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Project leader: Dr Richard Colgan - Natural Resources Institute, University of Greenwich

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Key staff: Debbie Rees, Chris Atkinson, Clare Hopson, Karen Thurston NRI - University of Greenwich

Julien LeCourt - NIAB-EMR

Abi Dalton, Katie Hunt - FAST LLP

Mehrdad Mirzaee, Mark Tully, Colin Carter - Landseer

Location of project: NIAB/EMR, FAST LLP, Selected Gala orchards in Kent

Industry Representatives: Nigel Jenner, Paul Smith and Nigel Stewart

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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

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GROWER SUMMARY

Increasing Fruit Dry Matter content in Gala requires a combined approach of manipulating crop load, ensuring maximum light penetration into the middle and lower canopy, and ensuring tree nutrition is well managed.

Tall spindle trees were converted into trees with a centrifugal habit to allow light to penetrate a central column surrounding the trunk allowing increased light interception in the lower and middle canopy

This was achieved by removing buds from branches located near the trunk and removing buds from the underside of the branches which encouraged more fruit to form at the terminal end of branches. Once trees had recovered from the process of conversion, centrifugally pruned trees allowed greater light penetration in the lower canopy. Combining centrifugal pruning with the positioning of reflective covers lead to a higher rate of fruit growth in August and raised %FDM by 1% in fruit in the bottom of the canopy when compared with Gala harvested from the lower canopy in tall spindle trees where no reflective covers were positioned. The combination of the two techniques evened out %FDM of apples across the tree.

Fruit thinning techniques that reduced crop load generally improved accumulation of FDM- the impact was partly dependent on the weather in terms that in years of high sunshine hours (2020) then difference between thinning treatments was less obvious. Implementation of hand thinning early in fruitlet development (10-20 mm) increased fruit size at harvest. No single thinning treatment was consistent in increasing %FDM as weather played a part in determining final FDM. Hand thinning practices where fruitlets were removed early (10-20 mm) to single fruits per cluster across the tree, singles above 1.5M and doubles below 1.5 M, or doubles across the tree along with thinning to size were effective in raising %FDM in 2019. However, in 2020 where higher levels of irradiance were encountered then FDM content in hand thinned trees (15.5-16.1 %FDM) were similar to unthinned trees (16.1%). The use of Exilis and Brevis to chemically thin crop load was variable between years. Efficacy of spray treatments is very much dependent on the timing of application and the temperatures around the time of application. In terms of increasing %FDM at harvest Brevis raised %FDM in 2 years out of 4 and Exilis 1 year in 4. Brevis application resulted in a slight reduction in class 1 fruit in 2 years out of 4 years tested, while Exilis caused a slight drop-in class 1 yield in 1 out of 4 years. The main loss of quality in Exilis and Brevis treated fruit was a slightly higher proportion of diseased, or mishappen (Exilis) fruits at harvest.

The implementation of chlorophyll fluorescence (CF) as a method to monitor changes in fruit maturity were tested over a 3-year period. The method based on repeat monitoring of CF profile starting with baseline measurements taken from developing fruits at the end of July followed by 2-3 assessments in August to track the decline in CF outputs. The rate of change in CF declined formed the basis for predicting a harvest window for Gala providing a 7–10-day advanced warning that fruits were ready to be harvested.

Headline

- Centrifugal pruning combined with positioning of reflective covers in alleyways may increase FDM content in lower canopy fruit.
- Thinning strategies are more likely to increase FMD in fruit from the upper canopy.
- Early thinning events increased fruit size at harvest.

Background

Fruit dry matter (FDM) content is considered a good indicator of high sugar and acid content (% Brix^o) and eating quality of apples at harvest. Apples high in FDM tend to retain quality attributes over extended periods of storage. The extent to which orchard management practices during flower bud and fruit development affects FDM at harvest requires further attention. Moreover, the relationship between FDM and fruit ex-store quality throughout the storage season is of interest to the UK apple industry and may afford the opportunity to identify orchard consignments that can be stored for longer.

Several research groups, including the work of Palmer (1999) in New Zealand have linked high FDM at harvest to good quality and good storage potential. These studies were reviewed in AHDB-Horticulture (TF 222) and although previous research highlights the potential to use FDM as a proxy measure of fruit quality, much of this work was correlative. FDM is a reflection of fruit carbohydrate content, where soluble solids content, measured as % Brix and starch are the major constituents.

The underlying basis of this relationship needs to be better understood so that it can be manipulated to deliver premium fruit quality. This will be achieved through a combination of a meta-analysis of existing data sets to obtain a greater understanding of the factors controlling both FDM and quality, and the development of practical strategies in terms of novel pruning strategies, reflective mulches and manipulation of crop load through bud and fruit thinning to help growers to improve the quality of stored apples.

The breakdown of starch into soluble solids content during fruit ripening makes FDM a valuable and accurate indicator of potential post-harvest soluble solids content. Improving the quality of stored apples and pears is an important priority area for AHDB Horticulture. A key indicator of fruit quality and storability is thought to be fruit dry matter content (FDM) as recent studies have suggested there is a good correlation between the FDM of apples and the ex-store sugar levels and eating quality

FDM is influenced by tree and fruit physiology and significantly affected by environmental conditions within and between seasons and cultural practices. Further research in this area is required to determine how environmental conditions and management practices employed during growth and development affect FDM at harvest and during storage and to determine the relationship between FDM and fruit ex-store quality for UK fruit.

In order to deliver 'Best Practice' to the top-fruit industry to improve FDM a series of work packages have been set up initially working on discrete aspects of husbandry with the aim of bringing together different components of each work package in the later stages of the project to form a single trial plot.

Work package 1 summarised existing data sets of FDM content acquired from orchard sample analysis over 2 years linked to mineral analysis of fruits, leaves and soil analysis. Additional work packages centred on increasing light penetration through the canopy and reflecting available light back up into the lower canopy (WP 2). While in WP3 the impact of crop load and thinning practices on buds, flowers and the timing of fruitlet removal was investigated. In WP 4 the use of chlorophyll fluorescence was investigated to quantify changes in advancing fruit maturity in a selected range of Gala orchards with the aim to increase the prediction window to schedule harvesting.

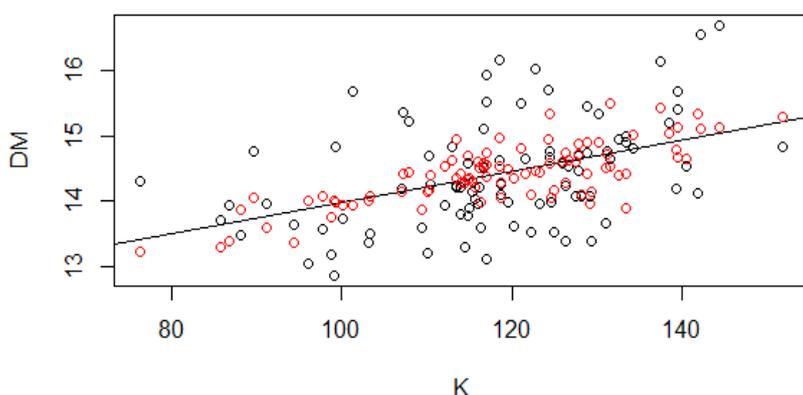
Summary

Work Package 1: Meta Analysis

Orchard surveys compiled by FAST have been collated on dry matter and mineral analysis from fruitlets, soil and leaves of 56 Gala orchards.

