layer where the clay layer is closer to the surface, thus providing more water to the tree and increasing the tendency to granulate. The correlation between moisture content and depth to clay on the date of sampling (June 2009) was good ($r=-0.5866$, $n= 25$, $p=0.0020$), however, on an individual tree or plot basis there was no correlation between either depth to clay and granulation or moisture content and granulation.

The reason for spatial variations thus remains unclear, providing another example of the complexity of factors affecting granulation incidence. This is an area that may warrant further methodical attention in future research.

4 Conclusions

4.1 Understanding of granulation

Project activities have made it clear that granulation is triggered in early fruit development. Competition for carbohydrates between young fruit, flowers and expanding flush may be the initial cause. This means in most years it is the first fruit to develop, that is, the largest fruit by the time of harvest, that are more prone to granulate.

On the basis of the data available, the seasonal conditions that might increase the incidence of granulation appear to be those that exacerbate early competition for carbohydrates: warm winters that trigger early flowering, and warmer than average temperatures and/or higher than average rainfall in early fruit development that stimulates vegetative flush.

Trials suggest the best strategy for reducing granulation is to support the development of young fruit through adequate nutrition that increases supply and reduces competition for photosynthates.

This explanation of the conditions that engender granulation does not in itself provide direct confirmation of the research hypothesis, that is, that the underlying cause of granulation is high water potential in juice cells due to lower soluble solid concentrations and/or high turgor pressure. This would require investigations at the cellular level. However, it is in alignment with the view that low soluble solid concentrations are an underlying cause. The lack of strong correlations between granulation and low Brix levels, on the other hand, weakens the argument for the hypothesis. There is a clear correlation between less-granulated fruit and high acid levels, but it is unclear whether this is due simply to the osmotic potential of high soluble acid concentrations, or more indirectly by the availability of acids as an energy source for some unspecified metabolic process.

At this stage the lack of consistent results from irrigation frequency trials compel the conclusion that nutrient applications are the more reliable management approach. In addition, it may suggest that it is the cellular solute levels rather than cell turgor or pressure that has the greater influence on granulation.

4.2 Recommended management practices

It is clear that management action should be targeted to supporting early fruit development. The question of whether or not granulation can be arrested in later fruit development remains unanswered.

The key management strategy for this is likely to be adequate nutrition. This project had substantial success with supplying additional nitrogen to the tree during Stage I of fruit development. While some of this nitrogen may be going directly into fruit growth, it is more likely supporting the expansion of new flush, so that leaves move more quickly from being a 'sink' to being a 'source' of photosynthates. Supplying other macro and micro-nutrient needs to ensure healthy trees, high levels of photosynthetic efficiency and storage of minerals and carbohydrates over the autumn and winter to support flowering and flush is also important in managing granulation.

Trials on reducing irrigation frequency had mixed success: but at this stage reducing irrigation frequency appears a risky strategy for growers. Growers are recommended to first try nutrition strategies to see if gains can be made.

Low crop loads contribute strongly to granulation, so management to reduce biennial bearing patterns is critical.

4.3 Future research

This project has provided a sound basis for future research into management of granulation in clarifying the importance of early fruit development and suggesting the seasonal conditions that may influence it.

The variability inherent in this problem has been a particular challenge for this project and the 60 years of research that preceded it. A particular challenge is the uncertainty as to whether seasonal conditions would be favourable or unfavourable. Consequently, some trials in climate-controlled greenhouses would be worth pursuing, using the most likely climate scenarios outlined in Section 3.1.9.

The multiplicity of factors involved and the strength of climate influences suggest that it is unlikely that management practices will eliminate granulation entirely. The enhanced greenhouse effect may also mean climate change such as warmer winters and summers may exacerbate the problem in future years. This suggests that it would be wise for the industry to invest in breeding new non-granulating varieties that could replace or supplement Imperials in the early season market. This work has commenced under the project CT0914 at Bundaberg Research Station. Rootstock research may also be part of the solution.

This project has made it clear, however, that there are potential gains from improved management practices. Further follow-on work is needed on the appropriate volumes and timing of nitrogen applications. This would provide insight into the balance between nitrogen application in late autumn and winter to support storage of nitrogen and carbohydrates to use in subsequent flush and flowering, and the 'top up' application in Stage I of fruit development (spring) which was successful in trials. This should include work on extra nutrition during leaf flush in late Stage II (February), as data on seasonal conditions suggest this may also be contributing to increased granulation.

Irrigation strategies need further research under controlled conditions, particularly as this may be the only strategy that can be used later in fruit development. Treatments that reduce both frequency and volume need to be tested, through scheduling irrigations to meet a range of targeted soil moisture levels. The possible confounding effects of rainfall would need to be removed through the use of plastic mulch or some similar technique.

In view of the importance of early fruit development and the effect of low crop loads on granulation, research into improved and reliable methodologies for crop regulation to reduce the demands on the tree at flowering and to reduce biennial bearing patterns may be warranted. To date, most Burnett growers have been reluctant to adopt such strategies, preferring manual thinning once natural fruit drop has ceased and the risk of crop losses and damage by hail and storms is past. While this preference may prevail, provision of a reliable crop regulation methodology would at least provide more options than currently available. Such technologies would have other benefits in terms of smoothing annual volumes supplied to the market and in more efficient inputs.

Technology transfer

Throughout the project, work has been conducted in collaboration with a range of growers and findings have been communicated progressively to the nine owners of trial blocks and to the 40 growers that cooperated each year in surveys. In addition, the four main citrus crop consultants in the region have been actively involved in providing advice and feedback as the project progressed, and have passed information on project results onto their clients.

Project results and recommendations were formally reported to growers at a series of meetings run in conjunction with Citrus Australia Ltd. This included reporting the results of first year of trials at a meeting to report the results of DPI&F research to growers in the Central Burnett at Gayndah on 5 March 2009. This was attended by ~60 growers/industry participants.

An article in the *Australian Citrus News*, vol. 76, Apr/May 2010, p. 5, 'Industry warned to stop immature fruit hitting market early', include mention of the possible climate scenarios contributing to increased granulation in 2010.

Final results covering the whole of the project were reported at a range of "Imperial Mandarin Quality Improvement" forums hosted by Citrus Australia Ltd in all Australian growing regions, as follows:

- Central Burnett: On 26 August 2010, two detailed sessions, one on the knowledge gained about the nature of granulation and its causes, and one on trials of management practices were presented to ~ 50 growers at a forum at the Golden Orange Hotel, Gayndah.
- Riverland: A single session combining both aspects of the project was presented to ~20 growers at the Berri Hotel on 16 November 2010.
- Sunraysia: Around 25 growers and industry stakeholders attended a forum at Mildura at the Dareton Research Station on 17 November 2010, where project results were presented and discussed in the context of other mandarin-related research in the region.
- Western Australia: 25 October 2010, two sessions as above, were presented to around 20 growers who attended at field day at Harvey.

These fora were heavily promoted by Citrus Australia Ltd and designed to ensure grower awareness and facilitate maximum grower participation. Many growers travelled long distances to hear the results, including one group from Loxton in South Australia who journeyed to Gayndah, Queensland, to attend the first forum. These information session were further reinforced with an article in *Australian Citrus News*, 'Citrus industry plans improvement to quality of Imperial mandarin', vol. 87, Aug/Sept, prepared by Andrew Harty, General Manager Market Development, who commented, 'In a methodical and detailed double presentation, Ms Hofman defined likely physiological causes of drying and discussed the orchard research she has carried out over the past three seasons….[S]everal practices were described which could immediately be trialled by growers, including the application of additional nitrogen in early spring' (p. 19). A second article by Mr Harty, which will include a summary of the main findings of the project, 'More Imperial quality forums around the regions', will be published in the November edition.

Copies of the data presented at these meetings have been provided on request to those who were unable to attend meetings and for a range of communication purposes.

A paper outlining the research hypothesis, 'Grappling with Granulation in Imperial mandarins' was presented at the 11th International Citrus Conference in Wuhan China 2008 (Hofman and Smith 2008).

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References

Agusti M, Almela V, Aznar M (1994) Satsuma mandarin fruit size increased by 2,4-DP. *HortScience* **29**, 279-281.

Agusti M, El-Otmani M, Aznar M, Juan M, Almela V (1995) Effect of 3,5,6-trichloro-2-pyridyloxyacetic acid on clementine early fruitlet development and on fruit size at maturity. *Journal of Horticultural Science* **70**, 955-962.

Agusti M, Zaragoza S, Iglesias DJ, Almela V, Primo-Millo E, Talon M (2001) The synthetic auxin 3,5,6-TPA stimulates carbohydrate accumulation and growth in citrus fruit. *Plant Growth Regulation* **36**, 43-49.

Agusti M, Zaragoza S, Iglesias DJ, Almela V, Primo Millo E, Talon M (2002) The synthetic auxin 3,5,6-TPA stimulates carbohydrate accumulation and growth in ctirus fruit. *Plant Growth Regulat.* **36**, 141-147.

Albrigo LG (1978) Rootstocks affect 'Valencia' orange fruit quality and water balance. *Citrus Industry* **59**, 31-44.

Awasthi RP, Nauriyal JP (1972a) Studies on granulation in sweet orange (*Citrus sinensis* Osbeck) - IV - Physical characteristics of granulated and non-granulated fruits. *Indian J. Hort.* **29**, 40-44.

Awasthi RP, Nauriyal JP (1972b) Studies on granulation in sweet orange (*Citrus sinensis* Osbeck). VI. Differences in moisture, total soluble solids and ascorbic acid content of juice vesicles in different stages of granulation. *Indian J. Hort.* **12**, 203-211.

Awasthi RP, Nauriyal JP (1972c) Studies on granulation in sweet orange (*Citrus sinensis* Osbeck): II Effect of age, tree condition, tree aspect, fruit size, rootstock and tree variation on granulation. *J. Research* **10**, 62-70.

Awasthi RP, Nauriyal JP (1972d) Studies on nutritional status of trees and carbohydrates metabolism in relation to granulation in sweet orange (*Citrus sinensis* Osbeck). *Indian J. Hort.* **29**, 252-257.

Bain FM (1949) Citrus and climate. *California Citrograph* **34**, 382, 412-314, 426, 448-389.

Bain JM (1958) Morphological, anatomical, and physiological changes in the developing fruit of the Valencia orange, *Citrus sinensis*, (l.) Osbeck. *Australian Journal of Botany* **6**, 1-25.

Barry GH, Castle WS, Davies FS (2004) Rootstocks and plant water relations affect sugar accumulation of 'Valencia' sweet orange via osmotic adjustment. *Acta Horticulturae* **632**, 159-165.

Bartholomew ET, Sinclair WB, Turrell FM (1941) Granulation of Valencia oranges. *Bul. Univ. California College of Agr.*, 3-63.

Benton RJ (1940) Granulation. *Citrus News* **16**, 129-130.

Bevington KB (1988) Improving citrus fruit quality through nutrition. In 'Tasting the difference: the importance of citrus juice yield and quality'. (Ed. L Revelant) pp. 35-37. (Department of Agriculture New South Wales: Griffith).

Bevington KB, Florissen P, Gee A, Falivene S (1998) CT95031 Final report Improving Imperial mandarin fruit quality and marketability NSW Agriculture and Horticulture Australia Ltd, Dareton, NSW.

Burns JK (1990) Respiratory rates and glycosidase activities of juice vesicles associated with section-drying in citrus. *HortScience* **25**, 544-546.

Burns JK, Achor DS (1989) Cell wall changes in juice vesicles associated with 'section drying' in stored late-harvested grapefruit. *J. Amer. Soc. Hort. Sci.* **114**, 283-287.

Burns JK, Albrigo LG (1997) Granulation in grapefruit. *Proceedings of the Florida State Hort. Soc.* **110**, 204-208.

Burns JK, Albrigo LG (1998) Time of harvest and method of storage affect granulation in grapefruit. *HortScience* **33**, 728-730.

Bustan A, Goldschmidt EE (1998) Estimating the cost of flowering in a grapefruit tree. *Plant, Cell and Environment* **21**, 217-224.

Camacho-B SE, Kaufmann MR, Hall AE (1974) Leaf water potential response to transpiration by citrus. *Physiologia Plantarum* **31**, 101-105.

Cassin J, Bourdeaut J, Fougue A, Furon V, Gaillard JP, LeBourdelles J, Montagut G, Moreuil C (1969) The influence of climate upon the blooming of citrus in tropical areas. *Proc. First Int. Citrus Symposium* **1**, pp. 315-323.

Castle WS, Warrington IJ (1995) Rootstock as a fruit quality factor in citrus and deciduous tree crops. *New Zealand Journal of Crop and Horticultural Science* **23**, 383-394.

Chakrawar VR, Singh R (1977) Studies on citrus granulation. I. Physical and quality aspects of granulation. *Haryana Journal of Horticultural Sciences* **6**, 128-131.

Chakrawar VR, Singh R (1978) Studies on citrus granulation: IV Effect of some growth regulators on granulation and quality of citrus fruits. *Haryana J. Hort. Sci.* **7**, 130-135.

Chakrawar VR, Singh R, Subbaih BV (1980) Studies on citrus granulation. III. Sugar transport and fruit granulation in citrus. *Haryana Journal of Horticultural Sciences* **9**, 17-20.

Chanana YR, Nijjar GS (1984) Note on foliar mineral status of trees in relation to granulation in sweet orange. *Indian Journal of Horticulture* **41**, 240-243.

Chanana YR, Nijjar GS (1985) Note on effect of crop load on the granulation of 'Hamlin' orange. *Indian J. Hort.* **42**, 76-77.

Chapman HD (1968) Chapter 3: The mineral nutrition of citrus. In 'The Citrus Industry'. (Eds W Reuther, LD Batchelor, HJ Webber) pp. 127-289. (University of California.

Chapman JC (1982) The effect of potassium and nitrogen fertilizers on the yield, fruit quality and leaf analysis of Imperial mandarins. *Aust. J. of Exp. Agric. Anim. Husb.* **22**, 331-336.

Chapman JC (1986) Growth management of mature citrus with applied nitrogen. *Acta Horticulturae*, 173-177.

Chen K, Xu C, Li F, Zhang S (2005) Postharvest granulation of 'Huyou' (*Citrus changshanensis*) fruit in response to calcium. *Israel J. Plant Sci.* **53**, 35-40.

Crocker TE, Bell WD, Bartholic JF (1974) Scholander pressure bomb technique to assess the relative leaf water stress of 'Orlando' tangleo scion as influenced by several rootstocks. *HortScience* **9**, 453-455.

Daulta BS, Arora RK (1990) Studies on granulation in different cultivars of sweet orange (*C. sinensis* Osbeck). *Haryana Journal of Horticultural Sciences* **19**, 18-21.

Davies FS, Albrigo LG (1994) 'Citrus.' (CAB International: Wallingford, UK).

Ding J, Deng X, Zhang H, Cheng Y, Xu Q, Liu Q (2009) Somaclonal variation with early juice-sac granulation obtained by inter-specific protoplast fusion in citrus. *J. Hort. Sci. and Biotechnol.* **84**, 567-573.

El-Otmani M, Agustí M, Aznar M, Almela V (1993) Improving the size of 'Fortune' mandarin fruits by the auxin 2,4-DP. *Scientia Horticulturae* **55**, 283-290.

El-Otmani M, Ait Oubahou A, El-Hassainate F, Kaanane A, Lovatt CJ (2004a) Effect of giberellic acid, urea and KNO_3 on yield and on composition and nutritional quality of clementine mandarin fruit juice. *Acta Horticulturae* **632**, 149-157.

El-Otmani M, Ait Oubahou A, Xahra F, Lovatt CJ (2002) Efficacy of foliar urea as an N source in sustainable citrus production systems. *Acta Horticulturae* **594**.

