Horticulture Innovation Australia

# Final Report

## Optimising cherry fruit set, crop load, fruit nutrition and size - Phase 2

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# Summary

This report describes the findings and implications of research conducted in areas of commercial interest to Australian cherry growers, and is reported in key activity sections:

1. Use of plant bio regulators (PBRs) for optimal crop load management - improving fruit set of shy bearing varieties and thinning of heavy setting varieties;

2. Girdling and floral biology for prevention of fruitlet abscission and improved fruit quality; and,

3. Fertigation for fruit quality. The program was a continuation of the successful collaboration with Associate Professor Matt Whiting of Washington State University whose contribution was mainly to the crop load management component of this project.

## PBRs for optimal crop load management:

Fruit set affects both yield and fruit quality, hence is a core issue of profitability for growers. Inadequate fruit set leads to low yields and a high incidence of fruit cracking; excessive fruit set leads to over-cropping and undersized, soft fruit. This study has examined strategies for solving the two distinct problems in management of crop load in sweet cherry cultivars: (i) poor set in shy bearing cultivars such as 'Kordia' and 'Regina'; and (ii) excessive set leading to overcropping in other cultivars.

To improve fruit set in shy-bearing cultivars several plant bio regulators (PBRs) were examined on both 'Kordia' and 'Regina', including: Retain® (15% amino vinyl chloride, Valent BioSciences Corporation); paclobutrazol (Payback®, 250 g/L paclobutrazol, Crop Care Australasia Pty Ltd); potassium nitrate ($KNO_3$); foliar organic nutrients (Quadshot®, SLTec); sodium molybdate, 2,4-D (Commercial Citrus Stop Drop®, Kendon Chemical & Mnfg. Co. Pty Ltd); naphthalene acetic acid (20 g/L NAA, Kendon Chemical & Mnfg. Co. Pty Ltd); amino acids - Flower 'n' Fruit Maker® (635 g/L amino acids, Wilchem); Tops® (10% 3,5,6-trichloro-2-pyridyloxyacetic acid, Colin Campbell Chemicals Pty Ltd); and Cytolin® (19 g/L gibberellins $A_{4+7}$, 19 g/L 6-benzyladenine, Valent BioSciences Corporation). A comparison of two rootstocks was also undertaken.

Retain was consistent in improving fruit set in both cultivars (up to 65% in 'Kordia' and 48% in 'Regina'). A single application prior to full bloom was as effective as two applications. In wet seasons, Retain reduced the incidence of fruit cracking in 'Kordia' by up to 44%. A reduction in stem pull force was observed in all Retain trials, however stem pull force was still considerably higher than the 500 g force standard set for market; hence this does not pose a problem. It was concluded that Retain is beneficial in improving fruit set, but may impact slightly on fruit size in some years. Paclobutrazol improved fruit set by 11% when applied in Spring. Autumn application resulted in improved fruit quality, but did not improve fruit set. Soil drench application also improved fruit quality more than butt spray application. There were no carry-over effects the following season. Amino acids had no effect on fruit set, but when supplementing the Retain program with amino acids 'Regina' fruit set was increased by an additional 30% to 77%. Cytolin and sodium molybdate reduced fruit set, while fortnightly application of foliar organic nutrient had no effect on fruit set, but in some seasons increased fruit weight and fruit firmness. Other PBRs examined had no effect on fruit set.

In a comparison of 'Regina' fruit set on Colt and Gisela 6 rootstocks, there was no difference in number of buds or flowers between the two rootstocks, but fruit set on Gisela rootstocks was 52% higher than on Colt.

For reducing crop load in heavy setting cultivars both ethephon and ammonium thiosulphate (ATS) were examined. Shuck fall was the most effective application time for ethephon, and a rate of 200-300 ppm reduced crop load by 24-51% depending on season. Single application of ATS resulted in a small reduction in crop load, but two applications at 30 and 80% bloom reduced crop load by 34%. Ethephon reduced stem pull force in the 2013/14 season, but not in other seasons. The conclusions from this work are that both ethephon and ATS are effective thinners in sweet cherry, however in frost prone areas, ethephon may be a safer choice as it is applied later in the season when the risk of frost is reduced.

Further research is required to determine the feasibility of incorporating amino acids into a Retain program to further improve the efficacy of Retain in improving fruit set, particularly if Retain and amino acids can be applied as a tank mix. To confirm that fruit set is higher in dwarfing rootstocks, the comparison between Gisela and Colt rootstocks should also be repeated.

# Girdling and floral biology for prevention of fruitlet abscission and improved fruit quality:

Fruitlet abscission is an issue impacting the profitability of the cherry industry, with particular varieties showing enhanced susceptibility. This study examined the role of carbohydrate availability in fruitlet abscission and post-harvest fruit quality in two sweet cherry varieties, 'Kordia' and 'Lapins'. Starch and soluble sugar concentrations in branch, trunk and root tissue were measured regularly throughout the growing seasons of 2012/2013, 2013/14 and 2014/15. Normal transport of carbohydrates was disrupted 5 weeks after full-bloom by applying trunk girdling and limb defoliation treatments, and the rate of flower and fruitlet abscission was monitored. In 'Lapins', trunk girdling decreased the rate of abscission, but abscission was unaffected by girdling in 'Kordia'. Trunk starch concentrations were higher in 'Kordia' than 'Lapins', and shoot starch concentrations were very similar to trunk in both varieties. Root carbohydrate concentrations did not recover after the trunk girdling treatment in either variety. There was a high rate of abscission in 'Kordia' in the 2013/14 and 2014/15 season which did not appear to correlate with differences in carbohydrate status of the tree. Flower biology tests showed issues with synchronisation of 'Kordia' bloom with its pollinator variety 'Regina' at the trial site, as well as reduced pollen germination rates following rain events. Further research into the flower biology of the low yielding variety 'Kordia' is recommended, including the use of plant bio regulators to improve fruitset.

Girdling in 'Kordia' resulted in fruit that were significantly firmer, higher in TSS, and larger in diameter which was further pronounced after postharvest storage. Defoliation in 'Kordia' resulted in softer fruit, lower TSS, and smaller in both mass and diameter. The 'Lapins' treatments often didn't respond in the same way that 'Kordia' did and thus the results of this study indicate a distinct carbohydrate source: sink relationship in 'Kordia' relative to 'Lapins', which may be due to differences in sink competition. Girdling can lead to increased carbohydrate availability resulting in higher quality fruit, particularly following post-harvest storage which could improve outcomes when exporting.

## Fertigation for fruit quality:

Nutrient management is a critical component of commercial sweet cherry orchard production as each nutrient plays an important role in the development and quality of sweet cherry fruit. Whilst fertigation is commonly practised by cherry growers in Australia, research into optimal nutrient supply to meet tree demands is limited and the effects of oversupply of pre-harvest nutrition on fruit quality are virtually unknown. Furthermore, even less is known about the influence of micro-element nutrition on sweet cherry fruit quality and which is the best method of application. This study investigated the role of Nitrogen (N) and Potassium (K) fertigation on sweet cherry fruit quality and post-harvest shelf-life whilst at the same time shed light on the role of pre-harvest micro-element nutrition on fruit quality.

This report summarises data from five separate trials conducted over a four year period. The research commenced originally at Grove Research Station in the Huon Valley region of southern Tasmania where N fertigation and micro-element trials were established. Changes in orchard management jeopardised the reliability of ongoing trials at this orchard. Therefore new integrated N fertiliser and irrigation trials were established at Ridgy Didge Cherries, a commercial orchard in the same region.

Nitrogen fertigation results showed that N content in sweet cherry fruit could be manipulated by fertigation treatments when base levels of N were suitably low. In this case, when high levels of N were applied, detrimental effects on fruit firmness were observed. Sensory analysis demonstrated that consumers strongly preferred export grade over high N fruit and that perception generally matched instrumental fruit quality assessments for the range of parameters tested (sugar, acid) with the exception of firmness. However, when base levels of fruit N matched commercial standards, high levels of applied N only slightly increased fruit N content but had no detrimental influence on fruit quality at harvest or after 28 days in storage. Irrigation treatments had little effect on fruit quality other than increased cherry size with increased volume.

Pre-harvest foliar micro-element sprays were more effective in increasing respective nutrient content in leaves and fruit than fertigated treatments of the same elements. Yet limited influence of micro-element fertilisers were observed on fruit quality at harvest or after 28 days in storage. Pre-harvest K treatments increased fruit firmness at harvest and in stored fruit and again foliar applied K was the more effective fertiliser.

Recommendations for the management of N fertiliser and micro-element nutrition are described.

## Keywords: cherry, fruit quality, crop load, plant bioregulators, girdling, fertigation, floral biology, consumer perception, firmness, stem pull

# Introduction

## PBRs for optimal crop load management:

Plant bio regulator (PBR) applications during flowering are common for the varied goals of improving fruit set, reducing crop load, and/or improving fruit quality. Ethephon has been reported to hasten the senescence of ovules in fruit species (Crisosto et al., 1985). In addition, Lombard and Richardson (1982) reported increases in fruit set and cropping of 'Comice' pear with applications of 300 mg/L of AVG after full bloom. There are no reports of plant growth regulators improving fruit set in sweet cherry. Stosser and Anvari (1982) reported that ovule longevity in sweet cherry was generally 4 to 5 days after anthesis and that applied growth regulators, including $GA_3$, enhanced ovule senescence. This proposal builds logically from our previously funded project CY10002. We have good preliminary evidence for improving fruit set with PBRs (based on our research into factors limiting fruit set) and thinning in early stages of fruit development, post-bloom. Studies of rate-response and timing of application are needed now to develop reliable recommendations.

To manipulate crop load in heavy setting cultivars, growers are reliant on hand-thinning, an expensive time consuming task, and as hand thinning is carried out around pit hardening stage of fruit growth, the gains in fruit size and return bloom are less than if excess flowers were removed early in the season. This practice can also contribute to irregular bearing habits. Previous work has demonstrated that fruit quality aspects such as size, colour, sugar levels and firmness in both pome fruit and cherries are affected by crop load during the early part of the season.

Timing of thinning is critical to fruit quality, particularly size, firmness and sugar content. Maximising resources during the cell division period of fruit growth that occurs in the first weeks after flowering will maximise both fruit size and internal quality. Leaving excess fruit on the tree during this period is a waste of the trees resources, as growth is put into fruit that is later removed. It is more productive to channel this energy into fruit that will remain on the tree through to harvest. This is particularly important in drought years when water resources are scarce.

In cultivars that are prone to excessive fruit set, there are several strategies that can be used to control crop load:

a. Use of pruning strategies and spur/bud extinction;

b. Reducing the number of flowers through control of flower initiation;

c. Prevention of fruit set (bloom thinning);

d. Fruitlet thinning (post-bloom thinning).

Strategies such as the use of substantial removal of flower buds during dormant pruning and application of blossom thinners early in the flowering period provide a means of maximising fruit quality, and thus returns to the grower. Compared with fruitlet thinning, both floral inhibition and bloom thinning have clear advantages as inter fruit competition for assimilates is reduced early in the season. However due to the risk of frosts during the bloom period, many growers are reluctant to apply blossom thinners, hence there is still a need to examine post-bloom thinning agents.

## Girdling and floral biology for prevention of fruitlet abscission and improved fruit quality:

Inconsistency in yield is an important problem for sweet cherry producers in Australia. Inconsistency of cropping can be due to the abscission of unpollinated flowers, flowers that have failed to set, and fruitlets that are not fully mature (Blanusa et al., 2005). The drivers of abscission of fruitlets prior to maturity in *Prunus* are not known, but environmental influences and competition for resources such as photoassimilates and plant hormones are likely causal factors (Thompson, 1996; cited in Blanusa et al., 2005). Moreover, it has been suggested for citrus that there's a strong relationship between carbohydrate available to the fruitlets, especially soluble sugars, and the probability of fruitlet abscission (Iglesias et al., 2003).

There is circumstantial evidence that developing fruitlets compete for photoassimilates, and it has been suggested that fruits that do not reach a certain threshold carbohydrate concentration have a high probability of abscission (Mahouachi et al., 1995). In regions where flower buds develop earlier than vegetative buds, flower development must rely upon total non-structural carbohydrate reserves until enough photosynthetic leaf area is present to provide sufficient photosynthate (Keller and Loescher, 1989).

## Fertigation for fruit quality:

Deciduous fruit trees accumulate and store nutrients at the end of the growing season for remobilisation in the following spring (Loescher et al., 1990). This resource remobilisation is critical for growth of flowers, fruit, leaves and shoots, yet little is known about seasonal nutrient budgets and the storage and remobilisation of nutrients (Frak et al., 2006). This is particularly true in a region such as southern Australia where limited research into the seasonal, soil and cultivar implications on nutrient requirements of sweet cherry has been completed. However, principles gleaned from research in other regions can be used to guide nutrient management and identify region specific research questions.

In highly studied macro-nutrients such as Nitrogen (N), it is well recognised that increasing the rate of N application can increase vegetative growth and yield but adversely affect fruit quality by decreasing fruit colour and firmness (Oberly and Boynton, 1966, Neilsen et al., 2003, Fallahi et al., 1993). James (2011) suggested that applying too much before harvest can cause uneven ripening, delay ripening and reduce fruit shelf-life. In addition, studies have shown that the efficacy of N application in orchards is related to irrigation practice as excess water can leach N below the root zone (Neilsen and Neilsen, 2002) while soil-water stress may reduce the tree's capacity for nutrient uptake. Therefore, the regulation of N and water is a crucial management consideration for commercial orchard production. The effectiveness of matching nutrient supply with tree demand requires a sophisticated understanding of seasonal cherry tree N recycling to maximize the advantages inherent in being able to apply N and water simultaneously. Fertigation and foliar nutrient application are important tools in the management of cherry nutrition and provide a more precise solution to meeting tree nutrient demand.

Precision farming through fertigation can facilitate efficient utilization of resources and improve returns per unit area and time to growers. Fertigation delivers both water and essential nutrients such as N directly to the active root zone of growing crops through micro irrigation systems, thereby minimising water and nutrient loss and improving productivity (Klein et al., 1989). Whilst fertigation is

commonly practised by cherry growers in Australia, research and management guidelines for optimal supply of tree nutrient and water requirements are limited.

Foliar application of nutrients in tree fruit crops is becoming an increasingly popular fertiliser management strategy. Foliar applied fertilisers are now proven in their ability to correct nutrient deficiencies and to place required nutrients that are otherwise immobile in the desired location for efficient uptake. In the Australian cherry industry supplementary crop nutrient requirements are met often using foliar fertiliser sprays. Fruit responses to foliar applications are readily observed and for many cultivars, ideal elemental concentrations for optimum production are well established. Yet, when deficiencies are observed, there is limited understanding of optimum rates and timing to correct them. This is due to the variation in growing region and environmental characteristics which will influence the rate and efficiency of uptake. In addition, the ontology of fruit development influenced heavily by cultivar, latitude, altitude and soil type will add further uncertainty to the timing of foliar applied elements.

This study investigated the role of Nitrogen (N) fertigation on sweet cherry fruit quality and post-harvest shelf-life whilst at the same time shed light on the role of pre-harvest microelement nutrition on fruit quality with an emphasis on Potassium (K).

# Key Activity 1: PBRs for optimal crop load management

***Lead Sally Bound: See appendices 1 & 2 for detailed reports***

## Methodology and results

This study built on the findings of CY10003 (initial findings of increased fruit set of 'Kordia' with the use of Retain) and focused on:

**Improving fruit set in shy-bearing cultivars**

a) examination of the ethylene inhibitor Retain® (15% amino vinyl chloride, Valent BioSciences Corporation) to improve fruit set in 'Regina' and 'Kordia'

b) paclobutrazol (Payback®, 250 g/L paclobutrazol, Crop Care Australasia Pty Ltd) application timing (autumn vs spring) and method (butt spray vs soil drench) to improve fruit set in 'Regina'

c) potassium nitrate ($KNO_3$) to improve fruit set in 'Regina'

d) a range of other PBRs including foliar organic nutrients, sodium molybdate, 2,4-D (Commercial Citrus Stop Drop®, Kendon Chemical & Mnfg. Co. Pty Ltd) and naphthalene acetic acid (20 g/L NAA, Kendon Chemical & Mnfg. Co. Pty Ltd) foliar organic nutrients (fortnightly) and amino acids (at 30% &75% bloom ), Triclopyr (20 ppm Triclopyr (Tops) at pit-harden) and Cytolin (50% bloom, full bloom (FB) & 2 weeks after FB) to improve fruit set in 'Regina' and 'Kordia'

**Reducing fruit set in heavy-setting cultivars**

e) Examination of ethephon application rate and timing and comparison with ATS for reducing crop load in 'Van'


*Figure 1: Ethrel trial on Vans in the Derwent Valley*

## Year 1 trials (2012/13)

### (i) examination of ethephon application rate and timing for chemical thinning

Ethephon was applied at three concentrations (100, 200 or 300 ppm) and three application times (full bloom, shuck fall, or 2 weeks after shuck fall) to mature 'Van' trees. Sprays were applied with an hydraulic hand gun to runoff. The wetter Kendeen 20 was added to all spray at the label rate. These treatments were compared with an untreated control and a handthin treatment. The lowest application rate of 100 ppm had no thinning effect, but both 200 and 300 ppm rates reduced crop load. Shuck fall was the most effective application timing.

Both fruit flesh firmness and stem retention force were reduced in ethephon treated fruit compared with the untreated control. At harvest, rate of ethephon had no effect on fruit flesh firmness, but at 42 days post harvest, fruit flesh firmness increased with increasing concentration of ethephon.

**(ii) Examination of the use of AVG (Retain) to improve fruit set in 'Regina'**

Following on from 'Kordia' trial in the 2011/12 season under project CY10002, a similar trial was established on 'Regina' in the 2012/13 season. To evaluate the impact of application timing, whole-tree applications of Retain® were made by hydraulic hand lance at 30% and 80% bloom, or as a double application at 30 and 80% bloom. On each application date, Retain® was applied at 500 or 1,000 g/ha.

Retain® applications increased fruit set by 33% (from 23.1 to 30.7%), compared to untreated control trees. We found no significant differences between the two application rates, nor was there any effect of application timing. Fruit size was increased slightly (2%) by the lower, but not the higher, rate of Retain ($p<0.001$).

**(iii) comparison of 'Regina' cropping on 2 different rootstocks: Colt and Gisela 6.**

There was no significant difference in number of buds or flowers between the two rootstocks, but fruit set on Gisela rootstocks was 52% higher than Colt.

Trees on Colt rootstock produced significantly smaller fruit than Gisela, however this may have been due to reduced water availability in the Colt block, as these trees received reduced water during the second part of the growing season. Fruit from Colt trees showed higher malic acid content but there was no difference between rootstocks on sugar content or juice pH. Colt fruit was firmer that Gisela, but had reduced skin puncture force and lower stem retention force that Gisela fruit.

While the results of this rootstock comparison suggest that fruit set is higher in Gisela rootstock, the comparison needs to be repeated to confirm that the difference is not due to the restricted water supply in the Colt trees. This lack of water is also most likely to have been the cause of the reduced fruit size in the trees on Colt rootstock and may also have affected other fruit quality parameters.

## Year 2 trials (2013/14)

Trials were established as follows:

### Improving fruit set in shy-bearing cultivars

- further examination of the ethylene inhibitor Retain® (15% amino vinyl chloride, Valent BioSciences Corporation) to improve fruit set in 'Regina' and 'Kordia'
- paclobutrazol (Payback®, 250 g/L paclobutrazol, Crop Care Australasia Pty Ltd) application timing (autumn vs spring) and method (butt spray vs soil drench) to improve fruit set in 'Regina' (including follow up for carry-over effects)
- potassium nitrate ($KNO_3$) to improve fruit set in 'Regina'
- a range of other PBRs including foliar organic nutrients, sodium molybdate, 2,4-D (Commercial Citrus Stop Drop®, Kendon Chemical & Mnfg. Co. Pty Ltd) and naphthalene acetic acid (20 g/L NAA, Kendon Chemical & Mnfg. Co. Pty Ltd) to improve fruit set in 'Regina' and 'Kordia'

*Figure 2: Flower counts for subsequent fruit set assessments*

## Reducing fruit set in heavy-setting cultivars

- Further examination of ethephon application rate and timing and comparison with ATS for reducing crop load in 'Van'

## Improving fruit set in shy-bearing cultivars

### *(i) Retain® to improve fruit set in 'Kordia' and 'Regina'*

Retain® was applied to both 'Kordia' and 'Regina' trees at the rate of 500 g Retain®/ha as a single application at either 50% bloom or 4 weeks after full bloom (wAFB). Sprays were applied by hydraulic hand lance as a fine mist at 1,000 L/ha water volume.

Bloom application of Retain® increased fruit set by 47% in 'Regina', while application at 4 wAFB had no effect; results for 'Kordia' were not significant, however similar trends were observed. Shedding of fruit was completed by 3rd December in both varieties.

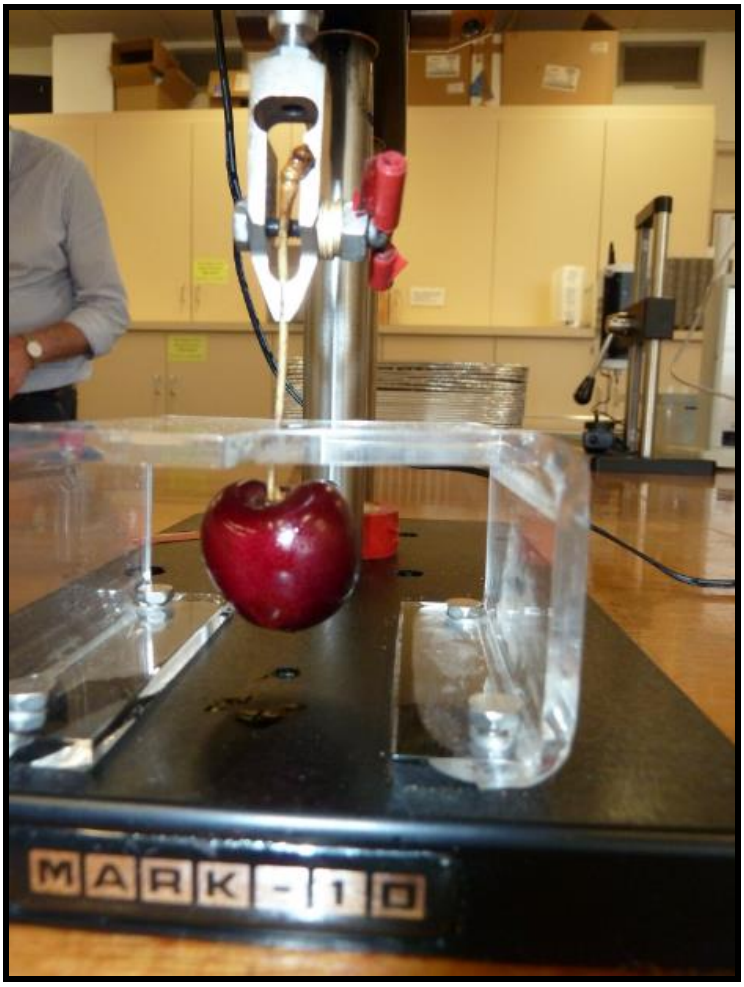


*Figure3: Modified Mark-10 stem pull force gauge*

***(ii) Examination of paclobutrazol to improve fruit set in 'Regina'***

Many growers use the systemic growth regulator paclobutrazol in an attempt to improve fruit set, however there is no evidence available to confirm that it does improve fruit set, and also a lack of information on application rate, optimal application time or application method. When soil applied, paclobutrazol also has a long residual life. This trial was designed to answer some of the questions surrounding the use of paclobutrazol for improving fruit set in sweet cherry.

Payback™ (250 g/L paclobutrazol, Crop Care Australasia Pty Ltd) was used in this study. Two application times (autumn vs early spring) were examined. At each time, Payback™ was applied at either 1, 2 or 4 ml product per tree as a soil drench or as a spray directly to the tree trunk (butt spray) to mature 'Regina' trees. For the soil drench treatments, the required dose of Payback™ was applied in 1 L water per tree to the soil around the base of the tree using a watering can; for the butt sprays, the Payback™ dose was applied to each tree trunk in 50 ml water in three vertical swipes of 20 cm length starting 10 cm above the soil. The autumn application was applied on 5th April 2013, and the spring application on 16th September 2013.

Spring application of paclobutrazol increased fruit set by 11%, autumn application had no effect. A-grade fruit was increased in the autumn treatments compared with the untreated control, while both autumn and spring applications reduced the percentage of reject fruit. Mean fruit weight was

increased by paclobutrazol applied in both autumn and spring, but the autumn application had the greatest effect. Rate of paclobutrazol had no significant effect on fruit pack-out or size, but the fruit set was 12.9 % higher in the 4 ml/tree treatments compared with the untreated control. Application method had no effect on pack-out but mean fruit weight was highest in the soil drench treatments.

Weight and diameter of A-grade fruit, fruit firmness, skin puncture force and stem retention force were all increased with autumn application of paclobutrazol. Application rate had some effect on most fruit quality parameters, with the 2 ml/tree treatments in general having the greatest effect. Weight and diameter of A-grade fruit and skin puncture force were higher in the soil drench treatments than the butt spray treatments.

### *(iii) Potassium nitrate ($KNO_3$) and ethephon to improve fruit set in 'Regina'*

Like paclobutrazol, winter application of potassium nitrate and autumn application of ethephon have been touted to increase flower initiation, thus leading to improved fruit set in shy bearing sweet cherry cultivars.

This trial was set up on 'Regina' in the same block as the paclobutrazol trial, and consisted of three treatments:

1. Untreated control
2. 75 kg/ha $KNO_3$ late winter application applied in 1,000 L/ha water volume
3. 750 ml/ha Ethrel® (720 ml/L ethephon, Bayer Crop Science) autumn application applied in 2,000/ha water volume

Ethrel® was applied as an autumn application on 5th April 2013, and $KNO_3$ as a late winter application on 27th August 2013. All sprays were applied with an hydraulic handlance. Flower counts were undertaken on 3 tagged limbs on each tree between 10-15 October, and fruit set counts on 3rd December 2013. Fruit was harvested from tagged limbs on 17th January 2014 and stored at 1°C prior to quality assessment.

There was no difference in number of flowers per tree between treatments or in fruit set, pack-out or mean fruit weight.

Compared with the untreated control, A-grade mean fruit weight and diameter was reduced by ethephon, but increased by $KNO_3$. Fruit compression force was increased by $KNO_3$. Ethephon reduced fruit flesh firmness and stem retention force, but $KNO_3$ had no effect.

### *(iv) Preliminary assessment of PBRs to improve fruit set in 'Kordia' & 'Regina'*

A range of PBRs were examined for their efficacy in improving fruit set of 'Regina' and 'Kordia'. In addition to an untreated control to determine the baseline fruit set in each cultivar, 500 g Retain®/ha applied at 50% bloom was included as a standard treatment as this has been shown to be effective in previous years. Other treatments included:

1. Fortnightly application of an organic nutrient mix (Quadshot, Sustainable Liquid Technology Pty Ltd (SLTEC)) containing fish emulsion, kelp, molasses, humic acid, fulvic acid, N, P, K, Ca, B and Zn. Quadshot was applied at 1.2 L/ha in 1,000 L/ha water volume, and treatment commenced approx. 3 weeks after flowering.

2. Molybdate (Mo) in the form of sodium molybdate ($Na_2MoO_4$) applied at 50% bloom at the rate of 0.36 g sodium molybdate per tree applied in 1,000 L/ha water (equivalent to 170 ppm Mo). Mo is used to improve fruit set in grapes.

3. 10 ppm 2,4-D (Commercial Citrus Stop Drop®, Kendon Chemical & Mnfg. Co. Pty Ltd) applied to drip at pit-harden stage of fruit development. This product is used to prevent fruit abscission in citrus.

4. 10 ppm naphthalene acetic acid (20 g/L NAA, Kendon Chemical & Mnfg. Co. Pty Ltd) applied to drip at pit-harden stage of fruit development. The wetter Kendene was added at 1 ml/L of spray solution. NAA prevents fruit abscission in pome fruit when applied prior to harvest.

Both retain and 2,4-D treatments increased fruit set in 'Regina' by 48% and 46% respectively, while Mo reduced fruit set by 30%. There were no significant treatment effects on 'Kordia'.

| Planned treatments | Actual treatments |
|---|---|
| 1. control | control |
| 2. 250ppm Ethrel at shuck fall (SF) | 250 ppm Ethrel at SF |
| 3. 1% ATS at 30% bloom | - |
| 4. 1% ATS at FB | 1% ATS at 100% bloom |
| 5. 1% ATS at SF | 1% ATS at SF |
| 6. 1 % ATS at 30% & FB | - |
| 7. 1% ATS at FB & SF | 1% ATS at 100% bloom & SF |

*Figure 4: Treatments applied in Trial vix*

## Reducing fruit set in heavy-setting cultivars

### *(v) Efficacy of ethephon and ATS in reducing crop load of 'Van'*

Following on from work with ethephon in year 1 (2012/13), the aim of this study was to confirm the efficacy of Ethrel® (720 ml/L ethephon, Bayer Crop Science) in reducing crop load of 'Van' and undertake an assessment of ammonium thiosulphate (ATS). Treatments applied are shown – the treatments planned for 30% bloom were unable to be applied due to severe wind and heavy rain during the flowering period, hence a modified trial design was used.

Relative to the untreated control, fruit set was reduced by ethephon and the bloom ATS applications. The proportion of A-grade fruit was reduced with ethephon and increased with the single ATS bloom treatments. Mean fruit weight was increased by ATS applied during bloom, while all treatments except for the SF ATS application increased fruit weight in the A-grade fruit. There was no effect on cracking incidence.

## Year 3 trials (2014/15)

All trials were established in commercial orchards in September 2014.

In all trials, two limb sections were tagged on opposite sides of each treatment tree and the number of spurs, floral buds and individual flowers counted in late September / early October for each tagged section; fruit set counts on each tagged limb were completed in early December. All trials were harvested at normal commercial harvest times. Two subsamples of 30 A-grade fruit were randomly selected for laboratory analysis of harvest and post-harvest fruit quality.

## Improving fruit set in shy-bearing cultivars

### *(i) Improving fruit set with PBRs*

Following on from results in 2013/14 trials, the PBRs showing potential were re-assessed with the addition of other products which have been reputed to improve fruit set in other crops. Retain is currently used in the cherry industry for improving fruit set, so was included as a known 'standard' in this work.

Cultivars: 'Kordia', 'Regina' Rootstock: Colt

Site: Cherries Tasmania, Old Beach

Treatments: ('Regina')
1. control
2. 500 g Retain/ha at 50% bloom
3. Foliar organic nutrients fortnightly
4. Amino acid (AA) at 30% &75% bloom
5. Retain + AA
6. 10 ppm amine 2,4-D at pit-harden
7. 20 ppm Triclopyr (Tops) at pit-harden
8. Cytolin at 50% bloom, full bloom (FB) & 2 weeks after FB (wAFB)

Treatments: ('Kordia')
1. control
2. 500 g Retain/ha at 50% bloom
3. Foliar Organic nutrients fortnightly
4. amino acid (AA) at 30% &75% bloom
5. Retain + AA
8. Cytolin at 50% bloom, FB & 2 wAFB

### *(ii) Carryover effects of paclobutrazol*

Cultivar: 'Regina' Rootstock: Colt

Site: Cherries Tasmania, Old Beach

This study commenced in autumn 2013, and was designed to answer some of the questions surrounding the use of paclobutrazol for improving fruit set in sweet cherry, examining both application time (autumn vs spring) and method (butt spray vs soil drench) of paclobutrazol (Payback®) on 'Regina'. Payback® was applied at rates of 1, 2 or 4 ml/tree.

Return fruit set and fruit quality were assessed in the 2014/15 season to ascertain whether paclobutrazol treatments had any carry-over effects.

Details of PBRs used in trials:

1. Retain® (150 g/kg aviglycine HCl, Valent BioSciences Corporation)
2. Amino acids - Flower 'n' Fruit Maker® (635 g/L amino acids, Wilchem)
3. Foliar organic nutrients (Quadshot®, SLTec)
4. 2,4-D (Commercial Citrus Stop Drop®, Kendon Chemical & Mnfg. Co. Pty Ltd)
5. Tops® (10% 3,5,6-trichloro-2-pyridyloxyacetic acid, Colin Campbell Chemicals Pty Ltd)

6. Cytolin® (19 g/L gibberellins $A_{4+7}$, 19 g/L 6-benzyladenine, Valent BioSciences Corporation)

7. Paclobutrazol (Payback®, 250 g/L paclobutrazol, Crop Care Australasia Pty Ltd)

Treatment application:

All PBRs were applied as a fine mist with a backpack mister at a water volume of 1,000 L/ha. Retain was applied at 500 g product/ha; amino acids at 4 L/ha; Cytolin at 440 ml/ha; 2,4-D at 10 mg/L; Tops at 20 mg/L and foliar nutrients (Quadshot) at 6 L/ha. The wetter Kendeen 20 was included with the Tops and Cytolin treatments at 1 ml/L spray solution. Application dates and weather conditions at application are recorded in Table 2.

## Reducing fruit set in heavy-setting cultivars

### *(iii) examination of optimal application time for ATS to reduce crop load*

Following on from the 2013/14 study, ammonium thiosulphate (ATS) was re-examined as both a single and double application. Ethephon was used as a known 'standard' in this trial.

Cultivar: 'Van' Rootstock: F12/1

Site: Reid Fruits, Plenty

Treatments:
1. Control
2. 250 ppm ethephon at shuck fall (SF)
3. 1% ATS at 30% bloom
4. 1% ATS at 80% bloom
5. 1% ATS at 30 & 80% bloom

Origin and active ingredients of PBRs used:

1. ATS (Thin-It, 782 g/L ammonium thiosulphate, Agrinova NZ Ltd)

2. ethephon (Ethrel 720, 720 g/L ethephon, Bayer CropScience Pty Ltd)

Treatment application:

Ammonium thiosulphate (ATS) was applied at 1% v/v as a single application at 30% bloom or 80% bloom, and a double application at 30 & 80% bloom. Ethrel was applied at 250 ppm; the wetter Kendeen 20 was added to the Ethrel spray solution at 1 ml/L water. Both chemicals were applied to runoff.

Results:

### *(iv) Improving fruit set with PBRs*

Fruit set in untreated 'Kordia' was extremely low at 8.1%, while in the 'Regina' trees background set was 20.6%. Application of Retain improved 'Kordia' fruit set by 65% and 'Regina' set by 47% compared with the untreated controls. Amino acids had no effect on fruit set in either 'Kordia' or 'Regina'. Supplementing the Retain program with amino acids had no effect on 'Kordia', but increased 'Regina' fruit set by an additional 30% to 77%, although this was not significantly different to the Retain treatment. Cytolin resulted in a significant reduction in fruit set in both varieties, while regular application of foliar organic nutrient had no effect on 'Kordia' fruit set, but reduced set in 'Regina' by half. In both varieties, there was no significant difference in mean fruit weight between the control and treated trees; however in the 'Kordia' trial, mean fruit weight in trees treated with foliar organic nutrients was significantly higher than fruit from all other treated trees.

Pack-out (A-grade fruit) was less than 50% in both 'Kordia' and 'Regina', and there was no difference between treatments in marketable fruit (A and B-grade) in either variety.

The incidence of cracking was very high in 'Kordia' (46.8%), and high in 'Regina' (26.9%). Although there was no significant difference in the percentage of cracked fruit when compared with the control, cracked fruit in the Retain plus amino acid treatment was half the level observed in the untreated control for both varieties. Tops significantly increased cracking incidence compared with the untreated control.

Reject fruit was only 4% in 'Kordia', but in 'Regina' the level of reject fruit was higher at 18.3%. Amino acid treatment increased the level of reject fruit in 'Kordia' by 385% compared with the untreated control, but had no effect in 'Regina'. Although not significant due to the high level of variation within the trial, the percentage of reject fruit in the Tops treatment was double that in the untreated control.

### *(v) Carryover effects of paclobutrazol*

There were no significant carry-over effects between the untreated control and paclobutrazol treated trees for fruit set or fruit size. However soil drench resulted in larger fruit than butt spray applications.

### *(vi) Examination of optimal application time for ATS to reduce crop load*

Natural fruit set for trees in the 'Van' block in the 2014/15 season was 38.8%. Crop load was successfully reduced by most treatments; application of Ethrel at SF reduced crop load by 51%, a single ATS applied at 80% bloom reduced crop load by 23% and the double ATS application by 34%. There was no treatment effect on mean fruit weight.

# Evaluation and discussion

## Improving fruit set in shy-bearing cultivars:

- Retain was consistent in improving fruit set in both varieties
- Cytolin reduced fruit set
- 2,4-D, Tops and amino acids showed no improvement in fruit set
- No carryover effects were found from paclobutrazol treatments the previous season

Results from this project suggest that Cytolin, 2,4-D and Tops are of no benefit in improving fruit set in shy-bearing cultivars.

Retain has been consistent across seasons and varieties in improving fruit set. Although amino acids alone did not improve fruit set, there was a reduction in fruit cracking when amino acids were combined in a program with Retain. Hence there may be some benefit in incorporating amino acids into a Retain program, particularly if Retain and amino acids can be applied as a tank mix. However further studies are required to determine the feasibility of this approach.

It would also be worth examining regular foliar nutrition applications in conjunction with a Retain program. Bud strengthening through a post-harvest foliar nutrition program may also be worthy of examination to determine whether this would be beneficial to fruit set the following spring.

### Reducing fruit set in cultivars that benefit from thinning:

- Application of 250 ppm ethephon at shuck fall reduced crop load by 50%
- 80% bloom application timing for ATS was more effective than 30% bloom stage
- Double ATS treatment resulted in 34% crop load reduction

The conclusions from this work are that both ethephon and ATS are effective thinners in sweet cherry. ATS gave additional fruit quality benefits in previous seasons compared with ethephon, there were no differences observed in fruit quality in the final year of study. Hence fruit quality benefits may be subject to seasonal variation.

## Recommendations

Further research is required to determine the feasibility of incorporating amino acids into a Retain program to further improve the efficacy of Retain in improving fruit set, particularly if Retain and amino acids can be applied as a tank mix. To confirm that fruit set is higher in dwarfing rootstocks, the comparison between Gisela and Colt rootstocks should also be repeated.

# Key Activity 2: Girdling and floral biology for prevention of fruitlet abscission and improved fruit quality

***Lead Jo Jones: See appendices 3 & 4 for detailed reports***

In Australia, the variety 'Kordia' is widely grown, possessing good fruit size, flavour and quality; however, in many regions it is prone to inconsistency in yields. 'Kordia' trees are typically grown in this region on a modified Spanish Bush system, encouraging lateral growth, as this system has proved the most successful. 'Lapins' is a more consistently yielding variety, and abscission rates are generally low. The experiments in this study aimed to test the hypothesis that interrupting the transport of carbohydrates to the competing roots will assist in reducing the rate of abscission in the two varieties. By defoliating shoots, the trial will shed light on the contribution of current carbohydrates from young leaves on developing fruitlets versus stored carbohydrates from roots and wood structures.

The aims of this study were to:

- Monitor natural fruitlet abscission throughout the season in 'Kordia' and 'Lapins'
- Understand the seasonal pattern of carbohydrate availability in 'Kordia' and 'Lapins'
- Determine the influence of trunk girdling and limb defoliation on fruitlet abscission and the resulting fruit quality
- Measure aspects of flowering biology as a potential cause of poor fruitset

The trial was established at a commercial orchard in southern Tasmania, Australia, in the spring of 2012, and was maintained for the three harvest seasons of 2013, 2014 and 2015. The trees were 16-year old 'Lapins' and 'Kordia' varieties grown on Mazzard F12-1 rootstocks. 'Lapins' trees were pruned to a conventional Kym Green Bush system and the 'Kordia' to a modified Kym Green Bush system with lateral growth, both widely accepted as best practice tree training systems for the respective varieties. Row orientation was east-west with 4.25 m between rows and 2 m between trees. The two varieties were studied in parallel.

For the girdling treatment, a 2 mm girdling knife was used to create two semi-circular incisions 10 cm apart, overlapping by 2 cm on both sides, 20 cm below the crown of the trunk. The defoliated treatment involved completely defoliating one limb per tree.

*Figure 5: A girdled trunk*

*Figure 6: A defoliated limb*

Trees were managed commercially in terms of irrigation, nutrient and pest management. These same trees were used for the fruitlet abscission monitoring, carbohydrate analysis, fruit quality and flower biology testing.

# Methodology

## Carbohydrate analysis

Wood sample collection

Trunk, branch and root carbohydrates were sampled once every other week, extracted and analysed for sugars and starch – see appendix for full details.