A preliminary multiple regression analysis was used to determine whether mineral content of soils influences fruit development and dry matter accumulation and, moreover, the extent to which mineral content of the soil influences leaflet and fruitlet analysis. From the two years of available data (2015-2016) FDM variation across all the data sets ranged from 13.6% to 18.9%, a span of 5.3% FDM. The Gala dataset consisted of 56 measurements with a range from 13.8% to 16.0% FDM and a span of 2.2% FDM.

Multiple regression analysis of fruit mineral content and FDM identified that there was a weak positive relationship between higher fruit potassium and magnesium and higher FDM content while higher fruit zinc content led to lower FDM (Figure 1).



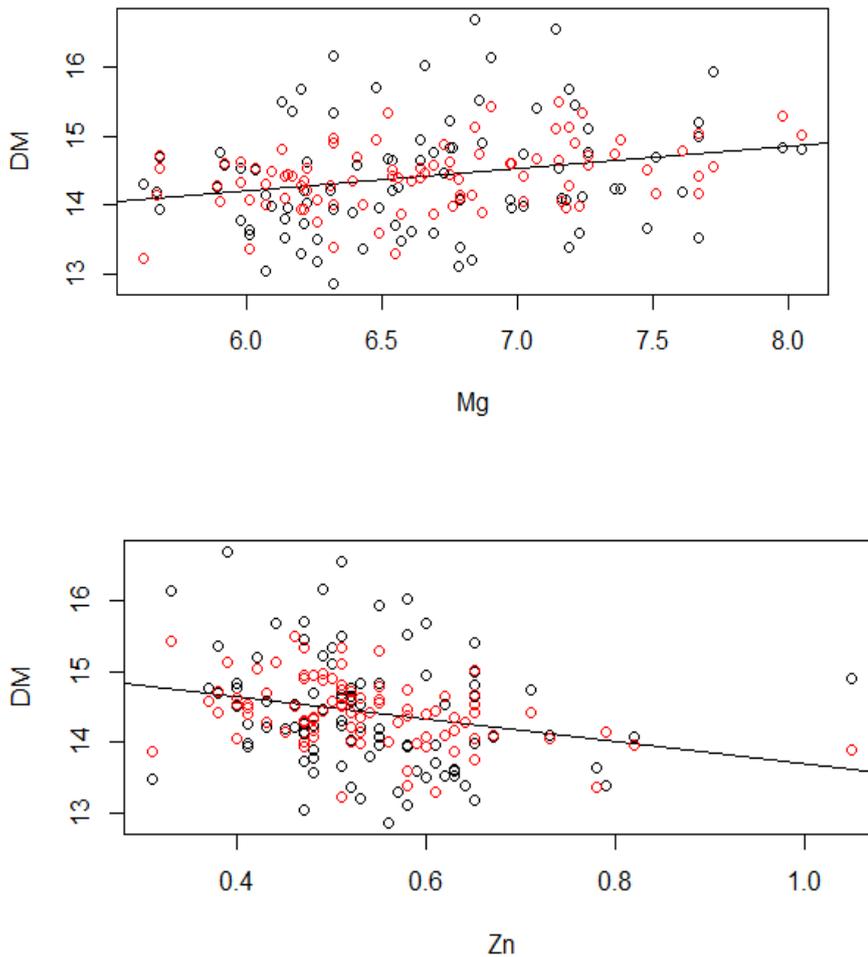


Figure 1: Multiple regression analysis depicting the positive relationship between K, Mg with FDM and a weak negative relationship with Zn. Concentration of mineral elements are in mg/100g FW.

Work Package 2: Manipulation of Light Interception into the Canopy (NIAB-EMR/NRI)

The reflective covers resulted in 29.45 % of the incident light to be reflected to the canopy against 4.27 % in the zones without (Figure 2). The results as shown below represent the mean of twenty measurements per treatment showing statistically significant differences between the treatments,

The Centrifugate System (CS) shows a greater amount of light penetrating the canopy recording 19.8 % light interception compared against 12.5 % in Tall Spindle (TS) (Figure 2). However, this difference was not statistically significant due to the large inter-tree variability.

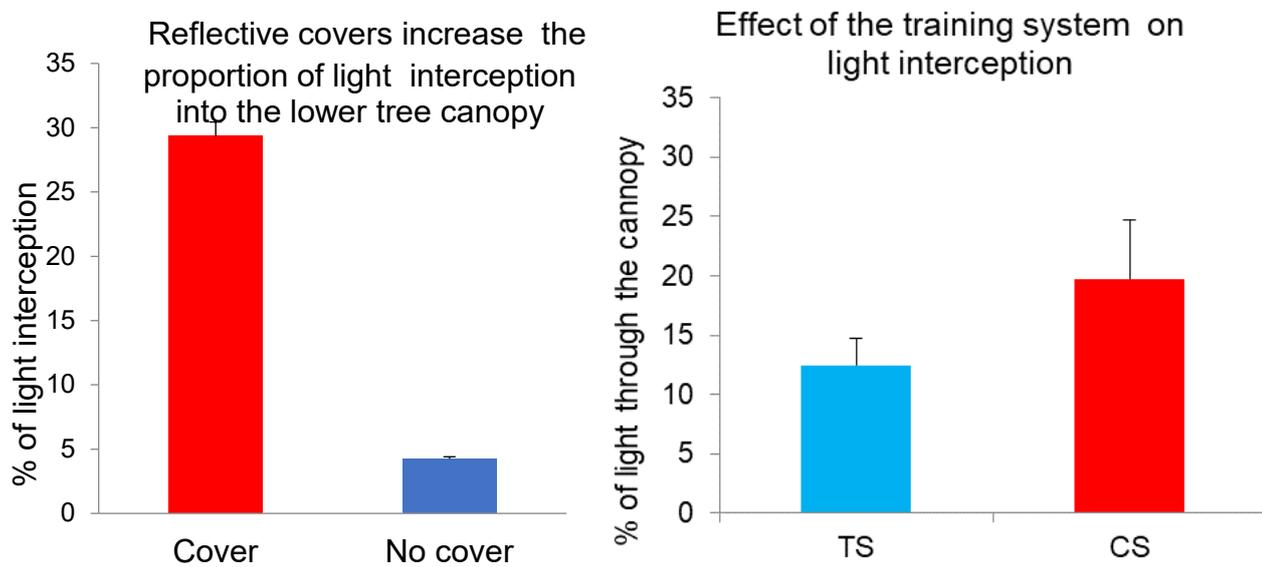


Figure 2: Light interception levels measured in the lower canopy (>1.5 M) where trees had been pruned using centrifugal pruning and/or where reflective covers had been added.