El-Otmani M, Taibi FZ, Lmoufid B, Ait Oubahou A (2004b) Improved use of foliar urea on clementine mandarin to manipulate cropping in a sustainable production system. *Acta Horticulturae* **632**, 167-175.

El-Zeftawi BM (1973) Granulation of Valencia oranges. *Food Technology Australia* **25**, 103-107.

El-Zeftawi BM (1978) Factors affecting granulation and quality of late-picked Valencia oranges. *J. hort. Sci.* **53**, 331-337.

Erickson LC (1968) Chapter 2, The general physiology of citrus. In 'The citrus industry'. (Eds W Reuther, LD Batchelor, HJ Webber). (University of California: Riverside, California).

Erickson LC, Richards SJ (1955) Influence of 2,4-D and soil moisture on size and quality of Valencia oranges. *Proc. Amer. Soc. Hort. Sci.* **65**, 109-112.

Fullelove G (2001) Dry imperial survey - season 2001. QDPIReport for "Mandelicious", the Queensland Mandarin Industry newsletter, Rockhampton.

Fullelove G, Walsh K, Subedi P, Shaw R, Pinnington G (2004) Final report: CT03029 Management of internal dryness of imperial mandarins. Horticulture Australia Ltd., Final project report No., Rockhampton.

Gallasch PT, Damiani J, Falivene S (1998) Citrus growing in Spain and California. South Australian Research and Development Institute, Adelaide.

Gardner FE (1969) A study of rootstock influence on citrus fruit quality by fruit grafting. *Proceedings of the First International Citrus Symposium* **1**, 359-364.

Gilfillan IM, Stevenson JA (1977) Postharvest development of granulation in South African export oranges. *International Society of Citriculture: Proceedings of the International Society of Citriculture* **I**, 299-303.

Goldhamer DA, O'Connell N (2005) Using regulated deficit irrigation to optimize fruit size in late harvest navels. In 'Citrus Research Board 2005 Annual Report'.

Goldschmidt EE, Golomb A (1982) The carbohydrate balance of alternate-bearing citrus trees and the significance of reserves for flowering and fruiting. *Journal of the American Society for Horticultural Science* **107**, 206-208.

González-Altozano P, Castel JR (1999) Regulated deficit irrigation in 'Clementina de Nules' citrus trees. I. Yield and fruit quality effects. *J. Hort. Sci. Biotechnol.* **74**, 706-713.

Goto A (1989) Relationship between pectic substances and calcium in healthy, gelated, and granulated juice sacs of Sanbokan (*Citrus sulcata* Hort. ex Takahashi) fruit. *Plant And Cell Physiology* **30**, 801-806.

Goto A, Araki C (1983) Chemical compositions and internal anatomy of the gelated and granulated juice sacs of Sanbokan (*Citrus sulcata* hort. ex. Takahashi) fruit. *Journal of the Japanese Society for Horticultural Science* **52**, 316-324.

Gravina A, Arbiza H, Telias A, Benzano F, D'Oliveira Flores V, Casanova S, Gambetta G (2004) Harvest date effect on fruit quality and return bloom in three citrus cultivars. *Proc. Int. Soc.Citricult. X Congress* **1**, 284-290.

Guardiola JL, Garcia-Luis A (1997) Thinning effects on citrus yield and fruit size. *Acta Horticulturae* **463**, 463-473.

Guardiola JL, Lazaro E (1987) The effect of synthetic auxins on fruit growth and anatomical development in 'Satsuma' mandarin *Scientia Horticulturae* **31**, 119-130.

Hearn CJ (1987) The 'Fallglo' citrus hybrid in Florida. *Proceedings of the Florida State Horticultural Society* **100**, 119-121.

Hield HZ, Erickson LC (1962) Plant regulator uses of 2,4-D on citrus. *California Citrographer* **47**, 308, 331-304.

Hirose K, Yamamoto M, Ikeda I, Ohata T (1972) Studies on thinning citrus fruits by applying chemicals. II. The effects of 2,4,5-trichlorophenoxyacetic acid and maleic hydrazide in thinning satsumas. *Bulletin of the Horticultural Research Station, B (Okitsu)*, 41-53.

Hofman H, Smith MW (2008) Grappling with granulation in Imperial mandarin. In '11th International Citrus Congress'. Wuhan, China. (Ed. X Deng). (Paper No. 149).

Hutton RJ, Landsberg JJ, Sutton BG (2007) Timing irrigation to suit citrus phenology: a means of reducing water use without compromising fruit quality and yield? *Austral. J. Expert Agr.* **47**, 71-80.

Jawanda JS, Uppal DK, Singh R, Arora JS (1978) Studies on granulation in citrus fruits. *Punjab Horticultural Journal* **18**, 180-188.

Jiminez-Cuesta M, Cuquerella, J., Martinez-Javaga, J.M. (1981) Determination of a colour index for citrus fruit degreening. *Proc. Int. Soc. Citriculture*, 750-753.

Jones WW, Embleton TW, Barnhart EL, Cree CB (1974) Effect of time and amount of fruit thinning on leaf carbohydrates and fruit set in Valencia oranges. *Hilgardia* **42**, 441-449.

Jones WW, Embleton TW, Coggins CW, Jr. (1975) Starch content of roots of 'Kinnow' mandarin trees bearing fruit in alternate years. *HortScience* **10**, 514.

Kaur H, Aulakh PS, Kapur SP, Singh SN (1990) Effect of growth regulators and micronutrients on granulation and fruit quality of sweet orange cv. Jaffa. *Punjab Horticultural Journal* **30**, 13-19.

Kaur H, Chanana YR, Kapur SP (1991) Effect of growth regulators on granulation and fruit quality of sweet orange cv. Mosambi. *Indian J. Hort.* **48**, 224-227.

Kotsias D (2004) Influence of *Citrus aurantium* L. and *Poncirus trifoliata* (L.) Raf. rootstocks and nutrient sprays on granulation of Valencia sweet orange [*C. sinensis* (L.) Osbeck] fruits. *European Journal of Horticultural Science* **69**, 244-249.

Kriedemann PE (1969) ^{14}C translocation in orange plants. *Australian Journal of Agricultural Research* **20**, 291-300.

Kriedemann PE (1970) The distribution of ^{14}C-labelled assimilates in mature lemon trees. *Australian Journal of Agricultural Research* **21**, 623-632.

Lenz F (1980) Photosynthesis and respiration of citrus as dependent upon fruit load. pp. 70-71.

Lloyd RH (1961) Granulation and regreening. *Citrus News* **37**, 21, 25.

Mahmoud SEM (1954) Prestorage treatment of grapefruit with 2,4-D and its effect on metabolism in storage. *Dissertation Abstracts* **14**, 8.

Malik MN, Akbar MJ, Ahmad I (1981) Granulation intensity in various citrus fruits and its response to different irrigation intervals. *Journal of Agricultural Research, Pakistan* **19**, 135-140.

Martinez JM, Banuls J, Quinones A, Martin B, Primo-Millo E, Legaz F (2002) Fate and transformations of 15N labelled nitrogen applied in spring to Citrus trees. *J. Hort. Sci. and Biotechnol.* **77**, 361-367.

Matsumoto K (1964) Studies on granulation in late-maturing oranges, especially the Sanbokan variety. *Mem. Ehime University Section VI (Agriculture)* **10**, 585-645.

McKinna D (2003) Fruit usage and attitudes study, Part B Comprehensive consumer usage, attitude and sensory study on fruit. Horticulture Australia Limited. Horticulture Australia Limited.

Miller JE, Bird PW, Maritz JGJ (1999) Effect of auxins and potassium on clementine fruit size. *Neltropika Bulletin*, 64-67.

Monselise SP, Goldschmidt EE, Golomb A (1981) Alternate bearing in citrus and ways of control. In 'Proceedings of the International Society of Citriculture' pp. 239-242.

Moon DG, Mizutani F (2002) Soluble solids and titratable acid contents in different portions in maturing satsuma mandarin fruit as affected by water stress. *Journal of the Japanese Society for Horticultural Science* **71**, 1-7.

Mooney PA, Richardson AC (1992) Seasonal trends in the uptake and distribution of nitrogen in Satsuma mandarins. *Proceedings of the International Society of Citriculture* **2**, 593-597.

Mostert PG, van Zyl JL (2000) Gains in citrus fruit quality through regulated irrigation. *Acta Horticulturae* **516**, 123-130.

Munshi SK, Singh R, Vij VK, Jawanda JS (1978) Mineral composition of leaves in relation to degree of granulation in sweet orange. *Scientia Horticulturae* **9**, 357-367.

Pan D, Chen G, Zheng G, Lin H, She W (1998) Effect of growth regulators on juice sac granulation in pummelo fruits (in Chinese, with English abstract). *Journal of Fujian Agricultural College* **27**, 155-159.

Peng YH, Rabe E (1998) Effect of differing irrigation regimes on fruit quality, yield, fruit size and net CO_2 assimilation of 'Mihowase' satsuma. *J. Hort.Sci. and Biotechnol.* **73**, 229-234.

Powell AA, Krezdorn AH (1977) Influence of fruit-setting treatment on translocation of ^{14}C-metabolites in citrus during flowering and fruiting. *Journal of the American Society for Horticultural Science* **102**, 709-714.

Raina JN, Lakhanpal SC (1997) Effect of levels and frequencies of irrigation on granulation in sweet orange (*Citrus sinensis* Osbeck.). *Horticultural Journal* **10**, 1-7.

Ritenour MA, Albrigo LG, Burns JK, Miller WM (2004) Granulation in Florida citrus. *Proc. Fla State Hort. Soc.* **117**, 358-361.

Romero P, Navarro JM, Perez-Perez J, Garcia-Sanchez F, Gomez-Gomez A, Porras I, Martinez V, Botia P (2006) Deficit irrigation and rootstock: their effect on water relations, vegetative development, yield, fruit quality and mineral nutrition of Clemenules mandarin. *Tree Physiology* **26**, 1537-1548.

Rosen JA, Pike CS, Golden ML, Freedman J (1978) Zinc toxicity in corn as a result of a geochemical anomaly. *Plant and Soil* **50**, 151-159.

Sagardoy R, Morales F, Lopez-Millan AF, Abadia A, Abadia J (2009) Effects of zinc toxicity on sugar beet (Beta vulgaris L.) plants grown in hydroponics. *Plant Biology* **11**, 339-350.

Sandhu AS, Singh K (1989) Effect of rootstocks and fruit size on granulation and fruit quality of Jaffa sweet orange. *Haryana Journal of Horticultural Sciences* **18**, 157-160.

Scuderi A (1970) The granulation of Ovale orange fruits in Sicily. *Phytopathologia Mediterranea* **9**, 182-184.

Sharma RR, Saxena SK (2004) Rootstocks influence granulation in Kinnow mandarin (*Citrus nobilis* x *C. deliciosa*). *Scientia Horticulturae* **101**, 235-242.

Sharma RR, Singh R, Saxena SK (2006) Characteristics of citrus fruits in relation to granulation. *Scientia Horticulturae* **111**, 91-96.

Shomer I, Chalutz E, Lomaniec E, Berman M, Vasiliver R (1988) Granulation in pummelo [*Citrus grandis* (L.) Osbeck] juice sacs as related to sclerification. *Proc. Int. Soc. Citricult. VI* **3**, 1407-1415.

Sinclair WB, Jolliffe WA (1961) Chemical changes in the juice vesicles of granulated Valencia oranges. *J. Food Sci.* **26**, 276-282.

Singh MI, Chohan GS (1982) Effect of nutritional sprays on granulation in citrus fruits cv. Wilking mandarin. *Punjab Horticultural Journal* **22**, 52-59.

Singh MI, Chohan GS (1984) Effect of various plant growth regulators on granulation in citrus cv. Dancy tangerine. *Indian J. Hort.* **41**, 221-224.

Singh R, Singh R (1979) Effect of granulation on respiration in citrus fruits. *Sci. and Culture* **45**, 491-493.

Singh R, Singh R (1980a) Effect of granulation on physical and chemical characters of the fruit of 'Kaula' mandarin. *Indian J. Agr. Sci.* **50**, 565-568.

Singh R, Singh R (1980b) Effect of irrigation frequency on granulation in Valencia Late sweet orange. *Punjab Horticultural Journal* **20**, 36-38.

Singh R, Singh R (1980c) Relationship between granulation and nutrient status of Kinnow mandarin at different localities. *Punjab Horticultural Journal* **20**, 134-139.

Singh R, Singh R (1981a) Effect of GA_3, Planofix (NAA) and Ethrel on granulation and fruit quality in 'Kaula' mandarin. *Scientia Horticulturae* **14**, 315-321.

Singh R, Singh R (1981b) Effect of nutrient sprays on granulation and fruit quality of 'Dancy Tangerine' mandarin. *Scientia Horticulturae* **14**, 235-244.

Sinha RB, Randhawa GS, Jain NL (1962) Seasonal changes in Hamlin and Valencia Late oranges. *Indian Journal of Agricultural Sciences* **32**, 149-161.

Sites JW, Reitz HJ, Deszyck EJ (1951) Some results of irrigation research with Florida citrus. *Florida Agricultural Experiment Station Journal Series*, 71-79.

Smith MW (2006) Better rootstocks. In 'R&D Forum, Queensland Citrus Growers Inc.'Gayndah, Australia).

Smith MW, Gultzow DL, Newman TK, Miles AK (2008) Better growing media in a citrus research nursery. In '8th International Congress of Citrus Nurserymen'. Chongqing, China. (Ed. C Zhou). (Poster No. 26 (C5)).

Spiegel-Roy P, Goldschmidt EE (1996) 'Biology of citrus.' (Cambridge University Press: Cambridge).

Stevens JW, Baier WE (1939) Refractometric Determination of Soluble Solids in Citrus Juices. *Industrial & Engineering Chemistry Analytical Edition* **11**, 447-449.

Stewart WS (1949) Effects of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid on citrus fruit storage. *Proceedings of the American Society for Horticultural Science* **54**, 109-117.

Storey R, Treeby MT (1999) Short- and long-term growth of navel orange fruit. *J. Hort. Sci. and Biotechnol.* **74**, 464-471.

Sweet JS, Newman VL, Arpaia ML, Kreuger RR (2009) Seasonal uptake of nutrients by mature field-grown 'Valencia' (Citrus sinesis O.) trees in California. *Proceedings of the International Society of Citriculture* **In press**.

Syvertsen JP (1981) Hydraulic conductivity of four commercial citrus rootstocks. *J. Amer. Soc. Hort. Sci.* **106**, 378-381.

Syvertsen JP, Albrigo LG (1980) Some effects of grapefruit tree canopy position on microclimate, water relations, fruit yield, and juice quality. *J. Amer. Soc. Hort. Sci.* **105**, 454-459.

Syvertsen JP, Graham JH (1985) Hydraulic conductivity of roots, mineral nutrition, and leaf gas exchange of citrus rootstocks. *Journal of the American Society for Horticultural Science* **110**, 865-869.

Treeby MT, Henriod RE, Bevington KB, Milne DJ, Storey R (2007) Irrigation management and rootstock effects on navel orange [*Citrus sinensis* (L.) Osbeck] fruit quality. *Agr. Water Mgt* **91**, 24-32.

Van Noort G (1969) Dryness in navel fruit. *Proc. First Int. Citrus Symp.* **3**, 1333-1342.

Verreynne JS, Rabe E, Theron KI (2001) The effect of combined deficit irrigation and summer trunk girdling on the internal fruit quality of 'Marisol' clementines. *Scientia Horticulturae* **91**, 25-37.

Vock N, Owen-Turner J, Smith D, Mayers P (1997) 'Citrus Information Kit ' (The Department of Primary Industries (Queensland): Brisbane).

Wang X (2005) Studies on the difference of some physiological indexes of vesicle collapse and granulation of Ponkan mandarin fruit (in Chinese, with English abstract). *Journal of Fruit Science* **22**, 216-219.

