Final Report

Reducing granulation in the production of Imperial mandarins

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Reducing granulation in the production of Imperial mandarins (CT19005)

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Abbreviations and definitions

B	boron
Ca	calcium
$Ca(NO_3)_2$	calcium nitrate
ese	estimated standard error of the mean. This indicates how much the sample mean could differ from the population mean.
ETo	evapotranspiration
GA	gibberellic acid
LoBi	low biuret urea
n	number in sample
ns	differences between sample means were not significantly different at the 95% confidence level (*see* 'P').
P	the *P* value or probability value is based on the variability of the data and describes how likely it is that the apparent difference between sample means is due to variability in the samples and not an effect of the treatments. Technically, it is described as the probability that the 'null' hypothesis, that there is no real difference between treatments, is true. The standard for statistical 'significance' or confidence is a *P* value of $\leq .05$ or 95% confidence that the treatment means are different. The reader may be prepared to consider that a lower level of confidence indicates a probable treatment effect e.g. a *P* value of 0.1 means we can be 90% confident that the treatment means are significantly different.
PRG	Project Reference Group
R	correlation coefficient. A value of 1 means a perfect correlation. A negative value means the relationship is inverse i.e. higher values for one variable mean lower values for the other.
% w/v	percentage of weight/volume e.g. weight of a compound (in kg) mixed with a volume of water (in L)
Stage I, II or III	Stage I, II or III refers to stages of fruit development, Stage I 'cell division and multiplication'— approximately Sep to Nov inclusive; Stage II 'cell expansion'— approximately Dec to Feb inclusive, Stage 3 'ripening' -- approximately Mar to harvest

Public summary

Granulation is a physiological disorder in which juice vesicles are hardened, gelled or granular. Severely granulated fruit are an opaque white in colour, with no or minimal extractable juice. Partially granulated fruit are 'crunchy', with less extractable juice and with less flavour than unaffected fruit.

Granulation of Imperial mandarins 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. Despite international research on this issue since the 1930s, until now little progress has been made in identifying the causes or providing solutions to this problem.

Granulation is more severe in years with high rainfall or with low crop loads. In research trials and surveys conducted as part of this project, we found granulation is associated with larger fruit size, lower acid content in juice, lighter (sandier) soils, vigorous rootstocks, and the position of fruit in the tree, with fruit inside the canopy granulating more. These factors are the basis of our hypothesis that granulation is caused by higher water potential in juice cells as the fruit develop. High water potential in juice cells is due to low soluble solid content (such as sucrose and acids) and/or higher turgor pressure. High water potential may lead to cell wall thickening and subsequent gellification or granulation of juice cells. Our hypothesis suggests that nutrition, irrigation and crop load management are key to managing this problem.

In this project, we conducted a range of on-farm trials of management techniques to give growers strategies for reducing the incidence of granulation in their crops. The most successful strategies were avoiding overwatering early in fruit development, maintaining consistently high crop loads and applying adequate nitrogen fertiliser in winter to support the spring flowering and flush.

The project included trials that attempted to answer in more detail the questions, 'How much water should we apply?' and 'Which are the critical times for reducing irrigation?". Unfortunately, frequent rainfall in several of the trial years meant these questions remain largely unanswered, although several aspects of our research suggest that the earlier stages of fruit development are more important. We tried to find management practices that might help after a wet spring, that is, strategies that can be applied later in fruit development, including foliar application of fertilisers. These were unsuccessful, emphasizing that early fruit development is the critical period. We found some influence of competition between spring flush growth and fruit quality, but our attempts to reduce this competition with the use of plant growth regulators, later pruning and branch girdling were also disappointing.

Growers are thus encouraged to focus on the three proven strategies of avoiding overwatering in early fruit development, applying sufficient nitrogen in winter and managing crop loads through thinning and timely picking of crops.

Keywords

Imperial mandarin, *Citrus reticulata*, granulation, fruit quality, vesicle drying, management practices, irrigation, crop load, noninvasive assessment

Introduction

Granulation is a physiological disorder in which juice vesicles are hardened, gelled or granular. Severely granulated fruit are an opaque white in colour, with no or minimal extractable juice. Less granulated fruit are 'crunchy', with less extractable juice and with less flavour than unaffected fruit. Industry guidelines suggest <25% juice content is considered severely granulated.

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 seasons (Figure 1).

Figure 1 External and internal images of five fruit showing varying levels of granulation. Photo H Hofman, 25/4/2023

Granulation is due to thickening of the walls of juice cells and vesicles, and the gelation of juice contents. Our research hypothesis, developed after a review of published literature, is that granulation is linked to higher water potential in juice cells. Appendix 7 summarises our review of published literature and outlines in detail the development of our hypothesis.

Water potential is a measure of the free energy of water per unit volume: water moves from an area of higher water potential into one of lower water potential. Water potential in plant cells is a function of three components: 'osmotic' or 'solute' potential, 'hydrostatic pressure' potential or cell turgor, and 'gravity' potential. Cells with lower total soluble solids and/or higher turgor pressure have higher water potential.

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 cell wall 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. There seems to be the possibility of a spiraling process, in which the thickening of cell walls 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, again increasing water potential. Thus water potential increase early in fruit development, from, for example, high rainfall in spring, may be more detrimental than water potential increases late in fruit development.

Our hypothesis suggests that granulation could be exacerbated by management practices that increase water potential in juice cells. Turgor pressure could be increased by frequent or excessive irrigation; soluble solid content could be too low if fertilizing is inadequate. Competition between flush expansion and fruit quality for carbohydrate and mineral resources could play a part in exacerbating granulation by reducing soluble solid content in juice cells. Strategies that have the potential to reduce flush growth could also hypothetically reduce granulation.

In an earlier project, *CT04002 Management of internal dryness of Imperial mandarin*, survey of ~40 commercial Imperial blocks over three years established a range of factors associated with granulation, including larger fruit size, lower crop loads, lighter (sandier) soils, vigorous rootstocks, position of fruit in the tree (with fruit inside the canopy granulating more). In on-farm trials in that project, we successfully reduced granulation through increasing nitrogen (N) nutrition in early fruit development. Citrus fruit development is considered to be in three main stages: Stage I is predominantly cell multiplication and division, Stage II is cell expansion and Stage III is fruit maturity and ripening. Broadcast N in Stage I of fruit development was the most effective application method and timing, but some reduction in granulation was also achieved with multiple foliar applications in Stage I. N applications in Stage II were not beneficial.

In this earlier project, treatments which reduced irrigation frequency but not overall volume did not reduce granulation in most trials. However, in a nursery trial of trees in pots, treatments which reduced both volume and frequency successfully reduced granulation compared to a well-watered control. Reductions in Stage I, or in all three stages of fruit development, were more effective than reductions in Stage II, Stage III, or Stages II and III, suggesting that Stage I is the more critical period. Several other strategies tested in this project were not successful, including increasing or reducing a range of nutrients (potassium, phosphorus, boron, zinc and calcium). Late thinning of fruit (in January) was not successful, nor was the application in late Stage I/early Stage II of the plant growth regulators 3,5,6-TPA, 2,4-DP or gibberellic acid (GA). Details of treatments and outcomes in this project and other Queensland research projects are summarised in table form in Appendix 8.

On the basis of this experience, we decided in this project to test reductions in irrigation volume, as well as further exploring winter N application. We hypothesised that the earlier success with Stage I applications of N was due to inadequate winter application, not to the Stage I timing *per se*. Nitrogen is the major mineral nutrient needed for leaf development in the spring flush, and the high demand in spring relies on stores taken up over winter.

We also decided to include in the project trials of another possible management approach: manipulating spring flush growth, with the aim of reducing competition between flush growth and fruit development in the early stages of fruit development. We hypothesized that competition for stored carbohydrates in spring might have the effect of reducing solutes in juice cells. This approach included the strategies of foliar and drench applications of growth retardants, and the use of GA in early winter to advance flush growth. We also determined to try later pruning to reduce pruning-stimulated flush growth, and branch girdling to reduce the movement of photoassimilates around the tree.

As the project progressed and the adverse effect of high rainfall in spring and summer became clearer, we also included trials of some 'late action' strategies to see if there were any management practices that would help in years when spring rainfall was high. This included late irrigation deficits, a late application of broadcast or foliar N, foliar application of a range of other nutrients (boron, zinc and potassium) and application of GA in late bloom to improve fruit set and retention.

The project also included a review of progress since 2011 in non-invasive detection of granulation on the packing line. This included the technologies of density sorting, visible and near-infrared spectroscopy, X-ray radiography and computed tomography, capacitance, acoustic vibration and magnetic resonance. This review is included as Appendix 9.

Methodology

The project consisted of field trials on commercial orchards in the Burnett and Central Queensland regions. Trials are listed below, numbered in order of date of commencement. All trials were randomized block designs with at least four replicates, except Trial 5. Details of any methodology common to all trials or to several trials, including fruit sampling and assessment, are outlined in Appendix 5.

We conducted a range of trials of reduced irrigation volumes, with one trial also including variable winter nitrogen applications in a factorial design. The trials were:

- Trial 1: 'Irrigation deficit × variable N applications': A trial of normal versus reduced irrigation and five rates of N application was conducted for five years on Imperials on 'Benton' rootstocks.
- Trial 3: 'Period of irrigation deficits': The objective of this trial, conducted over four years, was to test how long moisture deficits need to be applied to achieve optimum granulation results. In the trial, we applied four irrigation treatments: a control and 6, 12 and 18 week irrigation deficits, all commencing at the end of flowering.
- Trial 6: 'Severity of irrigation deficits': In this three-year trial, we aimed to investigate how severe water stress needs to be to reduce granulation. We wanted to impose 'little', 'medium' or 'high' water stress. In practice, in two of the three years of this trial, rainfall made it difficult to differentiate between the 'medium' and 'high' stress treatments.

Details of trial methodologies for irrigation and nitrogen nutrition trials can be found in Appendix 2.

Our hypothesis that granulation is due to high water potential in juice cells in early fruit development suggests that competition for resources between flush, flowers and fruit may contribute to granulation. We conducted three trials to assess whether we could manipulate flush growth and thus reduce granulation:

- Trial 2: 'Flush manipulation strategies trial'. A four-year trial testing the use of plant growth regulator treatments, foliar nitrogen (N) applications and spring girdling treatments to manipulate flush extent and/or timing. Plant growth regulator treatments included GA as 'ProGibb' and the growth retardants paclobutrazol, and procalcium hexadione, in the products 'AuStar' and 'RegalisPlus' respectively.
- Trial 4: 'Paclobutrazol applications trial'. This trial, conducted over three years, was established after initial promising results for paclobutrazol foliar sprays in Trial 2, with the aims of confirming these results and testing whether soil 'collar' drenches would be more efficacious than foliar sprays in reducing flush extent and/or changing time of flush expansion. A foliar treatment was compared to drenches with two timings: when flush first emerged and mid flush (approximately two weeks later).
- Trial 5: 'Late pruning trial'. The objective of this small single year trial (two blocked replicates) was to see if later pruning (late spring rather than winter) reduced granulation by reducing the competition between early fruit development and vegetative regrowth.

Details on methodology for flush patterns and flush manipulation trials can be found in Appendix 3.

At the request of the Project Reference Group, we conducted three single-year trials to explore whether 'late action' was effective after a wet spring. In these trials we used additional sprinklers to simulate higher rainfall in spring and/or in summer. Additional irrigation in summer irrigation was intended not as a remedial treatment but to see if late rainfall was as detrimental as early season rainfall. These trials were:

- Trial 7: 'First late treatments trial'. This trial included treatments of broadcast 'Quick-N' in November, four foliar sprays of N weekly from early November, a foliar spray of GA in early November, and a 'fruit retention' foliar spray mixture of GA + 2,4-D + calcium nitrate ($Ca(NO_3)_2$) in early November. We also aimed to reduce early summer irrigation for all treatments except the 'Quick-N'.
- Trial 8: 'Second late treatments trial'. In this trial we tested foliar treatments in a factorial design with four irrigation treatments. The irrigation treatments were control irrigation (standard farm practice), control irrigation in spring with a wet summer, wet spring with control irrigation in summer, and wet spring with deficit irrigation in summer. The foliar treatments were a control, a GA spray applied in late flowering (admittedly not a 'late' action), and three or four sprays of boron and calcium sprays in Stage I (September to November).
- Trial 9: 'Very late treatments trial'. Here we tested fortnightly sprays (total of four) of boron, calcium, boron and calcium, or potassium at an even later stage (December and January). This was overlaid on two irrigation treatments, a control and a late increase in irrigation (in February and March).

Details on methodology for 'late action' trials can be found in Appendix 4.

Results and discussion

Seasonal and crop load factors

Collation of climate and granulation data over this and the earlier project, CT04002, suggests that there is a strong association between high rainfall and granulation (Figure 2). In most years a wet spring also means a wet summer, so it is difficult to determine from the climate data which period is most important. However, the September to November total has been more variable in the last few years and follows the granulation pattern (in inverse) more closely than the December to January rainfall totals. Our hypothesis suggests that granulation can commence at any stage where water potential is high in juice cells. We suggest that earlier rainfall/irrigation is more detrimental because the earlier granulation is triggered, the longer it continues to develop and exacerbate.

There are no clear indications of an influence of temperature, except possibly where, as in 2022/23, colder winters increase flowering intensity and thus crop load.

Crop load also has a strong influence, with granulation more severe where the crop load is poor. This has become more apparent in recent years as labour difficulties due to the Covid-19 pandemic have meant hand-thinning has become less common and crops have become more biennial. In our trials, crop load influences appeared to be stronger than the effects of several treatments, including plant growth regulator applications. Growers need to manage for high crop loads each year by thinning fruit and avoiding late picking.

Climate and crop load influences are explored in more detail in Appendix 1.

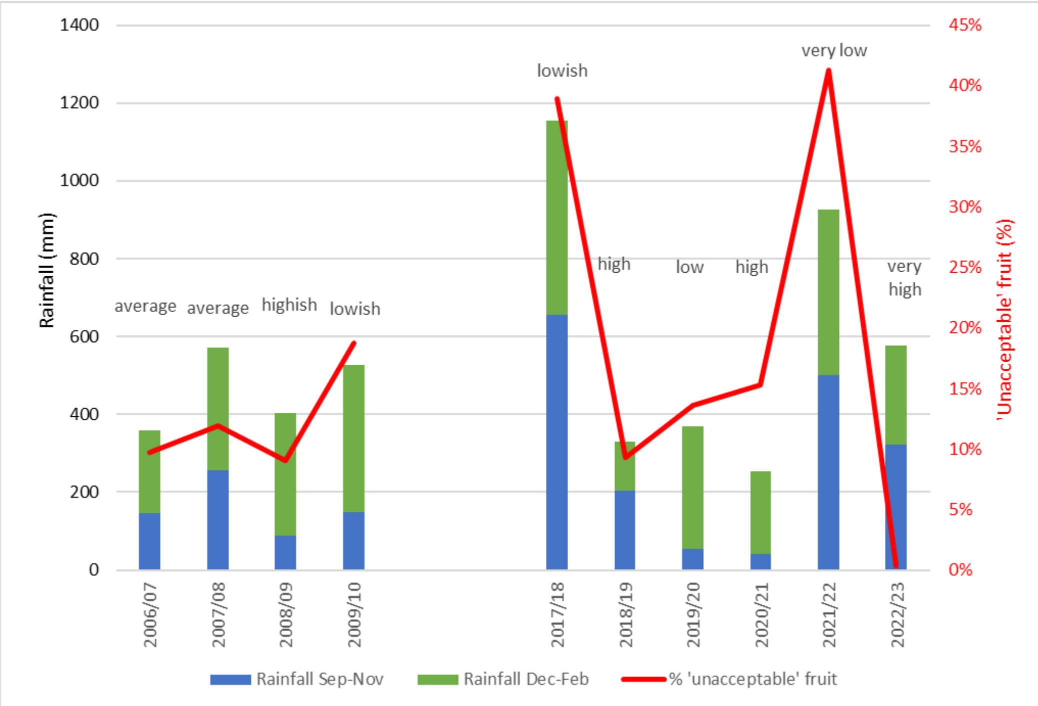

Figure 2 Rainfall totals and indicative granulation for projects CT04002 and CT19005. Data for 2006/7–2009/10 covers Gayndah rainfall, "% unacceptable fruit" for this period is the mean % of fruit rated at ≥2.5 in 35-40 surveyed blocks in Gayndah, Mundubbera and Childers. Data for 2017/18–2022/23 covers Bundaberg rainfall, "% unacceptable fruit" for this period is the mean % of fruit rated ≥3 in control treatments at 2, 3, 3, 4, 5 and 4 trials in Wallaville in 17/18, 18/19, 19/20, 20/21, 21/22 and 22/23 respectively. Text on graph summarises crop load trends, although these will differ from trial to trial. Trial 6 is not included in this latter data as Mundubbera has different rainfall patterns.

Irrigation and nitrogen nutrition trials

In Trial 1, the 'Irrigation deficit × variable N applications trial', reducing water (rainfall plus irrigation) from September to late January by just above half in 2018/19 and by 10-15 % in 2021/22 (a very wet year), reduced mean granulation from 1.55 to 1.06 (P= .009) in 2018/19 and 3.06 to 2.75 (ns, P= .147) in 2021/22 (Figure 3). 'Unacceptable' (severely granulated) fruit were reduced from 8.5% to 1.1% (P= .012) and from 62.3% to 47.6% (ns but close to significant, P= .080) in 2018/19 and 2021/22 respectively. The high granulation in 2021/22 was largely due to a very low crop load.

The other two trial years, 2019/20 and 2020/21, had low rainfall and in addition, the grower reduced the 'control' irrigation regime for Imperials on the property from 98% of evapotranspiration (ETo) (2018/19 data) to 50-60% of ETo. This was done in the light of good results by reducing irrigation in 2017/18 and 2018/19. In these low rainfall years, the applied deficit (~50% of the control treatment) had no additional benefit, that is, there were no significant differences in the irrigation treatments.

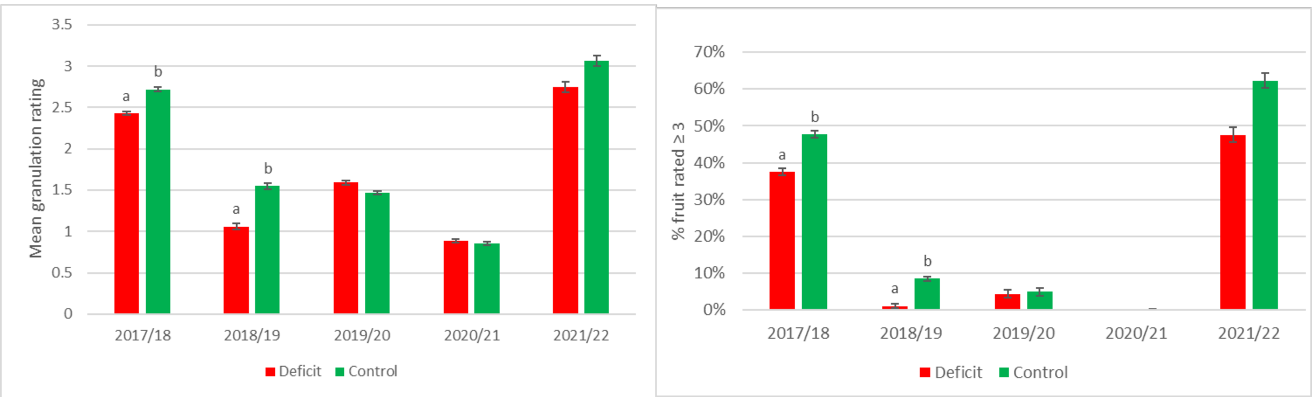

Figure 3. Trial 1: Mean granulation rating (left) and % 'unacceptable' fruit (rated ≥ 3) (right) by irrigation treatment 2017/18–2021/22. ***P*** **values for consecutive years for mean granulation were .013, .009, .147, .673, .147.** ***P*** **values for '% unacceptable fruit' for consecutive years were .017, .012, .665, .374, .080. Means within the one group marked with the same letter or no letter were not significantly different at the 95% confidence level. Error bars show estimated standard errors of the means.**

Higher winter nitrogen applications in Trial 1 tended to reduce granulation in the first four years of the trial, but differences between means were significant at the 95% confidence level only in 2018/19 (Figure 4). In 2021/22, the trend was reversed: the higher N treatments tended to have higher granulation than the lower N treatments, although differences were not significant at the 95% confidence level. This reversal may be due to the lower crop load for this treatment and/or the trend for higher N treatments to flush later.

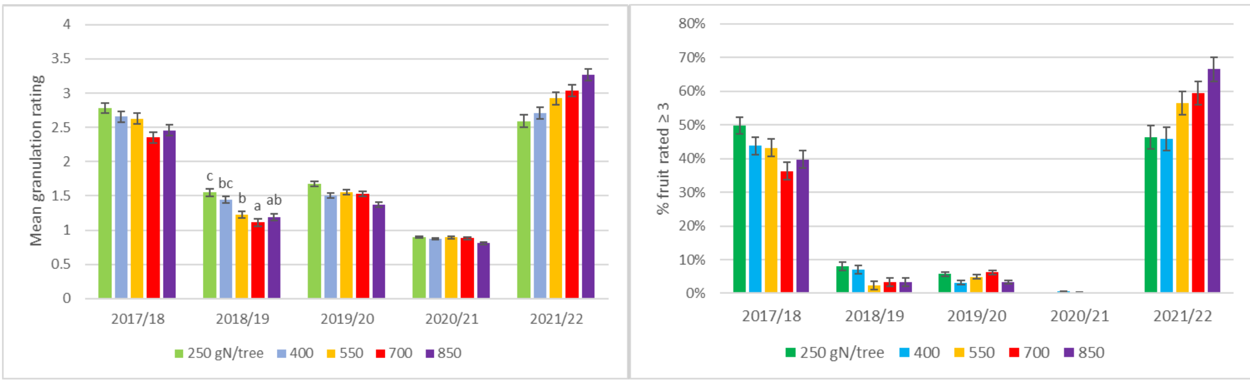

Figure 4. Trial 1: Mean granulation rating (left) and % 'unacceptable' fruit (rated ≥ 3) (right) by winter nitrogen treatment 2017/18–2021/22. ***P*** **values for consecutive years for mean granulation were .324, .022, .084, .376, .065.** ***P*** **values for 'unacceptable fruit' for consecutive years were .440, .348, .584, .487, .195. Means within the one group marked with the same letter or no letter were not significantly different at the 95% confidence level. Error bars show estimated standard errors of the means.**

In Trial 3, the 'Period of irrigation deficits trial', mean granulation rating was reduced in the longer deficit irrigation period (18 weeks) compared to the control from 1.63 to 1.26 in 2019/20, and 'unacceptable' fruit from 13.6% to 3.4% but differences between sample means were not significant at the 95% confidence level (*P*=.275, .217). In the following three years, there was no difference in granulation measurements nor any discernible trend with successive treatment times.

The lack of treatment effects in Trial 3 may be due to the overriding influence of soil profiles and drainage in the trial site. We drilled soil cores in a regular grid pattern at the site and found profiles varied widely: cores ranged from light sand to heavy clay, and there was often a heavy clay layer at varying depths under lighter soils. Higher granulation ratings were associated with lighter or sandier soils, particularly where there was a clay layer below the sand. We hypothesise this clay layer serves to 'trap' moisture which drains away in other profiles. The 'better' part of the block tended to have soil profiles of consistent clay loam. Clay loams have higher water content than sandy soils, but the moisture is less plant-available.

We also found that, regardless of treatment, tensiometer readings in Trial 3 correlated negatively with granulation means for the relevant eight plots, that is, higher average soil moisture tension meant lower granulation. In the dry year, 2019/20, the correlations were strongest with readings at the 15 cm depth; in 2022/23, a wetter year, correlations were strongest with the 60 cm depth. We suggest this reflects different water penetration. In all years, correlations for average readings in the first 6-week period were higher than the 7-12 or 13-18 week periods. This may be because excess water in early fruit development has more influence on granulation, or it may be that there was less rainfall in this period, so there was more range in the readings, giving stronger correlations.

In each of the three years of Trial 6, the 'Severity of irrigation deficits trial', the 'medium' and 'high' deficit treatments had less granulation than the 'little' stress treatment but there was no statistically significant difference between the two higher stress treatments (Figure 5). This is because rainfall made it difficult in practice to differentiate between the two treatments. In 2021/22, total water (rain plus irrigation) was reduced from 52% of ETo for the 'little' stress treatment to 31% and 26% for the 'medium' and 'significant' stress treatments respectively. In 2022/23 the 'little stress' treatment received 67% of ETo, and both the 'medium' and 'significant' stress treatments received 38% of ETo. Application of either level of deficit roughly halved the proportion of 'unacceptable' fruit. In 2021/22, the proportion of 'unacceptable' fruit was reduced from 24.4% in the 'little' stress treatment to 14.2% and 10.8% in the 'medium' and 'significant' stress treatments respectively (P=.034). In 2022/23, the proportion of 'unacceptable' fruit was reduced from 25.6% in the 'little' stress treatment to 14.1% and 12.7% in the 'medium' and 'significant' stress treatments respectively (P=.022).

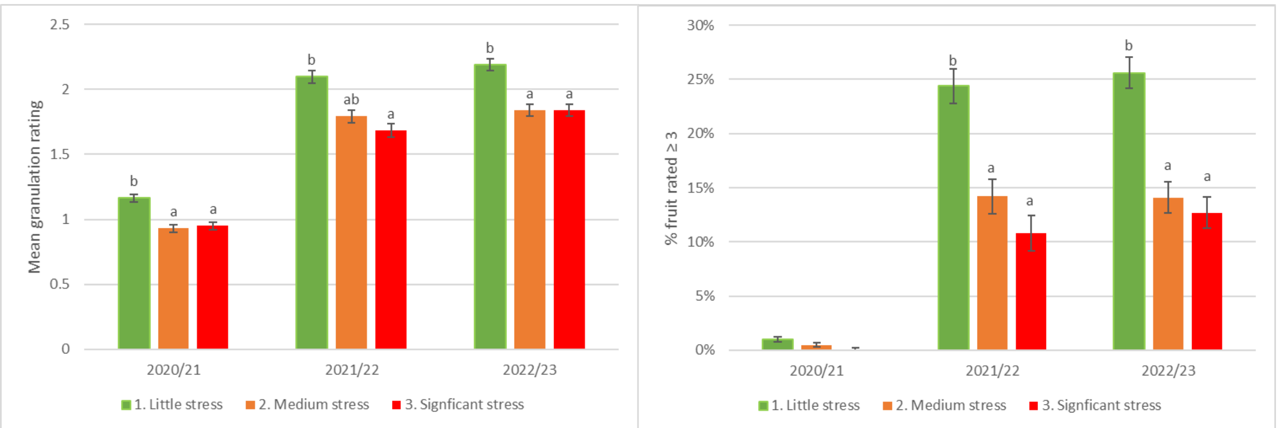

Figure 5. Trial 6: Mean granulation rating (left) and % 'unacceptable' fruit (rated ≥ 3) (right) by irrigation treatment 2020/21–2022/23. ***P*** **values for consecutive years for mean granulation were .034, .034, .038.** ***P*** **values for 'unacceptable fruit' for consecutive years were .254, .034, .022. Means within the one group marked with the same letter or no letter were not significantly different at the 95% confidence level. Error bars show estimated standard errors of the means.**

Overall, these three trials, along with our emerging understanding of the effects of high rainfall in increasing granulation, suggest that reducing irrigation is an important management practice for growers. Defining this quantitatively in terms of percentage of evapotranspiration has proved difficult, with good results achieved from a wide range of applications. Growers should trial various rates to find what is most efficacious in their own circumstances.

Further details on results of irrigation and nitrogen trials can be found in Appendix 2.

Spring flush competition and flush manipulation trials

Our hypothesis that granulation is due to high water potential in juice cells in early fruit development suggests that 'competition' for resources (carbohydrates and nutrients) between flush, flowers and fruit may contribute to granulation. In several trials we recorded flush growth on tagged twigs and rated the granulation levels of the mature fruit on that twig. In this data, granulation at the individual twig level was weakly associated with spring flush growth. This may in part explain the variability of granulation levels from fruit to fruit within the tree. As flush growth is more vigorous in low crop load years, it may also partly explain the higher granulation in low crop load years.

However, our attempts over four years in two trials to manipulate or support flush growth through potential management practices, including girdling, foliar N and plant growth regulators, were not promising.

In Trial 2, we applied two vegetative growth retardants, paclobutrazol and procalcium hexadione, as one or two foliar sprays on emerging spring flush or, in the case of paclobutrazol, as a soil drench. Other treatments included a foliar spray of GA in June; foliar sprays of nitrogen (N) in spring; a combination of N and GA; and branch girdling treatments in three dates in spring.

Results are shown in Figure 6. There was no improvement from girdling treatments, so we only tried these for one year. The foliar spray of gibberellic acid in early winter increased early flush growth and increased the proportion of vegetative shoots in comparison to mixed vegetative and floral shoots, but this had no discernible impact on granulation. The foliar spray of N, or the addition of N to the GA treatments, did not help (one year of treatments only). The most promising treatment was paclobutrazol, but gains were restricted to low crop load years and reductions in granulation were small. The procalcium hexadione treatments had no effect.

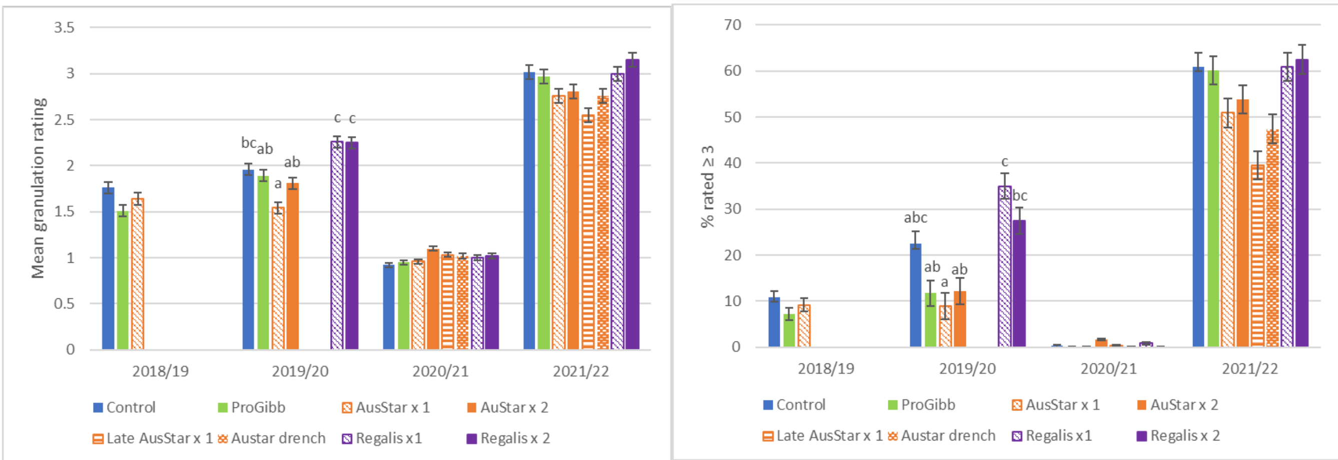

Figure 6 Trial 2 Mean granulation rating (left) and % 'unacceptable' fruit (rated ≥ 3) (right) by selected flush manipulation treatment 2018/19 to 2021/22. ***P*** **values for consecutive years for mean granulation were .175, .002, .326, .165.** ***P*** **values for 'unacceptable fruit' for consecutive years were .658, .013, .054, .126. Means within the one group marked with the same letter or no letter were not significantly different at the 95% confidence level. Error bars show estimated standard errors of the means.**

In Trial 4, we further tested a foliar spray of paclobutrazol and trialled the use of soil drenches. Results were again disappointing (Figure 7). In 2020/21, there was negligible granulation at the trial. In 2021/22, all paclobutrazol treatments had less granulation than the control but this may have been due to a lower crop load, as fruit from the control trees had been stripped later than the treated trees in the previous harvest (2020/21). In 2022/23, a high crop load year characterised by low granulation, there were no significant treatments differences in mean granulation rating or mean percentage of 'unacceptable' fruit, but there was a lower percentage of 'crunchy' fruit in the two drench treatments, although the difference between means was only significant at the 95% confidence level for the earlier drench.

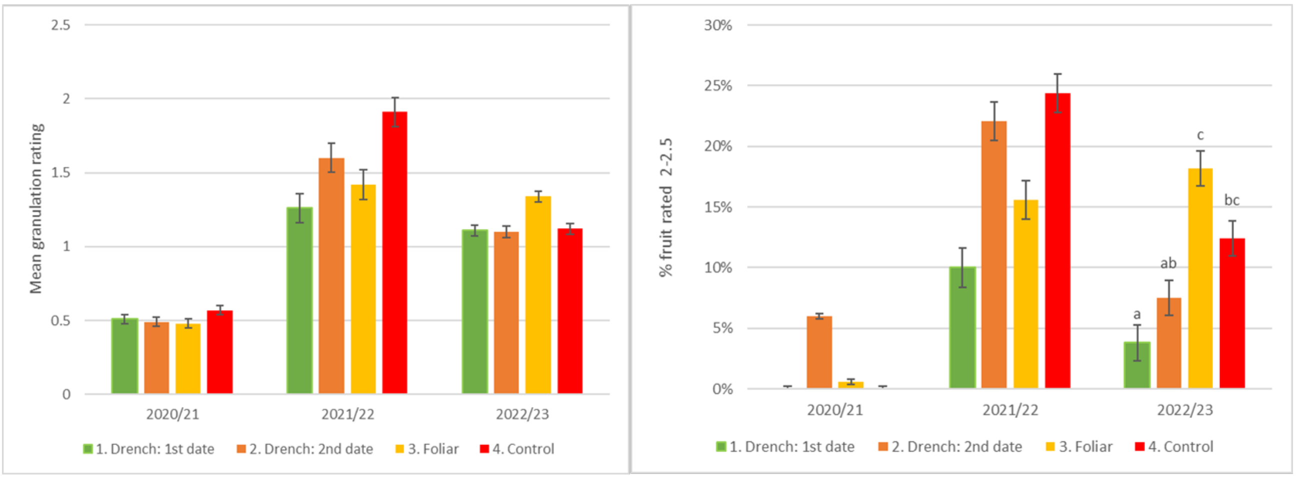

Figure 7 Trial 4 Mean granulation rating (left) and % 'crunchy' fruit (rated 2-2.5) (right) by paclobutrazol treatment 2020/21 to 2022/23. ***P*** **values for consecutive years for mean granulation were .744, .144, .850.** ***P*** **values for 'crunchy' fruit for consecutive years were .600, .228, .010. Means within the one group marked with the same letter or no letter were not significantly different at the 95% confidence level. Error bars show estimated standard errors of the means.**

Later pruning, that is, pruning in November rather than July (Trial 5), did not reduce granulation. The late-pruned trees had noticeably denser and darker canopies during early fruit set, particularly in the centre of the trees, with many water shoots. It is possible this growth competed with fruit for resources, and/or that the denser canopy reduced light for photosynthesis.

Overall, our results support the hypothesis that flush growth and granulation are associated, although the effect is not necessarily a simple causal one as both are affected by crop load. Our attempts to influence flush growth in Trials 2 and 4 had disappointing results. Overwatering appears to be a more influential factor. The most effective strategy for growers looking for the right balance between flush and fruit is to ensure they manage crop load by thinning and not picking late.

Further details on flush patterns and the results of flush manipulation trials can be found in Appendix 3.

'Late action' trials

In Trial 7, the 'First late action trial', none of the treatments reduced granulation, including broadcast 'Quick-N', foliar sprays of N, or foliar spray of GA. The 'fruit retention' foliar spray mixture of GA + 2,4-D + calcium nitrate increased granulation. High rainfall late in the season meant that we could not discern the effects of late reduction in irrigation.

In Trial 8, in the 'Second late action trial', none of the irrigation treatments – control spring plus wet summer, wet spring plus control summer, and wet spring with dry summer – reduced or increased granulation compared to the control. Of f the foliar treatments – boron and calcium in Stage I and GA in late bloom – only the GA treatment was effective. This reduced 'crunchy' fruit (rated 2 or 2.5) from 18.1% in the control to 10.7% in the GA sample (*P*=.023). The lack of effect from irrigation treatments may again be due to frequent rainfall, with ~616 mm of rainfall received in the treatment period. Nearly all treatments, whether 'control', 'wet' or 'dry', received total water close to or above evapotranspiration levels in spring. In summer, we were able to reduce total water in the control treatment from 90% of ETo to 65% in the 'deficit' treatment, but this did not produce any discernible effects.

In Trial 9, in the 'Very late action trial', there were no treatment effects from either the extra irrigation or the foliar spray treatments (boron, calcium, boron plus calcium or potassium). Note that crop load was high, and granulation overall was very low, so conditions were admittedly not ideal.

In summary, while these 'late action' trials were short term and conducted in less-than-ideal climatic conditions, we received very little support for the hypothesis that late action in terms of irrigation or nutrient foliar sprays would be beneficial. This may be because the effect of high rainfall was predominant. The one beneficial treatment was the GA sprayed at late petal fall, which increased crop load, a known factor in influencing granulation levels. Growers should consider use of GA if expecting a low crop load year.

Further details on the results of 'late action' trials can be found in Appendix 4.

Conclusion

Our trials suggest that the three key strategies for growers to minimise granulation are to maintain high crop loads, reduce irrigation, and apply sufficient nitrogen in winter.

Outputs

Table 1. Output summary

Output	Description	Detail
Advice to growers	Advice to growers based on trial results on management practices to reduce granulation	When approved, to be provided to Citrus Australia and DAF publications units for posting as appropriate format; copies to be sent to crop consultants and extension officers.
Publications	Article on project and findings to date	Article for Citrus News by Rosalea Ryan (Spring 2020). Draft scientific papers now in preparation.
Interviews in Citrus Australia podcasts	Interview for the Citrus Australia, 'An Imperial impact' on 'The Full Bottle Podcast', posted 29 May 2023	https://citrusaustralia.com.au/members-hub/the-full-bottle-podcast/ or https://open.spotify.com/episode/70dc7JbdloAuvBVIZLDqbP
Presentations at technical forums		Powerpoint presentations made available to members on the citrus Australia website https://citrusaustralia.com.au/members/2022/03/2022-citrus-technical-forum-presentations/: • End-of-season meetings organised by the Southern Queensland Regional Advisory Committee for Citrus Australia at Gayndah on 26 October 2021 and 26 October 2022. • Citrus Australia Technical Forum at Twin Waters, Queensland, on 8 March 2022.

Outcomes

Table 2. Outcome summary

Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence
Improved understanding of the effects of irrigation and nitrogen nutrition management and of patterns of flush growth on granulation.	Improved product quality and increased productivity from the application of innovation. Undertaking R&D and extension to enhance product quality (such as flavour and juiciness) including the development of non-destructive fruit	Improved understanding will inform development of appropriate management practices.	As reported in this report.

Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence
	testing and objective grading and waste reduction (*Citrus Strategic Investment Plan 2017-2021*)		
Available, accessible and clear guidelines to growers based on trial results into irrigation, N nutrition, pruning and PGR management practices that will help reduce granulation in Imperial mandarins, including preliminary guidelines for levels of N in leaf tissue	As above	Improved management practices should reduce the levels of granulation in marketed fruit.	Included in this report.
Increased capacity for growers to reduce granulation in Imperial mandarins through best practice.	As above	As above	Beyond the scope of project activities.
Improved consumer satisfaction through reduced incidence and severity of granulation in Imperial mandarins and more consistent quality from season to season.	As above	As above	Beyond the scope of project activities.

Monitoring and evaluation

Table 3. Key Evaluation Questions

Key Evaluation Question	Project performance	Continuous improvement opportunities
1. To what extent has the project achieved its expected outcomes?	The project has fully explored the areas listed in the project outline and contract. Research results in some cases were limited due to climatic conditions.	na (no follow-up project planned)
2. How relevant was the project to the needs of intended beneficiaries?	The project was extremely relevant. Granulation in this (and other) varieties continues to be an issue for the industry.	na
3. How well have intended beneficiaries been engaged in the project?	Growers in the Central Burnett have been engaged through Citrus Australia's preseason meetings and growers nationally through Citrus Australia's biennial Technical Forum and its publications. The Project Reference Group (PRG) includes four growers and a citrus-	na

Key Evaluation Question	Project performance	Continuous improvement opportunities
	industry consultant. That these were well-engaged is shown, for example in that several trials were added to the research program (the 'late action' trials) at the request of the PRG. In addition, the PRG requested a summary of Queensland research to date (now completed and available in Appendix 8)	
4. To what extent were engagement processes appropriate to the target audience of the project?	Engagement processes were those used throughout the citrus industry for research projects. Meetings were well attended.	na
5. What efforts did the project make to improve efficiency?	The project plan was reviewed every year to discard treatments showing no promise and add in new treatments with potential. Some time-consuming activities e.g. Brix and acid testing and fruit colour assessment were dropped after three years as no new understanding was likely with repeated measures.	na

Recommendations

Our trials suggest that the three key strategies for growers to minimise granulation are to avoid overwatering in early fruit development, apply sufficient nitrogen in winter and manage crop loads through thinning and timely picking of crops. More detailed information for growers is included in *Appendix 6: Summary for growers.*

Recommendations for future research are as follows:

a. Maintaining good crop loads is essential for management of granulation. Labour and cost of production pressures may incline growers towards using chemical rather than manual thinning to manage crop loads. An avenue for future research would be to assess differences in impacts between chemical and hand thinning. Most chemical thinning agents are applied earlier in the growing season than hand thinning and may thus, by reducing crop load early, promote granulation more than hand thinning, which tends to be later in the growing season (January). Both treatments tend to remove smaller fruit, leaving the larger fruit on the tree. It is the larger fruit that are most granulated. Research should cover different dates of application and/or products with different recommended application dates, and include effects on fruit size, taste and fruit quality.

b. The role of growth retardants in managing granulation needs further study. Paclobutrazol treatments appear to have some promise in low crop load years although the data from our trials is less than convincing and the scale of improvements is relatively small. We tried several applications and timings, but only used one application rate (the recommended rate for international usage). Trials which test a ranged of increased application rates as well as variable timings may be useful. Future trials would need to cover several years as crop load influence appears to be high. Note that there are currently no registered paclobutrazol products for citrus in Australia.

c. While the weight of evidence suggests that it is early fruit development that is the critical period for irrigation deficits, we were unable in our trials to demonstrate this convincingly, partly due to weather events. In a nursery trial with trees in pots in the earlier project (CT04002) (see Appendix 8), treatments reduced volume and frequency in Stage I of fruit development, Stage II, Stage III, Stages II and III, all stages, or watered frequently (the 'control'). In this trial granulation was lowest for both the 'Stage I' and the 'all stages' treatments, suggesting that Stage I was key. In Trial 3 in this project, this seemed to be supported by results in the first year of the trial, but we were unable to confirm this in the second, third and fourth year of the trial. In Trials 1 and 6, we have carried deficits through to mid- to late-January (late Stage II). This is because, in our trial locations, it is at this time that temperatures peak and natural water deficits are imposed simply through the difficulty of 'keeping up' the water across most orchards. But it may be that a deficit all season is beneficial: this remains to be tested.

d. A key question for growers is whether there are any effective late treatment strategies after a wet spring (see Appendix 4). While our trials showed clearly that late action in terms of nutrient foliar sprays were of little benefit, rainfall meant we were unable to fully test whether late irrigation deficits could still be beneficial. This is a management strategy that remains to be researched.

References

See *Appendix 7 Literature review and research hypothesis* and reference lists at the end of appendices.

Intellectual property

No project IP or commercialisation to report.

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Appendix 1: Seasonal and crop load factors

Summary

Over the life of this project and previous projects, granulation has been highest in our trials in growing seasons with higher rainfall. In most years, a wet spring also means a wet summer, so it is difficult to determine which period is most important. However, the September to November total rainfall (equivalent to Stage I of fruit development) has been more variable than the December to January rainfall (Stage II of fruit development) in the last few years and follows the granulation pattern (in inverse) more closely.

There are no clear indications of an influence of temperature, except possibly where, as in 2022/23, colder winters increase flowering intensity and thus crop load. The influence of low crop load has become increasingly apparent in recent years as labour shortages due to the Covid-19 pandemic meant there was no hand-thinning or only light thinning, and/or late picking of fruit. As a result, crops became more biennial in our trial sites.

Crop load influences appeared to be stronger than the effects of several treatments, including plant growth regulator applications.

Introduction

Our hypothesis is that wet growing seasons contribute to granulation by increasing water availability, which increases juice cell turgidity and thus water potential.

Temperature influences on flush and flowering may also be important, by increasing/decreasing competition for photosynthates and mineral nutrients between flush, flowering and fruit development. In addition, winter temperatures influence flowering and thus crop load. Cooler winters are associated with better flowering; and warm winters with poor flowering. Accumulation of lower temperature hours over winter reportedly shifts buds in citrus from vegetative to mixed to reproductive states, increasing flowering intensity (Moss, 1976; Valiente & Albrigo, 2004). Warm late winter or spring temperatures may lead to more leafy inflorescences as well as delaying flower development (Moss, 1969).

Reports on granulation indicate granulation is more severe in tropical than in cooler regions (Bain, 1949), and in coastal than inland areas, most likely due to increased humidity and/or rainfall (Bartholomew & Sinclair, 1947; Bartholomew et al., 1941; Benton, 1940; Lloyd, 1961). There are two published reports of specific climate patterns. Van Noort (1969) reported increased granulation when winter or early spring is warmer than average, followed by heavy rain in late summer or early autumn, i.e. Stage II of fruit development. Similarly, Ritenour et al. (2004) suggests that the severe granulation in Florida's 2003 navel orange crop was due to higher than average daily temperatures in February and March (early spring), higher maximum (but not average) temperatures in October and November (autumn), a compressed bloom period, low fruit set, and late summer and early autumn rains (August-September).

Crop load is an important factor. Surveys in the earlier project, *Management of internal dryness of Imperial mandarin* (CT04002), showed granulation is higher where crop loads are low. This aligns with other studies (El-Zeftawi, 1973, 1978; Gravina et al., 2004; Ritenour et al., 2004).

The physiological basis may be that a heavy crop load, particularly if picked late, means that carbohydrate production continues to be used for fruit development late into the season, and not stored. Low levels of carbohydrate stores mean there is insufficient reserves to supply demand in the spring of the following year. The demand in spring is high: Bustan and Goldschmidt (1998) estimate the processes of flowering and early abscission of fruitlets use about 27% of the annual photoassimilate production. Most of this is supplied by reserves rather than by 'current' or day-to-day production of photoassimilates from leaves.

These reserves include roots: roots actively accumulate carbohydrates in 'off' years (Goldschmidt & Golomb, 1982). A study by Monselise *et al.* (1981) of two Wilking trees – a strongly biennial cultivar -- show that the effect of 'on' years is to deplete starch from roots. In an 'off' year reserves accumulated in roots in very large amounts: the ratio of starch in roots of the 'off' year to the 'on' year was 17.2:1.

Several authors suggest that where reserves are short, the available carbohydrates may be preferentially partitioned to vegetative over fruit growth (Bartholomew et al., 1941; Benton, 1940; El-Zeftawi, 1978; Fullelove et al., 2004), or to roots as noted above. This may be because vegetative and root growth is more vigorous with higher demand (Kriedemann, 1969), or maybe it is simply a 'numbers game': more shoots than fruit, or more mass in shoots and roots than in fruit.

In addition, there is some evidence that in an 'off year', the overall photosynthetic capacity of the tree declines, so the insufficiency of carbohydrates continues even when the new flush has matured (Schaffer et al., 1987.; Wibbe & Blanke, 1995).

When crop load is low, fruit tend to be large, and larger fruit tend to be more granulated, as established in our surveys in CT4002. Matsumoto (1964) also found that granulation was more common in large fruit from branches bearing a light crop. We hypothesise that the relatively greater availability of water to the smaller number of fruit may increase cell turgor and be another cause of granulation. Crop load affects sap flow in branches: a heavy crop load has a lower sap flow, and increased water stress (Yonemoto et al., 2004). It is possible that water relations in the tree are affected by the lack of root growth in an 'on' year. Jones et al. (1975) record a reduction in feeder roots in 'on' years, leading to reduced root activity and increased water stress. A reduced water supply to fruit may be part of the reason for lower granulation in an 'on' year.

Seasonal data

Rainfall

Rainfall and granulation data show a clear pattern of higher granulation in wet years. Figure 1 demonstrates the association of rainfall with granulation levels in this project as well as the project CT04002 *Management of internal dryness of Imperial mandarin*.

An issue for management is whether rainfall is more detrimental in spring or in summer. This is a difficult question to answer: as can be seen from Figure 1, a wet spring generally also means a wet summer. Rainfall in spring and summer, and percentages above or below average are shown in Table 2, and suggest that the September to November total (equivalent to Stage I of fruit development) has been more variable than the December to January rainfall (Stage II of fruit development) and follows the granulation pattern (inverted) more closely. In our irrigation trials, we have successfully reduced granulation in some years by imposing irrigation deficits starting at the end of flowering and continuing to mid-January (see Appendix 2). However, in our 'late action' trials, we made little (negative) impact on granulation by applying extra water later in the growing season: December/January (see Appendix 4). These results support our view that that earlier rainfall is more detrimental than late rainfall, possibly simply because the earlier granulation is triggered, the longer it continues to develop, and the more severe it becomes.

Figure 1. Rainfall totals and indicative granulation for projects CT04002 and CT19005. Data for 2006/7 to 2009/10 covers Gayndah rainfall and "% unacceptable fruit" for this period is the mean % of fruit rated at ≥2.5 in 35-40 surveyed blocks in Gayndah, Mundubbera and Childers. Data for 2017/18 to 2022/23 covers Bundaberg rainfall and "% unacceptable fruit" for this period is the mean % of fruit rated ≥3 in control treatments at 2, 3, 3, 4, 5 and 4 trials in Wallaville in 17/18, 18/19, 19/20, 20/21, 21/22 and 22/23 respectively. Text on graph summarizes crop load trends, although these will differ from trial to trial (see Figure 4). Trial 6 is not included in this data as Mundubbera has different rainfall patterns.

Table 2. Rainfall totals and percentage above or below the mean (1942–2023) Bundaberg Airport (BOM Station no. 39128)

	Jun-Aug		Sep-Nov		Dec-Feb		Mar-May		Total	
	mm	**% mean**	**mm**	**% mean**	**mm**	**% mean**	**mm**	**% mean**	**mm**	**% mean**
Median	*75*		*143*		*364*		*177*		*759*	
Mean	*122*		*200*		*446*		*234*		*1002*	
2017/18	16	*- 87%*	656	*+ 227%*	498	*+ 12%*	46	*- 80%*	1216	*+ 21%*
2018/19	32	*- 74%*	203	*+ 1%*	126	*- 72%*	163	*- 30%*	524	*- 48%*
2019/20	33	*- 73%*	54	*- 73%*	315	*- 29%*	91	*- 61%*	493	*- 51%*
2020/21	86	*- 29%*	42	*- 79%*	212	*- 52%*	156	*- 22%*	497	*- 50%*
2021/22	113	*- 7%*	502	*+ 151%*	489	*+ 10%*	389	*+ 66%*	1492	*+ 49%*
2022/23	112	*- 8%*	*321*	*+ 60%*	*257*	*- 42%*				

** to end April 2023. Mean and median are for years 1942 to April 2023.*

Temperature

It is less clear whether there was any effect on granulation of temperature variations from year to year. Our hypothesis suggests that warmer/cooler winters may depress/enhance flowering; and that warmer/cooler springs may enhance/depress flush growth. Figure 2 shows cooler maximum temperatures in the winter months prior to the 2020/21 and 2022/23 seasons, and cooler winter minimums prior to the 2018/19 season. These may have contributed to the high crop loads in those years.

Higher spring maximum temperatures in September 2017/18 and 2019/20 also fit our hypothesis that warm springs may encourage flush growth and increase granulation (although winter temperatures were not particularly warm). Warmer spring temperatures, however, were not evident in the other low crop load year 2021/22. In that year, low crop loads are due to unthinned crops and/or late picking in 2021 due to labour shortages during the pandemic.

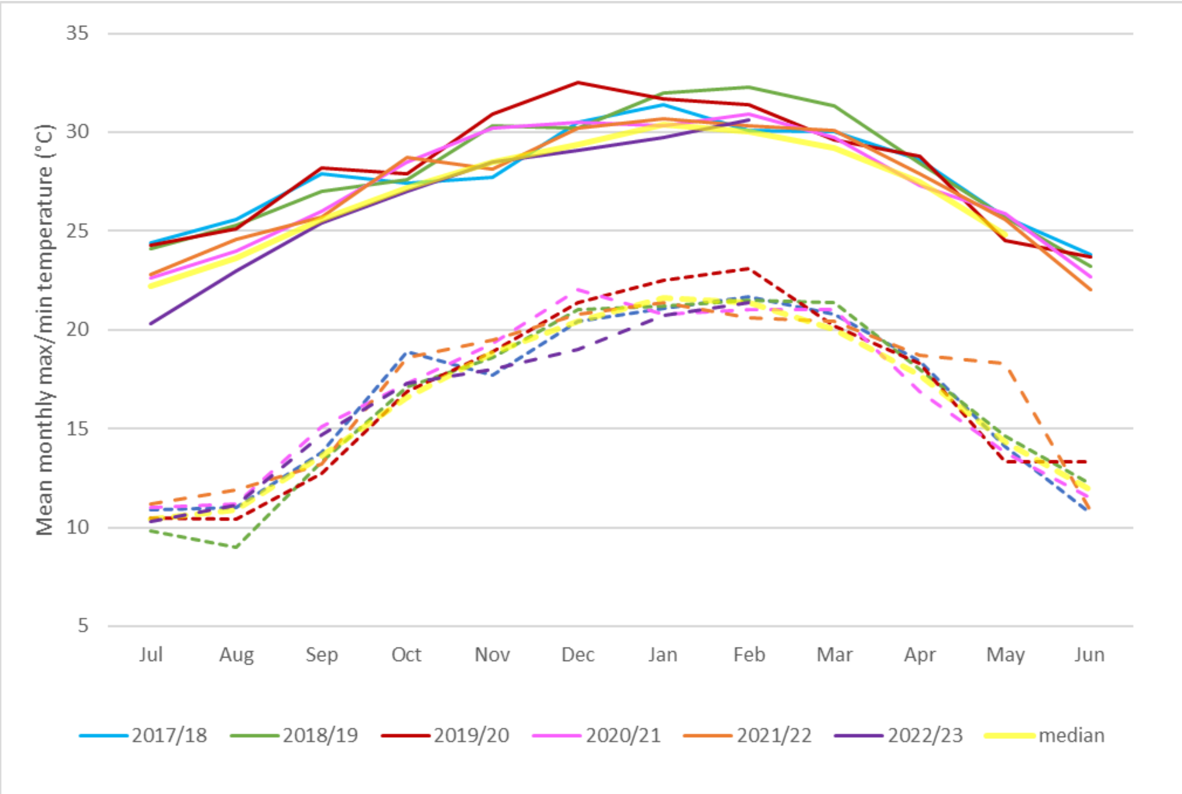

Figure 2 Monthly mean maximum (solid lines) and minimum (dashed lines) temperatures for Bundaberg. Source: Bureau of Meteorology data for Bundaberg Airport (Station no. 39128). Medians for years 1959 to 2023.

Another hypothesis for explaining some of the granulation trends in our trials is that dry periods affected root growth and root:shoot ratios. Poor flowering in the 2019/20 year may hypothetically be linked to low rainfall in the latter part of the previous season (November 2018 through to February 2019). This may have led to increased root growth in search of water and/or it may have limited the extent of the summer flush. Late summer was also hotter than usual. Then, in the following spring (2019/20), there was a heavy vegetative flush which may have been the reestablishment of the root:shoot ratio. This flush appears to have been at the expense of flowering and fruit set. This hypothesis remains untested.