More specially the regions of the canopy that light from centrifugal pruning techniques penetrates when either applied alone or in combination with reflective covers with was investigated. Light interception in the centre of the tree and the lower canopy most benefited from combining centrifugal pruning and reflective covers.

Centrifugal pruning alone increased light interception into the lower and mid-canopy and when combined with reflective covers there was an additive effect of raising light penetration into the lower and mid canopy of the Gala apple trees (Figure 3). The impact of greater light penetration was on increased rate of fruit growth. Improving light interception combining centrifugal pruning with reflective covers led to increased fruit growth in Gala measured in August though to Harvest in September (Figure 4.0).

Effect of the pruning system on the % of light intercepted by the canopy



Figure 4: The % light Interception measured by Acupar at different points in the tree canopy

Increase in Fruit Diameter (mm) Later Stages of Fruit Development and Maturation- 2019

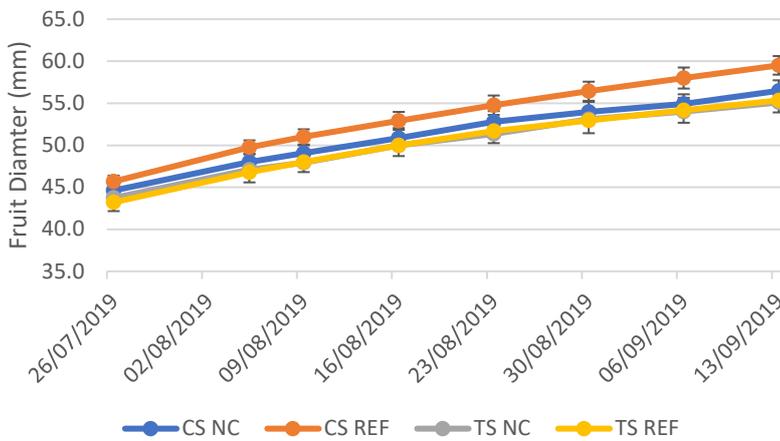


Figure 5: Fruit size increases in Gala under centrifugal training systems (CS) when light reflective covers (REF) were inserted in the alleyways during the later stages of fruit development and maturation compared to standard tall spindle training systems (TS).

For the first few years after trees were converted into centrifugally pruned trees yields and accumulation of dry matter content were lower than conventionally pruned (tall spindle) trees. In Year 4 of the project when trees have recovered sufficiently, the overall effect centrifugal pruning (+/- reflective covers) and where %FDM was averaged across fruit from the top and bottom of the canopy was o increased fruit dry matter (FDM) by 0.3% (Table 1). Overall, the impact of reflective covers (averaged across pruning system) was to

raise FDM matter across the tree by 0.3%. Apples harvested from the top of the tree (>1.5M) were higher in %FDM by 0.3%.

The combination of reflective covers, centrifugal punning was most effective in raising the %FDM in fruit from the lower canopy where light is most restricted, at this point in the canopy CS+ REF led to a 1% increase in %FDM (15.6%) compared to 14.6% in fruit from the lower canopy of Tall Spindle trees where no reflective covers were installed (Table 1.0).

Fruit maturity was affected by adoption of pruning system. Apples from the upper part of CS trees were less mature based on Internal Ethylene Concentration, this may be due to crop load still having an effect or tree architecture where more fruit on CS trees was borne on younger wood. The % Brix at harvest was not impacted by pruning, light interception or fruit maturity.

Table 1: The interaction between Training Systems, Reflective Covers and Fruit Position on Fruit Maturity attributes at Harvest

	Reflective Covers	Int. Eth. Conc. ppb		Starch CTIFL		% FDM		% Brix		Firmness (N)	
		Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
Tall Spindle	Yes	385	366	6.8	8.1	14.8	14.6	11.9	11.8	79.6	77
	No	348	377	6.2	6.5	14.4	14.6	12.1	11.8	83	81.3
Centrifugal	Yes	153	204	5.8	6.1	14.8	15.6	11.7	12	83.6	86.6
	No	179	330	6.7	6.8	14.6	14.9	12	12.1	82.5	82.7
F.prob		0.545		0.349		6.42		0.419		0.327	
LSD _{0.05}		80.5		0.35		0.3867		0.54		3.97	

The impact of pruning and increasing light interception had marginal impact on the sugar profile of fruit, with the exception that fruit subject to reflective covers had higher concentrations of sucrose which in previous trials has been an indicator of less advanced maturity with sucrose later being converted to glucose and fructose.

Table 2: Overall effects of training system, sampling position and the presence of reflective covers on % FDM and fruit maturity

Pruning	Tall Spindle	Centrifugal	F.prob	LSD _{0.05}
I.E.C (ppb)	369	216	<.001	42.5
% FDM	14.6	15.0	0.001	0.08

CTIFL starch	6.9	6.3	0.014	0.41
Fructose	130.6	122.7	0.082	12.92
Glucose	18.8	19.3	0.689	2.78
Sucrose	90.3	85.7	0.18	7.12
<i>Covers</i>	<i>Reflective Covers</i>	<i>No Covers</i>	<i>F.prob</i>	<i>LSD_{0.05}</i>
<i>I.E.C (ppb)</i>	<i>277</i>	<i>308</i>	<i>0.147</i>	<i>42.5</i>
% FDM	14.9	14.6	0.006	0.08
CTIFL starch	6.7	6.5	0.419	0.41
Fructose	128.4	124.9	0.393	12.92
Glucose	17.8	20.3	0.069	2.78
Sucrose	96.2	79.8	<.001	7.12
<i>Position</i>	<i>Top</i>	<i>Bottom</i>	<i>F.prob</i>	<i>LSD_{0.05}</i>
<i>I.E.C (ppb)</i>	266	<i>319</i>	<i>0.016</i>	<i>42.5</i>
% FDM	14.9	14.6	0.014	0.08
CTIFL starch	6.9	6.4	0.027	0.41
Fructose	130.6	122.7	0.084	12.92
Glucose	18.8	19.3	0.688	2.78
Sucrose	88.7	87.3	0.681	7.12

N.B numbers in bold represent values significantly different (P<0.05) in the same row

Work Package 3. Manipulation of Crop Load to improve Dry Matter Accumulation (FAST LLP, NRI)

The main aim of the project was to determine how manipulation of crop load either through reducing the number of developing fruit buds, reducing flower precocity and fruitlet number can impact on accumulation of fruit dry matter and increasing % Brix at harvest and development during storage. While thinning practices have an important bearing on increasing fruit size, the impact on raising fruit dry matter is less clear. In two out of the three years tested standard thinning practices were able to raise FDM by 0.7- 1.1% over unthinned trees (Table 3). Thinning to size and late thinning also in some years increase %FMD, however a strong seasonal effect was seen. In 2018 and 2020 were years of higher total sunshine hours accumulated during fruit development and in these years the difference between thinned fruit and unthinned in terms of %FDM accumulation was lower than in years 2017 and 2019 that were duller.