*Figure 7: Sampling wood from trunk*

*Figure 8: Sampling shoots*

*Figure 9: Sampling roots*

## Fruitlet abscission

In each season a monitor limb was selected at random and tagged. At late white bud stage, just prior to bloom, a section on each limb was tagged to include as close to 100 individual flowers as possible. This count was recorded. At predetermined intervals, each limb was recounted and the remaining number of flowers / fruitlets recorded, and the data was used to calculate percent abscission.

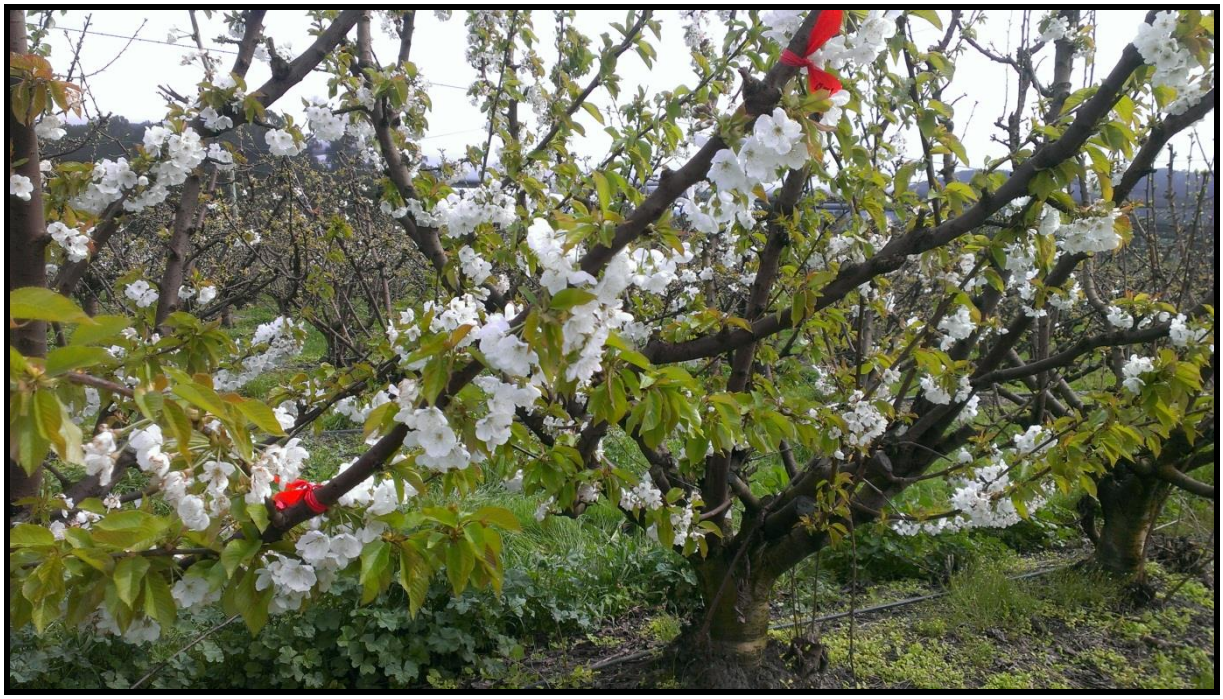

*Figure 10: A limb with 100 flowers marked between orange tape. These flowers / fruitlets were counted regularly and the percent abscission recorded.*

## Fruit quality and chemistry analyses

All fruit was harvested from the monitor limb on each tree at commercial maturity.

Firmness, colour, stem pull force, sugar and acids were analysed.

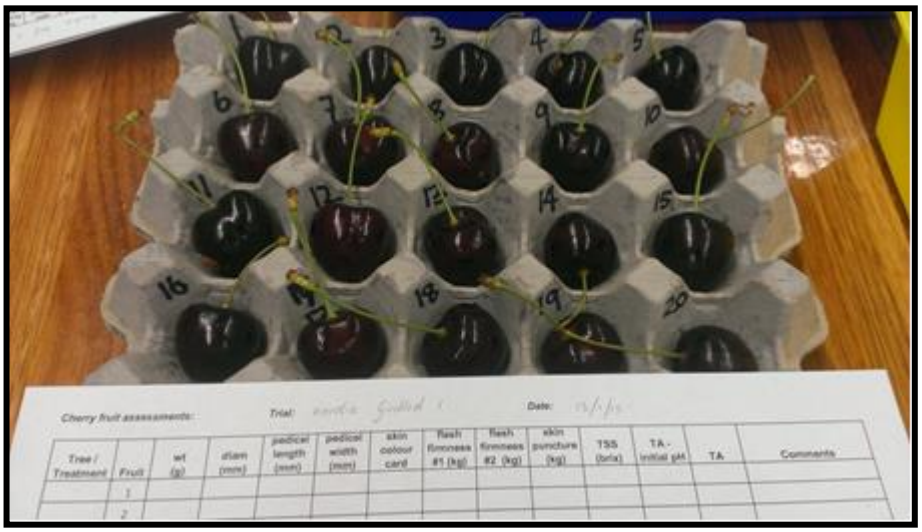


*Figure 11: fruit ready to be analysed for quality*

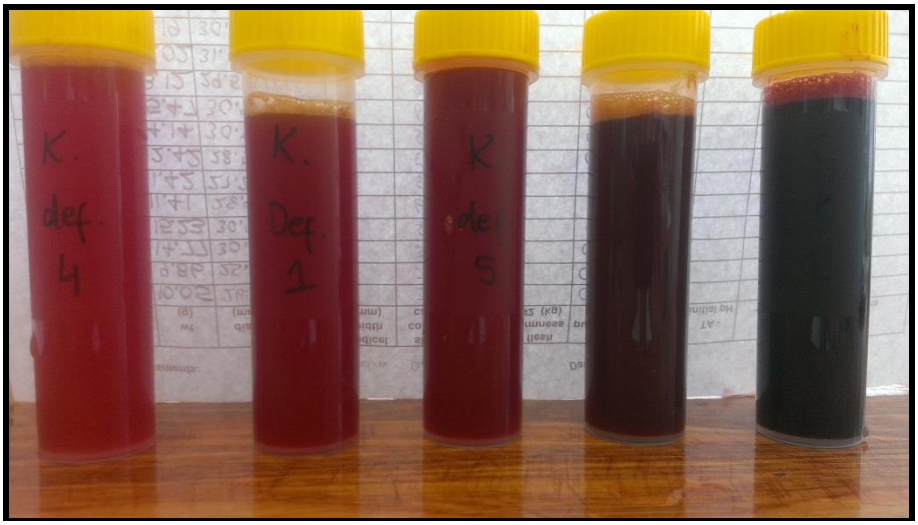


*Figure 12: Visual difference in juice colour, with three defoliated treatments on the left and a control and girdled samples respectively to the right.*

### Flower Biology

In the 2014/2015 season a detailed study of flower biology was undertaken. Pollen presence on the stigma, pollen viability, stigma receptivity, and *in vitro* pollen tube growth measures were made.

## Results

In the control treatment in all three years, 'Kordia' displayed greater rates of fruitlet abscission than 'Lapins'. Girdling reduced the rate of fruitlet abscission in 'Lapins', but not in 'Kordia'. In the 2013/14 and 2014/15 seasons 'Kordia' displayed very high levels of abscission across all treatments, prompting a study into the flower biology of the variety.

Similar patterns in seasonal carbohydrate levels were seen for both varieties. Starch concentrations were relatively high at the time of flowering, and declined to its lowest point around the time of pit hardening, which was followed by a gradual increase leading up to the final measurement taken just after commercial harvest. The concentrations of carbohydrates were also similar across the three seasons, and did not mirror the large increase seen in the rate of abscission which was seen in 'Kordia' in the latter two seasons.

The impact of trunk-girdling was clear for both varieties as carbohydrate flow from leaves to roots was restricted. Root carbohydrate concentration did not recover over the season, but instead remained low post-harvest. 'Kordia' displayed greater concentrations of carbohydrates in stem and trunk than 'Lapins'; however, the total volume of wood was lower for 'Kordia' due to the differences in tree structure, therefore the total available carbohydrates was lower for 'Kordia'.

For both 'Kordia' and 'Lapins', defoliation resulted in fruit with significantly lower total soluble solid (TSS) concentrations in all three seasons and less mass than fruit from control and girdled trees. In 'Lapins', defoliation also resulted in smaller fruit in 2012/13, higher flesh firmness measurements than the control and girdled treatments in all years, and less colour development in all years, emphasising the key importance of current-season photosynthate for developing optimal fruit quality.

Trunk girdling of 'Lapins' in all three seasons impacted negatively on stem retention as seen by lower stem pull force measurements (in 2013/14 girdled fruit was not different from the control, but was different from fruit from defoliated limbs). Fruit from girdled trees was also larger in the 2013/14 and 2014/15 seasons.

As seen in 'Lapins', defoliation of limbs in 'Kordia' resulted in decreased colour development in all years. Fruit from defoliated limbs also resulted in smaller fruit in 'Kordia' in all seasons, and both a lower titratable acidity and lower stem pull force in 2013/14 and 2014/15.

In 'Kordia', many fruitlets were seedless or had a seed trace, suggesting fruitset was a significant issue. 'Lapins' is a self-pollinated variety and 'Kordia' is pollinated by 'Regina' and 'Sylvia', with 'Regina' being planted in adjacent rows to 'Kordia' at the trial site. Bloom synchronisation between 'Kordia' and 'Regina' appeared to be an issue with 'Regina' trees reaching full-bloom after the majority of the 'Kordia' trees.

Pollen viability percentages were 55 % for 'Lapins' and 49 % for 'Regina'. 'Lapins' had a high pollen germination percentage (83 %) and very long tubes when grown in nutrient liquid agar. 'Regina' had a high pollen germination percentage also (78 %), except after a rain event when pollen germination was reduced to (28 %). After the rain events, the number of pollen grains present on the stigma was also low in some cases. It was not possible to distinguish between the variety's own pollen and the pollen from its compatible cultivar, so further research is needed to determine the true impact of rain on fertilisation.

# Evaluation and discussion

The results of this trial indicate that carbohydrate availability can have an important effect on fruitlet abscission (and yield) and the resulting fruit quality. When transport of carbohydrates is interrupted via girdling, resulting in greater availability of carbohydrates to the developing fruitlets, fruitlet abscission can be reduced and the final fruit quality improved in high yielding 'Lapins'. Conversely, fruit from defoliated limbs can result in inferior colour development, lower TSS, and less mass all due to the lack of carbohydrate availability. Girdling in 'Lapins' did appear to influence stem pull force negatively.

The discrepancy in the impact of the trunk girdling treatment on the two varieties may be explained by a distinct carbohydrate source:sink relationship in 'Kordia' relative to 'Lapins'. The tree structure whereby 'Kordia' fruit is born on lateral shoots, and the total stored carbohydrate available is lower due to the smaller branch cross-sectional-area, may also have had an impact.

A key difference between the two varieties studied here is that 'Lapins' are self-pollinated and 'Kordia' requires pollination by other varieties including 'Regina' and 'Sylvia'. The higher proportion of abscised, unfertilised flowers in 'Kordia', as well as the lack of synchronisation of bloom time with the pollinator variety at the trial orchard, suggests that a lack of successful fruitset in 'Kordia' may be a key driver of the low yields. This could also explain the lack of effect of trunk girdling in 'Kordia' compared to 'Lapins', as the girdling treatment was applied 5 weeks after full-bloom in this study.

# Recommendations

Future research should concentrate on the floral biology of 'Kordia' and the pollinator varieties, with a focus on pollen viability, pollen tube growth, stigma receptivity and ovule fertilisation. The use of plant bio regulators (PBR's) to aid synchronisation of pollinators could be investigated, including the impacts of PBR's on fruitset, yield and the resulting fruit quality.

# Key Activity 3: Fertigation for Fruit Quality

***Lead Nigel Swarts: See appendices 5 & 6 for detailed reports***

This key activity focussed on the following questions:

1. Does increased nitrogen application increase nutrient content in fruit, leaves and storage organs of sweet cherry trees?
2. Does increased nitrogen application have a negative effect on fruit quality?
3. Does irrigation quantity influence the nitrogen content in leaves and fruit and influence fruit quality?
4. Does foliar application of micro-elements increase nutrient content in fruit more effectively than fertigation?
5. Does increased micronutrient application improve fruit quality?

## Methodology

### Nitrogen Fertigation Grove Research Station

The fertigation trial was conducted at Grove Research Station in southern Tasmania on 10-year-old 'Lapins' trees, grown on F12-1 rootstock, pruned to a Spanish bush training system with tree spacing ranging from 1 m to 2 m and row spacing of 4.5 m. The variety was chosen to represent a commonly grown variety in the region and trees were subjected to standard orchard management with respect to irrigation, fertilisation and pest management. Row orientation was north-south with a very gentle slope downwards from the southern end of the row. Nitrogen application was ceased by the orchard manager in the experimental rows during the trial period and all other commercial orchard management continued.

*Figures 13 and 14: Establishment of Nitrogen fertigation trials at Grove Research Station*

Fertiliser application followed standard fertigation practise in the region with a 10 min water only wetting up period, followed by a 40 min application of N treatments and completed with a 10 min water only rinse. To assess the influence of pre-harvest fertigation on cherry fruit quality and post-harvest storage, N treatments included a 0N control, 25 g N/tree, 50 g N/tree and 75 g N/tree split into four weekly applications commencing approximately one month after bud burst when root uptake of N is considered to have replaced remobilised N as its dominant N source (Grassi et al., 2003).

Fruit quality assessments were completed at harvest, 25 and 50 days post-harvest. At the time of harvest, leaves were also sampled, dried at 600C and sent to a commercial laboratory for chemistry analysis. Fruit quality parameters of size, colour, firmness, sugars and acid were as described in Bound et al., (2013).

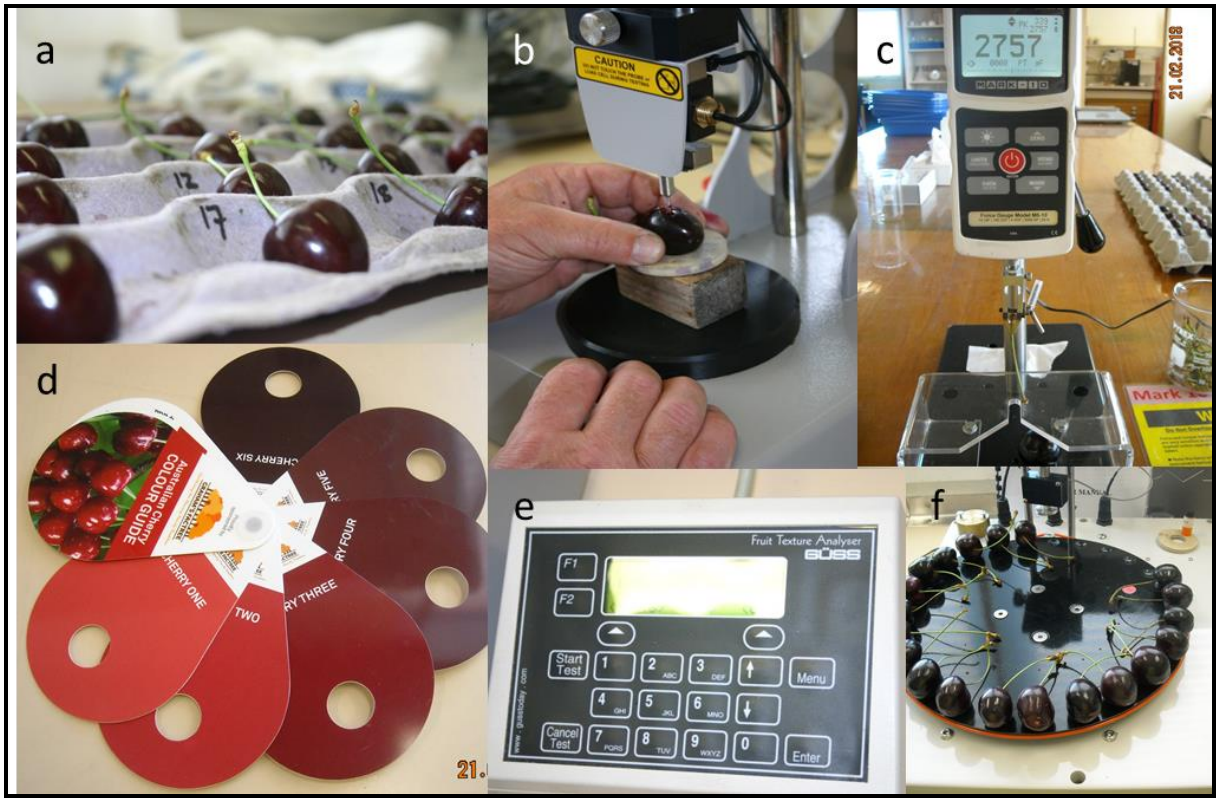


*Figure 15: Assessment of fruit quality using the a) Cherry trays, b) GUSS Fruit Texture Analyser; c) Stem Pull; d) Cherry colour chart; e) GUSS digital reader and f) Firmtek Machine. (Photographs by Justin Direen)*

## Micro-element Fertigation Trial

This trial was conducted at Grove Research Station as described above. Treatments included Potassium Sulphate (50kg/K/ha), Calcium carboxylate (30kg/Ca/ha), Zm$^2$ (20L/ha) and zero fertiliser water only control. Treatments were applied weekly for three weeks commencing in late November 2011.

## Micro-element Foliar Spray Trial

This trial was established on 'Lapins' at two orchards in the Huon Valley ('Grove Research Station' and 'Woodstock') in southern Tasmania. Foliar spray treatments of Ca, Mn, Zn and Zm$^2$ and water were applied on both sides of the rows. All treatments were applied using a motorized air blower backpack spray unit, following manufacturer's recommendations. Foliar sprays were applied as a fine mist covering leaves to drip point. The foliar sprays were applied in December 2012, in the morning, two weeks apart with the final application approximately three weeks before harvest. In both orchards, micro-element application was ceased for the duration of this trial in the experimental row, however all other commercial orchard management practices continued.

*Figures 16 and 17: Chemicals used in the micronutrient foliar spray trial*

*Table 1: Micronutrient products and rates used in spray trials*

| Micro-nutrient | Industry name | Analysis | Manufacturer | Recommended application | Applied quantity |
|---|---|---|---|---|---|
| Ca | Pitstop | 17%w/v Ca | Agrichem | 5-10 L/ha | 6ml/tree |
| Mn | Manni-Plex Mn | 6%w/v Mn; 2.8%w/v N as Nitrate; 5.6%w/v N as Urea | Barmac | 2-6 L/ha | 6ml/tree |
| Zn | Manni-Plex Zn | 8.8%w/v Zn; 2.8% w/v N as Urea; 3.6%w/v N as Nitrate | Barmac | 2-6 L/ha | 6ml/tree |
| Zn, Mn, Mg, Fe, S | Zm2 | 2.3%w/v Zn; 2.3%w/v Mn; 2.3%w/v Magnesium; 2% Fe; 5% Sulfur | Stoller's | 7 L/ha | 6ml/tree |

## Nitrogen Fertigation Ridgy Didge Cherries

Fertigation/irrigation trials were established in February 2013 at Ridgy Didge Cherries in Lower Longley, in southern Tasmania. The trial was conducted on 12-year-old 'Simone' trees, grown on Colt rootstock, pruned to a Spanish bush training system with tree spacing of 2 m and row spacing of 4.5 m. Nitrogen application was ceased by the grower in the experimental rows during the trial period and all other commercial orchard management continued.

The trial involved high (3.9 L/hr), medium (2.3 L/hr) or low (1.6 L/hr) drip irrigation treatments where the grower's existing irrigation infrastructure was modified to deliver the required irrigation rate. Irrigation was controlled by the grower and commenced in mid-October (mid-spring) and continued until late March (mid-autumn). Flow meters installed in the irrigation lines recorded the date and duration of each irrigation application.

Pre- and post-harvest N treatments included a 0N control, 1/3N (40 kg N/ha; grower's standard rate), 2/3N (80 kg N/ha) and 1N (120 kg N/ha) split into four weekly applications commencing in mid February 2013. Treatments in this study were applied February 2013 (post-harvest) and November 2013 (pre-harvest) before the February 2014 harvest; and February 2014 (post-harvest) and November 2014 (pre-harvest) before the February 2015 harvest.

### Potassium Trial Ridgy Didge Cherries

Potassium fertigation and foliar treatments were implemented pre-harvest in Dec 2014 along a single row of 12-year-old 'Simone' trees, grown on Colt rootstock at Ridgy Didge Cherries in Lower Longley, in southern Tasmania. Treatments included: foliar potassium nitrate ($KN0_3$), foliar potassium sulphate ($K_2SO_4$), fertigated $KNO_3$, fertigated $K_2SO_4$ and a zero K control. Potassium treatments were applied twice, one week apart commencing in December at a rate of 25kg/$K^+$/ha.

## Results

### Nitrogen Fertigation Grove Research Station

Total N concentration (%) in cherry fruit at harvest was significantly influenced by increased N supply under fertigation treatments where fruit harvested under the highest N treatment contained the greatest amount of total N. Average yield for trees within the trial approximated 5-6 kg/tree, which is substantially less than expected for 10-year-old 'Lapins' trees within the region, possibly contributing to the substantial treatment effect. Fruit harvested under the highest N treatment were significantly less firm than under the lower N treatments and 0N control. No other fruit quality variables at any of the assessment dates were significantly influenced by N treatments.

### Micro-element Fertigation Trial

Micronutrient fertigation treatments had little effect on increasing their respective nutrient content in the harvested fruit from each treatment. Fruit harvested from Ca treatments on average had higher levels of Ca than control fruit. This was also true of the treatment Mn and Zn at least for the Mn content in the fruit, however none of these results were significantly higher.

Fruit quality parameters from all micronutrient treatments at all assessment dates were not significantly different from the control with exception of Total Soluble Solids at harvest where fruit harvested from the potassium treatment were lower than the control treatment.

### Micro-element Spray Trial

Zinc and Mn foliar treatments significantly increased levels of their respective nutrients in fruit (Grove and Woodstock) and leaves (Grove). In both orchards, manganese levels in the Mn treatment were significantly higher than the control treatment and were significantly higher than the $Zm^2$ treatment which also contained Mn. Manganese levels in the $Zm^2$ treatment were significantly higher than the control treatment at Grove but not at Woodstock, yet were not significantly higher than any of the other treatments. A similar trend was found for Zn levels in fruit at both orchards under the Zn

treatment, however Zn levels under the $Zm^2$ treatment were not significantly different from the other treatments.

Leaf nutrient levels were only measured at the Grove Research Station. Zinc and Mn treatments had significantly higher levels of their respective nutrients in the leaves at harvest compared to the other treatments with exception to the $Zm^2$ treatment. Levels of Zn and Mn in the $Zm^2$ treatment were greater than the other treatments, yet they were also not significantly different from the control treatment. No increase in Ca levels under Ca treatments in leaves at Grove or fruit at both orchards were observed.

Analysis of fruit quality parameters at harvest and 25 and 50 days in post-harvest storage revealed little significant differences between micronutrient treatments despite the strong uptake of Mn and Zn micronutrients in the leaves and fruit of the sweet cherry trees. Cherry fruit harvested from Grove were significantly more coloured (red) in the Mn treatment than the $Zm^2$ treatment. The only other treatment effect was on Total Soluble Solids (TSS; sugars) content of stored fruit, 25 days post-harvest from Grove where Ca treatments had significantly greater sugars than the control and Zn treatments but not Mn and $Zm^2$ treatments.

## Nitrogen Fertigation Ridgy Didge Cherries

Fruit Total N content at harvest was significantly influenced by N fertiliser treatments in the 2013/14 season but not the 2014/15 season. In the former, only fruit harvested from the highest N treatment (1N = 120 kg N/ha), had N content significantly greater than the 0N control treatment, however fruit harvested from each of the N treatments did not have significantly different N content from each other. Leaf N content in both seasons, and at all times assessed, although appearing to trend higher with higher N applied was not significantly different from each other. Irrigation treatment did not have an influence on N content in fruit or leaves.

Weight of cherries at harvest was significantly influenced by irrigation treatment in both the 2013/14 and 2014/15 seasons, but not fertigation treatments. In 2013/14, fruit were significantly bigger in the high (3.9 L/hr) treatment compared to the low (1.6 L/hr) and medium (2.3 L/hr) treatments. In 2014/15, harvested fruit were significantly larger in the medium treatment compared to the low treatment, however fruit in the high irrigation treatment were not significantly larger or smaller than the others.

In the 2014/15 growing season, fruit firmness measurements of flesh firmness and skin puncture using the Guss Fruit Texture Analyser were significantly influenced by irrigation. Both measurements followed the same trend where penetration force (g/Kg) to fruit at harvest was significantly higher in the medium irrigation treatment compared to the high treatment, yet the low irrigation was not significantly different from the medium or high treatments. No influence of irrigation or N fertigation treatments were observed on fruit after 28 days in storage. Treatments did not have any influence on fruit chemistry at harvest or after 28 days in storage.

## Potassium Trials Ridgy Didge Cherries

Foliar and fertigation treatments had little influence over K content in leaves and fruit with no significant difference between them and the control zero K treatment.

For fruit quality assessments, only Firmtek measurements of fruit at Harvest and after 28 days in storage were significantly influenced by K treatments. At both assessment dates, all K treatments were not significantly different from each other, however on average, fruit from these treatments

were firmer than the control zero K treatment. At harvest, only the foliar $KNO_3$ treatment was significantly firmer than the control, but after 28 days in storage fruit from the foliar $K_2SO_4$ treatment were significantly firmer than the control treatment.

# Evaluation and Discussion

## *Does increased nitrogen application increase nitrogen content in fruit, leaves and storage organs of sweet cherry trees?*

Nitrogen fertigation treatments at Grove and Ridgy Didge in the 2013/14 season increased total N content in 'Lapins' and 'Simone' fruit respectively. In both trials, it was only in the highest N treatment where a significantly greater response was observed compared to the control zero N treatment. At Grove, where a marked response was observed, trees in the highest treatment received a pre-harvest quantity of 75 g N/tree (150 kg N/ha) which is three times higher than the recommended pre-harvest rate for the region. Total N content of the zero N control fruit was very low at <0.6% so it was not unexpected to see a marked response with high rates of N applied. Furthermore, changes in orchard management in the two seasons prior to trial commencement saw trees in the orchard receive less than the standard N application recommended by industry. Yet although fruit had an increased total N content, this did not lead to significantly increased storage in branches, trunk or root, nor did it result in higher bud N content for next season's vegetative and fruit growth.

At Ridgy Didge, fruit from the control trees averaged 0.7% total N in 2013/14 and over 0.8% in the 2014/15 season which possibly explains why the treatment effects were less pronounced. Furthermore, given that the majority of pre-harvest N requirement for deciduous fruit trees such as sweet cherry is provided by remobilised N, a carryover effect from previous season's fertiliser application is likely to have minimised the treatment effects.

## *Does increased nitrogen application have a negative effect on fruit quality?*

Our findings at Grove suggest that increased N application had negative consequences on fruit firmness but not the other quality parameters measured. Further, this trend was observed at Ridgy Didge (see Figure 18).

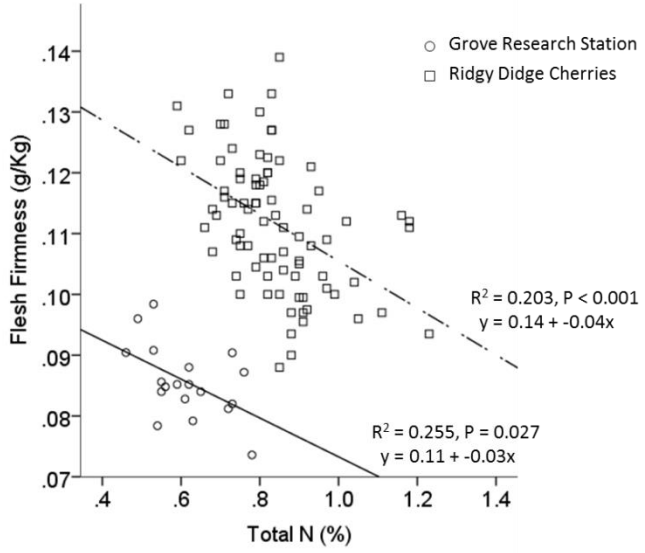


*Figure 18: Impact of increased N application*

Correlation between flesh firmness (Guss Fruit Texture Analyser) data, pooled from all treatments and seasons, and Total N (%) content of sweet cherry fruit at harvest from Grove Research Station and Ridgy Didge Cherries.

Obtaining the ideal balance of N supply to meet tree demand is a challenge for growers. Our data suggests that there is considerable flexibility in total N supply over a growing season given the lack of fruit quality response to even our highest N treatments of 240 kg N/ha at Ridgy Didge orchards. This may benefit growers who want to promote vegetative growth after strong winter pruning, yet also may be a risk to growers who want to keep trees in a reproductive state. For large old trees with capacity for considerable N uptake, it is likely that only minimal N is required to maintain adequate N content to avoid negatively influencing fruit firmness. For example, an orchard with 2000 trees/ha, an average crop load of 8 kg/tree, total N content of 0.9%, fruit dry matter content of 22% only requires a replacement rate of approximately 30 kg N/ha.

### *Does irrigation quantity influence the nitrogen content in leaves and fruit and fruit quality?*

In both seasons, irrigation quantity had no discernible influence on total N content in leaves and fruit of 'Simone' cherry trees at Ridgy Didge. No interaction effect of irrigation quantity on fertiliser treatment was observed when analysing total N content of leaves and fruit. This is surprising given that the irrigation volume of the high treatment (3.9 L/hr) was almost 2 and a half times that of the low irrigation treatment (1.6 L/hr).

Increased irrigation volume increased fruit size. Increasing irrigation volume prior to harvest appears to be a useful management strategy for increasing fruit size and therefore overall yield also in sweet cherry.

### *Does foliar application of micro-elements increase nutrient content in fruit more effectively than fertigation?*

Foliar Zn applications resulted in significantly increased Zn content in the fruit at both orchards, but fertigated Zn did not. Foliar Zn dramatically increased Zn content in the leaves at Grove Research Station when analysed at commercial harvest. Indeed the trees in the Zn treatments started to show symptoms of Zn toxicity (yellowing and defoliation) one week after the second application (see photo below). Yet, excessive Zn levels were not found in the fruit, suggesting that although some transport occurred, the majority of applied Zn remained in the leaves. This is not surprising given the poor mobility of Zn (Sánchez and Righetti, 2002). Zinc foliar spray was more effective at increasing leaf and fruit Zn content than $Zm^2$ however this was most likely due to the concentration of Zn in the Zn spray, being approximately 4x the amount of the $Zm^2$ spray. Alternatively, the uptake of Zn may have been compromised by other nutrients in the $Zm^2$ treatment.

*Figure 19: Leaf senescence following foliar application of zinc at Grove Research Station.*

Foliar application of Mn resulted in a significant increase in Mn content in harvested fruit at both orchards. Manganese was also applied in the $Zm^2$ treatment, but this treatment did not result in higher Mn levels perhaps due to the reasons described above for Zn. Manganese is a poorly mobilised nutrient (Marschner, 1997), which explains the greater treatment effect on fruit Mn concentration seen in foliar sprays compared with fertigated compounds. This demonstrates the effectiveness of Mn foliar sprays as a useful corrective fertiliser to balance micronutrient concentration when deficiencies are observed.

There was no significant increase in Ca levels in the fruit after foliar application although a slight average increase in fruit Ca levels following fertigation was observed. Research has shown that foliar application increases the Ca concentration in the leaves, but not necessarily in the fruit.

At Ridgy Didge both fertigated K forms increased K content in 'Simone' fruit and were more effective than foliar applications.

### *Does increased micronutrient application improve fruit quality?*

Although significantly increased concentration of Zn and Mn were found in fruit following foliar applications, there were surprisingly few fruit quality implications. No treatment influence on fruit size (weight or diameter) was observed with foliar or fertigated treatments. All fruit harvested from K treatments at Ridgy Didge on average were firmer than the control, however it was only the foliar K treatments which were significantly firmer at harvest and after time in storage. A stronger response

was seen at Ridgy Didge than Grove due to the earlier application time at the former and the greater quantity that was applied.

Our treatments showed no influence of fertigated or foliar applied Ca on fruit firmness. Application of Ca alone may not affect fruit firmness, but Ca applied with Copper, Boron or GA has been shown to improve fruit firmness in a range of tree fruit crops including sweet cherry (Mertes, 2011, Nielsen et al., 2000, Nielsen et al., 2004a, Patten et al., 1983, Marschner, 1997, Brown et al., 1996, Brown et al., 1995, Thurzó et al., 2010, Usenik et al., 2005b).

# Recommendations

There are a number of take-home messages from the data obtained from these trials.

- Higher levels of N found in fruit were strongly correlated with decreased firmness. The negative influence of high nitrogen application on sweet cherry fruit quality is probably overstated when N concentration in fruit is adequate (0.7-0.9%). When N is deficient (<0.6%), a strong response to fertigated N is observed.

- Nitrogen fertiliser rate/ha should match removed N from cropping and is based on crop load, fruit N status and dry matter content. Timing of N application should coincide with greatest root growth which commences approximately four weeks after bud burst. To avoid leaching of applied N, fertigation events should be staggered with up to one week break between fertigation events.

- Increased irrigation volume had a strong positive influence on fruit size. If available, additional irrigation prior to harvest may be an effective management tool in increasing overall yield (tonnes/ha) with no negative effects on fruit quality

- A strong response to foliar elemental sprays of Zn and Mn was observed in cherry leaves and fruit particularly when base nutrient levels were low, which suggest they are effective for correcting deficiencies. Rate of application must be carefully managed to avoid toxicity as seen in this research trial. Foliar applied Zn and Mn were more effective than fertigated Zn and Mn in increasing fruit nutrient content.

- Potassium (K) being readily transportable, was more effectively translocated to the fruit than via foliar applications. Yet on average foliar treatments had a stronger influence on fruit quality, namely fruit firmness, than fertigated treatments. Potassium in deciduous trees is recycled between seasons, however as fruits are a strong sink for K, adequate soil K is required to meet the demands of a developing crop. Fertigation with N in late spring would optimise the uptake of K.

- No dramatic effects on fruit quality were observed for all nutrients applied despite extremely high rates of some nutrients (particularly N) applied. When soil and tree nutrient status is adequate through ongoing monitoring and good soil management, there are significant cost and environmental benefits to minimising fertiliser application as much as possible.

# Outputs

## Cherry Growers Australia Newsletter stories

1. Optimising cherry fruit set, crop load, fruit nutrition and size (Dugald Close June 2013)
2. Too many fruit? Investigating post-bloom thinning of sweet cherry' – article on Washington State University component of the project – edited by Michele Buntain (TIA Extension officer) from progress report by Matt Whiting (WSU lead scientist) (October 2013)
3. Cherry shedding under the microscope (Jo Jones and Dugald Close June 2014)
4. Managing and predicting sweet cherry fruit quality and post-harvest shelf life (Eric Mertes and Dugald Close (July 2014)
5. Cherry fruit set and Crop load management (Sally Bound January 2015)

## Factsheets

1. Cherry shedding under the microscope by Jo Jones and Dugald Close
2. Fruitlet abscission in sweet cherry by Jo Jones and Dugald Close
3. Manipulation of nutrition for fruit quality and shelf life by Nigel Swarts
4. Investigating the influence of nitrogen on cherry quality and shelf life by Nigel Swarts
5. Understanding flowering, fruit set and crop load in cherries by Matt Whiting and Michele Buntain
6. Managing and predicting sweet cherry fruit quality and post-harvest shelf life by Eric Mertes and Dugald Close

All available on the Tasmanian Institute of Agriculture (TIA) web site:

http://www.utas.edu.au/tia/centres/perennial-horticulture-centre/fact-sheets-and-tools/fact-sheets-and-tools2

Victoria Cherry Growers Association Conference 2015: Provision of Fact Sheets

Fruit Growers Tasmania May Conference 2013, 2014: Provision of Fact Sheets

## Presentations

1. National Levy Payers Meetings: Dugald Close 2012, 2013. Sally Bound 2014
2. National Development Program (Penny Measham) in SA and WA 2013 Campaign
3. Fruit Growers Tasmania Cherry Night Time Seminar Series (Eric Mertes) - Tree Carbohydrate Availability and Fruit Quality' 14th November

4. Fruit Growers Tasmania May Conference 2014 (Sally Bound) - Cherry fruit set and Crop load management – can plant growth regulators help?

5. National 'HortLink' Agronomy Conference 2014 (Sally Bound) - Cherry fruit set and Crop load management – can plant growth regulators help?

# Outcomes

## PBRs for optimal crop load management

- Retain consistently improved fruit set by up to 65% in 'Kordia' and 48% in 'Regina'.
- Amino acids when supplementing the Retain program increased fruit set in 'Regina' by 30 to 77%
- Paclobutrazol marginally improved fruit set by 11% and had no carry over affect
- Potassium nitrate, foliar organic nutrients, NAA, sodium molybdate, 2,4-D, amino acids and Cytolin did not improve fruit set
- Fruit set was 52% higher on Gisela than Colt rootstocks
- Ethephon applied at shuck fall at 200-300 ppm reduced crop load from 24-51% in heavy setting varieties
- ATS at 30 and 80% bloom reduced crop load by 34% in heavy setting varieties

## Girdling and floral biology for prevention of fruitlet abscission and improving fruit quality

- Trunk girdling reduced fruitlet abscission from 43% to 30% in 2012/13, 52% to 36 % in 2013/14 and 65 % to 57 % in 2014/15 in 'Lapins' but had no effect in 'Kordia'
- Girdling significantly reduced root starch reserves
- Abscission of fruitlets in 'Kordia' was not related to starch reserves in 'Kordia'
- 'Kordia' had relatively few pollen grains on flowers indicating that synchronization with pollinator varieties is a significant issue
- Pollen germination rates were reduced following rain events
- Girdling in 'Kordia' resulted in firmer, sweeter and larger fruit that had better post-harvest shelf life

## Fertigation for fruit quality

- Fruit of high nitrogen content had relatively low firmness in a deficit situation
- Application of fertigation nitrogen did not negatively affect firmness or other quality parameters in a non N deficit situation
- Consumers could very accurately differentiate sugar and acid levels in different fruit in blind tastings
- Higher rates of irrigation resulted in bigger cherries
- Foliar micro-element (Mn, Zn, Ca) and K sprays were more effective for fruit and leaf uptake than delivery via fertigation
- Foliar micro-element sprays (Mn, Zn, Ca) had no measureable impact on fruit quality
- Potassium application increased fruit firmness at harvest

# Scientific Refereed Publications


- Bound SA, Close DC, Quentin AG, Measham PF and Whiting MD (2013) Crop load and time of thinning interact to affect fruit quality in sweet cherry. Journal of Agricultural Science 5: 216-230
- Quentin AG, Close DC, Hennen LMHP, Pinkard EA (2013) Down-regulation of photosynthesis via sink limitation is linked to foliar soluble sugar content in high- and low-yielding varieties of Sweet Cherry Acta Horticulturae accepted
- Quentin AG, Close DC, Hennen LMHP, Pinkard EA (2013) Down-regulation of photosynthesis following girdling, but contrasting effects of fruit set and retention, in two sweet cherry cultivars. Plant Physiology and Biochemistry 73: 359-367
- Bound SA, Close DC, Quentin AG, Measham PF, Whiting MD (2013) Regulating crop load of Sweetheart and Van sweet cherry for optimal quality. Acta Horticulturae accepted
- Bound SA, Close DC, Jones, JE, Whiting MD (2014) Improving fruit set of Kordia and Regina sweet cherry with AVG. Acta Horticulturae 1042: 285-292
- Swarts N, Mertes EF and Close DC (2014) Manipulation of fertigated nitrogen application influences cherry fruit content and quality. Acta Horticulturae accepted
- Mertes EF, Close DC, Jones JE (2015) The effect of carbohydrate availability on postharvest fruit quality in sweet cherry Acta Horticulturae accepted
- Jones JE, Mertes EF, Close DC (2015) Does carbohydrate availability play a role in sweet cherry fruitlet abscission? Acta Horticulturae accepted


# IP/Commercialisation

Nil

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# Appendices

## Appendix 1: CY12003 – Optimizing cherry fruit set, crop load, size and nutrition; Crop load management component

***Report on year 3 trials by Sally Bound***

Trials undertaken in year 3 (2014/15) of this project were based on the results of trials conducted in previous seasons, and included studies in two areas:

A. Improving fruit set in shy-bearing cultivars

B. Reducing fruit set in heavy-setting cultivars

Trial establishment and design:

All trials were established in commercial orchards in September 2014. Trial design was a randomised complete block design with 5 replicates per treatments. Blocks were based on position in the tree row. Each treatment tree was separated by a buffer tree to avoid overspray.