Crop load

The association between high granulation and low crop load on an individual tree basis is demonstrated in the example shown in Figure 3 (left hand graph), using data from Trial 1 in 2021/22, a low crop load year. Plotting the mean diameter of fruit for the same crop load ratings shows that the association is not solely due to larger fruit size (Figure 3, right-hand graph).

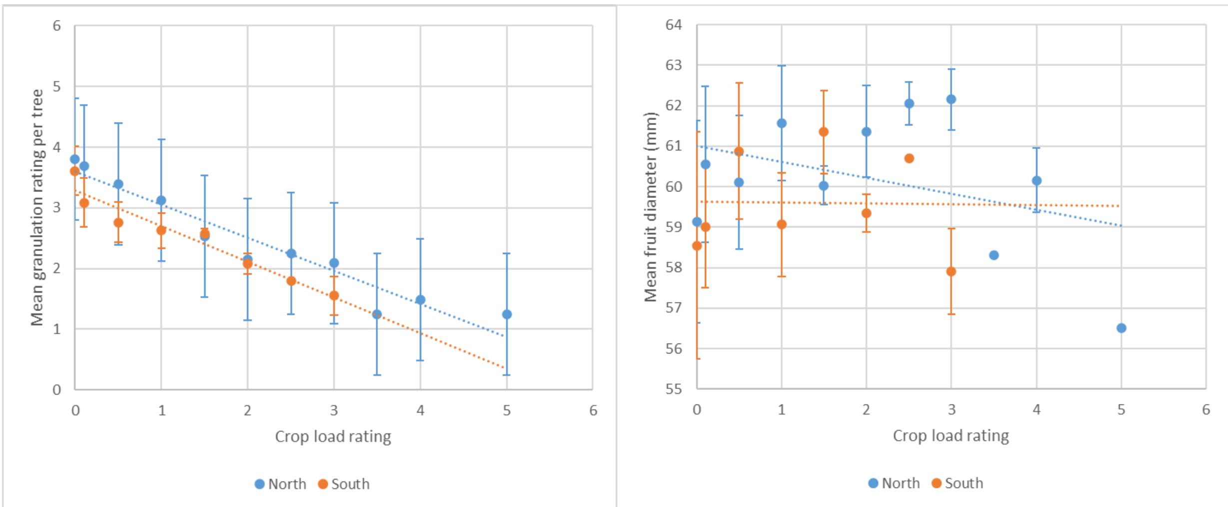

Figure 3 Trial 1 2021/22 Mean granulation rating (left) and mean fruit diameter (right) by crop load rating. Fruit from the north and south canopies of the tree shown separately. Total 150 trees. All treatments included. Error bars show standard deviations.

Figure 4 shows estimated crop loads in our trials since 2019/20. Trials 1, 2, 3 and 4, which were located in Wallaville, near Bundaberg, show a clear pattern of biennality with low crop loads for most trial sites in years 2019/20 and 2021/22, and higher crop loads in the alternate years. Trial 6, which was in Mundubbera, shows a different pattern, which may be due to climate differences between Mundubbera and Wallaville and/or thinning practices. Our observations suggest that biennality has become more pronounced in recent years due to labour shortages and cost pressures resulting from the Covid-19 pandemic, which meant that hand thinning was not done, or only lightly done, and, in some trials, picking was delayed.

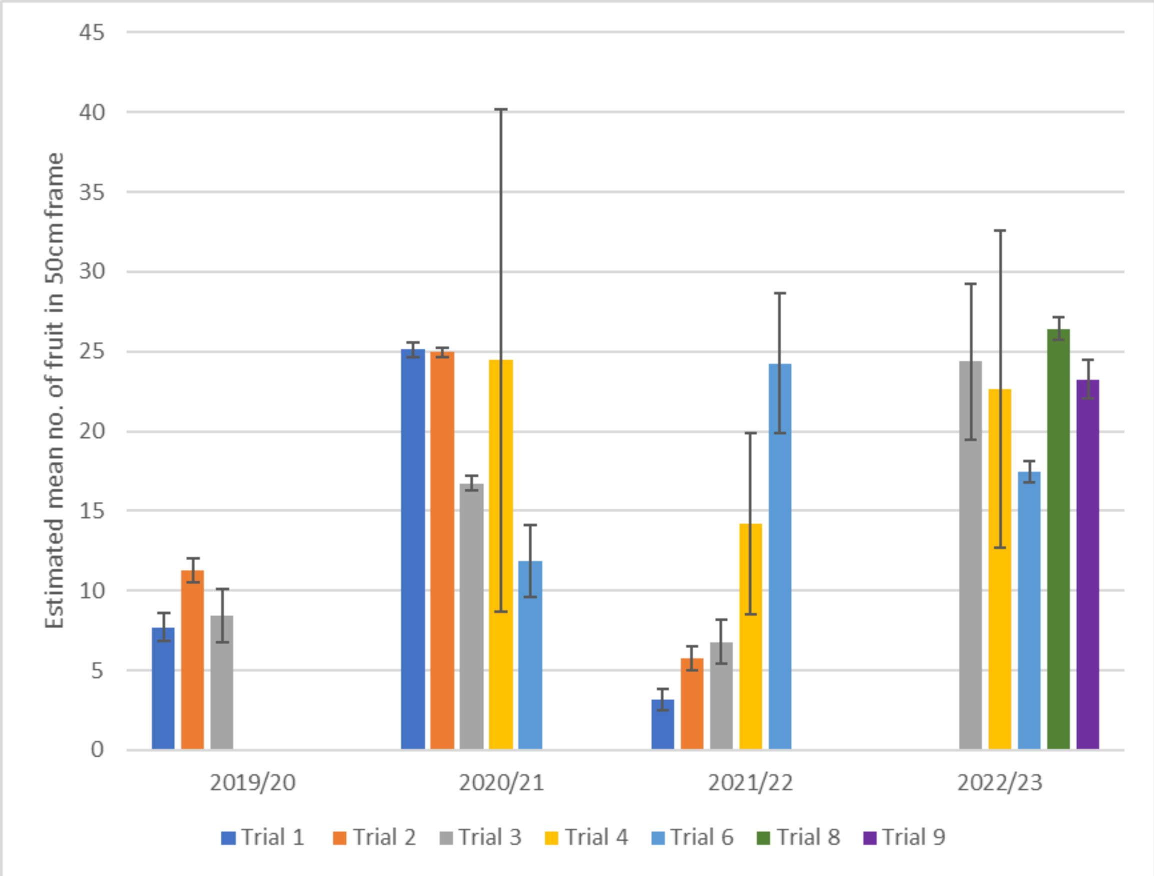

Figure 4 Estimated mean no. of fruit per 50cm cube per trial for 2019/20 to 2022/23. Fruit numbers estimated from mean ratings applied to a linear relationship derived between ratings and counts for a subsample of trees (see Appendix 5 General methodology). Error bars show 95% confidence interval for mean rating, converted to fruit per 50cm cube using the same linear formulae.

The trend in crop loads each year across all trials reflects (inversely) average granulation as shown in Figure 1. Crop load variations may explain some of the variations in the pattern of granulation which do not match rainfall patterns. For example, in 2022/23, rainfall was above average, but granulation at our trials was negligible because crop loads were high.

Discussion and conclusions

Which climatic characteristics influence granulation and why?

Over the life of this project and the previous project, granulation has been highest in our trials/survey blocks in growing seasons with higher rainfall.

Temperature influences seem to be mostly due to effects on flowering and thus crop load. Cooler maximum temperatures in the winter months prior to the 2020/21 and 2022/23 seasons, and cooler winter minimums prior to the 2018/19 season, may have contributed to the high crop loads in those years.

Our hypothesis that warm temperatures in spring increase or accelerate flush growth and this may also have an effect, as discussed in Appendix 3. However, the main effect of temperature seems to be through crop load.

What is the critical time for rainfall peaks?

In most years a wet spring also means a wet summer, so it is difficult to determine from the climate data which period is most important. However, the September to November total rainfall has been more variable than the December to January rainfall in the last few years and follows the granulation pattern (in inverse) more closely.

In a nursery trial in the project CT04002, deficits in Stage I of fruit development, or all season, had the most effect in reducing granulation compared to Stage II and/or Stage III deficits. In Trial 3 in this project, we tried to determine how long deficits need to be with no clear results. See the 'Discussion and conclusions' section in Appendix 2.

When we tried less or more water late in the season in December/January in our 'late action ' trials (Trials 7, 8 and 9) there was no adverse or beneficial effect on granulation (see Appendix 4). Published international observations suggest later rainfall, that is, in summer or autumn, is implicated in granulation of navel oranges (Ritenour et al., 2004; Van Noort, 1969). However, navels develop much more slowly than Imperials, so may have a different critical period. Our hypothesis suggests that granulation can commence at any stage where water potential is high in juice cells. We suggest that earlier rainfall/irrigation is more detrimental, possibly simply because the earlier granulation is triggered, the longer it continues to develop and increase in severity.

What is the effect of crop load?

A low crop load or 'off' year generally has a much higher incidence of granulation (El-Zeftawi, 1973, 1978; Gravina et al., 2004; Ritenour et al., 2004).

Crop load trends can explain the years when levels are higher or lower than might be expected from rainfall, as shown in Figure 1. For example, in 2022/23, rainfall was above average, but granulation at our trials was negligible because crop loads were very high.

What are the implications of climate and crop load aspects for management practices?

These climate and crop load trends indicate the importance of irrigation and crop load management in managing granulation.

While rainfall is beyond the control of growers, growers should plant on well drained soils and not exacerbate high natural rainfall with excessive irrigation.

Crop loads, while somewhat affected by climate, are also within management influence. A heavy crop that is not thinned, or late picking of the crop, will mean a lower crop load in the following season.

Labour and cost of production issues may incline growers towards using chemical rather than manual thinning to manage crop loads. This project has not examined if there are any differences in granulation incidence between chemical and hand thinning practices: this could be an avenue for future research. Note that most chemical thinning agents are applied earlier in the growing season than hand thinning and may thus, by reducing crop load early, promote granulation more than hand thinning. In addition, some chemical thinners tend to selectively remove smaller fruit, leaving the larger fruit on the tree. It is the larger fruit that are most granulated. Future research should cover different dates of application and/or products with different recommended application dates, and include effects on fruit size, taste and fruit quality.

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Appendix 2: Irrigation and nitrogen nutrition trials

Summary

In line with our hypothesis that granulation is due to higher water potential in juice cells, we conducted a range of on-farm management trials of reduced irrigation volumes, with one trial also testing the effects of variable winter nitrogen (N) applications.

Trial 1, of 'normal' versus 'deficit' irrigation and five rates of N application, was conducted on Imperials on 'Benton' rootstocks. Deficits varied with year and stage of fruit development but reducing combined irrigation and rainfall from September to end January by just above half in 2018/19 and by 10-15 % in 2021/22 (a very wet year), reduced mean granulation ratings in 2018/19 from 1.55 to 1.06 (*P*= .009) and in 2021/22 from 3.06 to 2.75 (ns, *P*= .147). 'Unacceptable' (severely granulated) fruit were reduced from 8.5% to 1.1% (*P*= .012) and from 2.3% to 47.6% (ns but close to significant, *P*= .080) respectively. In the two low rainfall years, 2019/20 and 2020/21, the grower reduced the 'control' irrigation regime for Imperials on the property from 98% of evapotranspiration (ETo) (2018/19 data) to 50-60% in the light of increasing evidence from project trials that overwatering is detrimental. In these years, the applied deficit (~50% of the control treatment) had no additional benefit.

Higher winter N applications in Trial 1 reduced granulation in four out of five years but differences between means were significant at the 95% confidence level only in 2018/19. In 2021/22, the trend was reversed: the higher N treatments had higher granulation than the lower N treatments, although differences between means were not significant. This reversal may be due to the lower crop load for these treatments.

A second trial (Trial 3), of Imperials on Troyer rootstocks, was conducted for three years in Wallaville, applying irrigation deficits for 6, 12 or 18 weeks after flowering compared to a 'no deficit' control. In 2019/20, the mean granulation rating for the longer deficit irrigation period (18 weeks) was reduced from 1.63 for the control to 1.26, and 'unacceptable' fruit from 13.6% to 3.4%, but differences between sample means were not significant at the 95% confidence level (*P*= .275, .217). In the following three years, there was no difference in granulation measurements nor any discernible trend with successive treatment times.

The lack of treatment effects in Trial 3 may be due to the overriding influence of soil profiles and drainage in this trial: we drilled soil cores through this trial and found that higher granulation ratings were associated with lighter or sandier soils, particularly where there was a clay layer below the sand. We hypothesize this clay layer serves to 'trap' moisture which drains away in other profiles. The soil profiles of plots with the lowest granulation tended to be consistent clay loam. Clay loams may have higher water content but the moisture is less plant-available than in lighter soils.

We also found that comparing mean granulation for the eight plots in Trial 3 where we had installed tensiometers correlated negatively with tensiometer readings, that is, higher average soil moisture tension meant lower granulation. Correlations for readings in the first 6 weeks were higher than in the 7-12 or 13-18 week period. This might support our hypothesis that early fruit development is the more critical period, or it may be because this period is somewhat drier, and thus there is more range in the variables, giving stronger correlations.

In a third trial, Trial 6, we aimed to apply three levels of water stress through irrigation deficits from the end of flowering to late January to see what level of stress is optimum. In each of the three trial years, the 'medium' and 'significant' stress treatments had less granulation than the 'little' stress treatment but there was no statistically significant difference between the two higher stress treatments. This is because rainfall made it difficult to differentiate between the two in practice in the field. In 2020/21, a low rainfall year, the main difference in treatment means was in the proportion of 'crunchy' fruit (rated 2 or 2.5), which was reduced from 11.1% in the 'little' stress treatment to 5.6% and 6.8% in the 'medium' and 'significant' stress treatments respectively ($P=$ .083). In 2021/22, 'unacceptable' fruit were reduced from 24.4% in the 'little' stress treatment to 14.2% and 10.8% in the 'medium' and 'significant' stress treatments respectively ($P=$ .034). In 2022/23, the reduction was from 25.6% to 14.1% and 12.7% respectively ($P=$ .022). These results mean the question, "How severe should deficits be?" remains largely unanswered, although they clearly confirm the benefit of reduced irrigation.

Introduction

Our hypothesis is that granulation is linked to higher water potential in juice cells, that is, cells with lower total soluble solids and/or higher turgor pressure. A key component of the project was trials that reduced irrigation to reduce cell turgor.

In on-farm trials in the previous project (CT04002), there was no consistent improvement in treatments that reduced irrigation frequency while maintaining the same volume as the control treatment. However, in a trial of trees in pots in controlled nursery conditions, in which both frequency and volume were reduced, granulation was much reduced. In this project, therefore, we focused in our on-farm trials on reducing volume by turning in-line taps on and off. Our first trial (Trial 1) simply applied a deficit compared to the control from the end of flowering to mid to late January. We also conducted trials to seek answers to the questions: "How long should irrigation deficits last?" (Trial 3) and "How severe do the deficits need to be?" (Trial 6).

Published literature on nutrient contents and nutrient applications generally report reduced granulation with applications of almost every mineral nutrient, but this may reflect deficiencies in the soils of growing regions (see Appendix 7 *Literature review and research hypothesis)*. In the project CT04002, we tried high and low levels of broadcast nitrogen (N), boron, zinc, potassium and phosphorus, and foliar N, calcium and zinc applications, in a range of trials with minimal effects in most cases with the exception of N. The most successful treatments, applied at several trial sites, were applications of N in Stage I of fruit development. Treatments that applied extra N in Stage II of fruit development had no effect. We hypothesised that the success of Stage I applications was due to inadequate N applications in winter rather than to the Stage I timing. Winter applications are important because N used during the spring flush is mostly sourced from storage organs; only 10% to 30% is supplied from the soil (Martinez et al., 2002; Mooney & Richardson, 1992; Sweet et al., 2009). Chapman (1986) established that applications at other times are wasteful and can be detrimental to fruit quality. It was common practice in the region at the time of the earlier project for growers to apply N below recommendations in order to encourage earlier skin colour and supply fruit to the early season, higher-priced market. In this project we included treatments of five rates of winter N application into Trial 1 to assess effects on granulation as well as any potential interactions between N and irrigation treatments.

In this report, the results of irrigation trials and treatments are first discussed in the section 'Results of irrigation trials', followed by a separate discussion of nutrient aspects, including the variable N treatments at Trial 1 and leaf tissue analysis at all trials ('Results of variable N treatments and leaf tissue analysis').

Materials and methods

Trial 1: Irrigation deficit x variable N applications

The objectives of this trial were to:

- test the hypothesis that water deficits during early fruit development will reduce granulation,
- test the effects of various rates of winter-applied N on granulation, and
- develop an understanding of any interaction between N and irrigation patterns.

This trial was on a commercial orchard at Wallaville, Central Queensland, using 'Benton' rootstocks, on a light, sandy soil, planted in 2009, at 7 x 3 m spacing. The trial design was factorial: the main plots consisted of two irrigation treatments and the split plots consisted of five rates of N. There were five replicates. The split plots had five trees, with data being collected from the middle three trees.

The two irrigation treatments were a 'control' treatment, that is, normal irrigation as practised at the orchard, and an 'irrigation deficit' treatment. Deficits were applied from the end of flowering through Stage I and early Stage II of fruit development (September to mid-January). The level of deficit each year varied with rainfall patterns and the practicalities of commercial orchard operations. Decisions to irrigate the deficit treatment were based on a visual assessment of stress in the trees and soil moisture monitoring using tensiometers. Table 1 shows key operative dates and Table 2 shows estimated evapotranspiration (ETo), water applied during treatment times, rainfall, and total water as a percentage of ETo. As a benchmark, the crop factor for citrus at 70% canopy cover as calculated by the UN's Food and Agriculture Organisation is 65-67% (FAO56). Note that in years 2019/20 and 2020/21 the grower reduced irrigation of all Imperial blocks on the property, so that, in these years, the control treatment represented a drier regime than standard industry practice.

The five rates of winter applications of N were 850, 700, 550, 400 or 250 g N/tree or 405, 333, 262, 190 and 119 kg N/ha respectively. In 2018/19 the lowest N rate was 340 g due to an application error. N applications were single broadcast applications of granular urea (46% N) in early winter with some minor adjustment for blends applied at approximately the same time. No additional N applications were made during the growing season.

Note that this trial report includes some data from the 2017/18 year, for which we have limited data on trial operation as the trial was unfunded at that stage.

Table 1 Trial 1: Key operative dates 2018/19 to 2021/22

Season	2017/18	2018/19	2019/20	2020/21	2021/22
N application date	1/6/2017	1/6/2018	10/6/2019	10/6/2020	22/6/2021
Irrigation deficit treatment begins	25/9/2017	20/9/2018	10/10/2019	25/9/2020	1/10/2021
Irrigation deficit treatment ends	16/1/2018	21/1/2019	13/1/2020	21/1/2021	31/1/2022
Sample assessment	4/4/2018	3/4/2019	14-15/4/2020	19-20/4/2021	20-21/3/2022

Table 2 Trial 1: Estimated irrigation, rain and evapotranspiration for Stage I and Stage II of fruit development 2018/19- 2021/22

			Hours of irrigation		Rain + irrig (mm)		(Rain+irrig)/ETo	
Stage of fruit development	**ETo (mm)[1]**	**Rain (mm)**	**Control**	**Deficit**	**Control**	**Deficit**	**Control**	**Deficit**
2018/19								
Stage I (wb 23/9 to wb 25/11)	395	306	38	0	431	306	**109%**	**77%**
Stage II (wb 2/12 to wb 20/1)	339	100	57	2	288	106	**85%**	**31%**
Total	734	405	94	2	719	412	**98%**	**56%**
2019/20								
Stage I (wb 7/10 to wb 25/11)	350	45	41	9	181	74	**52%**	**21%**
Stage II wb 2/12-wb 6/1	305	41	45	20	189	105	**62%**	**35%**
Total	656	86	86	28	369	179	**56%**	**27%**
2020/21								
Stage I (wb 21/9 to wb 23/11)	417	57	46	15	209	105	**50%**	**25%**
Stage II (wb 30/11 to wb 18/1)	334	191	55	21	371	260	**111%**	**78%**
Total	750	248	101	36	580	365	**77%**	**49%**
2021/22								
Stage I (wb 27/9 to wb 22/11)	333	358	49	25	510	434	**153%**	**130%**
Stage II (wb 29/11 to wb 24/1)	331	309	14	0	349	309	**105%**	**93%**
Total	664	667	63	25	859	743	**129%**	**112%**

ETo evapotranspiration; wb week beginning; conversion to ML/ha = mm/100. Data is not available for the 2017/18 year.
1 In 2018/9 and 2019/20, evapotranspiration data (ETo) is from Bureau of Meteorology, Bundaberg airport, Station no 39128. In 2020/21 and 2022/22 data is from the PhyTech alert system for Wallaville. Rainfall and irrigation hours were recorded by farm staff. Irrigation calculated at 3.33 mm per hour.

In 2018/19, 2019/20 and 2020/21, combined juice samples were assessed for Brix and acid content as outlined in Appendix 5. The same trees were used for colour samples, using five fruit per tree. Degreening and colour assessment processes are outlined in Appendix 5.

Trial 3: Period of irrigation deficits

The objective of this trial was to test how long moisture deficits need to be applied to achieve optimum granulation results.

The trial was established in a block of Imperials on 'Troyer' rootstock at Wallaville, Central Queensland. In this trial, we applied four irrigation treatments of 6, 12 and 18 week irrigation deficits and a normally-watered control. Deficits all commenced at the end of flowering. There were four blocked replicates of each treatment, with a minimum of nine trees in a single row in each plot, although by 2022/23, several trees had been removed due to ill health. We excluded the trees adjacent to these gaps in our analyses of plot means. The N nutrition program was 200 kg N/ha (as urea), except in 2020/21 when only 127 kg N/ha was applied.

Table 3 shows key rainfall, irrigation and evapotranspiration (ETo) data. This shows that our ability to differentiate between treatments varied from period to period and year to year. In 2019/20 and 2020/21, both low rainfall years, we were able to differentiate somewhat in all three six-week periods, but in 2021/22 we only achieved some differentiation in the first six-week period. In 2022/23, we were able to differentiate between treatments to a certain extent, but frequent rainfall meant that all treatments received well above the FAO benchmark level of 65-67% of ETo.

Table 3 Trial 3: ETo, water applied (combined irrigation and rainfall in mm) and water applied as % of ETo by period and treatment 2019/20- 2022/23

Period after end of flowering:	**Weeks 1-6**		**Weeks 7-12**		**Weeks 13-18**		**Total for treatment**			
Treatment:	**Control (tmt 1)**	**Deficit (tmts 2,3,4)**	**Control (tmts 1,2)**	**Deficit (tmts 3,4)**	**Control (tmts 1,2,3)**	**Deficit (tmt 4)**	**1. Control**	**2. 6-week deficit**	**3. 12-week deficit**	**4. 18-week deficit**
2019/20										
Rain (mm)	45		10		56		111			
Total ETo (mm)	285		302		222		808			
Total hours irrig	24	6	47	27	38	16	109	91	71	49
Total water	109	61	135	82	157	99	345	297	244	186
Water as % of ETo	**38%**	**22%**	**45%**	**27%**	**71%**	**45%**	**43%**	**37%**	**30%**	**23%**
2020/21										
Rain (mm)	55		67		80		201			
Total ETo (mm)	239		284		191		714			
Total hours irrig	28	6	33	18	23	9	84	62	47	34
Total water	128	71	153	114	140	104	421	364	324	289
Water as % of ETo	**54%**	**30%**	**54%**	**40%**	**73%**	**54%**	**59%**	**51%**	**45%**	**41%**
2021/22										
Rain (mm)	58		300		93		451			
Total ETo (mm)	229		219		254		702			
Total hours irrig	12	6	1	0	15	0	28	22	21	6
Total water	91	74	303	300	131	93	524	508	505	467
Water as % of ETo	**40%**	**32%**	**138%**	**137%**	**52%**	**36%**	**75%**	**72%**	**72%**	**66%**
2022/23										
Rain (mm)	177		186		142		506			

Period after end of flowering:	Weeks 1-6		Weeks 7-12		Weeks 13-18		Total for treatment			
Treatment:	**Control (tmt 1)**	**Deficit (tmts 2,3,4)**	**Control (tmts 1,2)**	**Deficit (tmts 3,4)**	**Control (tmts 1,2,3)**	**Deficit (tmt 4)**	**1. Control**	**2. 6-week deficit**	**3. 12-week deficit**	**4. 18-week deficit**
Total ETo (mm)	190		261		192		643			
Total hours irrig	26	0	17	4	19	5	62	37	23	10
Total water	245	177	231	197	192	157	668	603	567	531
Water as % of ETo	**129%**	**93%**	**89%**	**76%**	**100%**	**81%**	**104%**	**94%**	**88%**	**83%**

ETo data is from the PhyTech system. Conversion to ML/ha = mm/100. Irrigation calculated at 2.6 mm per hour.

Soil moisture was monitored using soil tensiometers in 2019/20, 2020/21 and 2022/23, using 15, 45 and 60 cm tensiometers in all treatments in two replicates. From 2020/21, we also used the PhyTech system based on dendrometers installed in three adjacent trees in one plot of each of the treatments.

Table 4 shows the number of days in the 'alert' colours used by the PhyTech alert system. These are described on the PhyTech system as green= no stress (optimal yield), yellow= low stress (not yet affecting plot yield), orange=mild stress (slightly affecting plot yield), and red=high stress (affecting plot yield). The anomalies in this table, e.g. where deficit treatments show higher alert levels than the control treatment, are probably due to declining tree health.

Table 4 Trial 3: Number of days in PhyTech alert system for 'control' and '18 week deficit' treatments by period and for all treatments for full trial periods in 2020/21–2022/23

Period after end of flowering:	Weeks 1-6		Weeks 7-12		Weeks 13-18		Total for full trial period			
Treatment:	**1. Control**	**4. 18-week deficit**	**1. Control**	**4. 18-week deficit**	**1. Control**	**4. 18-week deficit**	**1. Control**	**2. 6-week deficit**	**3. 12-week deficit**	**4. 18-week deficit**
2020/21										
Green	33	28	8	6	12	10	53	91	53	44
Yellow	9	13	16	19	6	2	31	21	36	34
Orange	--	1	18	15	14	7	32	5	16	23
Red	--	--	--	2	3	16	3	2	14	18
2021/22										
Green	34	38	41	32	35	23	110	95	79	93
Yellow	8	4	1	9	14	19	23	33	43	32
Orange	--	--	--	1	--	7	--	5	11	8
Red	--	--	--	--	--	--	--	--	--	--
2022/23										
Green	47	40	32	20	19	15	98	107	55	75
Yellow	--	7	11	16	11	13	22	17	18	36
Orange	--	--	4	12	5	12	9	9	51	24
Red	--	--	1	--	5	--	6	2	11	0

PhyTech data not available for 2019/20. Alert levels are described on the PhyTech system as green= no stress (optimal yield), yellow= low stress (not yet affecting plot yield), orange=mild stress (slightly affecting plot yield), and red=high stress (affecting plot yield).

In 2019/20 and 2020/21 juice samples were taken in the field and assessed for Brix and acid content as outlined in 'General methodology' in Appendix 5. The same trees were used for colour samples, using five fruit per tree. Degreening and colour assessment processes are outlined in Appendix 5.

Trial 6: Severity of irrigation deficits

The objective of this trial was to provide guidance on the level of water stress needed to reduce granulation significantly.

The trial was in Mundubbera, in the Central Burnett, of Imperials on 'Troyer' rootstocks, planted at 2.25 m spacing in rows 6 m apart. Sprinklers were located at every second tree. The trial was a randomised block design with six replicates. Plots were 8-10-tree plots within a single row.

We applied three levels of water stress, 'little', 'medium' or 'significant', through irrigation deficits from the end of flowering to late January. Moisture stress at the trial was monitored using dendrometers provided by PhyTech (see details in 'Methodology' for Trial 3). We aimed to irrigate the 'little' stress trees at or before the daily 'yellow' code was recorded on the PhyTech system; water the 'medium' stress treatment after at least one, preferably two, days at the 'orange' code; and water the 'significant' stress treatment at least one, preferably two, full days at the 'red' code.

Our ability to apply different stress levels was constrained by rainfall. While we were able to apply some deficits in 2020/21 and 2021/22, the difference between the 'medium' and 'high stress' treatments tended to be small, and in 2022/23 we could not differentiate between the 'medium' and 'significant' stress treatments at all (Table 5). In that year, all treatments remained unstressed for the whole of Stage I of fruit development, that is, every day registered 'green' in the PhyTech alert system (Table 6).

Table 5 Trial 6: Summary of estimated irrigation and rainfall received by treatment and as a percentage of evapotranspiration (ETo) 2020/21–2022/23

			Irrigation hours			**Rain + irrig (mm)**			**% ETo**		
Treatment/intended stress	**ETo (mm)**	**Rain (mm)**	**1. *little***	**2. *med.***	**3. *sig.***	**1. *little***	**2. *med.***	**3. *sig.***	**1. *little***	**2. *med.***	**3. *sig.***
				2020/21							
Stage I (28/9-29/11)	429	27	57	13	3	170	59	33	40%	14%	8%
Stage II (30/11-24/1)	382	113	58	34	29	257	199	185	67%	52%	49%
Total	810	139	116	47	32	427	257	219	53%	32%	27%
				2021/22							
Stage I (28/9-29/11)	409	556	47	5	6	170	59	33	42%	14%	8%
Stage II (30/11-24/1)	417	117	56	1	1	257	199	185	62%	48%	44%
Total	826	673	103	6	7	427	257	219	52%	31%	26%
				2022/23							
Stage I (28/9-29/11)	327	115	27	6	6	184	130	130	56%	40%	40%
Stage II (30/11-24/1)	470	161	79	4	4	347	172	172	74%	37%	37%
Total	798	276	106	10	10	531	302	302	67%	38%	38%

Irrigation calculated at 2.5 mm per hour. 'med'= 'medium', 'sig' = significant

Table 6 Trial 6: Number and % of treated days in PhyTech alert system by treatment for 2020/21–2022/23 seasons

Treatment/intended stress	Green			Yellow			Orange			Red		
	1 little	*2 med*	*3 sig.*	*1 little*	*2 med*	*3 sig.*	*1 little*	*2 med*	*3 sig.*	*1 little*	*2 med*	*3 sig.*
					No. of days 2020/21							
Stage I (28/9-29/11)	63	41	43	0	11	14	0	10	4	0	0	2
Stage II (30/11-24/1)	21	17	17	15	16	8	15	17	16	5	6	15
Total	84	58	60	15	27	22	15	27	20	5	6	17
					% of days 2020/ 21							
Stage I (28/9-29/11)	100%	66%	68%	0%	18%	22%	0%	16%	6%	0%	0%	3%
Stage II (30/11-24/1)	38%	30%	30%	27%	29%	14%	27%	30%	29%	9%	11%	27%
Total	71%	49%	50%	13%	23%	18%	13%	23%	17%	4%	5%	14%
					No. of days 2021/22							
Stage I (28/9-29/11)	70	69	66	0	1	4	0	0	0	0	0	0
Stage II (30/11-24/1)	68	65	49	2	4	7	0	1	14	0	0	0
Total	138	134	115	2	5	11	0	1	14	0	0	0
					% of days 2021/ 22							
Stage I (28/9-29/11)	100%	99%	94%	0%	1%	6%	0%	0%	0%	0%	0%	0%
Stage II (30/11-24/1)	97%	93%	70%	3%	6%	10%	0%	1%	20%	0%	0%	0%
Total	99%	96%	82%	1%	4%	8%	0%	1%	10%	0%	0%	0%
					No. of days 2022/23							
Stage	1	2	3	1	2	3	1	2	3	1	2	3
Stage I (28/9-29/11)	63	63	63	0	0	0	0	0	0	0	0	0
Stage II (30/11-24/1)	71	57	60	6	9	8	3	8	9	0	6	3
Total	134	120	123	6	9	8	3	8	9	0	6	3
					% of days 2022/23							
Stage I (28/9-29/11)	100%	100%	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Stage II (30/11-24/1)	89%	71%	75%	8%	11%	10%	4%	10%	11%	0%	8%	4%
Total	94%	84%	86%	4%	6%	6%	2%	6%	6%	0%	4%	2%

'med'= medium, 'sig' = significant. Alert levels are described on the PhyTech system as green= no stress (optimal yield), yellow= low stress (not yet affecting plot yield), orange=mild stress (slightly affecting plot yield), and red=high stress (affecting plot yield).

Leaf sampling – all trials

In 2020/21, 2021/22 and 2022/23 we took leaf samples from all treatments in all trials in February or March, the recommended sampling time for citrus. Leaves were sampled equally from all replicates. Tissue analysis was completed by the Chemistry Centre, Department of Environment and Science.

Carbon and nitrogen were extracted by Dumas Combustion, and other elements by nitric acid microwave digest.

Results of irrigation trials

Trial 1: Irrigation treatments

Granulation

In the years 2017/18 and 2018/19, the deficit irrigation treatment significantly reduced granulation (Table 7). Reducing water by ~ half in 2018/19 and by 10-15 % in 2021/22 (a very wet year), reduced mean granulation ratings in 2018/19 from 1.55 to 1.06 (P= .009) and in 2021/22 from 3.06 to 2.75 (ns, P= .147). 'Unacceptable' (severely granulated) fruit were reduced from 8.5% to 1.1% (P= .012) and from 2.3% to 47.6% (ns but close to significant, P= .080) respectively.

However, in 2019/20 and 2020/21, both low rainfall years, there was no significant differences between 'control' and 'deficit' treatments (Table 7). This may be in part due to the grower in these years reducing the 'control' irrigation regime from 98% of evapotranspiration (ETo) (2018/19 data) to 50-60% ETo in the light of increasing evidence from project trials that overwatering is detrimental. The high crop load in 2020/21 also meant minimal granulation in all treatments.

In 2021/22, the mean granulation rating was reduced but differences between means were not significant at the 95% confidence level (*P*=.147). Very low crop loads, due to late picking in 2021, meant very high granulation levels in this year, and also reduced the consistency between samples.

Fruit in the 'deficit' treatment samples were on average smaller in diameter. In most years, the mean treatment differences were <3mm but in 2019/20 the means differed by 7 mm (Table 7). Note that fruit were not thinned in 2019/20 due to low overall crop load. Crop load ratings were also lower in that year for the 'deficit' treatment than the control (1.0 v 1.9). Along with the poorer granulation response, the smaller fruit size and lower crop load suggest that the reduction in the deficit treatment to 27% of ETo, compared to 56% for the control, may have been too extreme.

Table 7 Trial 1: Mean granulation rating, percentage fruit in main classes, fruit diameter and crop load rating by treatment 2017/18–2021/22

Treatment	Mean rating	Mean % rated ≤1.5 'good'	Mean % rated 2-2.5 'crunchy'	Mean % rated ≥3 'unacceptable'	Mean fruit diameter (mm)	Mean crop load rating
			2017/18			
Irrigation treatment						
Deficit	2.43 a	34.3% b	28.3%	37.5% a	67	3.7
Control	2.72 b	27.1% a	25.3%	47.7% b	67	4
P	*.013*	*.003*	*.202*	*.017*	*.852*	*.613*
ese	*0.05*	*0.8%*	*1.4%*	*1.8%*	*0.3*	*0.3*
N treatment (N/tree)						
1. 850 g	2.46	34.3%	26.0%	39.7%	66 a	3.6
2. 700 g	2.35	33.5%	30.2%	36.3%	67 bc	3.5
3. 550 g	2.63	30.7%	26.2%	43.2%	66 ab	3.6

Treatment	Mean rating	Mean % rated ≤1.5 'good'	Mean % rated 2-2.5 'crunchy'	Mean % rated ≥3 'unacceptable'	Mean fruit diameter (mm)	Mean crop load rating
4. 400 g	2.66	29.7%	26.5%	43.8%	67 abc	4.5
5. 250 g	2.78	25.2%	25.0%	49.8%	67 c	4.0
P	*.324*	*.664*	*.361*	*.440*	*.034*	*.387*
ese	*0.15*	*4.7%*	*1.9%*	*5.1%*	*0.4*	*0.4*
P interaction	*.973*	*.967*	*.578*	*.996*	*.653*	*.833*
			2018/19			
Irrigation treatment						
Deficit	1.06 a	86.7% b	12.1% a	1.1% a	57 a	Not rated
Control	1.55 b	63.6% a	27.9% b	8.5% b	59 b	
P	*.009*	*.014*	*.017*	*.012*	*.011*	
ese	*0.07*	*3.9%*	*2.9%*	*1.2%*	*0.4*	
N treatment (N/tree)						
1. 850 g	1.19 ab	78.3% b	18.3% a	3.3%	58 ab	Not rated
2. 700 g	1.11 a	84.5% b	12.2% a	3.3%	57 a	
3. 550 g	1.22 ab	78.2% b	19.5% ab	2.3%	58 b	
4. 400 g	1.44 bc	71.8% ab	21.2% ab	7.0%	59 bc	
5. 340 g[1]	1.55 c	63.0% a	29.0% b	8.0%	59 c	
P	*.022*	*.035*	*.021*	*.348*	*.002*	
ese	*0.10*	*4.7%*	*3.3%*	*2.4%*	*0.4*	
P interaction	*.656*	*.629*	*.883*	*0.454*	*.149*	
			2019/20			
Irrigation treatment						
Deficit	1.59	66.1%	29.6%	4.4%	54 a	1.0 a
Control	1.47	72.1%	23.0%	4.9%	61 b	1.9 b
P	*.147*	*.296*	*.227*	*.665*	*.002*	*.002*
ese	*0.11*	*6.9%*	*5.7%*	*2.2%*	*1.1*	*0.2*
N treatment (N/tree)						
1. 850 g	1.37	76.8%	19.8%	3.3%	56 a	1.6
2. 700 g	1.53	70.7%	23.0%	6.2%	55 a	1.2
3. 550 g	1.56	67.6%	27.5%	4.8%	57 ab	1.4
4. 400 g	1.51	69.7%	27.2%	3.2%	59 bc	1.6
5. 250 g	1.68	60.5%	33.8%	5.7%	60 c	1.5
P	*.084*	*.204*	*.116*	*.584*	*.001*	*.215*
ese	*.07*	*5.0%*	*4.6%*	*1.2%*	*0.9*	*0.1*
P interaction	*.061*	*.199*	*.344*	*.128*	*.534*	*.987*
			2020/21			
Irrigation treatment						
Deficit	0.89	98.8%	1.1%	0.1%	57 a	*2.6*
Control	0.86	98.1%	1.7%	0.3%	60 b	*3.1*
P	*.673*	*.383*	*.466*	*.374*	*.002*	*.107*
ese	*0.05*	*0.5%*	*0.5%*	*0.1%*	*0.3*	*0.2*
N treatment (N/tree)						
1. 850 g	0.81	99.3%	0.7%	0.0%	*57*	2.6
2. 700 g	0.88	98.2%	1.8%	0.0%	*57*	2.7
3. 550 g	0.89	98.3%	1.3%	0.3%	*58*	3.0
4. 400 g	0.87	98.2%	1.3%	0.5%	*59*	3.0
5. 250 g	0.90	98.2%	1.8%	0.0%	*59*	3.1
P	*.376*	*.757*	*.645*	*.487*	*.066*	*.152*
ese	*0.03*	*0.7%*	*0.6%*	*0.3%*	*0.3*	*0.2*
P interaction	*.148*	*.602*	*.500*	*.540*	*.806*	*.235*

Treatment	Mean rating	Mean % rated ≤1.5 'good'	Mean % rated 2-2.5 'crunchy'	Mean % rated ≥3 'unacceptable'	Mean fruit diameter (mm)	Mean crop load rating
			2021/22			
Irrigation treatment						
Deficit	2.75	24.0%	28.3% b	47.6%	60	0.9
Control	3.06	14.5%	23.2% a	62.3%	61	0.7
P	*.147*	*.124*	*.044*	*.080*	*.057*	*.458*
ese	*0.123*	*3.5%*	*1.3%*	*4.4%*	*0.2*	*0.2*
N treatment (N/tree)						
1. 850 g	3.27	10.2%	23.3%	66.5%	60	0.7
2. 700 g	3.03	19.0%	21.5%	59.5%	60	0.5
3. 550 g	2.92	18.2%	25.2%	56.5%	60	0.7
4. 400 g	2.71	18.9%	35.2%	45.9%	61	1.0
5. 250 g	2.59	30.2%	23.5%	46.3%	61	1.1
P	*.065*	*.136*	*.106*	*.195*	*.100*	*.108*
ese	*.171*	*5.2%*	*3.8%*	*7.0%*	*0.4*	*0.2*
P interaction	*.452*	*.524*	*.629*	*.607*	*.310*	*.039*

[1] 340 g applied in 2018/19, 250 g in other years.
Treatment means within one group marked with the same letter or with no letter were not significantly different at the 95% confidence level.

There was no significant interaction in any year between irrigation and N treatments, although note that in 2019/20 the *P* value for mean granulation rating was .061, that is, almost significant at the 95% confidence level. The 'best' treatment combination in that year was the control irrigation treatment and the highest N level with a mean rating of 1.12 (Table 8). The 'worst' treatment was also the control irrigation treatment but the lowest N level with a mean rating of 1.73. Table 8 details the mean rating for the irrigation/nutrient combinations in years 2018/19 to 2021/22.

Table 8 Trial 1: Mean granulation ratings per tree by irrigation and nitrogen (N) treatment combinations 2018/19- 2020/21

Irrigation treatment	N treatment (N/tree pa)	2018/19	2019/20	2020/21	2021/22
Deficit	1. 850 g	0.92	1.62	0.81	3.40
	2. 700 g	0.93	1.52	0.92	2.78
	3. 550 g	1.08	1.59	0.96	2.76
	4. 400 g	1.10	1.60	0.91	2.50
	5. 340/250 g[1]	1.28	1.63	0.84	2.31
Control	1. 850 g	1.47	1.12	0.81	3.14
	2. 700 g	1.30	1.55	0.85	3.29
	3. 550 g	1.37	1.52	0.83	3.09
	4. 400 g	1.79	1.42	0.84	2.91
	5. 340/250 g	1.82	1.73	0.96	2.88
	P (interaction)	*.656*	*.061*	*.148*	*.452*
	ese	*0.15*	*0.10*	*.067*	*.25*

[1] 340 g applied in 2018/19 and 250 g in other years. Treatment combination means were not significantly different at the 95% confidence level.

Brix and acid levels

In all sample years (2018/19, 2019/20 and 2020/21), there were significant differences between the two irrigation treatments for mean acid levels in juice samples but not Brix levels, with the 'deficit' irrigation treatment having higher acid levels (Table 9, Table 10). Lower N treatments tended to have lower acid levels, but means were not significantly different. Brix did not vary between N treatments. The Brix:acid ratio was higher for the low N treatments in the first two of these years, although only significantly different when comparing the highest and lowest treatments (*P*=.006,.007).

Table 9 Trial 1: Brix and acid levels by treatment 2018/19 and 2019/20

	2018/19				**2019/20**			
	Mean Brix[1]	**Mean acid %**	**Mean Brix:acid**	**Mean ACS value**	**Mean Brix[1]**	**Mean acid %**	**Mean Brix:acid**	**Mean ACS value**
Irrigation treatment								
Deficit	9.45	0.867 b	11.06 a	98.6 a	9.104	1.641 b	5.68 a	41.9 a
Control	9.54	0.640 a	15.03 b	115.2 b	9.356	1.113 a	8.64 b	80.9 b
P	*.773*	*.008*	*<.001*	*.004*	*.426*	*<.001*	*<.001*	*.002*
ese	0.22	0.033	0.30	2.01	0.201	0.032	0.14	3.7
N treatment (g/tree)								
1. 850 g	9.37	0.77	12.57 ab	103.9	9.422	1.427	6.75 a	61.3 ab
2. 700 g	9.03	0.79	11.85 a	96.8	9.414	1.543	6.42 a	53.5 a
3. 550 g	9.61	0.773	12.75 ab	107.5	8.883	1.379	6.86 ab	55.5 a
4. 400 g	9.64	0.746	13.56 bc	109.8	9.127	1.245	7.98 c	68.4 b
5. 250/340 g [2]	9.83	0.691	14.5 c	116.6	9.306	1.291	7.79 bc	68.3 b
P	*.633*	*.223*	*.006*	*.137*	*.344*	*.063*	*.007*	*.033*
ese	0.38	0.032	0.48	5.3	0.211	0.074	0.33	4.0
P interaction	*.362*	*.439*	*.112*	*.140*	*.897*	*.854*	*.265*	*.341*

[1]Corrected for acid and temperature effects. [2] 340 g applied in 2018/19 and 250 g in 2019/20
ACS= Australian Citrus Standard (see Appendix 5 'General Methodology'). Treatment means within one group marked with the same letter or no letter are not significantly different at the 95% confidence level. These samples were taken some days before commercial harvest, and included fruit of all sizes, not just fruit of marketable size and colour, so do not represent the maturity of the first pick when marketed by the grower.

Table 10 Trial 1: Brix and acid levels by treatment 2020/21

	Mean Brix[1]	**Mean acid %**	**Mean Brix:acid**	**Mean ACS value**
Irrigation treatment				
Deficit	10.542	1.457 b	7.42 a	77.8
Control	10.637	1.117 a	9.84 b	101.8
P	*.714*	*.013*	*.009*	*.018*
ese	*0.172*	*0.056*	*0.36*	*4.4*
N treatment (g/tree)				
1. 850 g	10.768	1.334	8.38	89.7
2. 700 g	10.781	1.326	8.5	90.4
3. 550 g	10.362	1.358	8.01	81.4
4. 400 g	10.502	1.177	9.29	95.6
5. 250 g	10.535	1.241	8.96	91.9
P	*.491*	*.385*	*.348*	*.273*

	Mean Brix[1]	Mean acid %	Mean Brix:acid	Mean ACS value
ese	*0.194*	*0.073*	*0.47*	*4.5*
P interaction	*.316*	*.897*	*.915*	*.834*

[1]Corrected for acid and temperature effects.

ACS= Australian Citrus Standard (see Appendix 5 'General Methodology'). Treatment means within one group marked with the same letter or no letter are not significantly different at the 95% confidence level. These samples were taken some days before commercial harvest, and included fruit of all sizes, not just fruit of marketable size and colour, so do not represent the maturity of the first pick when marketed by the grower.

This pattern is evident regardless of whether there was a significant difference in granulation means in that year or not, so may reflect the immaturity or smaller size of the fruit in 'deficit' or low N treatments rather than its granulation status *per se*. This is supported by stronger correlations on a per tree basis of acid % with mean fruit diameter than with mean granulation rating in low-granulation seasons (2019/20 and 2020/21) (Table 11). In the higher-granulating season (2018/19), % acid was negatively correlated with both diameter and granulation rating.

Table 11 Trial 1: Correlation coefficients (r) for mean granulation and diameter of sampled fruit with Brix° and acid % 2018/19–2020/21

Year	2018/19		2019/20		2020/21	
Correlation of sample means for:	**r**	***P***	**r**	***P***	**r**	***P***
Granulation rating with Brix°	-0.013	*.928*	-0.198	*.168*	0.026	*.858*
Granulation rating with acid	-0.618	*<.001*	0.064	*0.660*	-0.150	*.300*
Diameter with Brix°	0.073	*.613*	0.048	*.739*	-0.037	*.800*
Diameter with acid	-0.678	*<.001*	-0.907	*<.001*	-0.691	*<.001*
Granulation rating with diameter	0.680	*<.001*	0.0511	*.724*	0.131	*.367*

Brix° and acid % are from a juice sample from 20 fruit per tree combined. P values represent 2-sided tests of correlations different from zero.

Fruit colour

In 2018/19 and 2019/20, when picked at harvest, there were colour differences for irrigation treatments, with fruit from the 'deficit' treatment measuring slightly lower (more negative) values of the Citrus Colour Index (CCI)(data not shown). After 48 hours, fruit from the control treatments had de-greened more successfully (Table 12). In 2020/21, there were no colour differences in irrigation treatment.

In 2018/19 the lower N applications tended to be slightly better coloured before and after degreening, although differences were small and were only significant for treatment 5 (250 g N) compared to the other treatments (Table 12). In 2019/20 there was a significant difference between some N treatments with the higher N treatments again degreening less effectively than the lower N treatments. In 2020/21 there were no treatment differences.

Table 12 Trial 1: Mean Citrus Colour Index values of treatments after 48 hours degreening 2018/19–2020/21

N treatment (N/tree)	**Deficit**	**Control**	Mean (N treatment)
	2018/19		
1. 850 g	0.169 ab	0.521 bc	0.345 a
2. 700 g	0.400 ab	0.307 ab	0.354 a
3. 550 g	0.239 ab	0.538 bc	0.389 a
4. 400 g	0.018 a	0.868 cd	0.443 a
5. 340 g	0.478 b	1.074 d	0.776 b
Mean (irrig treatment)	0.261 a	0.662 b	
P values 2018/19: =.001 for irrigation treatment, .031 for N treatment and .037 for the interaction of irrigation and N treatments.			
	2019/20		
1. 850 g	0.254	1.476	0.865 ab
2. 700 g	-0.862	1.586	0.362 a
3. 550 g	-0.137	1.480	0.671 ab
4. 400 g	0.587	1.793	1.190 b
5. 250 g	0.540	1.656	1.098 b
Mean (irrig treatment)	0.077 a	1.598 b	
P values 2019/20: <.001 for irrigation treatment, .030 for N treatment and .103 for the interaction of irrigation and N treatments			
	2020/21		
1. 850 g	1.142	1.070	1.106
2. 700 g	1.018	1.240	1.129
3. 550 g	0.960	1.205	1.083
4. 400 g	0.961	1.204	1.083
5. 250 g	1.118	1.436	1.277
Mean (irrig treatment)	1.040	1.231	
P values 2020/21: 0.423 for irrigation treatment, .906 for N treatment and .926 for the interaction of irrigation and N treatments.			

Treatment means within one group marked with the same letter or with no letters were not significantly different at the 95% confidence level.

Trial 3: Period of irrigation deficits

Granulation

In 2019/20, there was on average reduced granulation with the longer deficit irrigation period (18 weeks) but differences between means were not significant at the 95% confidence level (Table 13). In the following three years, there was no difference in granulation measurements, nor even any discernible trend in treatments.

Fruit on average were slightly smaller for the longer periods of deficit but differences were only significant in 2019/20 (Table 13). Crop load ratings were slightly higher in 2019/20 and 2020/21, with no significant differences in the other two years.

Table 13 Trial 3: Mean crop load rating, fruit diameter and granulation 2019/20–2022/23

Treatment	Mean crop load rating	Mean diameter (mm)	Mean granulation rating	Mean % rated ≤1.5 'good'	Mean % rated 2-2.5 'crunchy'	Mean % rated ≥3 'unacceptable'
			2019/20			
1. Control	1.9 c	61.2 c	1.63	64.4%	22.0%	13.6%
2. 6 weeks deficit	1.6 bc	60.3 bc	1.54	67.8%	23.1%	9.1%
3. 12 weeks deficit	1.4 ab	59.1 ab	1.50	71.1%	20.8%	8.1%
4. 18 weeks deficit	1.2 a	57.5 a	1.26	79.6%	17.0%	3.4%
P	*.013*	*.005*	*.275*	*.280*	*.621*	*.217*
ese	*0.1*	*0.5*	*0.13*	*5.3%*	*3.4%*	*3.1%*
			2020/21			
1. Control	3.6 b	59.3	0.98	91.1%	8.3%	0.6%
2. 6 weeks deficit	3.1 a	58.0	0.99	89.9%	9.1%	1.1%
3. 12 weeks deficit	3.1 a	57.5	0.95	92.7%	6.7%	0.6%
4. 18 weeks deficit	3.0 a	57.9	0.98	93.3%	5.6%	1.2%
P	*.026*	*.152*	*.968*	*.653*	*.569*	*.786*
ese	*0.1*	*0.5*	*0.06*	*2.1%*	*1.9%*	*0.5%*
			2021/22			
1. Control	1.4	64.2	1.99	56.4%	23.7%	19.8%
2. 6 weeks deficit	1.3	64.9	1.82	52.5%	30.3%	17.2%
3. 12 weeks deficit	1.5	64.6	1.82	56.4%	25.9%	17.7%
4. 18 weeks deficit	1.4	65.3	1.91	52.1%	29.2%	18.7%
P	*.962*	*.207*	*.709*	*.896*	*.390*	*.915*
ese	*0.2*	*0.3*	*0.12*	*5.3%*	*2.8%*	*2.9%*
			2022/23			
1. Control	5.9	57.0	1.33	83.8%	15.4%	0.7%
2. 6 weeks deficit	5.9	57.0	1.26	86.1%	13.4%	0.5%
3. 12 weeks deficit	6.1	57.9	1.34	81.7%	18.0%	0.3%
4. 18 weeks deficit	6.3	57.6	1.30	82.2%	17.3%	0.5%
P	*.262*	*.677*	*.868*	*.907*	*.885*	*.707*
ese	*0.2*	*0.6*	*0.07*	*4.6%*	*4.5%*	*2.9%*

Treatment means within one group marked with the same letter or with no letters were not significantly different at the 95% confidence level.

The following factors may have contributed to the lack of treatment effects on granulation in this trial:

- In the first three years (2019/20 to 2021/23), as a result of early project results, the grower at this orchard maintained a drier regime than the industry standard, particularly in early fruit development. For example, applications in the first six weeks of fruit development to control treatments were 38%, 54% and 40% ETo in these years respectively. This made it difficult to impose a sufficiently-differentiated deficit regime.
- Conversely, in 2022/23, high levels of rainfall in the first six weeks of fruit development meant it was again difficult to impose a deficit regime, with the control receiving 128% of ETo and the deficit treatments 93% (Table 3).

- There are variable soil profiles in this block which may be overriding treatment effects. Tree by tree representation of granulation data in rows shows a very strong spatial pattern in all trial years, irrespective of treatment effects (Figure 1, Figure 2). Each square in the figures is the mean granulation rating of fruit from one tree (sample of 20 fruit). Each column is a tree row. Blocks (replicates) are outlined in black, but treatments are not shown. Red shading means high granulation, green shading means low granulation.

2019/20							
1.28	1.40	0.88	0.75	0.88	1.13	1.28	1.18
1.63	2.00	0.90	1.10	1.28	1.23	1.28	1.00
1.15	1.13	0.78	1.55	0.98	1.55	1.00	1.65
0.98		0.60	1.28	1.23	1.23	0.98	1.53
1.25	0.45	0.70	1.10	1.40	0.90	1.68	1.43
1.48	0.73	0.85		0.98	1.58	1.60	1.23
1.28	0.85	0.98	1.05	0.90	1.45	1.58	1.28
1.60	0.65	1.08	0.93	0.63	1.43	2.23	1.23
1.50	0.98	0.85	1.05	0.95		1.83	1.53
1.13	0.85		0.85	2.20	1.45	3.15	1.88
1.05	1.08	0.88	0.90	0.98	1.30	2.03	2.30
0.97	1.63	0.87	1.43	0.73	1.83	3.15	0.93
1.75	0.80	0.98		1.30	2.65	2.68	1.58
1.03	1.48	1.10	1.28	1.13	1.98	2.28	2.43
0.68	1.00	0.98	0.90	1.45	1.90	2.13	
0.65		1.10	1.90	1.88	2.73	2.58	2.38
1.28	0.90	0.93	1.70	2.60	1.80	1.88	1.93
1.15	1.43	1.40	2.08	1.83	1.18	2.08	2.15
0.70	1.30	2.48	1.08	1.43	1.15	1.90	
1.13	1.78	2.48	1.23	1.33	1.20	1.85	2.58
1.55	2.15	2.50	1.73	1.80	1.45		2.18
1.83	3.28	1.30	1.08	1.23	1.70	2.03	2.18
0.75	2.63	1.35	0.95	1.95	1.98	2.58	2.28
				1.78	1.43	2.93	1.65

Figure 1 Trial 3: Spatial plot of mean granulation rating per tree 2019/20. Trees at irrigation taps, sick trees and trees adjacent to gaps are shown in this diagram but were not included in plot means for data analyses.