In the later years of the trials thinning to single fruitlets per cluster across the tree or singles (>1.5 M) and doubles (<1.5 M) or retaining double fruitlets per cluster throughout the tree, where applied at an earlier growth stage than normal had only a small impact on raising %FDM but did increase fruit size. Chemical thinning agents offer the potential to minimise labour costs in terms of achieving optimum crop load. %Brix content of fruit at harvest was a seasonally affected with %Brix oscillating 1-2 % between seasons (Table 4). No single thinning treatment led to higher sugars at harvest, but sugars continue to rise in store post-harvest

Total yields were generally slightly lower in thinned trees, but the amount of Class I fruit harvested was consistently higher in thinned trees. Of the approaches taken

Table 3: The impact of thinning practices on accumulation of % Fruit Dry Matter content in Gala apples

%FDM	Control	Bud	Mechanical	Exilis	Brevis	Std	Size	Late	Single	Singles / Doubles	Doubles
2017	16.4	15.9	16.0	16.6	16.3	16.4	16.7	17.0	x	x	x
2018	15.7	15.5	16.4	15.7	16.9	16.9	15.9	15.8	x	x	x
2019	15.4	x	x	15.7	16.1	16.5	16.5	x	16.4	15.7	16.1
2020	16.1	x	x	16.7	15.7	x	x	x	15.8	15.5	16.1

Table 4: The impact of thinning practices on % Brix

% Brix	Control	Bud	Mechanical	Exilis	Brevis	Std	Size	Late	Singles	Singles / Doubles	Doubles
2017	11.9	11.9	11.6	11.6	12.1	11.8	12.1	13.3	x	x	x
2018	12.6	12.7	12.6	12.8	13	13	12.8	12.6	x	x	x
2019	13	x	x	13.5	13.8	13.9	13.9	x	13.7	13.4	13.5
2020	12.3	x	x	12.6	12.1	x	x	x	11.9	11.4	12.2

Table 5: The effect of thinning practices on total yield of Gala trees

Total yield kg/ha	Control	Bud Thinning	Mechanical	Exilis Fixor	Brevis	Std Hand Thinning	Size hand thinning	Late thinning	Singles	Singles / Doubles	Doubles
2017	62.2	55.5	55.9	58	60.9	55	62	51.3	x	x	x
2018	78.3	80.5	83.2	68.2	77.4	85.3	84	87.8	x	x	x

2019	105.8	x	x	86. 4	65.6	80.7	83.0	x	71.3	87.9	72.5
2020	60.9	x	x	49. 8	62.7	x	x	x	56.5	68.3	62

N.B Yield was based on a total of 12 trees sampled across 4 plots- 3 trees per plot

Table 6: The impact of thinning practices on Class 1 fruit.

Class 1 kg /ha	Control	Bud Thinning	Mechanic al	Exili s & Fixo r	Brevi s	Std Hand Thinnin g	Size hand thinnin g	Late thinnin g	Singles / Double s	Singles	Double s	Double s
Year 2	33.5	33.6	34.7	35. 3	29.6	28.8	36.2	29.7	x	x	x	x
Year 3	53.6	56.5	55.8	44. 9	46.5	53.4	48.3	48.3	x	x	x	x
Year 4	53.6	x	x	53. 4	31.6	50.0	51.1	x	45.0	52.3	42.0	42.0
Year 5	24.4	x	x	22. 1	29.3	x	x	x	28.8	28.7	31.9	31.9

Table 7 (a-d): The impact of thinning practices on wastage categories for each of the 4 years (2017-2021) of the thinning trial

Table 7(a)

2017	Control	Bud	Mechanical	Exilis	Brevis	Std	Size	Late
Other	0.8	1	1	0	0	6.7	3.8	2.8
Lack % Red	15.1	19.8	40.2	25.7	37.7	28.3	19	9.3
Diseased	1.7	2	4.1	1	1.4	0	0	0
Small	20.2	8.9	4.1	16.2	12.3	10	10.5	13.5
Misshapen	2.5	4	2.1	7.6	2.2	10	5.7	2.8
Damage - pest/physic.	52.1	61.4	42.3	45.7	41.3	40.8	56.2	68.2
Scarring/Russet	7.6	3	6.2	3.8	5.1	4.2	4.8	3.7

Table 7 (b)

2018	Control	Bud	Mechanical	Exilis	Brevis	Std	Size	Late
Other	1.8	0	2.1	0	0	0	0	0
Lack % Red	5.3	3.7	2.1	2.2	7	0	5	0
Diseased	15.8	24.1	16.7	30.3	29.8	20.5	52.5	25
Small	42.1	42.6	20.8	25.8	10.5	30.8	7.5	50
Misshapen	7	3.7	20.8	24.7	12.3	7.7	2.5	10
Damage - pest/physic.	19.3	16.7	20.8	7.9	22.8	28.2	30	10
Scarring/Russet	8.8	9.3	16.7	9	17.5	12.8	2.5	5

Table 7 (c)

2019	Control	Singles	Singles/Doubles	Exilis	Brevis	Standard	Size	Doubles
Lack % Red	0.5	1	3	0.8	1.5	1.5	0.5	2
Diseased	9.8	10.5	7.3	7.8	12.3	11.8	11.5	9.5
Small	12.5	1.3	2.3	4.3	7.3	1	0.8	1.5
Misshapen	1.5	2	2.3	2.8	1.8	0.8	1	2.3
Damage - pest/physic.	7.5	5.5	8.5	6.5	9.3	8	9	9.3
Scarring/Russet	1.5	2.8	2	1.5	0.8	0.8	1	2

Table 7 (d)

2020	Control	Singles	Singles/Doubles	Exilis	Brevis	Doubles
Lack of % red	10.3	12.3	9.1	10.3	19.6	8.8
Diseased	25	47.7	46.9	37.4	29.4	30.2
Small	43.1	21.8	21.1	26.3	27	38
Mis-Shape	1.1	2.2	0	5.4	0.8	0
Damage - pest/physical	17.7	11.1	18.7	17.7	20.9	19
Scaring/damage	2.8	4.9	4.3	2.9	2.3	3.9

Table 8 (a-e): Fruit Size distribution (mm) of Gala subject to thinning treatments implemented at different stages of bud, flower, and fruitlet development