Yakushiji H, Nonami H, Fukuyama T, Ono S, Takagi N, Hashimoto Y (1996) Sugar accumulation enhanced by osmoregulation in satsuma mandarin fruit. *J. Amer. Soc. Hort. Sci.* **121**, 466-472.

Yonemoto Y, Matsumoto K, Furukawa T, Asakawa M, Okuda H, Takahara T (2004) Effects of rootstock and crop load on sap flow rate in branches of ‘Shirakawa Satsuma’ mandarin (*Citrus unshiu* Marc.) *Scientia Horticulturae* **102**, 295-300.

Zhang Z, Xie Z, Xu W (1999) Anatomical observation on differentiation and granulation processes of juice sacs in *Citrus grandis*. *Acta Botanica SInica* **41**, 16-19.

Zong RJ, Shao PF, Hu XQ, Dai LY (1979) Preliminary study on fluctuations of components in the juice sac and peel of citrus fruits with granulation. *Scientia Agricultura Sinica*, 60-64.

Appendix One: Estimating percentage granulation ratings

Levels of granulation were assessed in the field by cutting fruit in half along the horizontal axis and visually estimating the extent of granulation. Fruit were assessed on a scale of 0 to 5, in increments of 0.5 with 0 being fruit showing no evidence of the disorder and fruit rated 5 being totally or almost fully granulated. Fruit rated O were deep orange, with thin, barely visible vesicle walls, oozing juice when cut. Fruit rated 5 were white, or almost white, and highly gelled. Fruit in other ratings showed increasing thickness of vesicle walls, increasing whiteness in all or some vesicles, limited juiciness and increasing gelation of vesicles.

In 2010, 480 fruit from a range of orchards were used to assess whether the 0 to 5 ratings were an accurate assessment of the extent of granulation in terms of colour and available juice content. Fruit were cut in half equatorially and the diameter of the pulp area was measured. Peel thickness was calculated as half the difference between this measurement and that of the external diameter. Juice was extracted from both halves of the fruit using a domestic citrus juicer. Colour was assessed by use of a chromometer applied to the cut surface. Means of these key parameters were calculated for all ratings (Table xx).

Table 1: Mean values of key colour and juice content parameters by ratings

Rating	% rating	L*	*S*	A	*s*	Density	*s*	Juice/ weight	*s*	juice/ volume[1]	*s*
0	0%	44.81	*0.71*	4.62	*0.92*	1.01	*0.06*	47%	*7%*	74%	*11%*
0.5	5%	46.18	*1.24*	4.31	*0.81*	1.01	*0.06*	44%	*5%*	73%	*11%*
1	10%	47.47	*1.64*	3.85	*0.77*	0.99	*0.06*	42%	*6%*	68%	*11%*
1.5	15%	49.16	*1.76*	3.44	*0.68*	0.99	*0.07*	39%	*6%*	62%	*10%*
2	25%	51.72	*1.99*	3.01	*0.88*	0.98	*0.07*	36%	*6%*	57%	*11%*
2.5	35%	54.20	*2.56*	2.69	*0.80*	1.00	*0.06*	33%	*7%*	50%	*12%*
3	45%	55.19	*3.27*	2.29	*0.87*	0.96	*0.06*	27%	*7%*	42%	*12%*
3.5	55%	58.08	*2.44*	1.99	*0.81*	0.98	*0.05*	25%	*7%*	37%	*12%*
4	65%	60.57	*3.13*	1.41	*0.85*	0.95	*0.05*	19%	*9%*	28%	*13%*
4.5	80%	64.48	*3.52*	0.13	*0.94*	0.93	*0.06*	11%	*6%*	15%	*8%*
5	100%	72.33	*5.58*	-1.53	*1.61*	0.89	*0.10*	3%	*4%*	5%	*6%*

1. Volume calculated excluding peel and vacant core

Comparison of ratings and key parameters showed the best colour correlation was between ratings and L* values (r=0.9175). The correlation for *a* values was -0.8183 and for *b* values was 0.2328. The best correlation for a juice content parameter and ratings was juice % by estimated volume of pulp (-0.8470). The correlation between ratings and juice % by weight was -0.8455. Volume was calculated using the diameter and height of the flesh (i.e. excluding peel) and subtracting the estimated volume of the vacant core at the centre of the mandarin. The calculation assumed the mandarin was ellipsoid in shape.

The mean value for L values and for juice % by volume for each rating was converted to a percentage of the difference between the mean for 0 rated fruit and the mean for fruit rated at 5. Graphing these values showed that for both these parameters the ratings followed a more polynomial curve than the linear scale assumed by the 0-5

ratings (Figure i). Using the average of these two key parameters, an estimated percentage of granulation was assigned to each of the ratings and used in data analysis (Figure 1). These estimated percentage ratings were used in all calculations in this report.

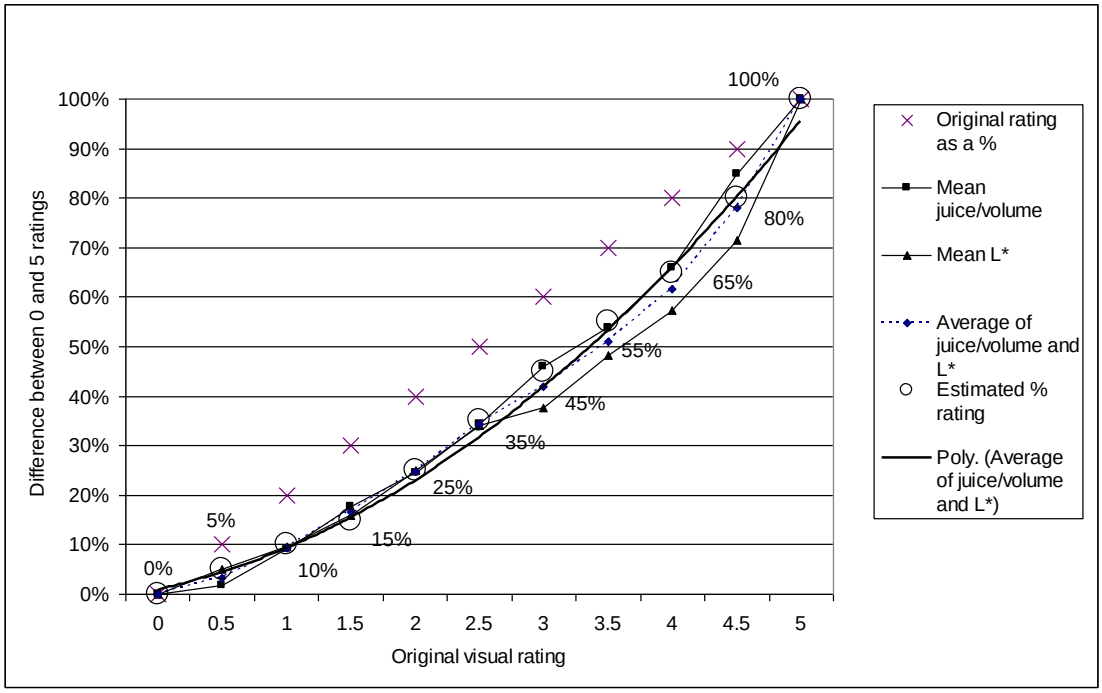


Figure i: Translating granulation scores to an estimated percentage granulation

Appendix Two: Characterisation and non-invasive assessment of granulation

Abstract

In previous years we had indicated that an optical method could be used to differentiate mandarin fruit with a dryness ('gelling') defect if the fruit were in 'breaking green stage' (with defect fruit being less mature than normal fruit). In the current year of activity, we were unable to validate this result. We had also reported no difference between interactance and transmittance optical geometries. Given that dryness defect affected tissue appears white (ie. is highly light scattering), it is reasonable to postulate that a transmission geometry should be advantageous. However, this was not demonstrated in a repeat experiment (interactance vs transmission geometries), nor in a laser scattering trial, with intact fruit. These results are attributed to the influence of the skin and flavedo. Other non-invasive techniques (nuclear magnetic resonance, computed tomography – X ray; on-line transmission X ray, firmness measurements by accelerometer, acoustic frequency and acoustic velocity) were also used unsuccessfully to differentiate defect from normal fruit. These results were attributed to the lack of chemical differences, density difference and the interference of the skin, respectively.

Given an indication in 06/07 that the severity of the defect increased in fruit in long term storage, a 14 week storage trial was established in 07/08 to investigate this possibility. The results did not support the hypothesis that defect severity increased during storage. Except in severely affected fruit, affected fruit were not different in water content relative to control fruit. Defect level was very poorly correlated to other measured fruit attributes (juice Brix, peel weight).

In our previous report, we provided preliminary results that in mature affected fruit, juice sacs were characterised by the presence of numerous small cells, located to the outside of the juice sac. It was postulated that this defect is established early in fruit growth as juice sac cell division presumably ends early in fruit development. This observation was confirmed using resin embedded sections examined using light and transmission electron microscopy, and cryoplaned frozen specimens, examined using scanning electron microscopy. Further, in severely affected tissue, cell wall thickness was increased.

Introduction

This section is repeated from an earlier report for this project.

'Internal dryness' of mandarin (particularly cv. Imperial) fruit is a major issue in the marketing of fruit from the Central Burnett Region of Queensland. The incidence of this 'dryness' defect varies with orchard location, soil type, season and rootstock, and also varies within a single tree (G. Fullelove, P. Rigden, Queensland Department of Primary Industries; unpublished report). This physiological disorder results in a dry, chewy and tasteless sensation when fruit is consumed. Affected fruits cannot be recognised on the basis of external attributes. Juice sacs of cut segments appear turgid, but 'gelled' (Fig. 1). Fruit juiciness (as indexed by extractable juice on a fresh weight basis, % w/w) is decreased from approximately 60 % in control fruit to as low as 4 % in severely affected fruit.

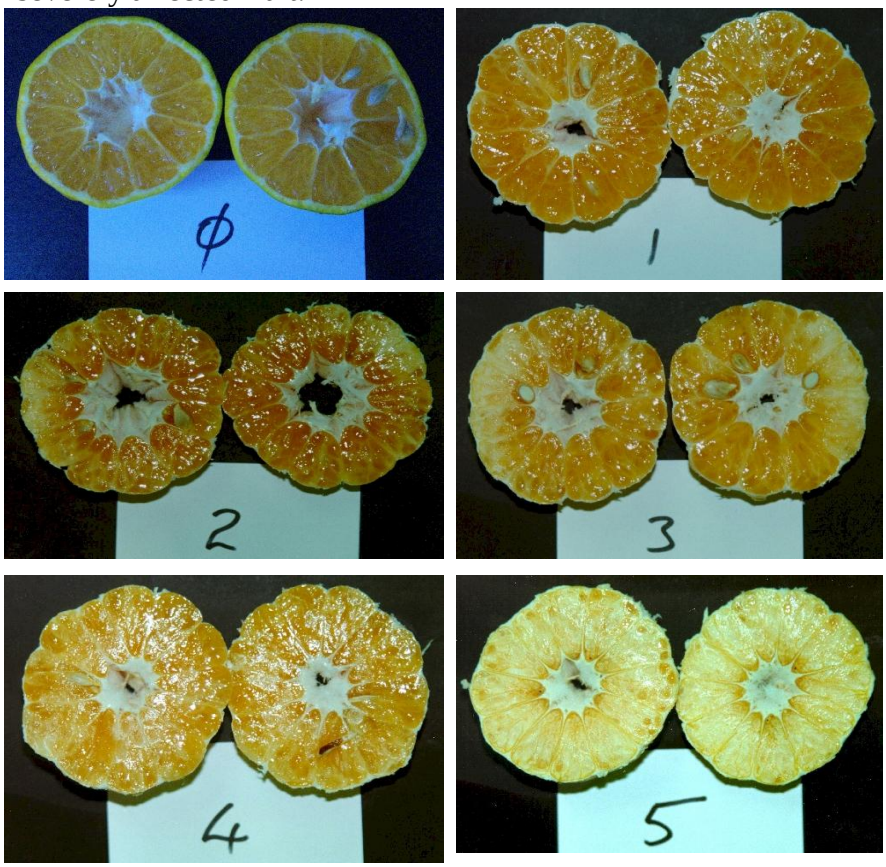


Figure 1. Images of fruit cut tangentially to reveal juice sacs within fruit segments. Fruit are ranked on a visual defect scale of 0 to 5, with increasing severity of disorder.

There are several disorders of citrus fruit which have been described under the broad term of 'internal dryness'. The 'internal dryness' defect under consideration in this study is distinct to the dryness defect caused by frost damage, in which juice sac cells are ruptured, and the entire juice sac subsequently collapses and dehydrates. In the latter case, defect fruit can be detected by increased density.

Albrigo *et al.* (1980) used the term 'section drying' to describe a 'dehydration disorder' in late-harvested grapefruit grown in Florida. This disorder was reported to be the result of either 'granulation' or collapse of juice vesicles. The anatomy of this disorder was studied by Burns and Burns and Achor (1989), who reported that

flattened juice sacs were characterised by collapsed internal parenchyma ('juice cells'). The collapsed cells also possessed thicker cell walls than those of non-collapsed cell walls.
For clarity, the 'internal dryness' disorder observed in the current study is referred to as 'gelling defect'.
A non-invasive sorting technique capable of separating fruit with gelling defect would be of use to the industry. For frost affected fruit with juice cell collapse, sorting techniques based on fruit density are appropriate (e.g. weight-volume measurement, or floatation), but this 'density' approach may not be valid for fruit with gelling defect. However, as gelling defect fruit appear to be more opaque (Fig. 1), an optical sorting technique would appear to be appropriate. We also speculated that the water in gelled fruit might be in a highly H bonded state, which would result in a shift in the position of the water absorption peaks. Such shifts are detectable using short wave near infrared spectroscopy (SWNIRS; 700 – 1050 nm) (e.g. Golic et al., 2003).

A technology based on SWNIRS, using partial or full transmittance of radiation through fruit, is already in commercial use on fruit packlines, primarily for assessment of total soluble solids, TSS, in thin skinned fruit) (e.g. Compac, Auckland, New Zealand; Colour Vision Systems, Bacchus Marsh, Australia). The use of SWNIRS has been reported by a number of authors for assessment of mandarin TSS content (e.g. Kawano *et al.* (1993), Miyamoto and Kitano (1995), Greensill and Walsh (2002); McGlone *et al.* (2003). These studies have employed both interactance and transmission optical geometries, but recommend against the use of a reflectance geometry.
In this project, we have considered whether such technology is appropriate for use in sorting mandarin fruit with gelling defect. Previous work poor, except for a good correlation noted in a population at 'colour break stage', with defect fruit being green, or immature, relative to control fruit.
It is expected that SWNIRS calibration model performance will be affected by fruit size for spectra collected using a transmission optical geometry. To address this issue, Kawano *et al.* (1993) identified absorption at 844 nm as related to fruit (Satsuma mandarin) diameter. Second derivative of absorbance data was first normalised by dividing by the second derivative of absorbance at 844 nm, before use in a multiple linear regression (MLR) regression model. However**,** Miyamoto and Kitano (1995) reported that this procedure hindered prediction ability when sample temperature was varied. These authors reported that it was possible to compensate for optical path-length by including the second derivative of absorbance data at or near the wavelengths of 740 or 840 nm as part of a four wavelength MLR calibration.
Peiris *et al.* (1998) reported on the use of the second derivative of absorbance at 768 and 960 nm to identify fruit with 'section dryness' disorder. These wavelengths are relevant to water absorption. Thus this approach would be appropriate for the identification of gelling defect fruit if such fruit have decreased water content.
In the current study (2007/08 season) we seek to further advance the work done previously (during the years 2004 – 2007) in the area of characterisation of the gelling defect, and to report on the application of SWNIRS and other non-invasive technologies for the detection of this defect in intact mandarin fruit. The performance of SWNIRS in assessment of TSS and dry matter (DM) content is also reported, as a benchmark against which to assess gelling defect results.