2020/21							
	1.15	1.18	0.90				
		1.15	0.78	0.7	0.725	0.775	0.5
0.88	1.28	1.00	0.88	0.43	0.78	0.58	0.98
1.00		1.15	0.88	1.03	0.35	0.53	0.63
1.05	1.28	0.80	1.38	0.93	0.33	1.28	0.60
0.83		0.85		0.83	0.33	0.78	0.58
0.65	0.73	0.80	0.85	0.68	0.53	0.93	0.83
1.00	0.80	0.80	1.00	0.53	0.68	1.65	0.70
1.03	0.60	0.80	0.90	0.55		1.20	0.78
0.78	0.78		0.73	0.18	1.43	1.45	1.15
0.85		0.92	0.63	1.00	0.65	1.18	2.20
0.75		0.45	0.68	0.35	1.33	1.28	0.73
0.58	0.63	0.73	0.65	0.70	1.23	1.05	0.90
0.80	0.43	0.93	0.80	0.95	1.28	1.10	1.08
0.88	0.63	0.80	0.58	1.43	1.05	1.28	
0.55	0.55	0.63	1.15	1.43	1.48	1.50	2.03
0.58	0.80	0.83	1.35	1.43	1.15	0.95	1.93
0.93	0.95	1.58	1.13	0.95	1.23	1.33	1.65
1.00	1.33	1.08		1.25	0.98	1.33	
0.90	1.20	1.50	0.95	0.80	0.85	1.13	1.55
1.43	1.23	1.45	1.00	1.43	1.15		1.35
1.53	1.35	0.88	1.10	1.05	1.43	1.58	1.00
1.10	1.48	0.93	1.43	1.40	1.68	1.10	1.50
				1.13	1.20	1.30	1.13

Figure 2 Trial 3: Spatial plot of mean granulation rating per tree 2020/21. Trees at irrigation taps, sick trees and trees adjacent to gaps are shown in this diagram but were not included in plot means for data analyses.

In July 2020, we excavated soil cores at the trial to a depth of 90-100 cm at every fourth tree in each row and described the soil profiles for each 20 cm layer. Profiles were highly variable. Table 14 compares mean granulation in 2019/20 with the soil profile characteristics that appeared to be most variable and associated with granulation: sand content (a subjective assessment) and the depth to the clay layer, if any. The higher granulation ratings were associated with lighter soils (that is, sandier), particularly where there was a relatively shallow clay layer below the sand. We hypothesize this clay layer serves to 'trap' moisture which in other profiles drains away. The better part of the block tended to have profiles mostly consisting of clay loams. Clay soils will have a higher water content but the moisture is less plant-available than in lighter soils.

Table 14 Trial 3: Spatial representation of soil cores across the trial block comparing mean granulation rating in 2019/20, rating of sand content of profiles and depth to clay layer

Tree 16	1.5	0.8	0.9	0.9	1.4	1.4	2.4	1.5	2.5	*Mean 3-tree granulation*
	2	0	0	1	0	1	2	3	5	*Sand content rating*
	100	*50*	*100*	*40*	*60*	*80*	*40*	*50*	*20*	*Depth to clay layer (cm)*
Tree 12	1.2	1.3	1.0	1.4	1.3	2.2	2.7	1.6	3.1	*Mean 3-tree granulation*
	1	0	0	0	3	3	4	2	5	*Sand content rating*
	80	*40*	*20*	*100*	*80*	*60*	*80*	*80*	*60*	*Depth to clay layer (cm)*
Tree 8	1.0	1.2	1.1	1.9	2.0	1.4	2.0	2.0	2.7	*Mean 3-tree granulation*
	3	1	2	5	5	3	4	1	2	*Sand content rating*
	60	*20*	*80*	*80*	*60*	*60*	*80*	*80*	*30*	*Depth to clay layer (cm)*
Tree 4	1.5	2.4	2.1	1.3	1.7	1.7	2.3	2.2	1.7	*Mean 3-tree granulation*
	4	5	5	2	1	3	2	3	2	*Sand content rating*
	20	*10*	*100*	*100*	*40*	*20*	*70*	*60*	*80*	*Depth to clay layer (cm)*
Row	1	2	3	4	5	6	7	8	9	

'Mean 3-tree granulation' is the mean granulation rating of samples from the listed tree (where we extracted the soil core) and its neighbours on either side in the row. 'Sand content rating' is a subjective rating of sand content of the profile from 0 (no sand) to 5 (very sandy). 'Depth to clay layer' indicates the depth at which heavy, impermeable clay was first detected in each core.

In addition, mean granulation for the eight plots where we had installed tensiometers correlated negatively with tensiometer readings, that is, higher average soil moisture tension (kPa) meant lower granulation (Table 15). A high kPa means soil water tension is high (drier soil), so a negative correlation means less granulation from drier soils, in line with our hypothesis. This data should be seen as indicative only as the number of data points is small (8 plots) and *P* values often high.

In 2019/20 and 2020/21, both relatively low rainfall years, correlations were stronger at the 15 or 45 cm depth readings than the 60 cm readings; in 2022/23 correlations were strongest with the 60 cm depth reading. This difference may simply reflect the depth of water penetration.

In 2019/20 and 2020/21, correlations were strongest with readings in the earlier stages of fruit development; in 2022/23 this was only evident at the 60 cm depth reading, possibly due to the consistent rain pattern in the first 12 weeks (Table 3), making the readings less variable from plot to plot at the shallower depths.

Table 15 Trial 3: Correlation coefficients (r) between mean granulation ratings and tensiometer readings per plot at three depths (8 plots) 2019/20, 2021/22 and 2022/23)

	1-6 weeks		**7-12 weeks**		**13-18 weeks**		**Overall whole treatment period**	
	r	***P***	**r**	***P***	**r**	***P***	**r**	***P***
				2019/20				
15 cm	-0.81	*0.014*	-0.46	*0.247*	-0.36	*0.383*	-0.69	*0.060*
45 cm	-0.68	*0.062*	-0.53	*0.178*	-0.16	*0.699*	-0.56	*0.148*
60 cm	-0.32	*0.435*	-0.65	*0.079*	-0.52	*0.188*	-0.56	*0.151*
Average of 3 depths	-0.80	*0.017*	-0.63	*0.096*	-0.40	*0.331*	-0.67	*0.069*
				2020/21				
15 cm	-0.41	*0.313*	-0.27	*0.525*	-0.53	*0.179*	-0.41	*0.309*
45 cm	-0.71	*0.049*	-0.63	*0.092*	+0.35	*0.396*	-0.55	*0.158*
60 cm	-0.53	*0.174*	-0.24	*0.563*	-0.13	*0.750*	-0.36	*0.384*
Average of 3 depths	-0.67	*0.071*	-0.48	*0.224*	-0.10	*0.817*	-0.52	*0.186*
				2022/23				
15 cm	-0.39	*0.344*	-0.23	*0.581*	-0.41	*0.311*	-0.46	*0.254*
45 cm	-0.43	*0.289*	-0.34	*0.410*	-0.59	*0.124*	-0.50	*0.204*
60 cm	-0.87	*0.006*	-0.82	*0.013*	-0.63	*0.093*	-0.73	*0.041*
Average of 3 depths	-0.70	*0.054*	-0.61	*0.107*	-0.75	*0.033*	-0.74	*0.036*

Mean granulation ratings used were for the total plot (9-14 trees). r = correlation coefficient. P= two-sided test of correlations different from zero.

Brix and acid levels

In 2019/20, juice samples showed higher acid content for the longer deficit irrigation treatments, but no pattern in Brix (Table 16). In 2020/21, there was very little difference in Brix and acid levels between the treatments.

Table 16 Trial 3: Mean juice content values 2019/20 and 2020/21

	2019/20				2020/21			
Treatment	**Brix[1]**	**% acid**	**Brix: acid ratio**	**Australian Citrus Standard value**	**Brix[1]**	**% acid**	**Brix: acid ratio**	**Australian Citrus Standard value**
1. Control	10.50	1.07 a	10.02	102.90	11.456	0.795	14.50	136.6
2. 6 weeks	10.05	1.04 a	9.96	96.90	11.388	0.879	13.49	129.9
3. 12 weeks	9.80	1.16 a	8.79	85.30	11.289	0.939	12.26	124.3
4. 18 weeks	10.40	1.39 b	7.69	80.00	11.124	0.893	12.93	124.6
P	*.459*	*.028*	*.061*	*.095*	.274	.158	.129	.154
ese	*0.33*	*0.07*	*0.59*	*6.18*	0.117	0.041	0.61	3.9

[1] Corrected for acid and temperature effects. Treatment means within one group marked with the same letter or with no letters were not significantly different at the 95% confidence level. These samples were taken some days before commercial harvest, and included fruit of all sizes, not just fruit of marketable size and colour, so do not represent the maturity of the first pick when marketed by the grower.

As for Trial 1 (Table 11), on a per-tree basis, in the two years of sampling, acid % but not Brix° was negatively correlated with mean granulation ratings and, more strongly, with mean fruit diameter (Table 17).

Table 17 Trial 3: Correlation coefficients (r) for mean granulation and diameter of sampled fruit with Brix° and acid % 2019/20–2020/21

Year	**2019/20**		**2020/21**	
Correlation of sample means for:	**r**	***P***	**r**	***P***
Granulation rating with Brix°	-0.022	*.880*	0.381	*.008*
Granulation rating with acid	-0.398	*.005*	-0.514	*<0.001*
Diameter with Brix°	-0.051	*.732*	-0.414	*.003*
Diameter with acid	-0.649	*<.001*	-0.727	*<.001*
Granulation rating with diameter	0.732	*<.001*	0.606	*<.001*

Brix° and acid % are from a juice sample from 20 fruit per tree combined. P values represent 2-sided tests of correlations different from zero. N = 48 trees

Fruit colour

In 2019/20, there were no significant differences in colour between treatments at the 95% confidence level, but the pattern was consistent with those for granulation and fruit size. The drier treatments were associated with greener fruit that degreened less successfully (Table 18). In 2020/21, there was a significant difference between treatments, but the deficit treatments did not vary consistently with length of deficit.

Table 18 Trial 3: Mean Citrus Colour Index values for 2019/20 and 2020/21

	2019/20		2020/21	
	Before degreening treatment	**After 48 hours degreening**	**Before degreening treatment**	**After 48 hours degreening**
1. Control	-3.57	1.21	-3.76 b	0.25 b
2. 6 weeks	-4.32	1.03	-5.57 ab	-0.53 ab
3. 12 weeks	-5.01	0.61	-7.08 a	-1.01 a
4. 18 weeks	-5.92	0.46	-6.11 a	-0.52 ab
P	*.313*	*.359*	*.027*	*.046*
ese	*0.85*	*0.32*	*0.62*	*0.26*

Data are the mean of 15 fruit per plot = 60 fruit per treatment. Treatment means within one group marked with the same letter or with no letters were not significantly different at the 95% confidence level.

Trial 6: Severity of irrigation deficits

In 2020/21, the two deficit treatments had less granulation than the control (*P*=.034), but there was no statistically significant difference *between* the two deficit treatments (Table 19). These results were achieved with 6 days in the 'red' alert zone for the 'medium' stress treatment and 17 in the 'significant' stress treatment, mostly in Stage II of fruit development (Table 6).

In 2021/22, mean granulation for the two stressed treatments was less than the control, but the difference between means was only significant for the 'significant stress' and 'control' treatments (Table 19).

In 2022/23, mean granulation for the two stressed treatments was less than the 'little stress' (control) treatment but again not different from each other, not surprisingly as both received the same water in that year (Table 5).

The lack of differentiation in granulation in the two higher stress treatments in the last two years is thus most likely due to the lack of treatment differentiation rather than demonstrating that the level of stress is irrelevant. Unfortunately, these results mean the question, "How severe should deficits be?" remains largely unanswered, although they clearly confirm the benefit of reduced irrigation.

Fruit on average were slightly smaller for the 'medium' and 'significant' stress treatments than for the 'little stress' treatment (*P*=0.045 in 2020/21, *P*=.100 in 2021/22, *P*=.462 in 2022/23, Table 19). Crop load ratings did not differ (Table 19).

Table 19. Trial 6: Mean crop load rating, granulation and fruit size by treatment 2020/21–2022/23

Treatment	**Mean crop load rating**	**Mean diameter (mm)**	**Mean granulation rating**	**Mean % rated ≤1.5 'good'**	**Mean % rated 2-2.5 'crunchy'**	**Mean % rated ≥3 'unacceptable'**
2020/21						
1. Little stress	2.6	61.6 b	1.16 b	87.9%	11.1%	1.0%
2. Medium stress	2.7	59.8 a	0.93 a	93.9%	5.6%	0.5%
3. Significant stress	2.3	59.7 a	0.95 a	93.4%	6.6%	0.0%
P	*.663*	*.045*	*.034*	*.059*	*.083*	*.254*

Treatment	Mean crop load rating	Mean diameter (mm)	Mean granulation rating	Mean % rated ≤1.5 'good'	Mean % rated 2-2.5 'crunchy'	Mean % rated ≥3 'unacceptab le'
ese	*0.3*	*0.5*	*0.06*	*1.7%*	*1.6%*	*0.4%*
2021/22						
1. Little stress	2.7	67.9	2.10 b	46.4% a	29.2%	24.4% b
2. Medium stress	2.3	66.8	1.79 ab	57.4% ab	28.4%	14.2% a
3. Significant stress	2.4	66.3	1.68 a	63.8% b	25.4%	10.8% a
P	*.675*	*.100*	*.034*	*.026*	*.543*	*.034*
ese	*0.3*	*0.5*	*0.10*	*3.8%*	*2.5%*	*3.2%*
2022/23						
1. Little stress	4.5	61.0	2.19 b	42.7%	32.3%	25.6% b
2. Medium stress	4.4	60.5	1.84 a	57.5%	28.6%	14.1% a
3. Significant stress	4.1	60.8	1.84 a	58.0%	28.5%	12.7% a
P	*.361*	*.462*	*.038*	*.092*	*.589*	*.022*
ese	*0.2*	*0.3*	*.09*	*5.0%*	*2.9%*	*2.9%*

Treatment means within one group marked with the same letter or with no letters were not significantly different at the 95% confidence level.

Results of variable N treatments and leaf tissue analysis

Trial 1: Variable N treatments

Higher winter N applications tended to reduce granulation but differences between means were significant at the 95% confidence level only in 2018/19. In that year, the 700 g N/tree application had significantly lower mean granulation than treatments of 400 g or 340 g N/tree ratings (*P*=.022) (Table 7).

In 2021/22, the trend was reversed: the higher N treatments tended to have *higher* granulation than the lower N treatments (*P* 'mean granulation' =.065; *P* '% fruit ≥3' = *.195*). This may be due to the lower crop load for this treatment although the trend for lower crop loads in the higher N treatments was also evident in other years without a parallel effect on granulation (Table 7).

Leaf tissue analysis

In the following analysis, we present leaf tissue concentrations for our trial treatments for three years of sampling (two high crop load years and one low crop load year). Because our samples were only from the nine trial sites, this must be considered a very limited sample. For many nutrients the range in nutrient concentrations across treatments and in granulation was very low, and the main contrasts in the data are the differences between trial sites.

Figure 3 shows the relationships for sampled treatments (all trials combined) between leaf tissue concentrations of total N and granulation in the year of sampling on the left, and leaf tissue concentrations and granulation in the year following sampling on the right. This shows different trends for the higher crop load years (2020/21 and 2022/23) than for the low crop load year (2021/22). In the low crop load year, high N in leaf tissue is associated with higher granulation; in the high crop load years, higher N was accompanied by lower granulation. If N levels are compared to granulation in the next season's crop, the trends are reversed. The physiological basis for this may be that, in a low crop load year, there is more flush growth and N is concentrated in leaves. In February/March, stores may already be accumulating to support the following year's crop.

The carbon (C) pattern is similar (Figure 4), and there is a similar, but much weaker pattern, for boron (B) (Figure 5).

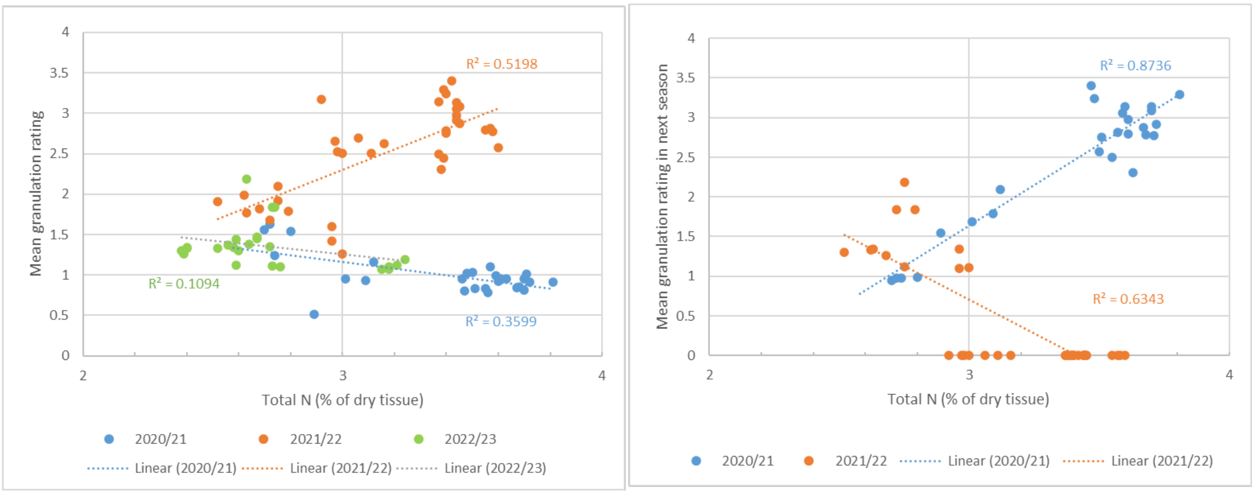

Figure 3 Leaf tissue concentrations of total N and granulation in the year of sampling (left) and in the next season (right). All sample trials are combined. Each data point represents a treatment mean. Current recommendations for N are 2.4-2.6%.

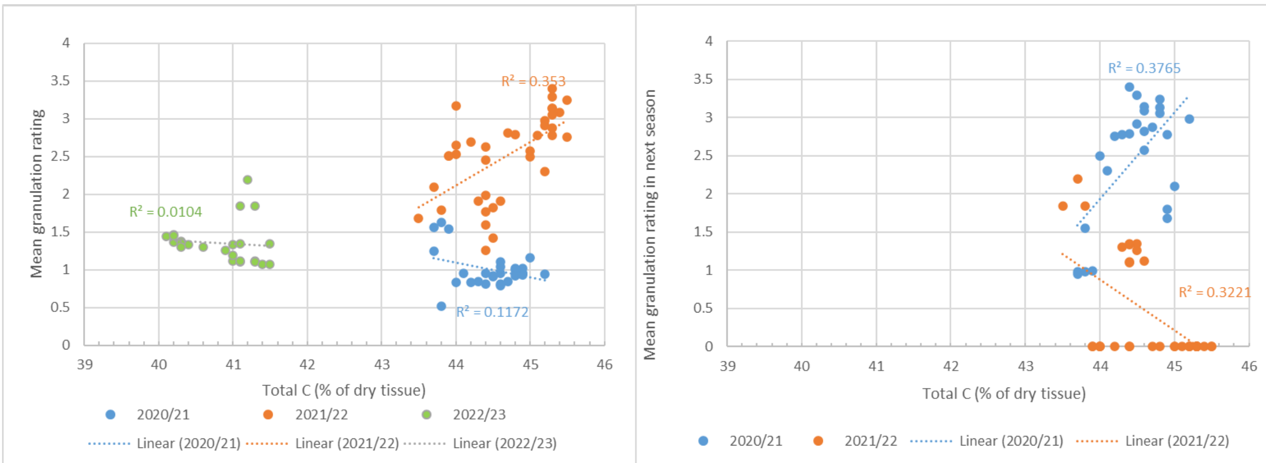

Figure 4 Leaf tissue concentrations of total C and granulation in the year of sampling (left) and in the next season (right). All sample trials are combined. Each data point represents a treatment mean.

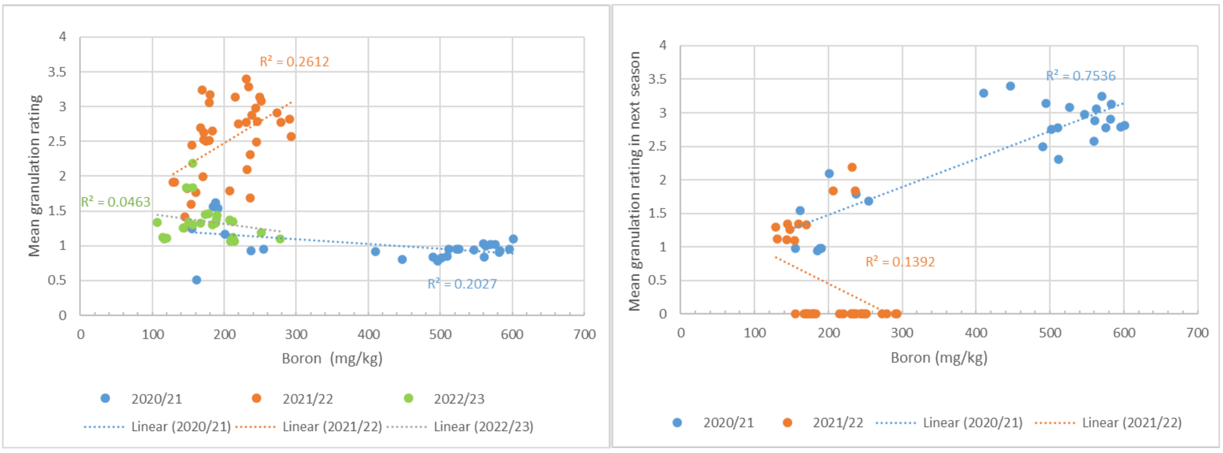

Figure 5 Leaf tissue concentrations of boron (B) and granulation in the year of sampling (left) and in the next season (right). All sample trials are combined. Each data point represents a treatment mean. Current recommendations for B are 30-100 mg/kg.

Other elements follow a pattern which shows concentrations affect the current year's crop but have little relationship with the following year. This includes potassium (K) (*Figure **6***) and calcium (Ca) (Figure 7). For K, the data for 2020/21 shows no trend: we don't have an explanation for why this year was different. For K, high levels are associated with increased granulation in this data, whereas for calcium low levels are associated with increased granulation, although only weakly.

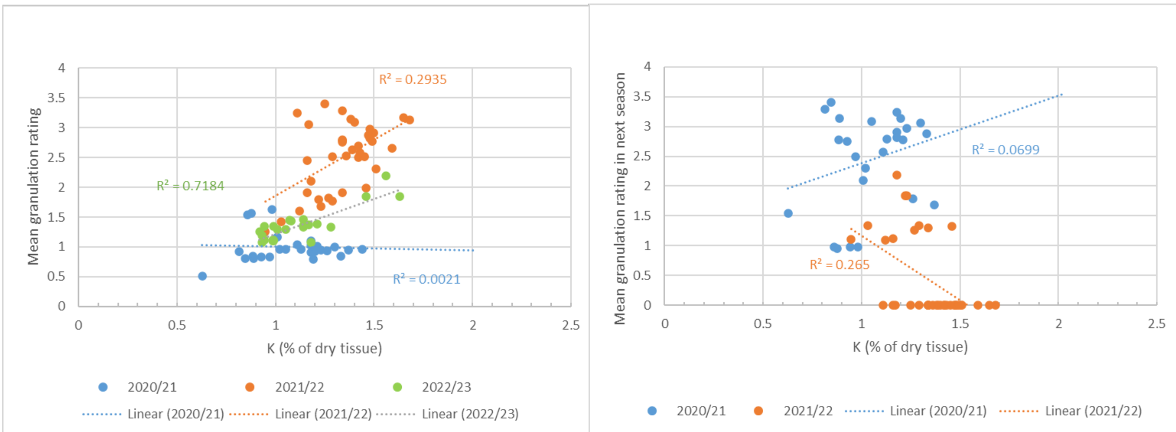

Figure 6 Leaf tissue concentrations of potassium (K) and granulation in the year of sampling (left) and in the next season (right). All sample trials are combined. Each data point represents a treatment mean. Current recommendations for K are 1.2-1.7%.

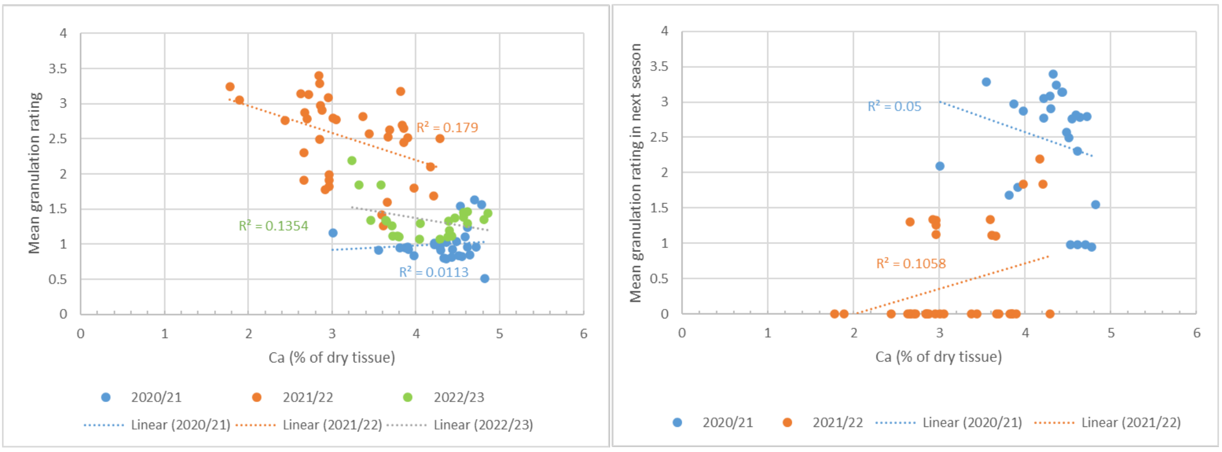

Figure 7 Leaf tissue concentrations of calcium (Ca) and granulation in the year of sampling (left) and in the next season (right). All sample trials are combined. Each data point represents a treatment mean. Current recommendations for Ca are 3-6%.

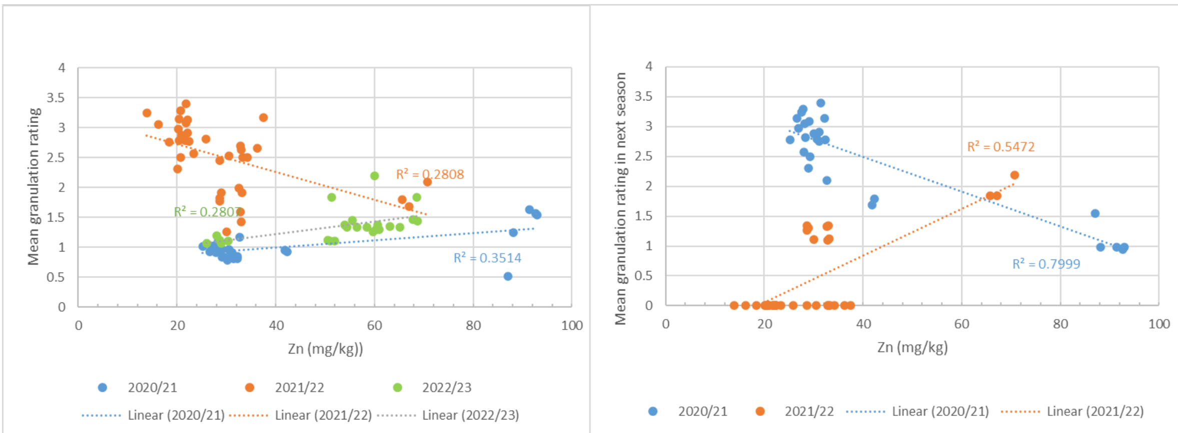

Figure 8 Leaf tissue concentrations of zinc (Zn) and granulation in the year of sampling (left) and in the next season (right). All sample trials are combined. Each data point represents a treatment mean. Current recommendations for Zn are 25-100 mg/kg.

Discussion and conclusions

How much water?

The results of these three trials, along with the data on climate presented in Appendix Two, indicate that management of soil moisture is important in reducing granulation.

Good results (~<5% 'unacceptable' fruit) were achieved in Trial 1 in high crop load years with 77% of ETo (2018/19 deficit treatment), 50% (2020/21 control treatment) and 21% (2020/21 deficit treatment) in Stage I, and 31%, 111% and 78% respectively in Stage II. In the lower crop load year of 2019/20, both 52%/62% and 21%/35% of ETo for the control and deficit treatments in Stage I/Stage II, respectively, produced good results.

Figure 9 shows granulation in terms of 'unacceptable' fruit for our irrigation trials in the two low crop load years 2019/20 and 2021/22, plotted against total water (rain + irrigation)(left-hand graph) and total water as a percentage of evapotranspiration (ETo) (right-hand graph). Only low crop load years are shown because in high crop load years, granulation tends to be negligible. Each dot represents a treatment. This shows a trend of increasing granulation with total water, which is more consistent for Stage I applications. However, the range of granulation results is very wide for a particular amount of water applied: this graph demonstrates the inadvisability of making specific volume recommendations. However, the graph on the right suggests that water/ETo above the UN's Food and Agriculture Organisation crop factor of 65-67% (FAO56) has a high probability of severe granulation.

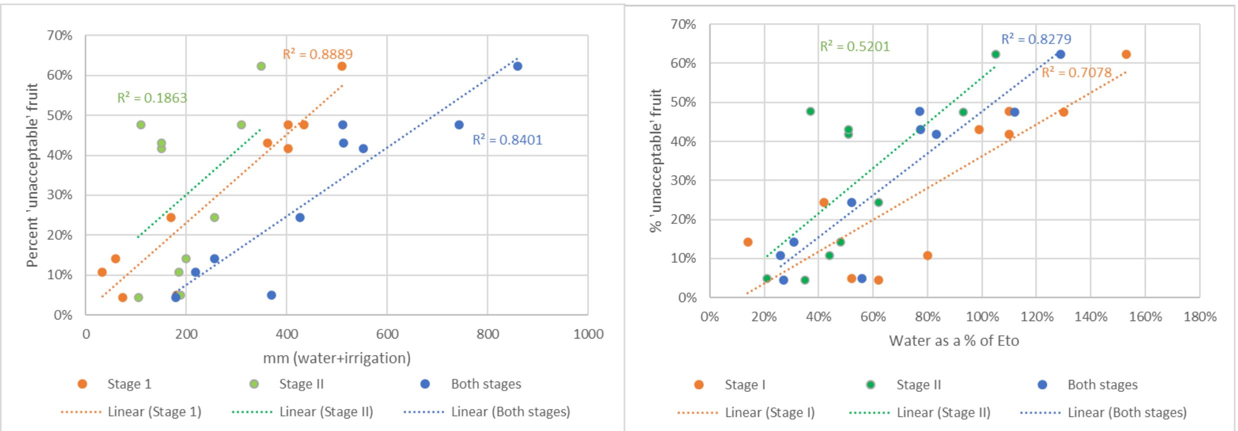

Figure 9 Percent 'unacceptable' fruit (rated ≥ 3) and water applied as mm (left) and as a % of ETo (right) for treatments in Trials 1,3,6 and 7 in low crop load years (2019/20, 2021/22). Trial 7 is discussed in Appendix 4. ML/ha = mm/100. Data should be considered as indicative only as treatment dates in each of the fruit development stages vary between trials and years.

In Trial 1 in 2019/20 reducing water to 21% of ETo in Stage I and 35% in Stage II may have been slightly detrimental. On the other hand, in 2020/21, 25% of ETo in the deficit treatment in Stage I had no significant effect compared to 50% in the control treatment: fruit in that year overall had very little granulation.

In Trial 6, low water in 2020/21 and 2021/22 in Stage I (in both years 14% of ETo for the 'medium' stress treatment and 8% for the 'significant' stress treatment) produced improvements compared to the control, but in the second year, granulation for all treatments was still quite high, due to lower crop load. In 2022/23, granulation levels across the trial were high despite a relatively heavy crop. This may be partly due to rainfall in the period, but also to high levels of stored water in the soil, as the winter preceding was quite wet (151 mm July to September). A high level of stored soil water may be the reason for the long period of 'green' water status recorded in the PhyTech system for all treatments.

In Trial 3, our deficit treatments over four years ranged from 22% to 137% of ETo, with little treatment effect on granulation. However, from our limited data, soil moisture tension correlates negatively with higher granulation. In this trial, the influence of the soil profile on soil moisture tension appears to have been the most important factor.

With this variability in results, it is not possible to provide black and white guidance to growers on irrigation amounts. We achieved mostly better results with treatments that reduced water availability, but crop load and soil profile (drainage) are also important management factors. Growers will need to experiment to suit their own conditions.

When is the critical period?

In a nursery trial with trees in pots in the earlier project (CT04002) (see Appendix 7), treatments reduced volume and frequency in Stage I of fruit development, Stage II, Stage III, Stages II and III, all stages, or watered frequently (the 'control'). In this trial, granulation was lowest for both the 'Stage I' and the 'all stages' treatments, suggesting that Stage I was key

In Trial 3, this seemed to be confirmed in the first year of the trial, but we were unable to confirm this result in the second, third and fourth year of the trial. In Trial 6 in 2020/21, most of the 'stress' as recorded by dendrometers developed in Stage II of fruit development, although water reductions began in Stage I. In Trials 1 and 6, we have carried deficits through to mid- to late-January (late Stage II) with good results. The end date of mid to late January was used because around this time temperatures peak and the trees become visibly stressed very rapidly. But it may be that a deficit all season is beneficial: this remains to be tested. Goldhamer and O'Connell (2006) tested varying the times of water deficits to early, middle and late season and found that granulation was lower than the well-irrigated control for all times, and particularly when stress (50% of evapotranspiration) was applied throughout the growing season.

What are the recommended nitrogen application rates?

The results of this project emphasise the importance of sufficient stores of carbohydrates and nutrients in the tree to support the spring flush. We recommend that for this reason, all N should be applied in winter. In the earlier project CT04002, we reduced granulation through applications of up to 200 g of N/tree in Stage I of fruit development (November), but we believe this was only successful due to inadequate winter applications (~500 g N/tree). Applications in Stage II (December) had no effect on granulation (see Appendix 8).

The results of Trial 1 suggest that the current recommendation of 800 g N/tree for mature trees is a good guide. Our leaf testing data emphasises the importance of managing crop load as well as N nutrient applications. The N leaf tissue results in this project tell us more about crop load status than they do about appropriate leaf N levels, with high levels of leaf N in 2020/21 associated with high levels of granulation in the following year, which we suggest relate mostly to crop load. With just three years of results from a restricted range of sites, there is insufficient data to be prescriptive. However, the current leaf tissue guidelines of between 2.2 and 2.4% N (Vock et al., 1997) appear to be too low. Levels ≥3 in the low crop load year of 2021/22 correlated with good results in 2022/23 (Figure 3).

Which is the more important management practice: N nutrition or irrigation? Is there any interaction between the two practices?

With the exception of 2019/20 noted above, there appeared to be no interaction between the two types of management practice in Trial 1. The scale of improvements from the two were similar e.g. in 2017/18, % of 'unacceptable' fruit was reduced by reducing irrigation from 47.7% to 37.5% of fruit, a 10.2% gain. The 700 g N treatment reduced 'unacceptable' fruit in the lowest N treatment (250 gN) from 49.8 % to 36.3%, a 13.5% gain. In 2018/19, % of 'unacceptable' fruit was reduced by reducing irrigation from 8.5% to 1.1%, a 7.4% gain. The highest N treatment reduced ‘unacceptable’ fruit in the lowest N treatment from 8 % to 3.3%, a 4.7% gain. In short, both aspects need to be managed for optimum results.

What about other elements?

This project did not explore treatments that applied other elements, with the exception of ‘late’ applications of Ca, K, and B, discussed in Appendix 4. In the first project (CT04002), there were no clear effects of either low or high B, K, Zn or P, or from Ca foliar sprays (see Appendix 8).

Some growers wonder if excessive potassium is a contributor to granulation. An early trial (Fullelove et al., 2004) found excess K may stimulate granulation but our trials in previous projects found no effects of either low or high potassium. However, in that project, as well as in this project, there was a weak association of high leaf potassium with high granulation in sampled treatments in some years (Goldhamer & O'Connell, 2006).

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Appendix 3: Spring flush competition and flush manipulation trials

Summary

Our hypothesis that granulation is due to high water potential in juice cells in early fruit development suggests that 'competition' for carbohydrate or mineral resources between flush, flowers and fruit may contribute to granulation.

In several trials we recorded flush growth on tagged twigs and rated the granulation levels of the mature fruit on that twig. This established that granulation at the individual twig level is weakly and positively associated with spring flush growth. Variability in flush growth may explain in part the variability of granulation severity from fruit to fruit within the tree. As flush growth is more vigorous in low crop load years, it may also partly explain higher granulation in low crop load years.

However, our attempts over four years in two trials to manipulate or support flush growth with girdling, foliar nitrogen (N) and plant growth regulators were not promising.

In Trial 2, over four years, we applied two vegetative growth retardants, paclobutrazol and procalcium hexadione, as one or two foliar sprays on emerging spring flush. In the case of paclobutrazol, we also tried application as a soil drench at two dates. We also applied a foliar spray of gibberellic acid (GA) in June; a foliar spray of nitrogen (N); a combination of N and GA; and branch girdling treatments in three dates in spring.

There was no improvement from girdling treatments, so we only tried these for one year. The foliar spray of gibberellic acid in early winter increased early flush growth and increased the proportion of vegetative shoots in comparison to mixed vegetative and floral shoots, but this had no consistent impact on granulation. The foliar spray of N, or the addition of N to the GA treatments, did not help (one year of treatments only). The most promising treatment was paclobutrazol, but gains were restricted to low crop load years and reductions in granulation were small. The procalcium hexadione had no effect.

In Trial 4, where we further tested the use of soil drenches and a foliar spray of paclobutrazol, results were also disappointing. In 2020/21, there was negligible granulation in the trial. In 2021/22, all paclobutrazol treatments had less granulation than the control but this may have been due to a lower crop load as the control trees had been picked later than the treated trees in 2020/21. In 2022/23, a high crop load year with little granulation overall, there were no significant differences between treatments in mean granulation rating or mean % of 'unacceptable' fruit, but there were less 'crunchy' fruit in the two drench treatments, although the difference between means was only significant for the earlier drench.

A trial of later pruning, that is, November rather than July (Trial 5), did not improve granulation results. The late pruned trees developed denser and darker canopies during early fruit set, particularly in the centre of the trees, with many water shoots. It is possible this growth competed with fruit for resources, or that the denser canopy reduced light for photosynthesis.

Introduction

Granulation is higher in low crop load years (see Appendix 1). Our hypothesis suggests that this could be due to 'competition' between flowering and flush, and/or to increased water availability to fruit. In addition, individual fruit vary in granulation severity which could also be partly due to less or more competition from less or more flush growth at the micro (shoot or twig) level.

'Competition' may not be an active process or a preferential allocation, sometimes referred to as 'sink strength', but a more passive insufficiency: stored resources or reserves may simply be inadequate to support both vegetative flush and fruit development, particularly before the flush matures and starts to photosynthesize and export, rather than consume, photoassimilates. Imperials are an early variety, that is, their growth is more rapid than other varieties. This may mean fruit development relies more heavily on stored carbohydrates and is more susceptible to competition from flush growth in early fruit development than other varieties.

On the other hand, once expanded, leaves become a source of photoassimilates for fruit rather than a 'competitor'. In citrus, flowers can be on 'old' wood (previous seasons' growth) or on axillary shoots that develop in the spring flush. The former are generally called 'leafless' inflorescences (although there will be leaves on the old wood), and the latter 'leafy' inflorescences. In citrus, nearby leaves appear to play a significant role in fruit development: leaves export their assimilates primarily to nearby fruits (Kriedemann, 1969, 1970). Logically, then, leafy inflorescences should have better rates of fruit set and quality (including less granulation), not only because the leaves are closer to the fruit but because the timing of leaf expansion and flower development are better synchronised than the development of leafless inflorescences, which tend to be earlier. The younger leaves feeding leafy inflorescences are also more photoefficient (once matured) than the older leaves on mature wood. Indeed, in most citrus varieties, leafy inflorescences reportedly have better fruit set and fruit size than leafless inflorescences (Erner & Shomer, 1996; Lenz, 1966; Moss, 1970). Studies by Lenz and Cary (1969) and Cary (1970) suggest this was particularly true if trees received adequate N. Gibberellic acid (GA) applied in early winter is known to inhibit flowering in citrus and to increase the proportion of leafy inflorescences and thus fruit size (Khurshid, 2005). In Trial 2, we investigated whether we could increase the proportion of leafy inflorescences by the use of gibberellic acid sprays, and if so, whether this would reduce granulation.

To investigate the effects of flush growth on granulation, and explore whether flush growth can explain variability of fruit within the tree, at several of our trials (not just Trial 2) we measured flush growth on tagged twigs at different dates and recorded whether fruit were from 'leafy' or 'leafless' inflorescences. At fruit maturity, we rated granulation of the fruit remaining on the twigs. We selected twigs in all treatments to assess the effect of treatments on flush growth. We have analysed the data to see if competition between flush and fruit explains treatment effects, including the irrigation and nitrogen treatments outlined in Appendix 2.

We conducted two trials (2 and 4) to attempt to reduce or support flush growth and reduce granulation. Treatments included gibberellic acid, the vegetative growth retardants paclobutrazol and procalcium hexadione, foliar N and girdling. In Trial 5 we tried later pruning (spring rather than winter) to see if reducing the 'pruning flush' response helped.

Methodology

Individual twig data – multiple trials

In Trials 1, 2, 3, 6, 7 and 8 we tagged twigs to improve our understanding of the effect of treatments on flush, fruit set and fruit drop. In all trials, there were either four or eight tags per tree, one or two in each quadrant of the tree. We tagged one tree per plot in most trials, but two trees per plot in Trials 3 and 6. Unless stated otherwise, twigs were tagged in late winter before any spring growth was evident. Twigs chosen were single growth units from the previous year's spring or summer flush.

For all trials we measured the length and number of new spring flush shoots on the tagged twigs when flush was fully elongated. In some trials/years we also measured growth approximately every two weeks in order to assess whether the timing of flush expansion is a factor in granulation. At several trials we recorded the inflorescence type ('leafy' or 'leafless') of fruit that had set (see Table 4). At harvest, the fruit on tags were cut and rated as for the main sample.

In this report we use the maximum length of the spring flush shoots. We have found this correlates well with the total flush on the twig (the sum of length of all new shoots), with r values of a sample of trial years shown in Table 1.

Table 1 Correlation coefficient between maximum flush length and sum total of flush on shoots for a selection of trials/years

Trial and year	Date of measurement	Correlation coefficient (r)
Trial 1 2020/21 (high crop)	24/9/2020	.83
	10/9/2020	.81
	27/8/2020	.75
	13/8/2020	.91
	3/8/2020	.87
Trial 2 2020/21 (high crop)	25/9/20	.84
	14/9/20	.83
	28/8/20	.85
	20/8/20	.92
Trial 2 2021/22 (low crop)	20/9/21	.83
	7/9/21	.79
	24/8/21	.89
	16/8/21	.89

Includes 0 values.

Data on flush growth from twigs that did not set fruit or had lost all fruit by harvest were excluded from analyses of flush effects on granulation, but not from treatment effects on flush. When more than one fruit was harvested from a twig, the same flush data was used for each fruit, not, for example, 'halved' where there were two fruit.

Flush manipulation trials

Trial 2: Flush manipulation strategies

The objective of this trial was to test the use of plant growth regulator treatments to manipulate flush extent and/or timing, or in the case of N treatments and spring girdling, to provide additional support to flush development in the critical spring period. The aim was to see if treatments reduced granulation by reducing competition between flowers, fruit and spring flush.

The trial was at Wallaville, Central Queensland, on a block of Imperials on 'Benton' rootstocks, planted in 2009. There were six replicates of three-tree plots with single guard trees between plots, in a randomized block design.

Treatments are shown in Table 2. Some treatments were changed in 2019/20 and 2020/21 after disappointing results in earlier years.

Treatments included vegetative growth retardants widely used on other tree crops: the triazole paclobutrazol, and procalcium hexadione, in the products 'AuStar' and "RegalisPlus' respectively. Neither of these products are currently registered for use on citrus in Australia. We sprayed one or two foliar applications onto emerging spring flush. We also tried a soil (collar) drench of paclobutrazol in the later years of this trial, and again for three years in a second trial (see below, Trial 4). We tried an N foliar spray to see if this reduced the competition from developing leaves.

We included a gibberellic acid treatment, and gibberellic acid plus foliar N treatment, to see if this reduced granulation by increasing the proportion of leafy inflorescences and/or advancing the timing of flush development relative to fruit growth.

Treatments included girdling at three dates in spring in 2018/19 to see if flowering or flush were limited by stores of carbohydrates in the roots or trunk and could be manipulated by reducing access to these stores. In 2019/20 we did not repeat the girdling treatments but at harvest in 2020 we sampled fruit to see if there was a carryover effect from the previous year. In this assessment we sampled eight fruit from each of three previously girdled branches and three ungirdled branches (using branches from the same limbs where possible). As there was no difference in the mean granulation rating for previously girdled and ungirdled branches (2.14 and 2.11 respectively, *P*=.839), we used a combined average for the tree in the comparison of treatment effects for the trial for 2019/20 shown in Table 10.

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Table 2 Trial 2: Treatment application dates 2018/19–2022/23

Treatment	2018/19	2019/20	2020/21	2021/22
Control	–	–	–	–
ProGibb in June	20/6/18	19/6/19	3/7/20	18/6/21
ProGibb in June, plus foliar N: two applications	ProGibb 20/6/18; foliar N as below	na	na	na
Foliar N: two applications	2/8/18 and 21/9/18	na	na	na

Treatment	2018/19	2019/20	2020/21	2021/22
AuStar: one foliar application when flush emerges	29/8/18	4/9/19	25/8/20	24/8/21
AuStar: one later foliar application	na	na	14/9/20	na
AuStar: two foliar applications	na	4/9/19 and 25/9/19	25/8/20 and 14/9/20	24/8/21 and 8/9/21
AuStar: drench when flush emerges	na	na	27/8/20	24/8/21
RegalisPlus: one application	na	4/9/19	25/8/2020	23/8/21
RegalisPlus: two applications	na	4/9/19 and 25/9/19	25/8/2020 and 14/9/20	23/8/21 and 7/9/21
50% of branches girdled with no foliar spray	2/8/18, 7/9/18 or 12/10/18	na	na	na
50% of branches girdled with 1 foliar N on day of girdling	As above	na	na	na

na =not applied

Application concentrations were as follows:

- In 2018/19, ProGibb at 6 ml per 10 L (active ingredient 0.1 g GA per litre)
- In 2019/20, ProGibbSG (soluble granules) at 2 g/L (active ingredient 400 g GA per kg)
- AuStar (active ingredient 250 g/L paclobutrazol) foliar sprays at 0.7%
- AuStar collar drenches 8.5 ml in 1 L water
- RegalisPlus (active ingredient 100 g/kg prohexadione-calcium) at 50 g/100L
- Foliar N at 1.5% LoBi urea (urea is 46% N)

For foliar treatments, trees were sprayed with a backpack sprayer at ~30 KPa. Trees were sprayed to the point of runoff, at approximately 1.5 L/tree. PrimaBuff was used to reduce pH to 4.5-5 for the GA treatment.

Trial 4: Paclobutrazol applications

This trial was established after promising results for paclobutrazol foliar sprays in Trial 2 in 2019/20, with the aim of further testing this treatment as well as testing whether soil drenches would be more effective than foliar sprays. Two drench timings were tested: when flush first emerged and ~two weeks later.

Trial 4 was at Wallaville, Central Queensland, on a block of mature Imperials on 'Troyer' rootstocks in a randomized block design with eight replicates of single tree plots over four rows. There was at least one guard tree between treated trees. Treatments and application dates are shown in Table 3. Collar drenches were applied around the trunk of each tree. The foliar treatment was applied with a backpack sprayer at ~30 KPa. Trees were sprayed to the point of runoff, at approximately 2 L/tree.

Table 3. Trial 4: Treatments and application dates 2020/21–2022/23

Treatment	Application	2020/21	2021/22	2022/23
1. Collar drench: first date	AuStar (250g/L paclobutrazol) at 9 ml in 1 litre of water each tree	3/9/2020	11/8/2021	5/8/2022
2. Collar drench: second date	As above	15/9/2020	24/8/2021	16/8/2022
3. Foliar spray: first date	AuStar at 0.7%, ~ 2 litres per tree	3/9/2020	17/8/2021	16/8/2022
4. Control	–	–	–	–

Trial 5: Late pruning trial

The objective of this trial was to see if late pruning reduced granulation by reducing the competition between early fruit development and regrowth.

Two rows were selected and two blocks of five trees were chosen for late pruning, one in each row. Control trees were assigned as trees that were pruned at the standard time in the same positions in the adjacent row. Control trees were pruned on 28 July 2020 and 'late pruned' trees on 20 November 2020. The 'late pruned trees' were pruned as they would have been in winter, not pruned to match the canopy level of control trees (Figure 1). This reduced overall crop on these trees.

Figure 1 Trial 5: Late pruned tree in November 2020 adjacent to a tree that had been pruned in winter. Photo: H Hofman.

Results were analysed using paired sample T-Tests, using trees paired at the same position in each row.

Results

Individual twig data – multiple trials

The analysis in this section disregards treatment effects. Treatment effects on flush are discussed in the following section.

Overall, at the individual twig and fruit level, there was an association between spring flush and granulation rating. Our analysis of data for Trials 2, 3, 4, and 6 shows a great deal of variation in patterns between the trials, and in some trials trends are weak, but in general there appeared to be two different patterns for low and high crop load years:

- In low crop load years, there is higher granulation. Flush is longer than in high crop load years. Stronger *early* flush growth is associated with higher granulation ratings. In most instances, but not all, stronger *later* flush was associated with *less* granulation. The clearest example of this is Trial 1 in 2019/20 (Figure 2).
- In high crop load years, there is less granulation. Flush is shorter. There appears to be no detrimental effect of *early* flush growth. The example shown in Figure 3 is also data from Trial 1, but in 2018/19, a high crop load year. In most trials, stronger *later* flush meant *more* granulation, as in Figure 3, although this was not evident in all trials.

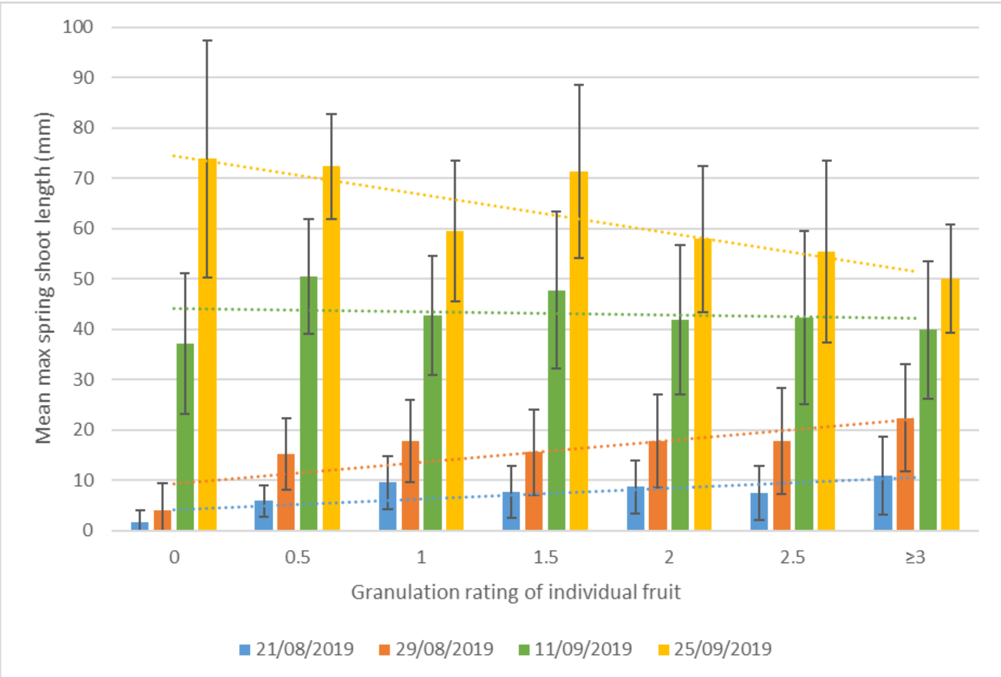

Figure 2 Trial 1: Mean maximum length of spring flush at four dates for rating of individual fruit in 2019/20 (low crop load year). All treatments combined. Error bars show standard deviations. Fruit of different ratings may come from the same twig. Data includes zero values (i.e. where twigs set fruit but had no spring flush). The numbers of fruit in each category were 0=10, 0.5=13,1=45,1.5=22, 2=14, 2.5=8, ≥3=8 (total 120 fruit). Mean fruit diameter (mm) in each category for rating 0=48.6, 0.5=53.8, 1=55.7, 1.5=57.7, 2=58.3, 2.5=62.4, ≥3=57.4.

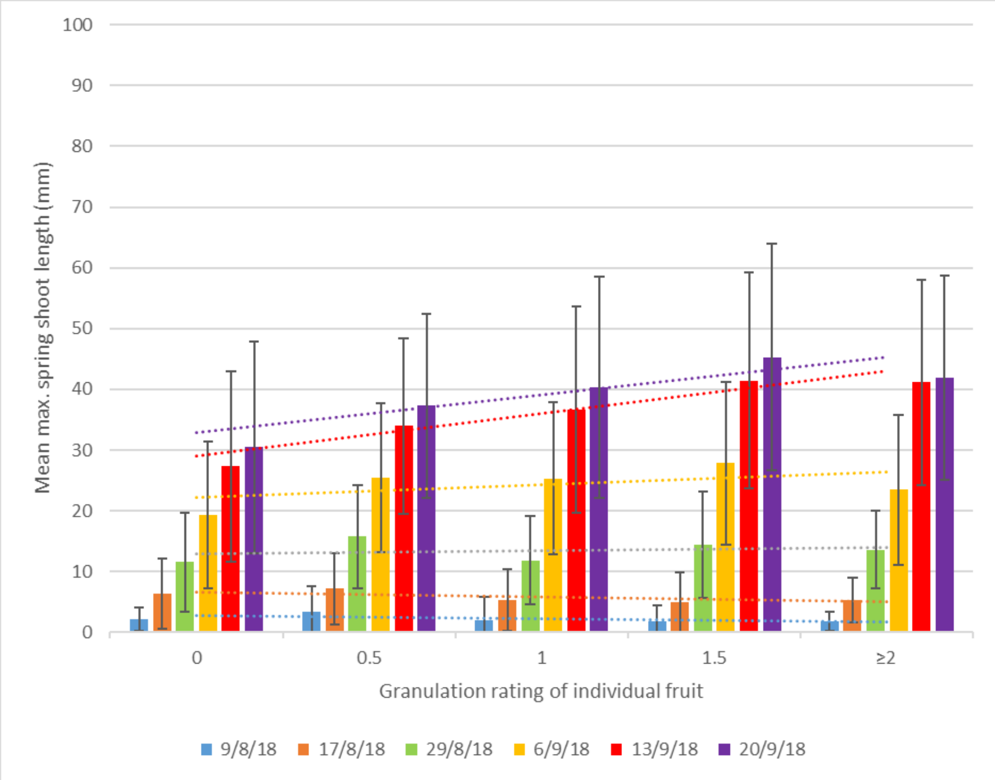

Figure 3 Trial 1: Mean maximum length of spring flush at five dates for rating of individual fruit in 2018/19 (high crop load year). All treatments combined. Error bars show standard deviations. Fruit of different ratings may come from the same twig. Data includes zero values (i.e. where twigs set fruit but had no spring flush). The numbers of fruit in each category were 0=25, 0.5=41, 1=33, 1.5=24, ≥2=14 (total 137 fruit). Mean fruit diameter (mm) in each category for rating 0=49.5, 0.5=51.4,1=52.2, 1.5=54.0, ≥2=57.4.