Table 8 (a)

55-60	Control	Bud	Mechanical	Exilis	Brevis	Std	Size	Late	Singles	Single/ Doubles	Doubles
2017	18.5	11.3	10.4	12.2	13.9	9.8	13.7	3	x	x	x
2018	31	23.7	10.7	9.3	20.8	12.7	2.5	16.1	x	x	x
2019	32.4	x	x	16.2	13.7	8.4	0.3	x	0.8	1.4	2.1
2020	36.7	x	x	15.8	23.8	x	x	x	18.2	24.8	35.4

Table 8 (b)

60-65	Control	Bud	Mechanical	Exilis	Brevis	Std	Size	Late	Singles	Single/ Doubles	Doubles
2017	32.2	25.5	24.3	28.8	33.3	21.1	17.3	16.4	x	x	x
2018	37	39.2	33.5	31.1	31.7	35.5	30.5	37.1	x	x	x
2019	36.8	x	x	49.1	46.9	42.0	29.3	x	19.3	21.1	12.4
2020	42.1	x	x	33	36.5	x	x	x	33.6	38.6	34.6

Table 8 (c)

65-70	Control	Bud	Mechanical	Exilis	Brevis	Std	Size	Late	Singles	Single/ Doubles	Doubles
2017	41.9	32.6	32.6	38.1	25.9	32.7	30.9	43.3	x	x	x
2018	19.6	28.5	33	35.8	33.3	38.6	47	32.7	x	x	x
2019	26.8	x	x	26.0	35.8	42.4	52.3	x	51.0	49.8	44.5
2020	16.8	x	x	35.7	26.1	x	x	x	27.3	22.6	23.9

Table 8 (d)

70-75	Control	Bud	Mechanical	Exilis	Brevis	Std	Size	Late	Singles	Single/Doubles	Doubles
2017	7.3	23.4	22.9	15.8	22.2	21.2	29.5	25.4	x	x	x
2018	12	8.6	19.8	20.5	12.6	12.2	18	14.1	x	x	x
2019	3.9	x	x	8.7	3.7	7.2	16.3	x	25.0	27.7	26.5
2020	4.4	x	x	15.5	10.6	x	x	x	17.8	11.3	3.4

Table 8 (e)

75-80	Control	Bud	Mechanical	Exilis	Brevis	Std	Size	Late	Singles	Single/Doubles	Doubles
2017	0	7.1	9.7	5	4.6	16.3	8.6	11.9	x	x	x
2018	0.5	0	3	3.3	1.6	1	2	0	x	x	x
2019	0.0	x	x	0.0	0.0	0.0	1.9	x	3.9	0.0	14.5
2020	0	x	x	0	0.6	X	x	x	3.2	2.8	2.7

Work Package 4. Prediction of harvest maturity of Gala using Chlorophyll fluorescence Landseer Ltd

Work package 4 focused on developing a non-destructive method to optimise harvest date and identifying the orchards suitable for long-term storage. This can be achieved by choosing the right fruit with high dry matter and balanced minerals that are picked at the right time for extended keeping quality during long term storage. If this process is carried out correctly then UK Gala should compete effectively with Southern hemisphere fruit both on fruit firmness and, more crucially, taste.

Current practice for harvesting of Gala for long term storage is to pick fruit when starch coverage has dropped to 85-80% coverage and where background red colour has developed sufficiently to satisfy the marketing desks. However, this narrow window does not afford growers sufficient time to organise harvesting crews in time to pick orchards before maturity advances observed through declining starch content. The results gathered between 2016-2018 confirmed that monitoring fruit using a chlorophyll fluorescence meter provided a non-destructive tool for fruit maturity and that changes in CF output were observed 7 to 10 days prior to changes in starch clearance patterns providing an early warning to growers to organise their picking schedules around the optimum harvest date for long-term storage. Where CF profiles was used on orchards where fruits had ample mineral content for long-term storage and high FDM then picking fruit at optimal maturity meant fruit performed well in long-term storage.

Sampling of Gala orchards for CF analysis was standardised to reduce variability, fruitlets (25-30mm) were picked in 9 selected orchards in Kent in the first week of July 2017. Samples were taken from each compass point on a tree, North, East, South, and West (4 fruitlets per tree, all samples picked from middle height of trees). Samples were taken in a “W” pattern across the orchard taking samples at appropriate points.

In the first week of August sample collection fruit (55-60 mm) from 9 orchards were repeated. After analysing CF, mineral profiles and FDM of fruit, according to the flow chart (see below) five of orchards were selected based on their suitability for long-term storage and were monitored with CF for a period of 2-3 weeks prior to commercial harvest. CF prediction model was restricted to fruit intended for long term storage (Table 9).

Table 9: Comparison of dry matter and mineral analysis in 9 orchard and selecting 5 orchards for the long-term storage (season 2017-18).

FIELDREF	Test	Clone	DMC	CF (Avf)	WT	Interpretation	N	Interpretation	P	Interpretation	K	Interpretation	Mg	Interpretation	Ca	Interpretation
Orchard 1	Fruitlet (July 2017)	Schneiga	15.6	4664	36.34	Normal	87.36	Normal	13.62	Low	147.78	High	11.02	High	16.59	High
	Fruit (August 2017)		16	4703	97.71	Normal	35.2	Low	9.4	Very Low	94.28	Sli Low	7.45	Normal	10.49	High
Orchard 2	Fruitlet (July 2017)	Mondial	13.2	5665	40.22	Normal	83.16	Normal	13.21	Low	135.24	Normal	10.04	Normal	14.42	Normal
	Fruit (August 2017)		14.6	4752	103.06	Normal	39.42	Sli low	7.55	Very Low	87.9	Low	6.92	Normal	10.24	High
Orchard 3	Fruitlet (July 2017)	Galaxy	14.2	4989	48.28	Normal	55.38	Sli low	14.73	Sli Low	129.68	Normal	9.41	Normal	14.86	High
	Fruit (August 2017)		13.6	4291	111.31	Normal	36.72	Sli low	11.08	Sli Low	93.09	Sli Low	6.55	Normal	10.71	High
Orchard 4	Fruitlet (July 2017)	Galaxy	13.2	4775	43.51	Normal	80.52	Normal	13.02	Low	145.7	High	9.83	Normal	16.34	High
	Fruit (August 2017)		13.6	4553	101.74	Normal	38.08	Sli low	8.39	Very Low	86.75	Low	6.95	Normal	10.06	High
Orchard 5	Fruitlet (July 2017)	Schneiga	14	5914	47.64	Normal	81.2	High	13.45	Low	125.9	Normal	8.78	Normal	14.15	Normal
	Fruit (August 2017)		13.6	6109	85.96	Normal	48.96	Normal	9.36	Very Low	69.26	Very Low	7.11	Normal	10.69	High
Orchard 6	Fruitlet (July 2017)	Mondial	12.8	5983	46.57	Normal	67.84	Normal	13.1	Low	103.43	Sli Low	8.08	Normal	15.43	High
	Fruit (August 2017)		13	5363	98.36	Normal	46.8	Normal	9.7	Low	85.76	Low	7.03	Normal	13.9	High
Orchard 7	Fruitlet (July 2017)	Mondial	14.2	4660	46.66	Normal	65.32	Normal	14.3	Sli Low	118.25	Normal	9.28	Normal	16.21	High
	Fruit (August 2017)		12.8	4726	114.86	Normal	42.24	Normal	10.45	Low	64.28	Very Low	6.04	Normal	10.17	High
Orchard 8	Fruitlet (July 2017)	Galaxy	13.6	6441	49.59	Normal	72.08	Normal	11.16	Very Low	124.99	Normal	9.3	Normal	14.59	High
	Fruit (August 2017)		13.2	5163	88.01	Normal	36.96	Low	6.48	Very Low	71.29	Very Low	6.16	Normal	10.85	High
Orchard 9	Fruitlet (July 2017)	Mondial	13.4	6602	58.42	Normal	73.7	High	15.01	Normal	144.37	High	8.82	Normal	14.53	High
	Fruit (August 2017)		14	5403	135.49	High	36.4	Sli low	8.7	Low	84.04	Low	6.04	Normal	8.99	High