Assessments:

In all trials, two limb sections were tagged on opposite sides of each treatment tree and the number of spurs, floral buds and individual flowers counted in late September / early October for each tagged section (Table 1); fruit set counts on each tagged limb were completed in early December. All trials were harvested at normal commercial harvest times. At harvest, fruit from each tagged limb section was placed into labelled bags, transported back to the laboratory where they were weighed and sorted into A-grade, B-grade and reject fruit. The number of cracked fruit was also counted; after counting, cracked fruit were allocated to B-grade or reject categories depending on the severity of cracking. Two subsamples of 30 A-grade fruit were randomly selected for laboratory analysis of harvest and post-harvest fruit quality. All fruit was bagged into polythene zip lock bags and placed into cool storage at 1°C until assessments were undertaken. Harvest assessments were completed within one week of harvest, while post-harvest assessments were undertaken approximately 5-6 weeks after harvest.

*Table 1: Dates of full bloom, flower and fruit set counts, trial harvest and laboratory fruit assessments for 2014/15 trials.*

| Activity | *Van* | *Kordia* | *Regina* |
|---|---|---|---|
| *Full bloom* | *6 Oct* | *9 Oct* | *10 Oct* |
| Bud/flower counts | 22-Sep | 26 Sep | 2 Oct |
| Fruit set counts | 2-Dec | 2 Dec | 2 Dec |
| Trial harvest | 26-Dec | 29 Dec | 19 Jan |
| Harvest quality assessments | 30 Dec | 5-6 Jan | 21-22 Jan |
| Post-harvest quality assessments | 10 Feb | 11 Feb | 23 Feb |

## A. Improving fruit set in shy-bearing cultivars

(i) Improving fruit set with PGRs

Following on from results in 2013/14 trials, the PGRs showing potential were re-assessed with the addition of other products which have been reputed to improve fruit set in other crops. Retain is currently used in the cherry industry for improving fruit set, so was included as a known 'standard' in this work.

Cultivars: *Kordia*, *Regina* Rootstock: Colt

Site: Cherries Tasmania, Old Beach

Treatments (*Regina*):
1. control
2. 500 g Retain/ha at 50% bloom
3. Foliar organic nutrients fortnightly
4. Amino acid (AA) at 30% &75% bloom
5. Retain + AA
6. 10 ppm amine 2,4-D at pit-harden
7. 20 ppm Triclopyr (Tops) at pit-harden
8. Cytolin at 50% bloom, full bloom (FB) & 2 weeks after FB (wAFB)

Treatments (*Kordia*):
1. control
2. 500 g Retain/ha at 50% bloom
3. Foliar Organic nutrients fortnightly
4. amino acid (AA) at 30% &75% bloom
5. Retain + AA
8. Cytolin at 50% bloom, FB & 2 wAFB

(ii) Carryover effects of paclobutrazol

Cultivar: *Regina* Rootstock: Colt

Site: Cherries Tasmania, Old Beach

This study commenced in autumn 2013, and was designed to answer some of the questions surrounding the use of paclobutrazol for improving fruit set in sweet cherry, examining both application time (autumn vs spring) and method (butt spray vs soil drench) of paclobutrazol (Payback®) on Regina. Payback® was applied at rates of 1, 2 or 4 ml/tree.

Return fruit set and fruit quality were assessed in the 2014/15 season to ascertain whether paclobutrazol treatments had any carry-over effects.

Details of PGRs used in trials:

1. Retain® (150 g/kg aviglycine HCl, Valent BioSciences Corporation)
2. Amino acids - Flower 'n' Fruit Maker® (635 g/L amino acids, Wilchem)
3. Foliar organic nutrients (Quadshot®, SLTec)
4. 2,4-D (Commercial Citrus Stop Drop®, Kendon Chemical & Mnfg. Co. Pty Ltd)
5. Tops® (10% 3,5,6-trichloro-2-pyridyloxyacetic acid, Colin Campbell Chemicals Pty Ltd)
6. Cytolin® (19 g/L gibberellins $A_{4+7}$, 19 g/L 6-benzyladenine, Valent BioSciences Corporation)
7. Paclobutrazol (Payback®, 250 g/L paclobutrazol, Crop Care Australasia Pty Ltd)

Treatment application:

All PGRs were applied as a fine mist with a backpack mister at a water volume of 1,000 L/ha. Retain was applied at 500 g product/ha; amino acids at 4 L/ha; Cytolin at 440 ml/ha; 2,4-D at 10 mg/L; Tops at 20 mg/L and foliar nutrients (Quadshot) at 6 L/ha). The wetter Kendeen was included with the Tops and Cytolin treatments at 1 ml/L spray solution. Application dates and weather conditions at application are recorded in Table 2.

**B. Reducing fruit set in heavy-setting cultivars**

(iii) examination of optimal application time for ATS to reduce crop load

Following on from the 2013/14 study, ammonium thiosulphate (ATS) was re-examined as both a single and double application. Ethephon was used as a known 'standard' in this trial.

Cultivar: *Van* Rootstock: F12/1

Site: Reid Fruits, Plenty

Treatments:
1. Control
2. 250 ppm ethephon at shuck fall (SF)
3. 1% ATS at 30% bloom
4. 1% ATS at 80% bloom
5. 1% ATS at 30 & 80% bloom

Origin and active ingredients of PGRs used:

1. ATS (Thin-It, 782 g/L ammonium thiosulphate, Agrinova NZ Ltd)
2. ethephon (Ethrel 720, 720 g/L ethephon, Bayer CropScience Pty Ltd)

Treatment application:

Ammonium thiosulphate (ATS) was applied at 1% v/v as a single application at 30% bloom or 80% bloom, and a double application at 30 & 80% bloom. Ethrel was applied at 250 ppm; the wetter Kendeen was added to the Ethrel spray solution at 1 ml per L water. Both chemicals were applied to runoff. Application dates and weather conditions at application are recorded in Table 2.

*Table 2: Summary of treatment application dates for 2014/15 trials on 'Van', 'Regina' and 'Kordia'*

| Treatment | | Treatment application date *Van* | *Kordia* | *Regina* | Weather conditions Tempera ture (°C) | Relative humidity (%) | Cloud cover (%) |
|---|---|---|---|---|---|---|---|
| 30% bloom: | ATS<br>amino acid | 26 Sept [a] | 3 Oct | 3 Oct [b] | 17<br>15 | 53<br>49 | 50<br>40 |
| 50% bloom: | Retain<br>Cytolin | -<br>- | 3 Oct [c]<br>3 Oct [c] | 3 Oct<br>3 Oct | 15 | 49 | 40 |
| 75% bloom: | amino acid | - | 6 Oct [d] | 6 Oct | 17 | 68 | 100 |
| 80% bloom: | ATS | 6 Oct | - | - | 17 | 44 | 80 |
| Full bloom: | Cytolin | - | 10 Oct | 10 Oct | 23 | 61 | 100 |
| Shuck fall: | Ethrel | 22 Oct | - | - | 24 | 81 | 70 |
| 2 wAFB: | Cytolin | - | 22 Oct | 22 Oct | 25 | 51 | 70 |
| Pit-harden: | amine 2,4-D<br>Triclopyr (Tops) | -<br>- | -<br>- | 10 Nov<br>10 Nov | 17 | 41 | 50 |
| Fortnightly foliar nutrients* | | - | 16-Sep<br>14-Oct<br>29-Oct<br>10-Nov<br>25-Nov<br>8 Dec | | 15<br>13<br>20<br>17<br>19<br>17 | 66<br>62<br>58<br>41<br>43<br>76 | 20<br>100<br>60<br>50<br>50<br>100 |

[a] trees at 20% bloom; [b] trees at 50% bloom; [c] trees at 30% bloom; [d] trees at 70% bloom

* no foliar nutrients applied during flowering

**Results:**

(iv) Improving fruit set with PGRs

Fruit set in untreated *Kordia* was extremely low at 8.1% (Table 3), while in the *Regina* trees background set was 20.6% (Table 4). Application of Retain improved *Kordia* fruit set by 65% (Table 3, Figure 1) and *Regina* set by 47% (Table 4, Figure 1) compared with the untreated controls. Amino acids had no effect on fruit set in either *Kordia* or *Regina*. Supplementing the Retain program with amino acids had no effect on *Kordia*, but increased *Regina* fruit set by an additional 30% to 77%, although this was not significantly different to the Retain treatment. Cytolin resulted in a significant reduction in fruit set in both varieties, while regular application of foliar organic nutrient had no effect on *Kordia* fruit set, but reduced set in *Regina* by half. In both varieties, there was no significant difference in mean fruit weight between the control and treated trees; however in the *Kordia* trial, mean fruit weight in trees treated with foliar organic nutrients was significantly higher than fruit from all other treated trees.

*Table 3: Effect of different plant growth regulators on fruit set and mean fruit weight of 'Kordia' sweet cherry.*

| Treatment | Fruit set (%) | No. fruit per floral bud | No. fruit per spur | Mean fruit weight (g) |
|---|---|---|---|---|
| Control | 8.1 bc | 0.23 bc | 0.85 bc | 14.4 ab |
| Retain | 13.4 d | 0.39 d | 1.50 d | 14.1 a |
| Amino acids (AA) | 8.9 c | 0.26 c | 1.06 c | 14.0 a |
| Retain + AA | 13.4 d | 0.39 d | 1.42 d | 13.3 a |
| Cytolin | 4.1 a | 0.12 a | 0.52 a | 14.1 a |
| Foliar organic nutrients | 5.5 ab | 0.16 ab | 0.58 ab | 15.5 b |
| *Lsd (P=0.05)* | *2.9* | *0.08* | *0.30* | *1.2* |
| *F Prob* | *<0.001* | *<0.001* | *<0.001* | *0.034* |

Within a single column means sharing the same letter are not significantly different at P=0.05 using the LSD test.

*Table 4: Effect of different plant growth regulators on fruit set and mean fruit weight of 'Regina' sweet cherry.*

| Treatment | Fruit set (%) | No. fruit per floral bud | No. fruit per spur | Mean fruit weight (g) |
|---|---|---|---|---|
| Control | 20.6 c | 0.74 c | 2.91 c | 11.4 |
| Retain | 30.4 d | 1.01 d | 4.30 d | 11.7 |
| Amino acids (AA) | 16.2 bc | 0.52 bc | 1.94 a | 11.0 |
| Retain + AA | 36.6 d | 1.11 d | 4.77 d | 10.5 |
| Cytolin | 7.3 a | 0.23 a | 0.82 a | 12.8 |
| Foliar organic nutrients | 10.5 ab | 0.36 ab | 1.55 ab | 12.5 |
| 2,4-D | 12.9 ab | 0.46 a | 1.67 ab | 11.8 |
| Tops | 15.1 bc | 0.51 bc | 2.13 bc | 10.7 |
| *Lsd (P=0.05)* | *7.1* | *0.24* | *1.25* | *ns* |
| *F Prob* | *<0.001* | *<0.001* | *<0.001* | *0.229* |

Within a single column means sharing the same letter are not significantly different at P=0.05 using the LSD test.

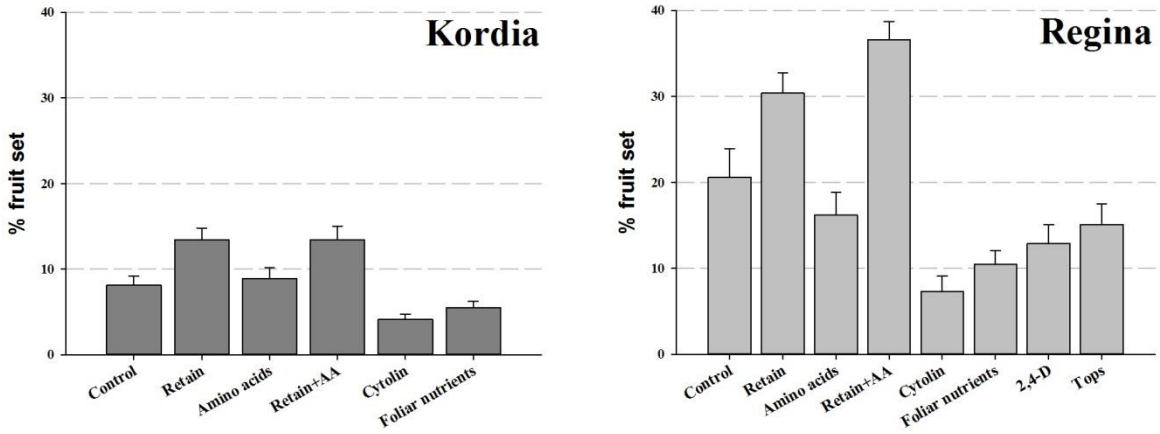


*Figure 1: The effect different plant growth regulators on percentage fruit set in (a) 'Kordia', and (b) 'Regina' sweet cherry.*

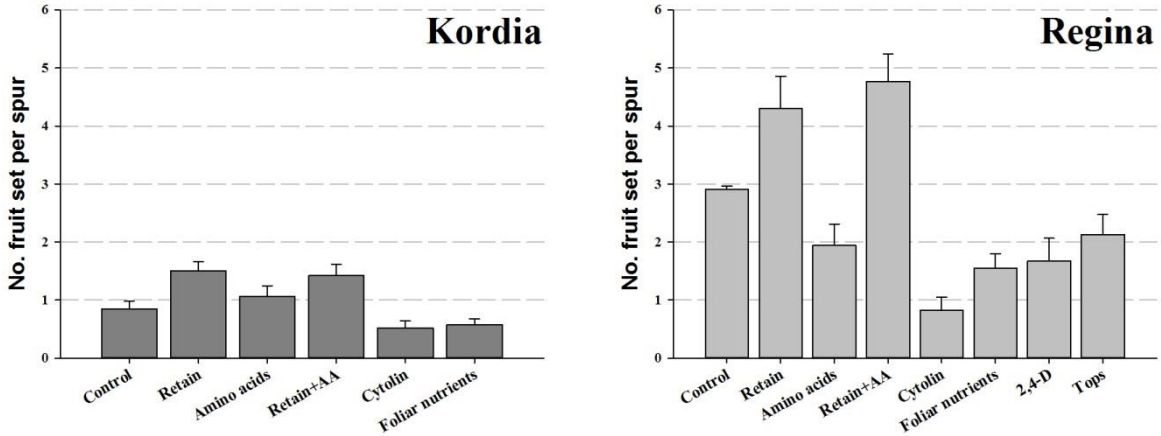


*Figure 2: The effect different plant growth regulators on number of fruit per spur in (a) 'Kordia', and (b) 'Regina' sweet cherry.*

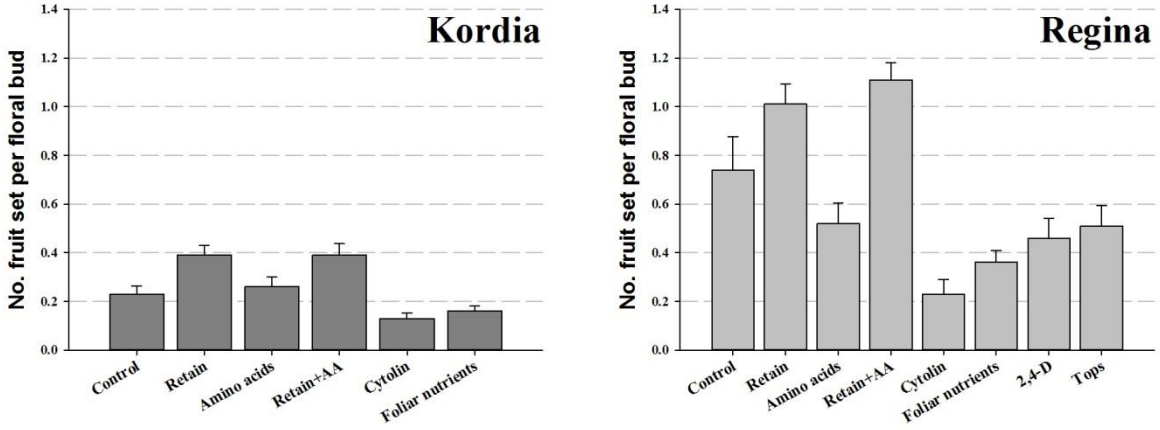


*Figure 3: The effect different plant growth regulators on number of fruit per floral bud in (a) 'Kordia', and (b) 'Regina' sweet cherry.*

Pack-out (A-grade fruit) was less than 50% in both Kordia and Regina, and there was no difference between treatments in marketable fruit (A and B-grade) in either variety (Table 5).

The incidence of cracking was very high in *Kordia* (46.8%) (Table 5, Figure 4), and high in *Regina* (26.9%) (Table 6, Figure 4). Although there was no significant difference in the percentage of cracked fruit when compared with the control, cracked fruit in the Retain plus amino acid treatment was half the level observed in the untreated control for both varieties. Tops significantly increased cracking incidence compared with the untreated control (Table 6).

Reject fruit was only 4% in *Kordia* (Table 5), but in *Regina* the level of reject fruit was higher at 18.3% (Table 6). Amino acid treatment increased the level of reject fruit in *Kordia* by 385% compared with the untreated control, but had no effect in *Regina*. Although not significant due to the high level of variation within the trial, the percentage of reject fruit in the Tops treatment was double that in the untreated control (Table 6).

*Table 5: Effect of different plant growth regulators on percentage A-grade, B-grade, cracked and reject fruit in 'Kordia' sweet cherry*

| Treatment | A-grade fruit % | B-grade fruit % | Cracked fruit % | Reject fruit % |
|---|---|---|---|---|
| Control | 45.9 | 50.0 | 46.8 | 4.0 a |
| Retain | 51.6 | 46.1 | 39.7 | 2.2 a |
| Amino acids (AA) | 44.4 | 40.2 | 49.9 | 15.4 b |
| Retain + AA | 55.9 | 36.6 | 27.6 | 7.4 ab |
| Cytolin | 43.4 | 48.9 | 54.7 | 7.6 ab |
| Foliar organic nutrients | 49.3 | 49.3 | 49.9 | 1.4 a |
| *Lsd (P=0.05)* | *ns* | *ns* | *ns* | *8.8* |
| *F Prob* | *0.940* | *0.830* | *0.579* | *0.039* |

Within a single column means sharing the same letter are not significantly different at P=0.05 using the LSD test.

*Table 6: Effect of different plant growth regulators on percentage A-grade, B-grade, cracked and reject fruit in 'Regina' sweet cherry*

| Treatment | A-grade fruit % | B-grade fruit % | Cracked fruit % | Reject fruit % |
|---|---|---|---|---|
| Control | 41.4 | 40.3 | 26.9 ab | 18.3 |
| Retain | 38.2 | 44.7 | 26.9 ab | 17.1 |
| Amino acids (AA) | 39.7 | 42.8 | 28.5 abc | 17.4 |
| Retain + AA | 77.9 | 44.1 | 12.4 a | 11.0 |
| Cytolin | 31.9 | 48.6 | 43.6 bc | 19.6 |
| Foliar organic nutrients | 36.0 | 40.7 | 47.1 bc | 23.4 |
| 2,4-D | 32.2 | 41.9 | 42.7 bc | 25.9 |
| Tops | 21.6 | 39.4 | 51.4 c | 39.0 |
| *Lsd (P=0.05)* | *ns* | *ns* | *23.3* | *ns* |
| *F Prob* | *0.208* | *0.959* | *0.028* | *0.090* |

Within a single column means sharing the same letter are not significantly different at P=0.05 using the LSD test.

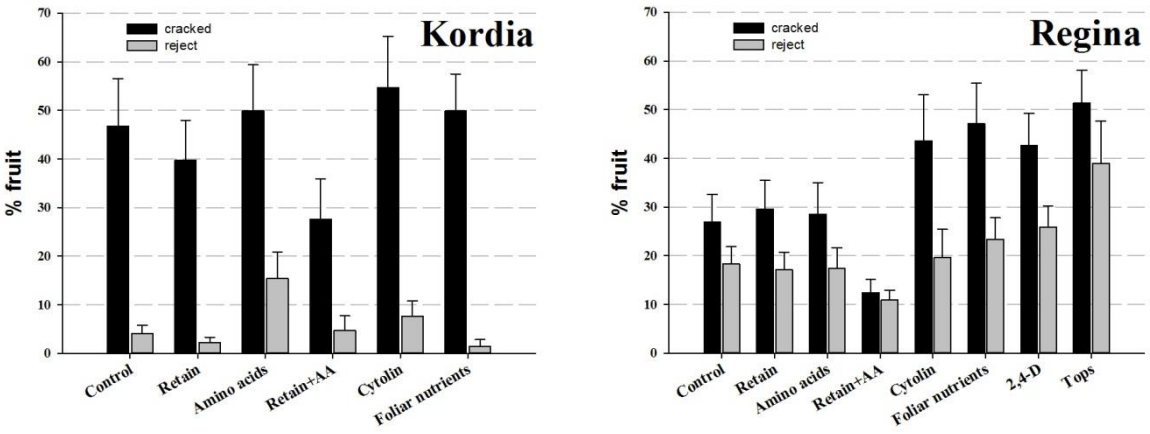


*Figure 4: The effect different plant growth regulators on the percentage of cracked and reject fruit in (a) 'Kordia', and (b) 'Regina' sweet cherry.*

In *Kordia*, weight of A-grade fruit was reduced by application of Retain, amino acids and Cytolin compared with the untreated control (Table 7), but there were no significant differences between treatments in the *Regina* trial (Table 8).

There was no effect on pedicel diameter in either variety (Table 7, 8), or on skin colour in *Kordia* fruit (Table 7). Skin colour in *Regina* was lighter in the Cytolin treatment compared with all other treatments (Table 8).

*Table 7: Effect of different plant growth regulators on fruit size, pedicel diameter and skin colour of A-grade fruit of 'Kordia' sweet cherry*

| Treatment | Weight (g) | Diameter (mm) | Pedicel diameter (mm) | Skin colour |
|---|---|---|---|---|
| Control | 15.6 b | 31.3 c | 1.19 | 4.76 |
| Retain | 14.7 a | 30.5 ab | 1.18 | 4.66 |
| Amino acids (AA) | 14.7 a | 30.8 bc | 1.19 | 4.43 |
| Retain + AA | 14.3 a | 30.5 ab | 1.15 | 4.12 |
| Cytolin | 14.2 a | 30.0 a | 1.16 | 4.59 |
| Foliar organic nutrients | 15.6 b | 31.4 c | 1.15 | 4.70 |
| *Lsd (P=0.05)* | *0.8* | *0.6* | *ns* | *ns* |
| *F Prob* | *0.004* | *0.003* | *0.709* | *0.146* |

*Within a single column means sharing the same letter are not significantly different at P=0.05 using the LSD test.*

*Table 8: Effect of different plant growth regulators on fruit size, pedicel diameter and skin colour of A-grade fruit of 'Regina' sweet cherry*

| Treatment | Weight (g) | Diameter (mm) | Pedicel diameter (mm) | Skin colour | |
|---|---|---|---|---|---|
| Control | 13.6 | 29.5 | 1.34 | 5.70 | b |
| Retain | 13.3 | 29.4 | 1.25 | 5.54 | b |
| Amino acids (AA) | 13.6 | 29.7 | 1.30 | 5.42 | b |
| Retain + AA | 12.6 | 28.7 | 1.24 | 5.82 | b |
| Cytolin | 14.2 | 30.4 | 1.28 | 4.93 | a |
| Foliar organic nutrients | 14.7 | 30.7 | 1.30 | 5.55 | b |
| 2,4-D | 13.9 | 30.0 | 1.28 | 5.43 | b |
| Tops | 13.7 | 29.9 | 1.30 | 5.48 | b |
| *Lsd (P=0.05)* | *ns* | *ns* | *ns* | *0.40* | |
| *F Prob* | *0.107* | *0.070* | *0.432* | *0.008* | |

*Within a single column means sharing the same letter are not significantly different at P=0.05 using the LSD test.*

There was no treatment effect on fruit compression firmness, flesh firmness or skin puncture force in either the *Kordia* or *Regina* trials (Table 9, 10).

Stem retention force in *Kordia* was not affected by treatment (Table 9), but in the *Regina* trial, stem retention force was reduced following treatment with Retain, amino acids, 2,4-D and Tops (Table 10). However, these reductions were relatively small, as all treatments still resulted in stem retention forces of more than double the required export standard of 500 g.

Postharvest assessment of fruit stored for 35 days again showed no treatment effect on *Kordia* fruit for fruit compression firmness, flesh firmness, skin puncture force or stem retention force (Table 11).

In the *Regina* trial, compression firmness was lower in the Retain plus amino acids treatment compared with the untreated control (Table 12), while flesh firmness and skin puncture force were higher in the foliar organic nutrient treatment than the untreated control. Tops treated fruit also showed higher skin puncture force than the control. With respect to stem retention force, post-harvest assessment showed similar patterns to harvest fruit, with Retain plus amino acids treated fruit having lower stem retention force than the control (Table 12).

*Table 9: Effect of different plant growth regulators on fruit firmness, skin puncture and stem retention force of 'Kordia' sweet cherry fruit*

| Treatment | Compression firmness ($g/mm^2$) | Flesh firmness (g) | Skin puncture force (g) | Stem retention force (g) |
|---|---|---|---|---|
| Control | 389 | 139 | 572 | 1,087 |
| Retain | 376 | 151 | 597 | 1,069 |
| Amino acids (AA) | 393 | 143 | 605 | 1,127 |
| Retain + AA | 402 | 156 | 617 | 1,035 |
| Cytolin | 363 | 139 | 578 | 1,160 |
| Foliar organic nutrients | 389 | 147 | 593 | 1,111 |
| *Lsd (P=0.05)* | *ns* | *ns* | *ns* | *ns* |
| *F Prob* | *0.571* | *0.593* | *0.662* | *0.202* |

*Within a single column means sharing the same letter are not significantly different at P=0.05 using the LSD test.*

*Table 10: Effect of different plant growth regulators on fruit firmness, skin puncture and stem retention force of 'Regina' sweet cherry fruit*

| Treatment | Compression firmness ($g/mm^2$) | Flesh firmness (g) | Skin puncture force (g) | Stem retention force (g) |
|---|---|---|---|---|
| Control | 349 | 90 | 415 | 1574 c |
| Retain | 331 | 84 | 382 | 1395 ab |
| Amino acids (AA) | 336 | 85 | 403 | 1561 b |
| Retain + AA | 306 | 76 | 352 | 1262 a |
| Cytolin | 360 | 93 | 413 | 1448 bc |
| Foliar organic nutrients | 362 | 100 | 452 | 1596 c |
| 2,4-D | 337 | 87 | 403 | 1531 b |
| Tops | 360 | 96 | 436 | 1517 b |
| *Lsd (P=0.05)* | *ns* | *ns* | *ns* | *176* |
| *F Prob* | *0.053* | *0.127* | *0.095* | *0.010* |

*Within a single column means sharing the same letter are not significantly different at P=0.05 using the LSD test.*

*Table 11: Effect of different plant growth regulators on post-harvest fruit firmness, skin puncture and stem retention force of 'Kordia' sweet cherry fruit*

| Treatment | Compression firmness ($g/mm^2$) | Flesh firmness (g) | Skin puncture force (g) | Stem retention force (g) |
|---|---|---|---|---|
| Control | 415 | 150 | 528 | 1,071 |
| Retain | 417 | 160 | 529 | 963 |
| Amino acids (AA) | 425 | 148 | 542 | 1,088 |
| Retain + AA | 431 | 150 | 523 | 946 |
| Cytolin | 399 | 145 | 516 | 1,041 |
| Foliar organic nutrients | 415 | 154 | 539 | 1,078 |
| *Lsd (P=0.05)* | *ns* | *ns* | *ns* | *ns* |
| *F Prob* | *0.855* | *0.766* | *0.984* | *0.201* |

*Within a single column means sharing the same letter are not significantly different at P=0.05 using the LSD test.*

*Table 12: Effect of different plant growth regulators on post-harvest fruit firmness, skin puncture and stem retention force of 'Regina' sweet cherry fruit*

| Treatment | Compression firmness ($g/mm^2$) | Flesh firmness (g) | Skin puncture force (g) | Stem retention force (g) |
|---|---|---|---|---|
| Control | 304 b | 87 ab | 350 ab | 1142 bc |
| Retain | 290 b | 78 a | 328 ab | 968 b |
| Amino acids (AA) | 298 b | 87 ab | 374 bc | 1129 b |
| Retain + AA | 262 a | 74 a | 306 a | 703 a |
| Cytolin | 317 b | 87 ab | 363 bc | 992 b |
| Foliar organic nutrients | 329 b | 106 c | 402 c | 1348 c |
| 2,4-D | 296 b | 89 ab | 363 bc | 1124 b |
| Tops | 325 b | 98 bc | 392 c | 1155 bc |
| *Lsd (P=0.05)* | *33* | *15* | *47* | *210* |
| *F Prob* | *0.009* | *0.008* | *0.006* | *<0.001* |

*Within a single column means sharing the same letter are not significantly different at P=0.05 using the LSD test.*

There were no significant treatment effects on soluble solids content or juice pH of *Kordia* fruit (Table 13), but Retain, Cytolin and foliar organic nutrients resulted in an increase in malic acid content. However in *Regina*, all treatments resulted in similar malic acid levels to the control, with the exception of Retain plus amino acids, which showed lower malic acid levels (Table 14).

*Table 13: Effect of different plant growth regulators on fruit total soluble solids (TSS), juice pH and malic acid content of 'Kordia' sweet cherry fruit at harvest and 35 days post-harvest,*

| Treatment | ----------Harvest---------- | | | ----------Post-harvest---------- | | |
|---|---|---|---|---|---|---|
| | TSS (°Brix) | Juice pH | Malic acid (g/L) | TSS (°Brix) | Juice pH | Malic acid (g/L) |
| Control | 15.6 | 4.06 | 7.10 a | 15.8 | 4.46 c | 5.45 |
| Retain | 15.9 | 3.99 | 7.86 cd | 15.7 | 4.41 ab | 5.55 |
| Amino acids (AA) | 15.7 | 4.05 | 7.47 ac | 15.1 | 4.47 c | 5.30 |
| Retain + AA | 14.9 | 4.01 | 7.35 ab | 14.7 | 4.45 bc | 5.32 |
| Cytolin | 16.3 | 4.02 | 7.94 d | 15.7 | 4.44 abc | 5.51 |
| Foliar organic nutrients | 16.4 | 4.01 | 7.65 bc | 16.2 | 4.40 a | 5.72 |
| *Lsd (P=0.05)* | *ns* | *ns* | *0.43* | *ns* | *0.04* | *ns* |
| *F Prob* | *0.261* | *0.160* | *0.006* | *0.484* | *0.038* | *0.127* |

*Within a single column means sharing the same letter are not significantly different at P=0.05 using the LSD test.*

*Table 14: Effect of different plant growth regulators on fruit total soluble solids (TSS), juice pH and malic acid content of 'Regina' sweet cherry fruit at harvest and 35 days post-harvest,*

| Treatment | ----------Harvest---------- | | | ----------Post-harvest---------- | | |
|---|---|---|---|---|---|---|
| | TSS (°Brix) | Juice pH | Malic acid (g/L) | TSS (°Brix) | Juice pH | Malic acid (g/L) |
| Control | 17.4 | 4.72 | 4.32 bc | 17.5 | 4.90 | 3.80 |
| Retain | 16.7 | 4.74 | 4.09 ab | 15.9 | 4.92 | 3.45 |
| Amino acids (AA) | 16.7 | 4.68 | 4.51 c | 16.5 | 4.85 | 3.81 |
| Retain + AA | 15.5 | 4.71 | 3.83 a | 15.2 | 4.89 | 3.26 |
| Cytolin | 16.9 | 4.67 | 4.50 c | 15.9 | 4.85 | 3.72 |
| Foliar organic nutrients | 18.2 | 4.69 | 4.38 bc | 18.2 | 4.91 | 3.68 |
| 2,4-D | 16.3 | 4.68 | 4.36 bc | 16.5 | 4.89 | 3.61 |
| Tops | 18.1 | 4.71 | 4.49 c | 17.5 | 4.86 | 3.72 |
| *Lsd (P=0.05)* | *ns* | *ns* | *0.34* | *ns* | *ns* | *ns* |
| *F Prob* | *0.171* | *0.675* | *0.004* | *0.090* | *0.641* | *0.094* |

*Within a single column means sharing the same letter are not significantly different at P=0.05 using the LSD test.*

(v) Carryover effects of paclobutrazol

This study was commenced in autumn 2013, and was designed to answer some of the questions surrounding the use of paclobutrazol for improving fruit set in sweet cherry. This trial was set up to examine both application time (autumn vs spring) and method (butt spray vs soil drench) of paclobutrazol (Payback®) on *Regina*. Payback® was applied at rates of 1, 2 or 4 ml/tree.

There were no significant carry-over effects between the untreated control and paclobutrazol treated trees for fruit set or fruit size (Table 15). However examination of the main effects for the paclobutrazol treatments showed a significant difference in fruit weight ($p = 0.040$) and diameter ($p= 0.049$) for application method, with soil drench resulting in larger fruit than butt spray applications. There were no significant interactions between paclobutrazol application time, rate or application method.

*Table 15: Effect of paclobutrazol (Payback®) on fruit set and fruit weight and diameter of 'Regina' sweet cherry*

| Treatment | Fruit set (%) | Fruit per spur | Fruit per floral bud | A grade fruit Mean fruit weight (g) | A grade fruit diameter (mm) |
|---|---|---|---|---|---|
| **(i)** | **Control vs treated** | | | | |
| Control | 7.3 | 0.89 | 0.22 | 15.2 | 30.7 |
| Treated | 7.8 | 0.97 | 0.24 | 16.1 | 31.5 |
| *Lsd (p=0.05)* | *ns* | *ns* | *ns* | *ns* | *ns* |
| *F Prob* | *0.799* | *0.759* | *0.773* | *0.070* | *0.079* |
| **(ii)** | **Paclobutrazol application time** | | | | |
| Autumn | 7.3 | 0.95 | 0.23 | 16.1 | 31.5 |
| Spring | 8.3 | 1.00 | 0.25 | 16.1 | 31.5 |
| *Lsd (p=0.05)* | *ns* | *ns* | *ns* | *ns* | *ns* |
| *F Prob* | *0.439* | *0.698* | *0.465* | *0.908* | *0.848* |
| **(iii)** | **Paclobutrazol rate** | | | | |
| 1 ml Payback™ / tree | 5.9 | 0.72 | 0.19 | 16.2 | 31.6 |
| 2 ml Payback™ / tree | 8.6 | 1.13 | 0.26 | 16.3 | 31.7 |
| 4 ml Payback™ / tree | 8.9 | 1.07 | 0.27 | 15.8 | 31.3 |
| *Lsd (p=0.05)* | *ns* | *ns* | *ns* | *ns* | *ns* |
| *F Prob* | *0.092* | *0.059* | *0.122* | *0.310* | *0.444* |
| **(iv)** | **Paclobutrazol application method** | | | | |
| Butt spray | 7.1 | 0.90 | 0.22 | 15.8 a | 31.2 a |
| Soil drench | 8.5 | 1.05 | 0.26 | 16.4 b | 31.8 b |
| *Lsd (p=0.05)* | *ns* | *ns* | *ns* | *0.5* | *0.5* |
| *F Prob* | *0.251* | *0.310* | *0.355* | *0.040* | *0.049* |
| **(v)** | **Interactions** | | | | |
| Time * Rate | ns | ns | ns | ns | ns |
| Time * Method | ns | ns | ns | ns | ns |
| Rate * Method | ns | ns | ns | ns | ns |
| Time * Rate * Method | ns | ns | ns | ns | ns |

*Within a single column and main effect only, means sharing the same letter are not significantly different at P=0.05 using the LSD test.*

There were no significant carry-over effects between the untreated control and paclobutrazol treated trees for fruit firmness, skin puncture or stem retention force (Table 16), total soluble solids, juice pH or malic acid content (Table 17). Fruit from trees treated with paclobutrazol the previous year showed darker skin colour than fruit from untreated trees (Table 17).

However examination of the main effects for the paclobutrazol treatments showed a significant difference in skin puncture force (p = 0.030) for application time, with spring application resulting in larger fruit than autumn applications. There were no significant interactions between paclobutrazol application time, rate or application method (results not presented).

*Table 16: Effect of paclobutrazol (Payback®) on firmness, skin puncture force and stem retention force of 'Regina' sweet cherry fruit*

| Treatment | Compression Firmness (g/mm) | Flesh pressure (g) | Skin puncture force (g) | Stem retention force (g) |
|---|---|---|---|---|
| **(i)** | | **Control vs treated** | | |
| Control | 286 | 96 | 418 | 1,760 |
| Treated | 286 | 99 | 453 | 1,807 |
| *Lsd (p=0.05)* | *ns* | *ns* | *ns* | *ns* |
| *F Prob* | *0.999* | *0.540* | *0.096* | *0.391* |
| **(ii)** | | **Paclobutrazol application time** | | |
| Autumn | 281 | 97 | 440 ab | 1,815 |
| Spring | 291 | 102 | 466 b | 1,800 |
| *Lsd (p=0.05)* | *ns* | *ns* | *43* | *ns* |
| *F Prob* | *0.221* | *0.159* | *0.030* | *0.635* |
| **(iii)** | | **Paclobutrazol rate** | | |
| 1 ml Payback™ / tree | 287 | 98 | 452 | 1,823 |
| 2 ml Payback™ / tree | 290 | 100 | 454 | 1,808 |
| 4 ml Payback™ / tree | 282 | 100 | 453 | 1,792 |
| *Lsd (p=0.05)* | *ns* | *ns* | *ns* | *ns* |
| *F Prob* | *0.636* | *0.797* | *0.993* | *0.713* |
| **(iv)** | | **Paclobutrazol application method** | | |
| Butt spray | 280 | 99 | 451 | 1,796 |
| Soil drench | 292 | 100 | 455 | 1,819 |
| *Lsd (p=0.05)* | *ns* | *ns* | *ns* | *ns* |
| *F Prob* | *0.130* | *0.723* | *0.727* | *0.440* |

*Within a single column and main effect only, means sharing the same letter are not significantly different at P=0.05 using the LSD test.*

(vi) examination of optimal application time for ATS to reduce crop load

Natural fruit set for trees in the *Van* block in the 2014/15 season was 38.8% (Table 18, Figure 5)). Crop load was successfully reduced by most treatments; application of Ethrel at SF reduced crop load by 51%, a single ATS applied at 80% bloom reduced crop load by 23% and the double ATS application by 34%. There was no treatment effect on mean fruit weight.

*Table 17: Effect of paclobutrazol (Payback®) on skin colour, sugar content, juice pH and malic acid content of 'Regina' sweet cherry fruit*

| Treatment | Skin Colour | TSS | Juice pH | malic acid (g/L) |
|---|---|---|---|---|
| **(i)** | **Control vs treated** | | | |
| Control | 5.5 a | 18.7 | 4.64 | 4.80 |
| Treated | 5.7 b | 19.5 | 4.63 | 5.03 |
| *Lsd (p=0.05)* | *0.19* | *ns* | *ns* | *ns* |
| *F Prob* | *0.028* | *0.255* | *0.690* | *0.234* |
| **(ii)** | **Paclobutrazol application time** | | | |
| Autumn | 5.7 | 19.3 | 4.63 | 4.96 |
| Spring | 5.6 | 19.8 | 4.62 | 5.09 |
| *Lsd (p=0.05)* | *ns* | *ns* | *ns* | *ns* |
| *F Prob* | *0.134* | *0.159* | *0.341* | *0.215* |
| **(iii)** | **Paclobutrazol rate** | | | |
| 1 ml Payback™ / tree | 5.6 | 19.5 | 4.63 | 5.03 |
| 2 ml Payback™ / tree | 5.7 | 19.8 | 4.63 | 5.02 |
| 4 ml Payback™/ tree | 5.7 | 19.3 | 4.61 | 5.03 |
| *Lsd (p=0.05)* | *ns* | *ns* | *ns* | *ns* |
| *F Prob* | *0.170* | *0.623* | *0.450* | *0.995* |
| **(iv)** | **Paclobutrazol application method** | | | |
| Butt spray | 5.7 | 19.4 | 4.65 b | 5.03 |
| Soil drench | 5.7 | 19.7 | 4.61 a | 5.02 |
| *Lsd (p=0.05)* | *ns* | *ns* | *0.03* | *ns* |
| *F Prob* | *0.963* | *0.406* | *0.039* | *0.954* |

*Within a single column and main effect only, means sharing the same letter are not significantly different at P=0.05 using the LSD test.*

*Table 18: Effect of the plant growth regulators Ethrel and ammonium thiosulphate (ATS) on fruit set and mean fruit weight of 'Van' sweet cherry*

| Treatment | Fruit set (%) | No. fruit per floral bud | No. fruit per spur | Mean fruit weight (g) |
|---|---|---|---|---|
| Control | 38.8 c | 1.37 c | 4.74 b | 10.3 |
| Ethrel at SF | 18.9 a | 0.65 a | 2.14 a | 10.7 |
| ATS at 30% bloom | 32.5 bc | 1.12 bc | 3.42 a | 11.1 |
| ATS at 80% bloom | 30.1 b | 1.01 b | 3.39 a | 11.0 |
| ATS @ 30&80% bloom | 25.6 ab | 0.94 ab | 2.83 a | 10.6 |
| *Lsd (P=0.05)* | *8.5* | *0.29* | *1.30* | *ns* |
| *F Prob* | *<0.001* | *<0.001* | *0.005* | *0.484* |

*Within a single column means sharing the same letter are not significantly different at P=0.05 using the LSD test.*

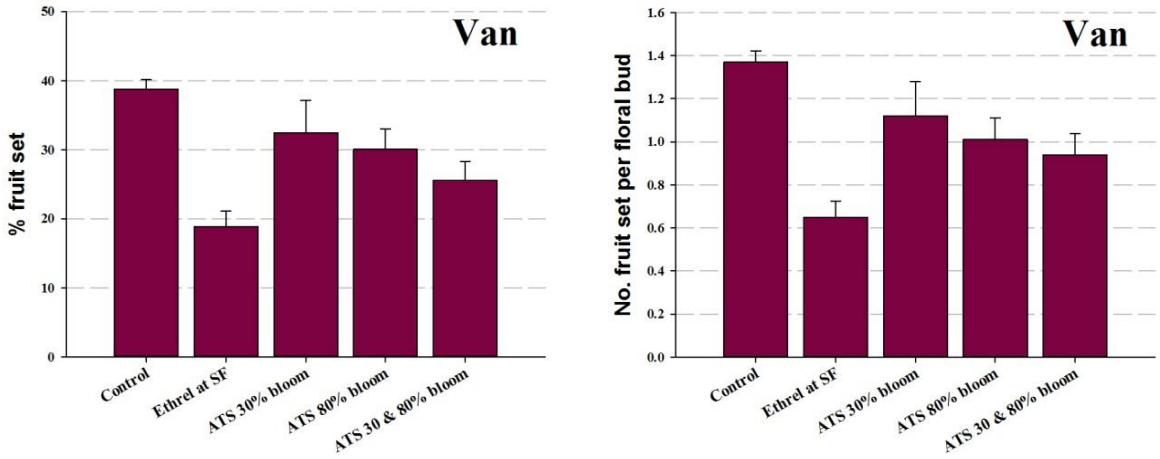


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*Figure 5: The effect of Ethrel and ammonium thiosulphate (ATS) on fruit set in 'Van' sweet cherry.*

Treatment with Ethrel or ATS had no effect on fruit pack-out or on the percentage of cracked or reject fruit (Table 19).