We suggest these patterns can be explained as follows:

- In low crop load years, carbohydrate and nutrient reserves in the tree are low (depleted by the previous season's crop) so there is competition between fruit development and *early* flush growth. On a twig-by-twig basis, later flush seems to support better fruit development. This may be because in low crop load years the fruit must rely more on current photosynthates produced by the flush once it hardens off, rather than, as in high crop load years, stores in the tree.
- In high crop load years, carbohydrate and nutrient reserves are high and sufficient to support both fruit growth and flush. In addition, heavy flowering and fruit set supresses flush growth, reducing potential competition. In the trials where stronger later flush on a twig was associated with more granulation, this may be due to inadequate nutrition: only the later flush growth is resource-limited and competes with fruit development.

Some of the factors which may influence these trends are as follows:

(1) *Farm management practices.* In particular, inadequate nutrition, lack of crop load management and late picking will reduce the carbohydrate stores in the tree that support the spring flush and fruit set.
(2) *The trend for more granulated fruit to be larger*. The larger size indicates that these fruit set earlier and are therefore more prone to competition in the early stages of the flush than later-setting fruit. Some severely granulated fruit can also be very small, i.e. very late setting, presumably these suffer because the tree has 'run out' of stores.

(3) *Whether the fruit set on leafy or leafless inflorescences*, as discussed below.

Leafy and leafless inflorescences

Our data showed that the proportion of fruit that were on leafy or leafless inflorescences depended on crop load. In high crop load years, more fruit were on leafless inflorescences (old wood) and there was minimal spring flush. In low crop load years, more fruit were on leafy inflorescences and there was more flush (Table 4).

In 5 of the 9 trials listed in Table 4, fruit on leafy inflorescences were less granulated than those on leafless inflorescences, with no pattern of high or low crop, but the difference were small and, in most years, not significant at the 95% confidence level. This may be because Imperials are more reliant on reserves than other more slowly-developing varieties, making the type of inflorescence largely irrelevant.

Table 4 Proportion of fruit set and mean granulation rating of fruit on leafy and leafless inflorescences in various trials/years

Year (crop load)	Trial	n	% fruit on inflorescences that were leafy	Mean granulation rating of fruit on inflorescences that were: Leafy	Leafless	*P*
2018/19 (high)	Trial 1	185	39%	0.95	1.05	.054
2019/20 (low)	Trial 1	116	58%	1.35	1.41	.728
2020/21 (high)	Trial 1	206	36%	0.66 a	0.82 b	.033
	Trial 2	56	38%	1.10 b	0.78 a	.004
2021/22 (low)	Trial 4	52	21%	0.38	0.27	.286[1]
	Trial 2	52	56%	1.55	1.96	.135 [1]
2022/23 (high)	Trial 3	124	15%	1.25	1.20	.753[1]
	Trial 4	83	22%	1.00	1.31	.247[1]
	Trial 6	73	21%	2.06	1.96	.712

n= no. of fruit remaining on tags at harvest. Treatment means within one group marked with the same letter or with no letters were not significantly different at the 95% confidence level.

[1] Means separated using a two-tailed t-test as there were not enough fruit of treatments/types/replicates left on tags to use analysis of variance.

Irrigation and N treatment effects on spring flush

Below we present the data from Trials 1 and 6. Methodology and granulation results for these trials are described in *Appendix 2: Irrigation and nitrogen nutrition trials*. The data for Trial 3, also an irrigation trial, showed no significant differences between treatments in any year in either flush lengths or granulation and is not presented here.

Trial 1: Irrigation deficit x variable N applications

In Trial 1 we tested reduced versus control irrigation and winter nitrogen applications (see Appendix 2). These treatments had some effects on flush and fruit set, which can be summarised as follows:

- The reduced irrigation treatment had lower maximum spring flush lengths in all years, and a lower mean number of spring flush shoots per tag in all years except 2020/21, but differences were not always significant at the 95% confidence level (Table 5).
- The fruit set data for the irrigation treatments shows the inverse of the flush growth data, with the deficit treatment, which had less flush, setting and retaining *more* fruit, although differences were not significant at the 95% confidence in two of the four years of data (Table 6).

This is in line with our hypothesis that flush and fruit development compete, but granulation data for these years was not fully aligned with these trends, with the deficit irrigation treatment only significantly reducing granulation in 2018/19 (high crop) and 2021/22 (low crop) despite having less flush and setting more fruit in all years. Other factors, such as irrigation + rainfall volumes, may have been more influential than any reduced effect of flush competition.

- The nitrogen treatments only showed different flush levels in low crop load years. In high crop load years, all treatments had similar levels of flush. In low crop load years, the higher N treatments had *less* flush growth, which aligns with our results showing less granulation from high N treatments (Table 5). There was no significant difference in initial or later fruit set by N treatment in any year (Table 6).

Table 5 Trial 1: Maximum new spring flush length and number of new shoots per tagged twig by treatment 2018/19–2021/22

	Max. flush length (mm)				**No. of new shoots**			
Year	**2018/19**	**2019/20**	**2020/21**	**2021/22**	**2018/19**	**2019/20**	**2020/21**	**2021/22**
Crop load	high	low	high	very low	high	low	high	very low
Date measured	5/10	10/10	24/09	5/10	13/09	10/10	24/09	13/09
Irrigation treatment								
Control	43.9	67.1 b	42.4	73.5	2.48 b	4.74	2.24	3.12
Deficit	39.9	56.0 a	33.0	67.9	1.67 a	4.05	2.41	3.13
P	*.624*	*.014*	*.144*	*.156*	*.030*	*.061*	*.531*	*.913*
ese	*5.7*	*1.9*	*3.7*	*2.7*	*0.25*	*0.19*	*0.18*	*0.09*
Nutrient treatment (N/tree)								
1. 850 g	51.6	60.5 ab	41.5	53.9 a	2.25	3.57 a	2.67	2.56 a
2. 700 g	32.8	44.8 a	29.3	73.6 bc	1.88	3.57 a	2.16	3.35 bc
3. 550 g	43.6	61.8 ab	39.3	61.1 ab	1.79	4.5 ab	2.23	2.66 ab
4. 400 g	42.5	75.3 b	39.7	83.7 c	2.21	4.77 ab	2.26	3.45 c
5. 250 g [1]	39	65.1 b	38.8	81.1 c	2.25	5.55 b	2.32	3.59 c
P	*.680*	*.027*	*.303*	*<.001*	*.853*	*.042*	*.820*	*.013*
ese	*9.0*	*6.2*	*4.3*	*4.2*	*0.39*	*0.50*	*0.33*	*0.25*
P interaction	*.872*	*.316*	*.618*	*.897*	*.853*	*.941*	*.618*	*.86*

[1] Treatment 5 received 340 g N/tree in 2018/19. Treatment means within one group marked with the same letter or with no letters were not significantly different at the 95% confidence level.

Table 6 Trial 1: Number of fruit per tagged twig in early October and November (after initial drop) by treatment 2018/19–2021/22

	No. of fruit early Oct				**No. of fruit early Nov**			
	2018/19	2019/20	2020/21	2021/22	2018/19	2019/20	2020/21	2021/22[2]
Crop load	high	low	high	very low	high	Low	high	very low
Date measured	11/10/18	10/10/19	8/10/20	5/10/21	2/11/18	7/11/19	5/11/20	
Irrigation treatment								
Control	4.50	1.05 a	3.48 a	0.04	1.42	0.63 a	0.59	Insufficient data
Deficit	4.76	1.79 b	5.17 b	0.07	1.80	0.91 b	0.76	
P	*.689*	*.002*	*.042*	*.591*	*.147*	*.003*	*.175*	
ese	*0.45*	*0.07*	*0.40*	*0.04*	*0.18*	*0.03*	*0.07*	
Nutrient treatment (N/tree)								
1. 850 g	6.13	1.85	4.51	0.06	1.88	0.88	0.70	
2. 700 g	4.17	1.67	4.10	0	1.46	0.71	0.60	
3. 550 g	4.40	1.48	4.44	0.04	1.88	0.85	0.75	
4. 400 g	4.13	1.15	4.41	0.03	1.12	0.73	0.67	
5. 250 g [1]	4.33	0.94	4.17	0.13	1.71	0.71	0.66	
P	*.281*	*.363*	*.970*	*.299*	*.316*	*.953*	*.821*	
ese	*0.72*	*0.35*	*0.50*	*0.04*	*0.28*	*0.20*	*0.09*	
P interaction	*.703*	*.373*	*.216*	*.118*	*.993*	*.637*	*.932*	

[1] Treatment 5 received 340 g N in 2018/19. [2] 2021/22 data on fruit set had many missing values due to very low fruit set. Treatment means within one group marked with the same letter or with no letters were not significantly different at the 95% confidence level.

The deficit irrigation treatment appears to have reduced the proportion of fruit on leafy inflorescences although the means were only significantly different in one year (Table 7). This is in line with the lower levels of flush from this treatment (Table 5). Nutrient treatments, however, did not appear to affect whether fruit were *retained* on leafy or leafless inflorescences (Table 7).

Table 7 Trial 1: Percentage of tagged fruit on leafy inflorescences by treatment 2018/19–2020/21.

Crop load	**2018/19 high**	**2019/20 low**	**2020/21 high**	**2021/22 very low**
Irrigation treatment				
Control	46%	53%	39% b	Insufficient data
Deficit	35%	48%	25% a	
P	*.325*	*.485*	*.015*	
ese	*7.6%*	*4.7%*	*2.4%*	
Nutrient treatment (N/tree)				
1. 850 g	32%	45%	32%	
2. 700 g	40%	23%	25%	
3. 550 g	46%	72%	40%	
4. 400 g	37%	53%	31%	
5. 250 g [1]	49%	59%	33%	
P	*.855*	*.110*	*.896*	
ese	*12.1%*	*12.4%*	*10.7%*	
P interaction	*.719*	*.917*	*.266*	

[1] Treatment 5 received 340 g N in 2018/19. [2] 2021/22 data on fruit set had many missing values due to very low fruit set. Treatment means within one group marked with the same letter or with no letters were not significantly different at the 95% confidence level.

Our findings that higher irrigation In Trial 1 increased fruit from leafy inflorescences but had higher granulation incidence suggests that total water has more influence than flush competition.

Only one year of data provides support for the hypothesis that leafy inflorescences may produce less granulated fruit where N is sufficient.

- In 2018/19, a high crop load year, there was no difference in the mean granulation rating of leafy or leafless inflorescences, respectively 0.95 and 1.05 (*P*=.450), but there was an almost-significant interaction of inflorescence type with N treatment (*P*=.054). This indicates lower granulation on leafy inflorescences for the higher N treatments 1 to 3, with the reverse pattern for the lower N treatments 4 and 5 (Figure 4).
- The pattern of lower granulation on leaf inflorescences only with higher N applications was not evident in 2019/20, a low crop-load year, when there was no difference in ratings by fruit type (*P*=.728) or differences in nutrient treatment/fruit type mean granulation (*P*=.618) (Figure 5).
- In 2020/21 (a high crop-load year) on average fruit from leafy inflorescences were less granulated than those from leafless inflorescences (means of 0.66 and 0.82 respectively, *P*= .033) but there was no clear trend in the N treatments. Overall granulation was very low, making it difficult to detect patterns (Figure 6).

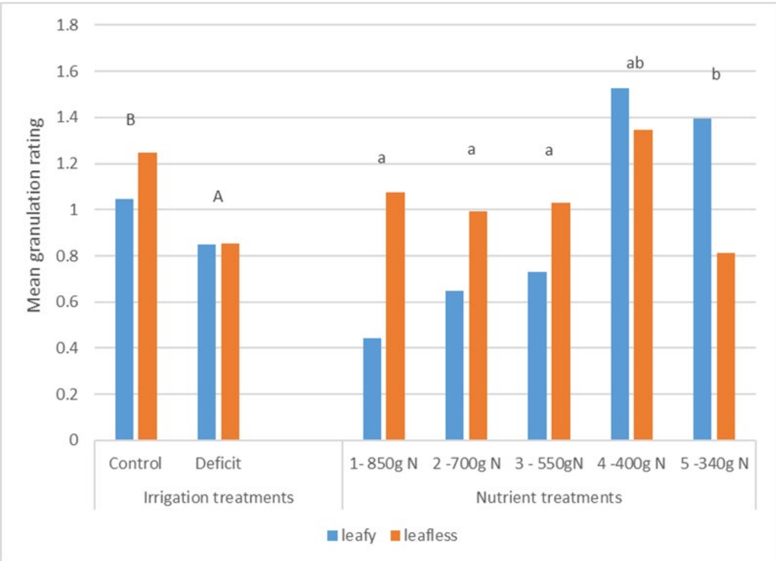

Figure 4 Trial 1: Mean granulation ratings by treatment and type of inflorescence in 2018/2019. This analysis uses three replicates only (total 185 fruit). P values: irrigation treatment =.034, nitrogen treatment =.019, fruit type =.45, irrigation treatment x fruit type=.468 and nitrogen treatment x fruit type =.054). For nitrogen treatments, means with the same letters shown on graph are not significantly different at the 95% confidence level.

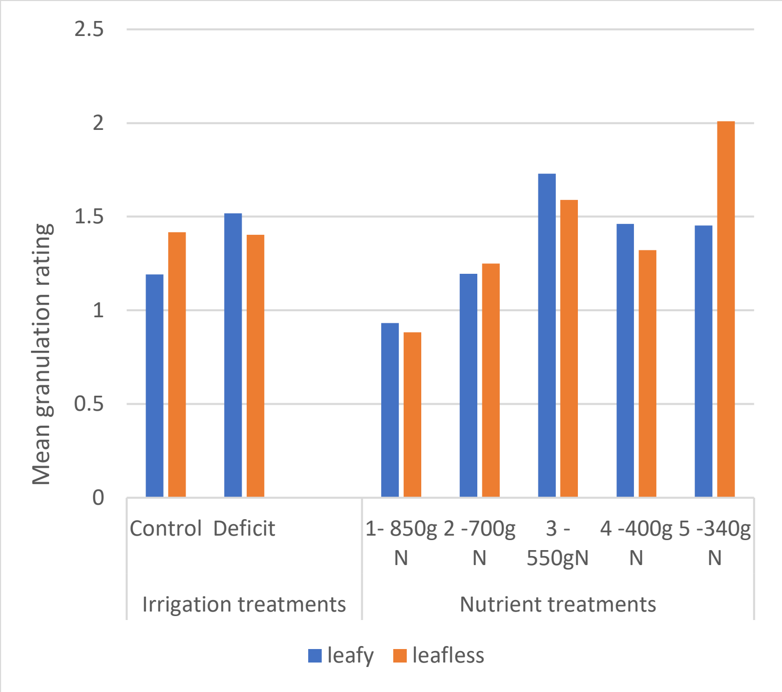

Figure 5 Trial 1: Mean granulation rating by treatment and type of inflorescence in 2019/20 (116 fruit). There were no significant differences between treatments, fruit types or their interactions (P values: irrigation treatment =.252, N treatment =.062, fruit type =.728, irrigation treatment x fruit type =.301 and N treatment x fruit type =.618).

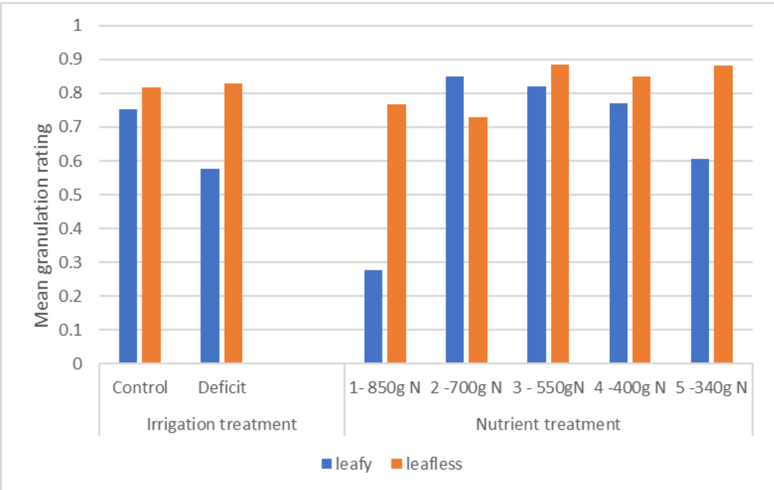

Figure 6 Trial 1: Mean granulation rating by N treatment and type of inflorescence in 2020/21 (n=206 fruit). There was a significant difference between fruit type (P=.033) but no significant difference between treatments or their interactions (P values: irrigation treatment=.600, N treatment =.103, irrigation treatment x fruit type =.185 and N treatment x fruit type =.100).

Trial 6: Severity of irrigation deficits

In Trial 6, we see the reverse trend to Trial 1 for irrigation treatments. Tagged shoots on average in the deficit treatments had more, rather than less, flush growth than the control treatment, although differences between means were not significantly different at the 95% confidence level (Table 8). If this means more competition between flush and fruit in the drier treatments, this conflicts with our findings that the deficit treatments had less granulation. Again, we suggest that total water has more direct influence on granulation than flush competition.

Table 8 Trial 6: Mean maximum length and number of spring flush shoots and mean granulation rating on tagged twigs by treatment 2021/22–2022/23

	2021/22			2022/23			
	Max. flush length (mm)	**Mean no. of shoots**	**Mean granulation rating**	**Max. flush length (mm)**	**Mean no. of shoots**	**Mean no. of fruit**	**Mean granulation rating**
Date measured	15/9/21	15/9/21	At harvest	11/10/22	11/10/21	11/10/22	At harvest
1. Little stress	30.3	1.3	2.41	34.4	1.7	10.3	2.30
2. Medium stress	30.4	1.7	1.76	31.4	1.7	9.8	1.87
3. Significant stress	37.2	1.8	1.76	40.1	2.1	8.9	1.71
P	*.571*	*.111*	*.385*	*.244*	*.107*	*.735*	*.466*
ese	*5.14*	*0.1558*	*0.363*	*3.47*	*0.1362*	*1.302*	*0.338*

Fruit on tags were not counted on 15/9/21. Treatment means within one group marked with the same letter or with no letters were not significantly different at the 95% confidence level.

We recorded inflorescence type of fruit in 2022/23: there were 73 fruit remaining on tags at maturity. The proportion of leafy inflorescences did not vary by irrigation treatment (*P*=.843). There was no significant difference or discernible pattern in granulation of tagged fruit by inflorescence type (*P*= .712), nor by irrigation treatment (*P*=0.199). There was no interaction (*P*=.731).

Trials of flush manipulation strategies

Trial 2: Flush manipulation strategies

Granulation results for all treatments are shown in Table 10 and are outlined below, in product or treatment groups, *i.e*. foliar nitrogen (N), paclobutrazol, gibberellic acid, prohexadione calcium and girdling. Of all the treatments, only the paclobutrazol treatments showed some promise in terms of reducing flush growth and granulation, but results were patchy.

Foliar N

In 2019/20, the foliar N treatment had no effect on granulation, whether singly or in combination with gibberellic acid or girdling treatments (Table 10). We did not continue with these treatments in the following years of the trial.

Paclobutrazol

In 2020/21 and 2021/22, the second and third years of treatment, only the 'AuStar foliar x 2' treatment showed significantly less growth in flush length than the control (Table 9). Growth in the 'AuStar foliar x 1' (the earlier date) was only significantly less in 2021/22. The 'Late Austar foliar x 1' treatment and the 'AuStar collar drench' were less successful in retarding flush growth.

However, granulation results for these treatments were less consistent (Table 10). There seemed to be some reduction in granulation from the AuStar treatments in low crop load years (2019/20 and 2021/22), but in 2019/20 means were not significantly different from the control at the 95% confidence level. In 2021/22, the effect of the 'AuStar foliar x 1', the 'Late AuStar foliar x1' and the 'AuStar collar drench treatments' were to shift fruit from the 'unacceptable' category into the 'crunchy' category – the proportion of 'good' fruit remained similar to the control.

Table 9 Trial 2: Mean number and mean maximum length of spring flush shoots on tagged twigs by treatment 2019/20–2021/22

	Mean maximum spring shoot length (mm)			**Mean no. of spring shoots**		
Year	**2019/20**	**2020/21**	**2021/22**	**2019/20**	**2020/21**	**2021/22**
Date measured	25/09/2019	25/09/2020	20/09/2021	25/09/2019	25/09/2020	20/09/2021
Control	56.6	50.2 bcd	68.4 bcd	4.7	2.9	2.7
ProGibb	70.2	63.8 d	72.4 cd	5.1	2.3	2.8
AuStar foliar x 1	55.1	37.0 ab	46.7 a	4.3	2.5	2.9
AuStar foliar x 2	46.9	22.9 a	44.8 a	3.3	2.3	2.8
Late Austar foliar x 1	na	55.0 cd	54.6 ab	na	3.4	3.3
AuStar collar drench	na	38.0 ab	59.3 abc	na	2.3	3
Regalis+ x 1	74.2	43.2 bc	81.8 d	4.2	2.4	3
Regalis+ x 2	70.6	39.2 abc	74.9 cd	5	2.6	3.1
P	*0.185*	*<.001*	*<.001*	*0.304*	*0.208*	*0.598*
ese	*8.4*	*5.7*	*5.88*	*0.6*	*0.33*	*0.2*

Table 10 Trial 2: Mean granulation rating, percentage fruit in main classes, fruit diameter and crop load rating by treatment 2018/19 – 2021/22

Treatment		**Mean crop load rating**	**Mean diameter (mm)**	**Mean granulation rating**	**Mean % rated ≤1.5 'good'**	**Mean % rated 2-2.5 'crunchy'**	**Mean % rated ≥3 'unacceptable)**
			2018/19				
Control		4.9	60.8	1.76	53.9%	35.3%	10.8%
ProGibb	*	4.8	60.5	1.51	69.7%	23.1%	7.2%
ProGibb plus foliar N	*	4.7	59.7	1.35	74.4%	22.2%	3.3%
Foliar N	*	4.9	59.9	1.58	64.7%	25.0%	10.3%
AuStar x 1	*	4.9	59.2	1.64	63.3%	27.5%	9.2%
Girdling *	Aug	4.8	59.2	1.28	72.3%	22.5%	5.2%
	Sep	4.8	60.0	1.56	64.6%	24.4%	11.0%
	Oct	4.9	60.2	1.31	72.9%	21.4%	5.7%
Girdling plus foliar N *	Aug	4.8	59.5	1.35	70.4%	23.7%	5.9%
	Sep	4.8	60.1	1.40	69.8%	24.9%	5.2%
	Oct	4.6	59.2	1.34	70.3%	23.7%	6.0%
P		*.431*	*.552*	*.175*	*.267*	*.259*	*.658*

Treatment		Mean crop load rating	Mean diameter (mm)	Mean granulation rating	Mean % rated ≤1.5 ‘good’	Mean % rated 2-2.5 ‘crunchy’	Mean % rated ≥3 ‘unacceptable)
ese		*0.1*	*0.5*	*0.13*	*5.2%*	*3.5%*	*2.8%*
			2019/20				
Control		2.3 c	64.0	1.96 bc	52.1% bcd	25.6% a	22.4% abc
ProGibb	**	1.8 ab	62.0	1.89 ab	48.9% abc	39.4% b	11.7% ab
AuStar x 1	**	2.3 bc	62.4	1.54 a	68.3% d	22.8% a	8.9% a
AuStar x 2	*	1.6 a	61.8	1.81 ab	57.2% cd	30.6% ab	12.2% ab
Regalis+ x 1	*	2.1 abc	63.0	2.26 c	34.4% a	30.6% ab	35.0% c
Regalis+ x 2	*	1.6 a	62.7	2.25 c	33.6% a	38.9% b	27.5% bc
No treatment (girdled 2018)		2.1 abc	62.2	1.94 bc	50.0% abc	28.5% ab	21.6% abc
No treatment (girdled plus foliar N 2018)		1.9 abc	61.6	2.25 c	36.2% ab	30.3% ab	33.5% c
P		*.044*	*.291*	*.002*	*.001*	*.049*	*.013*
ese		*0.1*	*0.7*	*0.12*	*5.8%*	*3.9%*	*5.7%*
			2020/21				
Control		2.8	60.8	0.92	98.3%	1.4%	0.3%
ProGibb	***	2.7	61.5	0.95	97.2%	2.8%	0.0%
AuStar foliar x 1	***	3.2	61.2	0.96	98.3%	1.7%	0.0%
AuStar foliar x 2	**	2.9	60.7	1.10	91.4%	6.9%	1.7%
Late Austar foliar x 1	*	2.8	61.4	1.03	97.1%	2.5%	0.4%
AuStar collar drench	*	2.7	60.3	1.02	95.8%	4.2%	0.0%
Regalis+ x 1	**	2.9	61.1	1.00	96.9%	2.2%	0.8%
Regalis+ x 2	**	2.7	61.0	1.02	95.8%	4.2%	0.0%
P		*.107*	*.890*	*.326*	*.212*	*.257*	*.054*
ese		*0.1*	*0.6*	*0.05*	*1.8%*	*1.6%*	*0.4%*
			2021/22				
Control		0.8 ab	63.4 c	3.02	14.0%	25.1% a	60.9%
ProGibb	****	1.3 bcd	62.9 c	2.97	12.5%	27.4% ab	60.1%
AuStar foliar x 1	****	1.5 cd	61.9 bc	2.76	15.9%	33.1% bc	50.9%
AuStar foliar x 2	***	1.7 d	59.2 a	2.81	14.1%	32.0% ab	53.8%
Late Austar foliar x 1	**	1.5 cd	59.4 a	2.55	20.2%	40.3% c	39.5%
AuStar collar drench	**	1.5 d	60.6 ab	2.76	18.0%	34.7% bc	47.4%
Regalis+ x 1	***	1.0 abc	62.4 c	3.00	14.2%	24.9% a	60.9%
Regalis+ x 2	***	0.6 a	62.4 c	3.15	4.8%	32.7% abc	62.5%
P		*.002*	*<.001*	*.165*	*.437*	*.004*	*.126*
ese		*0.2*	*0.5*	*0.15*	*4.5%*	*2.8%*	*6.2%*

*Treatment means within one group marked with the same letter or with no letters were not significantly different at the 95% confidence level. * 1st year of application ** 2nd year of application *** 3rd year of application **** 4th year of application*

Giberellic acid

The GA application (ProGibb) appeared to increase flush growth compared to all other treatments in 2018/19, 2019/20 and 2020/21, although treatment means were not significantly different from the control at the 95% confidence level (Table 10).

Figure 7, Figure 8 and Figure 9 show patterns for the 'ProGibb' treatment, the 'Austar foliar x 2' treatment and the 'control' for 2019/20, 2020/21 and 2021/22. Flush growth in the GA (ProGibb) treatment was earlier in 2019/20 and 2020/21 (only significant in 2020/21) but not in 2021/22, a very low crop load year.

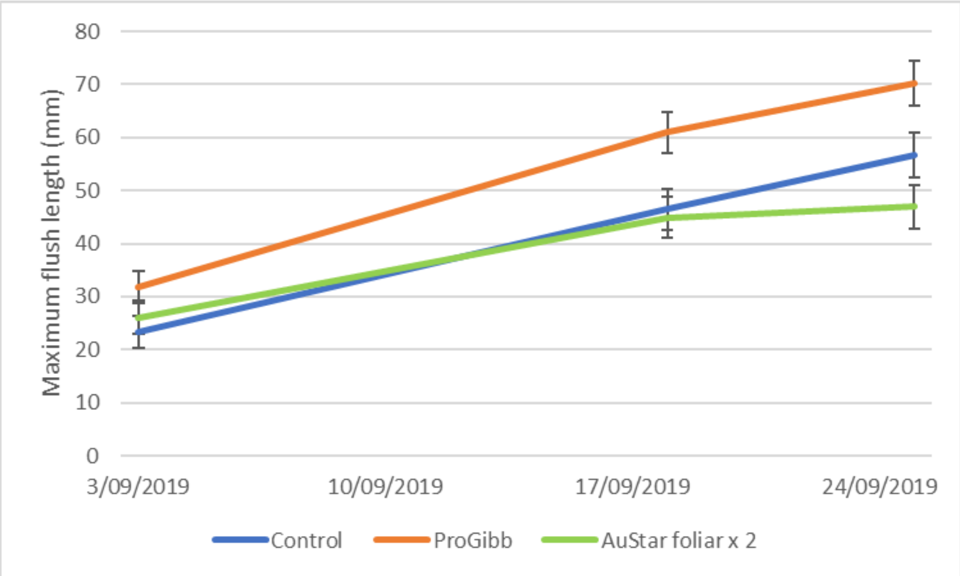

Figure 7 Trial 2: Timing of maximum flush growth by selected treatment 2019/20. There were no significant treatment differences at any date (P= 0.693, 0.194, 0.185 respectively). Error bars show estimated standard errors of the means.

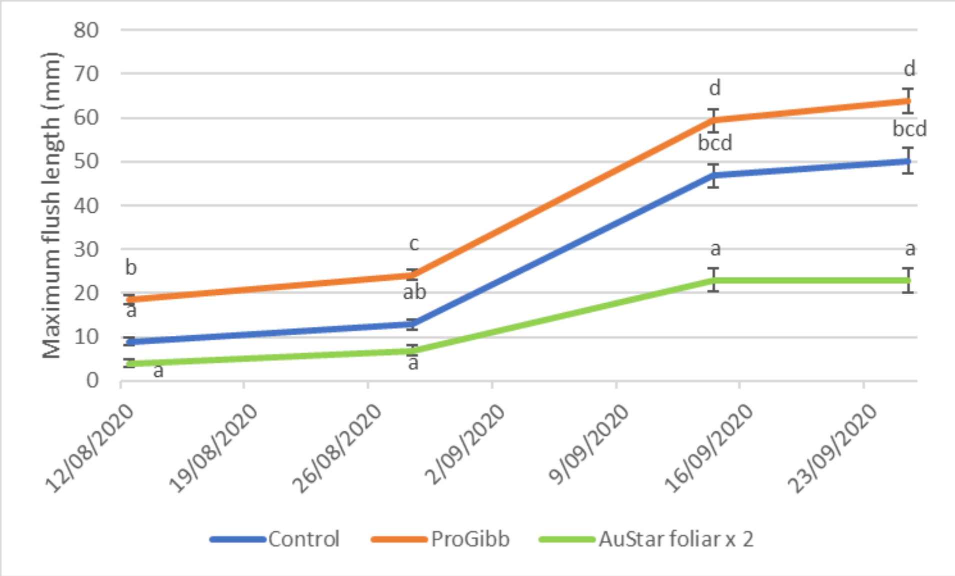

Figure 8 Trial 2: Timing of maximum flush growth by selected treatment 2020/21. Treatment means on the same date marked with the same letter or with no letters were not significantly different at the 95% confidence level. Error bars show estimated standard errors of the means.

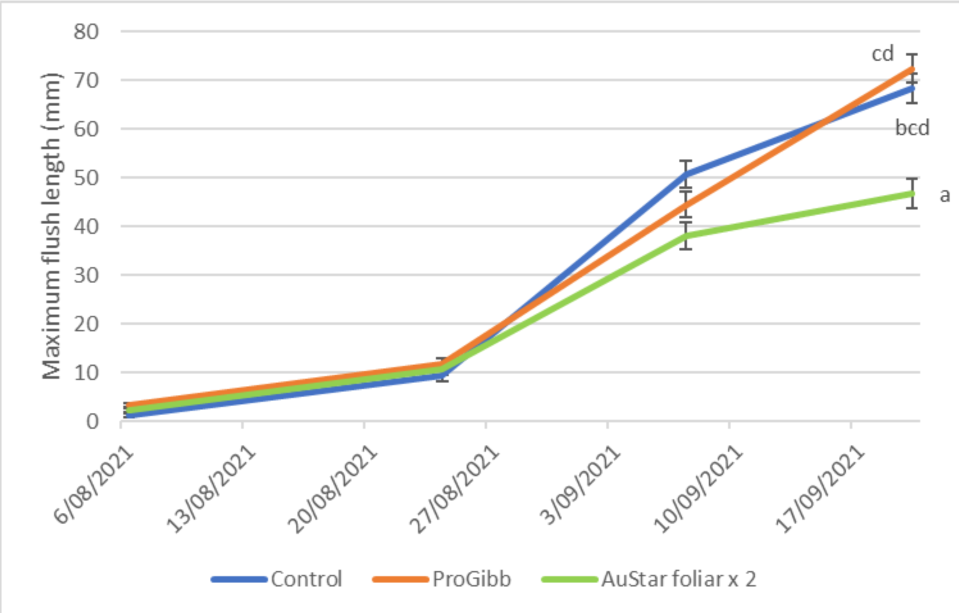

Figure 9 Trial 2: Timing of maximum flush growth by selected treatment 2021/22. Treatment means on the same date marked with the same letter or with no letters were not significantly different at the 95% confidence level. Error bars show estimated standard errors of the means.

In 2020/21 new growth for the GA treatment was more vegetative than other treatments, that is, fewer shoots were mixed shoots or 'leafy' inflorescences, with lower early fruit set (Table 11). This was not evident in 2021/22 as the flush was strongly vegetative and fruit set very low for all treatments. A high proportion of fruit on tagged twigs that remained to harvest were from leafy inflorescences (Table 11).

These trends -- earlier, longer and more vegetative growth -- had little effect on granulation, despite our findings that early flush growth can be detrimental in a low crop load year (see Section 'Individual twig data: flush and granulation'). In 2019/20, a low crop load year, the GA treatment had a higher proportion of 'crunchy' fruit than the control (39% compared to 26%, P=.04) but there was no significant difference in the proportion of ‘good’ fruit or in mean granulation rating (P=.175). In that year, the crop load was rated as lower than the control (P=.044).

Table 11 Trial 2: Percentage of mixed shoots and fruit set on tagged twigs in 2020/21 and 2021/22

	2020/21			**2021/22**		
	Mean % of flush shoots that were leafy inflorescen ces	**Mean no. of fruit per tag**	**Percent tagged fruit on leafy inflorescen ces**	**Mean % of flush shoots that were leafy inflorescen ces**	**Mean no. of fruit per tag**	**Percent tagged fruit on leafy inflorescen eces[1]**
Date measured	14/9/2020	8/10/2020	At harvest	20/9/2021	5/10/2021	At harvest
Control ***	89% b	4.3 bc	58%	9%	0.27 ab	71%
GA foliar ***	68% a	1.5 a	80%	5%	0.13 ab	100%
AuStar x 1 ***	91% b	4.7 bcd	39%	7%	0.21 ab	50%
AuStar x 2 **	97% b	6.7 e	23%	12%	0.90 c	37%
late AuStar foliar*	91% b	5.9 de	46%	9%	0.48 bc	57%
AuStar collar drench *	91% b	3.8 b	25%	8%	0.38 ab	25%
Regalis x 1 **	92% b	4.4 bcd	22%	9%	0.23 ab	86%
Regalis x 2 **	94% b	5.5 cde	11%	1%	0.02 a	100%
P	*.031*	*<.001*	*.069*	*.336*	*.014*	*na[1]*
ese	*5%*	*0.51*	*15%*	*3%*	*.16*	

Treatment means within one group marked with the same letter or with no letters were not significantly different at the 95% confidence level. [1] Simple means with low confidence: zero fruit retained on tagged twigs for many treatment/replicates meant insufficient data for analysis of variance.

Prohexadione calcium

The prohexadione calcium (RegalisPlus) treatments showed little potential to reduce granulation. In 2019/20, the ‘RegalisPlus x 2’ treatment had a higher percentage of ‘crunchy’ fruit than the control (38.9% compared to 25.6%, P=.049), but mean granulation was not significantly different. In 2020/21 and 2021/22 there were no treatment differences.

Girdling

In 2018/19, comparing limbs that were girdled with ungirdled limbs showed no significant differences in mean percentage granulation (Table 12). However, the mean proportion of fruit rated ≥2.5 on girdled branches was about 5% higher than ungirdled branches, that is, girdling had a slight detrimental effect.

Factors such as date of girdling and foliar application of N on the same date as girdling made no significant differences and there were no significant interactions between these factors (Table 12).

Table 12 Trial 2: Granulation by girdling treatment, foliar N application and date girdled 2018/19

Treatment		Mean granulation rating	Mean proportion of fruit rated ≥2.5
Girdled branch?	Yes	1.41	18.6% b
	No	1.32	13.8% a
		P =.179	*P=.027*
Foliar N on tree?	Yes	1.37	16.3%
	No	1.36	16.1%
		P=.962	*P=.952*
Date girdled	2/08/2018	1.34	15.1%
	7/09/2018[1]	1.42	18.5%
	10/10/2018	1.32	15.0%
		P=.469	*P=.325*

Treatment means within one group marked with the same letter or with no letters were not significantly different at the 95% confidence level. [1] The higher results may be due to substantial pre-existing watershoot growth from horizontal branches on nine branches, both girdled and ungirdled, on two of the trees.

Trial 4: Paclobutrazol applications

In the first year of the trial, there was no significant effect of treatments on flush. In the second year, 2020/21, the flush data from tagged twigs suggested the earlier of the two treatment dates -- both drench and foliar applications – was effective in reducing flush growth compared to the control, although this may have been due wholly or in part to later picking of the control fruit in the year before (Table 13). However, in 2021/22 and 2022/23, this pattern seems to be reversed, with the drench treatments having longer flush growth than the control, although overall flush was much lighter than previous years due to a heavy crop load. Overall, flush growth appears to be mostly determined by biennial bearing patterns rather than treatments.

There was no difference in fruit set between treatments (Table 13).

Table 13 Trial 4: Mean maximum new spring shoot length and fruit set on tagged twigs by treatment 2020/21–2022/23

	2020/21		**2021/22**		**2022/23***	
	Mean max flush (mm)	**Fruit set**	**Mean max flush (mm)**	**Fruit set**	**Mean max flush (mm)**	**Fruit set**
Date of measurement	1/10/2020	1/10/2020	20/9/2021	5/10/2021	14/9/2022	27/10/2022
1. Drench: first date	25.8	4.1 a	38.2 ab	1.2	19.9 b	1.2
2. Drench: second date	25.8	3.8 a	47.3 bc	0.9	12.2 ab	1.5
3. Foliar	20.8	6.8 b	33.8 a	1.4	7.2 a	1.3
4. Control	21.9	5.4 ab	58.3 c	0.5	8.5 a	1.4
P	*.777*	*.007*	*.003*	*.235*	*.013*	*.649*
ese	*4.3*	*0.6*	*3.9*	*0.3*	*2.64*	*0.2*

*Treatment means within one group marked with the same letter or with no letters were not significantly different at the 95% confidence level. * excludes two sick trees*

In 2020/21 there was negligible granulation at the trial (Table 14). In 2021/22, all paclobutrazol treatments had lower mean granulation ratings and lower proportions of 'unacceptable' fruit than the control, although differences between means were not significant at the 95% confidence level in either year. 'Control' trees had lower crop loads (although differences between drench treatment means and the control were not significant, *P*=.020) and larger fruit (*P*=.007). As noted above, later picking of the control trees at the end of the 2020/21 season may have influenced this result.

In 2022/23, a high crop load year with little granulation overall, there were no significant differences between treatments in mean granulation rating or mean % of 'unacceptable' fruit. However, there were less 'crunchy' fruit in the two drench treatments, although the difference between means was only significant for the earlier drench.

Table 14 Trial 4: Mean granulation rating, percentage fruit in main classes, fruit diameter and crop load rating by treatment 2020/21–2022/23.

	Mean crop load rating[1]	Mean fruit diameter (mm)	Mean granulation rating	Mean % rated ≤1.5 'good'	Mean % rated 2-2.5 'crunchy'	Mean % of fruit rated ≥3 'unacceptable'
			2020/21			
1. Drench: 1st date	2.2 a	56.4	0.51	100%	0%	0%
2. Drench: 2nd date	3.1 c	56.6	0.49	99.4%	0.6%	0%
3. Foliar	2.6 b	54.9	0.48	99.4%	0.6%	0%
4. Control	2.9 bc	55.6	0.57	99.4%	0%	0.6%
P	*<.001*	*0.12*	*0.744*	*.822*	*0.6*	*0.412*
ese	*0.08*	*0.52*	*0.062*	*0.6%*	*0.5%*	*0.3%*
			2021/22			
1. Drench: 1st date	2.7 ab	63.3 a	1.26	87.5%	10.0%	2.5%
2. Drench: 2nd date	2.7 ab	62.7 a	1.60	68.4%	22.1%	9.5%
3. Foliar	3.4 b	64.0 a	1.42	76.3%	15.6%	8.1%
4. Control	2.0 a	66.0 b	1.91	55.0%	24.4%	20.6%
P	*0.02*	*0.007*	*0.144*	*.096*	*0.228*	*0.207*
ese	*0.28*	*0.61*	*.198*	*8.8%*	*5.20%*	*5.9%*
			2022/23[2]			
1. Drench: 1st date	4.5	57.7	1.11	94.4% b	3.8% a	1.9%
2. Drench: 2nd date	4.3	57.4	1.10	90.6% b	7.5% ab	1.9%
3. Foliar	4.2	57.5	1.34	79.2% a	18.2% c	2.7%
4. Control	4.7	58.3	1.12	87.7% ab	12.4% bc	0%
P	*.368*	*.767*	*.085*	*.038*	*.01*	*.359*
ese	*0.19*	*0.67*	*0.074*	*3.49%*	*2.79%*	*1.55%*

[1] Crop load ratings are not directly comparable between years. Only 4 of 8 replicates were rated in 2021/22.

[2] Excludes two sick trees.

Treatment means within one group marked with the same letter or with no letters were not significantly different at the 95% confidence level.

Trial 5: Late pruning trial

Fruit were larger on average in the late pruned treatment (*P*=.018) and slightly more granulated (*P*=.045) (Table 15). The late pruned trees had denser and darker canopies during early fruit set, particularly in the centre of the trees, with many water shoots. It is possible this growth competed with fruit for resources, or that the denser canopy reduced light for photosynthesis. In addition, after pruning, the crop load on the trees was lower, a known risk for granulation.

Table 15 Trial 5: Mean granulation rating, percentage fruit in main classes and fruit diameter by treatment 2020/21.

Treatment	**Mean fruit diameter (mm)**	**Mean % of fruit ≥55mm**	**Mean granulation rating**	**Mean % of fruit rated ≤1.5 ('good')**	**Mean % of fruit rated 2-2.5 ('crunchy')**	**Mean % of fruit rated ≥3 ('unacceptable')**
Control	60.2	89%	0.79	96%	5%	0%
Late pruned	63.1	95%	0.96	95%	5%	1%
P	*.018*	*.051*	*.045*	*.743*	*.868*	*.343*

Discussion and conclusions

Does competition between flush and fruit set explain granulation?

Overall, our results support the hypothesis that flush growth and granulation are associated, although the effect is not necessarily a simple causal one as both are affected by crop load. Water availability appears to be a more influential factor.

Flush growth on individual twigs appears to help explain some of the variation of individual fruit across the tree, possibly because of competition between flush growth and early fruit development, but also, conversely, through 'nearby leaves' feeding the fruit in later fruit development.

The timing of flush growth also appears to be a factor, with differing effects depending on the extent of carbohydrate and nutrient reserves and crop load. Later flush appears to be detrimental in a high crop load year when reserves 'run out' and demand is high, but beneficial in a low crop year, when the many new leaves have expanded and begin to supply nearby fruit. In our trials, earlier flush was detrimental only in low crop years, presumably because reserves are low overall.

Whether the fruit set on leafy or leafless inflorescences also seems to have a small effect. The leaves on leafy inflorescences, once expanded, may be supporting nearby fruit. Such leaves, being younger than those feeding 'leafless' inflorescences, may also be more photoefficient. There is some evidence from our measurement of tagged fruit that this beneficial effect is dependent on sufficient supplies of N.

While these flush factors have an effect, our trials suggest other factors are 'in play' and are more important. Crop load has a strong influence both on flush and on granulation, and we suggest it also operates on fruit development directly or through other factors (not studied here), such as influencing storage of carbohydrates, improving photosynthetic efficiency (Palmer et al., 1997; Wünsche et al., 2000), and through competition for available moisture. The results in our irrigation trials suggest also that water availability is more important than flush/fruit competition.

Is the type of inflorescence a major factor in granulation, as it is for size and fruit quality in some other varieties of citrus?

The effect of inflorescence type ('leafy' or 'leafless') was not consistent from trial to trial, nor showed any pattern between low and high crop load years. The proportion of inflorescence type is largely determined by crop load, and our irrigation and some plant growth regular treatments mostly had only small or no effects on the proportion of leafy inflorescences. The higher irrigation treatments in Trial 1 had a higher proportion of leafy inflorescences, but in some years had higher granulation on average. Spraying GA in winter increased the proportion of leafy inflorescences, but this had no discernible effect on granulation.

We suggest that, as Imperials are a fast-growing fruit, fruit quality is more dependent on getting a good 'kick start' in early fruit development from carbohydrate and nutrient reserves, than on 'current' photosynthesis later in fruit development.

Can we influence flush extent and timing and thus reduce granulation?

Our attempts to influence flush growth in Trials 2 and 4 indicate this is not a simple task. Flowering intensity, and the resulting crop load, seems to be a much stronger driver than any of our treatments.

Paclobutrazol treatments appear to have some promise in low crop load years although the data is inconsistent and the scale of improvements is relatively small. We tried several applications and timings, but only used one application rate (the recommended rate for international usage). A trial which test a ranged of increased application rates as well as variable timings may be useful. Future trials would need to cover several years as crop load influence appears to be high. Note that there are currently no registered paclobutrazol products for citrus in Australia.

An application of GA (ProGibb) in early winter was effective in inducing early flush which we hypothesised would increase granulation. However, there didn't appear to be any clear negative, or positive, effect. In 2019/20 it seemed to increase the proportion of 'crunchy fruit', but this was at the expense of the 'unacceptable' fruit rather than reducing the proportion of 'good' fruit i.e. a 'good' effect.

In summary, the most effective strategy for growers looking for the right balance between flush and fruit is to ensure they manage crop load by thinning and not picking late.

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Appendix 4: 'Late action' trials

Summary

We conducted three single-year trials to explore whether 'late action' after a wet spring could reduce granulation. In these trials we used additional sprinklers to simulate higher rainfall in spring and/or in summer. The summer additional irrigation was to assess whether or not late rainfall is associated with higher granulation.

Trial 7, in 2021/22, included treatments of broadcast 'Quick-N' in November, four foliar sprays of nitrogen (N) weekly from early November, a foliar spray of gibberellic acid (GA) in early November, and a 'fruit retention' foliar spray mixture of GA + 2,4-D + calcium nitrate ($Ca(NO_3)_2$) in early November. We intended to reduce irrigation after the 'wet spring' for all treatments except the 'Quick-N', but this plan was undermined by high rainfall. None of the irrigation or foliar treatments reduced granulation, and the 'fruit retention' spray mixture increased granulation. The high rainfall meant that we could not discern the effects of late reduction in irrigation.

In Trial 8, in 2022/23, we tried foliar treatments in a factorial design with four irrigation treatments. The irrigation treatments were the 'control' (standard farm practice) in both spring and summer, control irrigation in spring with a wet summer, wet spring with control irrigation in summer, and wet spring with deficit irrigation in summer. The foliar treatments were a control (no additional sprays), a GA spray in late flowering (admittedly not a 'late' action), and three or four sprays of boron and calcium in Stage I of fruit development (September to November). Of these treatments, only the GA spray had an effect, and that was beneficial, reducing 'crunchy' fruit from 18.1% in the control to 10.7% in the GA sample (P=.023). The lack of effect from irrigation treatments may be due to frequent rainfall, with ~616 mm of rainfall received in the treatment period. Nearly all treatments, whether 'control', 'wet' or 'dry', received total water close to or above evapotranspiration (ETo) levels in spring. In summer, we were able to reduce total water from 90% of ETo (in the control) to 65% in the 'deficit' treatment, but this did not produce any discernible effects on granulation.

In Trial 9, in 2022/23, we tested fortnightly sprays (total of four) of boron, calcium, boron and calcium, and potassium at an even later time in the growing season (December and January). These treatments were overlaid on two irrigation treatments, a 'control' and a late increase in irrigation (in February and March). There were no treatment effects in this trial. Note that crop load was high and granulation overall was very low so conditions were not ideal.

In summary, while these trials were short term and conducted in less-than-ideal climatic conditions, we received very little support for the hypothesis that late action in terms of irrigation or nutrient foliar sprays would be beneficial. The one beneficial treatment was the GA sprayed in late petal fall, which increased crop load, a known factor in influencing granulation levels. Growers should consider use of GA if expecting a low crop load year.

Introduction

As the link between wet seasons, irrigation and high levels of granulation emerged during this project, the Project Advisory Group requested research into strategies that could be applied 'late' *i.e.* after a wet spring.

To date, the weight of evidence in our work suggests that the early stages of fruit development are more important. Some published literature supports the negative effect of late summer or early autumn rainfall on granulation with a later-developing variety, navel oranges (Ritenour et al., 2004; Van Noort, 1969). However, in the nursery trial in CT04002, water deficits in Stage II, Stage III or both Stages II and III did not reduce granulation as effectively as reductions in Stage I or all stages (Hofman, 2010). From a management perspective, applications of plant growth regulators containing auxin and gibberellic acid in Stage II have been reported to reduce granulation (Chakrawar & Singh, 1978; Kaur et al., 1990; Kaur et al., 1991) but our trials in CT04002 did not find any effect from Stage II applications of several auxin-based products or GA (Hofman 2010).

Nevertheless, because of its potential importance to on-farm management of granulation, we ran three one-year trials to test various 'late action' strategies in this project.

This focused on foliar applications of nutrients, nitrogen, boron, calcium, and potassium, and reducing irrigation. We also included treatments of additional irrigation in summer to assess whether or not late rainfall is associated with higher granulation. We incorporated some treatments to reduce fruit drop as an indirect strategy to increase crop load, using GA or GA plus 2,4-D. De Lima and Davies (1984) found 2,4-D at 10-20mg/L reduced drop. Bujanda (1984) found that GA plus 2,4-D reduced drop (summarised in El-Otmani et al., 2000). However, Rabe and Rensburg (1996) found that GA sprays on Ellendale tangors at three weeks after 100% petal fall – first week of October (20 mg/L or at the end of first fruit drop (end October) tended to delay fruit drop but did not increase final yield. We also used GA (applied late in the bloom period) with the aim of increasing initial fruit set (late bloom).

Methodologies

Trial 7: First 'late treatments' trial

This trial, in Wallaville, Central Queensland, was a randomised block design with eight treatments and five replicates. Plots had five trees with the central three trees used for data collection. Trees were Imperials on 'Benton' rootstock, closely planted in 2016 at 2.5 m spacing in rows 7 m apart.

Treatments, including total water applied, are described in Table 1. We simulated a wet spring with extra sprinklers and then applied six 'late management' treatments. These included additional nitrogen applications, broadcast as 'Quick-N' for faster uptake than standard urea (Treatment 1b), or as four foliar sprays (Treatment 1c). 'Quick-N' (Campbells Fertilisers Australia) is ammonium sulphate nitrate in a soluble granular form which claims to provide readily available nitrate nitrogen for rapid uptake, followed by sustained ammonium availability. The aim of the additional N treatments was to support leaf functioning and summer flush development.

Two treatments were attempts to reduce fruit drop and thus 'indirectly' affect granulation through crop load. These were a foliar spray of gibberellic acid (GA) (Treatment 1e) and a 'fruit retention' mixture of GA, 'StopDrop' and calcium nitrate (Treatment 1d). GA was applied at 15ppm using ProGibbSG. We applied 2,4-D at 10 ppm as 'StopDrop' (Kendon).

For most of these treatments we applied an irrigation deficit in early summer (December to January inclusive) by 'kinking off' sprinklers, intending to withhold irrigation until the soil dried out. The exception was Treatment 1b where we considered that irrigation was needed for uptake of the Quick-N. In the event, rainfall meant soil did not dry out very much.

In Treatment 2, we applied extra water in February and March to test the hypothesis that late summer rainfall (simulated in this trial) increases granulation.

Table 1 Trial 7: Treatment applications and summary of estimated total water received (rain plus irrigation) as mm and as a percentage of evapotranspiration (ETo) by treatment and period 2021/22

			29/9/2021 – 3/12/2021		**4/12/2021-25/1/2022**		**26/1/2022 – 25/3/2022**	
Tmnt	**Irrigation component**	**Additional applications**	**Total water (mm)**	**% of ETo**	**Total water (mm)**	**% of ETo**	**Total water (mm)**	**% of ETo**
1a	Wet spring + control Dec-Jan		402	110%	151	51%	407	156%
1b	Wet spring + control Dec-Jan	770g g/tree of Quick-N (=200gN) broadcast on 9/11/2021	402	110%	151	51%	407	156%
1c	Wet spring+ dry Dec-Jan	4 foliar sprays of N (Lo-Bi at 1.5% wv; applied on 8/11, 16/11, 3/12, 13/12/2021)	402	110%	109	37%	407	156%
1d	Wet spring + dry Dec-Jan	1 foliar spray of gibberellic acid (15ppm) + StopDrop (10ppm) a.i. 2-4,D) + $Ca(NO_3)_2$ (1%), applied on 7/11/2021	402	110%	109	37%	407	156%
1e	Wet spring + dry Dec-Jan	1 foliar spray of gibberellic acid (15 ppm) + Lo-Bi (1.5% wv) applied on 8/11/2021	402	110%	109	37%	407	156%
1f	Wet spring + dry Dec-Jan		402	110%	109	37%	407	156%
2	Control spring + control Dec-Jan + wet Feb-Mar		362	99%	109	62%	526	202%
3	Control all season		362	99%	151	51%	407	156%

'Total water' = rain plus irrigation

November and December 2021 saw high rainfall in the area, making total water received in Treatment 1 very similar to the control treatment (Treatment 3), that is, 402 mm and 362 mm respectively. During late fruit development (February and March), rainfall was also significant; however, there was a bigger difference in total water received between Treatment 2 and the control (Treatment 3), that is, 526 mm and 407 mm, respectively.

Shoots were tagged on the GA treatments (1d and 1e) and the control (3) in order to investigate whether treatments reduced fruit drop.

Trial 8: Second 'late treatments' trial

This trial was in the same location as Trial 7, *i.e.* young Imperials on 'Benton' rootstock, closely planted closely planted in 2016 at 2.5 m spacing in rows 7 m apart. The design was factorial with irrigation as the main factor (four treatments) and **'**late action**'** as the second factor (four treatments) in three tree plots. 'Irrigation' plots were separated by three guard trees; 'late action' plots by one guard tree. There were four replicates.

The irrigation treatments were applied by adding two sprinklers per tree for 'wet' periods and kinking off sprinklers for 'deficit' periods. The four irrigation regimes were:

A. Control irrigation in spring (to end November)→ control irrigation in summer (Dec-harvest)
B. Control irrigation in spring→ simulated wet summer
C. Simulated wet spring→ control irrigation in summer
D. Simulated wet spring→ deficit irrigation in summer.

Table 2 summarises total water (rain plus irrigation) in these treatments. PhyTech installed dendrometers in Treatments 1 and 4 to monitor tree stress. Soil moisture tensiometers were installed in two replicates of the same two treatments and monitored at each site visit.

Table 2 Trial 8: Summary of estimated total water received (rain plus irrigation) in mm and as a percentage of evapotranspiration (ETo) by treatment and period 2022/23

	Spring (19/9-29/12/22)		**Summer (30/12/22-19/3/23)**		**Total (19/9/22-19/3/23)**	
Treatment	**Total water (mm)**	**Total water/ ETo**	**Total water (mm)**	**Total water/ ETo**	**Total (mm)**	**Total water/ ETo**
A Control spring→ control summer	452	94%	360	90%	812	92%
B Control spring→ wet summer	452	94%	583	145%	1034	117%
C Wet spring→ control summer	620	128%	360	90%	980	111%
D Wet spring→ dry summer	620	128%	261	65%	881	100%

'Total water' = rain plus irrigation

The 'late' treatments were all foliar treatments applied as follows (~ 2L per tree, applied with backpack sprayers):

1. Control (no applications)
2. 'GA in September': 20 ppm GA *i.e.* 1 g of ProGibbSG (Sumitomo) (soluble granules, a.i. 400 g/kg GA) per 20L of water plus 0.5% w/v of LoBi (low biuret urea) urea on 20/9/22. The pH was reduced to ~4.5 by the use of AgriBuffa which includes a wetter. The application date was 20/9/2022.
3. 'Boron Oct-Nov': 100 g/100 L of 'Solubor' with LoBi at 0.2% w/v on 28/9/2022, 12/10/2022 and 8/11/2022. 'Solubor' (disodium octoborate tetrahydrate) is 21% boron.
4. 'Calcium Oct-Nov': 2% w/v of calcium nitrate $Ca\ (NO_3)_2$ with LoBi at 0.2% w/v on 28/9/2022, 12/10/2022, 27/10/2022 and 9/11/2022.