A comparison of CF outputs based on the formula below. The formula charting CF decline with advancing maturity requires construction of a baseline CF measurement at fruitlet (25-30 mm) stage and continuing measuring fruits with the PEA fluorimeter until the reduction passes 50% of the baseline CF level taken at fruitlet stage:

$$CF \text{ degradation} = \frac{(Fn - \sigma Fn)}{(F1 - \sigma F1)} < 50\%$$

Standard starch, firmness and % Brix readings were made for each pick date.

Fruits were harvested from each orchard samples based either on the prediction from the decline in chlorophyll fluorescence termed “CF pick” and or harvested when fruits had reached 80% Starch content based on standard iodine staining of an equatorial section of fruit termed “Starch pick”. Half of the samples were treated with SmartFresh. Samples were stored in two regimes and locations for 9 months:

5%CO₂: 1%O₂ (Control & +SF) at (Howt Green) (only CF pick samples).

5%CO₂: 1%O₂ (Control & +SF) at PQC (East Malling) (CF pick and starch pick samples).

Initial monitoring of fruit coming out of commercial stores (5%CO₂: 1%O₂) was limited to mid-April with subsequent assessments in mid-May 2018.

Samples stored in the PQC were stored until mid-June 2018 then all samples were tested for CF, dry matter, mineral analysis and quality assessments. Samples in May were sent for mineral analysis and FDM assessment to YARA analytic. Fruits were subject to CF measurements, fruit firmness, Brix^o and acidity analysis at Landseer.

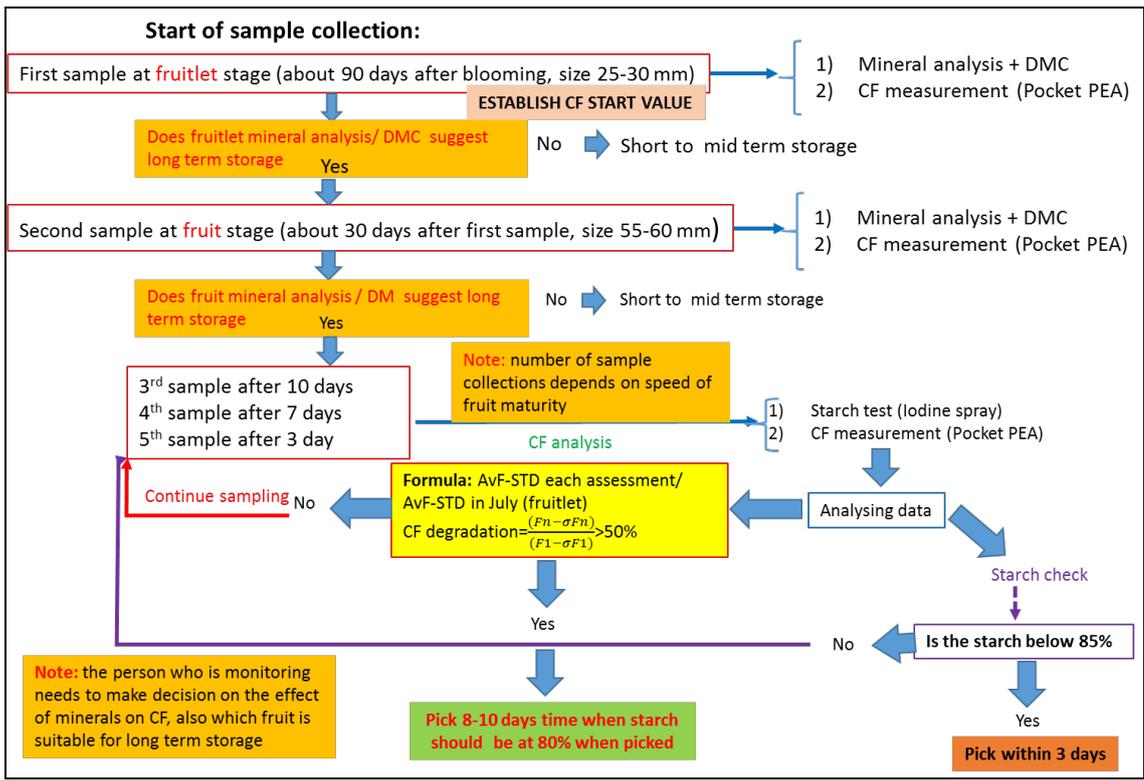


Figure 6: Decision tree flowchart for the process of sampling and analysing data for selecting the best orchards for long term storage and early warning for the best picking date.

Table 10: Comparison of fruit maturity warning by chlorophyll fluorescence Pocket PEA(CF) and starch staining patterns (2018).