*Table 19: Effect of the plant growth regulators Ethrel and ammonium thiosulphate (ATS) on percentage of A-grade, B-grade, cracked and reject fruit in 'Van' sweet cherry.*

| Treatment | A-grade fruit % | B-grade fruit % | Cracked fruit % | Reject fruit % |
|---|---|---|---|---|
| Control | 55.8 | 35.2 | 17.5 | 9.0 |
| 250 ppm Ethrel at SF | 59.0 | 31.5 | 18.3 | 9.5 |
| 1% ATS at 30% bloom | 62.2 | 30.3 | 19.5 | 7.5 |
| 1% ATS at 80% bloom | 50.6 | 38.1 | 25.1 | 11.3 |
| 1% ATS at 30 & 80% bloom | 63.2 | 31.6 | 15.5 | 5.3 |
| *Lsd (P=0.05)* | *ns* | *ns* | *ns* | *ns* |
| *F Prob* | *0.330* | *0.661* | *0.651* | *0.336* |

*Within a single column means sharing the same letter are not significantly different at P=0.05 using the LSD test.*
*SF = shuck fall*

There were no treatment effects on A-grade fruit weight or diameter, pedicel diameter or skin colour (Table 20), or on fruit firmness, skin puncture or stem retention force (Table 21). Fruit quality did not alter during storage (Table 22).

*Table 20: Effect of the plant growth regulators Ethrel and ammonium thiosulphate (ATS) on fruit size, pedicel diameter and skin colour of A-grade 'Van' sweet cherry fruit*

| Treatment | Weight (g) | Diameter (mm) | Pedicel diameter (mm) | Skin colour |
|---|---|---|---|---|
| Control | 11.3 | 28.9 | 1.10 | 4.08 |
| 250 ppm Ethrel at SF | 12.3 | 29.7 | 1.09 | 4.39 |
| 1% ATS at 30% bloom | 12.5 | 29.9 | 1.11 | 4.33 |
| 1% ATS at 80% bloom | 12.1 | 28.7 | 1.09 | 4.28 |
| 1% ATS at 30 & 80% bloom | 12.1 | 29.7 | 1.12 | 3.95 |
| *Lsd (P=0.05)* | *ns* | *ns* | *ns* | *ns* |
| *F Prob* | *0.346* | *0.382* | *0.817* | *0.596* |

*Within a single column means sharing the same letter are not significantly different at P=0.05 using the LSD test.*
*SF = shuck fall*

*Table 21: Effect of the plant growth regulators Ethrel and ammonium thiosulphate (ATS) on fruit firmness, skin puncture and stem retention force of 'Van' sweet cherry fruit*

| Treatment | Compression firmness ($g/mm^2$) | Flesh firmness (g) | Skin puncture force (g) | Stem retention force (g) |
|---|---|---|---|---|
| Control | 256 | 87 | 326 | 867 |
| 250 ppm Ethrel at SF | 266 | 92 | 345 | 892 |
| 1% ATS at 30% bloom | 254 | 90 | 329 | 872 |
| 1% ATS at 80% bloom | 270 | 96 | 346 | 853 |
| 1% ATS at 30 & 80% bloom | 269 | 92 | 356 | 866 |
| *Lsd (P=0.05)* | *ns* | *ns* | *ns* | *ns* |
| *F Prob* | *0.556* | *0.471* | *0.411* | *0.887* |

*Within a single column means sharing the same letter are not significantly different at P=0.05 using the LSD test.*
*SF = shuck fall*

*Table 22: Effect of the plant growth regulators Ethrel and ammonium thiosulphate (ATS) on **post-harvest** fruit firmness, skin puncture and stem retention force of 'Van' sweet cherry fruit*

| Treatment | Compression firmness ($g/mm^2$) | Flesh firmness (g) | Skin puncture force (g) | Stem retention force (g) |
|---|---|---|---|---|
| Control | 277 | 89 | 294 | 753 |
| 250 ppm Ethrel at SF | 296 | 97 | 347 | 712 |
| 1% ATS at 30% bloom | 301 | 97 | 327 | 737 |
| 1% ATS at 80% bloom | 291 | 94 | 339 | 773 |
| 1% ATS at 30 & 80% bloom | 307 | 99 | 332 | 814 |
| *Lsd (P=0.05)* | *ns* | *ns* | *ns* | *ns* |
| *F Prob* | *0.484* | *0.707* | *0.157* | *0.588* |

*Within a single column means sharing the same letter are not significantly different at P=0.05 using the LSD test.*
SF = shuck fall

Compared with the untreated control, fruit soluble solids content was increased in ethephon treated trees (Table 23), but there was no treatment effect on juice pH or malic acid level at harvest. Storage for 42 days resulted in a decrease in TSS and malic acid levels compared with fruit at harvest.

*Table 23: Effect of the plant growth regulators Ethrel and ammonium thiosulphate (ATS) on fruit total soluble solids (TSS), juice pH and malic acid content of 'Van' sweet cherry fruit at harvest and 42 days post-harvest*

| Treatment | ----------Harvest---------- | | | ----------Post-harvest---------- | | |
|---|---|---|---|---|---|---|
| | TSS (°Brix) | Juice pH | Malic acid (g/L) | TSS (°Brix) | Juice pH | Malic acid (g/L) |
| Control | 13.2 a | 4.00 | 6.64 | 12.4 a | 4.29 | 4.75 a |
| 250 ppm Ethrel at SF | 15.0 b | 4.07 | 6.53 | 14.9 b | 4.40 | 4.75 a |
| 1% ATS at 30% bloom | 14.8 ab | 4.03 | 6.60 | 14.2 b | 4.32 | 4.84 a |
| 1% ATS at 80% bloom | 14.5 ab | 4.00 | 6.57 | 14.2 b | 4.37 | 4.69 a |
| 1% ATS at 30&80% bloom | 13.8 a | 4.0 | 6.96 | 13.2 ab | 4.26 | 5.37 b |
| *Lsd (P=0.05)* | *1.1* | *ns* | *ns* | *1.2* | *ns* | *0.36* |
| *F Prob* | *0.033* | *0.262* | *0.471* | *0.003* | *0.056* | *0.008* |

*Within a single column means sharing the same letter are not significantly different at P=0.05 using the LSD test.*
*SF = shuck fall*

**Key points and conclusions:**

**(A) Improving fruit set in shy-bearing cultivars:**

- Retain was consistent in improving fruit set in both varieties
- Cytolin reduced fruit set
- 2,4-D, Tops and amino acids showed no improvement in fruit set
- No carryover effects from paclobutrazol treatments the previous season

Results from this project suggest that Cytolin, 2,4-D and Tops are of no benefit in improving fruit set in shy-bearing cultivars.

Retain has been consistent across seasons and varieties in improving fruit set. Although amino acids alone did not improve fruit set, there was a reduction in fruit cracking when amino acids were combined in a program with Retain. Hence, there may be some benefit in incorporating amino acids into a Retain program, particularly if Retain and amino acids can be applied as a tank mix. However, further studies are required to determine the feasibility of this approach.

It would also be worth examining regular foliar nutrition applications in conjunction with a Retain program. Bud strengthening through a post-harvest foliar nutrition program may also be worthy of examination to determine whether this would be beneficial to fruit set the following spring.

**(B) Reducing fruit set in cultivars that benefit from thinning:**

- Application of 250 ppm ethephon at shuck fall reduced crop load by 50%
- 80% bloom application timing for ATS more effective than 30% bloom stage
- Double ATS treatment resulted in 34% crop load reduction

The conclusions from this work are that both ethephon and ATS are effective thinners in sweet cherry, While ATS gave additional fruit quality benefits in previous seasons compared with ethephon, there were no differences observed in fruit quality in the final year of study. Hence, fruit quality benefits may be subject to seasonal variation.

# Appendix 2: Investigating post-bloom thinning

***Matthew Whiting, Washington State University - IAREC***

**Cooperators:** Bryan Peebles, Harold Schell, Chelan Fresh; Allyson Leonhard and Lu Zhang, Washington State University

**Objective:** To develop pragmatic, cost-effective post-bloom thinning strategies

## Significant Findings:

- Ethephon applications are effective at reducing fruit set in sweet cherry post-bloom (as great as 90% reduction)
- Thinning efficacy is largely rate-dependent
- Timing of application is important – greater thinning efficacy was observed with earlier applications
- Fruit quality improvements were inconsistent, irrespective of quality parameter
- Fruit soluble solids were improved consistently from thinning – size was not always improved, despite significant reductions in crop load
- There was no relationship between fruit set and fruit quality parameters

## Methodology

The need for post-bloom thinning tools is clear – one cannot assess fruit set until well after flowering. Currently, the only reliable means of post-bloom thinning in sweet cherry is manual fruit removal, an expensive operation. We propose to develop a post-bloom thinning strategy focusing on Ethephon because it showed promise in our previous work on 'Sweetheart', and 'Rainier'. Ethephon will be compared to hand thinning. There are two key elements that need to be determined – the best time for application and the rate-response.

**I – TIMING OF APPLICATION**

***Treatments:***

- unthinned control (water sprayed)
- hand thinning to about 30 fruit per foot
- Ethephon at 200 ppm

***Timing of application:***

- shuck fall
- shuck fall + 1 week
- shuck fall + 2 weeks
- shuck fall + 3 weeks

***Methods:***

Applications will be made using a pressurized spray gun or commercial airblast sprayer to 'Sweetheart', 'Rainier', and 'Skeena' trees that exhibit heavy fruit set. Two experiments will be conducted for each cultivar – one in a commercial orchard and one at the WSU-Roza experimental orchards. In addition, we will work opportunistically with additional growers interested in evaluating post-bloom thinning strategies by providing suggestions for protocols and helping with data collection on efficacy. On each application date, treatments will be made to entire trees, with 6 whole-tree replications. Hand thinning will be accomplished by manually removing fruit from throughout entire trees with a goal of leaving ca. 30 fruit per foot (preliminary work shows this is a reasonable target to balance fruit number with quality). Depending on the orchard, we will use either a completely randomized design or a randomized complete block design, with at least 2 border trees between adjacent treatments. We will require 96 trees in each orchard (4 treatments x 4 timings x 6 reps). Key environmental conditions (e.g., wind speed, temperature, humidity) during and following application will be monitored using AgWeatherNet stations in the vicinity.

Within a day of application, we will flag two limbs in every tree and count fruitlet density (fruitlets/limb cross-sectional area and length), measuring limb caliper as well. In addition, we will measure fruit diameter on 30 fruit per limb to record fruitlet size at the time of treatment – this will facilitate comparisons among cultivars with respect to timing). We will record the time required to hand thin and 'rake' thin each replicate tree. In addition, we will collect thinned fruit and measure fruit size and weight to see whether the population of thinned fruitlets differs significantly from the remaining unthinned fruitlets. A photo journal will be collected as well to visually document application timings and crop densities. At commercial fruit maturity we will make fruit counts to the same limbs and assess thinning efficacy as % fruitlet removal. Fruit subsamples (minimum 100 fruit per replication) will be collected and analyzed for quality attributes including color, weight, diameter, firmness, and surface damage.

***Scope of work:***

3 cultivars (Rainier, Skeena, Sweetheart)
2 sites for each cultivar (1 commercial orchard + WSU Roza farm)
16 'treatments' (4 timings and 4 treatments)
6 replicates

**II – RATE OF ETHEPHON**

***Treatments:***

- unthinned control (water sprayed)
- Ethephon at 100 ppm
- Ethephon at 200 ppm
- Ethephon at 300 ppm

***Methods:***

These experiments will be conducted as described above with respect to applications, experimental design, data collection, and analyses. Again, we will make applications to Rainier, Skeena, and Sweetheart in 2 locations (a commercial orchard + the WSU Roza farm), identifying commercial orchards once fruit density can be determined. The treatments will be made at shuck fall + 1 week by pressurized spray gun or commercial airblast sprayer. We will require 24 trees for these experiments (4 treatments x 6 reps).

In the second year, we will repeat post-bloom thinning experiments and generate outreach material describing the results from our post-bloom thinning trials. These may include videos (describing benefits of post-bloom thinning), presentations at winter meetings, and written reports for the Good Fruit Grower.

## Results

### *Fruit set*

In 2013 we conducted 5 distinct thinning trials, 4 with commercial growers and 1 at the WSU-Roza farm. In 2013 we included abscisic acid (ABA) in addition to the Ethephon treatments from 2012. The following will highlight the results from 3 of those trials – they are representative of the overall response.

In a 'Sweetheart' trial in the Yakima valley natural fruit set was about 80% of available flowers and fruit density was about 35 fruit/foot. Average fruit weight from untreated control limbs was 8.8 g (about 10.5 row). Ethephon treatment reduced fruit set proportional to rate, but only at the earliest application timing (FIG 1). Ethephon applied on the 6th of May (i.e., shortly after shuck fall, 11.9 mm mean fruit diameter) reduced fruit set by 4, 19, and 73% compared to the control at 100, 200, and 300 ppm, respectively. ABA was generally ineffective as a post-bloom thinner in this trial; in fact, later applications of ABA at 500 ppm improved fruit set by roughly 10-14%. Similarly, later applications of Ethephon were ineffective as thinners and, in some cases, increased fruit set by up to 17% (Ethephon at 300 ppm applied 20 May).

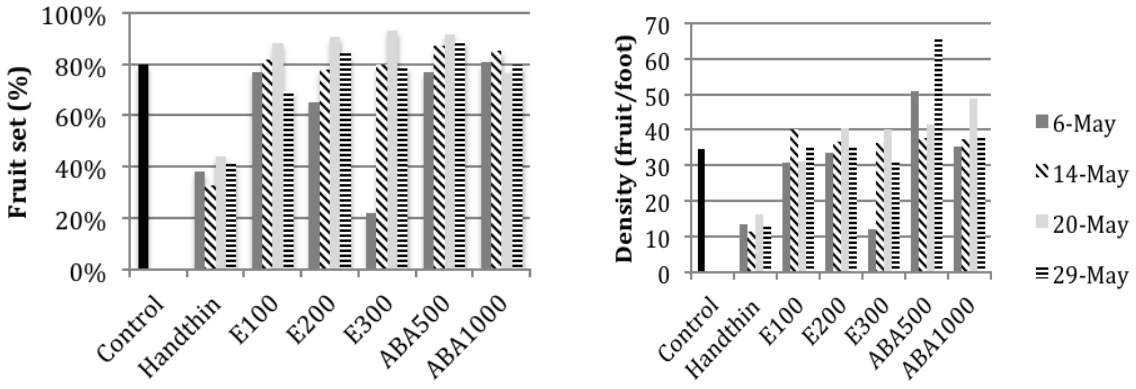



*Figure 1. Thinning efficacy of Ethephon or ABA applied to 'Sweetheart'.*

Hand-thinning treatments consistently reduced crop load, to about 12 fruit/foot. These thinning treatments did not improve fruit weight/size however (FIG). This suggests that fruit were not source-limited during growth and development; therefore, thinning was unnecessary. Fruit size from the first applications of Ethephon on 6 May (i.e., those treatments that did provide thinning) was improved 6% by 100 ppm, unaffected by 200 ppm, and reduced 25% by 300 ppm despite that treatment reducing fruit set 73%. Interestingly, similar to results in 2012 with different cultivars, several Ethephon treatments improved fruit quality without providing any thinning. The greatest improvements to fruit size/weight were from 100 ppm Ethephon at the first and second timings (+6 and 11%, respectively) and 300 ppm Ethephon on the second application date (+7%).

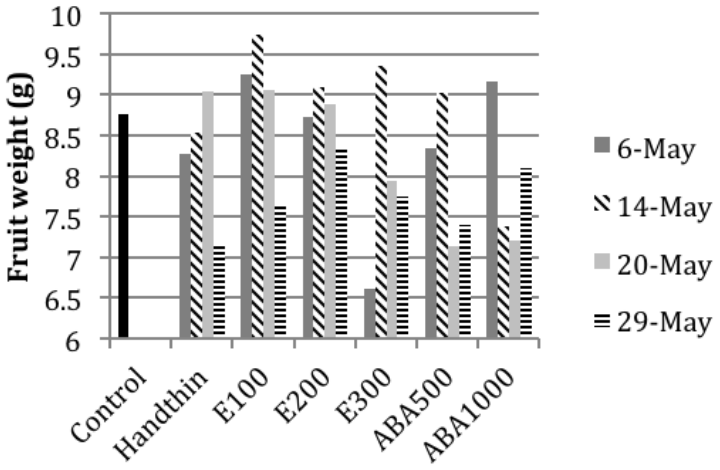



*Figure 2. Fruit weight of 'Sweetheart' following application of thinners.*

In a 'Lapins' trial in 2013 natural fruit set was high, about 90%, and fruit density was 46 per foot. Average fruit weight from untreated, control limbs was about 9.2 g (peaking on 10 row). Ethephon treatment at 100 ppm was ineffective as a thinner, average fruit set across all four application timings was about 91%. Ethephon at 200 ppm was effective for thinning but only on the first two application dates (5 and 14 May); later applications did not affect fruit set. Fruit set was reduced by Ethephon at 200 ppm to 45% on both the first two application dates. At 300 ppm Ethephon was an effective post-bloom thinner only on the first application timing when this treatment reduced fruit set to 20% (i.e., about 20% of control). Later applications of Ethephon at 300 ppm did not effectively thin fruit. ABA at 1000 ppm reduced fruit set by about 29% compared to unthinned control on the earliest timing, but was ineffective with later applications. ABA at 500 ppm was not an effective thinning agent at any timing. Ethephon applied at 100 ppm on the later two timings improved fruit set by 6%.

Fruit density was reduced by hand-thinning treatments consistently, to about 42% of the unthinned limbs (46 fruit/foot vs. 20 fruit/foot). The hand-thinning improved fruit size/weight by about 25%. Fruit weight was 11.5 g from all hand-thinned timings combined compared to 9.2 g in unthinned limbs (FIG).

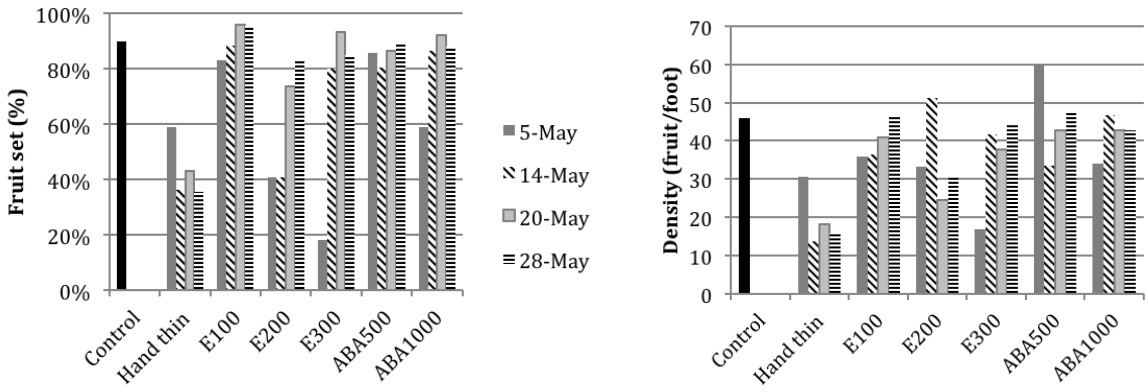



*Figure 3. Thinning efficacy of Ethephon or ABA applied to 'Lapins'.*

Interestingly fruit size/weight was improved by nearly every treatment, despite the inability of most treatments to thin the fruit. The greatest improvements in fruit weight were in response to Ethephon

at 100 ppm applied on the first two dates – these treatments led to improvements in fruit weight of 30 and 33%, respectively. This is similar to previous results from 2012 in which improvements in fruit quality were not associated with reductions in fruit set. The lack of relationship between fruit density and fruit weight suggests across all treatments and timings suggests that the PGR treatments are altering limb/tree source-sink relationships.

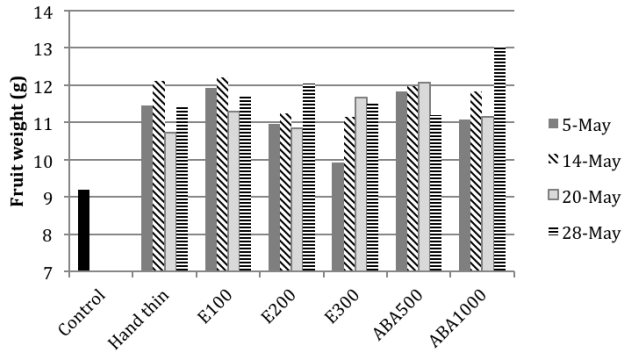



*Figure 4. Fruit weight of 'Lapins' following application of thinners*

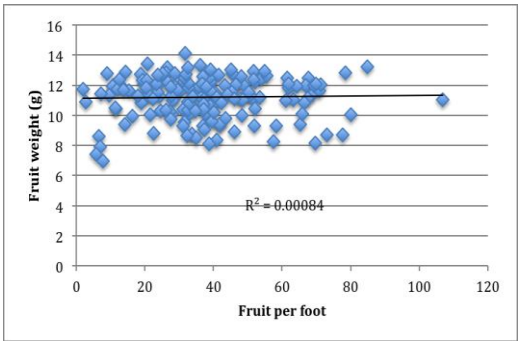



*Figure 5. Relationship between fruit density (fruit/ft) and individual fruit weight in 'Lapins'*

## Executive Summary

Ethephon showed potential to thin fruit after bloom. The thinning response was proportional to rate and earlier applications were more effective than late applications. ABA showed little potential as a post-bloom thinning agent for sweet cherry in the application timings we studied. We documented an interesting disconnect between thinning and fruit quality improvements – treatments that reduced fruit density did not always improve fruit quality, and, in many cases, Ethephon treatments of 100 or 200 ppm improved fruit quality without reducing fruit density. This is deserving of further study. It is recommended to conduct further trials with Ethephon at 100-200 ppm within 2 weeks after shuck fall.

**Abbreviated summary of results from 2012:**

Ethephon applications reduced fruit set significantly in every cultivar tested (data not shown). In Skeena, fruit set in untreated control was ca. 66%. Hand thinning treatments reduced final fruit by about half (fruit set = 31% overall), irrespective of timing of thinning (Figure 1). In comparison, mean fruit set across all timings was 68%, 50%, and 33% in response to treatment with 100 ppm, 200 ppm, and 300 ppm Ethephon, respectively. Therefore, 100 ppm was ineffective, and 300 ppm closely matched the hand thinning targets. Timing of Ethephon application was important – thinning efficacy was greatest on the first application and declined with each of the next two application dates (Figure 2). Expressed as a % of control fruit set, 300 ppm was effective at thinning on each application date, whereas 100 ppm was effective only on the first application date, and 200 ppm was effective only on the first two application dates (Fig. 2). These results suggest that there is a positive relationship between Ethephon rate and thinning efficacy, and that at higher rates, efficacy is greatest at early stages of fruit development.

In Sweetheart, fruit set of untreated limbs was similar to Skeena at about 66% (Figure 3). Hand thinning treatments reduced fruit set by about 65%, to 27% across all timings. In comparison, mean fruit set across all timings was 73%, 59%, and 34% in response to treatment with 100 ppm, 200 ppm, and 300 ppm Ethephon, respectively (each very similar to final fruit set in Skeena). Therefore, 100 ppm was ineffective, and 300 ppm most closely matched the hand thinning targets. Timing of

Ethephon application was important – thinning efficacy was greatest on the first application and declined with each of the next two application dates (Figure 2). Expressed as a % of control fruit set, 300 ppm was effective at thinning on the first three application dates, whereas 100 ppm was effective only on the first application date, and 200 ppm was effective only on the first two application dates (Fig. 4). These results suggest support our conclusion with Skeena that there is a positive relationship between Ethephon rate and thinning efficacy, and that at higher rates, efficacy is greatest at early stages of fruit development. Interestingly, Ethephon applied at 100 ppm and 200 ppm on the later application dates led to subtle improvements in final fruit set, with both treatments yielding about 40% more fruit than untreated control when applied on 8-June.

# Appendix 3: Cherry Fruitlet Abscission

***Jo Jones, Tasmanian Institute of Agriculture, University of Tasmania***

## Abstract

Fruitlet abscission is an issue impacting the profitability of the cherry industry, with particular varieties showing enhanced susceptibility. This study examined the role of carbohydrate availability in fruitlet abscission in two sweet cherry varieties, Kordia and Lapins. Starch and soluble sugar concentrations in branch, trunk and root tissue were measured regularly throughout the growing seasons of 2012/2013, 2013/14 and 2014/15. Normal transport of carbohydrates was disrupted 5 weeks after full-bloom by applying trunk girdling and limb defoliation treatments, and the rate of flower and fruitlet abscission was monitored. In Lapins, trunk girdling decreased the rate of abscission, but abscission was unaffected by girdling in Kordia. When fruit was assessed at commercial harvest, the fruit from the defoliated treatment was immature in both varieties. The highest sugar concentrations was found in fruit from the girdled trees in both Kordia and Lapins. Trunk starch concentrations were higher in Kordia than Lapins, and shoot starch concentrations were very similar to trunk in both varieties. Root carbohydrate concentrations did not recover after the trunk girdling treatment in either variety. There was a high rate of abscission in Kordia in the 2013/14 and 2014/15 season which did not appear to correlate with differences in carbohydrate status of the tree. Flower biology tests showed issues with synchronisation of Kordia bloom with its pollinator variety at the trial site, Regina, as well as reduced pollen germination rates following rain events. Further research into the flower biology of the low yielding variety Kordia is recommended, including the use of plant growth regulators to improve fruitset.

## Acknowledgements

The research team would like to acknowledge Hansen Orchards, Tasmania, for the use of their orchard for this three year trial and their guidance with this project. Technical assistance from Justine Direen, Widad Al Shawi, Eric Mertes, Caroline Claye and Elle Davis all of TIA is also acknowledged. Technical expertise from Dr Noel Davies at the University of Tasmania's Central Science Laboratory for soluble sugar analysis is appreciated. Advice and guidance from fellow TIA researchers Dr's Nigel Swarts, Sally Bound and Penny Measham is also appreciated.

## Introduction

Inconsistency in yield is an important problem for sweet cherry producers in Australia. Inconsistency of cropping can be due to the abscission of unpollinated flowers, flowers that have failed to set, and fruitlets that are not fully mature (Blanusa et al., 2005). The drivers of abscission of fruitlets prior to maturity in *Prunus* are not known, but environmental influences and competition for resources such as photoassimilates and plant hormones are likely causal factors (Thompson, 1996; cited in Blanusa et al., 2005). Moreover, it has been suggested for citrus that there's a strong relationship between carbohydrate available to the fruitlets, especially soluble sugars, and the probability of fruitlet abscission (Iglesias et al., 2003).

There is circumstantial evidence that developing fruitlets compete for photoassimilates, and it has been suggested that fruits that do not reach a certain threshold carbohydrate concentration have a high probability of abscission (Mahouachi et al., 1995). In regions where flower buds develop earlier than vegetative buds, flower development must rely upon total non-structural carbohydrate reserves

until enough photosynthetic leaf area is present to provide sufficient photosynthate (Keller and Loescher, 1989).

In Australia, the variety Kordia is widely grown, possessing good fruit size, flavour and quality; however, in many regions it is prone to inconsistency in yields (Bound et al., 2013). Kordia trees are typically grown in this region on a modified Spanish Bush system, encouraging lateral growth, as this system has proved the most successful. Lapins is a more consistently yielding variety, and abscission rates are generally low. The experiments in this paper aim to test the hypothesis that interrupting the transport of carbohydrates to the competing roots will assist in reducing the rate of abscission in the two varieties. By defoliating shoots, the trial will shed light on the contribution of current carbohydrates from young leaves on developing fruitlets versus stored carbohydrates from roots and wood structures.

The aims of this project were to:

- Monitor natural fruitlet abscission throughout the season in Kordia and Lapins
- Understand the seasonal pattern of carbohydrate availability in Kordia and Lapins
- Determine the influence of trunk girdling and limb defoliation on fruitlet abscission and the resulting fruit quality
- Measure aspects of flowering biology as a potential cause of poor fruitset

## Methods

### Trial design

The trial was established at a commercial orchard in southern Tasmania, Australia, in the spring of 2012, and was maintained for the three harvest seasons of 2013, 2014 and 2015. The trees were 16-year old Lapins and Kordia varieties grown on Mazzard F12-1 rootstocks. Lapins trees were pruned to a conventional Kym Green Bush system and the Kordia to a modified Kym Green Bush system with lateral growth, both widely accepted as best practice tree training systems for the respective varieties. Row orientation was east-west with 4.25 m between rows and 2 m between trees. The two varieties were studied in parallel.

A row of each variety was selected, and within each row, 5 trees were subjected to the girdling treatment, 5 trees to the defoliation treatment and 5 trees allocated as controls. Treatments were imposed 5 weeks after full bloom in each variety. For the girdling treatment, a 2 mm girdling knife was used to create two semi-circular incisions 10 cm apart, overlapping by 2 cm on both sides, 20 cm below the crown of the trunk. The defoliated treatment involved completely defoliating one limb per tree. The defoliated limb was selected using a random number table and did not require re-defoliation throughout the season, as leaf regrowth did not occur.


*A defoliated limb (above left) and a girdled trunk (above right).*

Trees were managed commercially in terms of irrigation, nutrient and pest management. These same trees were used for the fruitlet abscission monitoring, carbohydrate analysis, fruit quality and flower biology testing.

## Carbohydrate analysis

### *Wood sample collection*

Trunk, branch and root carbohydrates were sampled once every other week. Trunk samples were obtained by drilling into the trunk with a 10 mm boring bit half way up the trunk, and collecting the wood shavings. The same method was used for branch sampling but an 8 mm boring bit was used. Fine root samples were also taken. Samples were frozen then freeze-dried (Christ beta 1-8LD plus) for 48 hours at -45 °C and maintained at -20 °C for stored carbohydrate analysis. After drying, wood samples were ground sufficiently to be able to pass through a 1 mm sieve using an IKA Cutting Mill (A11 basic Analytical mill). Ground samples were stored in plastic zip-lock bags at -20 °C for further analysis using wet chemistry.

*Sampling of wood samples from trunk (above left), shoots (above right) and roots (left).*

Extraction

Approximately 100 mg of freeze dried, powdered wood samples were weighed into 10 ml plastic test tubes using an analytical balance (Mettler AE 200). A volume of 3 ml of 80% ethanol was added to each test tube. This was vortexed (Chiltern Scientific) and incubated at 60 °C in a water bath for 1 hour. The samples were then centrifuged at 4000 g for 10 min at 16 °C (Beckman rotor JA-20.1). The supernatant was pipetted into a plastic tube with lid. The extraction was repeated twice more. The supernatants were combined and frozen for soluble sugar analysis. The residue was also frozen and later analysed for Total Starch.

Sugars analysis

Using a 5 ml Hamilton syringe with a removable needle, the 9 ml of combined supernatant liquid was filtered through a 0.45 µm filter into a glass test tube. The filter was rinsed with approx. 0.5 ml of 80% ethanol. Between samples the syringe was rinsed once with 80% ethanol and three times with distilled water. 50 µm of Trehalose standard (250.3 mg/25 ml) was added to give a final concentration of 200 ppm.

The test tubes were placed in a boiling water bath and the liquid evaporated to less than 1ml. This was made up to 2.5 ml with distilled water and filtered through a SepPak Accell plus CM Cartridge into a 1.5 ml eppendorf centrifuge tube, which was filled to the line. Between samples the syringe was rinsed three times with distilled water.

The tubes were centrifuged at 10,000 rpm for 10 minutes, using an eppendorf bench top centrifuge. The supernatant liquid was filtered through a 0.45 µm and a 0.2 µm filter in tandem, into an HPLC vial. All samples were frozen and analysed for soluble fructose, glucose and sucrose, using HPLC – MS.

Starch Analysis

The starch level was determined enzymatically using a total starch assay kit (Megazyme Pty Ltd, Australia, Cat.#: K-TSTA, AOAC Method 996.11, AACC Method 76.13 ). The extracted frozen pellet was placed into a 20 ml glass test tube. A volume of 0.2 ml 80% aqueous ethanol was added to each test tube. A volume of 2 ml Dimethylsulfoxide (DMSO) was added immediately and vortexed. The tubes (with tops on) were then placed in a water bath with boiling water for 5 min.

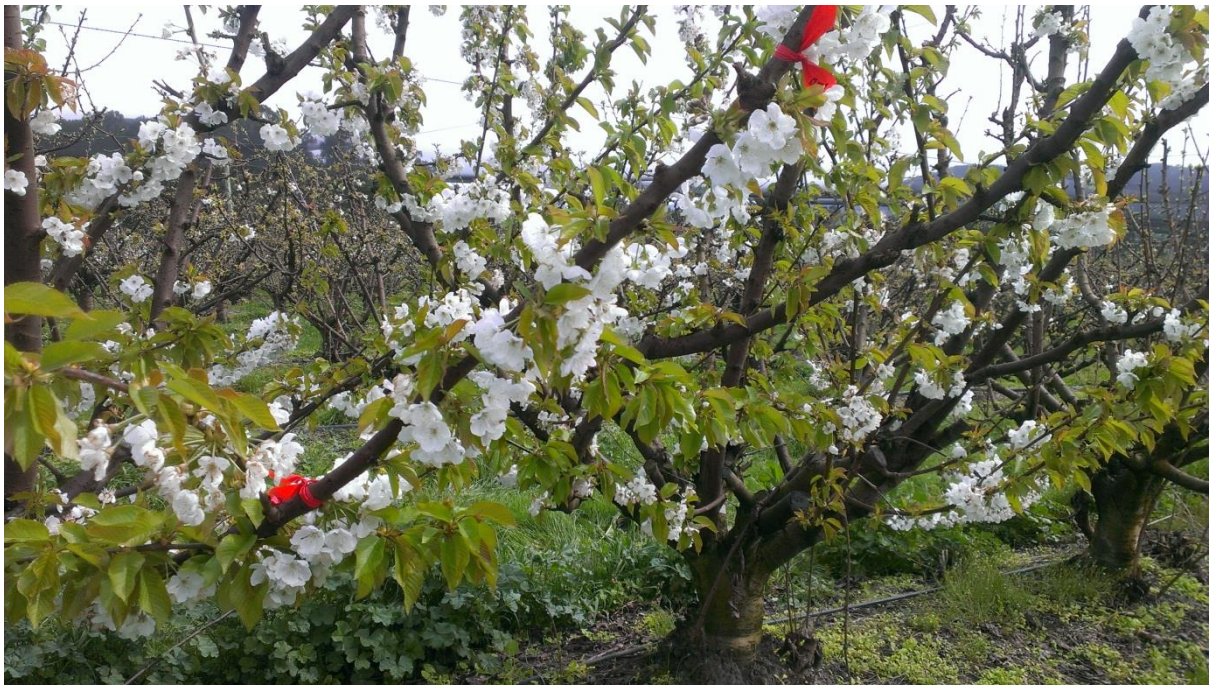
A volume of 3 ml of thermostable alpha-amylase in MOPS Buffer (1 ml of thermostable alpha-amylase in 30 ml of MOPS Buffer) was added and vortexed vigorously. The tubes were incubated in a water bath with boiling water for 6 min, stirring every 2 minutes (vortex mixer). A volume of 4 ml of Sodium Acetate Buffer (200 mM, pH 4.5) was added, followed by 0.1 ml of Amylo-glucosidase enzyme. The contents of the tubes were vortexed and incubated at 50 °C for 30 min. The mixture was then tipped into a plastic test tube and adjusted to the 10.0 ml volumetric mark using distilled water. These were then centrifuged at 3000 rpm for 10 min (4 °C) using the Avanti J- 301Beckman centrifuge.

Aliquots of 0.1 ml of the tube contents were transferred to glass test tubes (16 x100 mm) and 3.0 ml of GOPOD reagent (glucose oxidase, >12000 units/L; Peroxidase, >650 units/L; 4- Aminoantipyrine, 0.4 mM; Megazyme Australia Pty. Ltd.) added to each tube. The tubes, with tops, were incubated at 50 °C for 20 min. A glucose control was prepared with 0.1 ml of glucose standard and 3 ml of GOPOD reagent, whereas a reagent blank was prepared with 0.1 ml distilled water and 3 ml GOPOD. These were incubated together with the samples at 50 °C for 20 min. The tube contents for each sample were transferred to disposable 1.5 ml PMMA cuvettes and read within 60 min in a spectrophotometer (WPA light-wave Diode-array S 2000UV/Vis). Absorbance was read at 510 nm for each sample and the glucose standard, using a reagent blank.

Percent starch and mg/g dry weight starch in the sample were calculated.

## Fruitlet abscission

In each season a monitor limb was selected at random and tagged. At late white bud stage, just prior to bloom, a section on each limb was tagged to include as close to 100 individual flowers as possible. This count was recorded. At predetermined intervals, each limb was recounted and the remaining number of flowers / fruitlets recorded, and the data was used to calculate percent abscission.



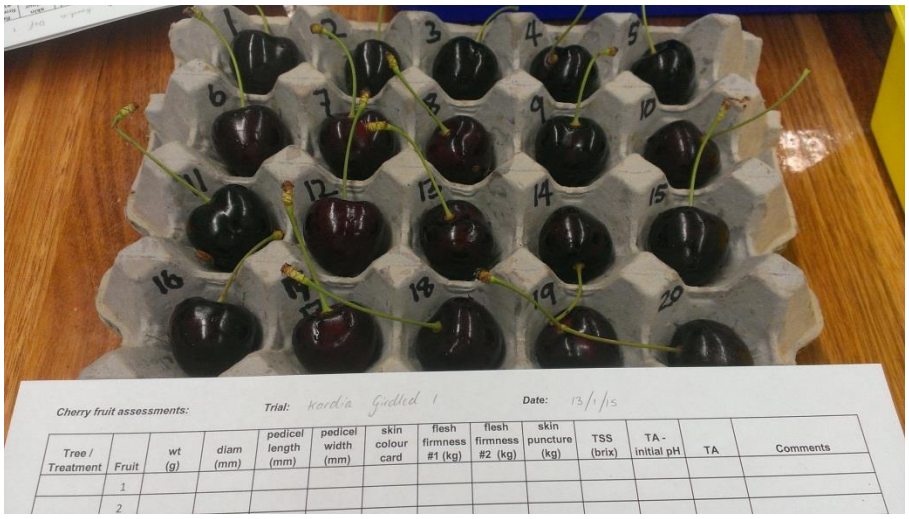
*A limb with 100 flowers marked between orange tape. These flowers / fruitlets were counted regularly and the percent abscission recorded.*

## Fruit quality and chemistry analyses

All fruit was harvested from the monitor limb on each tree at commercial maturity.