We added the non-ionic wetter 'Spreadmax' at 20 ml/100 L to the B and Ca sprays.

We tagged four small branches on one tree per plot in Treatments 1 and 2 ('Control' and 'GA in September') (total of 16 trees per treatment) to assess fruit retention. Because of a heavy crop load, all trees in this trial were chemically thinned by the grower on 4/11/2022 using Ethrel. We counted fruit on the tagged branches on 16/11/2022, after this Ethrel spray.

Mean fruit diameter (n=25) at the time of spraying was as follows: 28/9/2022 = 5.1 mm, 12/10/22= 6.6 mm, 27/10/22 =10.1 mm, 8/11/2022= 16.0 mm.

Trial 9: 'Very late' treatments trial

In a third trial we tested even later application of four foliar treatments, that is, fortnightly in December and January. The trial was in Wallaville, Central Queensland, using mature trees on 'Benton' rootstocks at 7 x 3 m spacing. The design was a randomised block design of two irrigation treatments, and five nutrient application treatments, with five replicates. Plots were single-tree plots, separated by one or more guard trees.

There were two irrigation treatments, 'control' and 'late wet' *i.e.* additional late irrigation. The late irrigation was applied from 1/2/2023 to harvest on 11/4/2023. In this period, there was 499 mm of rainfall. The 'control' received 569mm of total water or 152% of ETo. The 'wet' treatments received 640mm of total water or 171% of ETo.

The foliar treatments were four applications (on 13/12/2022, 28-29/12/2022, 11/1/2023, 24/1/2023) of the following:

1. 'Boron': 100g/100L of Solubor
2. 'Calcium': 2% w/v of $Ca(NO_3)_2$
3. 'Boron + calcium': 100g/100L of Solubor plus 2% w/v of $Ca(NO_3)_2$
4. 'Potassium': 3% w/v of KNO_3
5. Control

Low biuret urea at 0.2% w/v and non-ionic wetter were added to all sprays.

Results

Trial 7: First 'late treatments' trial

There was a very high level of granulation in this trial but granulation did not differ between treatments with the exception of Treatment 1d, 'Wet spring + fruit retention foliar mixture', which had an adverse effect (more granulation)(Table 3). Treatments that applied additional water, either early (October-November) or late (February-March), had no effect, and neither did drying out the plots (to the extent that we were able) in December-January. The lack of effects from varying irrigation may be due to the overall high rainfall: we suspect that there was not enough differentiation between irrigation treatments (*i.e*. 110% v 99% of ETo in October-November, 51% v 37% in December-January, and 156% v 202% in February-March).

Table 3. Trial 7: Granulation and fruit size by treatment 2021/22

Treatment	Mean granulation rating	Mean % rated ≤1.5 'good'	Mean % rated 2-2.5 'crunchy'	Mean % rated ≥3 'unacceptable'	Mean fruit diameter (mm)
1a Wet spring + control irrigation	2.53 a	22.7% bc	35.7%	41.7% a	65.0
1b Wet spring + Quick-N	2.45 a	27.8% c	33.1%	39.1% a	64.4
1c Wet spring + foliar N	2.63 a	21.3% bc	33.0%	45.7% a	65.1
1d Wet spring + fruit retention foliar mixture	3.17 b	9.3% a	23.8%	66.8% b	65.1
1e Wet spring + GA + foliar N	2.69 a	16.0% ab	36.3%	47.7% a	64.9
1f Wet spring + deficit irrigation	2.51 a	26.7% c	32.7%	40.7% a	63.9
2 Wet late	2.65 a	23.3% bc	29.3%	47.3% a	64.8
3 Control	2.51 a	26.0% bc	31.0%	43.0% a	64.7
P value	*.009*	*.014*	*.403*	*.016*	*.659*
ese	*0.124*	*0.035*	*0.038*	*0.050*	*0.477*

Treatment means within one group marked with the same letter or with no letters were not significantly different at the 95% confidence level.

Treatment 1d. 'Wet spring + fruit retention foliar mixture', had a significantly higher mean rating than all other treatments (*P*=.009), due to fewer 'good' fruit (rated ≤ 1.5) and significantly more 'unacceptable' fruit (rated ≥ 3). This was not related to crop load – there was no drop on our tagged shoots in any of the measured treatments (1d, 1e or the control) (data not shown). In fact, at no stage after spraying did we observe dropped fruit under the trees for any of the eight treatments.

There does not seem to be a clear explanation for this singular treatment effect. Treatment 1e 'Wet spring + GA + foliar N' also had a lower proportion of 'good' fruit, although this was not significantly different from most other treatments (Table 3), so it is possible that the GA had a negative effect, exacerbated by the 2-4,D. At three small PGR trials in CT04002 (the first granulation project), GA sprays (one spray in late November or three sprays monthly from late November onwards) tended to slightly increase granulation, although treatment means were in all cases not significantly different from the control (Hofman 2010). It also seems likely that a spatial trend exacerbated this result, as two of the five plots for each of the two treatments were in the two most granulated rows.

The lack of late fruit drop for any treatment was unexpected. Our data for other trials show fruit drop during this period. For example, in Trial 3 in 2018/19 (high crop load year), fruit drop from late October to harvest in the control treatment was 50%. In 2019/20, a low crop load year, it was 37%. In Trial 1, the rate was 13.2% in 18/19, 68.6% in 19/20 and 30.9% in 20/21 (note the high rate even in a low crop year). Drop during this period is generally explained by competition between the growing fruit and root flushes but we suspect that moisture stress may also play a role. Moisture stress would have been minimal in 2021/22, possibly explaining the total lack of drop.

The additional late N applications, both foliar and broadcast, had no effect on granulation. This result aligns with the results of nutrient trials in CT04002 project (Hofman, 2010). In that project, applications of urea in Stage I of fruit development reduced granulation (where winter applications were inadequate) but applications of urea in Stage II had no effect. In that project, foliar N applications (LoBi urea) (with various timings, some earlier than our treatments in this trial and some with similar timings) sometimes reduced granulation, but gains were neither large nor consistent.

Trial 8: Second ‘late treatments’ trial

There were no significant differences in mean granulation measurements between irrigation treatments. We attribute this to rainfall making it difficult to reduce total water. This was a wet season with ~616 mm of rainfall received in the treatment period. Nearly all treatments, ‘control’, ‘wet’ or ‘dry’, thus received total water close to or above evapotranspiration levels in spring (Table 2). In the summer period, in treatment D, we were able to apply 65% of ETo in summer, compared to 90% for the control (A). The wet season, along with the youth of the trees, accounts for the average granulation rating of ~1.5 in this trial, which is higher than we would expect from the high crop load.

Of the foliar treatments, the ‘GA in September’ treatment (2) had the lowest granulation, with 10.7% of fruit rated ‘crunchy’ compared to 18.1% of fruit in the control treatment (1) (*P*=.023)(Table 4). Higher crop loads are likely to be the reason for this. On our tagged branches, there was higher fruit retention for the ‘GA in September’ treatment compared to the control: 5.2 compared to 4.1 fruit per cm^2 of branch cross-sectional area (*P*=.03). The lower mean rating for the ‘GA in September’ treatment appears to be partly due to a higher proportion of smaller fruit in the sample compared to the ‘control’ treatment (Figure 1). Note, however, that fruit of comparable size (if below ~60mm) also had less granulation on average in the ‘GA in September’ treatment than in the ‘control’ (Figure 1). This supports our hypothesis that crop load itself has a beneficial effect, distinct from the higher proportion of smaller fruit that can be expected from a high crop load.

Table 4. Trial 8: Mean crop load rating, granulation and fruit size by treatment 2022/23

Treatment	Mean crop load rating	Mean granulation rating	Mean % rated ≤1.5 ‘good’	Mean % rated 2-2.5 ‘crunchy’	Mean % rated ≥3 ‘unacceptable’	Mean fruit diameter (mm)
Irrigation treatments						
A Control spring→ control summer	7.1	1.39	84.8%	14.5%	0.7%	59.1
B Control spring→ wet summer	7.2	1.46	78.3%	20.3%	1.4%	59.0
C Wet spring→ control summer	7.3	1.39	85.6%	14.4%	0	58.7
D Wet spring→ dry summer	7.0	1.39	84.4%	15.0%	0.6%	59.3
P value	*.491*	*.723*	*.527*	*.546*	*.414*	*.728*
ese	*0.11*	*0.051*	*3.75%*	*3.29%*	*0.54%*	*0.41*
Foliar treatments					.	
1. Control	7.2	1.45 b	80.8% a	18.1% b	1.0%	59.6 b
2. GA in Sept	7.3	1.30 a	88.5% b	10.7% a	0.7%	57.7 a
3. Boron Oct-Nov	7.2	1.40 b	84.7% ab	14.9% ab	0.4%	59.3 b
4. Calcium Oct-Nov	7.0	1.47 b	79.1% a	20.4% b	0.5%	59.5 b
P value	*.272*	*<.001*	*.022*	*.023*	*.642*	*<.001*

Treatment	Mean crop load rating	Mean granulation rating	Mean % rated ≤1.5 'good'	Mean % rated 2-2.5 'crunchy'	Mean % rated ≥3 'unacceptable'	Mean fruit diameter (mm)
ese	*0.11*	*0.027*	*2.2%*	*2.22%*	*0.37%*	*0.25*
P interaction	*.608*	*.369*	*.397*	*.485*	*.221*	*.341*

Treatment means within one group marked with the same letter or with no letters were not significantly different at the 95% confidence level.

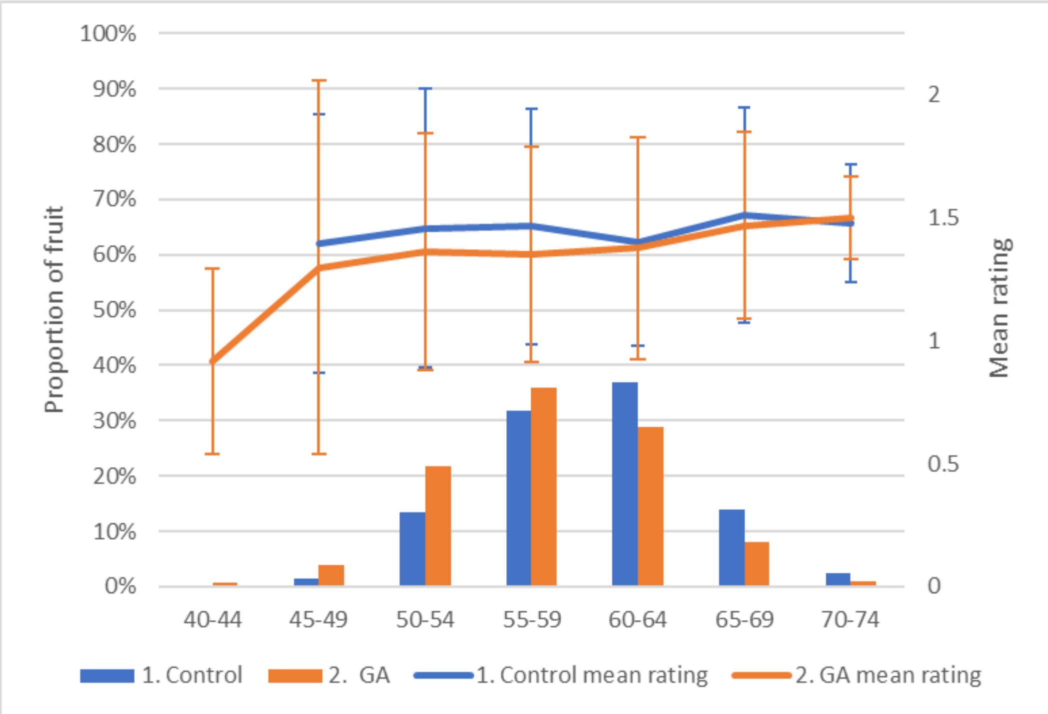

Figure 1 Trial 8: Proportion of fruit in size classes (columns) and mean granulation rating (lines) in classes for Treatment 1 'Control' and Treatment 2 'GA in September' in 2022/23. Error bars show standard deviations.

Trial 9: 'Very late' treatments trial

At this trial site, crop load was high and granulation on the site was very low, so not ideal for assessing treatment effects. There were no significant differences between mean granulation for irrigation treatments, or for foliar treatments (Table 5). The 'control' irrigation treatment seems to have performed slightly better than the 'wet' treatment in terms of the proportion of fruit that were 'good' or 'crunchy', but statistical confidence was low, with *P* values of 0.164 and 0.190, that is, 84% and 81% confidence, for the 'good' and 'crunchy' categories respectively. There was no significant interaction between irrigation and foliar treatments.

Table 5. Trial 9: Mean crop load rating, granulation and fruit size by treatment 2022/23

Treatment	Mean crop load rating	Mean granulation rating	Mean % rated ≤1.5 'good'	Mean % rated 2-2.5 'crunchy'	Mean % rated ≥3 'unacceptable'	Mean fruit diameter (mm)
Irrigation treatments						
Control	5.7	1.10	94.6%	5.4%	0.0%	60.4
Late wet	5.8	1.12	90.8%	9.0%	0.2%	60.2
P value	*0.663*	*0.850*	*0.164*	*0.190*	*0.324*	*0.588*
ese	0.161	0.0483	1.9%	1.9%	0.1%	0.233
Foliar treatments						
1. Control	5.9	1.07	95.5%	4.5%	0.0%	60.1
2. Boron	5.8	1.10	91.0%	9.0%	0.0%	60.7
3. Calcium	6.0	1.12	90.0%	10.0%	0.0%	60.2
4. Boron+ calcium	5.0	1.19	91.5%	8.0%	0.5%	59.9
5. Potassium	6.0	1.07	95.5%	4.5%	0.0%	60.7
P value	*0.06*	*0.796*	*0.557*	*0.582*	*0.420*	*0.416*
ese	*0.25*	*0.0764*	*3.0%*	*3.0%*	*0.2%*	*0.37*
P interaction of irrigation and foliar treatments	*0.749*	*0.623*	*0.877*	*0.904*	*0.420*	*0.890*

Treatment means within one group marked with the same letter or with no letters were not significantly different at the 95% confidence level.

Discussion and conclusions

Are there any effective late treatment strategies after a wet spring?

In summary, while our three trials were short term and conducted in less-than-ideal climatic conditions, we received very little support for the hypothesis that late action -- in terms of reduced irrigation, applications of foliar or broadcast nitrogen, or foliar sprays of boron, potassium or calcium -- would be beneficial. However, we were unable, because of rainfall, to completely dry out our trial blocks so this is a strategy that remains to be researched.

The one beneficial treatment that we have confidence in was gibberellic acid sprayed in late flowering (~90% petal fall), which increased crop load, a known factor in influencing granulation levels. Note that GA in late spring (November) was not beneficial in the year we tried it, as there was no fruit drop in any of our treatments after this date. While spraying at flowering is not a 'late' strategy, growers seeking to reduce granulation should consider use of GA if expecting a low crop or if flowering is poor.

Is late rainfall as detrimental as early rainfall?

While rainfall in 2021/22 and 2022/23 meant that the trial treatments that simulated additional late rainfall were not as differentiated from the control treatments as we would like, there was little indication that late rainfall is as detrimental as early rainfall. However, our hypothesis suggests that rainfall at any stage could exacerbate existing granulation, so we would advise growers to exercise irrigation restraint even at late stages in low crop load years or where there was high rainfall in spring or summer.

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Appendix 5: General methodology for all trials

Irrigation calculations

When calculating irrigation as estimated mm of rainfall, we have used the method followed by Phytech where mm = sprinkler output (L/hr)/ (row spacing (m) x sprinkler spacing (m)). Thus for a 70L/hr sprinkler system at a 7 x 3 spacing, mm per hour = 70/ (7 x 3) = 3.33 mm. This is a very conservative estimate as most sprinkler and root systems do not cover the interrow and are circular in radius.

Millimetres of rain and irrigation can be converted to ML per hectare by using the formula: ML/ha = mm/100.

The crop factor for citrus as calculated by the UN's Food and Agriculture Organisation is 0.65-0.67 for 70% canopy cover (FAO56).

Fruit sampling

The effects of treatments at all trials were assessed less than a week before the first commercial picking by the grower. Samples of 20 fruit were picked randomly from around the tree, five from each quadrant. Samples included fruit from both outside and inside the canopy, to a maximum of an arm's length within the canopy and height within the reach of the sampler from the ground. Where crop load was unevenly distributed around the tree, we adjusted sampling to better represent the whole crop.

Fruit were cut in half in the field along the equator and individually rated on an 11 point scale from 0 in 0.5 steps up to 5. Fruit rated 0 had no visible granulation, vesicles had thin translucent walls, and colour was deep orange. Fruit rated 5 were opaque and white, and when squeezed had no, or only a few millilitres, of extractable juice. Figure 1 provides an indicative example of ratings. This method allows large numbers of fruit to be quickly assessed but ratings are to some degree subjective. We tried to reduce bias in sampling wherever possible by ensuring harvest data sheets did not include treatment names. In addition, in trials where we had become familiar with treatment locations through repeated visits, we sampled rows in a blind and randomised pattern to reduce bias.

For trials with rows not planted north south (Trials 1, 2 and 4), we sampled 10 fruit from each of the north and south sides separately. These data are combined in this report, but note that in our trials the north side of the tree tended to flush and flower earlier, flush more strongly and to granulate more.


Figure 1 Indicative photo of a range of fruit with rating 5 at the top and 0 at the left. Photo: H Hofman

Fruit ratings and juice percentage

A sample of 300 fruit was collected from Trials 1 and 3 in April 2020, cut and a subsample selected when to cover as much of the range in ratings as possible. Very few '0' rated fruit were found in the sample, and no '5' rated fruit. The juice percentage and colour of each cut half of the subsample was measured. Juice percentage was calculated as ml of readily extractable juice (using a domestic juicer) per weight in grams of the half fruit. Flesh colour was assessed using a chromameter (Minolta Chroma Meter CR 400), taking three readings on each cut half. The bottom half of the fruit was used for both assessments.

Figure 2 shows the linear relationships and variability between granulation ratings and juice percentage. The mean values for juice percentage and the L value of fruit colour for each rating are shown in Table 1. The L value in the CIELAB or L*a*b* colour space is a measure of the luminosity or brightness of the light reflected from the fruit, or the perceptual lightness (a and b values relate to red, green, blue and yellow).

On the basis of this data, in this report we used the rating of ≥3 as a 'cut-off' for 'unacceptable' fruit or 33% juice.

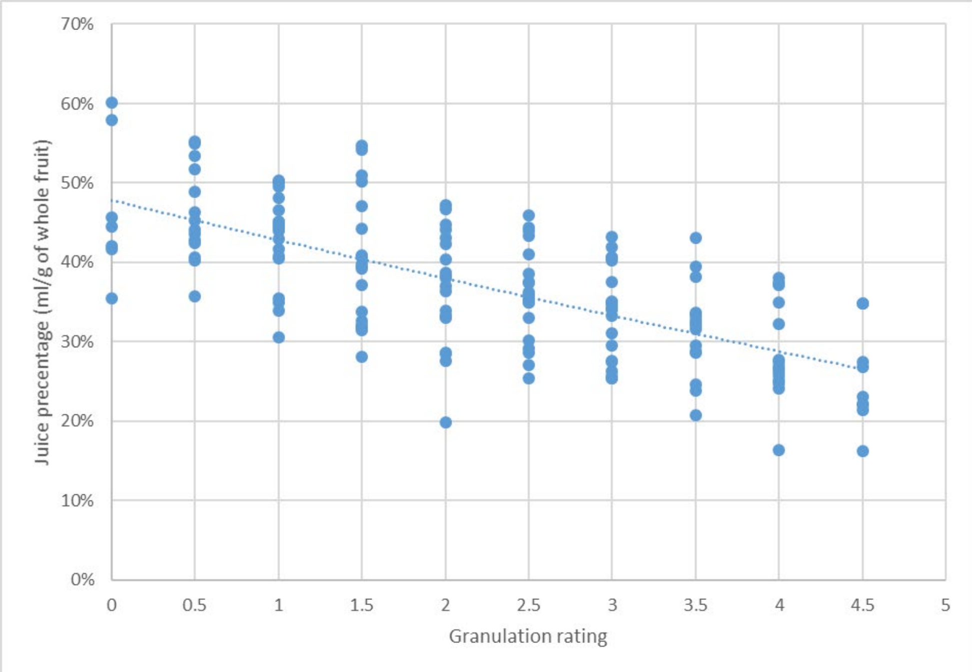

Figure 2 Juice percentages of individual fruit by rating in a sample of 162 fruit taken in 2020, showing the range present in each rating. The r value for this data is -0.679 (P<0.001).

Table 1 Mean juice percentage (ml/g of half of whole fruit) and mean L value of cut flesh on the surface of bottom half of fruit by ratings in 2020

Rating	**n**	**Mean juice percentage (ml/g)**	**s**	**Mean L value**	s
0	7	47%	9%	47.25	0.67
0.5	14	46%	6%	46.60	1.88
1	20	43%	6%	48.01	1.91
1.5	20	40%	8%	50.93	2.43
2	20	37%	7%	51.67	2.43
2.5	20	36%	6%	54.09	2.75
3	20	33%	6%	54.87	2.70
3.5	16	32%	6%	56.63	2.94
4	16	29%	6%	58.58	3.56
4.5	9	25%	6%	60.50	4.76
5	0	--	--	--	--
r (correlation coefficient)		-0.679		0.8942	s

Juice samples

Juice samples were taken in the filed on the same day as granulation was rated, using the same 20 fruit halves that we used to rate granulation. Fruit were squeezed manually to ‘extract’ 5 ml of juice. Samples were a combination of equal amounts of juice (5ml) from each half.

- In Trial 1, one sample was taken from one tree for each plot i.e., 5 samples for each irrigation/nutrient treatment combination. Samples were taken in 2018/19, 2019/20 and 2020/21.
- In Trial 3, we selected three healthy trees in each plot i.e., a total of 12 trees per irrigation treatment. Samples were taken in 2019/20 and 2020/21.
- In Trial 6, we used samples from two trees per plot i.e., a total of 12 trees per irrigation treatment. Samples were taken only in 2020/21.

Samples were analysed for Brix and acid content and the Brix:acid ratio and the Australian Citrus Standard (ACS) values were calculated. Acid percentage was calculated using titration with NaOH using the average of two 3 ml samples. The ACS is calculated as [Brix - (acid% x 4)] x 16.5. The current Australian Citrus Standard for marketing of Imperial mandarins is a minimum of 110.

Colour assessment

For Trials 1, 3 and 6, fruit for external colour measurement were selected from the same tree(s) as used for the 'juice sample', selecting five or six fruit per tree.

For Trials 1 and 3, fruit were measured before placement in a ripening or ‘degreening’ room, and 24 hours and/or 48 hours after exposure to 10 ppm ethylene at 24^{o} C. For Trial 6, only colour before degreening was measured for logistical reasons.

Colour was measured in three places on each fruit using a chromometer (Minolta Chroma Meter CR 400). Measurements were converted to an index value, the 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 differences 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. Figure 3 provides an example of the CCI values for a sample of varying fruit colours.

Figure 3 CCI values (mean of 6 measurements per fruit) of a sample of fruit shown were fruit 1 -9.3, fruit 2 -7.1, fruit 3-1.9, fruit 4 -1.5, fruit 5 +0.6, fruit 6 +2.7). Photo: H. Hofman

Crop load assessment

The crop load on trees was visually rated at harvest using a 10-point scale.

In addition to visual crop load ratings, we counted fruit in a 50x50x50 cm frame on a subset of trees in all years from 2020/21 onwards. From this data, we calculated a linear equation for the relationship between counts and the crop load rating per tree, and used this to calculate an overall estimate of the mean crop load in fruit per frame. The number of trees and counts varied with trial/year (Table 2). This formula was also used to calculate a 95% confidence interval for the mean. Note that in high crop load years, the r^2 value of the linear relationship is low because crop load ratings are similar from tree to tree.

This method of crop load assessment was done after any chemical or manual thinning, so may not accurately reflect crop load in early fruit development. However, since 2020/21, labour shortages, cost squeezes and climate factors affecting biennality have meant that in all trials crops were not manually thinned, or only lightly thinned. In 2022/23, Trials 8 and 9 were chemically thinned but as can be seen from Table 2, crop loads in these two trials were still high.

Table 2 Mean crop load ratings and estimated frame counts by trial and year

Trial	Year	Mean crop load rating all treatments	Mean calculated fruit count per frame	r^2	No. of counts
Trial 1	2019/20	1.5	7.7	*0.49*	Used Trial 2 counts
	2020/21	7.9	25.1	*0.12*	Used Trial 2 counts
	2021/22	0.8	3.1	*0.81*	22 trees x 3 frames
Trial 2	2019/20	1.9	11.3	*0.49*	37 trees x 1 frame
	2020/21	7.8	24.9	*0.12*	10 trees x 2 sides –2 people counting
	2021/22	1.2	5.8	*0.81*	Used Trial 1 counts
Trial 3	2019/20	1.6	8.4	*0.49*	Used Trial 2 counts
	2020/21	3.1	16.7	*0.05*	10 trees x 1 frame –4 people counting
	2021/22	1.4	6.8	*0.81*	Used trial 1 counts
	2022/23	6.1	24.4	*0.87*	15 trees x 2 counts
Trial 4	2020/21	7.7	24.4	*0.12*	Used Trial 2 counts
	2021/22	2.7	14.2	*0.81*	Used trial 1 counts
	2022/23	4.4	22.6	*0.72*	20 trees x 2 frames
Trial 6	2020/21	2.5	11.8	*0.58*	19 trees x 2 frames
	2021/22	2.4	24.3	*0.77*	15 trees x 2 frames –3 people counting
	2022/23	4.2	17.5	*0.72*	19 trees x 2 frames
Trial 8	2022/23	7.2	26.4	*0.32*	10 trees x 2 frames
Trial 9	2022/23	5.7	23.2	*0.69*	23 trees x 2 frames

Where there was more than one person counting, the average count was used.

Analysis and reporting

Our analyses were done using the Genstat statistical program (18th edition). Unless stated, treatment means are separated by analysis of variance using one- or two-way analysis as appropriate.

In this report we use a 95% confidence level for determining whether treatment means (that is, averages) differ 'significantly' from each other, that is, a 'P value' of 0.05 or less. If the P value is ≤0.05, we then indicate which of the treatment means can be considered statistically different within this confidence level by using different letters as suffixes: a, b, c etc. Where means in the same group of data are marked with the same letter, we can't be 95% confident that they are different from each other because of the variability in the data.

We report statistical error in these calculations as 'ese' or estimated standard error of the means. This provides an estimate of how likely the sample mean is different from the 'true' mean of the whole population. This calculation takes into account the variability of the data (the standard deviation) and the sample size.

Appendix 6: Summary for growers

Reducing granulation in the production of Imperial mandarins

Helen Hofman and Hanna Toegel, Queensland Department of Agriculture and Fisheries

This document summarises for growers the results of on-farm trials in management practices to reduce granulation in the production of Imperial mandarins in the projects CT04002, CT11007 and CT19005. These projects were funded by the Queensland Government and by Hort Innovation, using the citrus research and development levy and contributions from the Australian Government. Hort Innovation is the grower-owned, not-for-profit research and development corporation for Australian horticulture. Some trials were also funded by Nutrano Pty Ltd, Spencer Ranch Pty Ltd and the Mundubbera Fruit Growers Association. We acknowledge the generous contributions and cooperation of our grower collaborators in these projects.

What is granulation?

Granulation is a physiological disorder in citrus in which juice vesicles are hardened, gelled or granular. Severely granulated fruit are an opaque white in colour, with no or minimal extractable juice (Figure 1). Less granulated fruit are 'crunchy', with less extractable juice and reduced flavour. Industry guidelines suggest <25% juice is considered severely granulated.

Figure 1 Indicative photo of a range of Imperial fruit with rating 5 at the top and 0 at the left.

What causes granulation?

Despite international research into the problem since the 1940s, the cause of granulation is not yet understood. In our trials and surveys, we have found it is associated with larger fruit size, lower acid content in juice, lower crop loads, lighter (sandier) soils, vigorous rootstocks, and the position of fruit in the tree, with fruit inside the canopy more granulated.

These factors are the basis of our hypothesis that granulation is caused by higher water potential in juice cells, that is, juice cells with lower total soluble solid content and/or higher turgor pressure. The cell wall thickening in granulation may be a protection against moisture loss from cells that are high in water potential. The process of cell wall building may further deplete acids and sugars, causing a spiralling increase in granulation. Thus water potential increase early in fruit development, may be more detrimental than water potential increase late in fruit development.

This hypothesis suggests that granulation could be triggered by conditions or practices that increase turgor pressure in cells, such as frequent or excessive rainfall or irrigation, and/or practices that reduce soluble solid content, such as inadequate nutrition. Competition between flush expansion and fruit development, particularly in a low crop load year, could also reduce soluble solids in juice cells and exacerbate granulation.

Why are some years worse than others?

In our ten years of trials, granulation was higher in years with high rainfall in spring and summer (Figure 2). It was also higher in years with low crop loads. Crop loads tend to explain variations in granulation levels when they do not match the rainfall pattern.

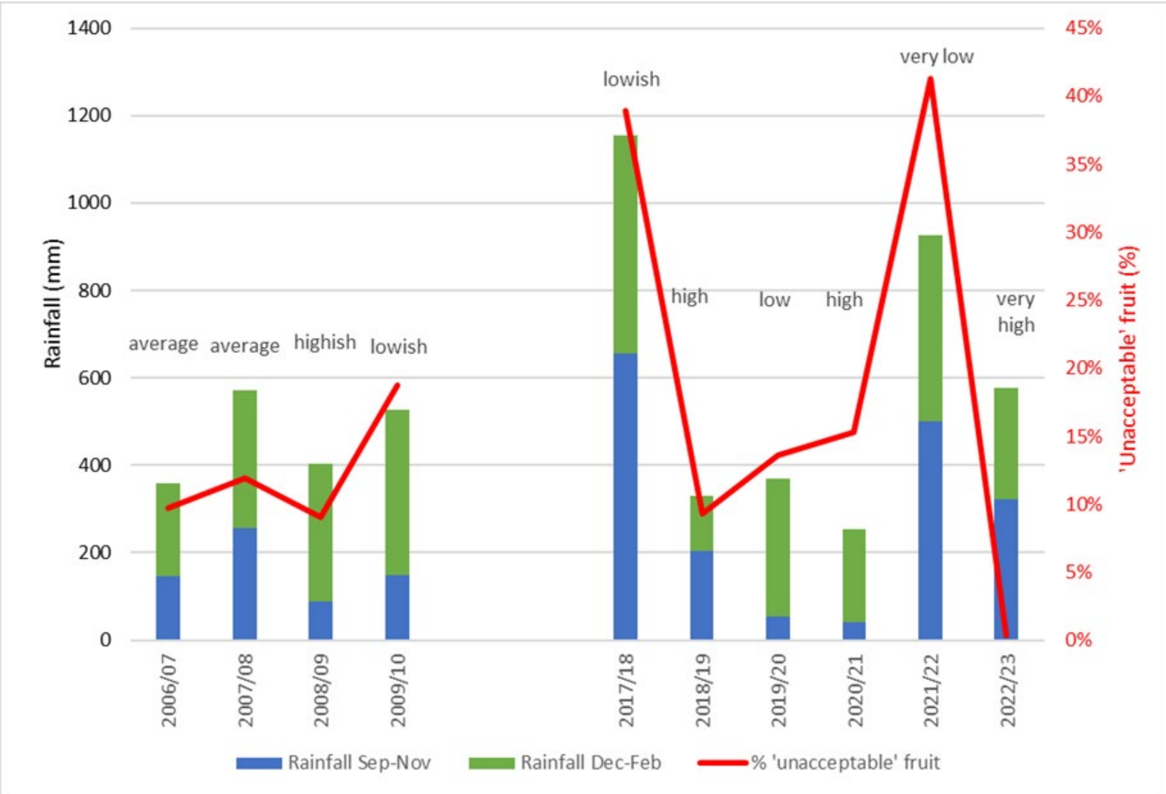

Figure 2 Rainfall totals and indicative granulation for projects CT04002 and CT19005. Data for 2006/7 to 2009/10 covers Gayndah rainfall, "% unacceptable fruit" for this period is the mean % of fruit rated at ≥2.5 in 35-40 surveyed blocks in Gayndah, Mundubbera and Childers. Data for 2017/18 to 2022/23 covers Bundaberg rainfall, "% unacceptable fruit" for this period is the mean % of fruit rated ≥3 in control treatments at 2, 3, 3, 4, 5 and 4 trials in Wallaville in 17/18, 18/19, 19/20, 20/21, 21/22 and 22/23 respectively. Text on graph summarizes crop load trends, although these will differ from trial to trial. Trial 6 is not included in this latter data as Mundubbera has different rainfall patterns. SOURCE: climate both projects

Why do low crop loads mean more granulation?

This is not well understood. There may be several reasons. A heavy crop load, particularly if picked late, means that carbohydrate production by photosynthesis in leaves continues to be used for fruit late into the season, and not stored. This means there are insufficient reserves to supply demand in the spring of the following 'off' year. The heavier vegetative growth in an 'off' year may also compete with the few fruit that do set. In low crop load years, trees have lower levels of photosynthesis. More vigorous root growth in 'off' years may also use more of the available photosynthates. We also hypothesise that a low crop may mean that there is plenty of water available to the few fruit that do set, increasing turgor and water potential in the juice cells.

What can I do to minimise granulation?

Our trials suggest that the three key strategies to minimise granulation are:

1. Maintain high crop loads,
2. Reduce irrigation, and
3. Apply sufficient nitrogen in winter.

How important is crop load management?

Maintaining consistent crop loads every season is essential for management of granulation in Imperials. Key aspects of this are thinning fruit and not picking too late.

Growers may be inclined towards using chemical rather than manual thinning because of labour and cost of production pressures. We did not do any trials that compared manual with chemical thinning, so cannot provide any information on relative effects on granulation. However, in considering your decision, be aware most chemical thinning agents are applied earlier in the growing season than hand thinning. By reducing crop load early, you may risk higher granulation compared to hand thinning, which tends to be later (January). In addition, some chemical thinners tend to selectively remove smaller fruit, leaving the larger fruit on the tree. It is the larger fruit that are commonly the most granulated.

In one of our trials, we had some success with using gibberellic acid at 90% petal fall to increase fruit set. This, or a similar strategy, may be of use if expecting a low crop load or if flowering is poor.

How much water should I apply?

Our trials all show that excessive water is a major cause of granulation. In our trials granulation was reduced where we cut back water compared to existing normal practice on the orchards.

However, our trials were not designed to provide advice on specific quantities required. Rainfall over the research period was so variable from year to year that with only four years of data we cannot specify quantities. For example, in one trial, good results (~<5% unacceptable fruit) were achieved in high crop load years with 21%, 50% and 77% of ETo (evapotranspiration) in Stage I of fruit development (includes rainfall), and 31%, 78% and 111% in Stage II. In a low crop load year, both 52%/62% and 21%/35% in Stage I/Stage II produced good results.

Figure 3 shows granulation in terms of percentage 'unacceptable' fruit for the treatments in our four irrigation trials in the two low crop load years 2019/20 and 2021/22 combined. The left-hand graph shows granulation against total water (rain + irrigation) in the first two stages of fruit development; and the right-hand graph shows total water as a percentage of evapotranspiration (ETo). Only low crop load years are shown because in high crop load years, granulation tends to be negligible. Each dot represents a treatment. This data should be considered indicative only as the trials, treatments and years were very different. It shows a trend of increasing granulation with total water which is more consistent for Stage I applications. However, the range of granulation results is very wide for a particular amount of water applied: this graph demonstrates the inadvisability of making specific volume recommendations. However, the graph on the right suggests that water/ETo above the UN's Food and Agriculture Organisation crop factor of 65-67% (FAO56) has a high probability of severe granulation.

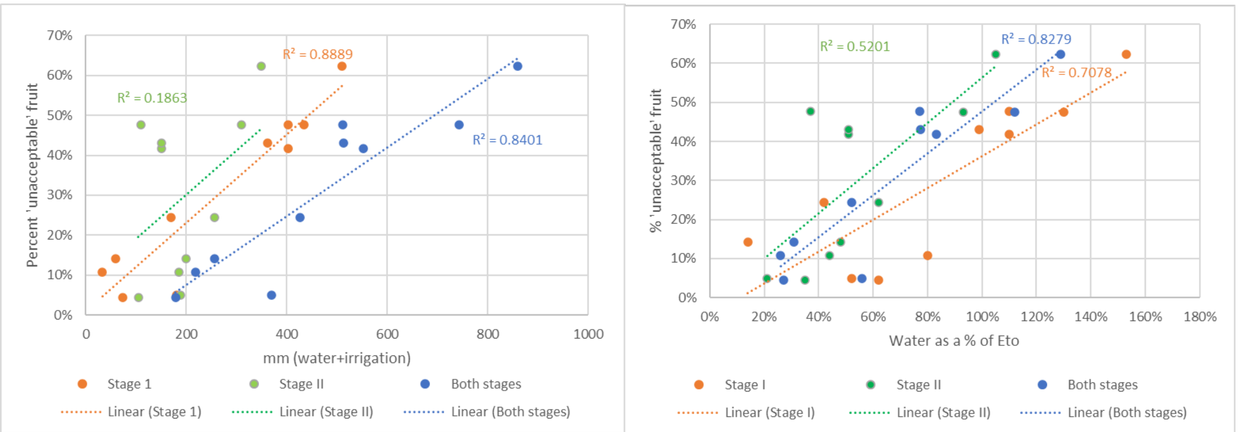

Figure 3 Percent 'unacceptable' fruit (rated ≥ 3) and water applied as mm (left) and as a % of ETo (right) for treatments in Trials 1,3,6 and 7 in low crop load years (2019/20, 2021/22). ***ML/ha = mm/100. Data should be considered as indicative only as treatment dates in each of the fruit development stages vary between trials and years.***

Growers will need to experiment to suit their own conditions, keeping in mind that excessive irrigation during early fruit development is likely to make granulation worse.

Soil profile and drainage are also important management factors: we found higher levels of granulation where clay layers slowed or prevented drainage.

When is the critical period?

Our results suggest that over irrigation is most damaging during Stage I of fruit development (~September to November inclusive). In a nursery trial with trees in pots in the earlier project (CT04002), treatments that reduced irrigation volume in Stage I of fruit development, or in all stages of fruit development, reduced granulation more than reductions in Stage II or III, or both Stages II and III. This suggested that Stage I was the most crucial.

However, some of our more recent trial results suggest that Stage II (December to February inclusive) is also important. For example, in one trial, there was no opportunity in 2017/18 to apply deficits in Stage I due to frequent rainfall, but deficits imposed in December through to mid-January still managed to reduce granulation. In a low rainfall year in another of our trials, most of the 'stress' recorded by dendrometers developed in Stage II of fruit development, even though water reductions began in Stage I. In these two trials, we carried deficits through to mid- to late-January (late Stage II) with good results. The end date of mid- to late- January was used because around this time temperatures peak and the trees become visibly stressed very rapidly. But it may be that a deficit all season is beneficial: this remains to be tested.

Our hypothesis suggests that granulation can commence at any stage where water potential is high in juice cells. We suggest that earlier rainfall/irrigation may be more detrimental, possibly because the sooner granulation is triggered, the longer it continues to develop and exacerbate.

In our trials we commenced deficits once there was full petal fall to ensure trees were not stressed during flowering to maximise fruit set.

Which N application rate and method produced best results?

Imperials are a fast-developing variety and are possibly more reliant than mid- or late-developing varieties on reserves of carbohydrates and minerals to support the spring flush and early fruit development. Slowly-developing varieties rely more on photosynthesis from newly expanded leaves for fruit development. The results of this project emphasise the importance of sufficient stores in the tree to manage granulation in Imperials. For this reason, all nitrogen should be applied in winter. Applications at other times are wasteful and can be detrimental to fruit quality. Our results suggest that the current recommendation of 800 g N/tree for mature trees is a good guide.

What leaf N is optimal for reducing granulation?

Our leaf tissue data (three years only) across our trials emphasises the importance of managing crop load as well as N nutrient applications. The N leaf tissue results in this project tell us more about crop load status than they do about appropriate leaf N levels, with high levels of leaf N in a high crop load year associated with high levels of granulation in the following year when crop load is low. We only have three years of results, but we suspect that the current leaf tissue guidelines of between 2.2 and 2.4% N are too low for Imperials. Levels ≥3 in the low crop load year of 2021/22 correlated with good granulation results in 2022/23.

What doesn't work?

Our trials showed little or no impact of high or low levels of the nutrients potassium, calcium, phosphorus, boron or zinc. We also tried various strategies to try and reduce the competition between fruit and flush growth in spring, including vegetative growth retardants, later pruning (that is, late spring rather than winter), branch girdling in spring, and winter applications of gibberellic acid. Results were disappointing. There was a small response in some low crop load years from a plant growth retardant, but results were unreliable.

What can I do if there is a wet spring?

In our trials, late (early or late summer) applications of foliar or broadcast nitrogen, or foliar sprays of calcium, boron or potassium, had no effect on granulation. We also tried to reduce irrigation but were unable, because of rainfall, to completely dry out our trial blocks, so this is a strategy that remains to be researched.

—OOO—

Appendix 7: Literature review and research hypothesis

Contents

Introduction

Imperial mandarins are an early mandarin variety, which is the first one to reach the domestic market each season. Therefore, their quality has an impact on the consumer behaviour for the rest of that season, with excessive amounts of granulated fruit reducing the likelihood of continued purchases of mandarins (Damiani, 2016). Citrus fruit that are 'granulated' taste dry, look white or colourless compared to normal mandarins, and are relatively tasteless. Granulation is a longstanding and major quality problem worldwide for a range of sweet orange, grapefruit, pummelo and mandarin varieties.

Since the 1930s, there has been extensive research in the main citrus growing regions of the world into the causes of granulation and into management practices for reducing the incidence and extent of granulation. It is generally believed that granulation is not caused by a bacterium, fungus, virus or other pest organism, but is a physiological disorder (Sinclair & Jolliffe, 1961). Research has established that a wide range of factors are associated with granulation, and that several management practices can affect its incidence and severity. However, there has been little attempt to present a holistic model that encapsulates all these factors, and no clear understanding of the underlying causes of granulation has yet emerged.

Terminology and other 'dryness' disorders

While granulation appears to be the most common term used for the disorder, a range of other terms have been proposed in the literature. These include 'sclerocystosis', 'segment drying', 'dry end' (Florida), 'dry juice sac' (Thailand), 'koa sarn' (raw rice)(Thailand), 'corkiness' (West Indies) and 'crystallisation' (California) (Awasthi & Nauriyal, 1971a; Bartholomew et al., 1941; Bitters, 1961; Boonyakiat & Yantarasri, 2001; Huang et al., 2023).

The existence of a number of unrelated 'dryness' problems encountered in citrus can also create confusion. These include:

- dryness due to sunburn or frost. The drying effects of sunburn can usually be distinguished from granulation as it is confined to the pulp below the sunburnt patch of the peel.
- dryness in which vesicles lose water and shrink. This disorder is most common during postharvest storage periods of several months, and is characterised by withered juice sacs, tasteless pulp, separation of rind and pulp and loose albedo (Bartholomew et al., 1941; Wang, 1993a). This has been variously named 'dry sac', 'xerocystosis' or 'withered juice sac' (Awasthi & Nauriyal, 1971a; Bartholomew et al., 1941). While this disorder has some similarities to granulation, including decline in sugars and acids in the pulp, the gelling, hydration and enlargement of the juice sacs common in early stages of granulation do not appear to be a feature. Wang (1993b) suggests that in the 'withered juice sac' disorder the water is transferred to the rind as it senesces because it has a lower water potential.
- dryness due to diseases. Desiccation, dry rot and premature fruit drop can be caused by the fungus *Nematospora (Eremothecium) coryli*, recently identified for the first time in Australia, but possibly affecting fruit in Australia for over 90 years (Shivas et al., 2005).

The term 'section drying' is sometimes used in the scientific literature and it is not always clear whether this means granulation and/or other drying disorders (Peiris et al., 1998; Shu et al., 1987). The various types of dryness disorders are also sometimes grouped together in discussions. This general grouping of drying effects might lead to poor management advice. For example, Browning et al. (1995) list freezing injury, sunburn, over-maturity, a lack of water, excessive tree vigour, severe mite damage and cool dry windy weather as possible causes of 'granulation'. This appears to be an unhelpful conglomeration of a range of dryness causes. They suggest that as water will be drawn from the fruit to supply the needs of the tree, keeping trees well irrigated will keep fruit from drying out and granulating.

This review is concerned only with the granulation disorder as described below in 'Granulation—what is it?' Other dryness disorders are not discussed.

Aspects of the ecology and physiology of citrus

Several aspects of citrus ecology and physiology are relevant to the problem of granulation.

Juice cells

Citrus fruit are unusual in that they consist of a collection of juice sacs or vesicles composed of cells that are dominated by a juice-filled vacuole. The structure of the juice sacs consists of an external layer of epidermal cells, then a layer of subepidermal cells, an elongated cell layer and, in the centre of the sac, a collection of progressively larger, thin-walled juice cells (Burns & Achor, 1989; Shomer et al., 1989).

Citrus fruit are 85-90% water by weight and juice sacs have notably high turgor and high concentrations of sugars, mostly sucrose, glucose and fructose (Koch & Avigne, 1990). Only a small proportion of the water used by the plant is transferred into the fruit, and the maintenance of supply appears to depend on low water potentials within the fruit (Mantell et al., 1980). That is, the maintenance of high levels of solutes creates an osmotic gradient which enables continuing water input into the juice cells.

Fruit development

Compared to other edible fruits, the development of a citrus fruit is a slow process, taking from six to over 11 months, depending on variety and climate.

There are considered to be three main stages in citrus fruit development (Spiegel-Roy & Goldschmidt, 1996):

- I – cell division, a period of 5-10 weeks (Marsh et al., 1999). This stage appears to commence even before anthesis. The juice sacs appear to be initiated very early in fruit development, at about the time that petals open (Bartholomew et al., 1941). Fruit size increases in this stage appear to be mostly due to peel growth (Spiegel-Roy & Goldschmidt, 1996).
- II – cell enlargement, a period of several months. In this stage, the juice sacs begin a rapid increase in moisture content (Lowell et al., 1989). Cell volume can increase 1000 times (Davies & Albrigo, 1994). The transition to stage III is not always clearly marked.
- III – fruit maturation and ripening. Some cell enlargement continues in this stage.

Granulation—what is it?

Granulation can be defined as the condition in which the juice sacs of citrus fruit progressively become enlargened, hardened or gelled, and nearly colourless. Soluble solids (sugars and acids) are at lower levels than normal fruit and the fruit as a consequence becomes 'tasteless'. While moisture content is as high or higher than normal fruit, the juice is harder to extract.

Physical changes to granulating vesicles

The three key physical changes to vesicles are (i) enlargement, (ii) development of opaque white tissue and (iii) hardening or gelation of the vesicles giving them apparent 'dryness'.

Enlargement of the vesicle

The vesicles in granulated fruit segments are enlargened (Bartholomew et al., 1941; Hwang et al., 1990; Sinclair & Jolliffe, 1961; Singh, 2001). Bartholemew et al. (1941), Hwang et al. (1990) Kang et al. (2022) report that the juice cells within the sacs are also larger. Sinclair and Jolliffe (1961) suggest that increase in vesicle size may be due to an increase in water content or to a change in chemical constituents. The increased moisture content usually reported, as noted below, suggests that this is the likely explanation.

It is possible that enlargement of the vesicles may be a trigger for granulation and not just a characteristic of it. Burns and Albrigo (1997) observed that large vesicles are the first to show granulation symptoms i.e. there is a tendency of large vesicles in a heterogeneous population to granulate before smaller vesicles.

Conversely, an Australian study (Fullelove et al., 2004) does not support the view that juice cells are enlarged. It suggests rather that granulation involves proliferation of small cells in the epidermal cell layer of the juice sac. These outer cells had thicker cell walls. This implies that granulation occurs during the cell division stage rather than the cell enlargement stage, that is, granulation is triggered early in fruit development.

Opaque, white tissue

Granulated vesicles are characterised by zones of opaque white tissue, which can extend partway or throughout the entire vesicle. This white tissue appears to be thickened cell walls (Shomer et al., 1989). Several studies indicate that juice cells within granulated vesicles have considerably thickened walls (Bartholomew et al., 1941; Burns, 1990; Burns & Achor, 1989; Goto & Araki, 1983; Kang et al., 2022; Mahmoud, 1954; Shomer et al., 1988; Zhang et al., 1999).

Shomer et al. (1988; 1989) conclude that granulation in pummelo is a result of sclerification, that is, the process of strengthening cell walls to provide support and strength.

The possible reasons for cell wall thickening are not clear from these studies. Thickening of cell and/or vesicle walls could be adding rigidity to prevent loss of turgor, adding strength, providing a barrier to water or dissolved solutes, or providing a defence against infection. Burns and Achor (1989) suggest that the cell wall thickening in granulating vesicles may occur as response to stress such as increased dehydration. Shomer et al. (1989) note that the opaque white tissue appears to be sclerenchymatous in nature, that is, similar to tissues that provide strength and/or rigidity to plant tissues.

Hardening or gelation of the vesicles ('dryness')

The hardening of the juice sacs has been generally attributed to thickening of the cell walls and/or gelation of the juice within the cells.

Granulated fruit have a lower extractable juice percentage (Awasthi & Nauriyal, 1972a; Chakrawar & Singh, 1977a; Daulta & Arora, 1990; Kaur et al., 1991; Kotsias, 2004; Sharma & Saxena, 2004; Singh & Singh, 1980a; Zong et al., 1979). However, several studies found that, in actuality, the moisture content is higher than normal (Awasthi & Nauriyal, 1972b; Chakrawar & Singh, 1977a; Singh & Singh, 1980a; Zong et al., 1979). Awasthi and Nauriyal (1972b) and (Ding et al., 2009) found reduced moisture content in the late stages.

It is likely that the extractable juice levels are lower because moisture is either bound in walls or other portions of the sac (Bartholomew et al., 1941), or bound in gels. Most studies support the view that moisture is bound in gels created by pectins. In pectic gels, water is tightly bound to the pectin fraction, making it difficult to extract (Burns & Achor, 1989).

It is thus possible that the processes of cell wall hardening and gelation of cell contents are related. The gelation of cell wall contents may be a side effect of the process of cell wall thickening. 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; Li et al., 2022).

Changes to juice content

The main changes to the components of juices in granulated fruit are decreased soluble solids, including decreased sugars and decreased acids. Changes to ascorbic acid are less well-established.

Decreased TSS

A decrease in total soluble solids (TSS) in granulated vesicles is well established (Awasthi & Nauriyal, 1972b; Ding et al., 2009; El-Zeftawi, 1978; Gilfillan & Stevenson, 1977; Kotsias, 2004; Sandhu & Singh, 1989; Sharma & Saxena, 2004; Sharma et al., 2006; Shomer et al., 1988; Sinclair & Jolliffe, 1961; Singh & Singh, 1980a; Sinha et al., 1962). Kaur et al. (1991) found the difference was not significant.

The significance of decreased TSS

Several authors have focused on decreased TSS as the most significant factor in understanding granulation. The most commonly advanced hypothesis for the causes of the lower levels of sugars in granulated fruit is that these are due to 'competition' between 'sinks' in the plant (Agusti et al., 2001; Chakrawar & Singh, 1978; 2004; Kaur et al., 1990; Kaur et al., 1991). 'Sink competition' refers to the portioning between plant organs (e.g. fruits, roots, leaves) of available carbohydrates, both stored or new products of photosynthesis. Organs are considered to have a greater 'sink strength' if they receive a greater allocation of carbohydrates and/or appear to have 'priority' over other organs, when carbohydrates are limited.

A second hypothesis for the decline in TSS is that granulation is a normal maturation process and is due, particularly of fruit in storage, to the consumption of nutrients, sugars and organic acids in the pulp during respiration (Tan, 1989). This hypothesis also fails to account for the phenomena of cell wall thickening and gelation, and the onset of granulation well before maturation.

Burns (1990) provides a third hypothesis for the link between cell wall thickening and carbohydrate supply. She provides evidence that the thickening of cell walls occurs at the expense of soluble sugars and acids. Similarly, a study following the path of ^{14}C-labelled glucose in granulated Ponkan mandarins found that a high number of glucose molecules were transformed into insoluble materials (Wang, 2005).

These three hypotheses for reduced TSS are not mutually exclusive. Initial low TSS levels could be further consumed in the process suggested by Burns, further reducing TSS levels.

Distribution of granulation

In granulated fruit, not all juice sacs will granulate: granulated vesicles can be found adjacent to healthy vesicles (Burns & Albrigo, 1997; Hwang et al., 1988; Sinclair & Jolliffe, 1961). Individual vesicles will also vary in granulation (i.e. at the juice cell level) as shown by the variable distribution of white patches within vesicles (Bartholomew et al., 1941).

Some studies suggest that granulation begins in the stem end of fruit and progresses to the stylar end (Bartholomew et al., 1941; Goto & Araki, 1983). The progression of granulation in some cases from the stem to the stylar end of fruit suggests that granulation is an issue of under- or over-supply of some element(s): water, photosynthate, mineral and/or hormone.

The process of granulation

Stages in granulation

Several studies outline stages in granulation (Awasthi & Nauriyal, 1972b; Bartholomew et al., 1941; Sinclair & Jolliffe, 1961):
(1) vesicles hydrate and enlarge abnormally
(2) vesicles gelatinize and continue to enlargen
(3) vesicles harden and cell walls thicken, with continuing enlargement; and
(4) gas bubbles (CO_2) appear within cells and finally cell walls disintegrate, moisture declines and there is progressive disintegration of the vesicles. Vesicle disintegration is not recorded in all studies.

The progressive whitening of the vesicles suggests that granulation is a gradual process, with the extent of granulation in the vesicles varying from one vesicle to another. It does not appear to be reversible, although as most studies involve destructive assessment, this is not possible to ascertain without doubt.

Exacerbated by maturity or storage

This increase with maturity has led several authors to suggest that granulation is a senescence process (Chen et al., 2005; Singh & Singh, 1979a). Sharma et al. (2016) found granulation was linked with high levels of senescence-related enzymes. Chen et al. (2005), in studying postharvest granulation of Huyou fruit, suggest that juice sacs undergo senescence during granulation while the peel does not. Bain (1949) suggested that granulation occurred as the fruit matures and acid content reaches a certain low point, so that starches are formed instead of sugars. He suggests granulation thus appears to be a 'normal stage of maturity, for all citrus fruit, that is reached when the required number of heat units for the variety affected have been utilized in growth' (p. 414).

Factors associated with granulation

Since the earliest studies of granulation, it has been recognised that many and various factors are associated with granulation, and that the effects of these factors in a given situation may be varied by interaction with other factors (Bartholomew et al., 1941). Climatic, nutritional, genetic and individual plant factors, as well as management factors, are involved.

The major factors that have been studied to date are outlined below.

Tree vigour

Trees that are considered more 'vigorous' are more prone to granulation (Jawanda et al., 1978; Matsumoto, 1964).