2018										
	AvF-STD (11 July)	AvF-STD (14 Aug)	AvF-STD (17 Aug)	AvF-STD (20 Aug)	AvF-STD (23 Aug)	AvF-STD (27 Aug)	AvF-STD (31 Aug)	AvF-STD (3 Sept)	AvF-STD (7 Sept)	AvF-STD (10 Sept)
Barnyard	4761	3685	3145	2911	3041	1838	1492	1634	797	696
Ratio to July		0.77	0.66	0.61	0.64	0.39	0.31	0.34	0.17	0.15
	DMC:15	DMC:13.4				CF alert		CF pick	DMC:12.6	
Starch		92%	90%	92%	90%	85%	82%	80%	75%	70%
Monks	4566	3180		3097	3054	2034	1198	1227	644	334
Ratio to July		0.70		0.68	0.67	0.45	0.26	0.27	0.14	0.07
	DMC:14.8	DMC:15				CF alert		CF pick	DMC:14.2	
Starch		94%		94%	92%	90%	86%	80%	75%	70%
Gibbens	4959	3773	3805	3348	3194	2639	1905	1738	1214	897
Ratio to July		0.76	0.77	0.68	0.64	0.53	0.38	0.35	0.24	0.18
	DMC:16.6	DMC:15.4				CF alert			DMC:14.6	CF pick
Starch		95%	94%	92%	92%	92%	92%	90%	85%	82%
Hill Top	4549	3038	2842	2642	2584	1585	1042	1069	416	304
Ratio to July		0.67	0.62	0.58	0.57	0.35	0.23	0.23	0.09	0.07
	DMC:15.6	DMC:13.6				CF alert		CF pick	DMC:13.4	
Starch		93%	91%	91%	90%	87%	86%	80%	75%	70%
Mystole	4665	3410		3040	3158	2412	2069	1652	1292	696
Ratio to July		0.73		0.65	0.68	0.52	0.44	0.35	0.28	0.15
	DMC:16.2	DMC:15.6				CF alert			DMC:14	CF pick
Starch		94%		94%	92%	92%	91%	86%	87%	82%

CF analysis predicted that Gala orchards from Barnyard, Monks and Hill Top should be picked in advance of orchards Gibbens and Mystole. Samples of apples from these orchards were analysed for Internal Ethylene Concentration (IEC) at the Produce Quality Centre. Based of IEC values the maturity of apples from selected orchards was ranked Monks>Barnyard>Hill top> Gibbens>Mystole, with Gala from Monks being the most mature and Mystole the least (Figure 7)

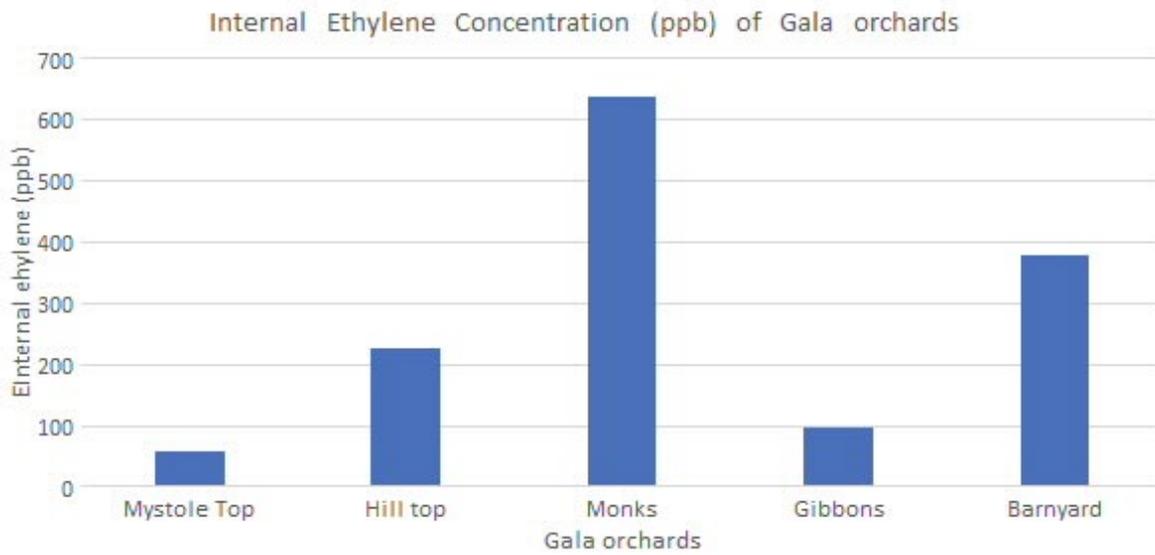


Figure 7: comparison of internal ethylene production in samples from each orchard 10 days before harvest.

SCIENCE SECTION

Introduction

Improving the quality of stored apples and pears is an important priority area for AHDB Horticulture. A key indicator of fruit quality and storability is thought to be fruit dry matter content (FDM) as recent studies have suggested there is a good correlation between the FDM of apples and the ex-store sugar levels and eating quality (Harker et al., 2009; Jordan et al., 2000; Palmer et al., 2010).

Several research groups have linked high FDM at harvest to good quality and good storage potential; FDM reflects fruit carbohydrate content, where soluble solids content (SSC) and starch are the major constituents. The hydrolysis of starch into SSC during fruit ripening makes FDM a valuable and accurate indicator of potential postharvest SSC, or of actual SSC once hydrolysis is complete (Jordan et al., 2000; McGlone and Kawano, 1998; McGlone et al., 2003).

FDM is influenced by tree and fruit physiology and significantly affected by environmental conditions within and between seasons and cultural practices. Further research in this area is required to determine how environmental conditions and management practices employed during growth and development affect FDM at harvest and during storage and to determine the relationship between FDM and fruit ex-store quality for UK fruit.

Fruit and tree development is the result of the interaction of diverse cultural practices (eg pruning, thinning, pest and disease management), environmental inputs (eg water, nutrition, light, [CO₂]) and physiological processes (eg light interception, photosynthesis, respiration, transpiration) (Wünsche and Lakso, 2000a), overlaid on the inherent genetic traits of the cultivar. These processes affect preharvest fruit development and influence how fruits at harvest appear, taste and perform in storage (Kader, 2002). Increasing FDM in fruit must not be at the detriment to other quality parameters; consumer preferences for sweeter apples is only true where fruit firmness is retained (Harker et al., 2008).

Approximately 90 % of FDM is composed of soluble and insoluble carbohydrates (Suni et al., 2000). The main soluble carbohydrates determining SSC of apple juice contains a mixture of fructose, glucose, sucrose, sorbitol, organic acids, and inorganic salts (Kingston, 1992; Wills et al., 2007). The ratio of sugars varies depending on the cultivar (Wu et al., 2007) and influences taste. Fructose is sweeter than sucrose, which is sweeter than glucose (Kader, 2002). The proportion of sugars depends on the source/sink relationship between leaves and adjacent fruits and on the proportion of sorbitol and sucrose entering fruit. Sorbitol makes up 80% of the photosynthate entering fruit, the balance being sucrose. Sorbitol breaks down inside the cells to fructose, while the disaccharide sucrose breaks down in equal measures of fructose and glucose. Often glucose is more readily metabolised than fructose, leaving the concentration of available glucose (0.8 - 1.0% fresh weight (FW)) inside cells rather small compared to fructose (3.9 - 5.7% FW) with sucrose concentrations between 3.5 and 4.6% FW (Ackermann et al., 1992).