Materials and Methods

Fruit populations and attribute characterisation

Twelve lots of mandarin (*Citrus reticulata* cv. 'Imperial') fruit were sourced from commercial harvest from orchards in Gayndah (25.6º S, 151.6º E) and Bundaberg (24.9º S, 152.3º E), Queensland, during the 2007 - 08 mandarin seasons.

Fruit from these populations were used in several exercises related to the non-invasive detection of the disorder, or further characterisation of the disorder. Fruit were cut in an equatorial plane and a subjective score (visual ranking) on fruit gelling defect (0 to 5 scale) based on the visual appearance of the flesh surface of horizontal cut fruit (as shown in Fig. 1) was made by a panel of two to three people. The flesh colour of the cut surface was also assessed using a colorimeter (Minolta, Chroma Meter CR-400/4, adopting the CIE 1976 L*a*b* colour system), using single readings taken from the positions of the cut fruit surface, on each side of fruit from which a SWNIR spectrum was obtained.

For spectroscopy scan, except population 7, one half of each fruit was used for determination of juice % Total Soluble Solids (TSS) and % juiciness and other fruit half for dry matter (DM) content analysis. In population 7, DM content was not measured. Following extraction of juice from fruit halves using a domestic juicer, juice TSS content was determined by refractometry (Bellingham and Stanley RMF 320). The % Juiciness was calculated from the weight of extracted juice and the initial fruit weight. Juice TSS content, fruit DM content, and cut surface colour was assessed for that half of the fruit facing the detector in 0^o geometries, and as the average of values from both halves of the fruit for the 180^o geometry. The %DM content was estimated after skin removal, as weight loss following oven drying at 65º C for 72 h.

Table 1. Description of 2007/08 fruit populations used in various experiments, in terms of date of harvest, experimental use, sample number, and mean and SD of TSS, DM, L*, score and % juiciness.

Population	Date	Experiment	n	Attribute	TSS	DM	L*	Score	% Juiciness
1	24/04/07	spectroscopy	100	Mean	9.83	11.56	53.67	2.45	44.35
				SD	1.01	1.20	7.03	1.45	17.58
2	26/4/07	CT X ray	20	Mean	-	-	-	2.10	-
				SD	-	-	-	1.41	-
3	01/5/07	CT X ray; spectroscopy	40	Mean	10.28	11.96	53.37	2.33	46.13
				SD	1.00	1.30	7.31	1.53	18.32
4	9/04/07	NMR	7	-	-	-	-	-	-
5	01/05/07	spectroscopy	40	Mean	9.85	11.58	53.63	2.44	44.42
				SD	1.01	1.18	7.1	1.45	17.67
6	04/06/07	spectroscopy; laser scattering	100	Mean	10.58	NA	51.82	2.10	NA
				SD	0.96	NA	8.18	1.42	NA
7	24/08/07	CT X ray	12	Mean	-	16.32	-	2.67	-
				SD	-	1.78	-	2.10	-
8	09/11/07	cell wall characterisation	20	10 control 10 severe fruit	-	-	-	-	-
9	12/4/12	Firmness	31	Mean	-	-	-	2.21	-
				SD				1.03	-
10	17/04/08	long term storage	350	-	-	-	-	-	-
11	17/04/08	Spectroscopy ; laser scattering	50	Mean	9.12	10.07	52.06	2.20	41.79
				SD	0.47	0.62	4.70	1.00	11.54
12	17/04/08	X-ray on line	50		-	-	-	XX	-

Light scattering (Laser)

Intact fruit were illuminated with a Nippon Electron Corporation GLG2058 (red) laser. The beam diameter of this laser was approximately 1 mm in diameter. The back-scattered light from the laser was re-emergent from the fruit from in a zone around the point of laser illumination. The extent of this zone was imaged using a CCD sensor (Olympus), and the diameter of the zone assessed.

4.3.1.1 Spectroscopy

For five populations, visible-short wave near infrared spectra (wavelength range 300 – 1156 nm) were acquired using a 100 W tungsten halogen light (in the interactance and transmission optical configurations reported by Greensill and Walsh, 2000b) illumination source, and a NIR enhanced Zeiss MMS1 spectrometer as the detector. Fruit were held with a constant distance between the fruit surface and the optical front end. A 180^0 (light source – sample – detector) geometry were used to collect spectra of fruit for all populations. The 0^0 configuration geometry was used as reported by Walsh *et al.* (2000a), with four 50 W lamps surrounding a detector probe (50 mm diameter) placed in contact with the fruit. The 180^o configuration consisted of a 100 W lamp at 180^o across the fruit from a detector probe (50 mm diameter) placed in contact with the fruit. The integration time used with the geometry used, being varied to maintain a signal level below saturation but above 66% of detector saturation value (e.g. 30 - 70 ms for 0^o and 750 - 1000 ms for 180^ogeometry).

Spectra were collected a position on the equator of the fruit, equidistant from proximal (peduncle) and distal ends. In some of the samples spectra were acquired of intact fruit, fruit with skin removed, and of single segments from the fruit. For the latter condition, a single segment was taken and spectra were acquired of the segment of the surface point at which spectra of intact and peeled fruit had been acquired.

The chemometrics software package 'The Unscrambler' v. 9.1 (Camo) was used for calibration model development based on partial least squares regression (PLSR) analysis. Calibration model performance was assessed in terms of R_{cv}, RMSECV, SD and standard deviation ratio (SD/RMSECV = SDR), while model performance in validation of an independent population (i.e. from a population not represented in the calibration set) was considered in terms of R_p, RMSEP, SDR, slope and bias. Cross-validation was performed using 20 segments with random selection of samples.

A Matlab based scrip developed by C. Leibenberg (Guthrie, Walsh *et al.* 2005) was used to 'map' PLS model performance (root mean square error of calibration, RMSEC) by start and end wavelength (using 8 PLS factors for all models). A spectral window of 300-1050 nm was adopted for Luminosity (L*) and score analysis, and 550-1050 nm for TSS and DM content.

Firmness

Several fruit firmness assessment technologies were used to assess 'firmness' of intact mandarin fruit. Fruit were then cut open and visually assessed for gelling defect. 'Firmness' was assessed using the Sinclair (accelerometer based), Aweta (acoustic frequency based) and our in-house (acoustic velocity based) techniques.

Nuclear magnetic resonance (NMR)

Intact juice sacs and juice from normal fruit, and from fruit with severe symptoms of gelling defect were inserted into D_2O in 5 mm diameter glass tubes. Magnetic resonance spectra of these samples were acquired using a Bruker Spectra-spin 400, and data analysis conducted using Bruker TOPSPIN1.3 software.

X- Ray – mass absorption assessment

Two lots of fruit were characterised using a computed tomography (CT) X-ray facility at the Rockhampton Base hospital (populations 2, 3 and 7, Table 1). Fruit were placed into an industry standard packing tray, with plastic insert. A small metal pin was

inserted into some fruit to aid orientation of the image. The CT scan was acquired using a Philips operated at 80 kV with 60 mA, and visualised at 2.0 mm/slice. This technique required an imaging time of approx. 5 minutes. Following the scan, fruit were cut transversely and given a visual score on the extent of dryness defect.
For line-scan imaging, fruit (population 12, Table 1) were imaged on the premises of Applied Sorting, Bulleen, Victoria, using a variable energy transmission X-ray unit (XR3000), with fruit moving at 132 mm/s. This oil filled/air cooled system is capable of operation between 36 and 60 kV. Accelerating voltage and current was varied to optimise image contrast.

Long term storage behaviour

Fruit were sourced from two blocks from grower 1, two blocks from grower 2, and 1 block from grower 3. All five blocks were located near Mundubbera, Queensland. Equal numbers of fruit were randomly taken from each of these five blocks to create eight populations of 50 fruit (n = 400). All fruit (all populations) were assessed in terms of the following characters:

(a) Skin colour (visual observation on a 1 – 4 scale for 'greenness'; 1 = green, 2 mostly green and 3 = mostly yellow and 4 = mostly orange scores).
(b) Fresh weight in air
(c) Fresh weight in water (for density measurement)

To assess water displacement, fruit was placed in a plastic net weight with a steel rod, and hung from the bottom linkage of an AND HF200 electronic balance (weighing to 1 mg). The weight of this assembly was recorded, and then re-recorded after submersion into a beaker of distilled water.

One population was destructively sampled at the start of the storage period. Five populations were kept at 6^{o} C in a humidified cold room, while a further two populations of fruit were maintained at room temperature. At intervals (3, 7, 10 and 14 weeks for fruit in cold store, and 3 and 7 weeks for fruit stored at room temperature), all fruit were assessed in terms of the three attributes mentioned above. At each time, a population of 50 fruit was also destructively sampled, with the following attributes recorded:

(a) Dryness defect score (visual observation, 0 – 5 scale)
(b) Peeled weight
(c) Pulp weight (after juice extraction)
(d) Juice weight

4.3.1.2 Microscopy

Juice sacs from normal and dryness affected tissue were fixed in a 3% glutaraldehyde in 10% sucrose solution, dehydrated through an alcohol/acetone series, and embedded in LR White resin. Resin embedded sections were sectioned using glass and diamond knives, and examined using light microscopy following toluidine blue staining and transmission electron microscopy (JEOL JSM – 1010) following lead citrate and uranyl acetate staining.
Juice sacs were also cryo-sectioned and transferred to a freezing stage within a JEOL JSM – 630 scanning electron microscopy.

4.3.1.3 High pressure liquid chromatography (HPLC)

4.3.1.4 A preliminary exercise on fruit chemical composition particularly sucrose: glucose: fructose levels of normal and gelled fruit was carried out in this exercise. The soluble sugar composition of the fruit juice was analysed by HPLC method. Single fruit of each category was used for this exercise.

4.4 Results and Discussion

Attribute inter-correlations

The attributes assessed in this study were not independent of each other.

For individual segments, defect score was correlated with L* and b* values, and with % juiciness, as expected (Table 2). These attributes were poorly correlated with juice Brix. Both raw transmitted light level (at 638 nm) and this value, corrected for segment thickness, were poorly related to all assessed variables.

Table 2. Correlation coefficient (R) between attributes of individual segments with a range of defect levels (population 7).

Attribute	CNT	D corr CNT	Seg wt	Juice/seg	Juiciness	L*	a *	b*	Score	Brix
Raw CNT (638 nm)	1	**0.99**	-0.19	0.03	0.34	-0.38	0.08	-0.40	-0.44	0.01
D. corr. CNT		1	-0.22	-0.01	0.31	-0.34	0.05	-0.36	-0.40	0.00
Seg. wt (g)			1	**0.83**	0.13	-0.17	0.28	-0.16	-0.22	0.03
Juice (g)/seg.				1	0.64	-0.56	0.35	-0.51	-0.58	0.20
Juiciness					1	**-0.80**	0.29	-0.69	**-0.78**	0.34
L*						1	-0.35	**0.87**	**0.88**	-0.34
a *							1	-0.06	-0.34	0.44
b*								1	**0.80**	-0.23
Score									1	-0.37
Brix										1

For intact fruit, again defect score was strongly correlated to the L* of the cut surface of fruit. However, there were no strong correlations between juice Brix (Fig. 1), fruit diameter, height or weight and fruit defect level (as assessed by visual score or L* of the cut flesh) (Table 3, 4).

Thus, unfortunately, dryness defect was not correlated to any of the measured externally assessable attributes. Thus this work does not offer any 'handle' on approaches for non-invasive detection of the disorder. Brix was related to dry matter content, as expected (Table 4).

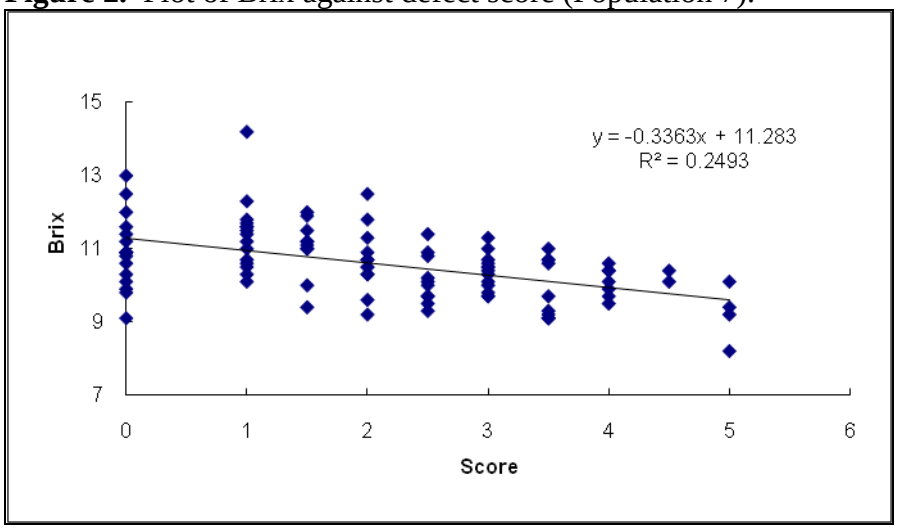
Table 3. Correlation coefficient (R) between various attributes measured of intact mandarin fruit (Population 7).

Attribute	L*	a *	b*	Score	Brix	Diameter	Height	Weight
L*	1	-0.75	0.62	**0.91**	-0.46	0.13	0.37	-0.04
a *		1	-0.10	-0.73	0.60	-0.16	-0.25	0.00
b*			1	0.59	-0.11	-0.02	0.20	-0.10
Score				1	-0.50	0.08	0.44	-0.04
Brix					1	-0.14	-0.35	0.10
Diameter						1	0.15	0.09
Height							1	-0.04
Weight								1

Table 4. Correlation coefficient (R) between various attributes measured of intact mandarin fruit (Population 4).

Attributes	Brix	DM	L*	Score	% Juice	Diameter
Brix	1	**0.86**	-0.22	-0.29	0.30	-0.24
DM		1	0.13	-0.04	-0.08	-0.03
L*			1	0.75	-0.83	-0.09
Score				1	-0.87	0.03
% Juice					1	-0.02
Fruit Diameter						1

Figure 2. Plot of Brix against defect score (Population 7).

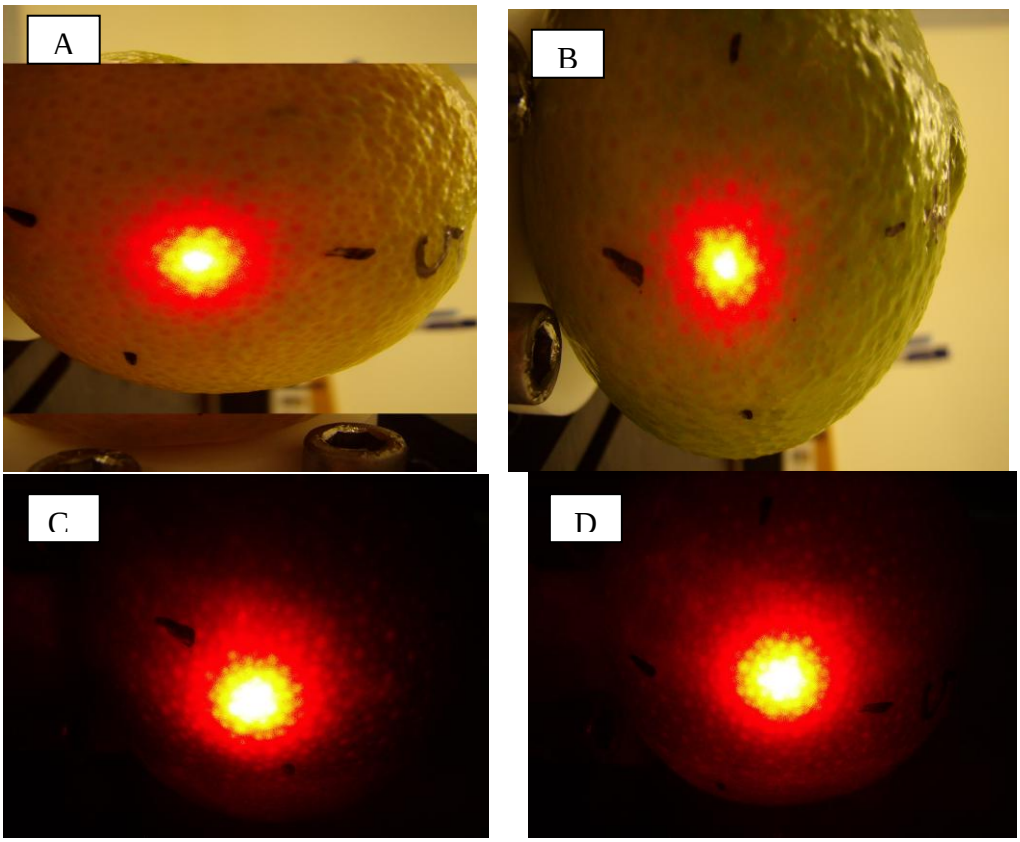


5

(Laser) Light scattering

Mandarin fruit are relatively translucent, with light from the laser visibly penetrating the skin, scattering within the fruit, and re-emerging through the skin. The oil glands represented a path of high transmissivity through the skin. However, there was no difference in the diameter of the visible emergence pattern between control and gelling affected fruit.