The 'vigour' conferred by nitrogenous (N) fertilisation or heavy pruning, resulting in 'luxuriant' vegetative growth, has also been correlated with increasing granulation (Bartholomew et al., 1941; Matsumoto, 1964). Lloyd (1961) found that split application of N also adversely affected quality. However, the Californian grower survey mentioned above found no relationship between the quantity of N or organic manures applied and granulation in Valencia oranges, but the authors note that the fertility of soils in this study varied (Parker et al., 1943).

The underlying reason for the association of tree vigour and granulation is usually considered to be carbohydrate partitioning between sinks, that is, that photosynthates produced by the plants are used in root and/or vegetative growth flushes, rather than fruit development.

Interestingly, Benton (1940) states that heavy fertilising alone will not increase granulation, but will do so in conjunction with ample irrigation. This suggests either the need for ample water to sustain root and shoot flushes, or it may contribute to the dilution of sucrose supply to the fruit.

Poor tree health

Conversely, the declining health of trees may also foster granulation. Awasthi and Nauriyal (1972c) found that the extent (but not the incidence) of granulation was higher in declining sweet orange trees of some varieties than in healthy trees.

Larger fruit

Most researchers agree that, in the main, larger fruit are more likely to develop granulation (Awasthi & Nauriyal, 1972c; Bartholomew et al., 1941; Burns & Albrigo, 1997; 1998; Gravina et al., 2004; Hearn, 1987; Lloyd, 1961; Sandhu & Singh, 1989; Sharma & Saxena, 2004; Sharma et al., 2006; Sinclair & Jolliffe, 1961; Van Noort, 1969), although this may not be true for all varieties (Awasthi & Nauriyal, 1972c).

The underlying reason for the association of larger fruit with granulation has been attributed to a higher metabolic activity of such fruit (Bartholomew et al., 1941; El-Zeftawi, 1978), or to the possibility that such fruit are senescent sooner (Sinclair & Jolliffe, 1961). Burns and Albrigo (1997) considered the possibility that larger fruit may be of a more advanced age, and that granulation thus may be related to maturity, but suggest that as juice sacs vary in granulation levels despite being initiated at the same time, age differences cannot account for granulation.

A possible relationship between large fruit and low levels of TSS is not considered in the literature. Large fruit generally have a lower juice percentage, lower sugar levels, lower acidity but a higher sugar: acid ratio (Bevington et al., 1998). Further, it is not clear whether or not large fruit have larger juice cells or have more juice cells, and how this might link to the prevalence of granulation.

Low crop load

Several studies demonstrate that granulation is more prevalent in an 'off year' on trees with alternate bearing habits or on trees with low crop loads (El-Zeftawi, 1973, 1978; Gravina et al., 2004; Ritenour et al., 2004). One study, of oranges in Ghana, however, suggested that granulation was more severe in 'major' rather than 'minor' seasons (Atubra, 1982).

The underlying reason for the linkage of low crop load with granulation is seen as similar to that of tree vigour: the competition for carbohydrates between fruit and vigorous vegetative growth (Bartholomew et al., 1941; Benton, 1940; El-Zeftawi, 1978; 2004). That is, in trees with a low crop load photosynthates appear to be preferably partitioned to vegetative growth. A new vegetative growth flush has a stronger demand on assimilates than fruit (Kriedemann, 1969). Roots are also a strong, actively accumulating carbohydrate sink in 'off' years (Goldschmidt & Golomb, 1982). A study by Monselise et al. (1981) of two Wilking trees – a strongly biennnial cultivar -- show that the effect of an on-season is to deplete starch from roots, while after an off-season reserves accumulate in roots in very large amounts: the ratio of starch in roots of the off-season to the on-season was 17.2:1.

The effect of low crop load on granulation may be linked to the size of the fruit. Matsumoto (1964) found that granulation was more common in large fruit from branches bearing a light crop. El-Zeftawi

(1973) considered that light crop loads were more granulated because they included large fruit of high metabolic activity, that is, less juicy and of poorer quality.

The three factors— low crop load, low TSS, and large fruit size—appear to be closely interrelated. Another possible factor may be involved that has not been considered in the literature on granulation is the effect on 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). It is also possible that tree water status is affected by the lack of root growth in an 'on year': water supply to the fruit in an on year may be relatively more restricted. 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 fruit in an on year may help avoid granulation.

While 'natural' load adjustment by the tree through reduced flowering and/or fruit and flower drop seems to have a clear effect on granulation, the effect of 'artificial' load adjustment through chemical applications or hand-thinning is less certain. It is possible that thinning does not encourage significant root or vegetative flushes and thus the supply of photosynthates to the fruit is largely unaffected. Indeed, Bevington et al. (1998) reported that hand thinning had no effect on juice content, Brix or acidity.

Fruit thinning can moderate the alternate bearing cycle (El-Zeftawi & Thornton, 1975) and may thus have indirect effects on granulation.

Varietal differences

To some extent varietal differences vary from region to region, suggesting other factors interact.

One possibility is that the 'early' maturing cultivars may be more susceptible. Takebayashi et al. (1993) noted that granulation seemed to occur earlier in the season in early maturing species and cultivars of citrus than in later maturing ones. This suggests that granulation might be a process associated with maturity. However, it may be more relevant that 'early' maturing cultivars are generally associated with lower TSS and acid concentrations.

Rootstock differences

Choice of rootstocks also has a significant effect on levels of granulation. The association with granulation has been attributed to rootstock vigour in several studies (Awasthi & Nauriyal, 1972c; Bain, 1949; Jawanda et al., 1978). In vigorous rootstocks there may be greater competition between sinks for minerals, water and photosynthates, and/or more efficient water extraction and conductivity to support growth (Albrigo 1977). The view that granulation is linked to rootstocks that produce fruit of low soluble solid content appears to have some credence. Rootstocks can also contribute to earlier maturity within a variety and may thus influence granulation in this way.

A further aspect of rootstock characteristics is the water potential status they confer on the plant. Not surprisingly, rootstocks have important effects on plant water relations, which strongly affect sucrose levels and other quality parameters (Albrigo, 1978; Castle & Warrington, 1995). A potential link to granulation is mentioned by Albrigo (1978), who suggests there may be a link between juice vesicle 'drying' and the water potential status associated with various rootstocks. While his suggestion that fruit may lose water to the leaves via the peel does not seem to be supported by the higher levels of water in granulated vesicles, the role of water status may be significant and is further discussed in the section 'Hypotheses for underlying causes' below.

Rootstock effects can be variable across citrus growing regions (Awasthi & Nauriyal, 1972c; Bartholomew et al., 1941); and from season to season (Roose, 2006), suggesting that the influence of rootstock can be affected by climatic or other site-specific factors. Castle and Warrington, in a review of rootstock effects noted that climate factors ‘can easily overwhelm any rootstock effect’ (1995, p. 388). Further, individual trees can show significant variations in granulation within the same rootstock (Roose, 2006).

Position of fruit on tree

Bartholomew et al. (1941) found a higher percentage of granulation in fruit from the ‘inside bottom’ portion of trees and on the northern half of the tree (in California) than on the outside east and west. This held true even though fruit were smaller on the northern half than the southern half of tree and could thus, , be expected to have a lower incidence of granulation. The researchers concluded that position may be a more important factor than size. Awasthi and Nauriyal (1972c) also found the incidence of granulation was higher on fruit inside the tree (in some study years only and in some varieties only), followed by fruit on northern side of the tree. In the southern hemisphere, correspondingly, Lloyd (1961) found Valencia fruit on the southern side of trees in Mildura were more prone to granulation.

However, Tominaga and Iwahori (1987) found granulation in Ponkan mandarins in Japan to be higher on the south side of the tree.

Bartholomew et al. (1941) found that reducing direct sunshine by enclosing trees in cheesecloth tents did not increase incidence or extent of granulation in young trees, but may possibly have had a slight effect in older trees. Lee et al. (2015) found granulation reduced in Ponkan mandarins when grown under white shade netting, but this may have been associated with reduced temperatures.

It may be that position of the fruit is linked with granulation through light availability to the fruit, or, more feasibly, to nearby leaves, increasing the supply of photosynthates. Leaves export assimilates primarily to nearby fruit (Kriedemann, 1969). The effect may depend on the denseness of the canopy. Outside canopy fruit have been found to have higher °Brix and lower acid than inside positions (Syvertsen & Albrigo, 1980). Note that Barry et al. (2004a) found that the position of the fruit on the tree was a more significant variable in explaining juice soluble solid content than whether the fruit were born on leafy or leafless inflorescences. The inflorescence type, however, can influence fruit size, with larger fruit borne on leafy inflorescences.

Location, climate and seasonal conditions

The effects of climate may be through temperature effects on development of fruit, that is, the rate of accumulation of heat units; and/or the effects of rainfall and humidity on plant water relations. Climate stress (drought or heat) is conceivably of particular significance.

Humidity

Granulation is generally more severe in tropical than in cooler regions (Bain, 1949). This could be due to humidity and/or to temperature. Granulation is also reportedly greater in coastal areas than inland areas in California and in Australia (Bartholomew et al., 1941; Benton, 1940; Lloyd, 1961), presumably due to increased humidity and/or moderation of temperature extremes. Buds from the same parent tree grafted on to similar stocks showed more granulation when grown in a coastal area than in the interior (Bartholomew & Sinclair, 1947). However, Chanana et al. (1984) found less granulation in climates of high humidity rather than the drier and hotter locations of the Punjab. Similarly, lower average relative humidity in the later part of fruit development has been found to correlate with increased extent, but not increased incidence, of granulation of oranges in the Punjab (Awasthi & Nauriyal, 1971b).

Bartholomew et al. (1941) note that coastal Valencias usually have a lower content of TSS, possibly due to the dilution effects of lower transpiration rates. Higher relative humidity is generally associated with increased juiciness of fruit (Bain, 1949). Fruit grown near the coast tends to have a higher percentage of water in pulp and peel (Bevington & Castle, 1985).

Temperatures

Cooler temperatures do not appear to be an important factor. Bartholomew et al. (1941) suggest that in California, low temperatures (that is, frosts or close to freezing) do not cause granulation but may augment it in those fruit which are inclined to granulate. Similarly, Lloyd (1961) suggests that freezing can increase but does not cause granulation of Valencia oranges in Mildura.

Effects of higher (but not extreme) temperatures on accelerating fruit development may be a possible factor. Awasthi and Nauriyal (1971b) found that increased heat units throughout the year, and more especially during the early stage of growth, leads to an increase in the incidence and extent of granulation. Matsumoto (1964) also found that higher temperatures in the early season of growth exacerbated granulation.

This may be linked to lower levels of acids and sugars. Albrigo (2004) provides a useful summary in his review of effect of warmer climates on fruit development:

> Regarding internal quality, high temperatures accelerate fruit growth, the fruit has less time to accumulate soluble solids, and the high respiration rate leads to uses of carbohydrates in respiration, which further reduces available sugars for accumulation in the fruit. The high respiration may lead to faster turnover of acids….(p. 280)

A study examining the effects of high temperatures on fruit in the Coachella Valley desert in California found that fruit grown under full exposure to the sun were smaller, weighed less, had less juice, more soluble solids and more granulated carpels than fruit grown in the shade (Ketchie & Ballard, 1968). The association of reduced size and increased TSS with granulation run counter to general trends. It may be possible that the granulation is due to the effects of extreme temperatures, as maximum temperatures in July reached 119°F (48.3°C). Optimum temperatures for citrus varies from 25°C to 30°C, and temperatures above 35°C can reduce photosynthetic activity (Spiegel-Roy & Goldschmidt, 1996). The effects of sunburn may also have been confused with granulation. Veste et al. (2000) report that high temperatures (above 45°C) reduce transpiration in citrus due to its relatively low hydraulic conductance which triggers stomatal closure. Consequent increases in leaf temperatures can decrease photosynthesis due to photoinhibition.

Warm springs, late summer rainfall

One of the most interesting climate patterns associated with granulation was first mentioned by Van Noort (1969) who reported increased granulation when winter or early spring is warmer than average, followed by heavy rain in late summer or early fall. Similarly, Ritenour et al. (2004) suggests that the severe granulation in Florida’s 2003 navel oranges crop was possibly due to higher than average daily temperatures in February and March, higher maximum (but not average) temperatures in October and November, a compressed bloom period around 10 March, low fruit set, late summer, and early autumn rains (August-September).

The season preceding a harvest of notably granulated fruit in the Burnett region of Queensland was characterised by high average maximum temperatures in early summer, high average minimum temperatures throughout summer, as well as high amounts of rain in spring and late summer (Fullelove et al., 2004).

Van Noort (1969) expresses the view that the underlying reason why early heat increases granulation is that it accelerates the development of the fruit, so that it is more advanced at harvest time. This is in line with the studies noted above that suggest that increased heat units in the early stages of growth exacerbated granulation (Awasthi & Nauriyal, 1971b; Matsumoto, 1964).

Van Noort does not suggest an explanation for the connection to early autumn rainfall. Carbohydrate partitioning in favour of root growth may be a factor when there are heavy rains. Citrus typically has two root flushes late in spring and late in summer (Davies & Albrigo, 1994). Bustan and Goldshmidt (1999) demonstrated that while older, larger fruit have a strong sink competitiveness, this is not to the exclusion of significant root growth. In subtropical climates, citrus fruit typically accumulate high levels of sugars in autumn, possibly because competing vegetative growth is slowed by cooler temperatures (Reuther & Rios-Castano, 1969). Rain in autumn may extend vegetative and root growth and reduce supply to the fruit. It may also be possible that sucrose supply to the fruit is diluted and/or the water potential in the fruit increases dramatically in this period, leading to granulation. Transpiration rates can be significantly reduced during rainy periods: Bevington et al. (1998) recorded reduced transpiration rates of nearly 50% for a day of rain in February for Imperial mandarins in Sunraysia.

Management strategies

Irrigation

A large number of 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; Goldhamer & O'Connell, 2006; Lloyd, 1961; Malik et al., 1981; Raina & Lakhanpal, 1997; Scuderi, 1970; Singh & Singh, 1980b; Sites et al., 1951).

Bartholomew et al. (1941) noted that some trees were more affected than others. Bevington et al. (1998), in their report on improving mandarin fruit quality in Sunraysia, note that fruit ‘dryness’ (which may not necessarily equate with granulation) is less of a problem on sites irrigated at longer intervals (10-14 days rather than 7 days). Singh and Singh (1980b) found granulation less common when trees were irrigated at 30 day intervals rather than at 10 day intervals (15 day intervals produced intermediate results). They attribute this to higher luxuriant growth when soil moisture levels are high. A grower survey (Valencia oranges) in 1939 and 1940 in California found lightly irrigated (in terms of volume) orchards produced less granulated fruit in 1939 (but not in 1940) but there was no clear relation between granulation and number of irrigations (Parker et al., 1943). Note, however, that irrigations at this time were much less frequent than contemporary regimes– they ranged from four to seven per year.

The discussion on early autumn rainfall noted in the section ‘Location, climate and seasonal conditions’ above might suggest that the critical period to reduce irrigation would be late in the cell expansion stage. However, Goldhamer and O’Connell (2006) tested varying the times of water deficits to early, middle and late season and found that granulation was lower than the well-irrigated control for all times, and particularly when stress (50% of evapotranspiration) was applied throughout the growing season.

While it may be a tempting solution, it does not appear that the influence of irrigation is necessarily through fruit size or load. While all studies agree that dryer regimes had a lower incidence of granulation, some studies found that dry trees produced more large fruit (Bartholomew et al., 1941; Parker et al., 1943); and others that dry trees produced fewer large fruit (Goldhamer & O'Connell, 2006; Sites et al., 1951). Similarly, studies differ on whether or not dryer trees produce less fruit (Bartholomew et al., 1941) or had the same load (Goldhamer & O'Connell, 2006).

It has been suggested that the effect of long irrigation intervals in reducing granulation may be because short interval sites (higher frequency) are underwatered (Bevington et al., 1998). However, this does not seem logical and studies such as Hutton et al. (2007) demonstrate that long rather than short irrigation intervals mean depleted soil moisture, that is, greater levels of water stress. As discussed in the section ‘Hypotheses for underlying causes’ below, some levels of water stress encourage higher levels of TSS, which appear to be associated with reduced incidence of granulation.

Plant growth regulators

The use of plant growth regulators (PGRs) to reduce granulation has been extensively studied. Discussion of the theoretical basis in these studies is generally scanty, but appears to be on the basis that PGRs, along with nutrition, play a role in regulating source-sink relationships by increasing photosynthate partitioning to developing fruits. Most studies of auxin and giberellic acid application report favourable results. In general, although there are some exceptions, spraying later in fruit development (that is, late Stage II and/or through Stage III) is reportedly more effective than spraying early in fruit development.

Auxins

The use of auxins has been studied on the basis that auxins may (i) increase the ‘sink strength’ of fruit, that is, induce carbohydrate accumulation and mobilisation of minerals to fruit and/or increasing fruit stem transport capacity (Agusti et al., 2001; Chakrawar & Singh, 1978; Kaur et al., 1990; Kaur et al., 1991), (ii) prolong the growth period of the fruit and delay senescence (El-Zeftawi, 1973), or (iii) influence some enzyme system involved in the aerobic respiration cycle (Mahmoud, 1954).

Applications of auxins (2,4-D; 2,4,5-T; 356-TPA; NAA) are generally reported as reducing granulation (Chakrawar & Singh, 1978; El-Zeftawi, 1973; Erickson, 1968; Erickson & Richards, 1955; Hield & Erickson, 1962; Kaur et al., 1991; Singh & Chohan, 1984; Singh & Singh, 1981a; Stewart, 1949). Foliar applications of 356-TPA on eight treatment dates found that there was a trend for less incidence of granulation at the later date treatments, that is, mid-season (early Stage II fruit development), but overall the season had low incidence of granulation (Fullelove et al., 2004). The mechanism of this effect may be similar to the known effect of auxin applications increasing fruit size: Auxins applied early in Stage II of fruit development have been shown to be effective in increasing fruit size and appear to function by increasing vesicle and cell size rather than number (El-Otmani et al., 1993). El-Otmani, et al. (1993) suggest that this is due to increased sink strength, and/or to enhanced CO_2 uptake by leaves resulting in increased availability of assimilates to fruit.

Some studies report *increased* granulation with auxin applications. Miller et al. (1999) and Hirose et al. (1972) found that spraying 2,4,5-T 30 days after full bloom increased granulation. Pan et al. (1998) found that spraying pummelo at full bloom or in the fruitlet stage ‘blocked’ granulation, while spraying later accelerated granulation. Gallasch et al. (1998) noted that auxins (2,4-DP and 3,5,6-TPA) are used in Spain to increase fruit size, but can cause decreases in juice content and early granulation of fruit in light cropping years. Hield and Erickson (1962) found that spraying small green fruit to increase size at maturity delayed the onset of granulation, but after the initial effect, there were no further effects evident.

Erickson and Richards (1955) provide a rare example of a study of the interactions of two factors—the effects of a PGR application (2,4-D) and irrigation. They found that spraying with 2,4-D when fruit (Valencia oranges) were small (average 11mm in diameter) and reducing soil moisture gave the highest content of soluble solids, with a significant reduction in granulation. The ‘wetter’ treatment meant irrigation when tensiometers at 1 foot depth reached 300 cm of water (this meant irrigation at intervals of around three weeks in summer); ‘drier’ treatments when tensiometers at the 2 foot depth exceeded 700 cm of water (this meant irrigation at intervals of around five or six weeks). Granulation tended to be lower in drier trees but was significantly so only in the presence of 2,4-D. 2,4-D significantly reduced the number of fruit per tree, but significantly increased (at the higher moisture level) the number of large-sized fruit per tree. Reduction of soil moisture, with or without 2,4-D, reduced the size, but not the number of fruit per tree.

Giberellic acid

Sprays of giberellic acids (GAs, usually GA_3) are widely reported to reduce granulation. Unlike the body of work with auxins, there does not appear to be any studies that report negative or inconclusive effects. Kaur et al. (1991) and Pan et al. (1998) suggest that GA_3 sprays are more effective than auxin sprays. However, Singh and Singh (1981a) found the reverse.

GAs are used with some citrus to delay maturation of the fruit, thus extending harvest time (Spiegel-Roy & Goldschmidt, 1996). Hypotheses for the effectiveness of giberellic acids include the possibility that this effect might reduce granulation (Chakrawar & Singh, 1978). More frequently, it is suggested that GAs decrease granulation by increasing the amount and movement of assimilates and minerals to fruit, that is, by increasing its ‘sink strength’, particularly in early development (Chakrawar & Singh, 1978; Kaur et al., 1991; Powell & Krezdorn, 1977). Several studies on carbohydrate partitioning in citrus demonstrate the role of GA_3 in increasing sink strength of fruit. Garcia-Martinez and Garcia-Papi (1979) established that the leaves of GA-treated Clementine mandarins had reduced N, P and K contents compared to non-treated plants, and that fruit from treated plants had higher levels of these macro-elements than non-treated plants, that is, the sink strength of the fruit for these macro elements increased. Mauk et al. (1986) demonstrated that both BA (benzyladenine, a cytokinin) and GA_3 enhanced ^{14}C-labelled assimilate export from foliage to developing fruit, with GA_3 being especially effective. NAA, ABA and paclobutrazol, in contrast, lead to a sharp decline in fruit growth. It is worth noting that BA and GA_3 also improved carbohydrate partitioning to fruit lower down the branch, whereas NAA acted in the reverse. Fidelibus and Davies (2002), however, found that fruit on trees sprayed with GA_3 at a late stage (colour break) sometimes had lower juice Brix than non-sprayed trees, although juice yield increased.

Including GA_3, NAA or BA in the medium of vesicles grown *in vitro* promoted the growth of juice vesicles but did not stimulate sugar accumulation (Harada et al., 2001). This supports the view that GA works through improving transport of assimilates rather than acting within the fruit itself.

As with auxins, most studies indicating effectiveness spray in Stage II and/or through Stage III. Note that application of exogenous GA can induce the production of endogenous auxins (Taiz & Zeiger, 2006). It may be these having the effect on granulation rather than GA per se.

Other PGRs

Applications of ethephon (2-chloroethylphosphoric acid), an ethylene releasing agent, in the cell expansion or cell ripening stage have been shown to reduce granulation of tangerines in India (Chakrawar and Singh 1978; Singh and Singh 1981; Singh and Chohan 1984). Cytokinins have not been much studied as potentially management tools for granulation. Pan et al. (1998) provides the only published study of the use of a cytokinin, kinetin, on pummelo. They found that spraying at full bloom or during the fruitlet stage ‘blocked’ granulation. Spraying later accelerated granulation.

Nutrients

Granulation has been associated with higher levels of Ca, K and, in some studies, of Mg in the fruit, and lower levels of Zn, Cu and B. Several studies have also examined levels of nutrients in leaves and/or stems. The majority of publications on the effectiveness of nutrient applications in reducing granulation were studies in northern India in the 1970s and 80s (Kaur et al., 1990; Manchanda, 1967; Singh & Chohan, 1982). These covered Ca, Mg, K, Zn, Mn, Cu, B and Fe. Most of these authors report that almost all micronutrient applications tested had positive effects, although Manchanda

(1967) and Manchanda et al. (1972) found Fe, Cu, and Mn ineffective, and Zn effective only in combination with Cu. Although the soil nutrient levels before and after applications in these studies is not described, Manchanda (1967) notes that multiple deficiencies are common in the Punjab. Only one study, Singh and Singh (1979b), relates granulation to soil nutrient levels at four sites in northern India. In this study no relationship was found between soil levels of N, P, K, Zn, Mn, Cu, Fe and granulation.

Nitrogen

Munshi et al. (1978) found an association between higher leaf nitrogen (N) levels and granulation but Chanana and Nijjar (1984), Singh and Singh (1980c) and Awasthi and Nauriyal (1972d) found no relationship with granulation and N contents of the plant. Singh and Singh (1979b) found no relationship between available N levels in soil and the extent and incidence of granulation at four sites in northern India.

The role of N in granulation appears to be largely indirect, that is, through increasing fruit size and/or stimulating vegetative growth. Bevington et al. (1998) found that higher leaf N content weakly negatively correlated with juice content (i.e. higher leaf N means 'drier' fruit), and weakly positively correlated with larger fruit.

Calcium

Calcium deficiencies are associated with a number of postharvest disorders in other fruits including bitter pit, lenticel blotch, cork spot, lenticel breakdown, cracking, low temperature breakdown, internal breakdown, senescent breakdown, Jonathon spot and water core in apples; cracking in cherry; soft nose in mango; and cork spot in pear (Wills et al., 1998). These effects appear to be due to calcium's role in binding with pectin substances in cell membranes. Abundant calcium thus reduces the incidence of many postharvest disorders by strengthening the structural components of cells, that is, delaying the loss of compartmentalisation and enzyme reactions that cause browning and pitting symptoms (Ferguson, 1984; Wills et al., 1998).

However, the reverse situation is pertinent in granulation. Increased levels of calcium (Ca) could enhance granulation because it is an essential component of cell walls. High levels of calcium have been noted in most studies of mineral composition of granulated fruit (Awasthi & Nauriyal, 1972a; Bartholomew et al., 1941; Gilfillan & Stevenson, 1977; Goto, 1989; Sinclair & Jolliffe, 1961).

However, calcium deficiencies may also play a role. Calcium is known to affect the translocation of carbohydrates (Davies & Albrigo, 1994). Calcium is also needed for a range of enzyme systems and metabolic sequences in plant tissues (Ferguson, 1984; Wills et al., 1998). However, individual enzymes respond differently to different calcium concentrations with some inhibited at higher concentrations that enhance the activities of others (Wills et al., 1998). Calcium is needed for amylase activity and thus supports increased levels of sugars in citrus (Chakrawar & Singh, 1977b).

Higher concentration of calcium in leaves of granulated trees has been recorded (Awasthi & Nauriyal, 1972c, 1972d; Singh & Singh, 1980c). However, others have found decreased Ca in leaves of granulated shoots (Munshi et al., 1978), and others no significant relationship (Chanana & Nijjar, 1984). Similarly, application of calcium has been effective in significantly reducing incidence of granulation in some studies (Singh & Singh, 1981b) but not in others (Kaur et al., 1990; Kaur et al., 1991). The multiple roles of calcium could explain these different results.

Note that Ca deficiency is rare in most citrus growing regions: where it is not abundant in the soil it is added though lime when controlling pH (Davies & Albrigo, 1994).

Phosphorus

Citrus require relatively low levels of phosphorus (P) (Davies & Albrigo, 1994). Phosphorus could be indirectly associated with granulation because excess P produces low acid, low sugar fruit (Chapman, 1968). Excess P also exacerbates Zn deficiency (Spiegel-Roy & Goldschmidt, 1996). Again, available information on the association between P levels and granulation shows variable results with Bartholomew et al (1941), Gilfillan and Stevenson (1977) and Sinclair and Joliffe (1961) finding higher levels of P in granulated fruit and Awasthi and Nauriyal (1972a) finding lower levels.

Potassium

Potassium (K) could conceivably be associated with granulation because K, which is 40% of total mineral content of the fruit, is known to be important in fruit development and size. K is involved in the translocation of carbohydrates from the leaves. It plays an important role in controlling (increasing) acidity of the juice, and has functions in membrane transport.

High levels of K have been noted in some studies of mineral composition of granulated fruit (Bartholomew et al., 1941; Gilfillan & Stevenson, 1977; Sinclair & Jolliffe, 1961). Munshi et al. (1978) found higher levels of K in leaves of shoots bearing granulating fruit, but notes that K in the soils in the study area were at quite high levels, and caused an increase in fruit size. Awasthi and Nauriyal (1972c), Chanana and Nijjar (1984) and Singh and Singh (1980c) found that leaf K levels seemed to have no definite relationship with granulation. Singh and Singh (1979b) found no relationship between available K levels in soil and the extent and incidence of granulation at four sites in northern India.

If there is an effect of K, it may be through increasing fruit size (Chapman, 1982b; Spiegel-Roy & Goldschmidt, 1996). Over-application of K produces large, coarse fruit with thick peels (Davies & Albrigo, 1994). Bevington et al. (1998), in a survey of Imperial mandarin fruit in Sunraysia, found that Imperial mandarins are heavy users of K. While there was no direct evidence that additional K improved fruit size, most growers producing a high proportion of large fruit applied K fertiliser annually and some also applied KNO_3 foliar sprays. Miller et al. (1999) found that treatments of KNO_3 + 2,4-D gave significantly larger fruit than other treatments (including K alone) but resulted in excessive granulation.

On the other hand, some studies suggest that deficiencies of K can exacerbate granulation. Several studies have found that foliar applications of K reduced granulation (Kotsias, 2004; Singh & Chohan, 1982; Singh & Singh, 1981b). Singh and Chohan (1982) found that sprays of Zn + Cu + K combined was more effective in reducing the degree of granulation than the same elements applied individually (from 23.85% in the control to 0.25%). This also produced the highest values of TSS. The authors suggest that K deficiencies interfere with translocation and cause loss of chloroplast functioning, leading to depressed photosynthesis and increased respiration.

Boron

Boron (B) could conceivably be associated with granulation because it is known to facilitate translocation of photosynthates. Boron deficiency shows in abnormal abortion of young fruits, albedo discolouration of fruit and dieback of growth (Chapman, 1968; Spiegel-Roy & Goldschmidt, 1996). Chapman (1968) writes that boron deficiency can produce low sugars as well as low juice content and 'dry interiors', but it is not clear if he is referring to granulation or to another dryness disorder.

There is a narrow range between deficient, adequate and toxic levels of boron (Davies & Albrigo, 1994).

Granulation has been associated with lower levels of boron in plant tissue (leaf or fruit) (Awasthi & Nauriyal, 1972d; Gilfillan & Stevenson, 1977; Munshi et al., 1978; Singh & Singh, 1980c). Accordingly, applications of boric acid have been found to significantly reduce incidence of granulation (Kaur et al., 1990; Kotsias, 2004; Singh & Singh, 1981b). Kaur et al. (1990) found that boron, or boron in combination with Fe, Zn or Mn, gave the highest TSS contents. Thus the link to reduced granulation may be through increasing sugar contents.

Boron deficiencies can be associated with igneous rocks (*e.g.* granite soils) (Chapman, 1968). Deficiencies are commonly addressed with boron sprays in the industry, and should thus not be as widespread a problem as granulation appears to be.

Other micronutrients

There are a number of elements that could conceivably be associated with granulation through various pathways and there are studies investigating many of them. However, the results are often inconclusive.

Many enzymes require zinc (Zn) for their activity, and some species require Zn for chlorophyll biosynthesis. Zn deficiency can also result in loss of capacity to produce sufficient endogenous auxins (IAA). It has been suggested that decreased auxin may increase oxidisation of sugars (faster respiration) thus decreasing the TSS (Singh & Chohan, 1982; Singh & Singh, 1981a). Some studies have found low levels of Zn in granulated fruit (Awasthi & Nauriyal, 1972d; Gilfillan & Stevenson, 1977).

Magnesium (Mg) is needed as a cofactor for enzymes that catalyse formation of sucrose. Mg is also an activator of photosynthesis and respiration (Spiegel-Roy & Goldschmidt, 1996). Thus Mg deficiency may exacerbate granulation through reducing sucrose supply to the fruit. However, magnesium deficiency is rare except in seedy cultivars or on calcareous soils, where Ca competes with Mg for uptake sites on the root (Davies & Albrigo, 1994). High levels of calcium have been noted in some studies of mineral composition of granulated fruit (Bartholomew et al., 1941; Goto, 1989; Sinclair & Jolliffe, 1961) and low levels in others (Awasthi & Nauriyal, 1972a; Gilfillan & Stevenson, 1977).

Manganese (Mn) is required in respiration and photosynthesis activities (Spiegel-Roy & Goldschmidt, 1996), and thus deficiencies could conceivably contribute to reduced sucrose supply. Singh and Singh (1980c) suggests high Mn lowers auxin content.

Copper (Cu) is widely used in pest management sprays in Australian orchards. Therefore, copper deficiencies are unlikely to be contributing to incidence of granulation (Chapman, 1982a). Similarly, Sulfur (S) deficiency is rare as it is provided in many fertiliser formulations (Davies & Albrigo, 1994). Studies examining the relationship between S and granulation have not found any significant relationship (Awasthi & Nauriyal, 1972d; Chanana & Nijjar, 1984).

Summary

Overall, the results of these studies appear to indicate that mineral deficiencies or toxicities, with the possible exceptions of N and K, are unlikely to be primary factors in granulation. The multiple functions of minerals in plant growth and fruit development may possibly explain the wide variation in results found in these studies, particularly as there appears to be a similarly wide variation in factors affecting granulation. The major effect of nutrients, as suggested by Singh and Singh (1981b) and Kaur et al. (1990), may be in affecting TSS content through increased translocation or other processes. Decreased granulation in the studies noted above is often accompanied by increased juice, TSS and acid content (Kotsias, 2004; Manchanda, 1967; Singh & Singh, 1981b).

Hypotheses for underlying causes

Despite extensive research over many years on granulation and its management, there has been little attempt to present a holistic model that caters for all or most of the factors established through empirical research, or to provide a comprehensive management approach.

Hypotheses that are raised, mostly in partial and undeveloped form, fall into three categories. These are that granulation is due to (i) stress (temperature and/or water stress), or (ii) a natural senescence process, or (iii) sink competition for carbohydrates i.e. reduced sink strength of granulated fruit compared to other sink strengths in the plant, or to inadequate translocation of sucrose into the fruit.

We advance a fourth and new hypothesis: that plant water relations, particularly in affecting water potential of the juice cells and/or hydraulic conductivity from the roots, are a key contributor to the process of granulation.

The hypotheses about underlying causes are explored in detail below.

The stress hypothesis

The ‘stress’ hypothesis, that is, that water and/or temperature stress cause granulation, has been proposed by Burns and Achor (1989). It is one that arises largely from considering a possible reason for cell wall thickening, that is, a response to limit water loss. While it is generally considered that the main mechanism for response to water deficits of plants is osmotic adjustment, Neumann (1995), suggests that cell wall hardening (or in some cases loosening) plays a greater role in the adjustment of growing plant tissues than osmotic adjustment, or is at least a ‘primary’ mechanism preceding the slower onset of conventional osmotic adjustment via solute accumulation. Note that Neumann’s work relates to root, stem and leaf tissue, and impacts on fruit tissue are not mentioned.

If this also applies to citrus juice cells, it could explain reports that granulation is triggered by high average temperatures very early in fruit development. Temperature or drought stress could be stimulating the cell wall thickening found in granulated vesicles. Fruit do not transpire as much as leaves and thus may be more subject to heat stress. Temperature stress can be exacerbated under high humidity and still air conditions (i.e. by reduced transpiration) in humid coastal areas. Citrus trees have relatively shallow root systems and thus could be considered to be relatively exposed to water or heat stress. Some citrus varieties may be more susceptible to stress than others. Bustan et al. (1996), in comparing the relative growth rate of Murcott mandarins and grapefruit, noted that the mandarin seemed ‘much more sensitive to transient environmental stresses, such as temperature and water deficiency’ (p. 83).

Assigning a role to heat/drought stress as a trigger for granulation does not explain the frequent occurrence of granulation in citrus trees that are apparently well-watered and not subject to any temperature or water stress. It runs counter to the finding that frequent irrigation exacerbates granulation, and the association of granulation with rootstocks of high hydraulic conductivity (see section ‘The possible role of rootstocks’ below). It does not explain why some fruit and vesicles within fruit granulate and others do not. Nor does it explain why some varieties, individual trees and specified rootstocks granulate and others do not when all could be expected to experience similar climatic conditions.

The senescence hypothesis

The increasing severity of granulation with storage, the increasing incidence of granulation if fruit is harvested late, and the occurrence earlier in the season with early maturing species and cultivars of citrus than in later maturing ones have suggested to some authors that granulation may be a senescence process (Chen et al., 2005; Grierson, 1981; Tan, 1989).

This hypothesis does not satisfactorily account for the range of granulation found within fruit, within trees and within an orchard. Nor does it explain the apparent onset of granulation prior to fruit maturity. Further research is needed to establish whether earlier developing fruit are more inclined to granulate, and if so, whether this is due to maturity per se, or to increased size, lower TSS or other factors better explained by the water potential hypothesis.

Huang et al. (2023) review hypotheses about the various metabolic pathways proposed for granulation, including both "regrowth" and "excessive senescence" of peel. They conclude with the hypothesis that granulation is due to a disruption of the cell wall metabolism. They do not, however, provide any hypotheses for the underlying cause of this disruption.

Sucrose partitioning and competitive sinks hypothesis

The lower levels of TSS found in granulated vesicles appear to be an important aspect of granulation. Several of the established factors discussed above strongly suggest that sucrose partitioning to the fruit ('sink competition') is an important causative factor. This is the view of several authors (Agusti et al., 2001; Chakrawar & Singh, 1978; Chakrawar et al., 1980; Fullelove et al., 2004; Kaur et al., 1990; Kaur et al., 1991).

The factors that support this hypothesis can be summarised as follows.

- Granulated vesicles are lower in TSS, including sugars.
- Fruit that are larger are more likely to be granulated, suggesting inadequate supply to the fruit. It is known that size is generally inversely related to TSS, even in normal fruit.
- Fruit on the shaded or inner side of the tree may be more likely to be granulated. This may be because nearby leaves produce fewer photosynthates.
- Increased vigour (young trees) increases granulation, possibly due to competition for photosynthates from vegetative and root growth.
- The increased granulation in an 'off' (low crop load) year can be explained if it is considered that photosynthates are 'diverted' into supporting a new vegetative growth flush and/or root flushes.
- Granulation appears to be more common in the stem ends of fruit in some varieties, where TSS is lower.
- The reported effectiveness of PGR applications in reducing granulation, which may be acting to enhance fruit sink strength.
- Root growth may also be a factor when there are heavy spring rains.

Translocation efficiencies

A variation on the competitive sink hypothesis is that granulation is associated with variations in translocation efficiencies for carbohydrates, water and/or minerals (Chakrawar & Singh, 1978; Chakrawar et al., 1980).

Bustan et al. (1995) suggested that limitations in the transport capacity in the pedicel may affect fruit growth, although increases in demand and/or photassimilate supply are also important and interacting factors. Fruit overcomes some of these transport limitations by inducing secondary thickening of its conductive tissues in the phloem up to 90 days after anthesis. Small advantages in improving transport capacity in the early stages of development appear to be 'crucial later, when a limiting condition occurs' (p. 665). However, García-Luis (2002) found that the transport capacity of the pedicel does not limit fruit growth, and increases in demand from the fruit were matched by an increase in supply at all stages of growth.

Plant water relations and cell water potential hypothesis

While the ‘competitive sink’ hypothesis explains many of the factors empirically associated with granulation, it does not fully explain factors such as the reduction of granulation where irrigations are less frequent; the increased incidence of granulation in humid climates; and the effects of early heat. Neither does the effect of low sucrose concentrations in the fruit explain why cell walls thicken and vesicles gelate, or why some vesicles granulate while adjacent vesicles do not.

We suggest that plant water relations and its effects on cell water potential may explain these factors.

Cell water potential

Granulation may be associated with water potential (Ψ) in the juice cells. Water potential is a measure of the free energy of water per unit volume. Free energy, a thermodynamic concept, is the potential for performing work. Thus water moves from an area of higher water potential into one of lower water potential (from high to low free energy status).

Water potential differentials drive a wide range of plant metabolic and development processes. Water potential in plant cells is a function largely of three components: ‘osmotic’ or ‘solute’ potential (Ψ_s), ‘hydrostatic pressure’ potential (Ψ_p) and ‘gravity’ potential (Ψ_g). At the cellular level, the gravitational component is generally negligible compared to osmotic potential and hydrostatic pressure (Taiz & Zeiger, 2006). In the case of fruit juice cells, Ψ_s is conferred by their TSS (sugars and acids) content, and Ψ_p by the size of the cell and the rigidity/elasticity of the cell membranes and cell wall.

As water moves from an area of higher Ψ into one of lower Ψ, cells with higher water potential may be at risk of losing water. A cell with a high concentration of solutes has a lower water potential, that is, it will ‘attract’ water, assuming that osmosis (diffusion across a permeable membrane) is possible.

Solute concentration in juice cells

The unusual anatomic isolation of the vesicles within citrus fruit means that fruit cell water potential remains more stable than leaf cell water potential. That is, citrus fruit are less affected by transpiration and translocation of water than leaves (Albrigo, 1978; Kaufmann, 1970). However, citrus fruit are composed of a high volume of water and continuing water supplies are needed for fruit growth. Juice cells could be expected to be strongly influenced by water potential differentials.

The main studies of the effects of water potential differences have been on the effects of water deficits on juice quality. It is well established that fruit from citrus trees under mild water deficits (drought stress) will have comparatively higher TSS (including acid) concentrations (González-Altozano & Castel, 1999; Hutton et al., 2007; Moon & Mizutani, 2002; Peng & Rabe, 1998; Romero et al., 2006; Spiegel-Roy & Goldschmidt, 1996; Treeby et al., 2007; Verreynne et al., 2001). This phenomenon is not simply dehydration but is due to changes in osmotic concentration: plants under water deficits can reduce water potential, minimise water loss and maintain cell turgor by osmotic adjustment or ‘osmoregulation’, that is, by accumulating osmotica or solutes such as sugars (Barry et al., 2004b; Yakushiji et al., 1996). Citrus is not alone in this: during water deficits, assimilates are directed to fruit and away from the roots in many plants (Taiz & Zeiger, 2006).

Yakushiji et al. (1996) demonstrated that, in satsuma mandarins, most of the accumulated solutes in fruit under water stress are monosaccharides i.e. glucose and fructose. Barry et al. (2004b) found that this increase in fructose and glucose concentration does not affect sucrose concentration, suggesting that sucrose hydrolysis takes place, allowing a continuing osmotic gradient in favour of increasing sucrose movement to fruit.

Thus, fruit under water deficit regimes is typically higher in sugar concentration. Note that studies indicate that increased sugar levels are independent of fruit size and juice content, indicating that this is not due simply to passive dehydration of juice sacs or concentration effects (Barry et al., 2004b; Romero et al., 2006; Yakushiji et al., 1998; Yakushiji et al., 1996).

Albrigo (1978) and Romero et al. (2006) found that fruit on trees with less water stress have lower juice content at maturity (the latter related to water stress in Phase III). Albrigo suggests that such fruit have lower TSS and thus have less ability to hold water against long term movement to the peel and then to leaves, and thus over time the fruit loses water.

Low solutes plus high turgor increase cell water potential

It is possible that in granulation, the characteristic enlargement or hydration of vesicles and the reduced levels of TSS may have the opposite effect on juice cells than effects on fruit under mild water deficits. Large (or enlargened) vesicles may have a higher water potential because they (i) have a higher Ψ_s as they are low in solutes such as sucrose, glucose, fructose and/or (ii) have a higher Ψ_p as the large size may mean that the cell is highly hydrated and turgid and thus the cell wall and possibly the vesicle epidermis exerts higher turgor pressure. The cell size may not even need to be large if the water potential increase caused by solute dilution is sufficient.

High water potential effects on cell wall thickening

The question as to how water potential might be linked to cell wall thickening and gelation characteristic of granulation needs to be considered. There are at least two possible answers.

Firstly, it may mean that enlargened cells and vesicles are at risk of losing water to tissues such as peel or leaves with lower cell water potential. The phenomenon of cell wall thickening may thus be a response to reduce the risk of water loss. Cell wall thickening may be triggered at a certain level of water potential or soluble solute concentration. However, the anatomical isolation of citrus juice cells from the rest of the plant that reduce the risk of water loss might make this explanation seem unlikely.

An alternative explanation is that cell wall thickening is due to the limitations to growth of juice cells within the fruit, driven by increased turgor pressure. While cell growth has in the past generally been explained by a process of cell wall extensibility or 'loosening' induced by auxins, turgor pressure has been shown to have an important role in inducing growth in plant cells and in promoting cell wall assembly as volume increases (Proseus & Boyer, 2005, 2006; Zonia & Munnik, 2007). Turgor pressure causes high polysaccharide concentrations, drives polysaccharide deposition and insertion into cell walls, and gel formation in pectins, thus promoting cell wall assembly as the cell expands (Proseus & Boyer, 2006). This mechanism, which operates even at 'normal' turgor pressure of 0.5MPA, has been described only for cell growth processes, but it is possible that turgor pressure may have a role in the cell wall thickening processes in granulation. It is possible that the juice cells have limited room to expand, thus polysaccharide deposition may have the effect of thickening cell walls without further cell expansion.

In the process of cell wall thickening, it may be that soluble sucrose is converted into cell wall components as suggested by Burns (1990). The conversion of sugars into cell walls would in turn increase water potential by further reducing osmotic concentration. If the reduction in water potential is the trigger for cell wall thickening, then this presents an increasing spiral of reduced sugars → thickening of cell walls → reduced sugars and so on. Thus water potential increase early in fruit development, from, for example, high rainfall in spring, may be more detrimental than water potential increase late in fruit development.

How water potential might explain key factors associated with granulation

The two key processes in granulation may thus be hydration of the juice cells and 'dilution' of solutes. If cells and vesicles become enlarged and hydrated through rapid growth of the fruitlet and/or ready availability of water, or are large because of genetic traits, they will have a higher turgor pressure (Ψ_p). Secondly, if osmotic potential (Ψ_s) increases due to dilution of TSS, or generally lower TSS levels as a result of competition between sinks or inadequate translocation, this may serve to exacerbate granulation.

The water potential hypothesis thus explains the additional factors noted above that have not been explained by the competitive sink hypothesis, and strengthens some others i.e.

- why some vesicles granulate while adjacent vesicles do not, if vesicles differ in size and thus in turgor pressure and possibly also solute concentration, and because vesicle walls are relatively impermeable, limiting exchange of solutes from vesicle to vesicle;
- why frequent irrigation exacerbates granulation, if it affects cell turgor. This may be of more relevance than the effect on fruit size. Most studies suggest an increase in irrigation equates to an increase in citrus fruit size (Albrigo, 1978; Erickson & Richards, 1955; Hutton et al., 2007; Sites et al., 1951; Spiegel-Roy & Goldschmidt, 1996). However, Verreynne et al. (2001) found that irrigation deficit had no major effect on fruit size, although they note that while 'deficit' irrigation amounted to 66% of 'normal' irrigation volumes, water content of the soil was the same for the deficit and the normal block, suggesting routine overwatering of the normal block.

In addition it may be possible that high water potentials are an alternative explanation for factors that may also be explained by the competitive sinks hypothesis; alternatively, the two hypotheses may be working together. These include:

- the increased granulation in an 'off' year crop. Yonemoto et al. (2004) established that crop load affects the sap flow in branches: a heavy crop load has lower sap flow. The reduced flow in an 'on' year could be expected to reduce the risk of higher water potentials.
- increased granulation incidence in large fruits. Large fruit are likely to have a larger proportion of large, hydrated vesicles.
- increased granulation of fruit on the shaded side of the tree. Leaf water potential is lower on the sunny side, as the gradient for transpiration is higher (Albrigo, 1978; Camacho-B et al., 1974).
- the increased prevalence of granulation in coastal areas. Fruit grown near the coast tends to have a higher percentage of water in pulp (and peel). This may mean higher turgor (Bevington & Castle, 1985).
- granulation at the stem end before the stylar end in some varieties. As well as the (marginally) lower concentrations of sugars at the stem end, Hwang et al. (1990) notes that juice vesicles at stem and stylar ends are generally larger than those in the centre of the fruit (grapefruit). However, Kaufman (1970) found that water potential (osmotic potential) increased slightly (around 1 bar from -12.9 to -11.9) from the stem to the stylar end in Navel oranges, which is the opposite to what would be expected if granulation is associated with higher water potential. Granulation tends to occur in the centre of navel oranges, making this a curious finding.

Note that this hypothesis incorporates the 'competitive sinks' hypothesis outlined above, in that sink competition and/or translocation factors would drive increases in solute water potential (Ψ_s).

Sucrose concentration in sap

While sink competition has attracted most comment from researchers, overall availability of sucrose in the sap (how diluted it is) is likely to also be a significant contributor. *In vitro* studies of cultured juice vesicles suggest that the sucrose concentration in the supply to vesicles is important: vesicles cultured on lower sucrose medium (5% sucrose) grow in size but absorb less sugar than those on higher concentrations (10% sucrose) (Harada et al., 2001; Mukai et al., 2001).

Lower overall availability of sucrose would explain why declining trees increase in granulation. This is not a product of competition between sinks per se but an overall lack of carbohydrate availability due to reduced photosynthesis and/or use of photoassimilates to fight infection/disease. It would also help explain why granulation is more prevalent in coastal areas as coastal fruit generally has a lower content of TSS.

Two factors, rootstock effects and seasonal effects, are considered further in the light of these four hypotheses.

The possible role of rootstocks

The effects of rootstocks on granulation are generally explained as affecting the vigour of the tree and thus the competitive carbohydrate supply to the fruit.

TSS content

The view that granulation is linked to rootstocks that produce fruit of low sugars appears to have some credence. Both rough lemon and Volkameriana lemon rootstocks, which show high frequencies of granulation, are considered to be rootstocks that produce low sugar fruit (Albrigo, 1978; Castle et al., 1993). Trifoliate orange has high levels of soluble solids and, with the exception of Bartholomew et al. (1941), most studies have found that trifoliate orange has relatively low or intermediate rates of granulation (Awasthi & Nauriyal, 1972c; Benton, 1940; El-Zeftawi, 1978; Lacey & Foord, 2006).

The contribution of the rootstock to low sugar levels, considering that it plays little part in the process of photosynthesis, may be through rootstock influence on production of relevant PGRs, the efficiency of nutrient uptake and/or the water status conferred by the rootstock as it affects the water potential and/or the osmotic adjustment mechanisms of the fruit. Water status could be affected by the hydraulic conductivity of the rootstock and/or its characteristic root extent and distribution.

Hydraulic conductivity

Syvertsen (1981) established that hydraulic conductivities of rootstocks differ. Hydraulic conductivity is how readily water will move through a membrane or a system. Carizzo citrange and rough lemon (seedlings in tubes) were found to have greater hydraulic conductivities than Cleopatra and sour orange. The ranking of rootstocks in order of their hydraulic conductivity reflects the relative vigour of these rootstocks (Syvertsen & Graham, 1985). Work with rough lemon and sour orange seedlings found that rough lemon seedlings had higher growth rates, greater root conductivity and leaf conductance, and greater leaf water potential (Syversten et al., 1983).

Root quantity and distribution also need to be considered alongside hydraulic conductivity. Syvertson (1981) found that:

- rough lemon has a higher hydraulic conductivity and a more extensive root system;
- sour orange has a lower hydraulic conductivity and the largest root system;
- Carizzo citrange (similar to Troyer) has a higher hydraulic conductivity and a less extensive root system; and
- Cleopatra has a lower hydraulic conductivity and a less extensive root system.

As this corresponds well to the general finding that rough lemon rootstocks are more inclined to granulation, and that Cleopatra rootstocks less so, it provides some support for the water potential hypothesis raised above.

Castle and Krezdorn (1973) examined the root distributions of Orlando tangelo trees on well-drained sandy soil and found that rough lemon and Palestine sweet lime rootstocks had a maximum rooting depth of five metres and 50% of roots were below 76 cm i.e. these rootstocks are deep rooted (the trees were also the largest). In contrast, Rusk citrange, Troyer citrange, trifoliate and Cleopatra rootstocks had maximum rooting depths of 3 to 3.5 metres and over 60% of roots above 76 cm, that is, relatively shallow root systems. These results are similar to those of Syvertsen. Tests with Tahiti lime scions in Brazil found that C13 citrange and *P. trifoliata* rootstocks had larger root systems than rough lemon and Volkamer lemon rootstocks in a clayey soil, but the latter (and *P. trifoliata*) had roots that exceeded the tree canopy and there were no significant differences in effective depth between all rootstocks (Neves et al., 2004). On the other hand, Romero et al. (2006) found that trees on Cleopatra had more efficient soil water extraction than trees on Carizzo, and maintained a higher plant water status.

Treeby et al. (2007) tested deficit and partial rootzone irrigation strategies for navel oranges and found that citrange and trifoliate orange rootstocks were most affected in terms of fruit numbers (but not weight or size) than Cleopatra and sweet orange.

Studies that assess both granulation and hydraulic conductivities and root distributions would be needed to further test this hypothesis.

Stem and leaf water potential

Some studies on stem and leaf water potentials also support the water relations hypothesis. Although it is not known whether stem and leaf water potentials correlate strongly with fruit water potential, they may provide some indications of relativities between rootstocks. Crocker et al. (1974) found higher stem and leaf water potentials on Palestine sweet lime and rough lemon rootstocks than on sour orange, Cleopatra mandarin, Carizzo citrange or Trifoliate orange. Camacho-B et al. (1974) found the rootstocks Cleopatra mandarin and Rangpur had lower leaf water potential than Troyer citrange rootstock at high transpiration rates under mild soil water deficits. Barry et al. (2004b) found that under early water stress, fruit from Carrizo citrange rootstocks had higher soluble solids concentration and lower water potential than trees on rough lemon rootstocks, which they attribute to the latter's greater root distribution, density and hydraulic conductivity. Effects of drought stress also manifested later on rough lemon. Albrigo (1978) found, correspondingly, that leaf water potentials were higher on rough lemon rootstock than on sour orange or Carrizo citrange, and also found that juice content was lower and more juice vesicle 'drying' occurred. If 'drying' equates to granulation, this provides support for the hypothesis that water potential and granulation are related.

Inverse relationship between TSS and hydraulic conductivity

The work of Barry et al. (2004) suggests an inverse association between soluble solid content of fruit and hydraulic conductivity/tree water status of rootstocks. Similarly Yonemoto et al. (2004) found for Satsuma mandarin that sap flow was lowest on the dwarf rootstock Flying Dragon compared to trifoliate orange rootstock, but soluble solid contents were higher. They suggest this may be due to lower hydraulic conductivity of roots.

Queensland rootstock trials with Imperial mandarins support this hypothesis (Smith, 2006). Granulation on Volkameriana rootstocks is severe: Brix values for Imperial and Nova fruit on Volkameriana rootstocks are very low. There are high levels of granulation on Troyer rootstocks, which is considered vigorous but intermediate in TSS. Granulation is less common on Swingle and Benton rootstocks. Benton is a vigorous stock but has high Brix levels. Swingle is a vigorous stock but has intermediate TSS levels

Overall, there appears to be strong evidence for a hypothesis that increased levels of granulation are experienced on 'vigorous' rootstocks with high hydraulic conductivities and that produce fruit low in TSS.

The possible role of seasonal patterns

The association of increased granulation with high temperatures in late winter/early spring, followed by heavy summer rainfall, is one of the more difficult factors to explain.

Stress?

Stress could explain why granulation appears to be triggered by high average temperatures very early in fruit development if it triggers cell wall hardening, or if, more indirectly it causes low fruit set, large fruit size and vigorous vegetative or root growth, all of which are associated with granulation. Increased temperatures, and lower relative humidities, can reduce leaf photosynthesis through reduced transpiration as well as photo-inhibition (Guo et al., 2006; Hu et al., 2007; Veste et al., 2000).

The effect of late summer rains, however, is not explained by this theory.

Hastening development?

High temperatures early in fruit development could act not as a stress but to promote fruit development, so that the juice cells are relatively large and may grow even larger at the onset of heavy summer rainfall. Marsh et al. (1999) found increases of 2.4 to 6.5°K (imposed by tunnel houses) during the first stage of fruit development (the cell division stage) increased fruit size by approximately 50% and increased growth rates. Higher growth rates were maintained when temperatures were reduced to ambient temperatures. They also noted that the increase of temperature in these early stages increased water uptake into the fruit, and pedicel diameter, that is, vascular capacity, increased.

However, Marsh et al. (1999) found the effect of increased temperatures on healthy citrus fruit is early accumulation of TSS if temperatures are increased in the early stages of fruit development, and accelerated loss of acid if the increase is in the later stages of fruit development. (Interestingly, increasing temperature during anthesis has a greater effect on subsequent sugar levels than increasing temperatures just after anthesis.) Others have also noted that increased heat units in late spring increases the Brix: acid ratio; but increased heat units in late summer reduces acid (Spiegel-Roy & Goldschmidt, 1996).