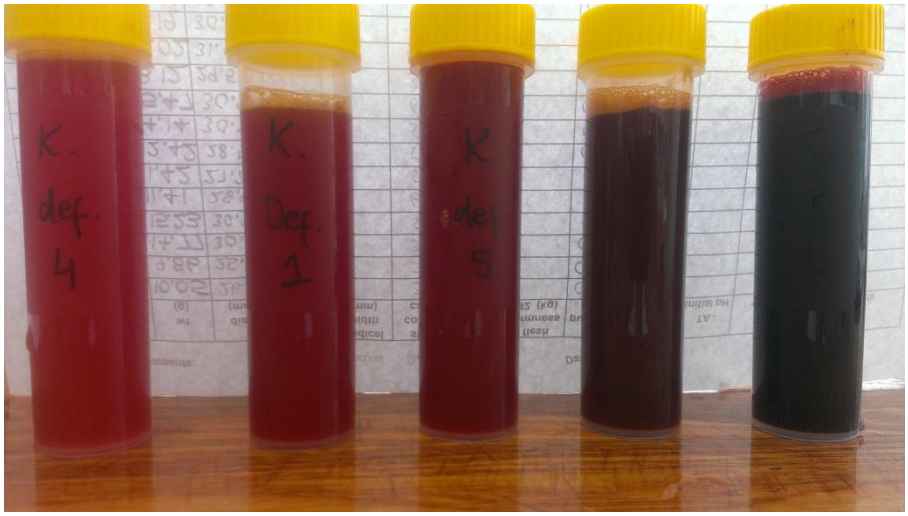
Twenty fruit from each sample were randomly sampled and analysed for fruit diameter using digital Vernier callipers, fruit weight with stems still attached, colour using the Australian Cherry Colour Chart and stem pull force using a stand mounted Mark 10 force gauge to assess stem retention. Fruit firmness was also assessed using a Bio Works FirmTech II Fruit Firmness Tester. A GÜSS model GS-20 Fruit Texture Analyser fitted with a 2 mm penetrometer probe, operating at a penetration speed of 10mm/second and a penetration depth of 4mm, was used to measure flesh firmness (on pared flesh) and skin puncture force. All fruit were allowed to warm to room temperature before analyses were undertaken to ensure that temperature effects were minimised.

A further 30 fruit from each sample were juiced collectively for total soluble solid (TSS), pH, and titratable acidity (TA). TSS concentration (°Brix) was assessed using an Atago PR-1 digital refractometer, and pH and TA were determined using a Metrohm 702 SM Titrino titrator.




*Top: fruit ready to be analysed for quality*

*Bottom: Visual difference in juice colour, with three defoliated treatments on the left and a control and girdled samples respectively to the right.*




## Flower Biology

In the 2014/2015 season a detailed study of flower biology was undertaken. Pollen viability, stigma receptivity, and *in vitro* pollen tube growth measures were made.

Flower sampling

Flowers were removed from the trees in early morning and kept in a sealed vial in an insulated container for transport to the laboratory, where they were refrigerated at 4°C for up to 4 hours.

Abscised flowers and fruitlets were collected for the presence of an embryo / seed.

Pollen viability

The Fluorochramatic Reaction Test (FCR) was used.

Stock of Fluorescein diacetate solution (FDA) 2mg/mL was prepared in acetone and stored at 4ºC. The stock solution of FDA was added drop by drop to 2 mL of 10% sucrose solutions, until the mixture showed persistent turbidity. The mixture was used within 30 minutes of preparation to prevent the FDA from precipitating. A drop of this solution was placed on a microscope slide and a small number of pollen grains were suspended in it to ensure uniform distribution of the pollen in preparation. A glass coverslip was lowered over the drop and the pollen grains that fluoresced brightly were counted under a fluorescence microscope. The preparation was observed immediately as the fluorescein eventually leached from the pollen grains (Shivanna and Rangaswamy, 1992). At least 250 pollen grains were counted in randomly selected fields under a fluorescence microscope (a Leica Leitz DM RBE microscope).

Presence of pollen on stigma

Excised stigmas were mounted in a drop of basic fuschin gel and examined under a light microscope for counts of pollen deposition in both opened and capped flowers. Using this technique, grapevine pollen stained red against a background of unstained stigmatic tissue. The basic fuschin gel was prepared, after the method of (Spurr, 2003). Forty grams of hydrated gelatin was melted in a microwave oven and mixed with 60mL of glycerol. Basic fuschin powder was gradually added to the mixture until a transparent mid red gel matching a colour standard determined in preliminary trials to

differentiate pollen and stigmatic tissue of grape flowers was obtained. The resulting mixture was filtered through glass wool and stored at 4ºC.

*In vitro* pollen germination

The hanging-drop germination method was used. Pollen was germinated in a liquid medium consisting of a suitable sucrose concentration, 1mM boric acid, and 1mM calcium nitrate (Shivanna etal, 1991). A drop of the prepared liquid was placed on a glass cover slip, and pollen placed onto the droplet. A rubber ring just less than the diameter of a slide was secured with Vaseline to a glass slide, with Vaseline also spread over the top surface of the ring. The coverslip was turned upside down and rested on the top of the ring, so that the droplet hung down within the ring, undisturbed. The slides were left the incubated in the dark at 20±1ºC for 24 hours. Pollen grains were classified as viable when the pollen tube length was twice as long as the diameter of the pollen grain. At least 300 pollen grains per dish were counted in randomly selected fields under a light microscope.

Stigma receptivity

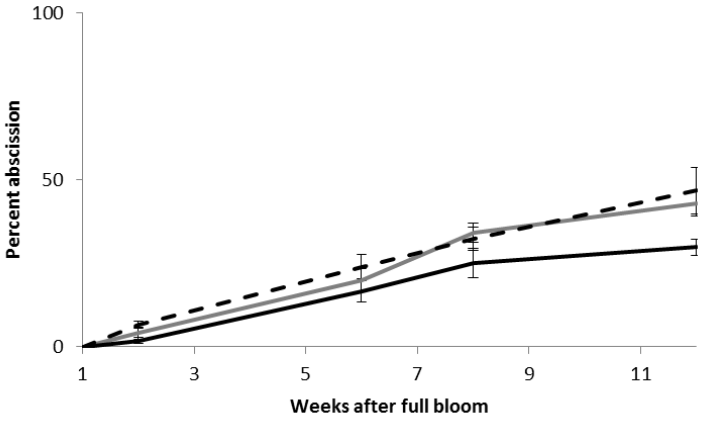
Stigmas were excised, placed on a slide, covered with a drop of aniline blue stain, and a cover slip and left at room temperature for four hours. A fluorescence microscope was used to view the mounts and the number of pollen tubes counted (Shivanna and Rangaswamy, 1992). The aniline blue stain was made by dissolving 5.75g of $K_3PO_4$ and 0.125g of water-soluble aniline blue in 250ml of distilled water. The fluorescence microscope was used to view the mounts and the total number of pollen grains and the number with pollen tubes counted (Shivanna and Rangaswamy, 1992).

## Data analysis

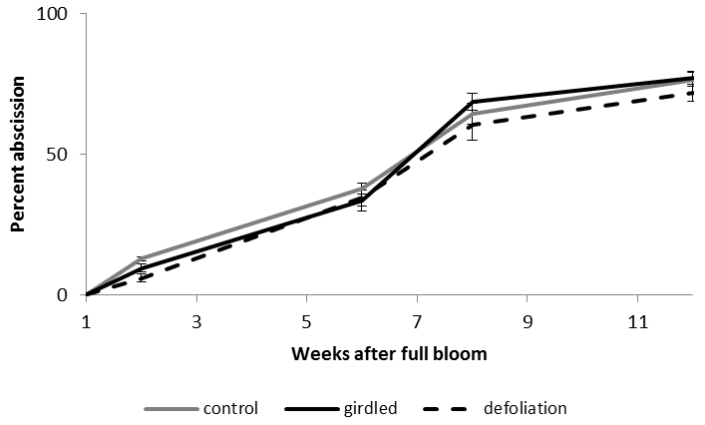
Fruit data were averaged for each tree and analysed assuming a completely randomized design with a repeated measures component performed separately for each variety. Analyses of variances and least squares means were calculated using SPSS statistical analysis software.

## Results and Discussion

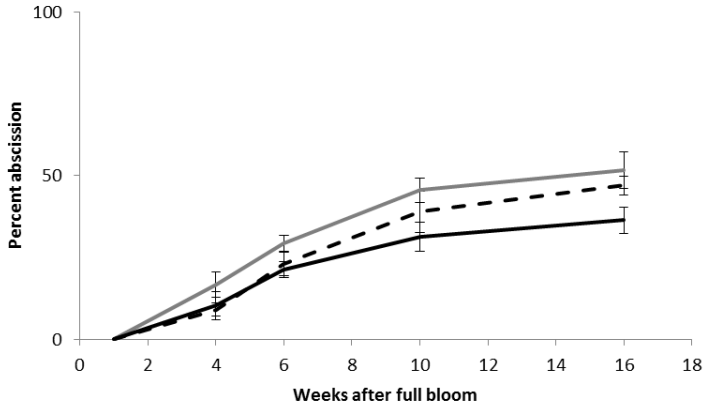
In the control treatment in all three years, Kordia displayed greater rates of fruitlet abscission than Lapins. Girdling reduced the rate of fruitlet abscission in Lapins, but not in Kordia as shown in Figure 1. In the 2013/14 and 2014/15 seasons Kordia displayed very high levels of abscission across all treatments, prompting and study into the flower biology of the variety.



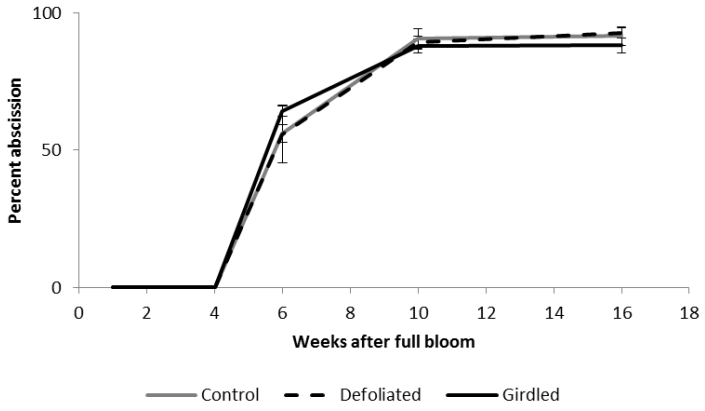

**1a.**




**1b.**

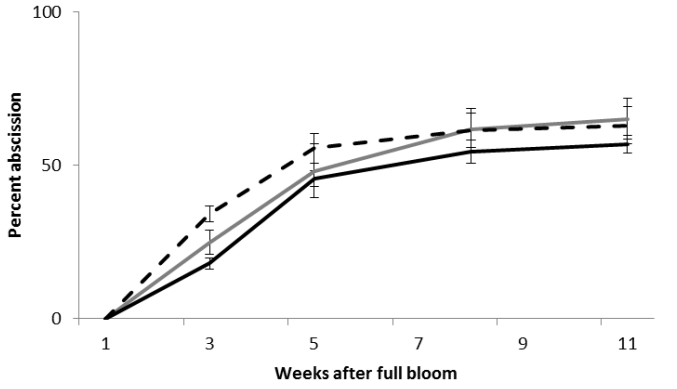



**2a.**

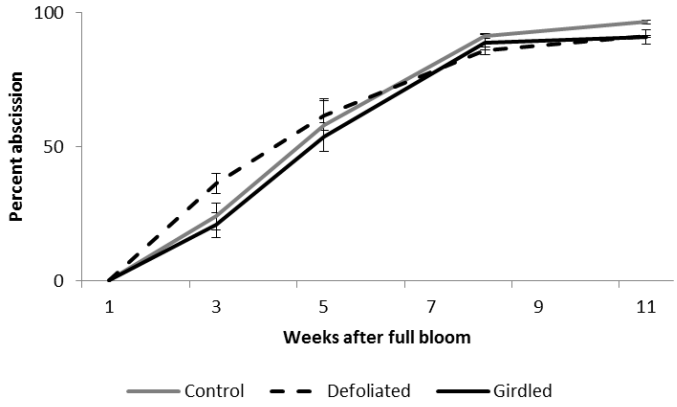



**2b.**

*Figure 1: Percent abscission for (a) Lapins and (b) Kordia in the (1) 2012/13 (2) 2013/14 and (3) 2014/15 seasons for control, defoliated and girdled trees. Error bars represent standard errors.*




**3a.**




**3b.**

It is possible that the sensitive period of early fruitlet development coincides with a period of active root growth and that the girdling disrupts the strong pull for carbohydrates by the roots. This is consistent with other studies (Iglesias et al., 2003) where increased carbohydrate availability to growing citrus fruitlets was associated with a decreased probability of abscission, resulting in a greater number of fruits at the end of the growing period. The difference in training systems and resulting tree architecture between Kordia and Lapins may partly explain why the effect was seen in one variety but not the other. It is possible that girdling earlier in the season for Kordia may result in a stronger impact on abscission rates, such as that seen in the study of Quentin et al. (2013).

Similar patterns in seasonal carbohydrate levels were seen for both varieties. Starch concentrations were relatively high at the time of flowering, and declined to its lowest point around the time of pit hardening, which was followed by a gradual increase leading up to the final measurement taken just after commercial harvest. The concentrations of carbohydrates were also similar across the three seasons, and did not mirror the large increase seen in the rate of abscission which was seen in Kordia in the latter two seasons.

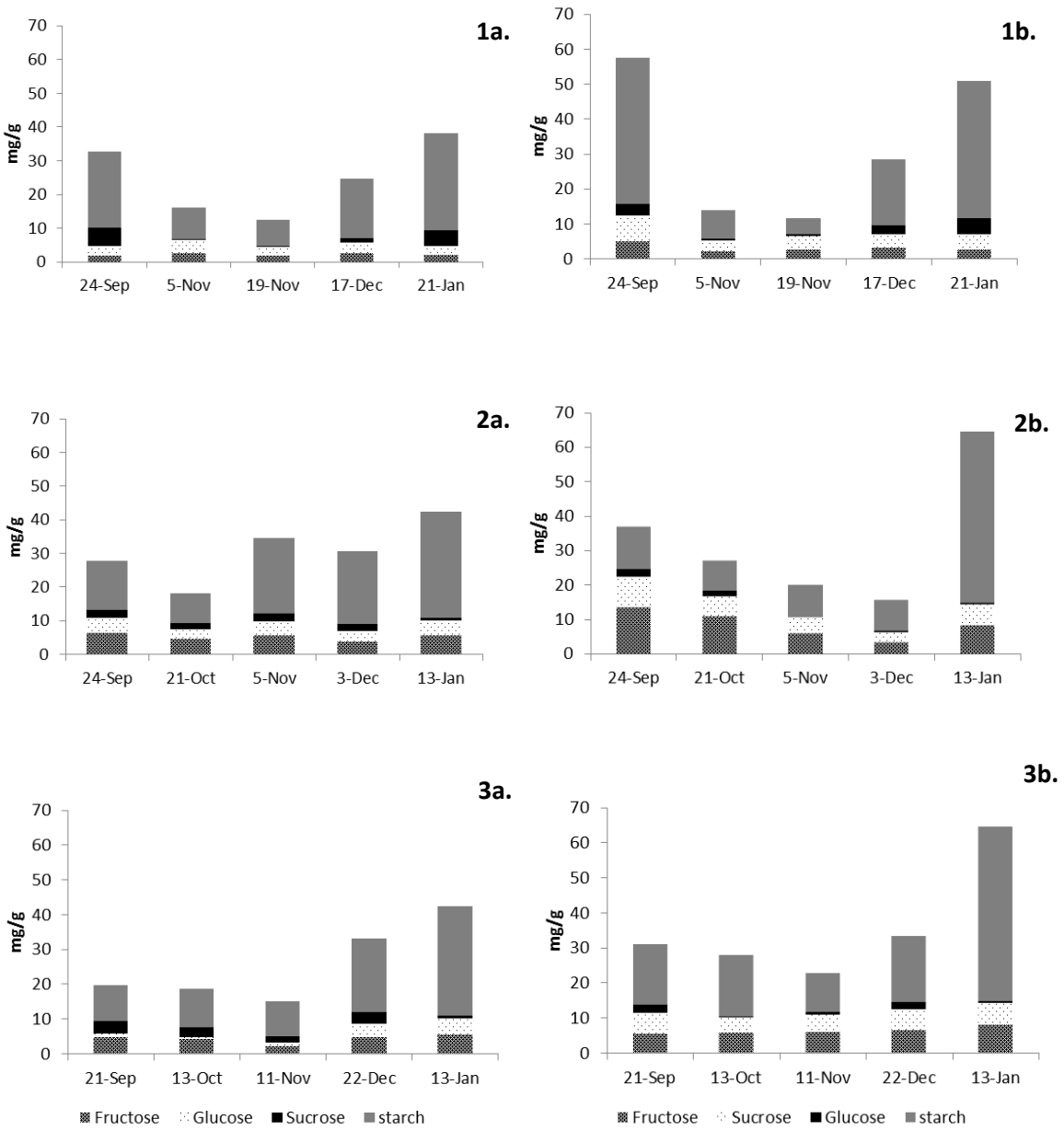



*Figure 2. Starch and soluble sugar concentration for (a) Lapin and (b) Kordia trunks in the (1) 2012/13 (2) 2013/14 and (3) 2014/15 season.*

The impact of trunk-girdling was clear for both varieties as carbohydrate flow from leaves to roots was restricted. Root carbohydrate concentration did not recover over the season, but instead remained low post-harvest, as illustrated in Fig. 3. Kordia displayed greater concentrations of carbohydrates in stem and trunk than Lapins; however, the total volume of wood was lower for Kordia due to the differences in tree structure, therefore the total available carbohydrates was lower for Kordia.

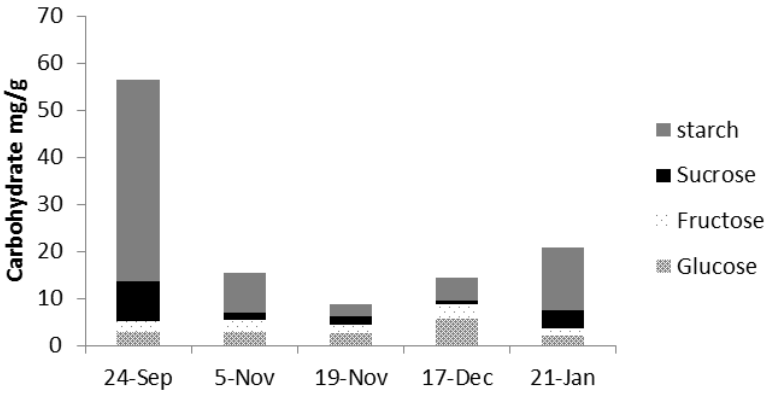



*Figure 3. Starch and soluble sugar concentration for Kordia Roots in the 2012/13 season, where the girdling treatment was imposed after the 5th November sampling*

Goldschmidt (1999) reported that treatments such as defoliation and girdling, in addition to altering the carbohydrate pool, also appear to modify different physiological processes such as hormone signalling, water relations and gas exchange. Other studies have demonstrated links between carbohydrate availability, hormones and abscission (Blanusa et al., 2005), polar auxin transport in sweet cherry (Iglesias et al., 2006) and ethylene and giberellins in citrus (Mahouachi et al., 2009). In the current study the impact of trunk girdling and limb defoliation could be seen in aspects of the resulting fruit quality.

For both Kordia and Lapins, defoliation resulted in fruit with significantly lower total soluble solid (TSS) concentrations in all three seasons (Fig. 4) and less mass than fruit from control and girdled trees (Tables 1 and 2). In Lapins, defoliation also resulted in smaller fruit in 2012/13, higher GÜSS flesh firmness measurements than the control and girdled treatments in all years, and less colour development in all years, emphasising the key importance of current-season photosynthate for developing optimal fruit quality.

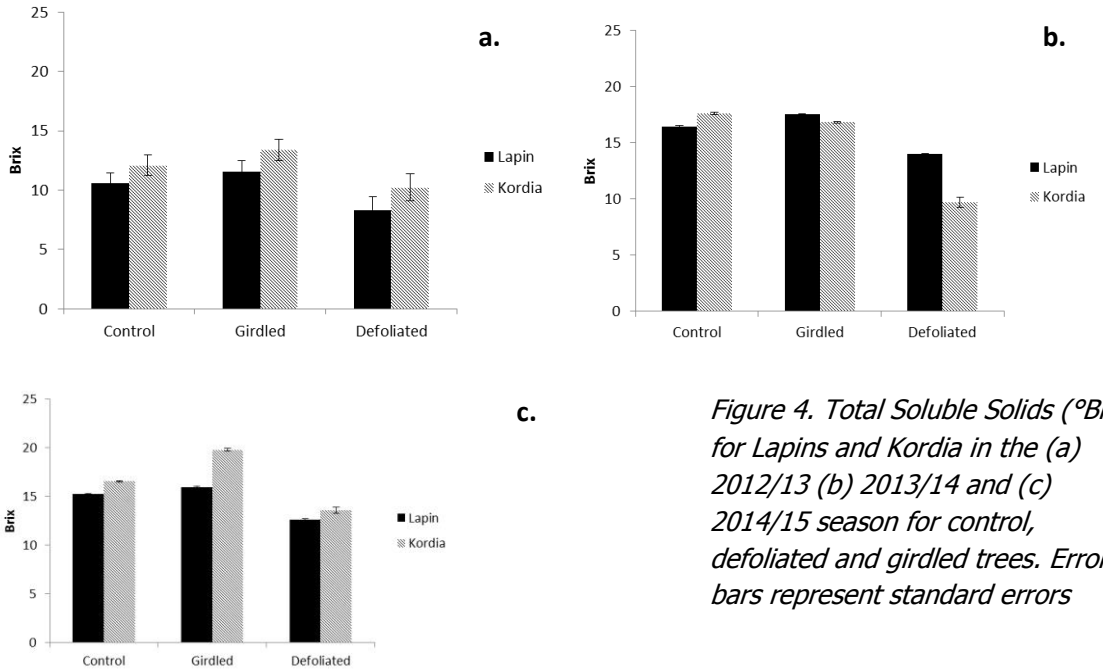



*Figure 4. Total Soluble Solids (°Brix) for Lapins and Kordia in the (a) 2012/13 (b) 2013/14 and (c) 2014/15 season for control, defoliated and girdled trees. Error bars represent standard errors*

*Table 1: Fruit quality measures for control, defoliated and girdled Lapins over the three seasons 2012/13, 2013/14 and 2014/15. Means within columns followed by different letters differ significantly at $p < 0.05$. Fruit mass, fruit diameter, stem pull, and flesh firmness data shown are means of 20 fruits for each of five replicates. TSS, TA, pH and colour data shown are means of 5 replicates.*

| | **Fruit Mass (g)** | | | **Fruit Diameter (mm)** | | | **Stem Pull Force (g)** | | | **Flesh Firmness (g/kg)** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2012/13 | 2013/14 | 2014/15 | 2012/13 | 2013/14 | 2014/15 | 2012/13 | 2013/14 | 2014/15 | 2012/13 | 2013/14 | 2014/15 |
| Control | 16.79$^{a}$ | 17.11$^{a}$ | 12.14$^{ab}$ | 33.38$^{b}$ | 28.20$^{a}$ | 29.47$^{a}$ | 648.08$^{a}$ | 592.76$^{a}$ | 813.46$^{a}$ | 0.092$^{a}$ | 0.09$^{a}$ | 0.12$^{a}$ |
| Defoliated | 13.79$^{b}$ | 12.59$^{b}$ | 11.31$^{b}$ | 31.03$^{a}$ | 30.09$^{a}$ | 28.63$^{a}$ | 861.53$^{b}$ | 784.17$^{b}$ | 802.86$^{b}$ | 0.11$^{b}$ | 0.11$^{b}$ | 0.10$^{b}$ |
| Girdled | 16.57$^{a}$ | 16.94$^{a}$ | 13.86$^{a}$ | 32.97$^{b}$ | 33.49$^{b}$ | 31.22$^{b}$ | 537.0$^{c}$ | 571.27$^{a}$ | 717.75$^{c}$ | 0.093$^{a}$ | 0.09$^{a}$ | 0.12$^{a}$ |

| | **TSS (°Brix)** | | | **TA (g/L)** | | | **pH** | | | **Colour** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2012/13 | 2013/14 | 2014/15 | 2012/13 | 2013/14 | 2014/15 | 2012/13 | 2013/14 | 2014/15 | 2012/13 | 2013/14 | 2014/15 |
| Control | 10.6$^{a}$ | 16.40$^{a}$ | 15.2$^{a}$ | 2.46 | 4.28 | 6.16 | 4.53 | 4.37 | 4.05 | 5.66$^{a}$ | 5.12$^{a}$ | 4.80$^{a}$ |
| Defoliated | 8.3$^{b}$ | 13.97$^{b}$ | 12.6$^{b}$ | 2.62 | 5.22 | 5.90 | 4.57 | 4.35 | 4.30 | 4.8$^{b}$ | 3.70$^{b}$ | 4.31$^{b}$ |
| Girdled | 11.6$^{a}$ | 17.50$^{a}$ | 15.9$^{a}$ | 2.52 | 4.29 | 5.35 | 4.2 | 4.40 | 3.98 | 5.68$^{a}$ | 5.60$^{a}$ | 4.74$^{a}$ |

*Table 2: Fruit quality measures for control, defoliated and girdled Kordia over the three seasons 2012/13, 2013/14 and 2014/15. Means within columns followed by different letters differ significantly at $p < 0.05$. Fruit mass, fruit diameter, stem pull, and flesh firmness data shown are means of 20 fruits for each of five replicates. TSS, TA, pH and colour data shown are means of 5 replicates.*

| | **Fruit Mass (g)** | | | **Fruit Diameter (mm)** | | | **Stem Pull Force (g)** | | | **Flesh Firmness (g/kg)** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2012/13 | 2013/14 | 2014/15 | 2012/13 | 2013/14 | 2014/15 | 2012/13 | 2013/14 | 2014/15 | 2012/13 | 2013/14 | 2014/15 |
| **Control** | 15.15[a] | 16.95[a] | 15.33[a] | 30.74[a] | 32.51[a] | 31.33[a] | 815.4 | 866.04[a] | 835.02[a] | 0.092 | 0.10 | 0.11 |
| **Defoliated** | 14.51[b] | 12.5[b] | 11.11[b] | 29.06[b] | 28.83[b] | 26.97[b] | 890.70 | 611.26[b] | 671.75[b] | 0.11 | 0.09 | 0.12 |
| **Girdled** | 15.15[a] | 16.25[a] | 15.11[a] | 30.59[a] | 32.57[a] | 30.74[a] | 827.44 | 836.34[a] | 736.63[c] | 0.093 | 0.09 | 0.17 |

| | **TSS (°Brix)** | | | **TA (g/L)** | | | **pH** | | | **Colour** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2012/13 | 2013/14 | 2014/15 | 2012/13 | 2013/14 | 2014/15 | 2012/13 | 2013/14 | 2014/15 | 2012/13 | 2013/14 | 2014/15 |
| **Control** | 12.08[a] | 17.58[a] | 16.54[a] | 3.81 | 5.93[a] | 6.04[a] | 4.01 | 4.16 | 4.05 | 6.0[a] | 5.88[a] | 5.44[a] |
| **Defoliated** | 10.23[b] | 9.7[b] | 13.54[b] | 3.61 | 3.91[b] | 5.90[b] | 3.61 | 4.11 | 4.30 | 5.9[b] | 5.72[b] | 4.08[b] |
| **Girdled** | 13. 38[a] | 16.8[a] | 19.76[c] | 3.55 | 6.42[c] | 7.13[c] | 4.42 | 3.85 | 3.98 | 6.0[a] | 5.94[a] | 5.78[a] |

Trunk girdling of Lapins in all three seasons impacted negatively on stem retention as seen by lower stem pull force measurements (in 2013/14 girdled fruit was not different from the control, but was different from fruit from defoliated limbs). Fruit from girdled trees was also larger in the 2013/14 and 2014/15 seasons.

As seen in Lapins, defoliation of limbs in Kordia resulted in decreased colour development in all years (Table 2). Fruit from defoliated limbs also resulted in smaller fruit in Kordia in all seasons, and both a lower titratable acidity (Table 2) and lower stem pull force in 2013/14 and 2014/15 (Fig. 5).

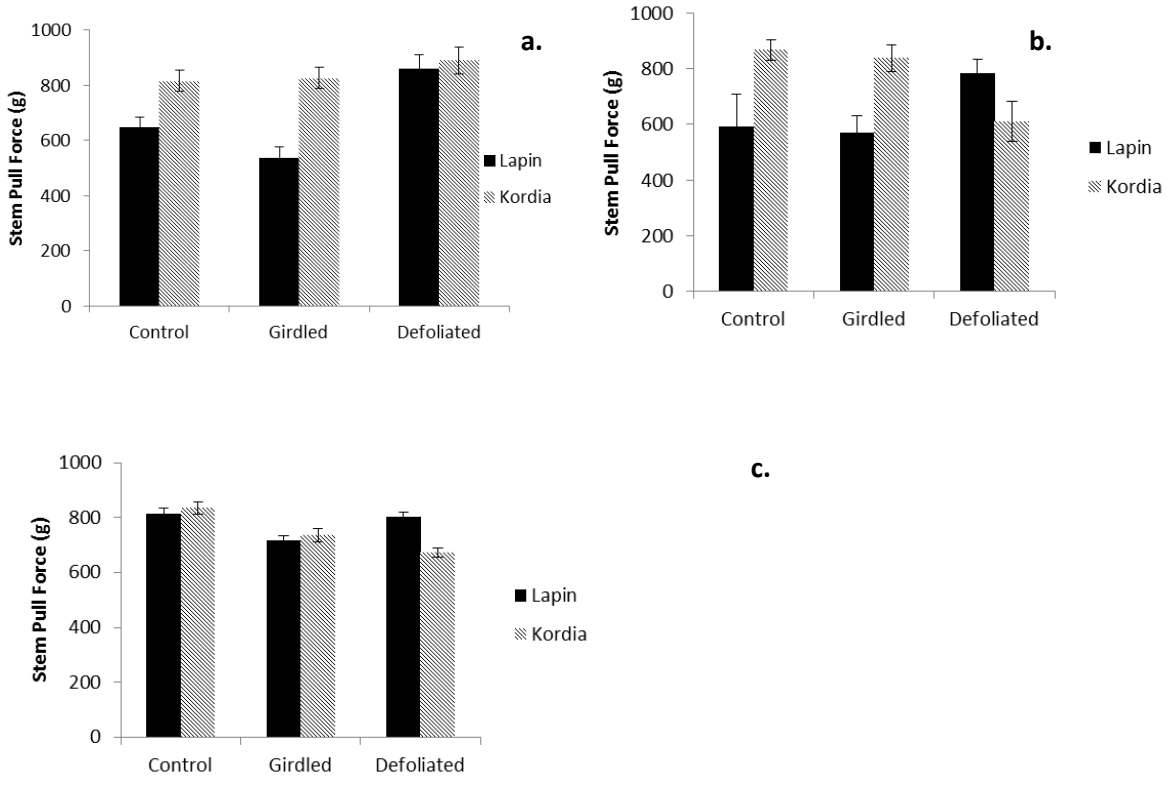


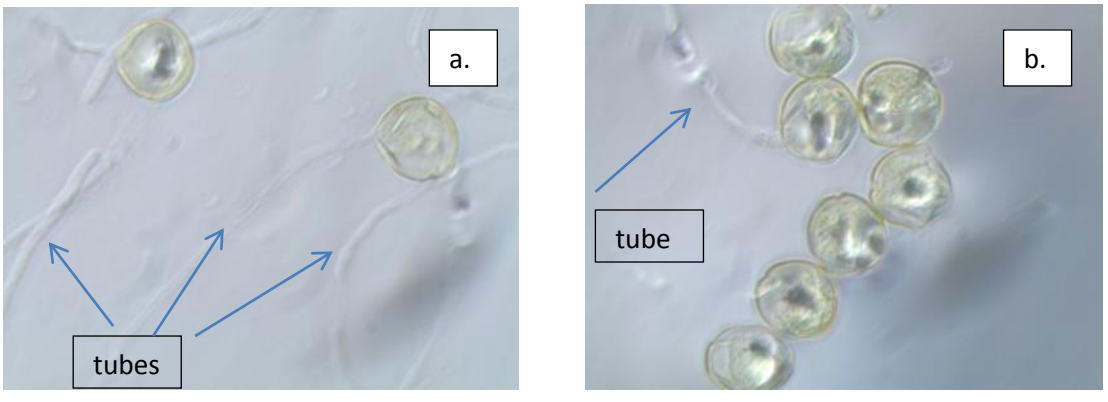

*Figure 5. Stem Pull Force (g) for Lapins and Kordia in the (a) 2012/13 (b) 2013/14 and (c) 2014/15 seasons for control, defoliated and girdled trees. Error bars represent standard errors.*

The one-season flower biology study indicated a large variability in maturity of abscised organs, including un-fertilised flowers, frost or cold-damaged fruitlets, fruitlets with a seed trace and a small number of fruitlets which had reached pit-hardening stage.

In Kordia, many fruitlets were seedless or had a seed trace, suggesting fruitset was a significant issue. Lapin is a self-pollinated variety and Kordia is pollinated by Regina and Sylvia, with Regina being planted in adjacent rows to Kordia at the trial site. Bloom synchronisation between Kordia and Regina appeared to be an issue with Regina trees reaching full-bloom after the majority of the Kordia trees.

*A selection of naturally abscised flowers and fruitlets showing the diversity of growth stages.*

Pollen viability percentages were 55 % for Lapins and 49 % for Regina. Lapins had a high pollen germination percentage (83 %) and very long tubes when grown in nutrient liquid agar. Regina had a high pollen germination percentage also (78 %), except after a rain event when pollen germination was reduced to (28 %). After the rain events, the number of pollen grains present on the stigma was also low in some cases. It was not possible to distinguish between the variety's own pollen and the pollen from its compatible cultivar, so further research is needed to determine the true impact of rain on fertilisation.




*Pollen grains germinating in vitro a) long tubes of Lapin pollen and b) a lack of tubes in Regina after a rain event.*

## Conclusions

The results of this trial indicate that carbohydrate availability can have an important effect on fruitlet abscission (and yield) and the resulting fruit quality. When transport of carbohydrates is interrupted via girdling, resulting in greater availability of carbohydrates to the developing fruitlets, fruitlet abscission can be reduced and the final fruit quality improved in high yielding Lapins. Conversely, fruit from defoliated limbs can result in inferior colour devlopment, lower TSS, and less mass all due to the lack of carbohydrate availability. Girdling in Lapins did appear to influence stem pull force negatively.

The discrepancy in the impact of the trunk girdling treatment on the two varieties may be explained by a distinct carbohydrate source:sink relationship in Kordia relative to Lapins. The tree structure whereby Kordia fruit is born on lateral shoots, and the total stored carbohydrate available is lower due to the smaller branch cross-sectional-area, may also have had an impact.

A key difference between the two varieties studied here is that Lapins are self-pollinated and Kordia requires pollination by other varieties including Regina and Sylvia. The higher proportion of abscised, unfertilised flowers in Kordia, as well as the lack of synchronisation of bloom time with the pollinator variety at the trial orchard, suggests that a lack of successful fruitset in Kordia may be a key driver of the low yields. This could also explain the lack of effect of trunk girdling in Kordia compared to Lapins, as the girdling treatment was applied 5 weeks after full-bloom in this study.

Future research should concentrate on the floral biology of Kordia and the pollinator varieties, with a focus on pollen viability, pollen tube growth, stigma receptivity and ovule fertilisation. The use of plant growth regulators (PGR's) to aid synchronisation of pollinators could be investigated, including the impacts of PGR's on fruitset, yield and the resulting fruit quality.

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# Appendix 4: The effect of carbohydrate availability on postharvest fruit quality in sweet cherry

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**Keywords:** *Prunus avium*, fruit firmness, girdling, defoliation, Tasmania, 'Lapins', 'Kordia'

## Abstract

Major export countries use a matrix of quality attributes of fruit at harvest to support decisions on the travel potential of key varieties. However there is uncertainty whether levels of firmness and soluble sugars at harvest are indicative of these attributes postharvest. The objective of this study was to determine the effects of manipulating tree carbohydrate availability through girdling or defoliation on fruit quality at harvest and postharvest. Trunk girdling and limb defoliation treatments were applied 5 weeks after full-bloom in trees of 'Kordia' and 'Lapins' varieties in order to manipulate carbohydrate supplies to fruit. Fruit were harvested at commercial maturity and analysed for fruit quality and chemistry parameters at harvest and after 28 days of postharvest storage.

Girdling in 'Kordia' resulted in fruit that were significantly firmer, higher in TSS, and larger in diameter which was further pronounced after postharvest storage. Defoliation in 'Kordia' resulted in softer fruit, lower TSS, and smaller in both mass and diameter. The 'Lapins' treatments often didn't respond in the same way that 'Kordia' did and thus the results of this study indicate a distinct carbohydrate source: sink relationship in 'Kordia' relative to 'Lapins', which may be due to differences in sink competition.

Therefore this study indicates that girdling can lead to increased carbohydrate availability resulting in higher quality fruit, particularly following post-harvest storage which could improve outcomes when exporting.

## Introduction

Carbohydrate partitioning is an important process in trees and depends on the strength of sinks and the availability of sources during the season. The growth rate of fruit (sink tissues) can be limited by an insufficient supply of photoassimilates from the source leaves (source-limited) or by the inability of the sink tissues to fully utilise the available photoassimilates from the source leaves (sink-limited) (Patrick, 1987; Atkinson et al., 2001). In cherries carbohydrates partitioned to fruit are mainly provided by root sourced carbohydrates initially, followed by leaves of reproductive and vegetative spurs and current season shoots (Ayala, 2004). Whilst the spatial and temporal relationships among carbohydrate sources (e.g., leaves and stored reserves) are not well understood in cherries (Whiting and Lang, 2004), Quentin et al. (2013) and Bound et al. (2013) determined that competitive sink limitation (girdling and thus removing the roots of the tree as a competitive sink) can lead to increased fruit set, decreased fruitlet abscission and improved quality characteristics in cherries.

It is essential to produce fruit of a high quality that attracts a premium price in export markets, given the high cost of production in Australia. As the majority of exported fruit is shipped overseas, postharvest fruit quality is a key component in the overall return to growers. It is generally considered that the production of large, firm fruit with high sugar levels have the best postharvest fruit quality. Given that fruit size and sugar levels are highly dependent on resource availability and translocation from tree reserves and current photosynthates in sweet cherries (Ayala and Lang, 2004), the objective of this study was to determine the effects of manipulating tree carbohydrate availability on fruit quality at harvest and after postharvest storage.

## Materials and Methodoloy

### *Trial design*

The carbohydrate manipulation trial was conducted at Hansen's Orchards in Huonville southern Tasmania, Australia. The trees were 16-year old 'Lapins' and 'Kordia' varieties grown on Mazzard F12-1 rootstocks, pruned to a Spanish Bush system. Row orientation was east-west with a tree spacing of 2m and row spacing of 4.25m. These varieties were chosen as 'Lapins' is self-fertile and high-fruit setting whereas 'Kordia' is prone to shedding and is low-fruit setting. Both are major export varieties grown in the region and the trees were subject to commercial orchard management practices in terms of irrigation, nutrient and pest management.

One row of each variety was selected in a block with five rows of trees as a buffer between each row, and each treatment row had 15 trees randomly allocated a carbohydrate treatment. These treatments included 5 control trees, 5 trunk girdled trees and 5 limb defoliated trees. A 2mm girdling knife was used to create a partial double girdle which has the bottom girdle overlapping where the top girdle ends creating a temporary interruption of the phloem. Girdles were spaced 10cm apart on the tree trunk and weren't re-opened again during the season.

One limb per treatment tree was defoliated and these limbs weren't defoliated again during the season. Girdling and defoliation treatments were applied 5 weeks after flowering as this is when they are most susceptible to shedding due to carbohydrate competition in the tree.

### *Fruit quality and chemistry analyses*

Fruit was harvested from the middle of each tree at commercial maturity with approximately 3kg of fruit picked from each control and girdled treatment, and all the fruit from the treatment limb of each defoliated treatment. Harvested fruit was taken to the laboratory where each treatment sample was randomly split in half, with one half analysed for fruit quality and chemistry measurements at harvest. The other half was placed into polyethylene bags which were sealed to maintain the ambient environment at close to 100% relative humidity and stored at 2 °C with no atmospheric control. These samples were analysed at 28 days postharvest.