Figure 3. Photographs of fruit illuminated by a laser, illustrating emergence pattern of scattered light. (A, C) normal, (B, D) fruit with gelling defect (Population 11).



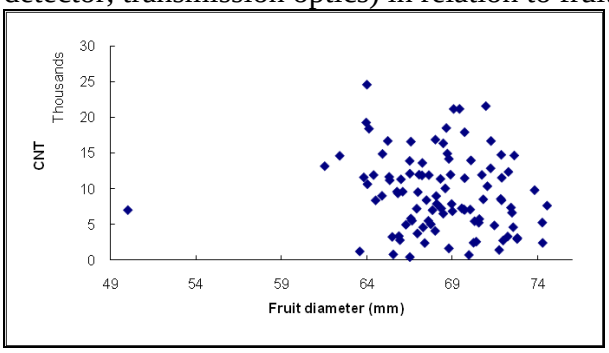
This result is counter intuitive. The experiment should also have been conducted of fruit with the skin removed, for which scattering should be measurable. There is a possibility that the camera auto-exposure may have been activated, invalidating the above result.

VIS – SWNIR Spectroscopy

Single wavelength assessment

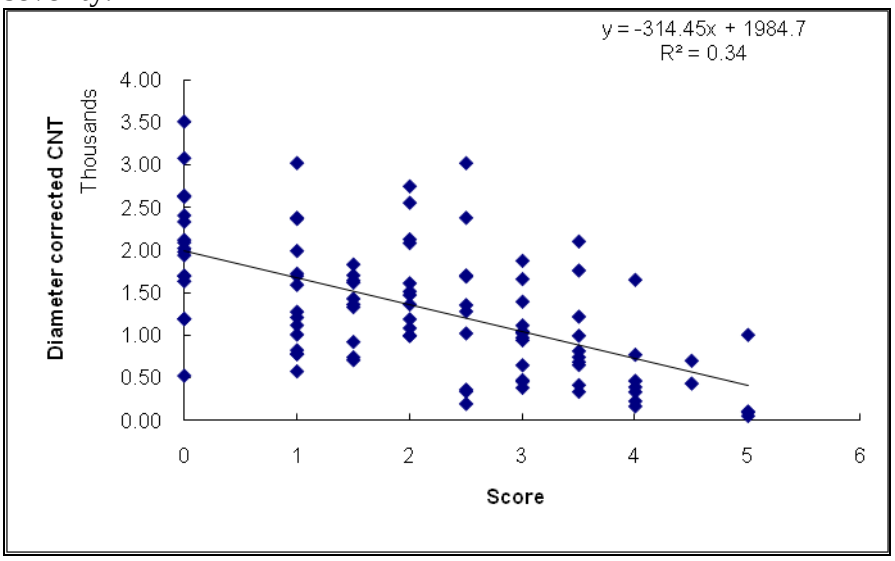
As the flesh of dryness affected fruit appears opaque, we hypothesised that transmissivity for a given wavelength, normalised for fruit diameter might allow a measure of defect severity in intact fruit. However, transmissivity of light through intact mandarin fruit at 638 nm was not related to fruit diameter (Fig. 4), and poorly related to defect score (Fig. 5).

Figure 4. Transmissivity (at 638 nm, as indexed by the A to D count level of the detector, transmission optics) in relation to fruit diameter (Population 6).



Neither raw transmissivity (638 nm, intact fruit) or transmissivity corrected for (divided by) measured fruit diameter were strongly related to visual defect score (Fig. 5).

Figure 5. Transmissivity (at 638 nm, indexed by the A to D count level of the detector, transmission optics) divided by fruit diameter, in relation to fruit defect severity.



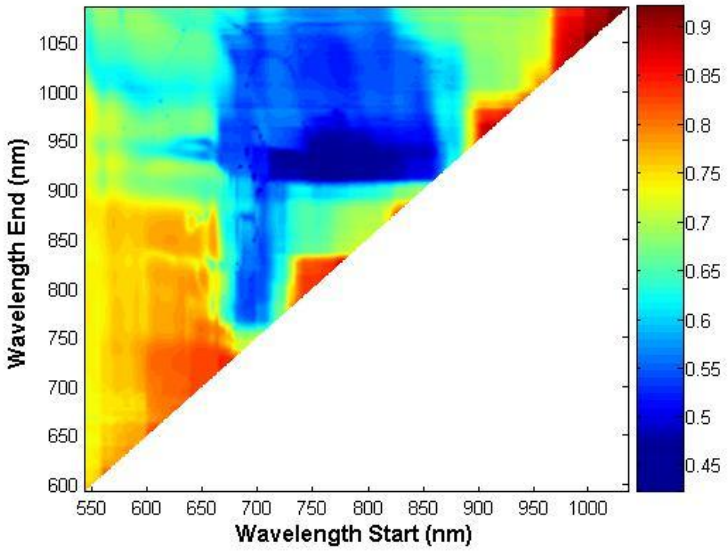
Interactance optics - wavelength region selection

SWNIR spectroscopy using interactance optics has been previously used for the assessment of TSS and DM in intact Imperial mandarin (e.g. Guthrie et al., 2005). Statistics for PLS regressions are reported here for these attributes as a means of confirming that the instrumentation and techniques used were performing to specification.

The moving window approach documented in Guthrie et al. (2006) was used to optimise the wavelength window used for model development. For both TSS (Fig. 6) and DM (data not shown), models with minimal RMSECV (of approx. 0.53 %TSS)

were achieved when using a wavelength region starting around 700 to 720 nm and ending around 960 nm (Fig. 6). These results are consistent with previous reports (e.g. Guthrie et al., 2006), indicating that the equipment and protocols followed were 'in specification'.

Figure 6. RMSECV (colour coded, see index on right) for TSS - PLSR models varying in start (x axis) and end (y axis) wavelengths (Population 1).



The best wavelength range for PLS regression models on the L* value of the mandarin flesh was from around 550 to around 695 nm (Fig. 7), and from around 550 nm to about 720 nm for the visual score of dryness defect in fruit flesh. This visible wavelength range is expected for an attribute visually assessed, probably light scatter. The best wavelength range for PLS regression models on % juiciness of mandarin flesh was from around 550 to around 950 nm (Fig. 8).

Figure 7. RMSECV (colour coded, see index on right) for L * Value - PLSR models varying in start (x axis) and end (y axis) wavelengths (Population 1).

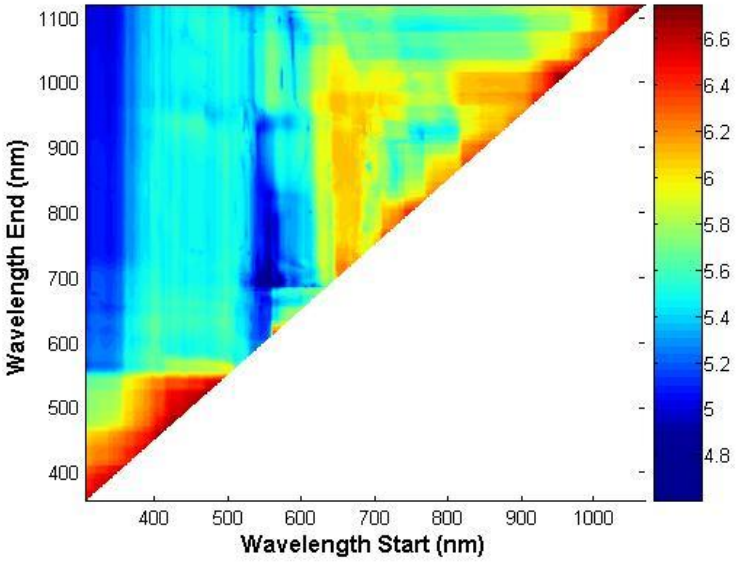
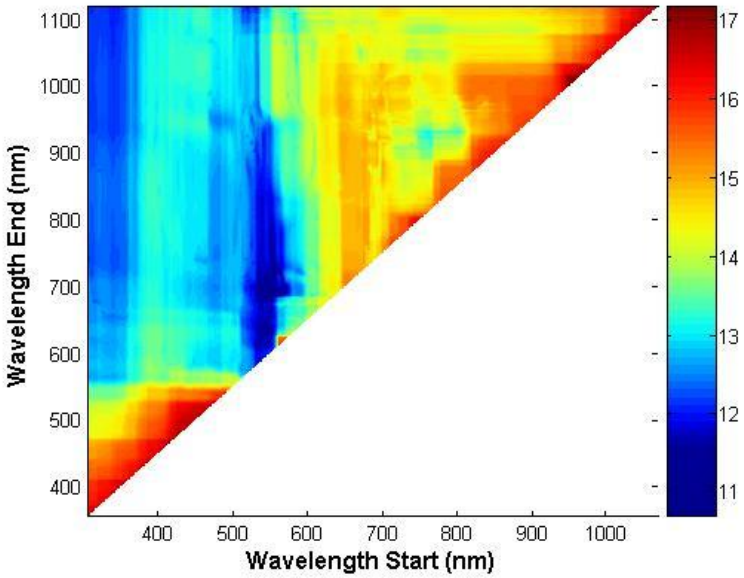


Figure 8. RMSECV (colour coded, see index on right) for % juiciness - PLSR models varying in start (x axis) and end (y axis) wavelengths (Population 1).



Interactance optical geometry- PLSR modelling

The correlation of spectra with TSS and DM was satisfactory in all the populations (Table 5). These results were consistent with previous year's exercises, and indicate that equipment function and protocols used in these exercise were correct. However, PLSR calibration model performance on L* value, score and juiciness was poor in all the populations and correlation coefficient of determination (R^2) = 0.75 was always less than 0.75, even for fruit harvested in 'breaking green' stages (Populations 1 and 11) (Table 5).

Table 5. Calibration Statistics (interactance geometry, second derivative of absobance data, intact fruit). R values >0.87 (i.e. $R^2 > 0.75$) have been bolded.

Attributes	Population	PCs	R	RMSECV	SDR	Slope
TSS	1	7	**0.88**	0.48	2.12	0.77
DM	1	7	**0.89**	0.55	2.18	0.8
L*	1	7	0.74	4.26	1.65	0.58
Score	1	6	0.66	1.06	1.37	0.47
Juiciness	1	7	0.75	11.2	1.57	0.58
TSS	3	6	**0.89**	0.44	2.30	0.79
DM	3	6	**0.89**	0.58	2.24	0.81
L*	3	9	0.81	4.41	1.66	0.71
Score	3	9	0.77	0.95	1.61	0.67
Juiciness	3	9	0.85	9.64	1.90	0.79
TSS	6	6	**0.87**	0.5	2.02	0.79
DM	6	5	**0.88**	0.54	2.17	0.78
L*	6	5	0.72	4.26	1.67	0.54
Score	6	5	0.78	10.9	1.62	0.63
Juiciness	6	9	0.75	0.94	1.54	0.59
TSS	1+3+6	7	**0.89**	0.47	2.18	0.8
DM	1+3+6	5	**0.90**	0.51	2.36	0.814
L*	1+3+6	9	0.76	4.08	1.74	0.59
Score	1+3+6	9	0.83	9.61	1.84	0.69
Juiceness	1+3+6	10	0.78	0.9	1.63	0.61
TSS	11	6	0.83	0.26	1.81	0.76
DM	11	7	0.85	0.33	1.88	0.77
L*	11	1	0.11	3.34	1.41	0.03
Score	11	4	0.30	0.97	1.03	0.16
Juiceness	11	5	0.50	7.36	1.14	0.33

TSS and DM models validated well when used in prediction of an independent population, as expected, and again indicating the technology was working well (Table 6). However, L*, score and % juiciness did not validate well (Table 6).

Table 6. PLSR model validation statistics. R values >0.87 (i.e. $R^2 > 0.75$) have been bolded.

Attributes	Cal set	Pred set	PCs	R	RMSECV	SDR	bias	Slope
TSS	1+3	5	5	**0.86**	0.52	1.94	0.02	0.74
DM	1+3	5	5	**0.90**	0.52	2.27	0.002	0.85
L*	1+3	5	8	0.78	4.10	1.73	0.6	0.60
Score	1+3	5	9	0.74	0.98	1.49	0.07	0.58
Juiceness	1+3	5	5	0.76	11.44	1.55	1.01	0.52

We conclude that interactance geometry SWNIR spectroscopy is not appropriate as a technique for the differentiation of normal and gelling defect fruit.

Transmission optical geometry- PLSR modelling

We hypothesised transmission geometry should do a better job than the interactance geometry. However, poorer statistics obtained, even for Brix and DM (Table 7). The poor results for this geometry could be attributed to effect of seed and central cavity and pith materials present in the fruit.

Table 7. Calibration Statistics (transmission, skin on)

Attributes	Population	PCs	R	RMSECV	SDR	Slope
TSS	1	5	0.82	0.5	2.01	0.72
DM	1	5	0.82	0.65	2.03	0.67
L*	1	1	0.49	4.96	1.42	0.27
Score	1	1	0.59	1.19	1.22	0.34
Juiceness	1	1	0.59	13.6	1.30	0.36
TSS	3	7	0.82	0.55	1.75	0.73
DM	3	9	0.81	0.6	2.10	0.73
L*	3	2	0.6	4.63	1.49	0.4
Score	3	1	0.6	1.1	1.27	0.38
Juiceness	3	3	0.62	12.6	1.36	0.41
TSS	5	6	**0.89**	0.46	2.26	0.84
DM	5	5	**0.87**	0.52	2.49	0.82
L*	5	1	0.53	6.2	1.19	0.32
Score	5	1	0.73	1.04	1.44	0.32
Juiceness	5	2	0.79	10.1	1.83	0.64
TSS	1+3+5	7	0.86	0.54	1.99	0.78
L*	1+3+5	2	0.65	5.2	1.48	0.46
Score	1+3+5	3	0.70	1.04	1.39	0.51
TSS	6	5	**0.87**	0.47	2.05	0.78
L*	6	3	0.78	4.64	1.76	0.64
Score	6	1	0.78	0.86	1.65	0.62
TSS	11	6	0.77	0.29	1.62	0.64
DM	11	6	0.75	0.26	2.38	0.83
L*	11	5	0.34	4.45	1.06	0.18
Score	11	2	0.54	0.77	1.30	0.34
Juiceness	11	3	0.63	5.62	1.50	0.46

Poor validation results also obtained using transmission geometry (Table 8), with R^2<0.87 in all the attributes considered in this experiment.