Thus, if the water potential hypothesis holds, initial high temperatures would most likely be insufficient to trigger granulation because osmotica levels would be maintained or enhanced: it would need to be followed by some factor that increases water uptake or reduces sugar levels. Some possible additional factors, that is, heavy rainfall or compensatory growth, are discussed below.

Depletion of carbohydrate reserves?

The effect of early heat may be explained by a depletion of carbohydrate reserves. Citrus trees under cultivation outside the tropics utilise large amounts of carbohydrates in a single flowering event. Bustan and Goldschmidt (1998) estimate the processes of flowering and early abscission of fruitlets use about 27% of the annual photoassimilate production. High temperatures during flowering periods work to increase the daily demand for carbohydrates, by accelerating flower development and by increased respiratory demand. It is possible that carbohydrate needs are then drawn from reserves from other tree organs, depleting these for later supply to developing fruit. However, this seems to be unlikely to be a major contributor to granulation in view of the increased TSS levels that result as noted above, and the ability of leaves to supply nearby fruit as it develops.

However, carbohydrate reserves may have an indirect effect on granulation across seasons by regulating the crop load. Goldschmidt and Golomb (1982) found that there was a large difference in root starch content in late winter between an ‘on’ tree (fruit-bearing) and an ‘off’ tree (non-bearing) of the absolute alternate bearing mandarin variety ‘Wilking’. These reserves in the roots have been shown to decline quickly once vegetative growth and flower development start in spring in trees entering an ‘on’ season (Monerri et al., 2011). However, when reserves were low in trees that had finished an ‘on’ season and were entering an ‘off’ season, the subsequent flowering was reduced and accumulation of reserves continued as vegetative growth developed. This implies that fruit set and development draw substantial amounts of carbohydrates and that reproductive organs are a priority sink for carbohydrate reserves.

There is uncertainty how this correlation between the amount of carbohydrate reserves and the subsequent extent of flowering functions and on which signalling pathway it operates. However, whether the carbohydrates themselves act as a signal, or whether hormonal signals are involved, the winter carbohydrate status of a tree is an important indicator of the crop load, and thus granulation, in the following season.

Compensatory growth?

If early heat causes mild water stress, it is possible that a mechanism known as compensatory growth could be contributing to granulation. Huang et al. (2000) established that fruit subjected to a period of mild water stress (approximately one month) in the early juice sac expansion stage grow faster and larger after rewatering than those on regularly watered trees—this phenomenon is referred to as ‘stored growth’ or ‘compensatory growth’. ‘The lasting effect of water stress made the stressed fruit a stronger competitor for water’ (p. 234, Huang et al. 2000). Cohen and Goell (1988) also found that when irrigation of grapefruit was resumed after drought stress of one to two months over various periods in the summer and autumn, fruit from stressed trees grew faster than those from regularly irrigated trees. They suggest this may be due to excess dry matter accumulated during periods of stress.

The phenomenon of compensatory growth may explain the effects of higher temperatures in spring and late summer rains, if these rains lead to rapid cell hydration and thus increased water potential.

Late summer rains contribute to hydration and/or dilution of sugars?

Late summer rains could simply have a hydration and/or dilution effect at later stages of development, regardless of early heat. If water is available, the sucrose content of the liquids supplied by the phloem could be diluted. Similarly, photoassimilates may be directed into root or vegetative growth at this time, making it less available to the fruit. It may also be that humidity and overcast skies at this time reduces the transpiration and photosynthetic capacity of the tree, making photoassimilates less available while water supplies are abundant. It would appear that this hydration effect causes granulation because the fruit are at an advanced stage of development and relatively large.

In summary, possible explanations for the effect of this pattern are that high temperatures may be providing either a stress or a stimulus at the early stages of fruit development; and the later rainfall may increase fruit water potential and/or reduce carbohydrate supply through stimulating root and/or vegetative growth.

Conclusions

It is clear that climatic, water relations, varietal and rootstock differences, crop load and individual tree factors play a part. A range of factors such as minerals and PGRs appear to be more indirect or secondary to these major factors.

The fundamental causes of granulation remain uncertain. The main hypothesis advanced by researchers to date is that granulation is the result or cause of low TSS levels in the fruit due to sink competition, and the research data supports that this is, at least, a contributing factor.

Our hypothesis after reviewing the literature is that the low soluble solids in juice cells are acting to increase granulation through increasing cell solute water potential (Ψ_s). High levels of pressure water potential (Ψ_p) due to the increased turgor of hydrated cells may also increase cell water potential. High water potential may cause cell wall thickening as a mechanism to reduce water loss to other plant tissues, or may be due to increased polysaccharide deposition due to restricted growth of juice cells in a hydrated environment.

If the water potential hypothesis is valid, key management strategies would involve influencing the two key components of high water potential: fostering high sugar levels to decrease Ψ_s and/or reducing excessive hydration and enlargement of the juice cells to reduce Ψ_p. The main strategies would include irrigation management, selection of rootstocks that are characterised by high TSS but low water conductivity, nutrition management to increase TSS, and minimising low crop load years.

Glossary and abbreviations

2,4,5-T	2,4,5-trichlorophenoxyacetic acid. 2,4,5-T is an auxin.
2,4-D	2,4-dichlorophenoxyacetic acid. 2,4-D is an auxin.
356-TPA	tryclopyr (a synthetic auxin)
Albedo	or mesocarp is the white material between the fruit's pulp and peel
Extent of granulation	in most studies used to refer to the proportion of individual fruit affected i.e. severity, degree within the fruit (*cf* 'incidence')
IAA	indole-3-acetic acid
Incidence of granulation	in most studies used to refer to the proportion of crop affected (*cf* 'extent')
NAA	naphthaleneacetic acid
TSS	total soluble solids (acids and sugars)
Water potential	a measure of the free energy of water per unit volume. Water moves from an area of higher water potential into one of lower water potential (from high to low free energy status).

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Appendix 8 Granulation in Imperials:

Summary of Queensland research trials

The Australian citrus industry, Queensland Government and Hort Innovation have funded several research projects on farm management practices to reduce the incidence and severity of granulation in Imperials since 2003.

This document aims to provides a succinct summary of this research. It should be read in conjunction with "Advice to growers". Details of results are available in the following final reports (copies are available on request):

- CT03029 Management of internal dryness of Imperial mandarin. Final report September 2004, G. Fullelove, K. Walsh, P. Subedi, R. Shaw, G Pinnington,
- CT04002 Management of internal dryness of Imperial mandarin Final report 31 December 2010, H. Hofman.
- CT11007 Management of internal dryness of Imperial mandarin—extension. Final report 31 May 2013, H. Hofman.
- 2015/16 DAF trial (unfunded, in kind contributions from Nutrano P/L). Effects of varied applications of nitrogen and potassium on granulation in Imperial mandarin, unpublished report, June 2016, H. Hofman.
- CT19005 Reducing granulation in the production of Imperial mandarins (includes initial trials funded by DAF, Spencer Ranch, Nutrano and Mundubbera Fruit Growers Association). Project currently underway.

For abbreviations used, see list at the end of this document.

This document has six parts:

Unless stated otherwise,

- All trial designs were randomised block designs, with a minimum of 4 replicates, and with appropriate guard trees.
- Treatment means are generally '% unacceptable fruit' (*not* mean granulation rating), abbreviated as 'unacc%'. 'Crunchy%' is used in some instances where there were negligible levels of unacc%. See 'Definitions' for details.
- Treatment comparisons (i.e. 'reduced' or 'increased' granulation) in 'Results' are to the 'control' treatment in the trial outlined.
- Control treatments received the same agronomic inputs as other Imperial blocks on the property in which the trial was located. This may vary from year to year.

Table 1 Nutrition trials

Trial, project, dates and treatments	Results	Comments
Identifying key nutrients (CT04002 Trial A 2007/08, 08/09 and 09/10) • **extra N in Stage I** (0.65 kg urea in Yrs 1&2, 0.35 in Yr3) • **extra N in Stage II** (0.65 kg urea in Yrs 1&2, 0.35 in Yr3) • **N foliars** (x 2 in Yr2, x 5 in Yr 3), treatment added in Yr 2 • **high K** (addl 1.8 kg SoP in 2 appns) • **low K** (0.9 kg SoP) • **high P** (addl 2.6 kg SuperMo in 2 appns in Yr 1, 0.75L/tree of Polyphos in Yrs 2 and 3) • **high B** (addl 2 foliars) • **low B** (no foliar), treatment discontinued after Yr 1 • **soil-applied extra Zn**, treatment added in Yr 2 • **high Zn** (addl 2 foliars) • **low Zn** (only 1 x Zn foliar), treatment discontinued after Yr 1 • **control**	• 'Extra N in stage I' reduced granulation the most (only sig. in Yr 2 when unacc% reduced from 22% to 9%, *P*=.011; Yr 1 was 50%→ 44%, *P*= .513; Yr 3 was 20%→12%, *P*= .414). • 'High Zn' (foliars) looked promising but not sig. (Yr 1 unacc% 50%39%, *P*=.513; Yr 2 22%→14%, *P*=.011; Yr 3 20%→16%, *P*=.414) . • 'Soil-applied extra Zn' increased granulation although not sig.	• These results, along with that for Trial B, suggest that, except for N, specific deficiencies or toxicities are not the main issue in granulation. • The efficacy of additional N in Stage I most likely due to insufficient application in winter rather than timing of application in spring. • Lack of improvement from N application in Stage II points to importance of early fruit development. Analysis of soil N levels suggests Stage II applications were not taken up by trees. • The early promise of Zn was not supported in subsequent trials (see Trial D, K, M). • 'Control' applications/tree were urea =.65 kg in Yr1 and .96 in Yrs 2 and 3 (recommended application is 800gN = 1.7kg urea), SoP=1.5 kg in Yr 1, 1.8 in Yr 2 and 1.9 in Yr 3, 2 x Zn foliar in Yrs 1 and 3, 1 x Zn foliar in Yr 2, and B foliar in Yrs 1 and 3.
Identifying key nutrients (CT04002 Trial B 2007/08) • **low N** (no broadcast urea) • **extra N in Stage I** (addl 1.2 kg urea) • **extra N in Stage II** (addl 1.2 kg urea) • **high K** (addl 1.8 kg SoP in 2 appns) • **low K** (0.9 kg SoP) • **high P** (addl 3.6 kg SuperMo in 2 appns) • **high Zn** (= 2 foliar sprays) • **low Zn** (no foliar) • **control**	• Only sig. difference in treatment means was for 'low N' which produced **higher** granulation (unacc% for control was 24%, for 'low N' was 40%, *P*<.001).	• This trial confirms the importance of N nutrition over other elements. • N applications for the control were closer to recommended rate than in Trial A: this may help explain the lack of treatment differences in this trial. • 'Control' applications/tree were urea =1.2 kg, SoP=1.5 kg in 2 appns, 1 x Zn foliar.
Extra N, K and Zn (CT04002 Trial K 2008/09) • **Extra foliar Zn in Stage I** (3x foliars) • **broadcast Zn in Stage** I (.5 kg of $ZnSO_47H_2O$) • **extra broadcast N in Stage I** (addl 0.6 kg urea) • **extra double broadcast N in stage I** (addl 1.2 kg urea) • **foliar N Stage I** (2x 1.5% LoBi foliars) • **extra K in Stage I** (2kg SoP in 2 appns)	• Best result from 'extra broadcast N in Stage I' but not sig. (unacc% reduced from 50% for control to 35%, *P*=.097).	• As for Trial A, this trial suggests the importance of N in early fruit development. • The inefficacy of 'extra double N' treatment may be due to increased pH inhibiting nitrification at a critical period. • Control applications were urea =1 kg, SoP=1.5 kg, Zn foliar, KNO_3 foliar. 1 kg urea = 470gN, less than

Trial, project, dates and treatments	Results	Comments
• **extra NPK in Stage I** (1.5 kg of Nitrophoska + 0.22 kg urea) • **control**		recommended for mature trees. So again, the efficacy of additional N in Stage I may be due to insufficient application in winter.
Foliar nutrients (CT04002 Trial M 2009/10) • **Foliar N in Stage 1** (1.5% LoBi x 5 appns) • **foliar N+Zn in Stage I** (x 5 appns of $ZnSO_4 7H_2O$ at 100g/100L or Zinc100 at 50ml/100L plus 1% LoBi) • **foliar Ca in Stage I** ($Ca(NO_3)_2$ at 1% x 5 appns) • **broadcast N in Stage I** (addl 0.6 kg urea in Sep) • **control**	• 'Foliar N in Stage 1' and 'broadcast N in Stage I' sig. reduced granulation compared to the control (unacc% = 19%, 17% and 47% respectively, *P*= .011). • Note 'foliar N+Zn in Stage I' did not have same effect (unacc%=42%). • No effect of Ca treatment.	• Control applications prior to treatments were urea =1 kg, SoP=1.5 kg, Zn foliar, KNO_3 foliar and $Ca(NO_3)_2$) foliar. 1 kg urea = 470gN, less than recommended for mature trees. So again, the efficacy of additional N in Stage I may be due to insufficient application in winter. • Foliar N applications were surprisingly effective – compare to less promising results in Trial A and Trial C.
Timing and rate of broadcast N (CT04002 Trial P 2009/10) • **Control N** (1.2kg urea broadcast in July) • **low N in July** (0.6 kg urea) • **August N** (1.2kg urea) • **split July/August** (0.6 kg urea each date) • **zero N**	• 'Zero N' sig. **increased** granulation (unacc% increased from 45% to 94%, *P*=.049). • 'Low N in July' had higher granulation on average (unacc%=63%) but not sig. • No sig. difference between other treatments.	• No guards between 3-tree plots. • 'Zero N' also ~ halved crop load. • Confirms importance of sufficient winter N. • Timing of July or August application of N seems unimportant: volume more important.
Timing of broadcast N (CT11007 Trial B(2) 2012/13) • Single early winter (700gN July) • early winter and pre-flowering (470 gN July, 230gN Aug) • early winter and early Stage I (470gN July, 230gN Oct) • autumn (700g N May) • equal autumn, winter, pre-flowering (167 gN each May, July, Aug) • single early winter (470gN July)	• No sig. treatment differences (*P*=.688).	• Single early winter application of 470gN July considered common industry practice at the time (promotes early fruit colour). • Rate of N application made very little difference in this trial, possibly because crop load was uniformly high. • In conjunction with Trial P and Trial C(2), results suggest that as long as N goes on pre-flowering, specific timing will make little difference.
Broadcast N rate X timing (CT11007 Trial C(2) 2012/13) • 500 g N broadcast • 700 g N broadcast **X** • single early winter application • early winter and pre-flowering • early winter and early Stage I • autumn • autumn and pre-flowering • equal applications autumn, winter, pre-flowering	• No sig. treatment differences (in mean granulation) for either factor: *P*(rate)=.312, *P*(timing)=.180. • Interaction between rate and timing factors is sig. (*P*=.009) but results don't make sense.	• 40 of 193 trial trees lost in flooding or burning of debris after flood. • Probable that soil moisture overrode any N application effects on granulation in this trial. • In conjunction with Trials B(2) and P, appears that as long as N goes on pre-flowering, specific timing will make little difference.

Trial, project, dates and treatments	Results	Comments
Winter N rate X K rate (Unfunded 1-year trial 2015/16) • 700, 450 or 333+200 (see comment) gN per tree **X** • 350, 500 or 700 gK/tree (base appn to all tmnts in July of 94gK in Nitrophoska, then equal split appns in Sep and Dec)	• 333gN/tree, topped up with 200gN in December, granulated **more** than 450g and 700g treatments (unacc%= 60%, 31% and 15% respectively, *P*<.001). • No difference between K treatments (unacc%=41, 31, 34% for 350g, 500g or 700g respectively, *P*=.283). • No interaction between N and K treatments (*P*=.863).	The lowest rate of N applied in winter was 333gN/tree: this resulted in leaf yellowing in December, so an additional 200gN/tree was applied in December. This split treatment had the highest rate of granulation, suggesting the December appn, while it regreened the trees, was too late to improve fruit quality. This reinforces the need for N to be applied before flowering and at the recommended rate (100gN/tree per year of age up to a maximum of 800gN).
See also irrigation treatments in Table 4 Late action trials		

Table 2 Irrigation, and irrigation X nutrition trials

Trial, project, dates and treatments	Results	Comments
Irrigation frequency X foliars (CT04002 Trial C 2007/08) **Reduced irrigation frequency** in Stage I, Stage II, Stage III, , Stages II+III, all stages or control **x** **Foliars** in Nov, Dec, Jan and Feb: N, P, B, Zn or control	• Best result for 'Reduced frequency in all stages' but not quite sig. (unacc%=73%, control=93%, *P*=.075). • Best foliar treatment results were for N and Zn (unacc% of 80% and 82% respectively compared to 89% for control, *P*=.01). • No interaction between irrigation and nutrient treatments.	• An extra sprinkler per tree was used in low frequency treatments and there were 5 'taps off' v 6 'taps on' irrigations so effectively ***volume was not reduced.***
Irrigation frequency X extra N (CT04002 Trial J 2008/09 and 2009/10) **Reduced irrigation frequency** in Stage I, Stage II, Stage III, , Stages II+III, all stages or control **X** • **Extra broadcast N in Stage** I (0.6 kg urea) • **control**	• 'Extra N in Stage 1' reduced granulation, only sig. in Yr 2 (unacc% reduced from 57% to 50% in Yr 1, *P*=.106; and 42% to 16% in Yr 2, *P*<.001). • No benefit from reducing irrigation frequency in any treatment. • No interaction between irrigation and nutrient treatments.	• An extra sprinkler per tree was used in low frequency treatments. There may not have been sufficient difference between treatments: 2008/09 was OK (6 'taps off' v 9 'taps on' irrigations and 6 rainfall events >10mm), but in in 2009/10 there were 4 'taps off' v 7 'taps on' irrigations and 11 rainfall events >10mm. • Yr 1 appn of extra N was in late Oct; Yr 2 in early Sep. The Yr 1 date may have been too late. • 'Control' appn of N was 1 kg of Granam = 202gN – well below recommendations.

Trial, project, dates and treatments	Results	Comments
Irrigation frequency X extra N and Zn (CT04002 Trial D 2008/9 and 2009/10) **Reduced irrigation frequency** in Stage I, Stage II, Stage III, Stages II+III, all stages, or control **X** • **Extra broadcast N in Stage I** • **extra broadcast N in Stage I + foliar Zn** (2-3 appns) • **control**	• in Yr 1 best results for 'Reduced frequency in Stage I' and 'Stage III' but not sig. (unacc%=19%, 21% respectively, control=27%, *P*=.100). • This trend **reversed** in Yr 2 (unacc%=29%, 26% respectively, control=14%, *P*=.016). • In Yr 1 but not Yr 2, 'extra N in stage 1 +foliar Zn' sig. reduced unacc% (from 31% to 21%, *P*=.024). • No interaction between irrigation and nutrient treatments.	• Similar constraints to the above. • The reversal in irrigation outcomes between the two years suggests an overriding crop load influence: however, our measurements do not show any treatment differences in crop load in either year. A puzzle….
Irrigation volume and frequency- pot trial (CT04002 Trial N 2009/10) • **Reduced irrigation volume and frequency** in Stage I, Stage II, Stage III, Stages II+III, all stages or control • Treatment trees irrigated at -55kPa in applicable times; non-treated/control trees watered every 2 days till early November, then daily	• Granulation was lowest for 'reduced volume and frequency in Stage I' and 'all stages' (unacc%=52%, 38% respectively, control=106%, *P*=.002). (Note: control is >100% due to adjustment of results for crop load variations).	• 2-year old trees on Trifoliata rootstock in 40cm pots under polyethylene. • Nutrient applications ceased in May before trial commenced in order to exacerbate granulation. • Fruit size most affected in 'all stages' (60.2mm, v. 68.5 for control) but not sig. (*P*=.072) • Best indication of trials in the first project that less **volume** of irrigation, perhaps even some water stress, reduces granulation: reducing **frequency** without also reducing volume appears insufficient.
Irrigation volume (CT11007Trial A(2) 2012/13) • Control • Control with 'Tyvek' rain protection, • **Reduced irrigation volume (~50%) in Stage I** with 'Tyvek' • **Reduced irrigation volume (~50%) in Stages II&III with 'Tyvek'** (until flood late Jan)	• Granulation **higher** for 'control with Tyvek rain protection' but ns (unacc%=56.5 compared to 'control'=20.6% (*P*=.128). • No improvement from other treatments.	• Flooding in late January destroyed trees, Tyvek, tensiometers and irrigation infrastructure. • Tensiometers indicate Tyvek acted to **retain** soil moisture rather than prevent water infiltration until late November. This may explain higher granulation. • Tyvek also caused some leaf yellowing, possibly due to lack of N uptake due to excessive soil moisture.
Irrigation deficits x 5 rates of winter nitrogen (CT19005 Trial 1 2017/18 to 2021/22) • Control and deficit irrigation **X** • 250, 400, 550, 700 or 850 gN/tree	• Overall, in three out of five trial years, deficit irrigation reduced granulation (unacc% in Yr 1 48% → 38%, *P*=.017; Yr 2 9% →1%, *P*=.012; Yr 5 62%→48%, not quite sig. *P*=.08). • In Yrs 1-4, **lower N** treatments had **higher** granulation but not sig. in any year. In Yr 1 unacc% ranged from 50% to 40% (*P*=.440); in Yr 2 from 8% to 2% (*P*=.348); in Yr 3 from 6% to 3% (*P*=.584), and in Yr 4 from 1 to 0% (*P*=.400).	• In Yrs 3 and 4, where irrigation deficits were ineffective, irrigation volume in the control was low compared to common industry practice. • The Yr 5 'reversed' trend for N is probably due to low crop loads increasing flush growth, although this was not clear from our observations. • In each year, the N treatments and the irrigation treatments have a similar range in possible improvements, but the data for N treatments is much more variable.

Trial, project, dates and treatments	Results	Comments
	• In Yr 5, **higher** N treatments had higher granulation; range 46% to 67% (*P*=.195).	
Staged irrigation deficits (CT19005 Trial 3 2019/20 to 2022/23) Reduced volume of irrigation for 6, 12 or 18 weeks after flowering cfed control (no deficit)	• In 2019/20 reduced granulation with progressively longer deficit treatments (but *P*=.275) • In the following three years, there was no difference between treatments	• Strong spatial trends to granulation in this trial. Soil investigations show granulation is higher in sandier plots, particularly where above a clay layer • Granulation correlates well with tensiometer measurements i.e. lower soil tension
Irrigation deficit levels (CT19005 Trial 6 2020/21 to 2022/23 • Control (little stress) • Medium stress • Signficant stress	• In all years, deficit irrigation reduced granulation but there was no difference between 'medium' or 'sig.' stress treatments. In Yr 1, 1% v 0.5% v 0% for 'little', medium' and 'sig' stress , *P*=.254; Yr 2 24.4% v 14.2% v 10.8%, *P*=.034; Yr3, 25.6% v 14.1% v 12.7% (*P*=.022).	• Lack of separation of results between 'medium' or 'sig.' stress due to the difficulty of differentiating between these in the amount of water applied.

Table 3 Plant growth regulator trials

Trial, project, dates and treatments	Results	Comments
Winter GA (CT03029 Exp 1 2003/4) • mid May 10 ml/100L Pro Gibb • early June 10 ml/100L Pro Gibb • control	• early June GA treatment reduced granulation but not mid-May treatment (78%, 84%, 76% 0-rated fruit in order of tmts listed at left).	• 3 replicates, 10-tree plots. • Low incidence of granulation in this season. • Contrasts with lack of improvement with June GA application in 'Flush manipulation trial' (CT19005 Trial 2).
356-TPA (auxin) on 8 dates in Stage I (CT03029 Exp 2 2003/4) • 9 treatments: • Maxim @ 10 ppm on 8 dates 1 week apart from 21/10/2003 to 8/12/2003 • control	• % 0-rated fruit in control =78%, on spray dates from earliest to latest = 86%, 85%, 96%, 85%, 82%, 99%, 94%, 94%.	• Only 3 trees per 3 treatment, non-randomised design. • Low incidence of granulation in this season. • Author states 'trend for less granulation with later treatments' but no statistical confidence reported and this conclusion may be optimistic in light of variability of results.
Plant growth regulators (CT03029 Exp 3 2003/4) • Uniconazole (Sunny at 1L/100L) on 10/12/2003 • Handthinning plus 300g KNO_3 (broadcast) on 16/12/2003 • Handthinning plus 300g KNO_3 (broadcast) plus 20 ppm Maxim (#56-TPA) on 16/12/2003	• no sig. treatment differences. % 0-rated fruit in order of treatment list on left = 59%, 63%, 46%, 48%, 67%.	• 5 replicates, appear to be single-tree plots, but not stated.

Trial, project, dates and treatments	Results	Comments
• Ethrel (ethylene) at 1L/100L on 9/3/2004 (at onset of vegetative flush) • control		
Uniconazole (CT03029 Exp 4 2003/4) • Sunny (uniconazole) at 1L/100L in early Stage II (10/12/2003) • control	• no sig. treatment differences. % 0-rated fruit for Sunny= 61%, for control=48%.	• 5 replicates, appear to be single-tree plots, but not stated.
PGRs applied Nov, Dec, Jan (three applications) (CT04002 three duplicate Trials F, G, H 2007/08) • Auxin: 356-TPA (triclopyr) as 'Tops' at 20pp a.i. • Auxin: 2,4-DP as 'Corasil' at 200 ml/100L • GA_3 as 'Ralex' (=40g/L GA) at 50ml/100L • Seaweed concentrate 'Kelpak'at 200 ml/100L Trial F also included 1 application on 20/11 of the same products	• No reduction in granulation from any treatments at any trial. • At one trial (H), 'Tops' treatment **increased** granulation (unacc%=53% compared to 14% for the control, not sig. *P*=.054)	• Effect of 'Tops' treatment at Trial H may be linked to fruit growth which was greater for 'Tops' treatment. This higher growth rate was not observed in the other two trials.
Flush manipulation (CT19005 Trial 2 2019/20 to 2021/22) • Control • Girdling – *see Table 5 Other strategies* • GA (ProGibbSG at 2g/L) 1 appn June • Paclobutrazol (AuStar at 0.7%) 1 or 2 appns • Paclobutrazol (AuStar at 8.5ml in 1L water): collar drench • Prohexadione-calcium (RegalisPlus st 50g/100L) 1 or 2 applications	• GA increased early flush growth in 3 of 4 years; however, had no effect on granulation. • In Yr 2, 1 x foliar paclobutrazol had sig. less granulation (unacc% 9% compared to 22% (*P*=.013). • In Yr 4, some paclobutrazol treatments had sig. less flush and 10-20% less unacc% than the control which had 61%, but ns (*P*=.126). • No improvement from prohexadione-calcium treatments in any year.	• We did not experiment with different rates of PGRs in this trial, focusing instead on dates, so it is possible higher rates may have some success. • The paclobutrazol results were hopeful in lower crop load years, but results are inconsistent, which is not encouraging.
Flush manipulation (CT19005 Trial 4A 2019/20) • Control • Paclobutrazol (AuStar at 0.7%) 1 or 2 appns early/ late Sep • Prohexadione-calcium (RegalisPlus st 50g/100L)1 or 2 applications early/late Sep	• No sig. difference between treatments (*P*=.909).	• High crop load in this trial year probably helps explain complete lack of treatment effects, but adds to the evidence that growth retardants are not the silver bullet.
Flush manipulation with paclobutrazol (CT19005 Trial 4 2020/21 to 2022/23)	• In 2020/21, high crop, no unacc% in any treatment. • In 2021/22 all paclo treatments had less granulation than the control (but see comment right). Unacc% was 20.6% (control) v 2.5% (1st drench date) v 9.5% (2nd drench date) and 8.1% (foliar)(*P*=.207)	• 2021/22 results may be biased by the later picking of the control treatment by about 2 weeks in the 2020/21 year. The control had the lowest crop load rating of all treatments in 2021/22 (*P*=.020)

Trial, project, dates and treatments	Results	Comments
	• In 2022/23 unacc% not sig. different but 'crunchy'% was 12.4% (control) v 3.8% (1st drench date) v 7.5% (2nd drench date) and 18.2% (foliar)(*P*=.01)	

Table 4 Late action trials

Trial, project, dates and treatments	Results	Comments
Late management action (CT19005 Trial 7 2021/22**)** Wet spring, wet autumn and control irrigation tmnts. In 'wet spring' treatment, 6 late action treatments: • Control • Quick-N (9/11) • 4 x foliar N (LoBi at 1.5% w.v.) applied 8,11, 16/11, 3/12 and 13/12 + deficit irrigation from 4/12-25/1 • GA at 15ppm+ LoBi at 1.5% w.v. applied 8/11 + deficit irrigation from 4/12-25/1 • Fruit retention mixture (GA at 15ppm as ProGibbSG, 2-4,D at 10ppm as StopDrop and 1% $CaNO_3$) applied 7/11 + deficit irrigation from 4/12-25/1	No sig. treatment differences except for **higher** granulation from 'fruit retention mixture' (*P*=0.016).	• Not much difference between irrigation treatments due to frequent and high rainfall. • Nevertheless, granulation across the trial was high and **none** of the late-action treatments helped. • GA treatment applied later than is recommended for fruit retention (i.e. late bloom).
Simulated high rainfall x late management action (CT19005 Trial 8 2022/23) • Control • Simulated wet summer • Simulated wet spring • Simulated wet spring, deficit irrigation in summer. **X** • 20 ppm GA (as ProGibbSG at 90% petal fall • 4 x foliar B (100g/100L of Solubor) (28/9 - 9/11). • 4 x foliar Ca (2% w/v of $Ca(NO_3)_2$) (28/9 - 9/11).	• No treatment effects from irrigation treatments • GA treatment the only one of the foliar tmnts that sig reduced granulation with crunch% 10.7% compared to 18.1% in control (*P*=.023.	• The GA treatment was not applied 'late' but during flowering. • The GA treatment increased crop load.
Very late action (CT19005 Trial 9 2022/23 Control x simulated late rainfall (1/2/2023 to harvest) x Foliar treatments 8 4 sprays fortnightly from 13/12 to 24/1 1. Control	No sig. treatment effects from the irrigation or any of the foliar treatments.	Crop load high and granulation very low overall in this trial so may not have been the best material.

Trial, project, dates and treatments	Results	Comments
2. Boron (100g/100L of Solubor) 3. Calcium (2% w/v of Ca $(NO_3)_2$) 4. Boron+ calcium (as above) 5. Potassium (3% w/v of KNO_3)		

Table 5 Other strategies (thinning, girdling, late pruning)

Trial, project, dates and treatments	Results	Comments
Thinning (CT04002 Trial E 2007-08) Thinning mid January (mean fruit diameter = 41mm) • control = normal commercial (leaving 10-15 fruit per .125m^3) • heavy thinning = 5-7 fruit per .125m3 • strip half tree • strip alternate limbs • heavy thinning alternate limbs	• 'Heavy thinning' and 'strip alternate' limbs had **higher** granulation but not sig. (unacc% = 89%, 92% respectively, control=76%, *P*=0.177). • **BUT** these treatments had sig. **lower initial crop loads** (8.3, 9.6 fruit per .125m^{3}respectively, control=13.6, *P*=.023).	• Thinning was quite late in season, but clearly didn't help. • Other trials (See CT04002 Trial I) and surveys make clear that naturally low crop load generally means higher granulation. • The stronger influence of natural crop load suggests, hypothetically, that the trees' ability to support flowering and early fruit development with stored resources is more important than carbohydrate flows later in the season.
Thinning (CT04002 Trial I 2007-08) • Naturally high crop load • Naturally low crop load • Naturally variable pattern • Heavy thinning of high crop (end January) • Stripping alternate limbs of high crop (end January) Block thinned commercially in same week before treatments applied (mean fruit diameter = 43mm)	• Treatment 2 'Naturally low crop' was **more granulated** than 'naturally high crop', 'heavy thinning' or 'stripping alternate limbs' (unacc%=86% compared to 63%, 70% and 78% respectively, not sig. *P*=0.077).	• The results emphasize influence of natural crop load. • Results, including no improvement from 'stripping alternate limbs', suggest little movement of resources between branches at later stages of fruit development.
See also 'hand thinning plus KNO_3' treatments in Table 3 Plant growth regulator trials		
Girdling in late winter and spring (included in flush manipulation trial 4A CT19005 2017/18 only)	• On whole tree basis, limb girdling in 2018/19 had no effect on granulation whether done in August, September or October (*P*=.325), and addl foliar N made no difference, (*P*=.952).	• Girdling dates were all quite late to try to affect flush rather than flowering. Earlier timing to promote flowering and its effect on granulation remains untested.

Trial, project, dates and treatments	Results	Comments
• Girdling of ~50% of branches on 2/8/18, 7/9/18 or 12/10/18). Both 2mm and 5 mm girdles were tried. • Girdling as above + foliar N (1.5% in August, 1% in September and October)	• Girdled limbs had slightly higher unacc% (18.6% v 13.8% *P*= 0.027). • No effect on granulation in the following season.	
Late pruning (CT19005 Trial 7 2020/21) • pruning in winter (control) • pruning in late November	Late pruned treatment had sig. higher mean rating of 0.96 compared to 0.79 for the control (*P*=.045).	• Two replicates of 5-tree plots (3 data trees per plot). • Overall granulation very low in this trial due to high crop load (no fruit were rated ≥3). • November pruning brought canopy back to a winter structure rather than matching the canopy of control trees which had grown by this date. • Our hypothesis was that delaying pruning would mean less vegetative growth in spring to compete with early fruit set, but in the event late pruned trees had denser and darker canopies in spring, particularly in the centre of the trees, with many water shoots. In addition, after pruning, the crop load on the trees was lower.

Table 6 Survey results and investigations within trials

The sample numbers for CT04002 surveys were 287, 277, 332 and 333 trees in the years 2007 to 2010 respectively. Correlation coefficients cited (r) are between parameters on a per tree basis with mean granulation rating of a 12 fruit sample from the tree.

Factor under investigation, project and activity	Results	Comments
Fruit position (branch no. away from the trunk) (CT03029 Exp 5)	Slight tendency for fruit on branches closer to the trunk to granulate, but relationship very poor. R^2 between fruit score and branch level = 0.0019.	• Single tree, 2328 branches, 182 fruit.
Fruit position – distance from trunk (CT04002 Trials A and J, 2008/9 and 2009/10)	Fruit inside canopy granulated more than fruit on the outside regardless of size. R values were -0.43, -0.31 in Yr 1 and -0.74 and -0.12 in Yr 2 for trials A and J respectively.	• Hypothetically, this may be due to shade reducing photosynthetic production from leaves and/or less competition for water from xylem close to trunk.
Rootstocks (CT04002 surveys, 2007-10)	Troyer had higher granulation than Cleopatra or Swingle in all years.	• More granulation from vigorous rootstocks that produce earlier fruit, due to, hypothetically, less competition with vegetative growth and/or greater water uptake from vigorous root systems.

Factor under investigation, project and activity	Results	Comments
		• Less prone rootstocks can granulate if crop loads are low.
Crop load (CT04002 surveys, 2007-10 and trial data 2009 and 2010)	• Crop loads were measured **after** thinning in surveys so correlations are not strong. Stronger in off years where there is a greater range of crop loads (r=+.06, -.23, -.15, -.29 for years 2007-2010 respectively). • Correlations with crop loads measured **before** thinning (on trial sites) tend to be slightly stronger, ranging from +.05 to – 0.50 (may be due to more extreme values).	• Range of loads >15 fruit per 0.125m^3 quadrat show little difference. • Low crop loads also linked to larger fruit size.
Fruit size (CT04002 surveys, 2007-10 and trial data 2009 and 2010)	• Positive correlation between mean fruit size per tree (sample of 12) and mean granulation evident in all years (r=.27, .25, .45, .42 in Yrs 1-4 respectively). • Correlations (r) in trials ranged from 0.16 to 0.66.	• Severely granulated fruit can be both large and small. • Measurement of growth of tagged fruit in trials suggest that final fruit size is determined mostly by date fruit begin to grow (~ date of anthesis) rather than to variable growth rates.
Brix and acid (CT04002 surveys in 2007 and 2010, and trial data in 2009 and 2010)	In surveys: • Weak negative correlation between acids and granulation (r=-.37 in 2007 and -0.39 in 2010). • Even weaker correlations between Brix° and granulation (r=-.10 in 2007 and -0.25 in 2010). In trials: • Clearer negative correlation between acids and granulation, ranging from weak to strong depending on year and trial (r=-.37 to -.81). • Weaker correlations between Brix° and granulation (ranging from r=+.31 to-.58, depending on year and trial).	• Weakness of correlations partly due to methodology: samples are of combined juice from 12 fruit. It is difficult to extract juice from highly granulated fruit so these will contribute less to the combined sample. Vesicles within the one fruit also vary and less granulated vesicles will release more juice than others. • Survey data taken at different dates and thus maturities, whereas trial sample dates tended to be more consistent, thus explaining better correlations for acid from trial data.
Tree health (rating) (CT04002 surveys, 2007-2010)	No consistent relationship.	• Unhealthy trees produced on average more granulated fruit, but some produced perfect fruit.
Rootstock/scion circumference and 'benching' (i.e. difference in cross sectional area (CSA) of rootstock and scion as a % of rootstock CSA) (CT04002 surveys, 2007-2010 and trial data selected trials 2008-2010)	• Weak negative correlations between scion and rootstock circumferences and granulation in surveys (r values range from -.13 to -.35). • **No** correlation between differences in CSA (benching) and granulation (in surveys, r values range from +.02 to-.15; even within trials, i.e. with trees of the same age, r values range from +.06 to -.12)	• Larger and older trees less likely to granulate. • Benching clearly not the cause of granulation.

Factor under investigation, project and activity	Results	Comments
Soil types (CT04002 surveys, 2007-2010)	• Trends not clear. • Loam has the lowest granulation on average, but clay loam blocks had the highest granulation in 2010; as did sandy clay blocks in 2008.	• Sand=1 block, sandy loam=19 blocks, loam=11 blocks, sandy clay=2 blocks, clay loam=5 blocks.
Harvest date- tagging of pairs of fruit of the same size and harvesting/assesing the second at a later date (CT04002, 7 sample sets: trials A, J, K in Yr 2, Trials A, J, M, P in Yr 3)	• No clear trend: in some trials granulation was lower on second date, in others higher, and in others there was no difference. • No correlation with growth of second fruit. • In 4/7 sample sets, larger fruit got worse.	• These results suggest that granulation is triggered early in fruit development but continues to develop throughout fruit growth.
Granulation of thinned fruit (on the ground) in late Jan/early Feb (CT04002 4 trials in year 3, 20-27 plots per trial, 2 sample dates each)	Correlation coefficients (r) between granulation of thinned fruit with fruit at harvest ranged from 0.32 to 0.73, average =.55).	• Thinners target smaller fruit (=less granulated) so correlations are unlikely to be high. • While granulation is detectable at this stage, it is more difficult to differentiate between ratings. • Results supports view that action to minimise granulation needs to be before this date.
Individual sprinkler output (CT04002 Trials B, P, K, M and J in 2008/09 or 2009/10)	In 2009 at Trial B, output of sprinklers correlated well with granulation of individual trees (r=.67) i.e. more water, more granulation, but when measurements were taken at Trial P, K, M and J in 2010 there was no correlation, despite similar variability in outputs.	

Key to abbreviations

addl	additional
a.i.	active ingredient
appn	application
B	boron
Crunchy%	The % of fruit rated at 2 or 2.5, compare 'unacc%'.
Exp	experiment
K	potassium
N	nitrogen
P	phosphorus
P=	The P value indicates the probability that the apparent difference in treatment means is due to natural variability in the samples and not an effect of the treatments. The standard for statistical 'significance' or confidence is a P value of ≤ .05 or 95% confidence that the treatment means are different. The reader may be prepared to consider a lower level of confidence indicates a probable treatment effect e.g. a P value of 0.100 means we can be 90% confident that the treatment means are significantly different. See 'sig' below.
r	correlation coefficient. A value of 1 means a perfect correlation. A negative value means the relationship is inverse i.e. higher values for one variable mean lower values for the other.
sig.	(or 'not sig.') refers to whether (or not) the treatment means were statistically significantly different at the 95% confidence level. See "*P=*" above.
SoP	sulphate of potash
Stage I, II or III	refers to stages of fruit development, Stage I 'cell division and multiplication'—approximately Sep to Nov inclusive; Stage II 'cell expansion'—approximately Dec to Feb inclusive, Stage 3 'ripening' -- approximately Mar to harvest
unacc%	The percentage of fruit rated as unacceptable i.e. rated at ≥2.5 or <~35% juice. For the project CT19005, this was changed to rating ≥3 as recalibration of rating and juice % revealed that we were rating fruit 'harder' than in the earlier research i.e. rating ≥3 =<35% juice.
X	if used in the list of treatments: this means the trial had a factorial design, in which the second factor is superimposed over the first factor e.g nutrient treatments are superimposed over irrigation treatments.
Yr	year of trial
Zn	zinc

Appendix 9:

Report on non-invasive technologies for detection of mandarin granulation

Prepared by: Kerry Walsh, Phul Subedi, Dipendra Aryal, Bed Khatiwada, Central Queensland University

Prepared for: Helen Hofman, Department of Agriculture and Fisheries

Servicing: Reducing granulation in the production of Imperial mandarin

(Hort Innovation, CT19005)

30/5/2021

Item 6 Services: (clauses 3.1 and 19.1)

1. Review and document CQU activities since 2011 to test the use of near infrared spectroscopy for non-invasive assessment of internal flesh granulation in Imperial mandarins
2. Review the status of international development of other technolgies that have potential for non-inavasive detection, using published scientific literature
3. Extend assessment using current technology, if warranted.
4. Provide recommendations for future research, if warranted

Program of works

Milestone No.	Achievement Date	Description of Milestones
1	1/6/2021	Report on non-invasive technologies completed as outlined in services (Item 6).

1. Introduction

The Australian industry has adopted a visual symptom scorecard chart (Fig. 1) and the lot acceptance criteria of a maximum of three fruit with severe granulation (more than 55% area of cut surface affected, i.e., stage 1-4, and less than 25% juice extraction, by weight) in a 30 piece sample (Hancock, 2019). This assessment relies on the destructive sampling of fruit.

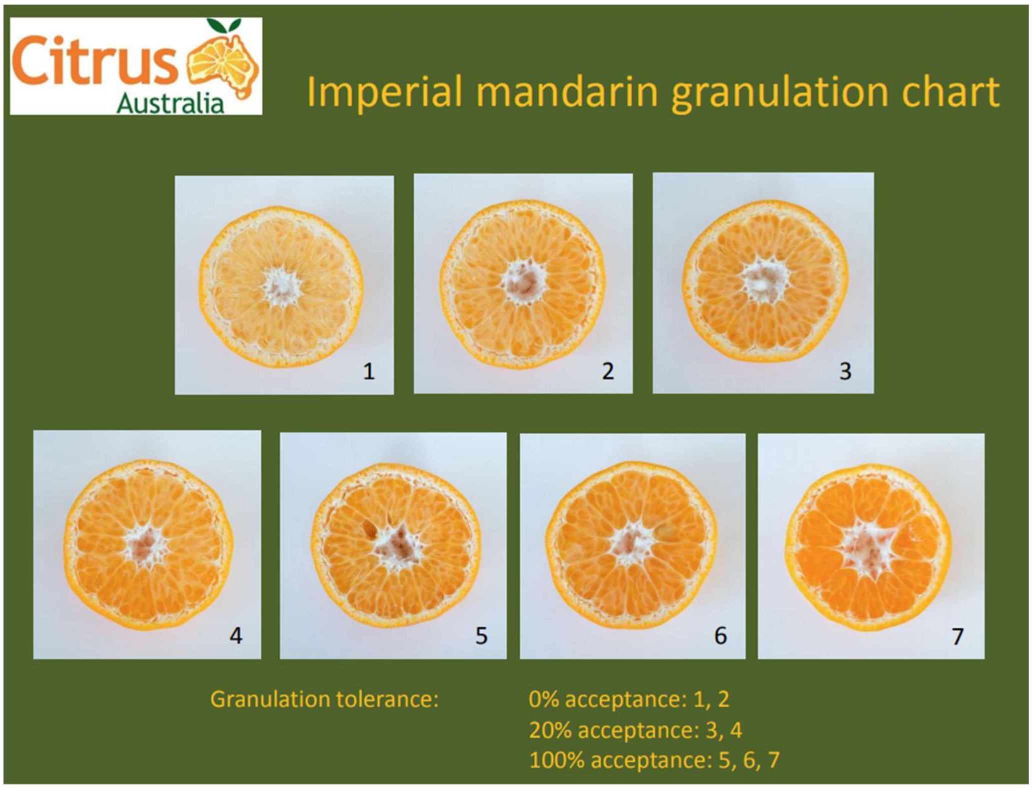

Figure 1. Imperial Mandarin granulation chart (Hancock, 2019)

In the granulation disorder of citrus fruit, the subepidermal cell walls of juice sacs become swollen with secondary thickening, resulting in the visible appearance of opaque white regions in juice sacs in cut fruit of pummelo (Shomer et al. 1989). In severe granulation, total dry weight, cellulose, pectin, lignin, and hemicellulose content is increased compared to non-affected tissue (Shomer et al., 1989; Wu et al., 2020), with activities of pectin methylesterase (PME) and the antioxidant enzymes peroxidase (POD), superoxide dismutase, and catalase increased, while those of polygalacturonase (PG) and cellulose (CL) are decreased (Wu et al., 2020). However, dry weight (the inverse of moisture content) and fruit density is unaffected at low to moderate levels of granulation. Water is retained in a gel-like matrix of the expanded cell walls at low to moderate levels of granulation, such that juice extractability from fruit is decreased.

With the primary visual symptom of the disorder being tissue opaqueness, i.e., increased light scattering, it is logical to suggest that the disorder could be assessed in intact fruit based on the proportion of incident light transmitted through the fruit. Fruit moisture content is substantially decreased in severe but not in moderate or mild granulation, thus assessment techniques based on the assessment of water content will only be useful for detection of severe levels. Another symptom varying with granulation across all severity levels is juice extractability. A technique that could detect the level of bound, as opposed to free, water in the apoplast could provide a measure of granulation. This could either involve a direct measure, e.g., free water has less H bonding than bound water, impacting near-infrared absorption spectra and nuclear magnetic resonance, or an indirect measure, e.g., if the gelling of water impacted electrical conductivity or material stiffness of the fruit, which is related to the Youngs modulus, and thus to vibration behavior following impact.

For many potential methods for the detection of granulation in intact fruit, the fruit skin represents an 'interference', as skin thickness and 'looseness' (level of adhesion to juice segments) can be variable (e.g., Fig. 2). Such variation in skin properties may affect measurements of attributes that may act as an index of granulation in flesh tissue, e.g., light transmission or acoustic properties. For example, Fraser et al. (2003) reported high absorption of light by the skin of mandarin fruit relative to endocarp, and that the skin had significant internal reflective properties.

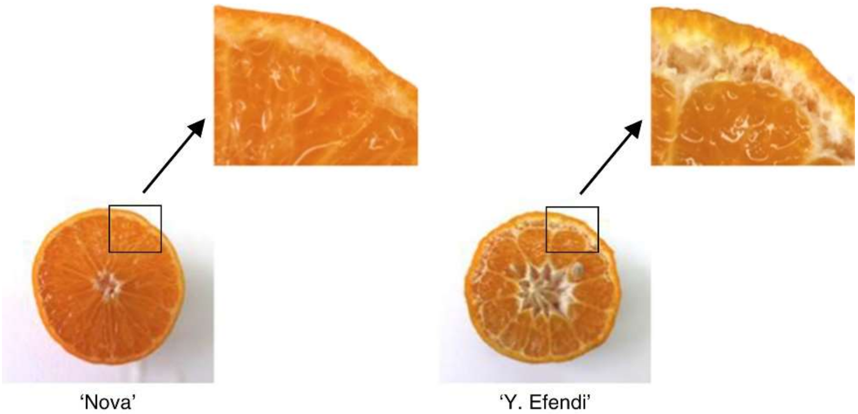

Figure 2. Rind separation in mandarin (Goldenberg et al., 2018)

It is therefore important that reports on the applicability of a technique for non-invasive assessment of granulation in fruit consider:

(i) multiple test populations of fruit drawn from different growing conditions,
(ii) fruit with granulation at the lower levels of consumer non-acceptance, i.e., levels 3 and 4, as well as severe granulation (levels 1 and 2).

These use of a range of technologies for detection of granulation was considered as part of the HIA/DAF/CQU project CT04002 (2007-2010), but results were either inconsistent between populations or fruit or the technology was impractical to deploy in a packhouse or orchard environment. Technologies tested included interactance and transmittance optical geometries, nuclear magnetic resonance, computed tomography – X ray; on-line transmission X ray, firmness measurements by 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.

Consideration is given to assessment in context of use: (i) on packing-lines for assessment of all fruit; (ii) in an at-line quality control station in a packing-house, enabling assessment of many more fruit than destructive sampling; and (iii) in a handheld device for use in orchard, again allowing assessment of more fruit than destructive sampling.

Section 2 of this report reviews relevant literature on the assessment of various technologies in the detection of granulation, with a focus on reports published since 2015. The availability of instrumentation on packing lines is also reviewed. Section 3 is a report of unpublished work undertaken at CQUniversity on the use of two higher light intensity systems for optical assessment of granulation in fruit, an in-house constructed and a commercially available system. This work formed part of a PhD thesis by Bed Khatiwada supervised by K. Walsh and P. Subedi. Section 4 presents recommendations.

2. Current status of technologies for non-invasive assessment of citrus granulation – a review

Density sorting

Ritenour et al. (2004) reported the use of automatic sizing and grading equipment to select for small-sized, high-density fruit as that grade was reported to have the highest proportion of non-granulated, packable fruit navel orange. To the extent that such correlations apply, modern pack lines equipped with vision systems for volume estimation and load cells for weight estimation could be used. However, change in fruit density in granulated Imperial mandarin is only evident in severely impacted fruit, limiting the usefulness of this approach.

Visible and Near-infrared spectroscopy

Light transmission

The simple measure of light transmission through an intact tangerine (cv ‘Keaw Dumnuan’) as detected by a single photodetector was reported to allow separation of most moderately granulated and all severely granulated fruit from unaffected fruit (Teerachaichayut et al., 2019) (Fig. 3).

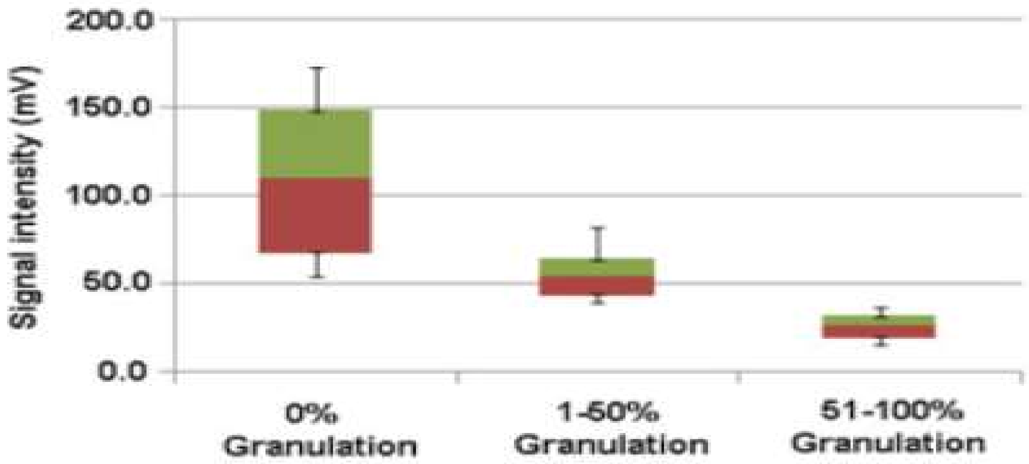

Figure 3. The signal intensity of tangerines varying in level of granulation defect (from Teerachaichayut et al., 2019).

However, this study involved only 52 fruit from a single collection event, i.e., presumably a single growing condition. Fruits were selected to be of similar size, avoiding the issue of optical path length on % light transmitted. The study is therefore not conclusive. If the technique was robust, a visible – near infrared spectroscopic (Vis-NIRS) assessment would also be successful.

Visible - near-infrared spectroscopy

The topic 'Nondestructive Assessment of Citrus Fruit Quality and Ripening by Visible-Near Infrared Reflectance Spectroscopy' was recently reviewed by Cavaco et al. (2021), however only passing attention was given to the granulation defect. Two reports were cited, both from the same laboratory (Xu et al., 2020; Sun et al., (2020).

Sun et al. (2020) reported discrimination of granulation in 'Honey' pomelo at a classification accuracy of >95% using a principal component analysis based on a generalized regression neural network (GRNN). Best results were obtained using a fusion of spectral data with fruit weight and machine vision estimated fruit volume. The trial involved 600 fruit. Limitations of this work include the use of fruit from a single parent population (i.e., both calibration and validation sets were from a single growing condition). There was also a clear correlation between defect level and fruit size, providing an ancillary variable related to granulation level (Fig. 4).

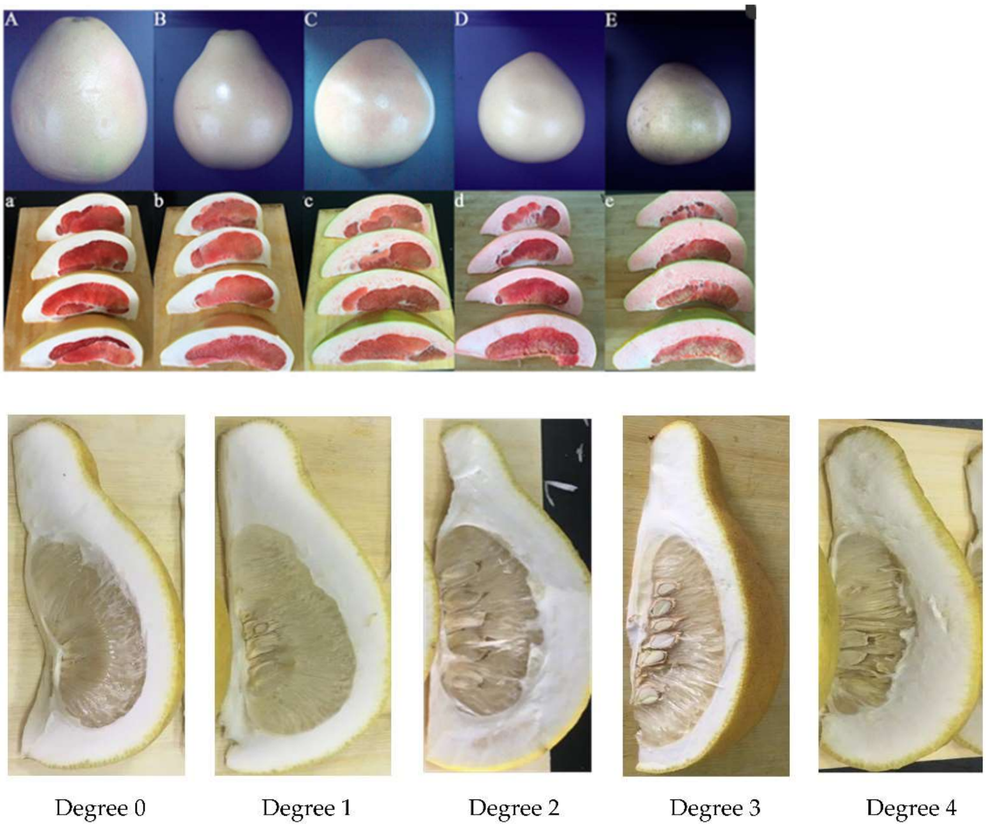

Figure 4. Fruit categorized into 5 levels of granulation defect (Sun et al., 2020) (top panel) and (Xu et al., 2020) (bottom panel).

The same group (Xu et al., 2020) also reported on the detection of the level of granulation in pomelo using Vis-NIRS using Savitsky Golay smoothed absorbance spectra as input to a Partial Least Squares Regression (PLSR) model. Complete discrimination of medium to severely affected fruit (degree 3 and 4 in Fig. 4 was claimed, with an R^2 of 0.97 and RMSEP (root mean of square of errors of prediction) of 1.0 (on the 0-4 scale) for a quantitative prediction of granulation level. Similar limitations to this study apply as for Sun et al. (2020), including the use of a small sample set (120 fruit) drawn from one harvest population and use of a test set that was randomly selected from the parent population, i.e., selected to represent the calibration set.