On each date 10 fruit from each sample were randomly sampled and analysed for fruit diameter using digital Vernier callipers, fruit weight with stems on using an A&D GX-4000 digital balance, colour using the Australian Cherry Colour Chart and stem pull force was measured using a stand mounted Mark 10 force gauge to assess stem retention. Fruit firmness was also assessed using a Bio Works FirmTech II Fruit Firmness Tester. A GÜSS model GS-20 Fruit Texture Analyser fitted with a 2 mm penetrometer

probe, operating at a penetration speed of 10mm/second and a penetration depth of 4mm, was used to measure flesh firmness (on pared flesh) and skin puncture force. All fruit were allowed to warm to room temperature before analyses were undertaken to ensure that temperature effects were minimised.

A further 30 fruit from each sample were juiced collectively for total soluble solid (TSS), pH, and titratable acidity (TA). TSS concentration (°Brix) was assessed using an Atago PR-1 digital refractometer, and pH and TA were determined using a Metrohm 702 SM Titrino titrator.

### *Data analysis*

Cherry data were averaged for each tree and analysed separately for each variety assuming a completely randomized design with a repeated measures component. Analyses of variances and least squares means were calculated using proc mixed in SAS 9.3 statistical analysis software. Random effects were assigned to trees to allow for repeated measures. Multiple comparisons adjustments of least squares means were done by simulation using proc plm in SAS 9.3 statistical analysis software (Westfall et al., 1999).

## Results

### *Fruit Quality*

There were no significant differences in FirmTech fruit firmness at day 0, but at 28 days postharvest the girdled 'Lapins' treatment measured significantly firmer than the defoliated treatment ($p=0.0096$) (Fig. 1(a)). The 'Lapins' defoliated treatments decreased significantly ($p=0.0177$) in Firmtech firmness postharvest (Fig. 1(a)). At day 0 the defoliated 'Lapins' treatment had a significantly higher GÜSS flesh firmness measurement than the control ($p=0.0428$) and girdled ($p=0.0494$) treatments, and at day 28 the girdled 'Kordia' treatment flesh firmness was close-to-significantly firmer than the defoliated 'Kordia' treatment ($p=0.0513$) (Fig. 1(b)). The girdled 'Kordia' treatment had a significantly higher skin puncture force measurement than the defoliated treatments at day 0 ($p=0.0060$), and at 28 days postharvest the girdled treatment was significantly higher than both the control ($p=0.0346$) and defoliated treatments ($p=0.0013$). Although 'Lapins' treatments did not significantly differ in skin puncture force between treatments, both the control ($p=0.0079$) and girdled ($p=0.0081$) treatments did increase postharvest (Fig. 1(c)).

At day 0 the control 'Lapins' treatment fruit was significantly larger than the defoliated treatment ($p=0.0181$), but there were no significant differences between 'Kordia' treatments (Fig. 1(d)). However, at day 28 the girdled 'Kordia' fruit were significantly larger in diameter than both the control ($p=0.0040$) and defoliated ($p=0.0052$) treatments and there were no significant differences between 'Lapins' treatments (Fig. 1(d)). Also, whilst the control 'Kordia' ($p=0.0006$) and the control ($p=0.0004$) and girdled ($p=0.0056$) 'Lapins' treatments decreased significantly in diameter postharvest, the girdled and defoliated 'Kordia' and defoliated 'Lapins' treatments did not change significantly (Fig. 1(d)).

At day 0 the control 'Kordia' fruit was significantly heavier than the defoliated fruit ($p=0.0186$), but at day 28 the girdled fruit were significantly heavier than both the control ($p=0.0258$) and defoliated ($p=0.0157$) fruit which were now similar in mass (not shown). The 'Lapins' control and girdled fruit were both similar in mass but significantly heavier than the defoliated fruit at both dates (not shown). The control 'Kordia' and girdled and control 'Lapins' treatments all decreased in mass significantly

postharvest, whilst the girdled and defoliated 'Kordia' and defoliated 'Lapins' did not change significantly (not shown).

Overall there was a consistent decrease in stem pull over time, although the defoliated 'Lapins' fruit had a significantly higher stem pull than the girdled fruit at day 0 ($p=0.0129$). This dropped significantly postharvest ($p=0.0019$) with similar stem pull measurements to the control and girdled treatments (Fig. 2(a)).

There were no significant differences in colour measurements between treatments or dates (not shown).

***Fruit Chemistry***

Overall there was a consistent increase in TSS over time across both varieties and treatments. The girdled 'Kordia' treatment had a significantly higher TSS than the control ($p=0.0248$) and defoliated ($p=0.0446$) treatments at day 28 (Fig. 2(b)). The girdled ($p=0.0364$) and control ($p=0.0271$) 'Lapins' treatments had a significantly higher TSS than the defoliated treatment at day 28. Although TA results generally did not differ between treatments at day 0, at day 28 the defoliated 'Lapins' treatment was significantly higher compared to control ($p=0.0098$) and girdled ($p=0.0173$) treatments. Regardless of treatment TA was always higher in 'Kordia' than 'Lapins' (not shown).

## Discussion

The girdled 'Kordia' treatment had significantly higher skin puncture values and trended strongly towards higher flesh firmness values at both harvest and post-harvest assessments relative to controls. Furthermore, by day 28 the defoliated treatment was significantly lower in flesh firmness and skin puncture compared to the girdled treatment. This may indicate that the girdled treatment had greater flesh and skin structural strength, conferred through greater carbohydrate availability relative to the control and defoliated treatments. This speculation is consistent with the higher soluble sugar levels observed in the girdled Kordia treatment at day 28. Although there is no literature specific to this in sweet cherries, Saei et al. (2011) have reported that dry matter content positively relates to flesh firmness and soluble solids in apples. This was induced by higher carbohydrate availability to fruit via crop load manipulation and was especially well correlated at postharvest.

It is interesting that these trends were not observed in the same treatments in the 'Lapins' variety, although at day 28 when the girdled treatment FirmTech fruit firmness measured significantly firmer than the defoliated treatment. This may be due to different sink strength competition for carbohydrates between varieties, as referred to by Quentin et al. (2013) who found that the effect of girdling was less pronounced in 'Sylvia' than in 'Kordia', possibly related to a higher alternative (to fruit) sink demands in 'Sylvia' than in 'Kordia'.

There was a consistent increase in TSS over time observed in both varieties, and this was particularly clear in the girdled 'Kordia' treatment which was significantly higher in TSS at day 28 compared to the control and defoliated treatments. This may be due to the accumulation of carbohydrates above the girdle which were then sequestered for fruit development due to the strong sink strength, resulting in more mature fruit (Roper and Williams, 1989; Urban et al., 2004). This is believed to result in greater levels of hemicelluloses and pectins that are speculated to underpin the development of TSS in cherries postharvest.

TA was consistently higher in 'Kordia' irrespective of treatment, and this could be why 'Kordia' is considered by industry to be a superior export variety relative to 'Lapins'.

There was a consistent decrease in stem pull over time in both varieties, and this has been observed in other studies (unpublished) in which 5 orchards had a linear decrease in stem pull of approximately 9.5% every 10 days in postharvest storage irrespective of starting values, which varied considerably with cooler sites generally producing fruit of higher stem pull force. The defoliated 'Lapins' had a significantly higher stem pull at day 0 and significantly higher TA at day 28 which may indicate it's immaturity relative to other treatments.

The girdled 'Kordia' treatment lost significantly less in mass between day 0 and day 28 as well as being significantly larger in diameter and heavier in mass relative to the control and defoliated treatments. Fruit weight has been shown to consistently decrease with postharvest storage and induces drying and browning of peduncles (Drake & Elfving, 2002; Alique et al., 2006). These results show that the girdled treatment maintains a similar diameter and weight during postharvest, indicating that the fruit is dehydrating less than the other treatments. The girdled fruit were also significantly higher in TSS and therefore will be slower to lose water than smaller fruits which have less sugar (Kupferman, 1986). Conversely, the control and girdled 'Lapins' decreased in mass by approximately 2 grams each during the postharvest period.

## Conculsions

The results of this trial indicate that carbohydrate availability has an important effect on fruit quality postharvest. Girdling in 'Kordia' resulted in fruit that had significantly better firmness properties, higher TSS, and significantly larger in diameter and mass all of which are desirable for export. This was particularly evident after postharvest storage and suggests that girdling could lead to higher quality fruit for export.

Conversely, the defoliated 'Kordia' resulted in softer fruit, lower TSS, and was smaller in both mass and diameter. Therefore this study indicates increased carbohydrate availability results in higher quality fruit, particularly for export.

However, the 'Lapins' treatments often didn't respond in the same way that 'Kordia' did and thus the results of this study indicate a distinct carbohydrate source: sink relationship in 'Kordia' relative to 'Lapins', which may be due to differences in sink competition. Future research could further evaluate the effects of carbohydrate manipulation over several seasons and between different varieties.

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**Figures**

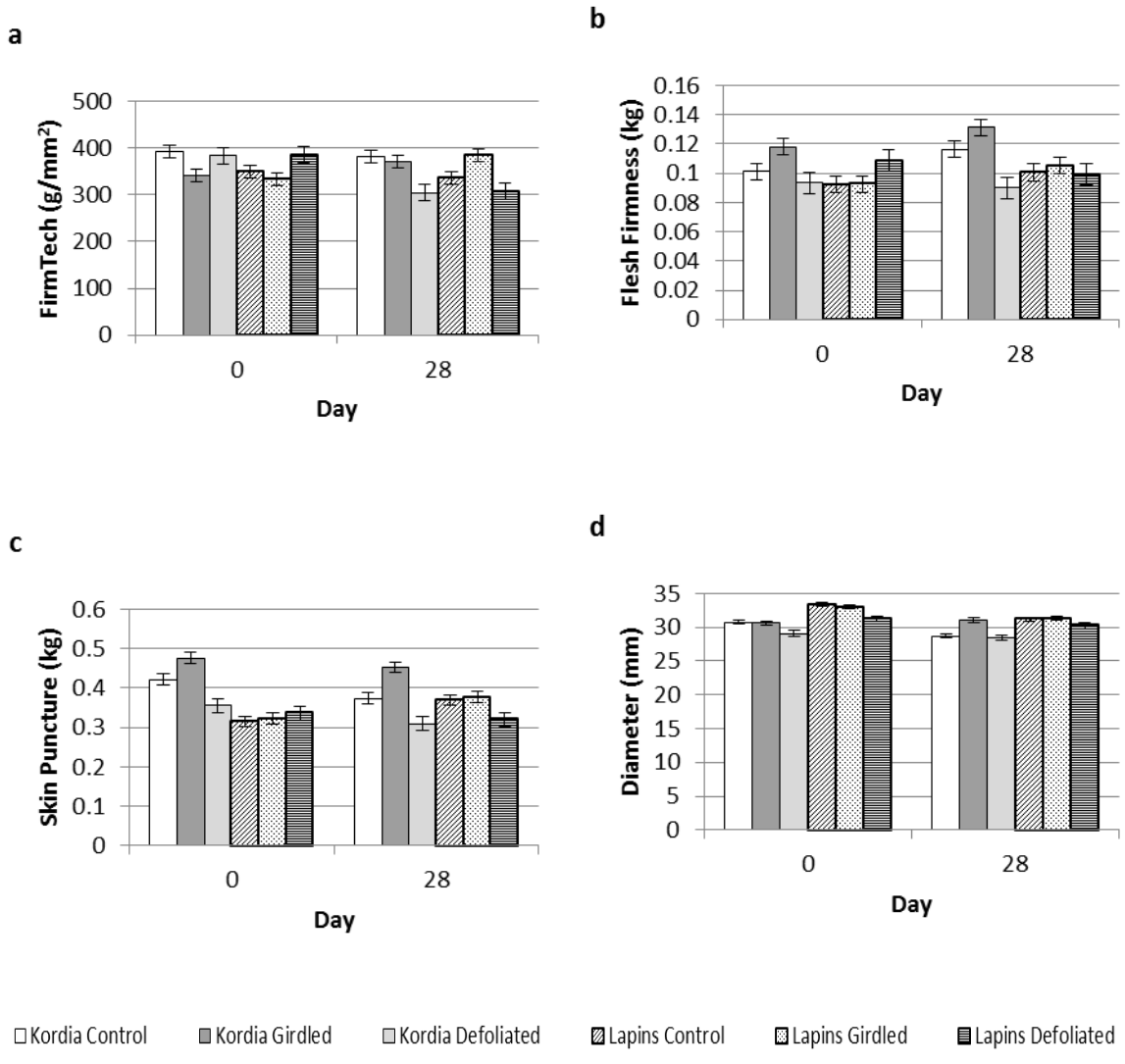


*Figure 1: Differences in measurements in control, girdled and defoliated treatments in 'Kordia' and 'Lapins' at day 0 (harvest) and day 28 (postharvest). Error bars represent one standard error of the mean. Shown are measurements of (a) FirmTech fruit firmness; (b) flesh firmness; (c) skin puncture force; (d) fruit diameter.*

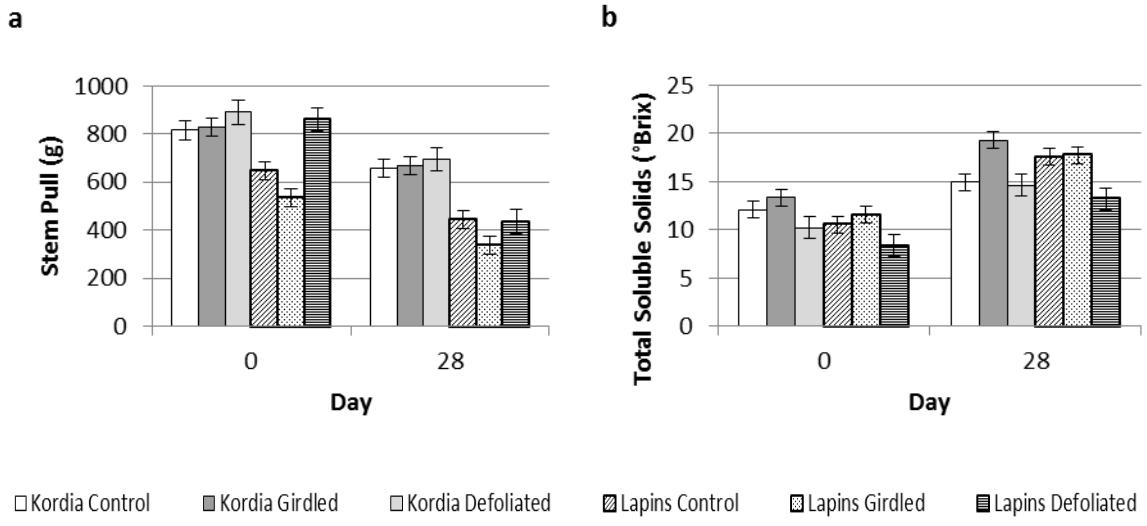


*Figure 2: Differences in measurements in control, girdled and defoliated treatments in 'Kordia' and 'Lapins' at day 0 (harvest) and day 28 (postharvest). Error bars represent one standard error of the mean. Shown are measurements of (a) stem pull; (b) total soluble sugars (TSS).*

# Appendix 5: Optimising sweet cherry fruit nutrition and quality through fertigation and foliar applied fertilisers

***Dr Nigel Swarts and Assoc. Prof. Dugald Close, Tasmanian Institute of Agriculture, University of Tasmania***

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## Executive Summary

Nutrient management is a critical component of commercial sweet cherry orchard production as each nutrient plays an important role in the development and quality of sweet cherry fruit. Whilst fertigation is commonly practised by cherry growers in Australia, research into optimal nutrient supply to meet tree demands is limited and the effects of oversupply of pre-harvest nutrition on fruit quality are virtually unknown. Furthermore, even less is known about the influence of micro-element nutrition on sweet cherry fruit quality and which is the best method of application. This study investigated the role of Nitrogen (N) fertigation on sweet cherry fruit quality and post-harvest shelf-life whilst at the same time shed light on the role of pre-harvest micro-element nutrition on fruit quality with an emphasis on Potassium (K).

This report summarises data from five separate trials conducted over a four year period. The research commenced originally at Grove Research Station in the Huon Valley region of southern Tasmania where N fertigation and micro-element trials were established. Changes in orchard management jeopardised the reliability of ongoing trials at this orchard. Therefore new integrated N fertiliser and irrigation trials were established at Ridgy Didge Cherries, a commercial orchard in the same region. The materials and methods and results sections are split into two parts accordingly.

Nitrogen fertigation results showed that N content in sweet cherry fruit could be manipulated by fertigation treatments when base levels of N were suitably low. In this case, when high levels of N were applied, detrimental effects on fruit firmness were observed. However, when base levels of fruit N matched commercial standards, high levels of applied N only slightly increased fruit N content but had no detrimental influence on fruit quality at harvest or after 28 days in storage. Irrigation treatments had little effects on fruit quality other than increase cherry size with increased volume.

Pre-harvest foliar micro-element sprays were more effective in increasing respective nutrient content in leaves and fruit than fertigated treatments of the same elements. Yet limited influence of micro-element fertilisers were observed on fruit quality at harvest or after 28 days in storage. Pre-harvest K treatments increased fruit firmness at harvest and in stored fruit and again foliar applied K was the more effective fertiliser.

Recommendations for the management of N fertiliser and micro-element nutrition are described.

## Background

Deciduous fruit trees accumulate and store nutrients at the end of the growing season for remobilisation in the following spring (Loescher et al., 1990). This resource remobilisation is critical for growth of flowers, fruit, leaves and shoots, yet little is known about seasonal nutrient budgets and the storage and remobilisation of nutrients (Frak et al., 2006). This is particularly true in a region such as southern Australia where limited research into the seasonal, soil and cultivar implications on nutrient requirements of sweet cherry has been completed. However, principles gleaned research from other regions can be used to guide nutrient management and identify region specific research questions.

In highly studied macro-nutrients such as Nitrogen (N), it is well recognised that increasing the rate of N application can increase vegetative growth and yield but adversely affect fruit quality by decreasing fruit colour and firmness (Oberly and Boynton, 1966, Neilsen et al., 2003, Fallahi et al., 1993). James (2011) suggested that applying too much before harvest can cause uneven ripping, delay ripening and reduce fruit shelf-life. In addition, studies have shown that the efficacy of N application in orchards is related to

irrigation practice as excess water can leach N below the root zone (Neilsen and Neilsen, 2002) while soil-water stress and may reduce the tree's capacity for nutrient uptake. Therefore, the regulation of N and water is a crucial management consideration for commercial orchard production. The effectiveness of matching nutrient supply with tree demand requires a sophisticated understanding of seasonal cherry tree N recycling to maximize the advantages inherent in being able to apply N and water simultaneously. Fertigation and foliar nutrient application are important tools in the management of cherry nutrition and provide a more precise solution to meeting tree nutrient demand.

Precision farming through fertigation can facilitate efficient utilization of resources and improve returns per unit area and time to growers. Fertigation delivers both water and essential nutrients such as N directly to the active root zone of growing crops through micro irrigation systems, thereby minimising water and nutrient loss and improving productivity (Klein et al., 1989). Whilst fertigation is commonly practised by cherry growers in Australia, research and management guidelines for optimal supply of tree nutrient and water requirements are limited.

Foliar application of nutrients in tree fruit crops is becoming an increasingly popular fertiliser management strategy. Foliar applied fertilisers are now proven in their ability to correct nutrient deficiencies and to place required nutrients that are otherwise immobile in the desired location for efficient uptake. In the Australian cherry industry supplementary crop nutrient requirements are met often using foliar fertiliser sprays. Fruit responses to foliar applications are readily observed and for many cultivars, ideal elemental concentrations for optimum production are well established. Yet, when deficiencies are observed, there is limited understanding of optimum rates and timing to correct them. This is due to the variation in growing region and environmental characteristics which will influence the rate and efficiency of uptake. In addition, the ontology of fruit development influenced heavily by cultivar, latitude, altitude and soil type will add further uncertainty to the timing of foliar applied elements.

This study investigated the role of Nitrogen (N) fertigation on sweet cherry fruit quality and post-harvest shelf-life whilst at the same time shed light on the role of pre-harvest microelement nutrition on fruit quality with an emphasis on Potassium (K). In this study we asked the following questions

- Does increased nitrogen application increase nutrient content in fruit, leaves and storage organs of sweet cherry trees?
- Does increased nitrogen application have a negative effect on fruit quality?
- Does irrigation quantity influence the nitrogen content in leaves and fruit and influence fruit quality?
- Does foliar application of micro-elements increase nutrient content in fruit more effectively than fertigation?
- Does increased micronutrient application improve fruit quality?

## Materials and Methods

### Part 1a. Nitrogen Fertigation Grove Research Station

#### *Trial Establishment*

The fertigation trial was conducted at Grove Research Station in southern Tasmania on 10-year-old 'Lapins' trees, grown on F12-1 rootstock, pruned to a Spanish bush training system with tree spacing ranging from 1m to 2m and row spacing of 4.5m. The variety was chosen to represent a commonly grown variety in the region and trees were subjected to standard orchard management with respect to irrigation, fertilisation and pest management. Row orientation was north-south with a very gentle slope downwards from the southern end of the row. Nitrogen application was ceased by the orchard manager in the experimental rows during the trial period and all other commercial orchard management continued.

*Figure 1. Establishment of Nitrogen fertigation trials at Grove Research Station*

The experimental row was split into five blocks of 12 trees, buffered by three trees between each block. Each block was considered a replicate and further split into four sub blocks of three trees to which N treatments were randomly allocated. To deliver the N treatments, four new 13mm polypipe lines were installed down the row, one for each treatment and 4L/hr pressure compensated drippers were fixed to the lines at approximately 15cm from the base of the treatment tree on the upward side of the slope. Nitrogen was applied as fertiliser grade $Ca(NO_3)_2$ mixed with water in a 44gn drum header tank and delivered via a fire-fighting pump at constant 10psi. Fertiliser application followed standard fertigation practise in the region with a 10min water only wetting up period, followed by a 40min application of N treatments and completed with a 10min water only rinse. To assess the influence of pre-harvest fertigation on cherry fruit quality and post-harvest storage, N treatments included a 0N control, 25g N/tree, 50g N/tree and 75g N/tree split into four weekly applications commencing approximately one month after bud burst when root uptake of N is considered to have replaced remobilised N as its dominant N source (Grassi et al., 2003).

#### *Soil Analysis*

Soil conditions and chemistry of the site were obtained by sampling three locations evenly spaced along the length of the row. At each sampling location, five holes were dug along the treeline using a hand auger at 0 - 20cm, 20 – 40cm and 40 - 60cm depths. Soils from the same depths were pooled and a subsample removed for air-dying and sieving to 2mm in the laboratory. Soil samples were sent to a commercial laboratory for chemistry analysis (Table 1).

*Table 1: Soil attributes at Grove Research Station averaged across three sampling locations*

| Depth | Ammonium Nitrogen | Nitrate Nitrogen | Phosphorus Colwell | Potassium Colwell | Sulphur | Organic Carbon | Conductivity | |
|---|---|---|---|---|---|---|---|---|
| cm | mg/Kg | mg/Kg | mg/Kg | mg/Kg | mg/Kg | % | dS/m | pH |
| 0-20 | 4 | 12.7 | 127.7 | 218.0 | 5.2 | 2.8 | 0.1 | 6.9 |
| 20-40 | 5 | 6.0 | 65.7 | 172.3 | 6.3 | 3.1 | 0.1 | 6.7 |
| 40-60 | 2 | 3.0 | 36.0 | 57.7 | 6.9 | 0.7 | 0.0 | 6.4 |

### *Fruit Quality Analysis*

Fruit was harvested from the middle tree of the treatment sub-block coordinated with commercial harvest for the orchard. Approximately 3kg of fruit from all parts of the tree were picked and five trees were randomly selected from the trial for where all fruit were picked to estimate crop yield for the row based on weight. Harvested fruit was returned to the laboratory, weighed and sorted as either marketable or otherwise (cracked, rotted). For at-harvest fruit quality assessments, a subsample of 25 blemish-free was taken from each replicate and another 20 fruit from each replicate were sent to a commercial laboratory for chemistry analysis. To investigate post-harvest fruit quality, two further subsamples of 25 blemish-free fruit were taken from each replicate and placed into polyethylene bags. Bags were sealed to maintain the ambient environment at close to 100% relative humidity and stored at 2°C with no atmospheric control. Fruit quality assessments using these fruit were completed at 25 and 50 days post-harvest. At the time of harvest, leaves were also sampled, dried at $60^0$C and sent to a commercial laboratory for chemistry analysis.

Fruit quality parameters measured only at harvest included fruit diameter using digital vernier calipers and fruit weight, recorded using an AND digital balance (model GX-4000). At all assessment dates, skin colour, flesh firmness (on pared flesh) and skin puncture force and stem retention were measured following methods described by Bound et al (2013). Total soluble solids (TSS), pH and titratable acidity (TA) were also measured for each assessment date using juice collected from crushing fruit from each replicate following Bound et al., (2013).

### *Data Analysis*

Fruit quality assessment data was analysed using a one way ANOVA in SPSS with N treatments considered a fixed factor with, block (replicate) considered as a random factor and with each fruit quality parameter considered as the dependant variable. *Post hoc* tests were computed using Tukey's Honestly Significant Difference (HSD) or Fishers Protected Least Significant Difference (LSD). Significance was calculated at P = 0.05 and standard error used for comparison of mean values in the tables and figures. No data transformations were necessary.

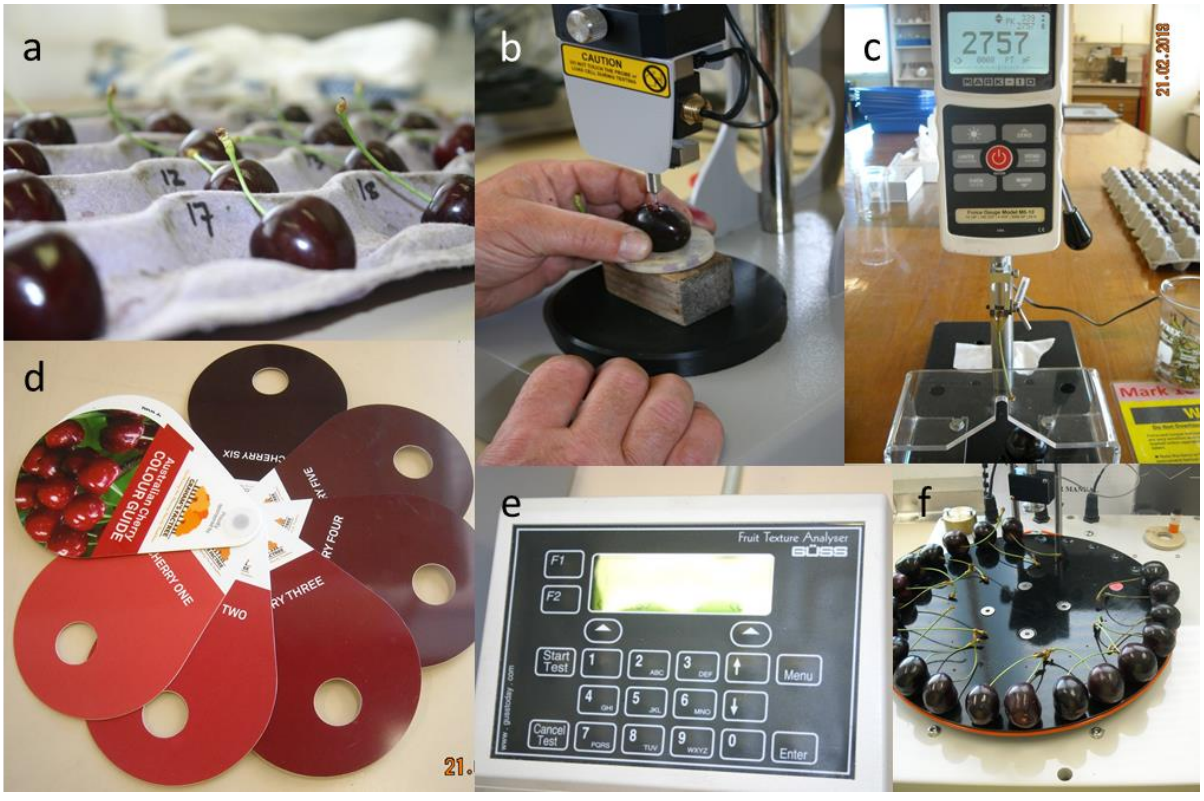


*Figure 2. Assessment of fruit quality using the (Photographs by Justin Direen). a) Cherry trays, b) GUSS Fruit Texture Analyser; c) Stem Pull; d) Cherry colour chart; e) GUSS digital reader and f) Firmtek Machine.*

**Part 1b. Micro-element Fertigation Trial**

***Trial Establishment***

This trial was conducted at Grove Research Station in southern Tasmania on 10-year-old 'Lapins' trees on F12-1 rootstock. Orchard rows were established north-south on a gentle slope downwards towards the northern end of the row. Trees were pruned to a Spanish Bush training system with tree spacing approximately 1.5m. Trees were subjected to standard orchard management with respect to irrigation and pest management. Nitrogen and micronutrient application was ceased by the orchard manager in the experimental rows during the trial period.

Treatments were applied down one experimental row split into four blocks of twelve trees with each block constituting a replicate. Each block was further split into four sub-blocks of three trees to which four treatments were randomly allocated. Of the three trees, the two outside trees although receiving the allocated treatment were guard trees with the centre tree sampled for fruit quality and chemistry. Treatments included Potassium Sulphate (50kg/K/ha), Calcium carboxylate (30kg/Ca/ha), $Zm^2$ (20L/ha) and zero fertiliser water only control. Treatments were applied weekly for three weeks commencing in late November 2011.

To deliver the four micronutrient treatments, four new 13mm polypipe lines were installed down the row and 4L/hr pressure compensated drippers were fixed to the lines at approximately 15cm from the base of the treatment tree on the upward side of the slope. The required amount of each chemical was in turn, mixed with approximately 40L of water in a 44gn drum header tank and delivered via a fire-

fighting pump at constant 10psi. Fertiliser application followed standard fertigation practise in the region with a 10min water only wetting up period, followed by a 40min application of fertiliser treatments and completed with a 10min water only rinse.

### *Fruit Quality Analysis*

Fruit was harvested at commercial harvest and quality was assessed as described above at harvest, 25 and 50 days post-harvest. Fruit was stored in sealed plastic bags at $2^0$C with no modified atmosphere conditions. A sub sample of harvested fruit and leaves were sent to a commercial laboratory for chemistry analysis

### *Data Analysis*

Analysis of fruit quality and nutrient composition was completed using ANOVA in SPSS. Each quality parameter or nutrient was considered as a dependent factor with treatment considered as the dependent factor. *Post hoc* tests were computed using Tukey's Honestly Significant Difference (HSD) or Fishers Protected Least Significant Difference (LSD).

## Part 1c. Micro-element Foliar Spray Trial

### *Trial Establishment*

This trial was established at two orchards in the Huon Valley ('Grove Research Station' and 'Woodstock') in southern Tasmania (Table 2).

*Table 2. Design characteristics of orchards used in spray trials.*

| | **Grove** | **Woodstock** |
|---|---|---|
| Orchard age (years) | 14 | 10 |
| Planting density (trees/ha) | 1500 | 3000 |
| Planting distance | 1.5 m | 0.75 m |
| Pruning system | V axis | Half V trellis |
| Rootstock | M-12 | Colt |

At both orchards treatments were applied down one experimental row with each row split into three blocks. Each block consisted of five sub-blocks of three and six trees at Grove and Woodstock respectively, to which the treatments were applied with two border trees separating treatment trees. The treatments were randomly ordered within the blocks. Foliar spray treatments of Ca, Mn, Zn and $Zm^2$ and water were applied on both sides of the rows (see Table 3, Fig. 3). All treatments were applied using a motorized air blower backpack spray unit, following manufacturer's recommendations. Foliar sprays were applied in a fine mist covering leafs to dripping point. The foliar sprays were applied in December 2012, in the morning, two weeks apart with the final application approximately three weeks before harvest. In both orchards, micro-element application was ceased for the duration of this trial in the experimental row, however all other commercial orchard management practices continued.

*Figure 3. Chemicals used in the micronutrient foliar spray trial*

*Table 3. Micronutrient products and rates used in spray trials*

| Micro-nutrient | Industry name | Analysis | Manufacturer | Recommended application | Applied quantity |
|---|---|---|---|---|---|
| Ca | Pitstop | 17%w/v Ca | Agrichem | 5-10l per ha | 6ml/tree |
| Mn | Manni-Plex Mn | 6%w/v Mn; 2.8%w/v N as Nitrate; 5.6%w/v N as Urea | Barmac | 2-6L per ha | 6ml/tree |
| Zn | Manni-Plex Zn | 8.8%w/v Zn; 2.8% w/v N as Urea; 3.6%w/v N as Nitrate | Barmac | 2-6L per ha | 6ml/tree |
| Zn, Mn, Mg, Fe, S | Zm2 | 2.3%w/v Zn; 2.3%w/v Mn; 2.3%w/v Magnesium; 2% Fe; 5% Sulfur | Stoller's | 7L per ha | 6ml/tree |

### *Fruit Quality Analysis*

Fruit was harvested at commercial harvest and quality was assessed as described above at harvest, 25 and 50 days post-harvest. Fruit was stored in sealed plastic bags at $2^0$C with no modified atmosphere conditions. A sub sample of harvested fruit and leaves were sent to a commercial laboratory for chemistry analysis.

### *Data Analysis*

Analysis of fruit quality and nutrient composition was completed using ANOVA in SPSS. Each quality parameter or nutrient was considered as a dependent factor with treatment considered as the dependent factor. *Post hoc* tests were computed using Tukey's Honestly Significant Difference (HSD) or Fishers Protected Least Significant Difference (LSD).

## Part 2a. Nitrogen Fertigation Ridgy Didge Cherries

### *Trial Establishment*

Fertigation/irrigation trials were established in February 2013 at Ridgy Didge Cherries in Lower Longley, in southern Tasmania. The trial was conducted on 12-year-old 'Simone' trees, grown on Colt rootstock, pruned to a Spanish bush training system with tree spacing of 2m and row spacing of 4.5m. Row orientation was north west-south east with a considerable slope downwards from the northern end of the row. Nitrogen application was ceased by the grower in the experimental rows during the trial period and all other commercial orchard management continued.

The trial was implemented along four rows, each row represented one replicate and were split into three blocks of 12 trees. Each block was randomly allocated to a high (3.9L/hr), medium (2.3) or low (1.6L/hr) drip irrigation treatment where the grower's existing irrigation infrastructure was modified to deliver the required irrigation rate. Irrigation was controlled by the grower and commenced in mid-October (mid-spring) and continued until late March (mid-autumn). Flow meters installed in the irrigation lines recorded the date and duration of each irrigation application.

Each block of 12 trees were further split into four blocks of three trees to which N treatments were randomly allocated. To deliver the N, new 13mm polypipe irrigation lines were installed down each row, one for each treatment and 4L/hr pressure compensated drippers were fixed to the lines at approximately 15 cm from the base of the treatment tree on the upwards side of the slope. Nitrogen was applied as fertiliser grade $Ca(NO_3)_2$ mixed with water in a 44gallon drum header tank and delivered via a firefighting pump at constant 10psi. Pre- and post-harvest N treatments included a 0N control, 1/3N (40kgN/ha; grower's standard rate), 2/3N (80kgN/ha) and 1N (120kgN/ha) split into four weekly applications commencing in mid February 2013. Treatments in this study were applied February 2013 (post-harvest) and November 2013 (pre-harvest) before the February 2014 harvest; and February 2014 (post-harvest) and November 2014 (pre-harvest) before the February 2015 harvest.

### *Soil Analysis*

Soil was sampled along the treeline of three experimental rows at locations receiving the highest N and zero N treatments in November 2013 and July 2014. In November 2013, soil was also sampled along the inter-row of the experimental rows at the grower's standard N fertiliser rate (Table 2).

*Table 2. Nitrate N, Total N and Total C percentage for soil sampled from various locations of the experimental rows in November 2013 and July 2014.*

| | | | | Nov-13 | | | Jul-14 | |
|---|---|---|---|---|---|---|---|---|
| | | | Depth | Nitrate N (mg/Kg) | Total N (%) | Total C (%) | Total N (%) | Total C (%) |
| Row 1 | High N | Treeline | 0-10cm | 4.51 | 0.23 | 3.5 | 0.19 | 2.68 |
| | | | 30cm | 2.81 | | | | |
| | | | 60cm | 1.28 | | | | |
| | Zero N | Treeline | 0-10cm | | | | 0.22 | 3.04 |
| | Standard N | Inter-row | 0-10cm | 3.30 | 0.27 | 3.67 | | |
| Row 2 | High N | Treeline | 0-10cm | 3.73 | 0.22 | 3.24 | 0.2 | 2.88 |
| | | | 30cm | 3.85 | | | | |
| | | | 60cm | 1.24 | | | | |
| | Zero N | | 0-10cm | | | | 0.23 | 3.36 |
| | Standard N | Inter-row | 0-10cm | 3.49 | 0.28 | 4.32 | | |
| Row 3 | High N | Treeline | 0-10cm | 4.56 | 0.24 | 3.31 | 0.25 | 3.53 |
| | | | 30cm | 3.72 | | | | |
| | | | 60cm | 2.33 | | | | |
| | Zero N | | 0-10cm | | | | 0.21 | 2.86 |
| | Standard N | Inter-row | 0-10cm | 2.51 | 0.3 | 4.43 | | |

All 0 – 10cm samples were collected using a 'pogo stick' where four soil cores were sampled approximately 20cm apart and pooled to form a sample Soil sampled from 30 and 60cm depths were collected using a hand auger and a measuring tape to determine depth. For each soil sample, a subsample was returned to the laboratory and air dried, gently disaggregated in a mortar and pestle and sieved to 2mm. Approximately eight grams of dried soil was placed in a 50ml centrifuge tube and roller extracted for 18 hours with 40ml of 2mol KCl . The tubes were centrifuged at 3000 rpm for 10 min and a 10ml subsample was filtered through a 0.45micron syringe filter and then analysed for nitrate. Nitrate analysis was performed on a Westco Smartchem 200 discrete analyser. Nitrate was reduced to nitrite by passing a filtered sample through a tubular copperised cadmium column. The resulting nitrite was then determined by diazotization with sulfanilamide followed by coupling with N-(napthyl)-ethylenediamine dihydrochloride to form an azo dye measured colourimetrically at 550nm.

For determination of total N and C, a small subsample of each composite (5 - 10 g) was ground for two minutes using a Retch MM200 ball mill. 20-30mg of the fine ground soil was then analysed using a Perkin Elmer CHN-S 2400 oxidative combustion analyser to determine total C and N.

### *Fruit Quality Analysis*

Fruit was harvested following the methods described above. Approximately 1kg of fruit was harvested from each tree, with a sub sample of 20 blemish-free fruit analysed immediately for fruit quality parameters (see above), a sub sample of 20 fruit diced to remove pips and then dried at $60^0$C for chemistry analysis at a commercial laboratory and a further sub sample stored in Modified Atmosphere Packaging bags for 28 days at $2^0$C for post-harvest fruit quality analysis. At the time of harvest, leaves were also sampled, dried at $60^0$C and sent to a commercial laboratory for chemistry analysis.

During winter dormancy (July 2012), the trunk, lower and upper branches, roots and buds were sampled from each treatment for their total N content. Samples were collected by drilling organs with a

spade bit to a depth of approximately 20mm into the tree material. Wood grinds were collected and dried at 60$^{o}$C for 48hrs before being analysed for Total N content at a commercial laboratory. The trunk was sampled at approximately 20cm above ground level. A lower branch was sampled at approximately 1m above ground level and the upper branch was sampled at approximately 2m above ground level. Roots greater than 20mm in diameter were sampled.

### *Data analysis*

Fruit quality assessment data were analysed using a general linear model approach in SPSS, with N, irrigation and N by irrigation included as fixed factors. Block and block by irrigation were included as random factors with block by irrigation used to test the irrigation effect. N and N by irrigation interaction effects were tested against the residual error. Assumptions of normality and homoscedasticity were assessed and met for each fruit quality parameter. *Post hoc* tests were computed using Tukey's Honestly Significant Difference (HSD) or Fishers Protected Least Significant Difference (LSD).

## Part 2b. Potassium Trial Ridgy Didge Cherries

### *Trial Establishment*

Potassium fertigation and foliar treatments were implemented pre-harvest in Dec 2014 along a single row of 12-year-old 'Simone' trees, grown on Colt rootstock at Ridgy Didge Cherries in Lower Longley, in southern Tasmania. Trees were pruned to a Spanish bush training system with tree spacing of 2m and row spacing of 4m. Row orientation was north west-south east with a considerable slope downwards from the northern end of the row. Other than the potassium fertiliser application, trees were managed following standard commercial orchard practise. The experimental row was split into five blocks of 15 trees, with each block treated as a replicate. Each block was further split into 5 sub-blocks of three trees randomly allocated to one of five potassium fertiliser treatments. These include: foliar potassium nitrate ($KN0_3$), foliar potassium sulphate ($K_2SO_4$), fertigated $KNO_3$, fertigated $K_2SO_4$ and a zero K control. Potassium treatments were applied twice, one week apart commencing in December at a rate of 25kg/$K^+$/ha. Each of the three trees in the sub-block were treated with the fertiliser but only the middle tree was sampled for fruit quality and fruit and leaf chemistry. Leaves were harvested one week following the second application and again at commercial harvest.