Table 8. Validation statistics (skin on) (transmission geometry)

Attributes	Cal set	Pred set	PCs	R	RMSECV	SDR	Slope
TSS	1	5	4	0.82	0.67	1.56	0.79
DM	1	5	14	0.75	0.92	1.40	0.99
L*	1	5	1	0.62	5.88	1.25	0.28
Score	1	5	1	0.66	1.181	1.27	0.36
Juiciness	1	5	2	0.71	12.88	1.43	0.51
TSS	1+3+5	6	5	0.81	1.86	0.51	0.75
L*	1+3+5	6	1	0.69	7.66	1.07	0.21
Score	1+3+5	6	1	0.73	1.43	0.99	0.31

With the fruit skin removed, calibration models were improved compared to that with skin on, with R^2 >0.85 obtained for TSS, L* and score (Table 9).

Table 9. PLSR calibration model statistics of whole fruit with skin removed (transmission geometry; n = 100).

Attributes	Population	PCs	R	RMSECV	SDR	Slope
TSS	6	8	**0.89**	0.465	2.05	0.81
L*	6	2	0.85	4.64	1.76	0.74
Score	6	2	**0.87**	0.86	1.65	0.76

A similar exercise was also conducted with intact segment for the same population (Table 10). Model performance of intact segments was similar to that of whole fruit with skin removed.

Table 10. PLSR calibration model statistics – spectra of single segments (transmission geometry; n = 100).

Attributes	Population	Mean	SD	PCs	R	RMSECV	SDR	Slope
TSS	6	10.72	0.87	6	**0.89**	0.35	2.48	0.807
L*	6	45.66	8.04	1	0.63	5.26	1.53	0.41
Score	6	1.68	1.40	1	0.76	0.87	1.62	0.58
Juiciness	6	59.53	14.16	3	0.63	8.00	1.77	0.45

We conclude that a transmission geometry is not preferable to an interactance geometry for TSS and DM measurement, and that this geometry is also inappropriate for the detection of gelling defect.

Reflectance optical geometry- PLSR modelling

Calibration model performance was inferior when based on reflectance spectra (Table 11), compared to the use of interactance or transmission spectra. This result is

expected, as reflectance spectra will primarily contain information from the skin of the fruit.

Table 11: PLSR calibration model statistics of the attributes using reflectance geometry.

Attributes	Population	PCs	R	RMSECV	SDR	Slope
TSS	11	7	0.66	0.29	1.31	0.53
DM	11	1	0.15	0.82	1.00	0.05
L*	11	1	0.24	3.60	1.31	0.06
Score	11	1	0.01	0.85	1.18	0.00

Firmness

Gelling defect score was not related to firmness as indexed by the Aweta, Sinclair or our in-house build technologies (Table 12). This result is ascribed to the effect of the relatively loose skin of a mandarin, such that the non-invasive measurements are not reflected of the fruit flesh firmness.

Table 12. Correlation coefficient (R) between various non-invasive measurements of firmness of intact fruit and visual defect score attributes (Population 9, Table 1). SV and Aweta measurement were recorded on the same day, fruit were then sent to another location for the Sinclair measurement, then returned, with a further measurement by the SV technique (week 2). The SV refers to the sound velocity technique. Aweta refers to the Aweta acoustic frequency technique. SIQ refers to the Sinclair accelerometer.

Attributes	SV (week 0)	Aweta	SIQ	SV (Week 2)	Defect score
SV (week 0)	1	**0.70**	0.39	0.50	0.26
Aweta		1	0.66	0.37	0.35
SIQ			1	0.33	0.40
SV (Week 2)				1	0.29
Defect score					1

The best correlation between instruments was achieved using the SV and Aweta units. The correlation between SV week 0 and week 2 readings was weak, indicating that fruit within the lot were not on the same 'trajectory' of loss of SV units. Further, the difference in SV values between week 0 and week 2 was not correlated with score (R= 0.04).

NMR spectroscopy

The spectra of seed coat material was distinctly different to that of juice sacs or juice (Fig. 9). The major spectral features of NMR spectra of intact healthy and defect juice sacs were similar, inferring similar chemical composition (Fig. 9, 10). Indeed

the spectra of juice alone was similar to that of whole juice sacs. Thus, presumably the juice sac spectra is dominated by features related to sugar in water, with the'crystalline' C in cellulose a brod underlying influence.

Figure 9. NMR spectra of an intact gelled juice sac (4, blue line), a control juice sac (5, red line); juice from a control fruit (6; green line) and seed coat (7; purple line).

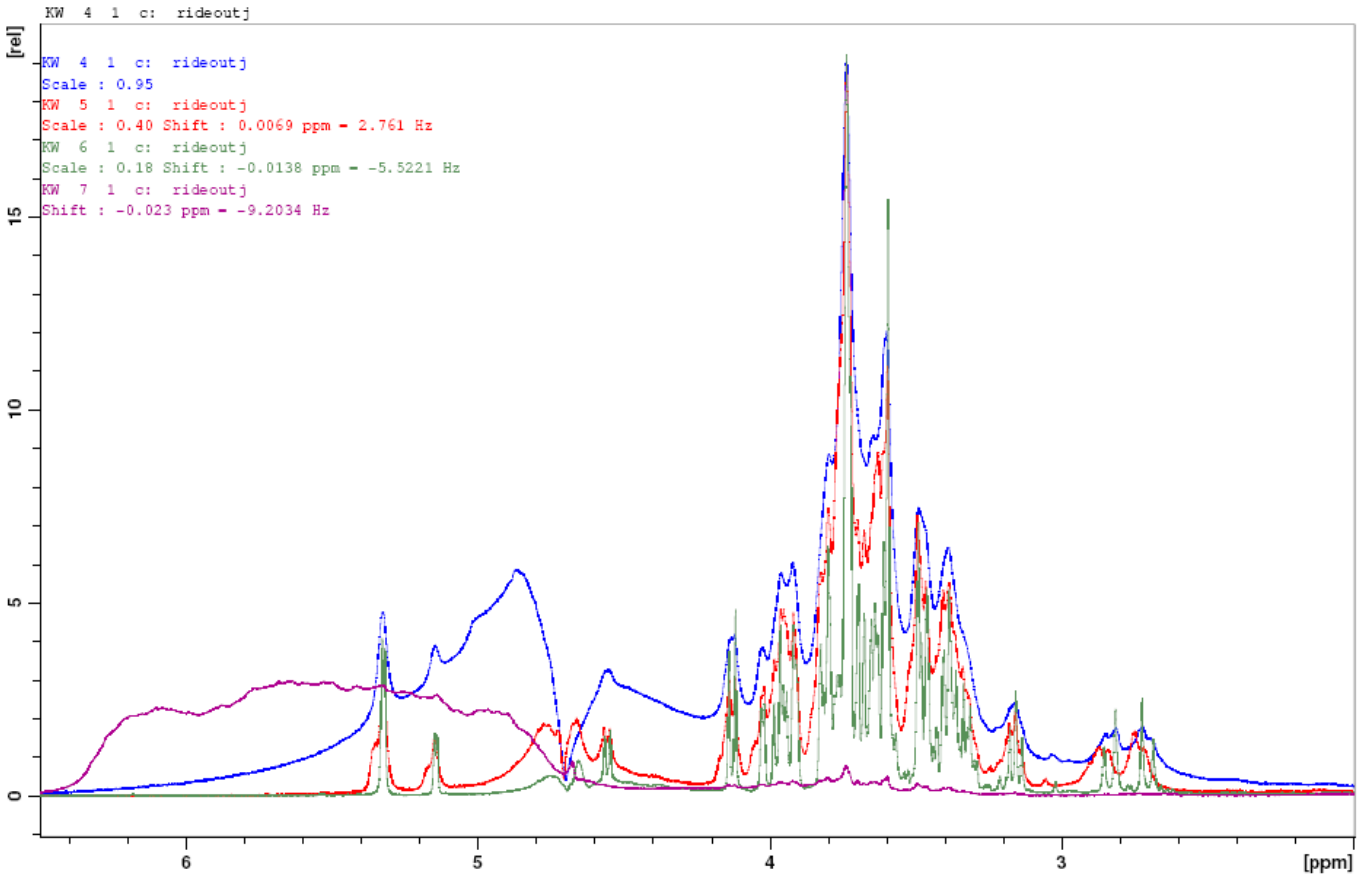
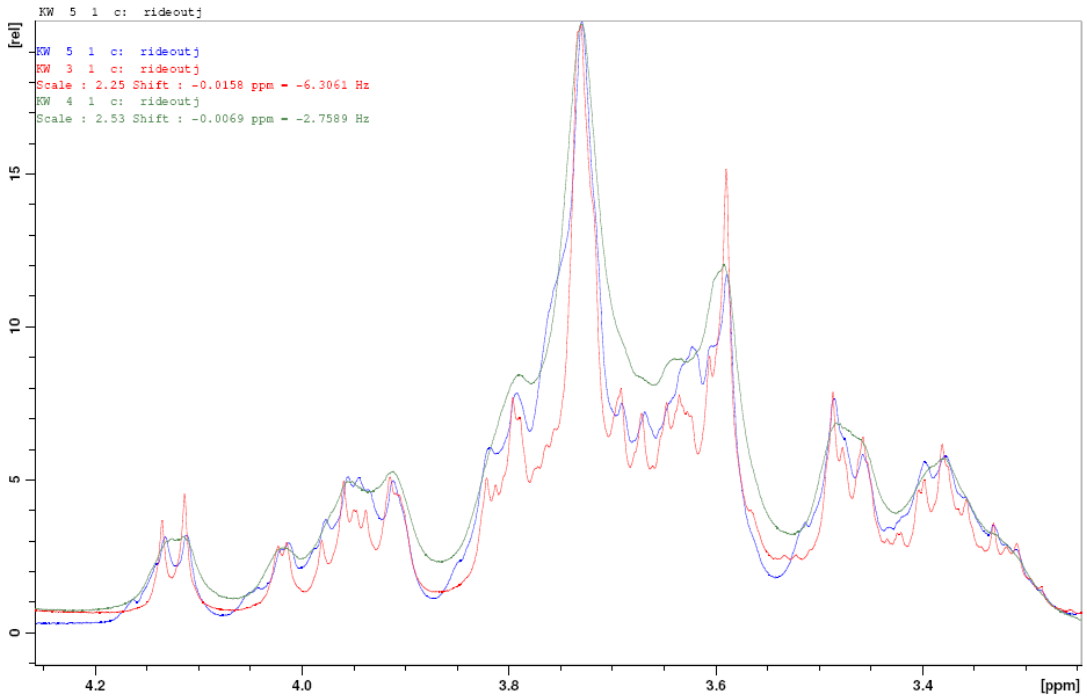


Figure 10. NMR spectra of intact gelled juice sacs (3 and 4, red and green lines, respectively), a control juice sac (5, blue line).



Given the similarity between NMR spectra of normal and gelling defect affected juice sacs, this technology is inappropriate for the non-invasive detection of this disorder.

X- Ray – mass absorption assessment

CT imaging

CT images of mandarin fruit reveal spaces of low density, interpreted as (a) pith tissue in the central fruit axis; (b) flavedo tissue between fruit segments and skin; (c) oil glands in the fruit skin; (d) air space around some seeds (Fig. 11). In some fruit, some juice cells were also of low density. This was not associated with any visible defect within the fruit. Seeds were not distinguished directly by their X-ray absorption, but only by the presence of an associated air gap.

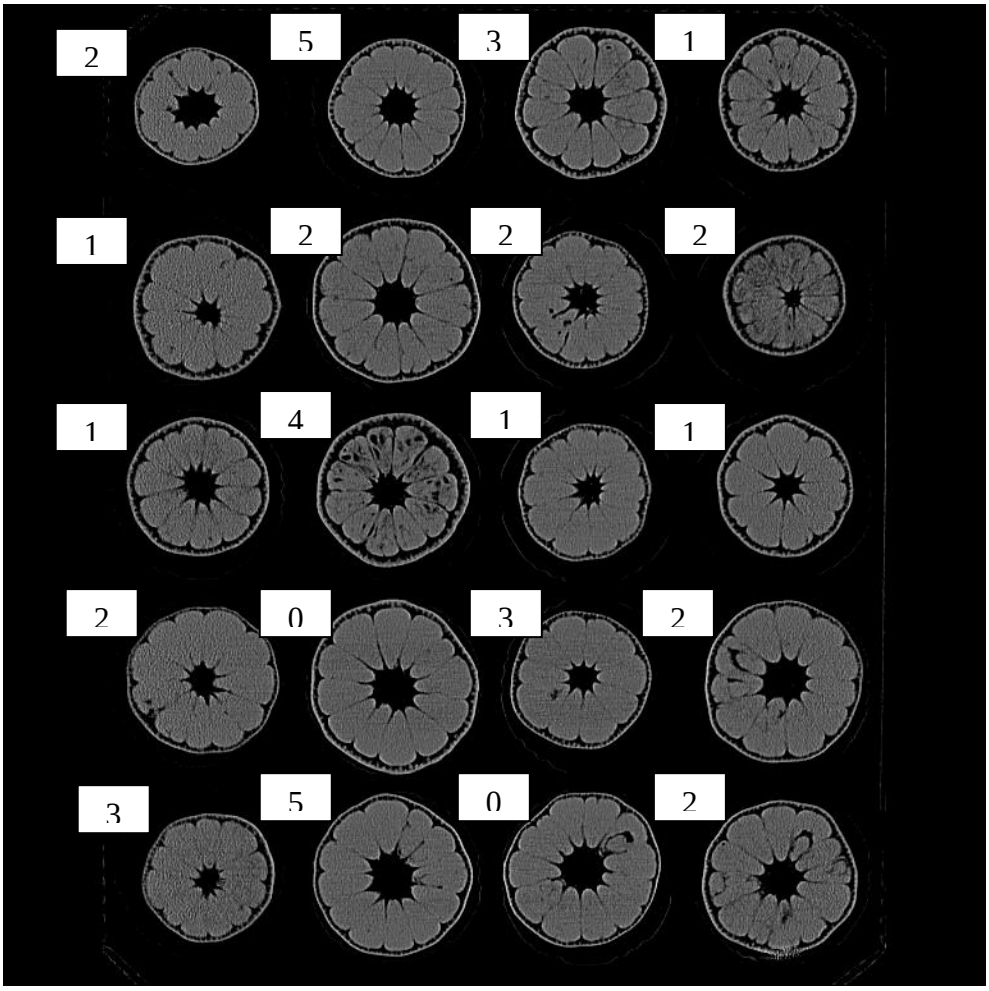


Figure 11. Medial 'transverse section' (TS) CT X-ray image of a tray of mandarin fruit, imaged using an accelerating voltage of 35 kV and a current of 2 mA. The figures from left to right, top to bottom indicates the visual scores of gelling defect.

Line-scan X-ray image

At higher operating energies (accelerating voltage), X-rays are capable of penetrating denser objects, but little contrast is achieved with lower density materials like fleshy fruit (mandarin). However, with low operating energy the noise of the detection system increases (leading to fuzzier images). The applied current is another variable that can be adjusted (X-ray flux). The result is that a compromise must be struck, with accelerating voltage and current optimized for a given application. For mandarin fruit, greater contrast in the image produced with the lower (35 kV cf. 55 kV) accelerating voltage.

An X-ray transmission system is able to image differences in mass absorption. This is a function of both density and thickness. Unfortunately, as fruit are not regular objects, both attributes vary and the resulting picture could not be interpreted in terms of density alone.

Unfortunately, the mass absorption differences within fruit are relative minor, even for structures such as a seed or stone (stone fruit). This result reflects two considerations: (a) relatively little density difference between such objects and fruit flesh, and (b) the uncertainty introduced by varying path lengths.

In practice, gelling defect could not be detected, indicating that little difference in mass absorption by healthy and gelled tissue. Seediness within fruit also could not be detected, unless the seed was surrounded by an air space (e.g. Fig. 12).