The thickness of the albedo layer and high seediness (as seen in Fig. 4) in the fruit under consideration are expected to make detection of granulation difficult, given the impact on light transmission and as they are likely to vary with growing condition. The results reported were surprisingly good, but there is a likelihood that the models have been over-fitted to the data, i.e., that results are over-optimistic and that the model will perform poorly in use with the fruit of different growing conditions.

Theanjumpol et al. (2019) reported on the assessment of granulation in ‘Sai Num Pung’ tangerine based on the use of NIR spectroscopy. The study involved 178 fruit samples ranked to five levels of granulation. A supervised self-organizing map (SSOM) was used to classify granulation classes, with 78.4% of a test set correctly classified. The test set, however, was drawn from the same harvest population as the calibration set.

Jie et al. (2021) reported on the use of a conventional convolution neural network (CNN) with a batch-normalization layer with NIR spectra in the assessment of granulation. A 100% accuracy in the classification of defect fruit was achieved with the training set and 98% with the validation set, respectively. With a restricted wavelength range of bands of 660–721, 709–750, and 807–847 nm, 90% and 85% accuracy were achieved in the training set and validation set, respectively. Again, however, the validation set was drawn from the same parent population as the training set, and thus results are likely to be optimistic relative to practical use, involving assessment of fruit from different growing conditions to those of the fruit used in training.

In summary, recent literature reports support the notion that citrus fruit can be assessed non-invasively using Vis-NIRS, a result likely to be principally based on the impact of light scattering by granulated tissue rather than light absorption. This technology would be suited to one-line or at-line application, rather than in-orchard. However, the scope of the studies has been limited to validation using ‘internal’ population sets, without testing across multiple harvest populations. A further focus is required on the detection of lower severity, but consumer unacceptable, granulation. This is needed as severe granulation is associated with the change in fruit density and water content attributes that can be assessed non-invasively, while moderate or mild granulation lacks such an association.

Commercial packing-line systems

The major international fruit grading equipment manufacturers Compac (Tomra) (https://www.compacsort.com/en/produce/citrus/) and Aweta (https://www.aweta.com/en/produce/citrus; https://www.agriculture-xprt.com/products/aweta-citrus-sorting-machine-576331) explicitly claim granulation assessment on their websites. Others (e.g., MAF Roda https://www.maf-roda.com/en/page/grading.php) have been used in granulation sorting, although no explicit claim is made on their websites.

In the section 3 of this report, unpublished work by Khatiwada, Subedi and Walsh is presented. In summary, this work explores several technologies that utilize Vis-NIRS, including instrumentation from a packing line manufacturer. The MAF Roda Insight2 unit was used in assessment of mandarin granulation, indexed by either visual score or % juice recovery, with good results achieved in discrimination of granulated fruit. The impact of the threshold setting on sorting operations was also explored, with compromise between true positive rate and false positive rate in the sorting operation.

X-ray radiography and computed tomography

Radiography

Radiography involves a measure of the attenuation of X-rays through an object, which is a function of object density and path length. Fruits are not uniform in thickness and the various internal tissues are is similar, except for internal air spaces and foreign objects (stones, etc.). Thus, radiographic visualization of fruit internal attributes is generally poor. Radiography is routinely used in the assessment of foreign material in fresh-cut packaged produce and has been commercially used for the detection of hollow hearts in potatoes (Abbott, 1999).

In a PhD thesis, Lebotsa (2017) has reported accurate classification of granulated fruit based on 'Satsuma' mandarin and Navel' orange radiographic and tomographic images, after exclusion of stylar-end and stem-end views. This work was also published within several papers, with the promotion of the CT X-ray solution, as described below.

CT X-Ray

X-ray CT (computer tomography) involves the measurement of X-ray projections at multiple positions around the sample, with the reconstruction of a 3D image of the internal structure. The technique is well used in medical applications where low acquisition speeds and high instrument costs are tolerated. Some work has been done promoting the use of the technology in an on-line application for fruit sorting, based on reconstruction from far fewer projections, and a patent has been taken on the concept (Van Dael et al., 2014).

The work that is based on this patented system has been published by Van Dael et al. (2016). Van Dael et al. (2014) reported on automated online detection for granulation in Washington Navel' orange from multiple scans in an X-ray system (75 kV, 468 mA, 60-ms exposure), with fruit placed on a stage and rotated within the beam of a microfocus CT X-ray scanner. An image processing algorithm was used to automatically segment the affected fruit tissue, with a classifier used to assign to granulated and sound classes (Van Dael et al., 2016) (Fig. 5). A 96% classification success was claimed, for work involving 38 fruit.

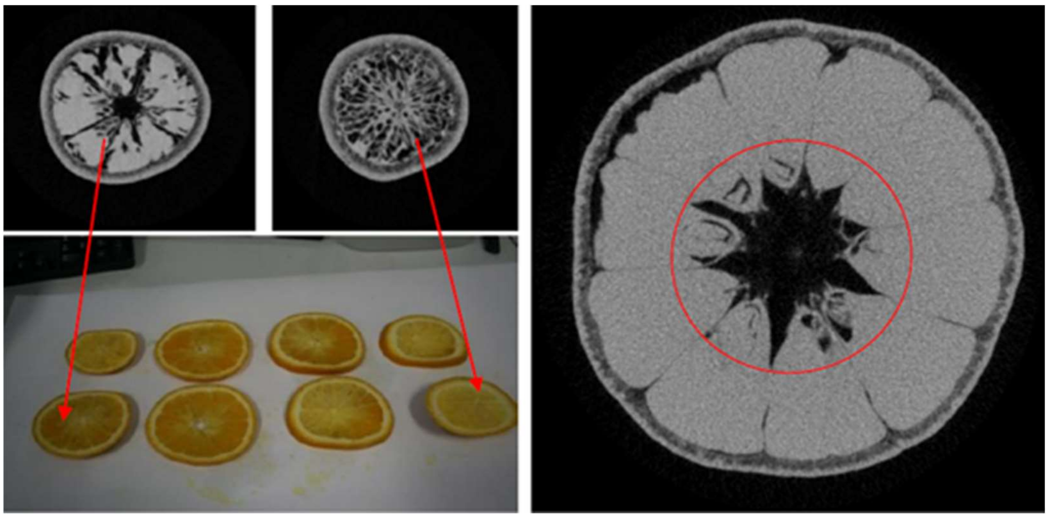

Figure 5. Results from Van Dael et al. (2016). CT X-ray images of granulated Navel orange (left) and seeded Nardorcott mandarin (right).

Recent work by the same group has considered the use of machine vision in the assessment of X-ray CT images for fruit sorting applications (Van De Looverbosch et al., 2020).

As for Vis-NIRS, this technology would be suited to on-line or at-line application, rather than in-orchard. However, commercial adoption into packing-line systems has not occurred in the five years since the Van Dael et al. (2016) patent was taken. Should instrumentation become available, cabinet X-ray licensing would be required (managed by respective state health departments).

Capacitance

Teerachaichayut et al., (2012) reported capacitance of granulated tangerine fruit (*Citrus reticulate*, 'Keaw Dumnuan' variety) to be higher than that of normal fruit in a non-invasive measurement, with 1.75 nF recommended as the cut off value. The work was preliminary, based on 100 fruit for calibration and a test set of 45 fruit. Classification accuracy of 75.6% was achieved on the test set. A limitation to the technique was that the measurement required 3 minutes per fruit.

This method could potentially be incorporated into a hand-held device, and thus used in orchard. Further evaluation of this approach is warranted to consider whether the measurement could be made in a shorter time and to validate the reported accuracy.

Acoustic

Zhang et al. (2018) and He et al. (2021) have reviewed the use of acoustic vibration methods to assess agricultural products. In essence, the technique relies on the application of vibration of set frequency to an object, with analysis of the speed of transmission or resonant frequency of the transmitted vibration. These characteristics are affected by the Youngs modulus of the material (i.e., the stiffness of the material).

The technology has been utilized in attempts to assess citrus granulation. Muramatsu et al. (1999) applied the laser Doppler technique to detect citrus fruit afflicted with internal defects. The phase shift associated with granulated fruit was significantly lower than in control fruit at all vibration frequencies. However, in this study the specific gravity of the granulated fruit was 20% less than that of unaffected fruit, and thus it seems that fruit with severe granulation was used. Whether the technology is capable of distinguishing fruit with moderate granulation is not clear.

Kittiyanpunya et al., (2017) suggest the use of backscatter from the fruit of two radio frequencies (1 and 2.2 GHz) to detect granulated pomelo fruit by the measure of phase shift and magnitude change. The use of two frequencies is claimed to allow the separation of peel information. However, this study presents only simulation data.

Magnetic resonance

Proton magnetic resonance imaging (MRI) is widely used in medical applications. The use of the technology has been reported for the detection of seeds in citrus (mandarins and oranges) using MRI (Barreiro et al., 2008). The level of detail in images is remarkable (Fig. 6) and it is expected that granulation would be easily detected. Thus, although no study was found demonstrating the use of MRI to detect granulation, the potential for success is very high. A limitation of the technique is the length of the acquisition period.

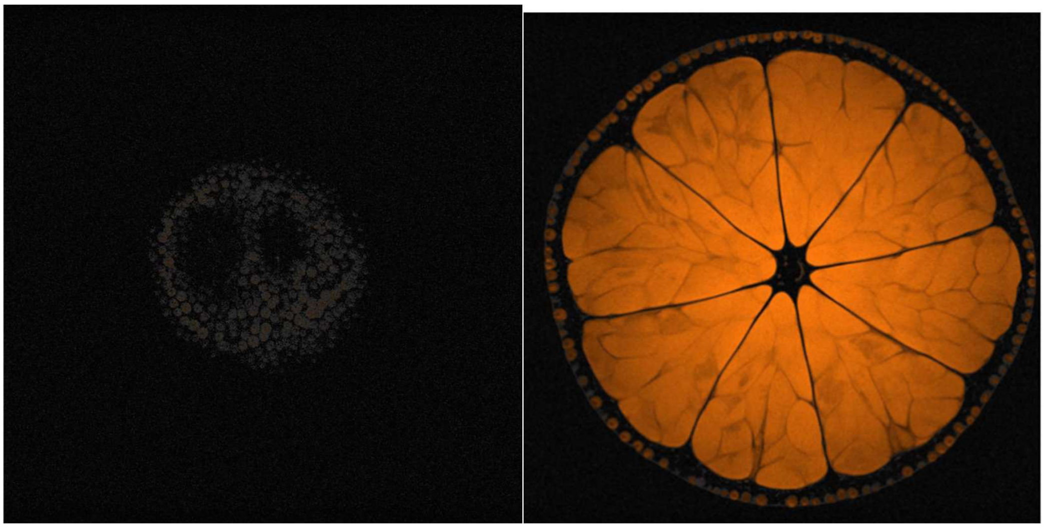

Figure 6. MRI slices through an unaffected citrus fruit. With juice sacs clearly delineated it is expected that granulation would be clearly detected. https://stacks.wellcomecollection.org/peering-through-mri-scans-of-fruit-and-veg-part-1-a2e8b07bde6f doa 15/05/2021

Aspect Imaging (Shoham, Israel) is a manufacturer of MRI equipment, with a focus to lower cost, faster acquisition time, industrial applications. Around 2015, a demonstration unit was produced that produced a single slice image in 2 seconds, with batches of approximately 10 fruit assessed in each capture (i.e., equivalent to 0.2 s per fruit) (Fig. 7). However, the work no longer features on their website and thus appears to have been discontinued.

Figure 7. The Aspect Imaging system for citrus that produces a single medial MRI image per fruit in approx. 2 seconds. (image from 2015 YouTube clip: https://www.youtube.com/watch?v=nV2Nuv9m4bU&feature=youtu.be)

Other groups may advance this technology to the point where translation to horticultural use becomes viable. For example, the University of Queensland is pursing the development of portable MRI systems for medical and other uses where high-resolution imaging is not required, and thus safer low strength magnetic fields can be used (https://www.uq.edu.au/news/article/2020/06/1-million-develop-portable-mri-device).

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3. Experimental exercises: Detection and characterisation of granulation in Imperial mandarin by using NIR spectroscopy.

Abstract

Spectroscopic instrumentation operating in full transmission optical geometry was employed for the non-invasive assessment of the disorder, using visual score, luminosity (L) and juice recovery as reference attributes. Classification based on wavelength, ratio-based algorithm and other classification algorithm yielded classification accuracy as high as 98 % for acceptable fruit while for defect fruit, the classification accuracy was lower. High prediction accuracy (92%) was reported for IDD0 instrumentation using the PLS-DA classification method.

3.1 Introduction

Citrus fruit can develop a range of physiological disorders including fruit cracking, sunburn, puffiness, rind breakdown, chilling injury and internal dryness (Munshi, Singh, Vij, & Jawanda, 1978; Peiris et al., 1998; Subedi, 2007). The descriptor of internal dryness and the associated terms granulation, section drying and gelling have been used somewhat interchangeable by several authors. Peiris et al. (1998) categorized internal dryness into two broad categories, namely dehydration and granulation (Fig. 1). Dehydration involves shrinkage of the tissue followed by a complete collapse of the affected vesicles due to loss of vesicle contents, e.g., following frost damage. In contrast, granulation begins with the hardening of the affected vesicles following the gradual collapse of the inner cells resulting in an empty cavity. Juice recovery rate is decreased proportionate to the extent of the disorder (e.g., 40% v/w for normal fruit to 5% in defect fruit), but water content and TSS is constant, except in severely granulated fruit, in which levels of these attributes are decreased (Subedi, 2007). Affected fruits become unfit for fresh consumption due to a chewy, dry, and tasteless mouthfeel.

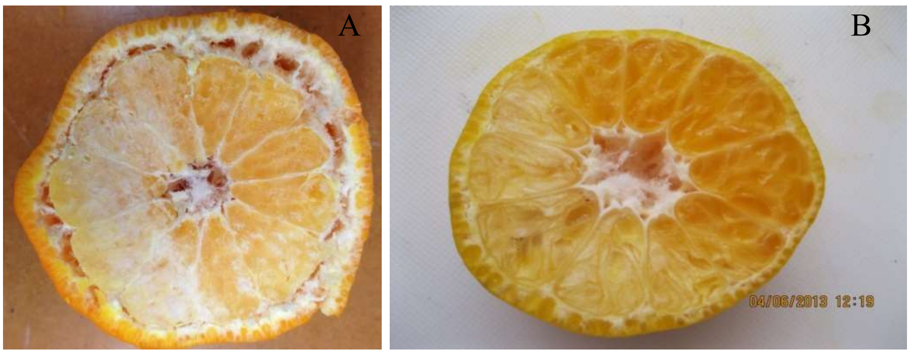


Figure 1. Internal defects of citrus: A. dehydration defect (white areas) following freezing injury B. granulation defect in 'Imperial' mandarin.

'Imperial', a brightly coloured and easy-to-peel cultivar, is prone to granulation disorder. An erratic incidence of this disorder is reported in Queensland, with seasonal variation and variation among soil types, nutrition, irrigation, rootstock and orchard locations (Hofman, 2011). There can be a difference in the incidence and severity of this defect within a single tree and indeed between fruit on a single twig. Hofman (2011) reported the incidence of granulation is associated with the very early fruit development stages with competition between fruitlets, flowers and flush for the nutrients particularly nitrogen. It was observed that granulation was decreased when winter nitrogen application was followed by an additional spring application. Current field research is also exploring the hypothesis that over- availability of water due to rainfall or irrigation is also a contributing factor.

Affected fruit cannot be recognized on visual external appearance. Only severely dry fruit can be detected by hand feel based on firmness. Given the difficulty in recognizing affected fruit and the unpredictable nature of incidence of the disorder, there is a clear need for the development of non-invasive sorting technology.

The difference in fruit optical properties (extent of light scattering) and water content offer promise for the application of non-invasive detection technologies. Peiris et al. (1998) reported the use of Vis-NIR absorption spectrometry and X-ray computed tomography for detection of the tangerine tissue drying disorder. Vis-NIR absorption spectra (500-1000 nm) were acquired using a 75 W tungsten halogen lamp as the light source and a silicon-based CCD spectrometer (Ocean Optics, SD 1000-TR). A multiple linear regression was undertaken, with a model based on second derivative absorbance values at 768 nm and 960 nm yielding a correlation coefficient of determination (R^2) of 0.77 on a two-point scale of visual granulation scores. The 960 nm absorbance feature of fruit is well known to be associated with the second overtone of O-H stretching, and thus to water content. This result is consistent with the detection of a dehydration defect, but this measurement may not be appropriate for the detection of granulation defect within a fruit given the lack of association of granulation level to water content (Subedi, 2007).

Our group has reported on the use of Vis-NIRS (500-1000 nm) spectroscopy to assess granulation of intact Imperial mandarin (Subedi, 2007; also CT04002). Defect level was scored by visual assessment and by a chromameter (luminosity, L*) reading of the cut surface of the fruit. For fruit without peel, the luminosity was reasonably well modelled ($R^2 = 0.84$). However, for whole fruit, calibration R^2 was decreased to 0.74 and validation of the model using an independent population was poor ($R^2 < 0.5$).

A limitation of this previous work was in the intensity of the light source used, requiring extended acquisition times. Further work was therefore undertaken using two high light intensity systems, one developed in-house and a commercially available system, in context of non-invasive detection of the granulation disorder in Imperial mandarin.

3.2 Materials and methods

Fruit

Fruits of the mandarin variety 'Imperial' were sourced from two commercial farms in Central Queensland, Australia. Fruits were harvested from areas which the farm manager reported as having a high incidence of granulation. Fruit were stored at 10^{o}C. Each fruit was marked at two locations on the equator of the fruit, opposite to each other.

Instrumentation and fruit measurements

Three vis-NIRS instruments were employed, the InSight2, IDD0 and IDD2. The Insight-2 (MAF Roda, France) unit utilises a 150 W tungsten halogen lamp and a spectrometer (600-973 nm) within a partial transmission (interactance) geometry. The unit was operated using an integration time of 4 ms. This unit was similar to that employed in CT04002. The IDD0 was developed in-house and utilized a 300 W tungsten halogen lamp and an MMS 1 Zeiss spectrometer (300-1100 nm with an interval of 3.3 nm) within a 180^{o} transmission geometry. The unit was operated using an integration time of 400 ms. The two units were characterized by the repeatability of less than 2 mA in the 600-900 nm range. The IDD2 unit (Fig. 2., MAF Roda, Bacchus Marsh, Vic.) is based on a sequential operation of LEDs at four peak wavelengths (700, 810, 780 and 880 nm) with detection of transmitted light by a single photodiode detector.

Figure 2. IDD2 test unit, from MAF Roda. Rotating carousel conveys fruit into light protected box for light transmission measurements.

For the IDD0 and InSight2 instruments, dark and white reference measurements were acquired at the initiation of each run, with integration time was set to achieve an analogue to digital conversion count with fruit samples of >50% of saturation level (32,000 for IDD0, 64,000 for InSight2). Four spectra were averaged from each scanned position per fruit. Example spectra (raw analogue to digital conversion count, ADCC) are displayed in Fig. 3.

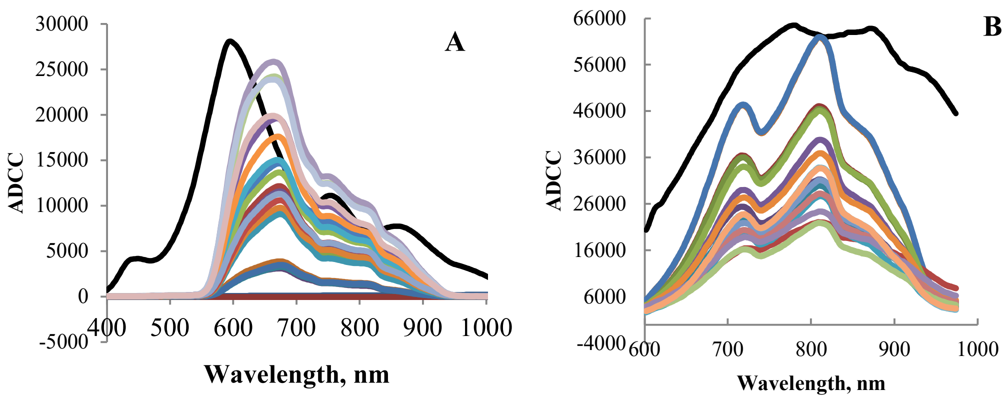


Figure 3. White reference and sample spectra from IDD0 (A) and Insight2 (B) units, with spectra acquired using an integration time of 400 ms and 2 ms, respectively, with samples stationary and moving, respectively. The black solid line represents a white reference.

Destructive reference methods

Visual dryness score

Each fruit was cut transversely at the equator of the fruit and the cut surface photographed using a Canon PC1474 digital camera (12.1 megapixel sensor).

The cut surface image was visually scored for the extent of dryness score, aided by reference images (Fig. 4). Visual scoring to a seven point scale was attempted but repeated assessments had was poor, and a five-point scale was used in this exercise. This subjective score depends on both the area affected by the defect and the degree of the defect (whiteness of the tissue). For the sorting exercises, visual scores 1 to 3 were considered consumer-acceptable while scores 4 and 5 were considered to be consumer-unacceptable.

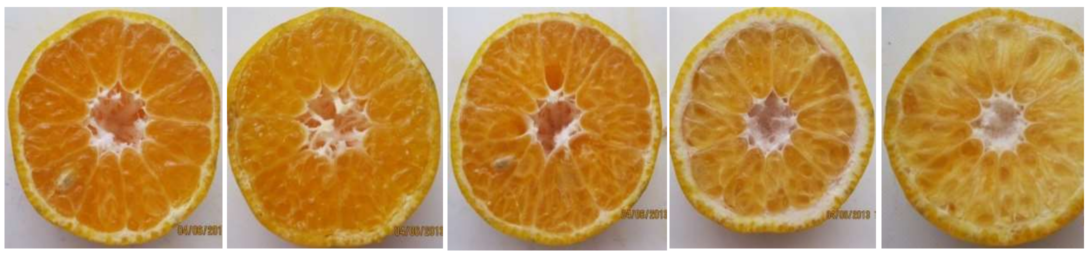


Figure 4. Visual score (1 to 5 scale) in granulation in 'Imperial' mandarin.

Other measurements

The fruit CIE Lab colour space was measured at four locations on the cut surface of each fruit using a Chromameter CR 400 (Konica Minolta; 2 degree observer). Values for a given fruit were averaged. The Chromameter was calibrated using a standard calibration procedure before each lot of Lab measurements.

The diameter and weight of each fruit were recorded. The juice was extracted from both hemispheres of the cut fruit using a manual juice extractor. Juice recovery was calculated as total juice weight divided by total fruit weight including peel weight. Total Soluble Solids (TSS) was measured using a refractometer (RFM320, Bellingham and Stanley Limited).

Data analysis and chemometrics

Chemometric analysis was undertaken using The Unscrambler 10.3 (Camo Inc. Oslo, Norway) and Matlab (Mathworks Inc.) software. Principal component analysis (PCA), partial least square regression (PLSR) and multiple linear regression (MLR) were utilised. The classification algorithms of linear discriminant analysis (LDA), partial least-square discriminant analysis (PLS-DA), support vector machine (SVM) classification and soft independent modelling of class analogy (SIMCA) and multiple logistic regression were trialed for classification of good and defect fruit.

3.3 Results and Discussion

Fruit sample and population structure

Statistics on the populations of fruit used in terms of reference-quality parameters are presented in Table 1. Different fruit populations were used with each instrument, due to instrument availability windows.

Table 1. Population statistics on the visual score (5-point scale) and juice recovery (% w/w, peel included) for calibration and prediction sets in season 1 and season 2. Data presented as mean ± SD.

	Season 1		Season 2	
Set	Calibration	Prediction	Calibration	Prediction
Sample	75	50	200	110
Visual score	3.0 ± 1.4	2.8 ± 1.4	2.2 ± 1.3	2.9 ± 1.4
Juice recovery	21.5 ± 7.9	21.8 ± 7.9	28.4 ± 7.9	29.0 ± 7.4
CIE L* value			51.9 ± 4.2	49.7 ± 4.1

Non-spectral measures as an index of granulation

As with any sorting operation, clarity on the attribute to be assessed is critical. Consumers object to the granulation defect on two grounds, a visual assessment (whiteness of the cut surface) and an eating quality basis (dry mouth feel). Visual score on a 5-point scale and cut surface luminosity and % juice recovery were assessed to provide relevant objective measurements of the defect. However, the linearity of such measures with a physical (e.g., scattering) or chemical (e.g., water content) fruit attribute associated with granulation defect is not clear.

Relationships between reference attributes were weakly to moderately correlated (e.g., correlation coefficient of determination between juice recovery and a visual score of $R^2 = 0.57$, and with L value of cut surface, of $R^2 = 0.83$, Fig. 5, Table 2). A non-linearity is evident in the relationship between score and % juiciness, with visual score 5 fruit having low juiciness. Cut surface L* value is a potential quantitative reference method for assessing this disorder, to mimic the human observation, while % juiciness is a measure related to eating experience.

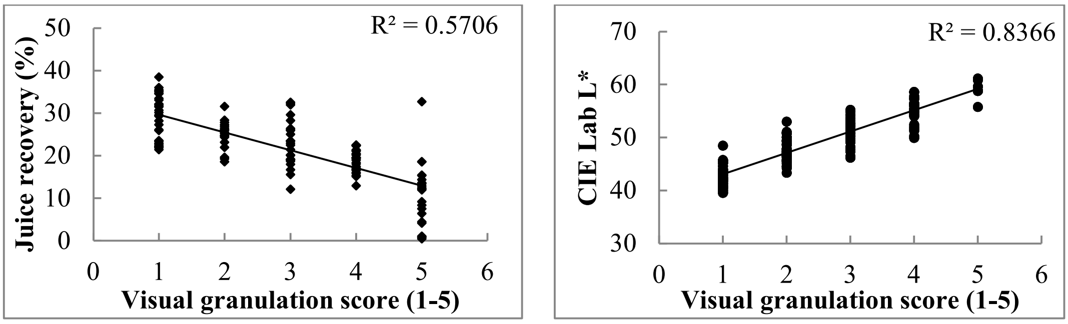


Figure 5. Scatter plot of juice recovery and surface Luminosity and visual score (Season 1, n=125).

Table 2. Correlation coefficient of determination (R^2) between reference parameters

	Score	Juice recovery	TSS
Score	1		
Juice recovery	- 0.57	1	
TSS	0.01	0.011	1
CIE Lab L*	0.84	0.34	0.002

Spectral features – linear regressions

The average absorbance values of defect (visual score 5, n= 88) fruit were higher than that of good (visual score 1, n = 248) fruit across the wavelength range 500 - 980 nm, with a maximum difference at 578 nm (Fig. 6; IDD0 instrument). This result is consistent with a higher scattering of light in defect fruit, as manifest in the increased L value of the cut fruit surface.

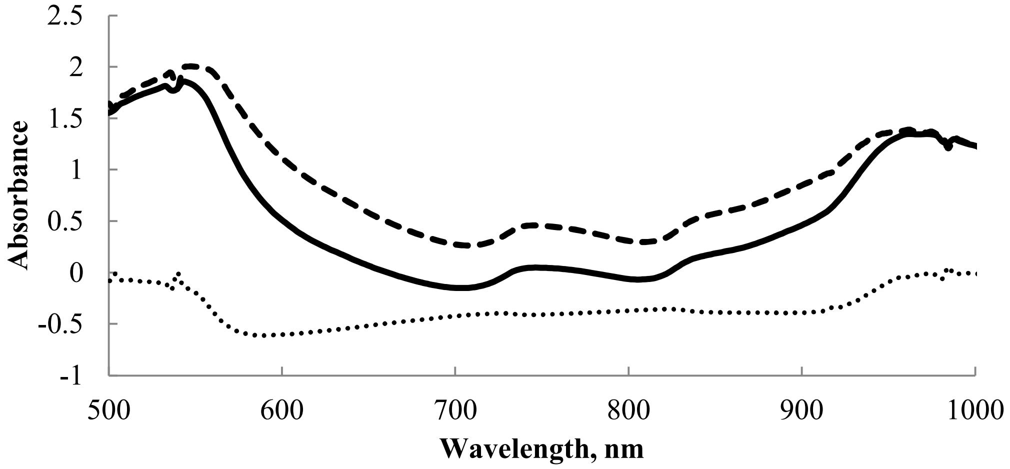


Figure 6. Average IDD0 absorbance spectra of acceptable (n = 30 fruit; solid line) and defect (n = 22 fruit; dashed line) fruit and the difference spectra (acceptable – defect; dotted line)

However, the linear correlation between the absorbance any single wavelength and the visual dryness score was relatively poor (e.g., at 578 nm, $R^2 = 0.3$) and is therefore not useful as an index for discriminating good and defect fruit (Fig. 7). The relationship between Abs578 nm and score demonstrated non-linearity, with higher absorbance associated with score 5 fruit.

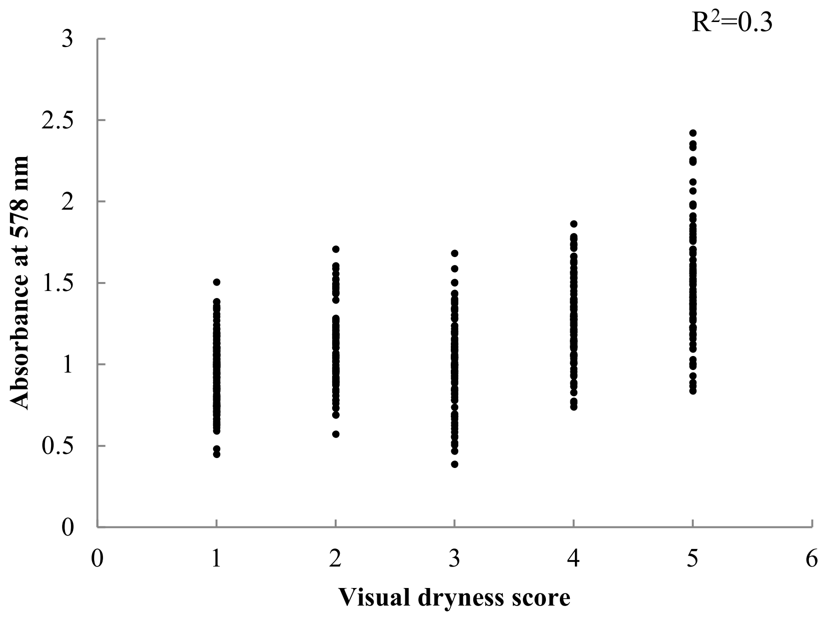


Figure 7. Scatter plot of apparent absorbance at 578 nm and the visual score of the cut surface.

This consideration of the relationship between defect attribute level and absorbance at a single wavelength was extended to all wavelengths (Fig. 8). A correlation coefficient R of approx. -0.75 existed between apparent absorbance at wavelengths between 600 and 920 nm and % juiciness (scale 1-5) (Fig. 8). The relationship between absorbance and score was similar, if slightly weaker. Water absorption features (e.g., as expected for the second overtone of the O-H stretch at 960 nm) were not weighted, consistent with spectral information relevant to this defect being related to scattering rather than water content.

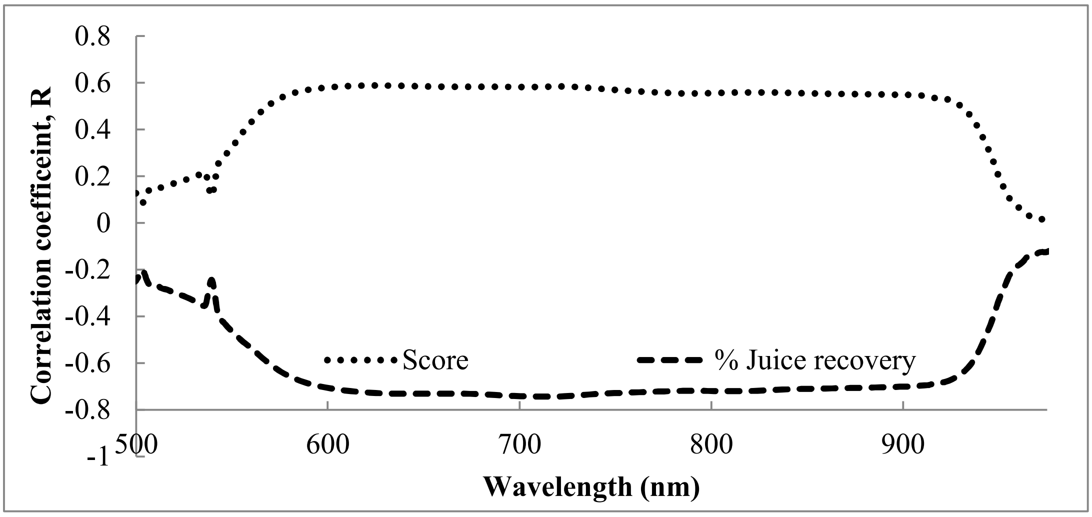
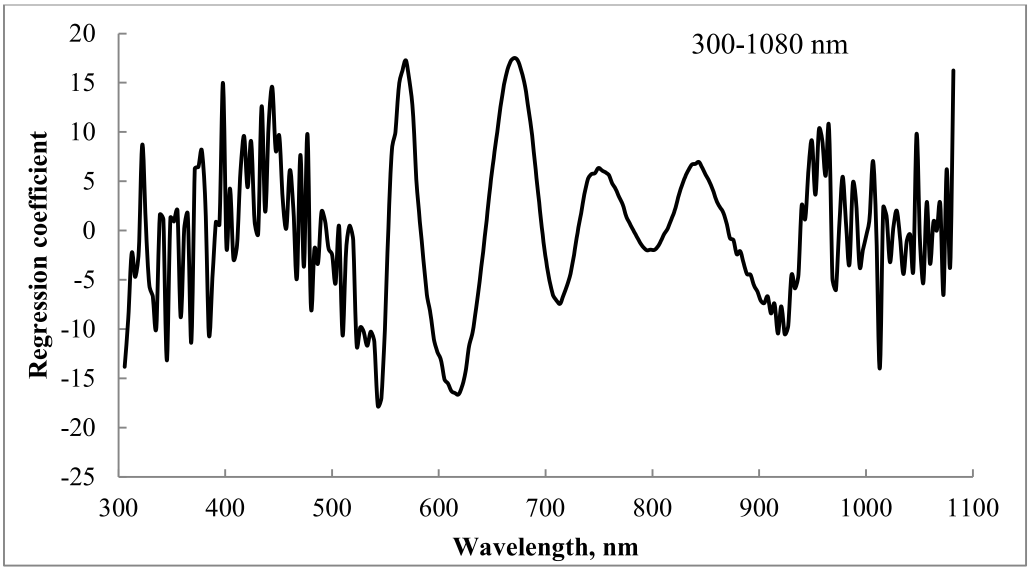


Figure 8. Correlation coefficient for the relationship between absorbance at a given wavelength and visual score and % juice recovery.

Multiple linear regression on score achieved a calibration $R^2_c = 0.41$, RMSEC = 0.97 with use of absorbance at of wavelengths 621, 634, 667 and 790 nm. For % juiciness, a result of ($R^2_c = 0.6$, RMSEC = 5.16) was achieved, using wavelengths 611, 617, 621, 732, 738, 832 and 903 nm.

The regression coefficients for a PLS regression model based on absorbance data and defect score using the entire available wavelength range were noisy at wavelengths below 550 and above 870 nm (Fig. 9), suggesting little information was carried in these regions. The PLS model awarded high positive coefficient values to 550, 575, 661 and 765 nm, and high negative values to 560, 621, 713 and 869 nm.



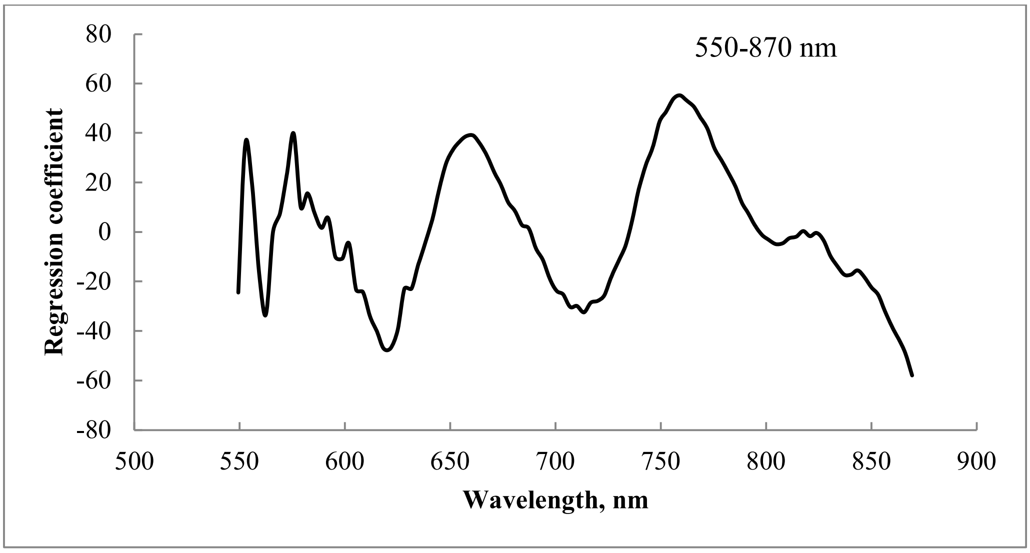


Figure 9. Regression coefficients for a PLSR model of the visual score using absorbance values over the wavelength range of 300-1080 nm (top) and 550-870 nm (bottom).

The predictive performance of the PLSR model was improved using pre-processing treatments (IDD0 spectra, Table 3). The best result, in terms of prediction of an independent set, was achieved with standard normal variate and second derivative pre-treatments. Similar results were achieved for PLSR models based on spectra of the Insight2 unit (data not shown). However, while better results were obtained for the % juiciness model than the visual score model with the IDD0 unit, the reverse was true for the Insight2 unit (Table 4). This result could be due either to the difference in wavelength ranges of the two instruments, or aspects of the two populations of fruit.

Table 3. IDD0 instrument: Partial least square regression (PLSR) model performance for spectra at 550-870 nm for visual granulation score and % juice recovery. Season 1 population.

Parameter	Calibration set (n = 75)			Prediction set (n = 50)		
Visual score	R^2_{cv}	RMSECV	PCs	R^2_p	RMSEP	bias
Abs	0.60	0.87	10	0.33	1.35	0.6
Abs SNV	0.56	0.93	9	0.45	1.1	0.27
Abs MSC	0.51	0.98	10	0.49	1.04	0.19
D2A	0.56	0.93	7	0.49	1.04	0.27
SNVd2A	0.55	0.94	8	**0.49**	**1.07**	**0.33**
MSC d2A	0.54	0.95	7	0.47	1.06	0.28
% juice						
Abs	0.76	3.64	9	0.43	6.49	-1.36
Abs SNV	0.76	3.61	7	0.49	6.01	-0.53
Abs MSC	0.65	4.23	11	0.6	5.22	-0.71
D2A	0.72	3.97	7	0.66	5.04	-0.75
SNVd2A	0.74	3.59	7	**0.68**	**4.87**	**0.13**
MSC d2A	0.66	4.37	7	0.66	4.86	-0.34

Table 4. InSight 2 instrument: Calibration and prediction statistics based on the partial least square regression (PLSR) of SNV d2A spectra at 550-870 for IDD0 and 600-973 nm using visual granulation score (1-5) and % juice recovery as reference parameters.

	Calibration statistics (n = 200)			Prediction statistics (n = 110)		
Parameter	R^2_{cv}	RMSECV	PCs	R^2_p	RMSEP	Bias
Granulation score	0.63	0.8	7	0.62	1.05	-0.57
% juice recovery	0.62	4.78	8	0.32	7.25	3.27
L* cut surface	0.57	2.75	8	0.23	3.65	0.82

Spectral features – discriminant analysis

MLR and PLSR are essentially linear regression techniques, although PLS can handle a degree of non-linearity in the data. However, the level of granulation defect in mandarin fruit as assessed by visual score, luminosity or % juiciness, does not necessarily link to a linear quantitative change in a physical or chemical attribute with an associated spectral feature, in the way that, e.g., water content is related to dry matter content, with water having clear absorbance features in the SWNIR. Therefore, the use of a discriminant technique rather than a regression technique is logical for the assessment of this attribute.

Various algorithms were trialed for the classification of fruit as defect or sound based on spectral information over the range 550-870 nm and 600-973 nm for the IDD0 and Insight 2 units respectively (Table 5). For IDD0 instrumentation, the highest prediction sorting accuracy was achieved with a PLS-DA routine, while for InSight2, the best results were obtained using a PCA-LDA-MD or a PLS-DA (undertaken in Matlab). Better results were obtained with the IDD0 instrument than the InSight 2 instrument (e.g., accuracy of 92 vs. 72% in classification of the validation set), as expected given the higher illumination and transmission geometry employed in the IDD0 instrument.

Table 5. Results of several algorithms for classification of good and defect fruit based on the visual score using IDD0 and Insight 2 instrumentation using raw absorbance spectra at 550-870 nm for IDD0 and 600-973 nm for Insight2 data. Units in percentage. TPR is True positive rate, TNR is true negative rate, FDR is false detection rate. Best prediction results are shown in bold.

	IDD0						Insight 2					
	Calibration statistics (n = 75)		Prediction statistics (n = 50)				Calibration statistics (n = 200)		Prediction statistics (n = 110)			
Classification methods	TPR	TNR	TPR	TNR	Accuracy	FDR	TPR	TNR	TPR	TNR	Accuracy	FDR
PLS-DA (Unsb.)	87.5	81.0	**87.0**	**97.0**	**92.0**	**3.33**	98.00	59.25	100	9.83	54.9	47.4
PCA LDA Linear 8 PCs	87.7	69.2	98.1	43.1	70.6	36.7	88.75	78.75	83.6	43.58	63.6	40.3
PCA LDA MD 5 PCs	82.9	75.5	96.2	50.0	73.1	34.2	98.75	37.5	**97.3**	**47.2**	**72.2**	**35.2**
KNN (4 neighbours)	94.9	81.6	85.4	42.0	63.7	40.4	98.3	86.9	92.2	34.0	63.1	41.7
SIMCA	97.8	48.6	89.2	43.75	66.5	38.6	98.8	30	100	2.77	51.4	49.3
SVM. (Csvc Linear)	98.0	40.1	89.6	25.00	57.3	45.6	98.8	37.5	100	4.16	52.0	48.9
PLS-DA-matlab	89.8	67.9	91.4	48.2	69.8	36.2	86.4	82.5	**80.7**	**52.8**	**66.7**	**36.9**

IDD2 two wavelength model

IDD 2 unit employs LEDs producing four wavelengths. Using a population of 160 fruit (a subset of Season 2 set), the use of various ratios of these wavelengths were assessed (data not shown), with the best result for the detection of granulation defect obtained using Abs 810/Abs 700 nm (Fig. 10).

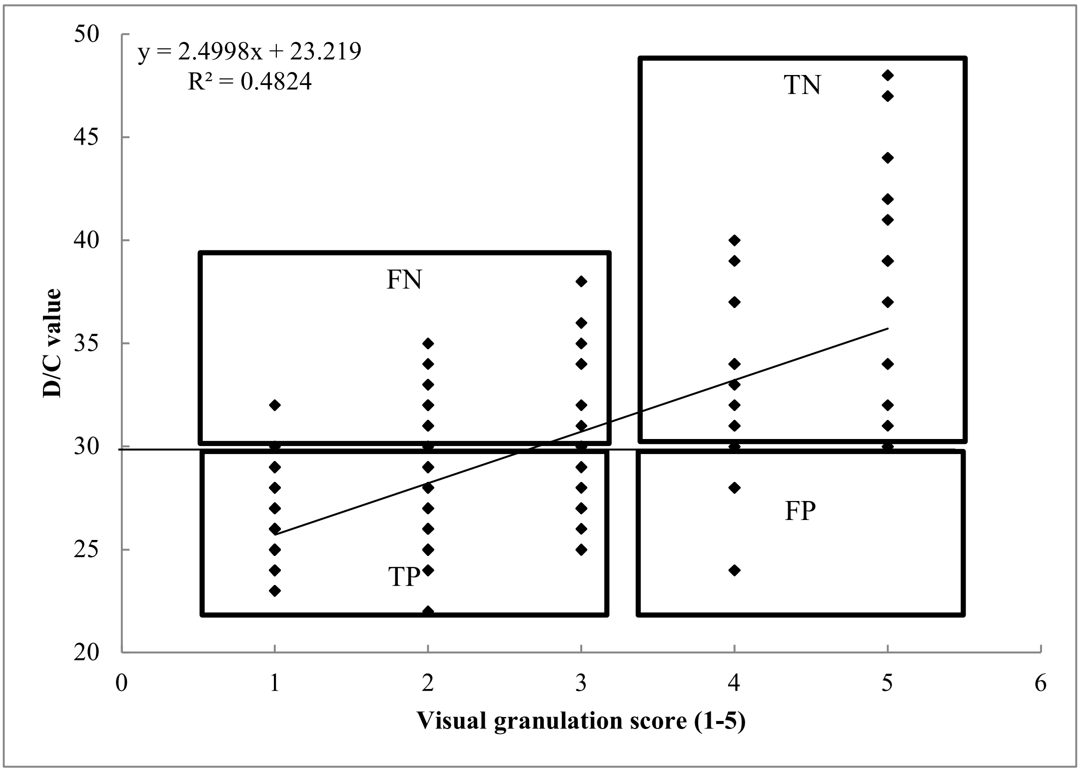


Figure 10. Scatter plot of the ratio of absorbance at 810/700 nm and visual score, for a population involving 160 fruit. FN = false negative, TN = true negative, TP = true positive, FP = false positive, where P is acceptable fruit and N is unacceptable fruit.

IDD2 sorting optimization

A sorting operation is intended to remove defect fruit, ideally eliminating but at least decreasing incidence in the output population. The sorting operation is impacted by the population distribution of the attribute under consideration and the threshold level chosen for the sorting operation.

The impact of varying the threshold set on a sorting function on output classes was considered for the IDD2 results for a population involving 160 fruit (with 126 fruit, i.e., 79% of population having a visual score of 1-3 and 34 fruit or 21% of fruit having a score of 4 or 5). As threshold level was decreased, the distribution of defect level in the output population was shifted to lower values, although this was at the expense of the number of accepted fruits (Fig. 11).

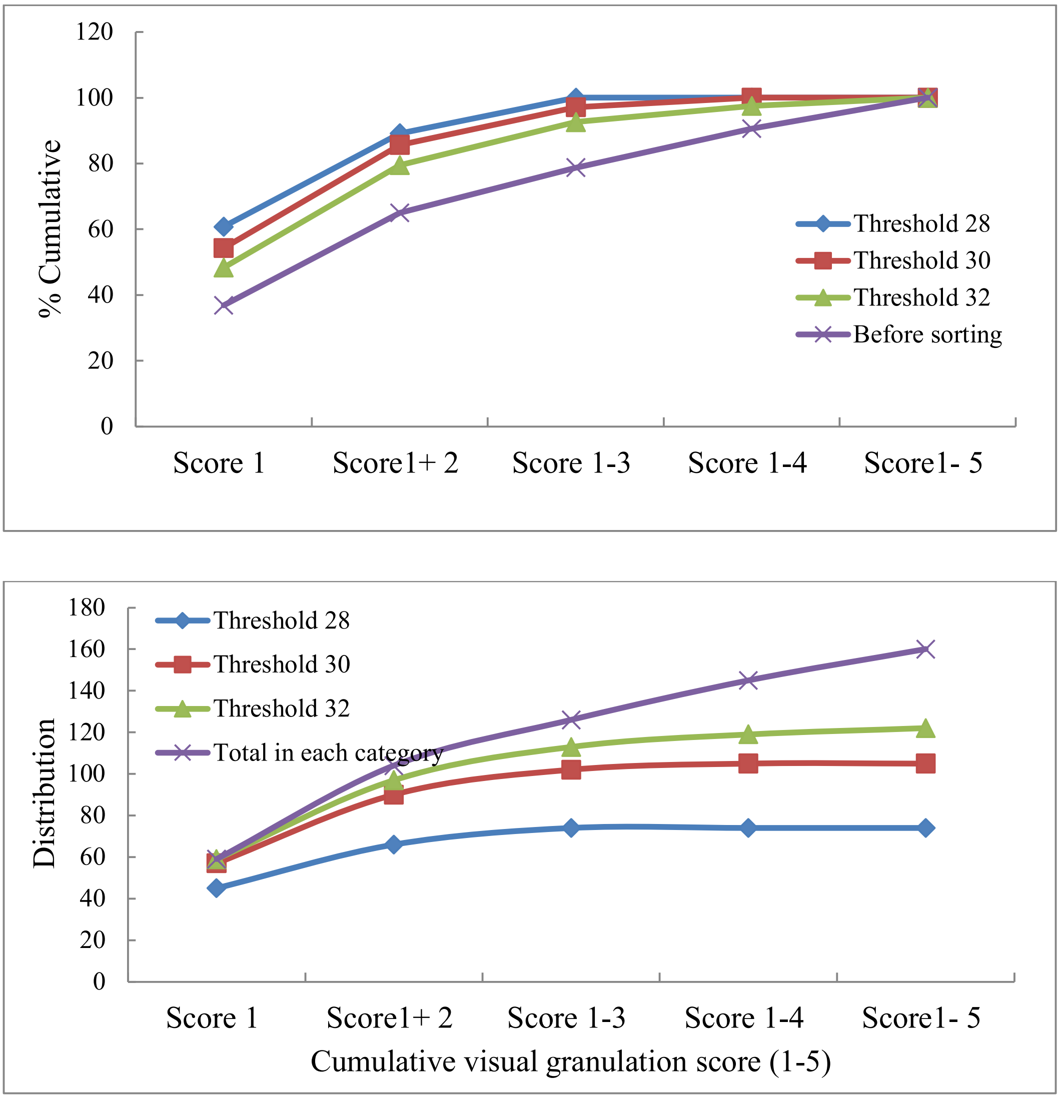


Figure 11. Cumulative distribution of mandarin defect for populations before and after sorting, to various threshold values on the IDD2 detection result. A. Distribution based on % cumulative result. B. Distribution based on fruit number.

The sorting results were re-presented to emphasise features of interest to the operator with a change in threshold value (Fig. 14). For this population, a false discovery rate (FP/(FP+TP)) of 5% (a possible market tolerance point) was achieved at a threshold of 29, associated with a 67% yield. If a false discovery rate of 10% is acceptable, a threshold of 31 can be used, achieving a yield of 78% (Fig. 12).

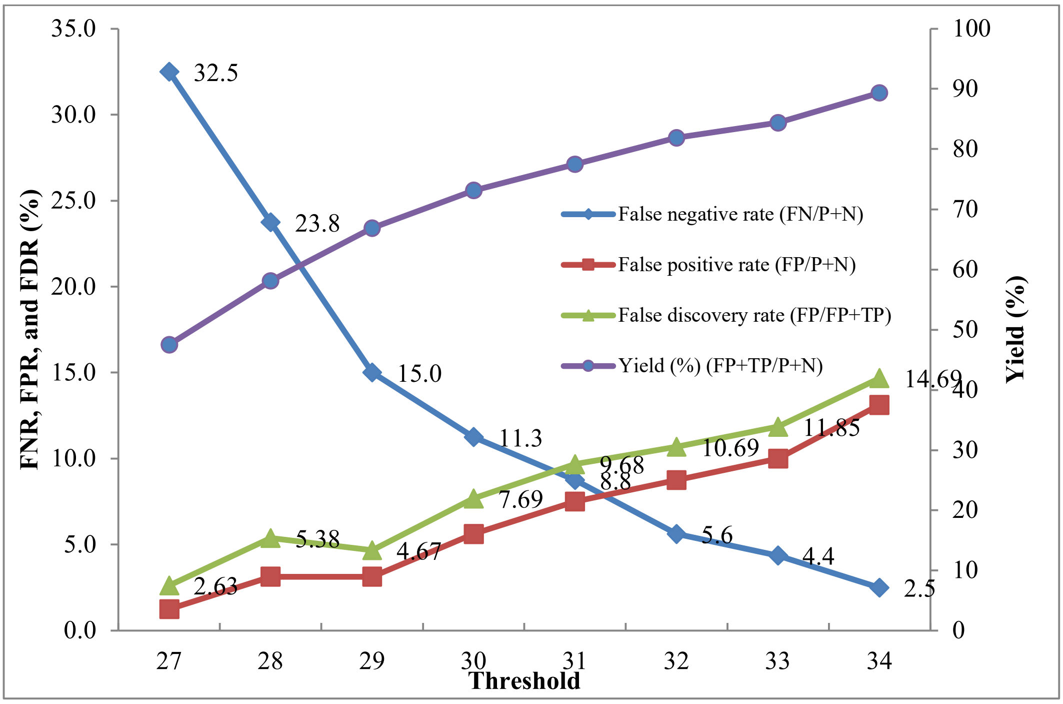


Figure 12. Effect of threshold sorting value on classification error for mandarin defect (with score 1 to 3 considered acceptable fruit and score 4 and 5 deemed defect fruit) for population 2 (involving 160 fruit).

3.4 Conclusion

For non-invasive sorting of fruit for granulation defect using Vis-NIR spectra, a discriminant analysis approach, such as PLS-DA, is recommended over linear regression techniques such as PLS and MLR, and the use of a high intensity illumination system and a full transmission optical geometry is recommended over partial transmission geometries. An in-house developed high illumination, full transmission geometry system (IDD0) achieved an accuracy of 92% on sorting of a test population, a significant advance on previous work (e.g., as reported in CT04002). A high illumination intensity, full transmission two-wavelength discriminator was also shown to be useful. With a sorting threshold of 29 (instrument D/C setting) an out-turn rate (yield) of 78% with a false discovery rate (defect fruit in out-turn population) of 5% for an input population with 21% defect fruit. This was achieved using the commercially available IDD2 packing line instrument. Further trials are recommended to demonstrate the robustness of detection for fruit of a range of growing conditions, and the utility of use in a packhouse sorting environment, with compromise between false negative and false positive rates impacted by the defect distribution of the population assessed and the threshold level chosen for the sorting operation.

3.6 Acknowledgements and Disclaimer

MAF Oceania (Bacchus Marsh, Victoria) is acknowledged for provision of the IDD2 test unit. The CQUniversity team have a longstanding interaction with MAF Roda.

3.7 References

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4. Recommendations

Several technologies have potential for non-invasive detection of granulation in Imperial mandarin fruit. Recommendations are made, in order of priority.

Vis-NIRS is available within existing packing-line systems, and an assessment of the MAF Roda IDD2 gave encouraging results. The technology has measurement errors, such that practical application becomes a statistical exercise, balancing error types.

Recommendations:

1. A third party assessment of the performance of on-line full transmission optical grading systems applicable to granulation assessment, e.g., from MAF-Roda, Compac, Aweta. Ideally such an assessment would use a common sets of test populations of fruit followed by a common manual assessment of defect level.
2. Given satisfactory results from (1), provision of a users tool to guide setting of threshold levels in a sorting operation with knowledge of input population granulation level and desired output levels.

MRI is not available in a form appropriate to on-line or at-line use in packhouses. However, the technology is rapidly advancing, and a point will come where translation to horticultural use is possible.

Recommendation:

3. Discussion with UQ MRI group regarding the potential for an at-line assessment tool with long term potential for use on-line.

Results of a single published study suggest capacitance technology has potential for use in a handheld device for assessment of granulation of fruit in field.

Recommendation:

4. Seek industry feedback on whether a non-invasive assessment tool for use in-orchard is of interest, to enable assessment of a greater number of fruit than manual destructive assessment. If so, a pilot R&D project could be undertaken to test the capacitance technology. Given positive outcomes, this could be followed by development of a field-portable device for use in-orchard, ideally in concert with a portable equipment manufacturer.