### *Fruit Quality Analysis*

Fruit was harvested and analysis for quality parameters as described above.

### *Data Analysis*

Fruit quality assessment data was analysed using a one way ANOVA in SPSS with K treatments considered a fixed factor; with block (replicate) considered as a random factor; and with each fruit quality parameter considered as the dependant variable. *Post hoc* tests were computed using Tukey's Honestly Significant Difference (HSD) or Fishers Protected Least Significant Difference (LSD). Significance was calculated at P = 0.05 and standard error used for comparison of mean values in the tables and figures. No data transformations were necessary.

## Results

### Part 1a. Nitrogen Fertigation Grove Research Station

Total N concentration (%) in cherry fruit at harvest was significantly influenced by increased N supply under fertigation treatments (Fig.1), where fruit harvested under the highest N treatment contained the greatest amount of total N. Average yield for trees within the trial approximated 5-6 kg/tree, which is substantially less than expected for 10-year-old 'Lapins' trees within the region, possibly contributing to the substantial treatment effect. There were significant differences in flesh firmness with level of N fertigation at 25 days post-harvest assessments (Fig.1). Fruit harvested under the highest N treatment were significantly less firm than under the lower N treatments and 0N control. No other fruit quality variables at any of the assessment dates were significantly influenced by N treatments.

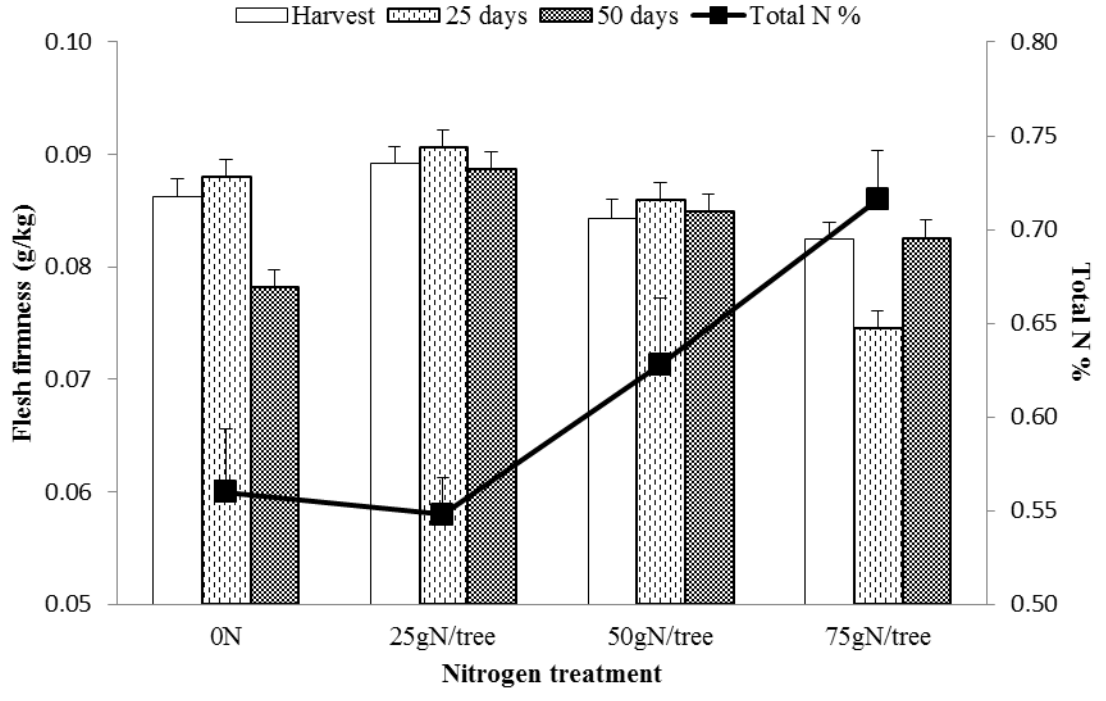


*Fig. 4. Flesh firmness under different nitrogen (N) application treatments with time in storage. Total N concentration of fruit analysed at harvest is overlayed. Error bars represent standard error. Chart copied from Swarts et al., (under review)*

Total N content of cherry tree storage organs sampled during winter dormancy ranged from 0.3% and 0.5% (Fig. 5). Buds had a much higher N content than the tree storage organs ranging from 2.5% and 2.8% between treatments. Although on average Total N content was higher in the buds and roots under the two highest N treatments, these were not significantly greater than the others.

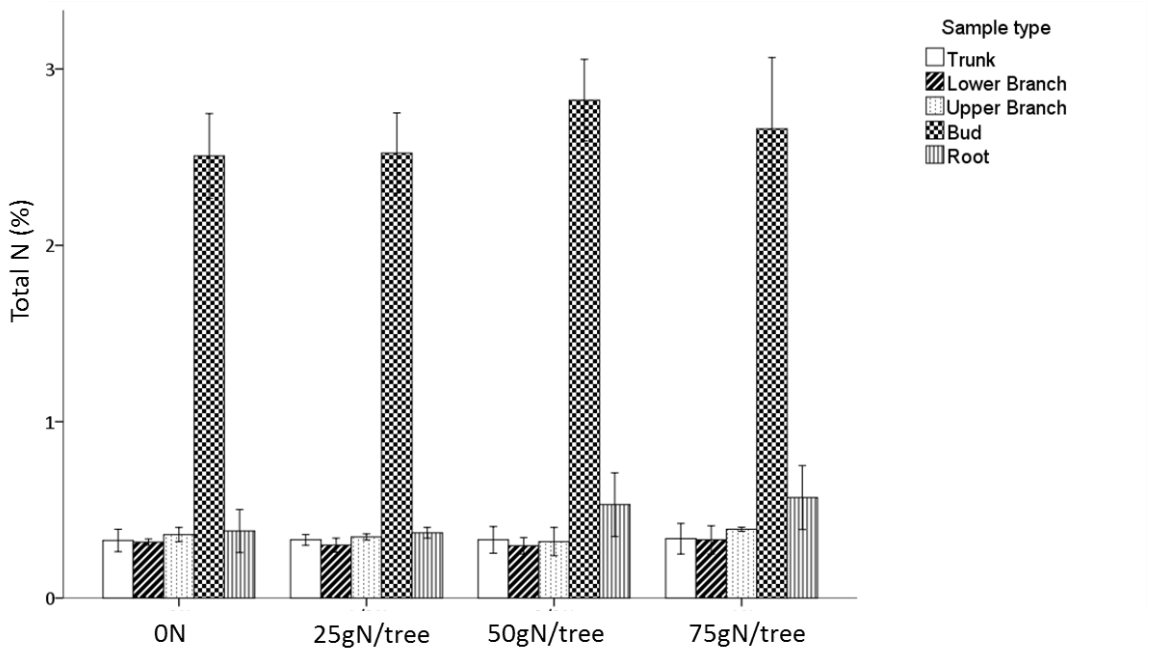


*Figure 5. Total N content of sweet cherry tree storage organs and buds sampled during winter dormancy. Error bars denote standard error.*

**Part 1b. Micro-element Fertigation Trial**

Micronutrient fertigation treatments had little effect on increasing their respective nutrient content in the harvested fruit from each treatment (Fig. 6a). Fruit harvested from Ca treatments on average had higher levels of Ca than control fruit. This was also true of the treatment Mn and Zn at least for the Mn content in the fruit, however none of these results were significantly higher.

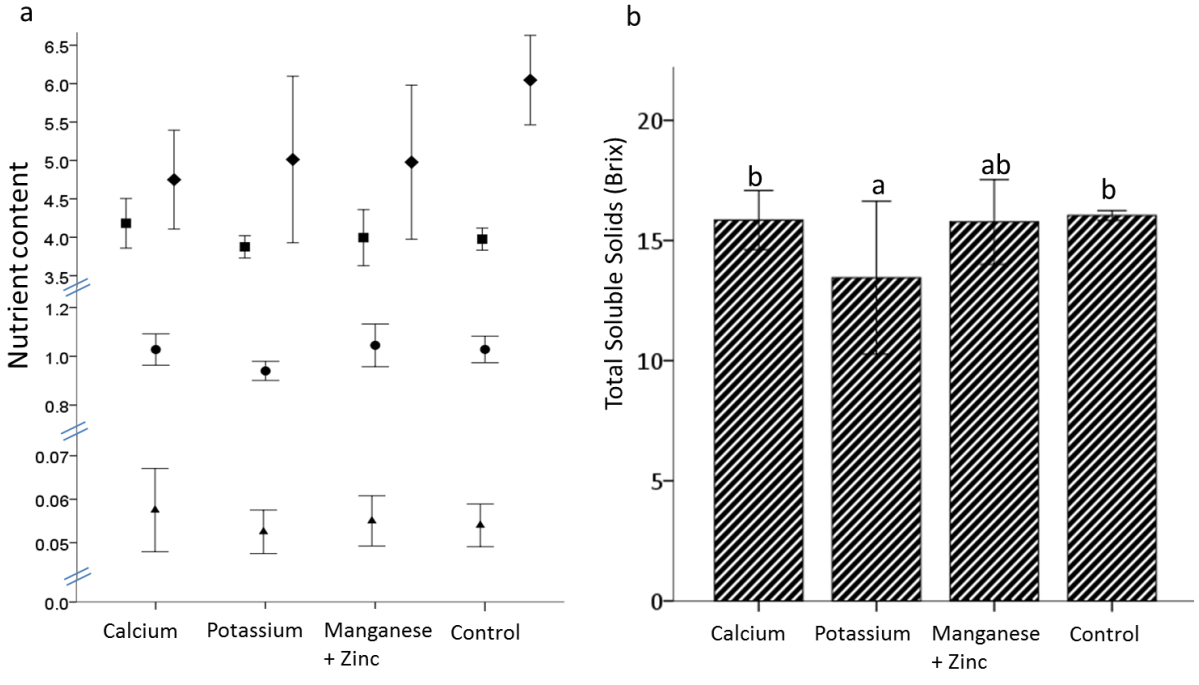

*Figure 6 (a) Nutrient content in harvested 'Lapins' fruit following micronutrient fertigation. Calcium (%) is indicated by triangles, Potassium (%) indicated by circles, Manganese (mg/Kg) indicated by squares and Zinc (mg/Kg) indicated by diamonds. Error bars denote standard error. (b) Total soluble solid content in harvested 'Lapins' fruit following micronutrient fertigation treatments. Error bars represent standard error. Letters above bars indicate significant differences between treatments.*

Fruit quality parameters from all micronutrient treatments at all assessment dates were not significantly different from the control with exception to Total Soluble Solids at harvest (Fig. 6b). Sugars in fruit harvested from the potassium treatment were significantly ($F = 4.558$, $P = 0.022$) lower than the control treatment.

**Part 1c. Micro-element Spray Trial**

Zinc and Mn foliar treatments significantly increased levels of their respective nutrients in fruit (Grove and Woodstock) and leaves (Grove) (Fig. 7). In both orchards, manganese levels in the Mn treatment were significantly higher than the control treatment and were significantly higher than the $Zm^2$ treatment which also contained Mn (Fig. 7a). Manganese levels in the $Zm^2$ treatment were significantly higher than the control treatment at Grove but not at Woodstock, yet were not significantly higher than any of the other treatments. A similar trend was found for Zn levels in fruit at both orchards under the Zn treatment, however Zn levels under the $Zm^2$ treatment were not significantly different from the other treatments (Fig. 7b).

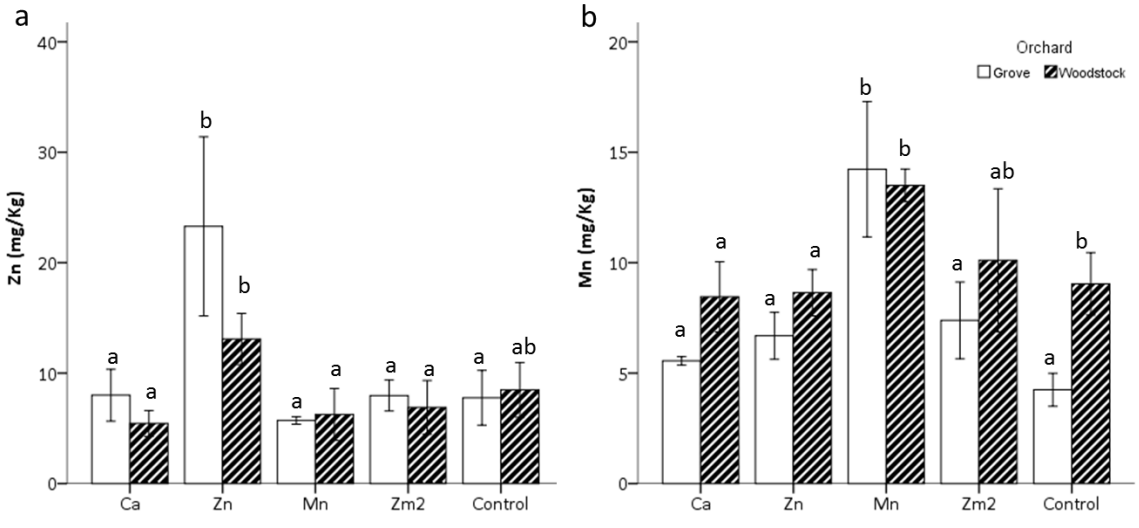


*Figure 7. Content of Zinc (a) and Manganese (b) in sweet cherry fruit at harvest after micronutrient spray treatments at Grove Research Station and Woodstock orchards. Error bars denote standard errors. Letters indicate significant differences between treatments.*

Leaf nutrient levels were only measured at the Grove Research Station. Zinc and Mn treatments had significantly higher levels of their respective nutrients in the leaves at harvest compared to the other treatments with exception to the $Zm^2$ treatment (Fig. 8). Levels of Zn and Mn in the $Zm^2$ treatment were greater than the other treatments, yet they were also not significantly different from the control treatment. No increase in Ca levels under Ca treatments in leaves at Grove or fruit at both orchards were observed.

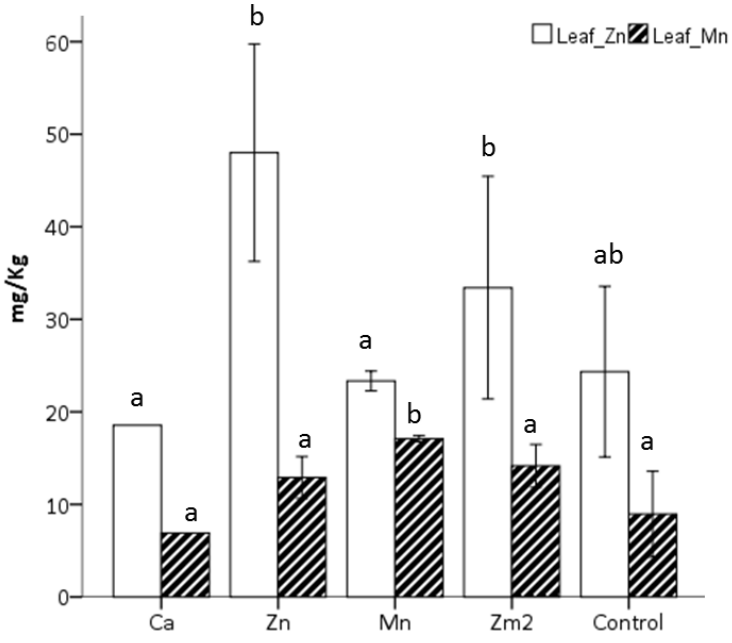


*Figure 8. Content of sweet cherry leaf Zinc and Manganese under micronutrient spray treatments at Grove Research Station. Error bars denote standard error. Letters indicate significant differences between treatments.*

Analysis of fruit quality parameters at harvest and 25 and 50 days in post-harvest storage revealed little significant differences between micronutrient treatments despite the strong uptake of Mn and Zn micronutrients in the leaves and fruit of the sweet cherry trees. Cherry fruit harvested from Grove were significantly more coloured (red) in the Mn treatment than the $Zm^2$ treatments (Fig. 9a) however, fruit from both these treatments were not significantly more coloured from any of the other treatments. The only treatment effect was on Total Soluble Solids (TSS; sugars) content of stored fruit, 25 days post-harvest from Grove where Ca treatments had significantly greater sugars than the control and Zn treatments but not Mn and $Zm^2$ treatments (Fig. 9b).

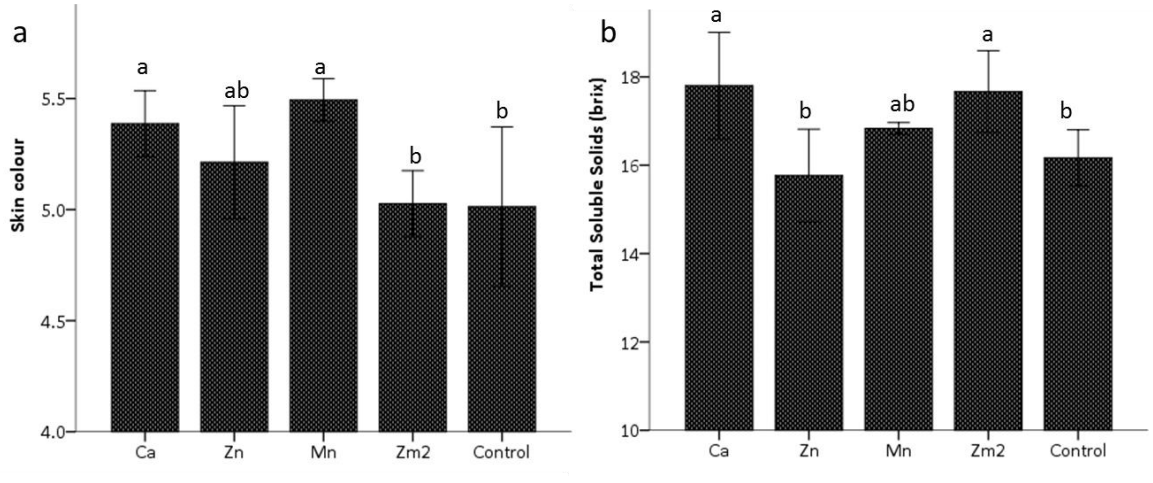


*Figure 9. Differences in Skin Colour at harvest (a) and Total Soluble Solids after 25 days in storage (b) of sweet cherry fruit at Grove Research Station. Error bars denote standard errors. Letters indicate significant differences between means.*

**Part 2a. Nitrogen Fertigation Ridgy Didge Cherries**

Fruit Total N content at harvest was significantly influenced by N fertiliser treatments in the 2013/14 season but not the 2014/15 season. In the former, only fruit harvested from the highest N treatment (1N = 120kgN/ha), had N content significantly (F = 4.653, P = 0.007) greater than the 0N control treatment, however fruit harvested from each of the N treatments did not have significantly different N content from eachother (Fig. 10). Leaf N content in both seasons, and at all times assessed, although appearing to trend higher with higher N applied was not significantly different from eachother. Irrigation treatment did not have an influence on N content in fruit or leaves.

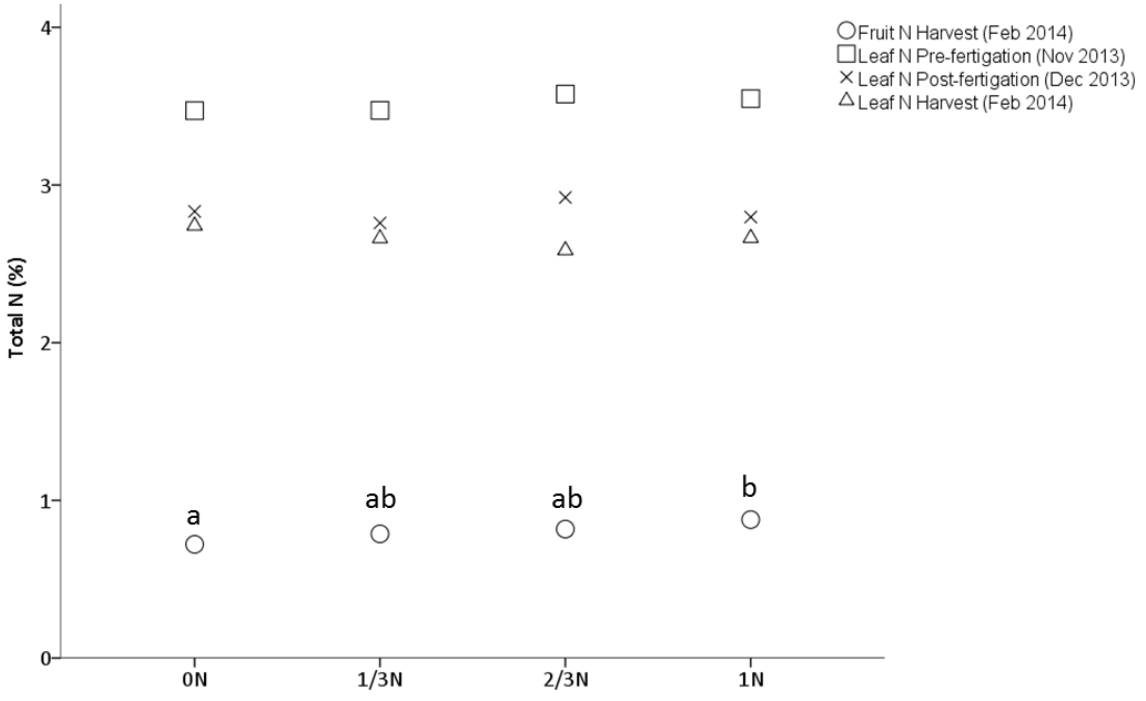


*Figure 10. Total N content (%) of fruit (harvest) and leaves sampled before fertigation, after fertigation and at harvest in the 2013/14 season. Letters indicate significant differences between treatments.*

Weight and diameter (data not shown) of cherries at harvest were significantly influenced by irrigation treatment in both the 2013/14 and 2014/15 seasons, but not fertigation treatments. In 2013/14, fruit were significantly bigger (F = 9.212, P = 0.015) in the high (3.9L/hr) treatment compared to the low (1.6L/hr) and medium (2.3L/hr) treatments (Fig. 11). In 2014/15, harvested fruit were significantly larger (F = 6.797, P = 0.029) in the medium treatment compared to the low treatment, however fruit in the high irrigation treatment were not significantly larger or smaller than the others.

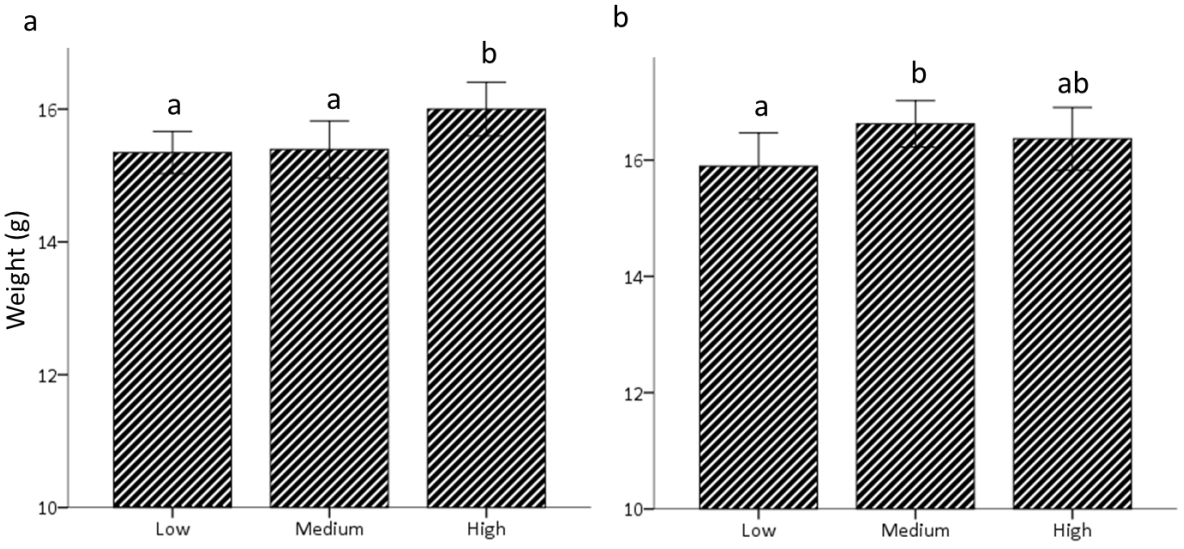


*Figure 11. Weight of fruit at harvest in the 2013/14 (a) and 2014/15 (b) seasons. Error bars denote standard error. Letters above bars indicate significant differences between treatments.*

In the 2013/14 growing season, flesh firmness measurements from the Firmtek and Guss Fruit Texture Analyser (skin firmness and skin puncture tests) were unaffected by irrigation or fertigation treatments at either harvest or after 28 days in storage. Stem pull force at harvest was significantly influenced by irrigation treatment ($F = 9.504$, $P = 0.015$) where fruit harvested from the high treatment required significantly more force to remove the stem than the medium treatment (Fig. 12a). However the force required to remove stems from the low irrigation treatment was not significantly different from the other two. Fruit chemistry measurements (TSS, TA and pH) at harvest was unaffected by irrigation treatments, however, TSS (sugars) of fruit after 28 days in storage was significantly ($F = 3.34$, $P = 0.035$) influenced by nitrogen fertigation treatments. Here, sugars in fruit harvested from each treatment receiving N were not significantly different from eachother, however, fruit sugars in the 0N, 1/3N and 1N treatments were significantly higher than in the 2/3N treatment (Fig. 12b).

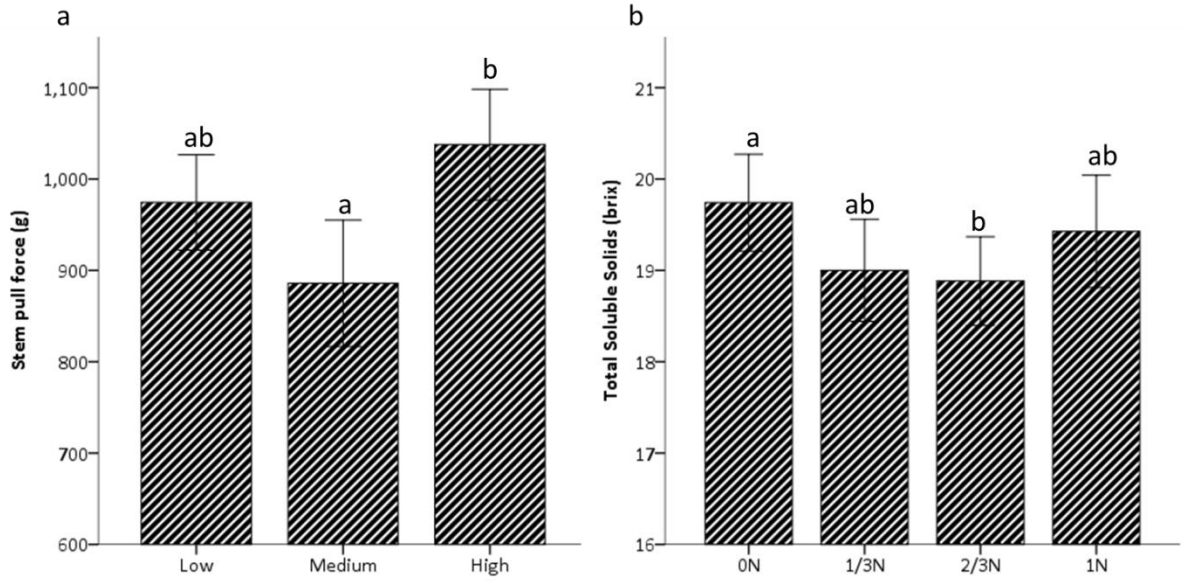

*Figure 12. Stem pull force of 'Simone' cherries at harvest under irrigation treatments (a) and Total Soluble Solid content of 'Simone' cherries after 28 days in storage under Nitrogen fertigation treatments. Error bars denote standard error. Letters above bars indicate significant differences between treatments.*

In the 2014/15 growing season, fruit firmness measurements (Fig. 13a,b) of flesh firmness (F = 5.06, P = 0.05) and skin puncture (F = 15.09, P = 0.005) using the Guss Fruit Texture Analyser were significantly influenced by irrigation. Both measurements followed the same trend where penetration force (g/Kg) to fruit at harvest was significantly higher in the medium irrigation treatment compared to the high treatment, yet the low irrigation was not significantly different from the medium or high treatments. No influence of irrigation or N fertigation treatments were observed on fruit after 28 days in storage. Treatments did not have any influence on fruit chemistry at harvest or after 28 days in storage.

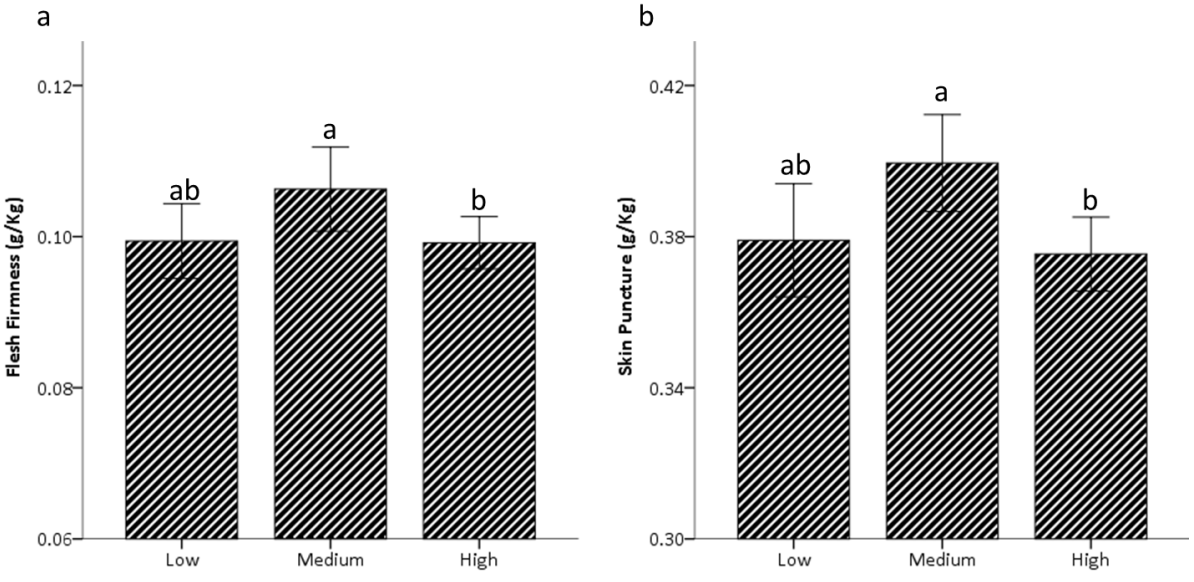


*Figure 13. Influence of irrigation treatment on Flesh Firmness (a) and Skin Puncture (b) of 'Simone' cherries using the Guss Fruit Texture Analyser. Errors bars denote standard error. Letters indicate significant differences between treatments.*

**Part 2b. Potassium Trials Ridgy Didge Cherries**

Foliar and fertigation treatments had little influence over K content in leaves and fruit (Fig. 14a) with no significant difference between them and the control zero K treatment.

For fruit quality assessments, only Firmtek measurements of fruit at Harvest (F = 3.66, P = 0.028) and after 28 days (F = 4.766, P = 0.011) in storage were significantly influenced by K treatments (Fig. 14b). At both assessment dates, all K treatments were not significantly different from eachother, however on average, fruit from these treatments were firmer than the control zero K treatment. At harvest, only the foliar $KNO_3$ treatment was significantly firmer than the control, but after 28 days in storage fruit from the foliar $K_2SO_4$ treatment were significantly firmer than the control treatment. Although not significantly different, on average, skin colour in the fertigated K treatments was darker at harvest than the foliar treatments, but lower in skin puncture force and in TSS (not shown).

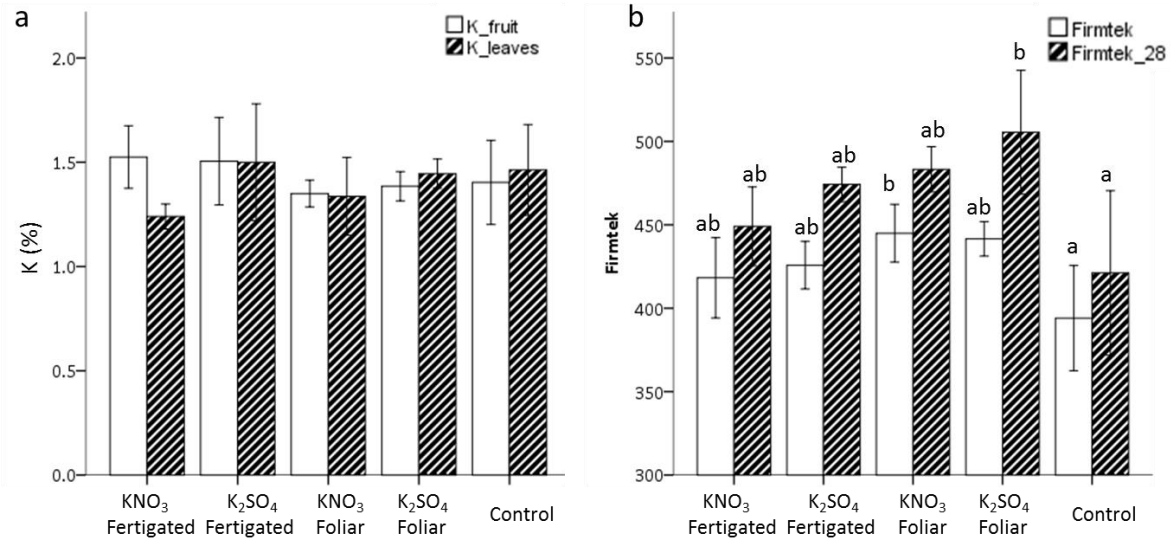


*Figure 14. Potassium (K) content in fruit and leaves at harvest after pre-harvest foliar and fertigated K treatments (a) and Firmtek results at harvest and after 28 days in storage following pre-harvest foliar and fertigated K treatments (b). Error bars denote standard errors. Letters indicate significant differences between treatments.*

## Discussion

### *Does increased nitrogen application increase nitrogen content in fruit, leaves and storage organs of sweet cherry trees?*

Nitrogen fertigation treatments at Grove and Ridgy Didge in the 2013/14 season increased total N content in 'Lapins' and 'Simone' fruit respectively. In both trials, it was only in the highest N treatment where a significantly greater response was observed compared to the control zero N treatment. At Grove, where a marked response was observed, trees in the highest treatment received a pre-harvest quantity of 75gN/tree (150kgN/ha) which is three times higher than the recommended pre-harvest rate for the region. Total N content of the zero N control fruit was very low at <0.6% so it was not unexpected to see a marked response with high rates of N applied. Furthermore, changes in orchard management in the two seasons prior to trial commencement saw trees in the orchard receive less than the standard N application recommended by industry. Yet although fruit had an increased total N content, this did not lead to significantly increased storage in branches, trunk or root, nor did it result in higher bud N content for next season's vegetative and fruit growth.

At Ridgy Didge, fruit from the control trees averaged 0.7% total N in 2013/14 and over 0.8% in the 2014/15 season which possibly explains why the treatment effects were less pronounced. Furthermore, given that the majority of pre-harvest N requirement for deciduous fruit trees such as sweet cherry is provided by remobilised N, a carryover effect from previous season's fertiliser application is likely to have minimised the treatment effects. Therefore in the 2014/15 season, we were anticipating a much stronger response to treatments as by harvest, trees would have received the 2013/14 pre-and post-harvest N and the 2014/15 pre-harvest N applications. In the maximum N treatment, this equates to an extra 300gN/tree than the zero N control treatment and yet treatment effects were even less obvious than the previous season. Fertigation treatment effects on leaves were not obvious either, particularly in the comparison before and after the 2013/14 pre-harvest fertigation period. In both seasons, leaf N at

harvest remained unaffected by fertigation treatments. There are a number of additional reasons why treatment effects on fruit and leaf N content were less than expected at the Ridgy Didge site.

- Trees were pruned heavily by the grower during winter dormancy in 2014 therefore a strong vegetative response in the spring and summer of 2014/15 may have limited the availability of N to fruit.

- Cherries at Ridgy Didge are harvested very late compared to other cherries in the region and research has demonstrated that transpiration rate and photosynthesis in cherry trees reduce dramatically after harvest (ref) meaning that the N applied post-harvest may not have been taken up by the trees.

- The cherry trees are large old trees which may have a strong dilution effect of N in the leaves

- Yield in both seasons were low (<4kg/tree) due to high shedding in 2013/14 and cracking/rot in 2014/15 implying that N demand was low.

- Little is known about the soil physical properties at the site. Applying N via pressure compensated drippers in one localised area may result in leaching of N below the root zone via preferential flow pathways before the trees have the opportunity for uptake.

- Nitrogen in the soil may have been sufficient to meet cherry tree demand therefore no extra N was required

- There are few studies comparing the influence of pre-harvest N application on N content in fruit and leaves, however Neilsen et al., (2007) found that N concentration in 6 – 8-year-old 'Lapin' fruit increased after increased pre-harvest N fertigation.

***Does increased nitrogen application have a negative effect on fruit quality?***

Studies have reported negative consequences of increased N application on fruit quality (Neilsen et al., 2004a). Our findings at Grove suggest that this is likely the case at least for fruit firmness as this was the only fruit quality parameter influenced by N treatments. Although fruit on average were firmer under lower N treatments, significant differences were only observed after 28 days in storage, not at harvest or after 50 days in storage. Yet at Ridgy Didge, flesh firmness using both analyses were unaffected by treatments in both the 2013/14 and 2014/15 seasons. Flesh firmness measured with a Guss Fruit Texture Analyser is indicative of fruit texture or crunchiness of the cherry when a consumer bites through the flesh.

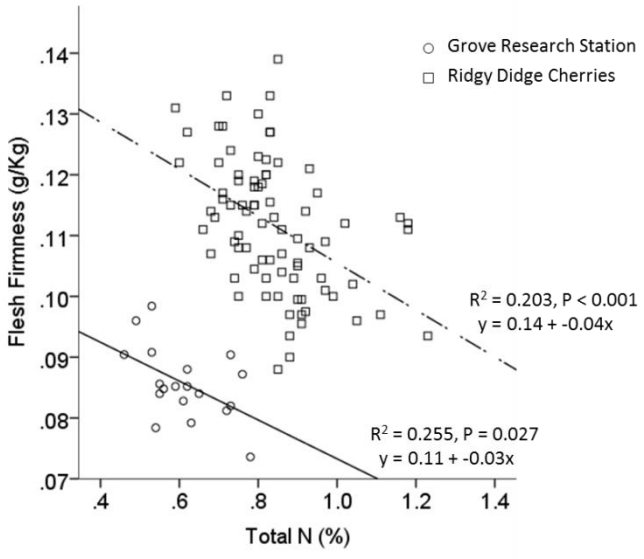


*Figure 15. Correlation between flesh firmness (Guss Fruit Texture Analyser) data, pooled from all treatments and seasons, and Total N (%) content of sweet cherry fruit at harvest from Grove Research Station and Ridgy Didge Cherries.*

When data was pooled from all treatments and seasons, there was a strong significant correlation between flesh firmness and total N content (Fig. 15). This suggests that when N content in fruit is inadequate (0.4 - 0.5%), high N application pre-harvest may have a detrimental effect on fruit firmness (e.g. as seen at Grove), yet when N content in fruit is adequate (0.7 - 0.8%), the application of additional N pre-harvest is unlikely to increase N content in the fruit (as seen at Ridgy Didge) or have a detrimental effect on fruit firmness. No other fruit quality parameters at Ridgy Didge Only Total Soluble Solid content in fruit after 28 days in storage in the 2014/15 and even here variation between the treatments was extremely low.

Obtaining the ideal balance of N supply to meet tree demand is a challenge for growers. Our data suggests that there is considerable flexibility in total N supply over a growing season given the lack of fruit quality response to even our highest N treatments of 240kgN/ha at Ridgy Didge orchards. This may benefit growers who want to promote vegetative growth after strong winter pruning, yet also may be a risk to growers who want to keep trees in a reproductive state. For large old trees with capacity for considerable N uptake, it is likely that only minimal N is required to maintain adequate N content to avoid negatively influencing fruit firmness. For example, an orchard with 2000 trees/ha, an average crop load of 8kg/tree, total N content of 0.9%, fruit dry matter content of 22% only requires a replacement rate of approximately 30kgN/ha. Whilst there is little information on the proportion of uptake of applied N in cherry orchards, it is well known as earlier stated that a large component of pre-harvest N requirement is provided by remobilised N (Millard et al., 2006). Therefore 30kgN/ha over the course of a season will enable trees to maintain adequate fruit N content for optimal fruit quality and for N withdrawal into storage organs prior to dormancy. Furthermore, reducing N application will minimise potential groundwater leaching as well as facilitate a significant fertiliser cost saving.