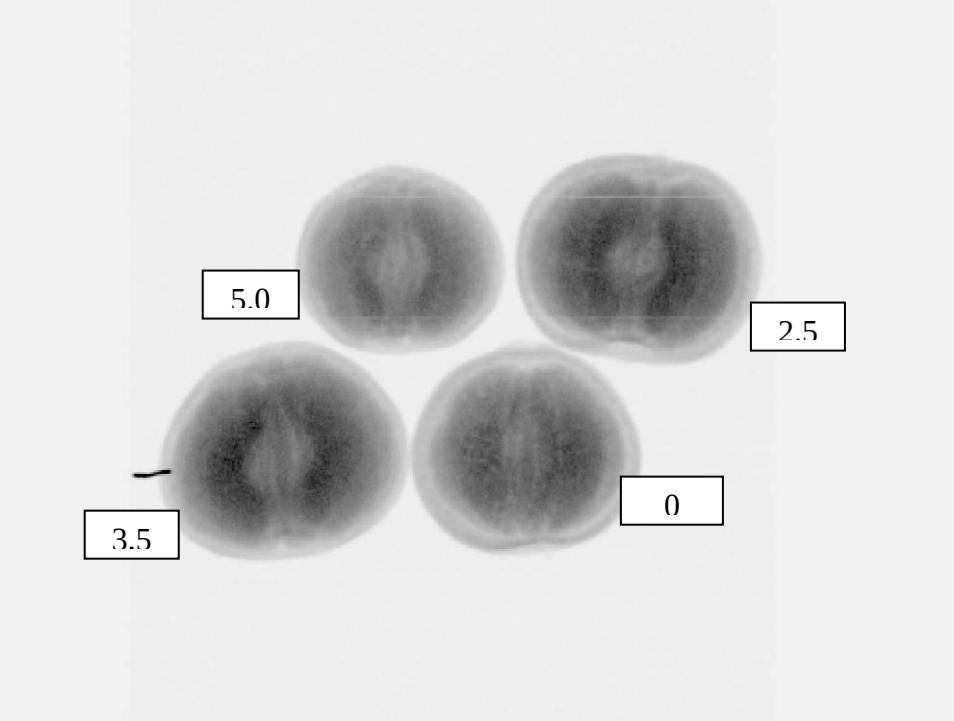


Figure 12. Medial 'transverse section' (TS) X-ray image of a four mandarin fruit travelling on a belt at 0.1 m/s, imaged using an accelerating voltage of 35 kV and a current of 2mA. Fruit on bottom left has a pin inserted for ease of image orientation (fruit # 13, 14, 15 and 16 with visual scores of 3.5, 5, 0, and 2.5 respectively).
We conclude that X-ray imaging is also unable to differentiate gelling defect affected fruit from normal fruit.

Long term storage (additional data provided in Appendix)

Skin colour and fruit density were not significantly different between the seven lots of fruit at the beginning of the long term storage trial (Table 13, columns marked week 0).
All fruit were fully coloured (skin colour score 4) after 3 weeks of storage at either 6 or 22^{o} C. However, there was no significant change in fruit density during storage for any of the seven lots. Further, there was no significant difference between lots (varying in storage time) for the attributes of % peel weight, % pulp weight, % juice weight or defect score.
Table 13. Attributes of fruit at the beginning and end of a post-harvest period of up to 14 weeks for fruit stored at 6^{o} C, and 7 weeks for fruit stored at room temperature (approximately 22^{o} C). Each harvest consisted of a population of 50 fruit, with 350 fruit used in total for the trial (population 10, Table 1). Dry matter content at week 0 was 10.07 +/- 0.62, and in fruit stored for 14 weeks at 6^{o}C was 11.89 +/- 1.84.

		Week 0			At harvest				
Storage condition	Statistics	Skin colour	Density (g/cc)	Week of harvest	Density (g/cc)	Defect Score	%Peel (g/gFW fruit)	%Pulp (g/gFW fruit)	%Juice (g / gFW

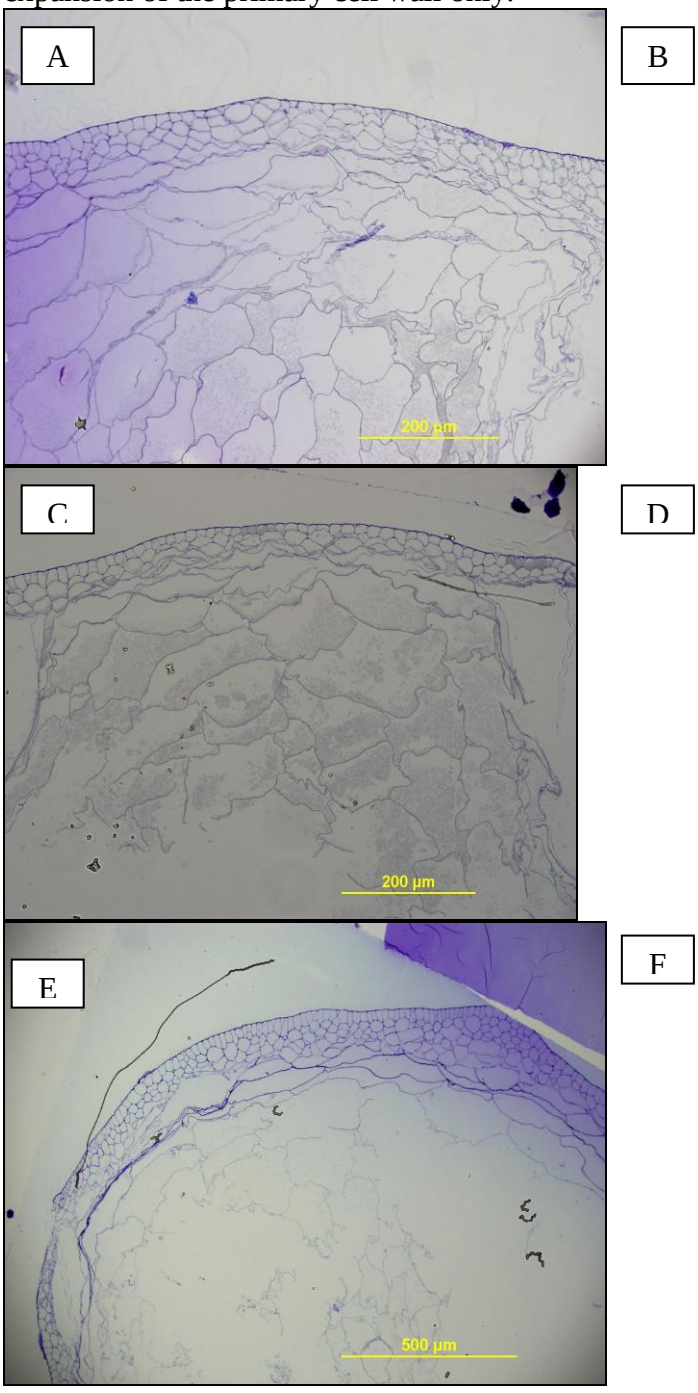
									fruit)
Cold Room	Mean	2.50	0.88	0	-	2.42	30.3	36.1	33.6
	SD	*0.76*	*0.04*		-	*1.26*	*5.1*	*11.1*	*13.5*
Cold Room	Mean	2.76	0.87	3	0.85	2.05	33.4	36.7	44.5
	SD	*0.74*	*0.04*		*0.05*	*1.43*	*6.6*	*9.9*	*15.3*
Cold Room	Mean	2.90	0.87	7	0.85	2.30	33.8	36.4	44.6
	SD	*0.74*	*0.05*		*0.05*	*1.40*	*7.8*	*10.8*	*16.9*
Cold Room	Mean	2.74	0.89	10	0.85	1.97	30.7	38.6	31.7
	SD	*0.69*	*0.04*		*0.05*	*1.21*	*5.9*	*9.6*	*11.0*
Cold Room	Mean	2.96	0.88	14	0.83	2.67	29.3	37.2	46.7
	SD	*0.67*	*0.04*		*0.04*	*1.22*	*8.5*	*11.0*	*16.9*
Room temp	Mean	2.58	0.88	3	0.88	0.57	22.3	19.6	74.6
	SD	*0.54*	*0.02*		*0.02*	*0.86*	*4.3*	*3.7*	*5.9*
Room temp	Mean	3.00	0.88	7	0.85	1.98	32.1	35.1	46.8
	SD	*0.67*	*0.03*		*0.07*	*1.56*	*7.3*	*9.4*	*18.3*

There was no significant difference for another of the attributes assessed at week 0 with that of subsequent harvesting times (up to 14 weeks in cold temperature and 7 weeks at room temperature) (Table 13).

We conclude that there was no increase in fruit defect severity during storage.

Anatomical characterisation

Juice sacs from control fruit were characterised by large internal cells, enveloped by an epidermal and hypodermal layers, approximately three to five cells deep (Fig. 13 A,B). These layers were composed of smaller cells, relative to the internal cells. This results was confirmed with SEM and TEM based observations (Figs. 14, 15).
In extremely affected fruit (fruit with no extractable juice), the cell wall thickness of juice cells was also increased (Fig. 14). These cell walls appeared to consist of an expansion of the primary cell wall only.



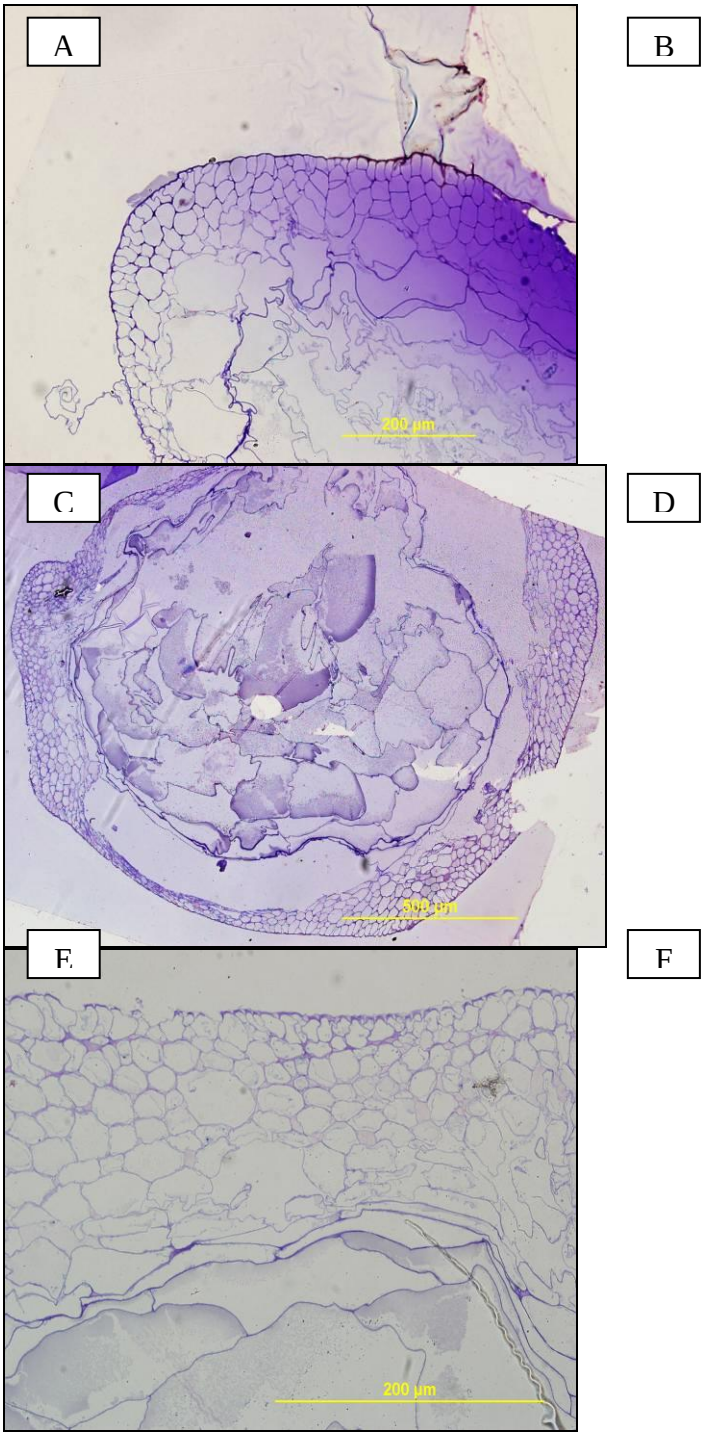
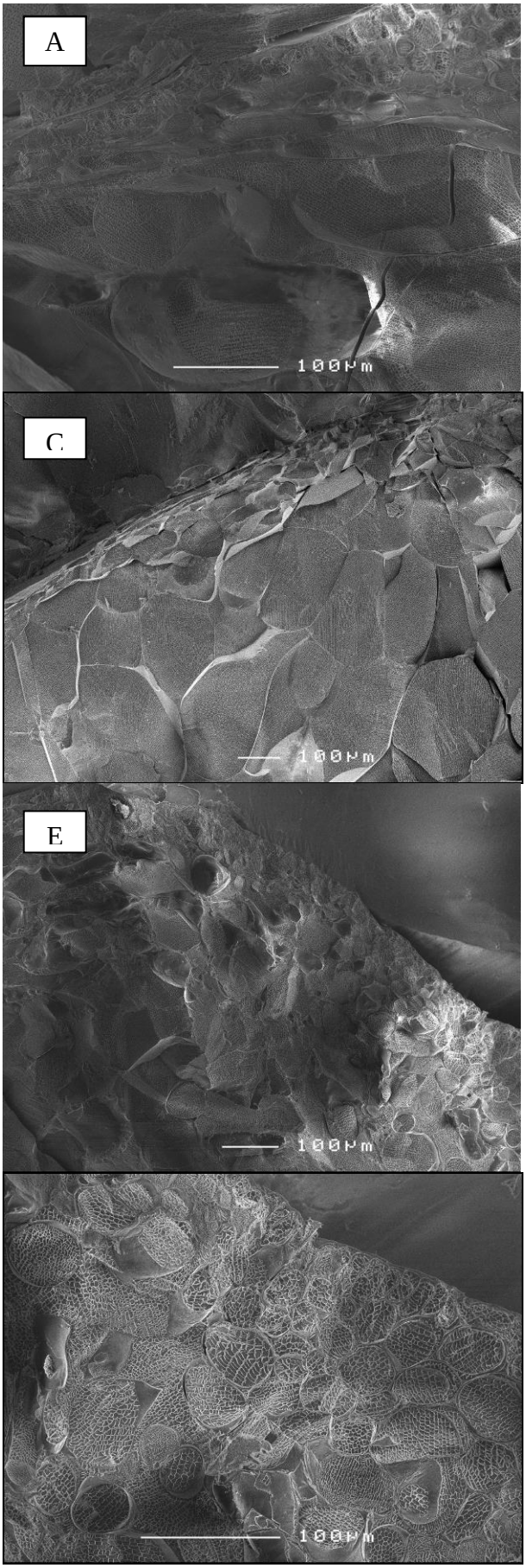


Figure 13. Light microcopy of touidine blue stained sections of resin embedded juice sacs, for (A, B) control fruit; (C,D), fruit with moderately severe symptoms of dryness defect, and (E,F) severely affected fruit. The number of cell layers in the hypodermal tissue increased in drynness affected fruit. However, there was no apparent difference in cell wall thickness.