In this trial, N treatments commenced approximately one month after bud burst when N supply has been shown to shift from N remobilisation to root uptake N (Grassi et al., 2002). This is also the period of greatest N uptake as demonstrated from comparisons between pre-and post-harvest applications (San-Martino et al., 2010, Azarenko et al., 2008). Timing of fertiliser application should coincide with active root growth maximising the uptake proportion of applied N.

***Does irrigation quantity influence the nitrogen content in leaves and fruit and fruit quality?***

In both seasons, irrigation quantity had no discernible influence on total N content in leaves and fruit of 'Simone' cherry trees at Ridgy Didge. No interaction effect of irrigation quantity on fertiliser treatment was observed when analysing total N content of leaves and fruit. This is surprising given that the irrigation volume of the high treatment (3.9L/hr) was almost 2 and a half times that of the low irrigation treatment (1.6L/hr). Increased irrigation volume may have leached additional nitrate-N through the soil profile via preferential flow pathways, or may have diluted the concentration of applied nitrate-N. Additional regular soil sampling following fertigation events would assist to confirm either of these scenarios.

Irrigation treatments had a consistent influence on fruit size in the 2013/14 and 2014/15 growing seasons where increased irrigation volume increased fruit size. Increasing fruit size in response to higher irrigation levels is commonly seen in a wide variety tree fruit crops including apples (Fallahi et al., 2010, Naor et al., 1997), Peach (Naor, 2006), pear (Naor, 2001) and citrus (Treeby et al., 2007). Increasing irrigation volume prior to harvest appears to be a useful management strategy for increasing fruit size and therefore overall yield also in sweet cherry. Furthermore, increasing irrigation volume has been reported to reduce the risk of cherry cracking after rain events as it equalises the water potential of the flesh with the outside skin (Measham et al., 2009).

***Does foliar application of micro-elements increase nutrient content in fruit more effectively than fertigation?***

The effectiveness of nutrient uptake through leaf cuticles is related to the anatomical structure of the leaf as well as the existing nutrient concentration, soil availability and other environmental factors. (Smoleń, 2012). Foliar elemental sprays are usually salts which enable penetration through cuticles by diffusion in aqueous pores of varying molecular dimensions (Schönherr, 2001). The concentration difference across the cellular membrane is described as the driving force for cuticular penetration (Schönherr, 2001). Interestingly, Schonherr (2001) also reported that rates of penetration were greatly affected by humidity over cuticles, and hygroscopicity of salts rather than temperature. In this study, although we did not assess rates of penetration of foliar applied nutrients, we did assess fruit and leaf nutrient composition following pre-harvest foliar and fertigated nutrition.

Foliar Zn applications resulted in significantly increased in Zn content in the fruit at both orchards, but fertigated Zn did not. Foliar Zn dramatically increased Zn content in the leaves at Grove Research Station when analysed at commercial harvest. Indeed the trees in the Zn treatments started to show symptoms of Zn toxicity (yellowing and defoliation) one week after the second application (Fig. 16). Yet, excessive Zn levels were not found in the fruit, suggesting that although some transport occurred, the majority of applied Zn remained in the leaves. This is not surprising given the poor mobility of Zn (Sánchez and Righetti, 2002). Zinc foliar spray was more effective at increasing leaf and fruit Zn content than $Zm^2$ however this was most likely due to the concentration of Zn in the Zn spray, being approximately 4x the amount of the $Zm^2$ spray. Alternatively, the uptake of Zn may have been compromised by other nutrients in the $Zm^2$ treatment. There is limited research comparing the nutrient content of fruit following pre-harvest foliar Zn application in sweet cherry. In other tree fruit crops, Zn foliar sprays increased Zn concentration in apples (Nielsen et al., 2004b), but not in avocado (Farré et al., 2001) or peach (Policarpo et al., 2001). In this study foliar applied Zn was far superior than fertigation in boosting fruit Zn content, a result also reported by earlier authors (Nielsen et al., 2004b, Swietlik, 2001).

*Table 4. Comparison of nutrient content in sweet cherry fruit at harvest following fertigation or foliar application*

| Nutrient | Method | Orchard | Rate applied (g/tree) | Rate applied (kg/ha) | Increase from base level (%) |
|---|---|---|---|---|---|
| Calcium | Foliar | Grove | 22 | 30 | 0 |
| | Foliar | Woodstock | 11 | 30 | 0 |
| | Fertigated | Grove | 22 | 30 | 1 |
| Potassium | Fertigated | Grove | 30 | 50 | 0 |
| | Foliar $KNO_3$ | Ridgy Didge | 22 | 50 | 19 |
| | Fertigated $KNO_3$ | Ridgy Didge | 22 | 50 | 0 |
| | Foliar $K_2SO_4$ | Ridgy Didge | 22 | 50 | 7 |
| | Fertigated $K_2SO_4$ | Ridgy Didge | 22 | 50 | 0 |
| Zinc | Foliar | Grove | 0.72 | 18 | 300 |
| | Foliar | Woodstock | 0.36 | 18 | 54 |
| | Fertigated | Grove | 0.35 | 25 | 0 |
| | Foliar ($Zm^2$) | Grove | 0.3 | 20 | 3 |
| | Foliar ($Zm^2$) | Woodstock | 0.15 | 20 | 0 |
| Manganese | Foliar | Grove | 0.72 | 18 | 235 |
| | Foliar | Woodstock | 0.36 | 18 | 49 |
| | Fertigated | Grove | 0.35 | 25 | 0 |
| | Foliar ($Zm^2$) | Grove | 0.3 | 20 | 74 |
| | Foliar ($Zm^2$) | Woodstock | 0.15 | 20 | 12 |

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*Figure 16. Leaf senescence following foliar application of zinc at Grove Research Station.*

Foliar application of Mn resulted in a significant increase in Mn content in harvested fruit at both orchards. Similar results were found in studies applying foliar sprays containing Mn where increased Mn concentrations were also observed in the leaves (Naseri et al., 2002, Marschner, 1997, Policarpo et al., 2002). Manganese was also applied in the $Zm^2$ treatment, but this treatment did not result in higher Mn levels perhaps due to the reasons described above for Zn. Manganese is a poorly mobilised nutrient (Marschner, 1997), which explains the greater treatment effect on fruit Mn concentration seen in foliar sprays compared with fertigated compounds. (Naseri et al., 2002) demonstrated that significant amounts of Mn could be absorbed within the first 24 hours of spraying. At Woodstock the Mn levels were higher than that at Grove, however the treatment seemed to have a greater effect on the fruit at Grove. This relationship may be a result of a cultivar dependent response, or might be a result of management practice differences between Woodstock and Grove where base levels of Mn were substantially higher (Fig. 7b). This demonstrates the effectiveness of Mn foliar sprays as a useful corrective fertiliser to balance micronutrient concentration when deficiencies are observed.

There was no significant increase in Ca levels in the fruit after foliar application although a slight average increase in fruit Ca levels following fertigation was observed. Research has shown that foliar application increases the Ca concentration in the leaves, but not necessarily in the fruit. Calcium is transported through the xylem and therefore abundant in the stem and leaves but not in low-transpiring sinks supplied via the phloem (Marschner, 1997). Transport of Ca is dependent of the respiration rate of the fruit which increases in the rapid growth stage, but low before and after (Hong-Qiang and Yu-Ling,

2005, James, 2011, Marschner, 1997). In this study, foliar sprays were applied late in the rapid fruit enlargement stage and because Ca is so immobile this might have been too late in the growing season to influence fruit Ca concentration. Low levels of Ca result in weak cell walls and (when wet) in fruit cracking (Hong-Qiang and Yu-Ling, 2005). Extra Ca can be added through fertigation or foliar spray, but foliar spray is only effective on young leaves (Marschner, 1997). Research on three commercial cherry orchards in Tasmania, Australia (1991-1993) showed that foliar application of Ca and Cu three weeks after full bloom significantly increased the percentage of intact fruit, suggesting that there was a higher Ca and Cu concentration in the fruit (Brown et al., 1995). A similar result was also shown by (Wojcik et al., 2013).

In the K trial at Grove Research Station, fertigation of $K_2SO_4$ was ineffective at increasing K content in 'Lapins' fruit. However in the more comprehensive trial at Ridgy Didge, on average, both fertigated K forms increased K content in 'Simone' fruit and were more effective than foliar applications, yet the treatment effects were not significantly greater. Although K is highly immobile in the soil, drip irrigated (fertigation) K has been shown to be more readily available to trees than broadcast K (Uriu et al., 1980) and strong evidence for fruits being a strong sink for K has been demonstrated for a number of tree crops (Neilsen et al., 1999, Zeng et al., 2001). Little research has been completed on foliar K treatments, however K deficiencies in soil and plants are regularly seen following fertigation with salts (Neilsen et al., 2004b).

***Does increased micronutrient application improve fruit quality?***

Although significantly increased concentration of Zn and Mn were found in fruit following foliar applications, there were surprisingly few fruit quality implications. No treatment influence on fruit size (weight or diameter) was observed with foliar or fertigated treatments. Perhaps not so surprising given factors such as the fruit to leaf area are more likely to influence fruit weight. Improved light penetration results in higher photosynthetic activity and may increase carbohydrate supply to the sink, allowing larger fruits (Romano et al., 2006, Patten et al., 1983). Another possibility is that fruit weight is not directly influenced by nutrients but more so by plant hormones such as Gibberellic Acid (GA). Usenik et al., (2005a) and Patten et al., (1983) reported that sweet cherry fruit treated with GA had an increase in weight and were wider however results were cultivar dependent.

Firmness was measured using the Guss Fruit Texture Analyser and the industry relevant Firmtek machine. The Guss Fruit Texture Analyser measures penetration force of the skin with a puncture test and the flesh after a slice of the skin has been removed. Flesh firmness is strongly indicative of the texture or crunchiness of the cherry which is a trait strongly desired by the consumer. All fruit harvested from K treatments at Ridgy Didge on average were firmer than the control, however it was only the foliar K treatments which were significantly firmer at harvest and after time in storage. A stronger response was seen at Ridgy Didge than Grove due to the earlier application time at the former and the greater quantity that was applied. Previous works have not identified a key role of potassium for increasing fruit firmness. Kaiser et al., (2009) reported an increase in fruit firmness of sweet cherry after pre-harvest soil drenching of potassium silicate, however this may be due to the increase in silicate which is known to strengthen cell walls. Potassium is often credited with facilitating photosynthesis in tree fruit crops thus the greater opportunity for accumulation of photosynthates into the fruit may indirectly influence fruit firmness. However this is also dependent on the leaf-fruit ratio and light penetration (Patten et al., 1983).

Although not directly compared as part of this trial, significant differences in skin firmness for different treatments were found between cherries harvested from Grove and Woodstock. Woodstock cherries were on average always firmer in every treatment (data not shown) due to the improved overall management practise, a result commonly reported in other studies (Choi et al., 2002, Toivonen et al., 2004, James, 2011, Usenik et al., 2005b).

Calcium is generally considered the most effective nutrient influencing fruit firmness, however there is great difficulty increasing leaf and fruit concentrations due to its immobility in transport. Our treatments showed no influence of fertigated or foliar applied Ca on fruit firmness. Application of Ca alone may not affect fruit firmness, but Ca applied with Copper, Boron or GA has been shown to improve fruit firmness in a range of tree fruit crops including sweet cherry (Mertes, 2011, Nielsen et al., 2000, Nielsen et al., 2004a, Patten et al., 1983, Marschner, 1997, Brown et al., 1996, Brown et al., 1995, Thurzó et al., 2010, Usenik et al., 2005b).

Decreased fruit firmness has been correlated to darker skin colour (Clayton et al., 1998, Romano et al., 2006), which is most likely a reflection on the ageing process of fruit, particularly in storage. Fruit at Grove harvested following treatment with foliar Mn were significantly darker than the control fruit, but not different to the Zn and Ca treatments. These results are in contrast with the understanding that Mn and Zn both influence a large range of enzymes including antioxidant containing enzymes which are thought to decrease the ageing of the fruit (Marschner, 1997).

There were no treatment effects on stem pull force at either orchard. There is little known on the influences of nutrients on the stem pull force of cherries. Recent research showed that higher K levels in fruit was correlated with greater stem pull force (Mertes, 2011). This contrasts with another study which found that stem pull force decreased as N, K, Ca and Zn levels increased (Azarenko, 2005). It is recognised that stem pull force is most likely influenced by other external factors such as rain fall and high temperatures (Azarenko, 2005). Further research is needed to gain insights on the factors influencing stem pull force.

A very slight negative response to fertigated K was observed at Grove in the total soluble solid content (sugars) of harvested fruit. Additionally there was a treatment response to foliar applied $Zm^2$ and Ca where greater fruit sugars than the control were recorded after 25 days in storage. These responses are difficult to explain given the little difference both fertigated K and foliar applied Ca had on their respective nutrient content of the fruit. Interestingly this was the only fruit quality response to the $Zm^2$ treatment in the trial so perhaps the constituents of the fertiliser worked favourably for this parameter compared to the other foliar sprays. Factors generally though to influence TSS are considered to be plant density and leaf-fruit ratio (Romano et al., 2006, Patten et al., 1983).

## Recommendations

There are a number of take-home messages from the data obtained from these trials.

- Higher levels of N found in fruit were strongly correlated with decreased firmness. The negative influence of high nitrogen application on sweet cherry fruit quality is probably overstated when N concentration in fruit is adequate (0.7-0.9%). When N is deficient (<0.6%), a strong response to fertigated N is observed.

- Nitrogen fertiliser rate/ha should match removed N from cropping and is based on crop load, fruit N status and dry matter content. Timing of N application should coincide with greatest root growth

which commences approximately four weeks after bud burst. To avoid leaching of applied N, fertigation events should be staggered with up to one week break between fertigation events.

- Increased irrigation volume had a strong positive influence on fruit size. If available, additional irrigation prior to harvest may be an effective management tool in increasing overall yield (tonnes/ha) with no negative effects on fruit quality

- A strong response to foliar elemental sprays of Zn and Mn was observed in cherry leaves and fruit particularly when base nutrient levels were low, which suggest they are effective for correcting deficiencies. Rate of application must be carefully managed to avoid toxicity as seen in this research trial. Foliar applied Zn and Mn were more effective than fertigated Zn and Mn in increasing fruit nutrient content.

- Potassium being readily transportable, was more effectively translocated to the fruit than via foliar applications. Yet on average foliar treatments had a stronger influence on fruit quality, namely fruit firmness, than fertigated treatments. Potassium in deciduous trees are recycled between seasons, however as fruits are a string sink for K, adequate soil K is required to meet the demands of a developing crop. Fertigation with N in late spring would optimise the uptake of K.

- No dramatic effects on fruit quality were observed for all nutrients applied despite extremely high rates of some nutrients (particularly N) applied. When soil and tree nutrient status is adequate through ongoing monitoring and good soil management, there are significant cost and environmental benefits to minimising fertiliser application as much as possible.

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# Appendix 6: Role of Nitrogen Fertigation in Sweet Cherry Fruit Quality and Consumer Perception of Quality: At- and Post-harvest

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**Keywords**: sensory analysis, post-harvest, firmness, storage, Tasmania, 'Lapins', *Prunus avium*

## Abstract

The management of nitrogen (N) is key for commercial orchard production as N plays an important role in the quality of sweet cherry fruit. Whilst fertigation is commonly practised by cherry growers in Australia, research into optimal N supply to meet tree demands is limited and the effects of oversupply of pre-harvest N on fruit quality are virtually unknown. This study aims to investigate (i) the role of pre-harvest N application on fruit N concentration and the implications for fruit quality post-harvest; (ii) consumer perception of stored fruit produced under a relatively high N supply compared with high grade export quality fruit and; (iii) how consumer perception aligns with objective quality measures of firmness (compression test by FirmTech and puncture text by Guss Texture Analyser), sugar and acid composition. Nitrogen treatments, applied as calcium nitrate, were imposed pre-harvest to 10 year old 'Lapin' trees on F12 rootstock in Southern Tasmania through a drip irrigation system. Nitrogen concentration in cherry fruit at harvest significantly increased under the high N treatments. Fruit firmness at harvest and in storage was significantly reduced under increased N supply. Results demonstrate an immediate effect of increased N supply on cherry N concentration and fruit firmness. Sensory analysis demonstrated that consumers strongly preferred export grade over high N fruit and that perception generally matched instrumental fruit quality assessments for the range of parameters tested with the exception of firmness. Pre-harvest N application must be carefully managed to avoid over-supply of N and consequent detrimental impacts on fruit quality at- and post-harvest.

## Introduction

Post-harvest fruit quality is a key component of tree fruit crop production and plays a major role in the overall crop value and return for growers. Sweet cherries (*Prunus avium*) are one of the highest value tree crops due to the price paid for a premium product, yet cherries are highly perishable and have one of the shortest shelf-lives of all tree crops.

An increasing body of research is revealing the complex dynamics of internal tree N-use and N-use-efficiency in deciduous tree crops (e.g. Millard and Grelet, 2010; Millard et al., 2006; San-Martino et al., 2010). Seasonal N fluxes are regulated by remobilisation from internal reserves, root uptake of N from the soil and phloem – xylem recycling. Nitrogen for spring flowering and growth is first supplied by the remobilisation of stored N from tree roots, trunk and branches (Grassi et al., 2002; Millard, 1996), then supplemented by root uptake of external sources such as ammonium, nitrate and organic N (Fallahi et al., 1993). As the season progresses, the simultaneous flush of vegetative growth with bud differentiation and fruit development creates competition between organs for available resources. In contrast to other tree crops, sweet cherry is known for bearing primarily on second year and older

fruiting spurs with a very short fruit development period. This has a significant effect on the timeframe and availability of nutrient supply to meet demands by the developing fruit and leaf populations. N is implicated as a major factor in the key pre-harvest yield components of fruit number per tree and fruit mass at maturity.

Post-harvest quality and taste attributes of fruit and vegetables can be influenced by N availability pre-harvest (Locascio et al., 1984). This has been clearly demonstrated in studies on apples, where high N applications were associated with a reduction in red blush (Marcelle, 1993; Neilsen et al., 2009), storage longevity (Fallahi et al., 2006), increased fruit softening (Marcelle, 1993) and N related physiological disorders such as watercore (Fallahi et al., 2005). In contrast, negligible effects of N fertilisation on fruit quality were reported in blackberries (Alleyne and Clark, 1997), apricots (Suman et al., 2003) and wine grapes (Neilsen et al., 1987). In sweet cherries, Neilsen et al., (2005) reported a similar negligible effect of pre-harvest fertigated N in young plantings of 'Lapins' trees. In mature trees, Hansen (1997) and Stanberry and Clore (1950) demonstrated that increased levels of N fertilization led to delayed maturity in sweet cherry and Neilson et al., (2007) reported that high N applications led to reduced fruit size and titratable acidity, while fruit total soluble solids and firmness were unaffected. What is unclear from this research is if the quantity of N applied pre-harvest influences total fruit N concentration and how it correlates with fruit quality and at- and post-harvest. Furthermore, all fruit quality assessments were made using instrumentation and non subjective measurements so very little is known about consumer perception to altered fruit quality attributes as influenced by nutrient management.

Sweet cherry is highly appreciated by consumers due to its quality attributes, most notably colour, flavour and texture (Díaz-Mula et al., 2009; Romano et al., 2006). Fruit quality is largely determined by visual appearance (including size and colour), stem colour, firmness and flavour (Webster and Looney, 1996). The flavour of sweet cherries is largely determined by sugar content and acidity, and during storage acidity changes more rapidly than does soluble solids such as sugar (Drake and Fellman, 1987). Consumer panels are used to assess these factors and can be used to develop prediction models to estimate sensory responses potentially allowing the use of analytical measurements as a proxy for sensory evaluation (Ross et al., 2009).

The objective of this study was to determine firstly if the N concentration in cherry fruit could be manipulated by pre-harvest N fertiliser application and whether this had an influence on fruit quality at- and post-harvest. Secondly, we investigated whether consumers could determine differences in fruit quality after storage in non export quality fruit (as influenced by N fertiliser application) and export quality fruit and how this related to objective estimates of fruit quality.

## Materials and Methods

### Trial design

The fertigation trial was conducted at Grove Research Station in southern Tasmania on 10-year-old 'Lapins' trees, grown on F12-1 rootstock, pruned to a Spanish bush training system with tree spacing ranging from 1m to 2m. The variety was chosen to represent a commonly grown variety in the region and trees were subjected to standard orchard management with respect to irrigation, fertilisation and pest management. Row orientation was north-south with a very gentle slope downwards from the southern end of the row.

The experimental row was split into five blocks of 12 trees, buffered by three trees between each block. Each block was considered a replicate and was further split into four sub blocks of three trees to which N treatments were randomly allocated. To deliver the N treatments, four new 13mm polypipe lines were installed down the row, one for each treatment and 4L/hr pressure compensated drippers were fixed to the lines at approximately 15cm from the base of the treatment tree on the upward side of the slope. Nitrogen was applied as fertiliser grade $Ca(NO_3)_2$ mixed with water in a 44gn drum header tank and delivered via a fire-fighting pump at constant 10psi. Fertiliser application followed standard fertigation practise in the region with a 10min water only wetting up period, followed by a 40min application of N treatments and completed with a 10min water only rinse. To assess the influence of pre-harvest fertigation on cherry fruit quality and post harvest storage, N treatments included a 0N control, 25g N/tree, 50g N/tree and 75gN/tree split into four weekly applications commencing approximately one month after bud burst when root uptake of N is considered to have replaced remobilised N as its dominant N source (Grassi et al., 2003).

**Fruit quality and chemistry analysis**

Fruit was harvested from the middle tree of the treatment sub block coordinated with commercial harvest for the orchard. Approximately 3kg of fruit from all parts of the tree were picked and five trees were randomly selected from the trial for where all fruit were picked to estimate crop yield for the row based on weight. Harvested fruit was returned to the laboratory, weighed and sorted as either marketable or otherwise (cracked, rotted). For at-harvest fruit quality assessments, a subsample of 25 blemish-free was taken from each replicate and another 20 fruit from each replicate were sent to a commercial laboratory for chemistry analysis. To investigate post-harvest fruit quality, at the time of sorting, two further subsamples of 25 blemish-free fruit were taken from each replicate, placed into polyethylene bags, which were then sealed to maintain the ambient environment at close to 100% relative humidity and stored at 2 °C with no atmospheric control. Fruit quality assessments using these fruit were completed at 25 and 50 days post harvest.

Fruit quality parameters measured only at harvest included fruit diameter using digital vernier calipers and fruit weight, recorded using an AND digital balance (model GX-4000). At all assessment dates, skin colour, flesh firmness (on pared flesh) and skin puncture force and stem retention were measured following methods described by Bound et al (2013). Total soluble solids (TSS), pH and titratable acidity (TA) were also measured for each assessment date using juice collected from crushing fruit from each replicate following Bound et al., (2013).

**Sensory analysis**

To investigate consumer perception of two grades of cherry fruit quality: non export quality vs export quality, a sensory panel of likely consumers was established. Sensory panel analyses were conducted in January and February 2013 at the School of Agricultural Science, University of Tasmania. 57 adults between the ages of 18 and 75 with no special characteristics were recruited via email. Participants visually analysed and consumed cherry samples and filled in a questionnaire related to the samples. The questionnaire was conducted at harvest and 28 days postharvest on a subsample of cherries harvested from 'Lapins' trees receiving the High N treatment at Grove Research Station (non export grade) and export grade' Lapins' fruit harvested from Campania. A representative sample of 20 fruit from each quality grade was submitted to instrumental quality analysis as described above, at both assessment dates to enable a comparison between sensory panel preferences and instrumentation.

Due to varied ripening between locations, fruit from the Grove Research Station were harvested 20 days before the fruit from Campania and were placed in storage at 2 °C prior to the sensory panel assessment. Between assessment periods, harvested fruit was stored at 2 °C with no atmospheric control.

At the time of assessment, blemish-free fruit were selected and randomly allocated into plastic bowls with five fruit to a bowl. Consumers were provided with savoury crackers and water to rinse their palate between samples. The questionnaire consisted of eleven assessment variables which consumers ranked fruit on a scale from 1 to 5. These variables included size, firmness, colour, stem appearance, sweetness, acidity, flavour intensity, overall texture, juiciness, ripeness and overall fruit quality.

### Data analysis

Fruit quality assessment data was analysed using a univariate general linear model approach in SPSS with treatment and time considered as fixed factors. Significance was calculated at $P = 0.05$ and standard error used for comparison of mean values in the tables and figures. No data transformations were necessary.

The data from the sensory panel was analysed using SAS 9.2 statistical analysis software to determine the mean rank and standard error of each variable. Paired t-tests of each quality variable for both fruit grades were used to determine if consumers identified differences in mean rank of fruit quality variables between: (i) harvest and storage and; (ii) the two grades of fruit over time. Significant differences between instrumental measurements of fruit quality variables between: (i) assessment dates for High N cherries and export grade cherries and; (ii) cherry grades, were completed using independent t-tests in SPSS.

## Results and Discussion

### Nitrogen fertigation

Total N concentration (%) in cherry fruit at harvest was significantly ($F = 6.8$, $P = 0.004$) influenced by increased N supply under fertigation treatments (Fig.1), where fruit harvested under the highest N treatment contained the greatest amount of total N. Average yield for trees within the trial approximated 5-6 kg/tree, which is substantially less than expected for 10-year-old 'Lapins' trees within the region, possibly contributing to the substantial treatment effect. Furthermore, changes in orchard management in the two seasons prior to trial commencement saw trees in the orchard receive less than the standard N application recommended by industry. This result demonstrates the effectiveness of fruit competing against vegetative growth for available N via root uptake. In this trial, N treatments commenced approximately one month after bud burst when N supply has been shown to shift from N remobilisation to root uptake N (Grassi et al., 2002). This is also the period of greatest N uptake as demonstrated from comparisons between pre-and post harvest applications (Azarenko et al., 2008; San-Martino et al., 2010), however available N is partitioned to first year growth and fruit rather than to storage organs. Studies comparing varying pre-harvest N application are limited, however Neilsen et al., (2007) found a similar result where N concentration in 6 – 8-year-old 'Lapin' fruit increased after increased pre-harvest N fertigation.

There were significant differences in flesh firmness with level of N fertigation at harvest and post-harvest assessments ($F = 19.377$, $P < 0.001$) but timing of assessment was not significant (Fig.1). A significant interaction effect between treatment and assessment date ($F = 6.189$, $P < 0.001$) was observed and similar trends were found for Firmtech measurements and skin puncture tests (data not shown). Fruit harvested under the highest N treatment were significantly ($F = 3.44$, $P < 0.001$) less firm than under the lower N treatments and 0N control. No other fruit quality variables were significantly influenced by N treatments, however, significant effects attributed to assessment date were found for decreased TSS in storage ($F = 6.88$, $P = 0.002$), darker skin colour in storage ($F = 321.018$, $P < 0.001$), reduced stem pull force in storage ($F = 17.704$, $P < 0.001$) and reduced titratable acidity in storage ($F = 8.776$, $P < 0.001$) (data not shown).

Whilst studies in cherries and other fruits often report decreased firmness under high N applications, the mechanism for this is largely unknown. The increased fruit growth potential of N fertilization is largely recognised (Saenz et al., 1997). For cherry fruit with a rapid cell expansion stage, cell wall integrity may be compromised under conditions of oversupply of N. Furthermore, increased enzymatic rate under greater N availability may lead to faster fruit respiration and decay. Further research is required to test these hypotheses.

**Sensory analysis**

For the flavour attributes of high N fruit, consumers perceived significant ($P < 0.05$) reductions in sweetness, acidity and flavour intensity between the two assessment dates, yet no significant differences were perceived for the export grade cherries (Fig. 2a). Further, consumers clearly preferred the export quality fruit relative to the high N fruit for each flavour attribute (Fig. 2a). Instrumental measurements of TSS corresponded with consumer perception of sweetness with a reduction observed between assessment dates for high N fruit, but not for export grade fruit (Fig. 2b). The contrasting changes in TSS between the two fruit grades can be attributed to the older age of the high N fruit at initial assessment where fruit respiration had already consumed some available substrate. Consumers perceived a significant reduction in acidity in the high N fruit between assessments, however TA measurements indicated a significant increase in acid content in storage (Fig. 2b). This is difficult to explain and is not consistent with other studies (e.g. Puniran et al., 2010). Consumer perception of acidity in export grade fruit matched corresponding TA measurements and the significant differences in acid content between the two fruit grades were easily recognised by consumers.

Consumers perceived a significant increase in firmness in the high N fruit between assessments, whilst a significant decrease was observed for overall texture (Fig. 3a). A decreasing trend was observed for juiciness, however this was not significant. For the export grade fruit, consumers observed no differences in firmness, texture or juiciness (Fig. 3a). Consumers perceived high N fruit to be significantly firmer than export grade fruit, however, texture and juiciness were significantly less (Fig. 3a). Compression (Firmtech) and puncture (GUSS Texture Analyser) tests of high N fruit were similar between assessments and contrasted strongly with consumer perception of firmness (Fig. 3b). An increase in firmness 28 days post-harvest relative to 0 days post-harvest has been reported and is attributed to dehydration of the fruit post-harvest (Drake and Elfving, 2002; Puniran et al., 2010). Flesh firmness measured by puncture tests can also be indicative of fruit texture, and these results corresponded well with consumer perception of overall texture for both fruit grades at the two assessment dates and the comparison between them.

## Conclusion

Our results indicate that N concentration in sweet cherry fruit can be manipulated through increased N fertiliser applied through a drip irrigation system. This is most likely due to the competitive effectiveness of cherry fruit at sourcing available N during periods of rapid root uptake. The increased N concentration in fruit as a consequence of higher N application was shown to have a detrimental effect on fruit firmness as measured by compression and puncture tests. Whilst time in storage did not further exacerbate the effects of high N applications on firmness, post-harvest storage time did influence TSS, TA, skin colour and stem pull force, as expected as the fruit ages. The effectiveness of fruit in sourcing available N could be underestimated by growers and pre-harvest N applications must be monitored carefully.

Consumers identified deterioration in sweetness, acidity, flavour intensity, overall texture and juiciness after storage in fruit receiving a high N treatment. In contrast, no significant deterioration in export grade sweet cherry fruit after storage was noted by consumers and the differences between the two fruit grades was stark. Given the older age of the high N fruit at initial assessment, and that management and environmental differences between the two orchards were also variables, we cannot argue that the stark consumer perceptions of the two fruit grades were solely due to high N applied to the trees. However, we can conclude that consumer perception of fruit quality components sweetness, acidity and firmness matched that of objective measures for export quality fruit and the comparison between fruit grades, but that perception of increased firmness with time in storage of high N fruit did not align with objective measures. This may be because fruit dehydration can create an impression of firmer fruit due to coarser texture of fruit flesh.

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## Figures

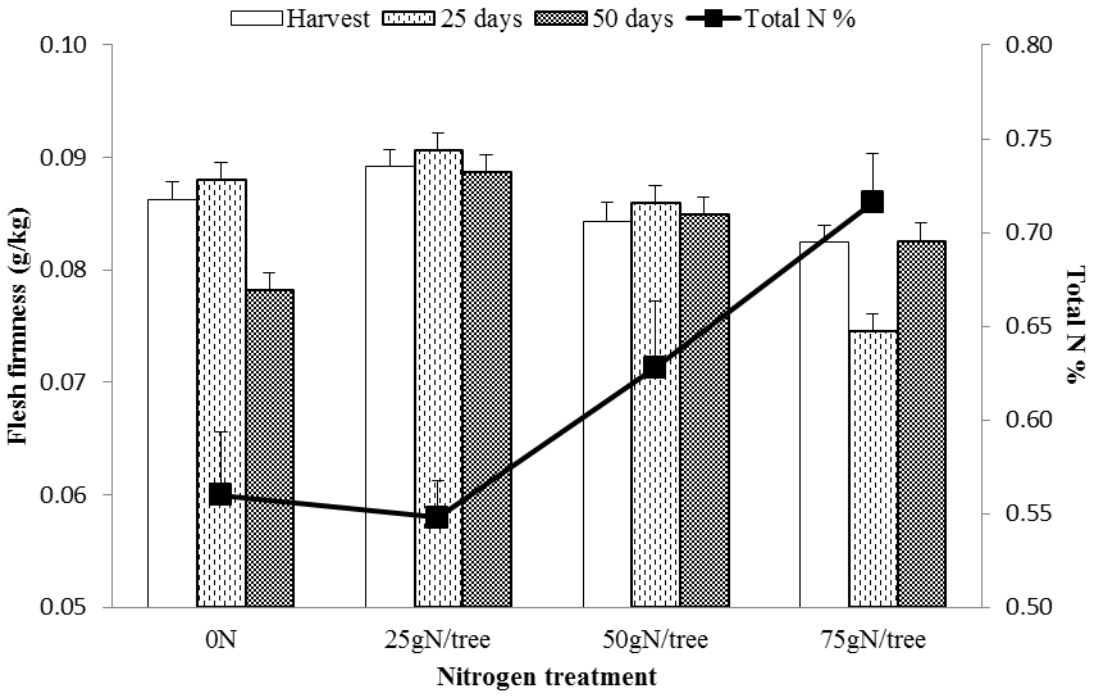


*Figure 1: Flesh firmness under different nitrogen (N) application treatments with time in storage. Total N concentration of fruit analysed at harvest is overlayed. Error bars represent standard error.*

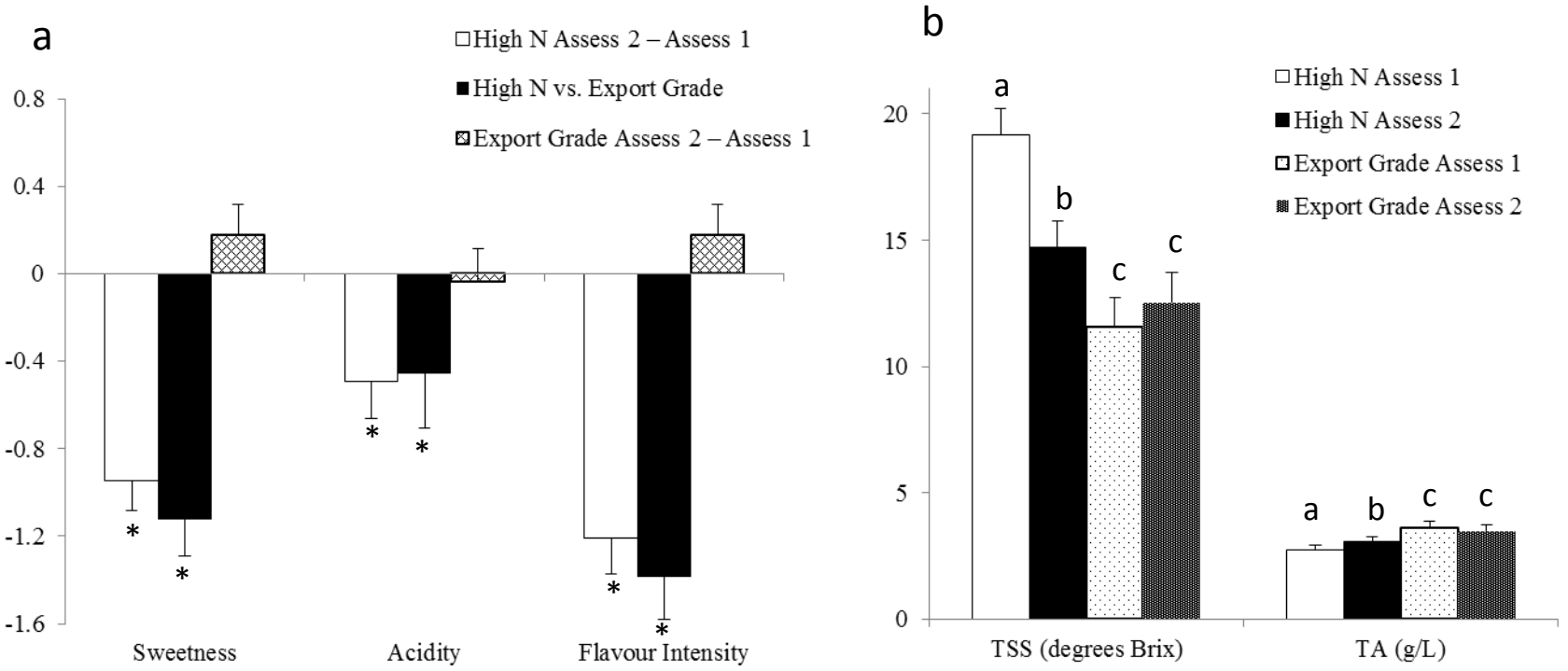


*Fig. 2 (a). Consumer perception of differences in sweetness, acidity and flavour intensity of High N and export grade sweet cherry fruit after storage. * represent significant (P < 0.05) differences in consumer perception between assessment dates. (b) Instrumental analysis of total soluble solids (TSS) and titratable acidity (TA) of High N and export grade sweet cherry fruit at two assessment dates. Letters indicate significant (P < 0.05) differences. Error bars represent standard error.*

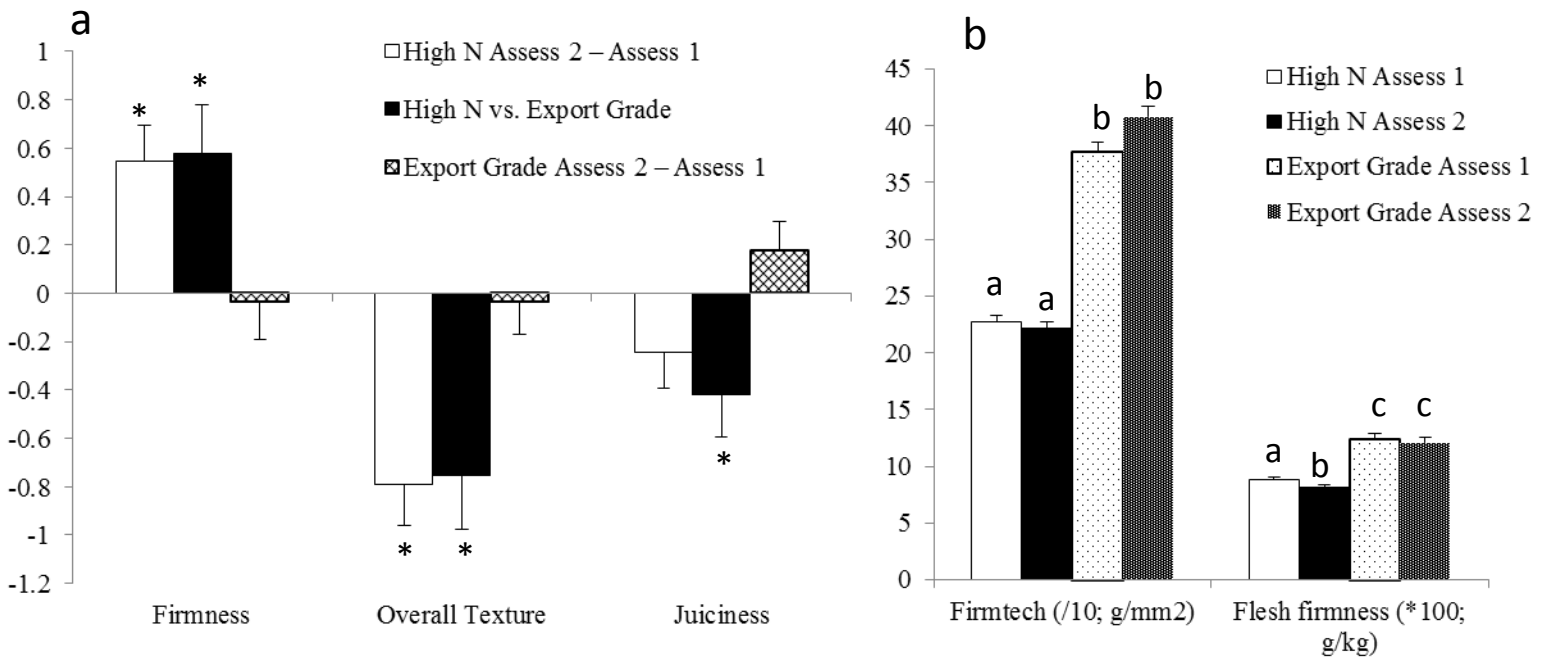


*Fig. 3 (a). Consumer perception of differences in fruit firmness, overall texture and juiciness of High N and export grade sweet cherry fruit after storage. * represent significant (P < 0.05) differences in consumer perception between assessment dates. (b) Firmtech and flesh firmness analysis of High N and export grade sweet cherry fruit at two assessment dates. Letters indicate significant (P < 0.05) differences. Error bars represent standard error.*