A
B
100µm
C
D
100µm
E
F
100µm
100µm

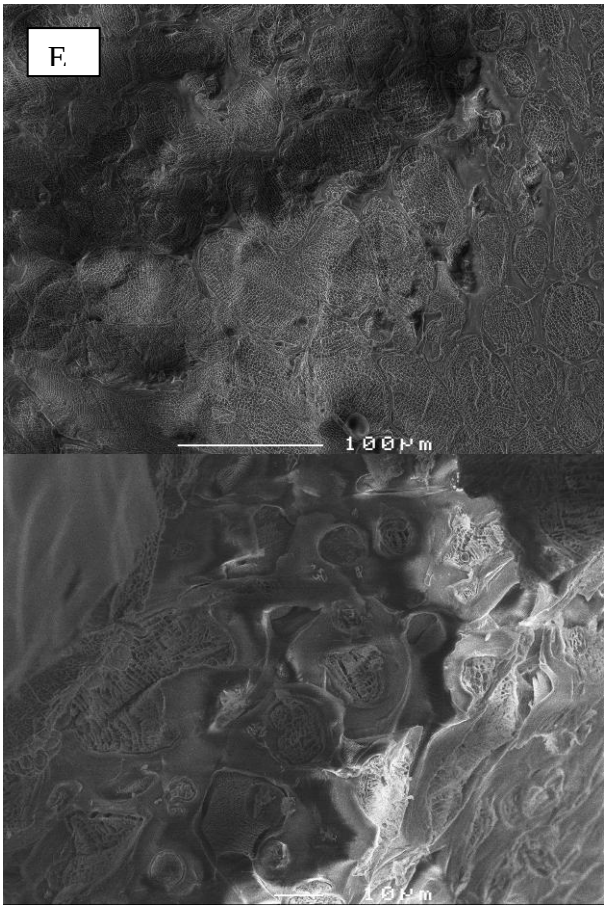


Figure 14. Scanning electron microcopy of cryo-section frozen juice sacs, for (A, B) control fruit; (C, D) fruit with moderately severe symptoms of dryness defect; and (E,F) extremely affected fruit (no extractable juice). The number of cell layers in hypodermal tissue was increased in dryness affected fruit. However, there was an apparent difference in cell wall thickness in the extremely affected fruit.

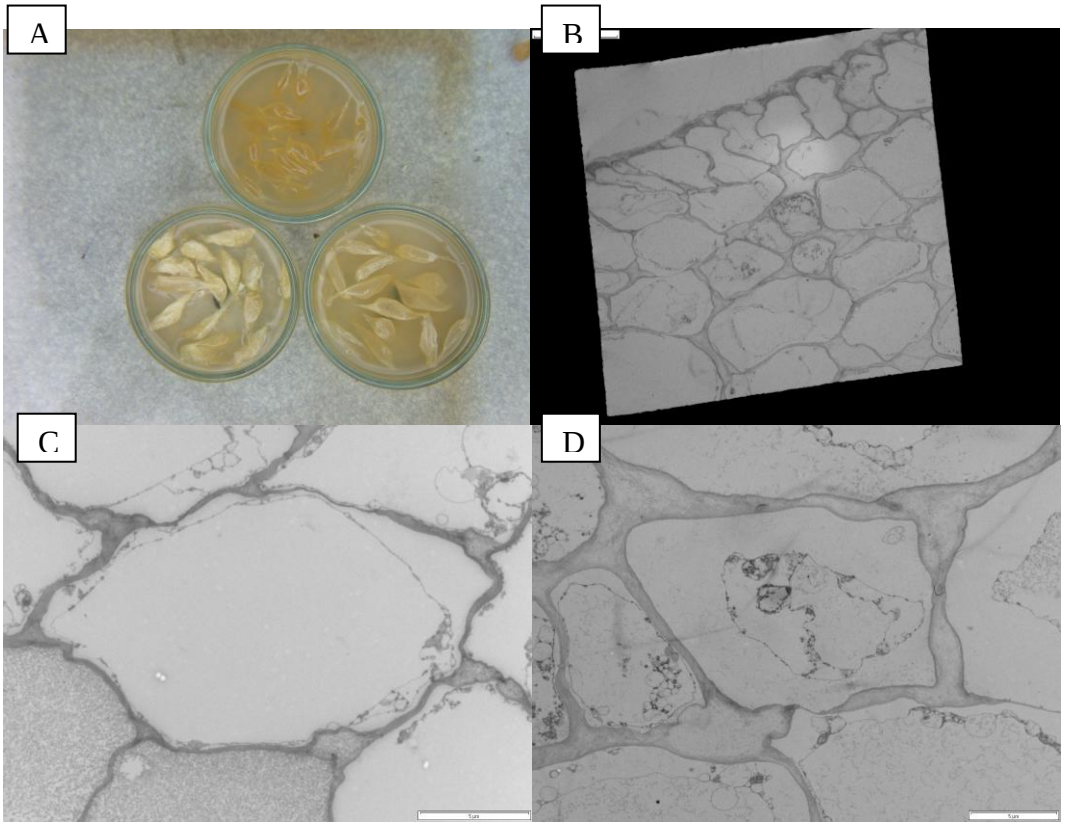


Figure 15. (A) Photograph of juice sacs used in microscopy work (clockwise from top: control, moderately affected, severely affected). Transmission electron microscopy of sectioned resin embedded juice sacs, for (B) control fruit; (C) juice cell within juice sac with moderately severe symptoms of gelling defect; and (D) juice cell within juice sac with severely affected symptoms of gelling defect.

Fruit chemical composition

We have previously indicated that gelling defect fruit were not different to control fruit in terms of callose, suberin or lignin content, as evidenced by histochemical stains. Also, there was no evidence of galactomannan content in these tissues, as evidenced by High Pressure Liquid Chromatography (HPLC) analysis of TFA digested insoluble material. However, glucose content of TFA digests of the insoluble fraction of gelled juice sacs was higher than that of control fruit.
This observation warrants further investigation. During 2008 we unsuccessfully attempted to locate other laboratories routinely involved in cell wall characterisation.
Juice from normal and gelled fruit was also analysed for juice soluble sugar composition by HPLC in a preliminary trial. The sucrose: glucose: fructose ratio was 1:0.41:0.50 in control fruit, 1.0:0.44:0.60 in defect level 2 fruit, 1.0:0.54:0.76 in defect level 4 fruit, and 1.0:0.94:1.5 in defect level 5 fruit (n=1 for each category). This observation also warrants further investigation.

Conclusion

Previous results indicated that an SWNIR technique could be utilised to differentiate normal and gelling defect mandarin fruit if the fruit could harvest in its breaking green stages. In the current year of activity, we were unable to validate this result. We had also reported no difference between interactance and transmittance optical geometries. Given that dryness defect affected tissue appears white (ie. is highly light scattering),

it is reasonable to hypothesize that a transmission geometry should be advantageous to detect this defect. This possibility was explored using several populations, however, this geometry was not demonstrated to improve performance over that experienced with interactance geometry.
Ideally, the laser light scattering experiment should be repeated, using fruit with skin and fruit with skin removed.
Other non-invasive technologies (acoustic, X-Ray and NMR technologies) were also unable to discriminate between normal and defected fruit.
The exercise in anatomical characterisation of a gelling symptom confirmed that this gelling defect is different to the citrus drying defects reported by Albrigo et al. (1980). The gelling defect is characterised by cell proliferation, creating numerous epidermal and hypodermal layers. Only in severely gelled fruit is primary cell wall thickening evidenced.
Further work on the chemical characterisation of the disorder, in terms of juice soluble sugar and cell wall composition, is warranted.

Acknowledgements

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References

Albrigo, L.G., Kawada, K., Hale, P.W., Smoot, J.J., Hatton, T.T., 1980. Effect of harvest date and preharvest and postharvest treatments on Florida grapefruit condition in export to Japan. Proc. Fla. State Hort. Soc. 93, 323-327.

Burns, K.K., Achor, D.S., 1989. Cell wall changes in juice vesicles associated with "section drying" in stored laee-harvested grapefruit. J. Amer. Soc. Hort. Sci. 114, 283-287.

Fullelove, G.D., Rigden, P., 2001. A survey to assess the incidence of internal dryness of Imperial mandarins. Report to the Central Burnett Horticultural Committee, Spring 2001.

Golic, M., Walsh, K.B., Lawson, P., 2003. Short-wavelength near-infrared spectra of sucrose, glucose, and fructose with respect to sugar concentration and temperature. Applied Spectroscopy 57, 139-145.

Greensill, C.V., Walsh, K.B., 2002. Calibration transfer between miniature photodiode array-based spectrometers in the near infrared assessment of mandarin soluble solids content. Journal of Near Infrared Spectroscopy 10, 27-35.

Guthrie, J.A., Walsh, K.B., Reid, D.J., Liebenberg, C.J., 2005. Assessment of internal quality attributes of mandarin fruit. 1. NIR calibration model development. Australian Journal of Agricultural Research. 56, 405-416.

Kawano, S., Fujiwara, T., Iwamoto, M., 1993. Non-destructive determination of sugar content in Satsuma Mandarin using near infrared (NIR) transmittance. Journal of the Japanese Society for Horticultural Science 62, 465-470.

McGlone, V.A., Fraser, D.G., Jordan, R.B., Kunnemeyer, R., 2003. Internal quality assessment of mandarin fruit by vis/NIR spectroscopy. Journal of Near Infrared Spectroscopy 11, 323-332.

Miyamoto, K., Kitano, Y., 1995. Non-destructive determination of sugar content in satsuma mandarin fruit by near infrared transmittance spectroscopy. Journal of Near Infrared Spectroscopy 3, 227-237.

Peiris, K.H.S., Dull, G.G., Leffler, R.G., Burns, J.K., Thai, C.N., Kays, S.J., 1998. Non-destructive detection of section drying, an internal disorder in Tangerine. HortScience 33, 310-312.

Walsh, K.B., Guthrie, J.A., Burney, J., 2000. Application of commercially available, low-cost, miniaturised NIR spectrometers to the assessment of the sugar content of intact fruit. Australian Journal of Plant Physiology 27, 1175-1186.

APPENDIX: Long term storage trial – attribute inter-correlations

A. Fruit stored at room temperature

Week 0

The average skin colour at the beginning of this trial was 2.5 (breaking green stage). Skin colour was poorly correlated to score in this population (Table 14). Peel weight was weakly correlated to % juiciness and defect score.

Table 14. Correlation coefficient (R) between attributes of intact fruit (n = 50) in week 0 with a range of defect levels (sub-sample of population 11)

Attributes	Skin colour	Density	Defect score	%Peel (g/g fruit)	%Pulp (g/g fruit)	%Juiciness (g/g fruit)	% Juiciness (g/g fruit with skin removed
Skin colour	1	-0.35	0.3	0.35	0.19	-0.29	-0.24
Density (0 week)		1	-0.34	**-0.88**	-0.12	0.43	0.30
Defect score			1	0.52	**0.87**	**-0.91**	**-0.90**
%Peel				1	0.3	-0.62	-0.49
%Pulp					1	**-0.93**	**-0.98**
%Juice of whole fruit						1	**-0.98**
%Juice of flesh only							1

Week 3

All the fruit stored in cold room turned into orange colour in three weeks time, so this observation was not recorded from week 3 and on ward.

Density week 0 to week 3: R= 0.97; week 7: R = 0.97
Density was negatively correlated with % Peel in both observations (week 0 and week 3)

Negative correlation of % juiciness with % pulp (R = -0.93) and score (R = -0.85)
Similar trend were observed in the subsequent weeks until week 14.

Table 15. Correlation coefficient (R) between attributes of intact fruit (n = 50) in week 3 fruit stored in 6^{o} C with a range of defect levels (sub-sample of population 11)

Attributes	Skin colour	Density	Density	Score	%Peel	%Pulp	%Juiciness
Skin colour	1	-0.32	-0.39	0.15	0.31	0.05	-0.15
Density (0 week)		1	0.**97**	-0.23	**-0.94**	0.13	0.20
Density (3 weeks in store)			1	-0.36	**-0.93**	-0.01	0.33
Score				1	0.24	**0.79**	-0.**85**
%Peel					1	0.08	-0.27
%Pulp						1	-0.**93**
%Juiciness							1

Week 7

Table 16. Correlation coefficient (R) between attributes of intact fruit (n = 50) in week 7 fruit stored in 6^{o} C with a range of defect levels (sub-sample of population 11).

Attributes	Skin colour	Density	Density	Score	%Peel	%Pulp	% Juiciness
Skin colour	1	-0.2	-0.24	0.16	0.15	0.07	0.11
Density (0 week)		1	0.**97**	-0.17	**-0.89**	0.19	0.15
Density (after 7 weeks)			1	-0.27	**-0.87**	0.20	0.23
Score				1	0.24	0.**82**	-0.**90**
%Peel					1	-0.16	-0.25
%Pulp						1	-**0.90**
%Juiceness (flesh)							1

Week 10

Table 17. Correlation coefficient (R) between attributes of intact fruit (n = 50) in week 10 fruit stored in 6^{o} C with a range of defect levels (sub-sample of population 11)

Attributes	Skin colour	Density	Density	Score	%Peel	%Pulp	%Juiciness	% Juiciness
Skin colour	1	-0.24	-0.25	-0.03	-0.2	0.01	-0.11	-0.05
Density (0 Week)		1	0.**93**	-0.19	-0.93	-0.03	0.52	0.35
Density (10 weeks in store)			1	-0.37	-0.**92**	-0.17	0.64	0.48
Score				1	0.16	0.**89**	-0.**86**	-0.9
%Peel					1	-0.03	-0.5	-0.31
%Pulp						1	-0.91	-**0.93**
%Juice of whole fruit							1	**0.98**
%Juiciness (flesh only)								1

Week 14

% DM was poorly correlated with all of the attributes considered in this exercise

Table 17. Correlation coefficient (R) between attributes of intact fruit (n = 50) in week 14 fruit stored in 6^{o} C with a range of defect levels (sub-sample of population 11)

Attributes	Skin colour	Density	Density	Score	%Peel	%Pulp	%Juiciness	% DM
Skin colour	1	-0.2	0.03	-0.03	-0.11	0.01	0.05	0.15
Density (0 Week)		1	0.**75**	-0.36	-0.68	-0.04	0.31	-0.37
Density (After 14weeks)			1	-0.52	-0.56	-0.35	0.54	-0.08
Score				1	0.37	0.72	-0.**84**	0.25
%Peel					1	-0.01	-0.34	0.37
%Pulp						1	-0.**93**	0.13
%Juiciness							1	-0.13
% DM								1

B. Fruit stored in room temperature

All the fruit stored in room temperature turned into orange colour in three weeks time, so this observation was not recorded from weeks 3 and 7.
Density week 0 to week 3 R= 0.95 week 7 R = 0.90

As in cold temperature in room temperature density was negatively correlated with % Peel but in lesser extend and % pulp was strong negatively correlated with % juiciness.
Similar trend was observed in week 7 as well.

Week 3

Table 18. Correlation coefficient (R) between attributes of intact fruit (n = 50) in week 3 fruit stored in 22^{o} C with a range of defect levels (sub-sample of population 11)

Attributes	Skin colour	Density	Density	Score	%Peel	%Pulp	% Juiciness
Skin colour	1	-0.42	-0.49	0.24	0.43	0.23	-0.29
Density (0 Week)		1	0.**95**	-0.42	**-0.72**	-0.23	0.37
Density (After 3 weeks)			1	-0.53	**-0.76**	-0.35	0.48
Score				1	0.52	0.54	-0.59
%Peel					1	0.55	-0.**71**
%Pulp						1	**-0.98**
%Juiciness							1

Week 7
Gelling defect was negatively correlated with density (R = -0.75)

Table 18. Correlation coefficient (R) between attributes of intact fruit (n = 50) in week 7 fruit stored in 22^{o} C with a range of defect levels (sub-sample of population 11).

Attributes	Skin colour	Density	Density	Score	%Peel	%Pulp	% Juiciness
Skin colour	1	0.14	-0.18	0.09	0.04	0.21	-0.14
Density (0 Week)		1	**0.90**	-0.63	**-0.89**	-0.43	0.68
Density (after 10 weeks)			1	**-0.75**	**-0.90**	-0.59	**0.80**
Score				1	**0.74**	0.**89**	**-0.94**
%Peel					1	0.54	**-0.80**
%Pulp						1	**-0.93**
%Juiceness							1