The balance between crop load and vegetative growth is key to maximising FDM. However, root biomass and the influence of carbohydrate reserves in roots should not be overlooked. Castle (1995) reviewed the literature on the impact of rootstocks on fruit quality for citrus and deciduous fruit crops; rootstocks will influence canopy management and nutrition uptake and thus will impact on crop load and fruit size and storage potential of fruit. The impact of thinning, pruning or rootstocks on fruit quality attributes is often difficult to estimate without considering the impact of crop load; statistical techniques such as analysis of covariance have helped to quantify the influence of rootstock on fruit quality, considering variability in trees crop load. Drake (1988) compared cv. Gold Spur apples grafted onto various rootstocks; M9 and M27 produced the firmness fruit and the highest % Brix in juice samples.

Some of these studies were reviewed in AHDB Horticulture (TF 222) and although previous research highlights the potential to use FDM as a proxy measure of fruit quality, much of this work was correlative.

The underlying basis of this relationship needs to be better understood so that it can be manipulated to deliver premium fruit quality. This is being achieved through a combination of a meta-analysis of existing data sets to obtain a greater understanding of the factors controlling both FDM and quality, a series of field-based experiments at NIAB-EMR and FAST LLP, trials on commercial grower sites and the development of practical strategies to help growers to improve the quality of stored apples.

Many studies have been undertaken on both thinning and pruning of apple trees, such that both the optimum crop load for good yield and pruning techniques to increase light interception are well known. We will take full advantage of this knowledge in designing our experiments and trials to understand the mechanisms for optimising quality for long-term storage.

The impact of dry matter accumulation on fruit maturity is less well documented; many of the factors that influence FDM (light intensity, rootstock, pruning and crop load) can influence the rate of fruit maturation.

Fruit maturity at harvest is vital in dictating post harvest storage life and future eating quality (Kader, 2002), therefore it is important to have a better method for predicting maturity on the tree. Gala destined for long-term storage should be picked at 85-90% starch content (based on iodine staining of equatorial slices). In many instances once fruit start to ripen and starch clearance starts, then a rapid decline in zonal starch patterns of 2% a day is often observed, giving growers little time to pick orchards at optimum maturity as they often have only 1 to 2 days' warning that fruits are starting to ripen. Identifying non-destructive techniques that allow growers and advisors the ability to assess maturity changes across orchards and even within the canopy of individual trees affords opportunities to have greater control of harvesting schedules and practices.

Recent work on fruit quality commissioned by AHDB

AHDB commissioned a series of reviews on the relationship between FDM and fruit quality on thinning methods and on future research needs for improving the storage quality of UK apples and pears. The objectives of this proposal have been developed based on these reviews and from the findings of a series of

projects commissioned by AHDB over the past few years that have focused on improving quality of apples and pears.

TF 213, 221 “Extend the marketing period of Gala apples” (led by NRI) studied the relationship between quality characteristics and volatile components on consumer acceptability as well as factors affecting quality after storage. Over a two-year period, consumer acceptability of UK Gala from a selection of Gala orchards found that fruit with higher FDM at harvest equated to higher % Brix^o at harvest and to a better % Brix^o coming out of store. Fruit with % Brix^o exceeding 13.5% were considered in many cases to have equal overall acceptability with imported fruit in late April/early May. UK fruits generally have better firmness and acidity and, where % Brix^o was equal to imported fruit (13.5%), were considered more acceptable despite being lower in the complement of volatiles. Taste-odour interactions lead to complicated changes in perceived flavour. Increasing sucrose concentrations can reduce perceived levels of bitterness and sourness and in addition increased sweetness can increase the perception of fruity aromatic flavours. The ability to market fruit into late May and early June is dependent on selecting the high FDM yielding orchards and storing them in regimes that maximise taste and flavour. Within project TF 221 alternative regimes were investigated that preserve taste. Several alternative CA regimes such as 3% CO₂ 2% O₂ (+ Smart Fresh (SF)) and 3% CO₂ (0.6-0.4% O₂) scored more highly than conventional regimes in taste panel assessments, despite having similar firmness, %Brix^o and acid ratios. Storage in oxygen <1% retained selected volatiles compared to conventional storage in 5% CO₂ and 1% O₂ where high CO₂ is known to restrict the esterification of alcohols to respective acetate esters.

TF 198 “Developing water and fertiliser saving strategies to improve fruit quality and sustainability of irrigated high-intensity, modern and traditional Conference pear production” (led by EMR) investigated the potential to develop water and fertiliser saving irrigation strategies that would also optimise Class 1 yields and fruit quality. Results over two seasons showed that FDM varied significantly between the four different growing systems in the AG Thames Concept Pear Orchard (CPO) at EMR, and that marketable yields and fruit quality were maintained or improved by alternate wetting and drying treatments. The scientifically derived irrigation scheduling guidelines developed in TF 198 are now being tested in a project funded by Worldwide Fruit Ltd and Marks and Spencer plc on a commercial pear farm in North Kent to optimise production efficiency of high intensity Conference pear production. The potential of using deficit irrigation strategies to manipulate resource partitioning and fruit FDM was being investigated in 2016.

TF 210 and TF 214, led by EMR, investigated the potential to use precision irrigation and targeted fertigation to improve marketable yields, consistency of cropping and fruit quality of Gala and Braeburn.

Description of Work Packages

To deliver ‘Best Practice’ to the top-fruit industry to improve FDM a series of work packages have been set up initially working on discrete aspects of husbandry with the aim of bringing together different components of each WP in the later stages of the project to form a single trial plot.

Work package 1: Meta analysis- analysing existing data sets.NRI, FAST, NIAB-EMR

A significant number of data sets exists on the manipulation of orchard crops to maximise fruit quality at harvest and a number of these are related to increasing partitioning of photosynthates from leaves into fruits. Moreover, within the UK industry a significant body of data exists of fruit dry matter content of Gala apples from commercial orchards along with corresponding mineral analysis and other quality attributes that could provide valuable insights into the variability of %FDM across the UK Gala crop as well as factors that might influence its accumulation.

Work package 2: To determine the impact of increasing light interception in vertically trained high-density orchards by pruning and/or using reflective mulches at different stages of Gala fruit development on fruit quality and FDM. NIAB-EMR/NRI,

Compared to many areas of tree fruit production, the productivity of UK orchards is limited by light levels ([Palmer 1999](#)). The close relationship between the amount of light intercepted by the tree canopy and fruit production is well known (e.g. Lakso, 1996, Figure 1A) and increased light interception promotes dry matter accumulation (e.g. Palmer et al. 1992, Figure 1B), TSS, fruit colouration and profitability ([Jackson 1970](#); [Robinson and Lakso 1988](#); [Kappel and Neilsen 1994](#); [and Lakso 1996](#); [Kappel and Brownlee 2001](#)). Therefore, optimising light interception in high-density orchards is critical and although different strategies are available to growers (see below), scientifically derived guidelines are needed to optimise their use in UK commercial intensive apple and pear orchards.

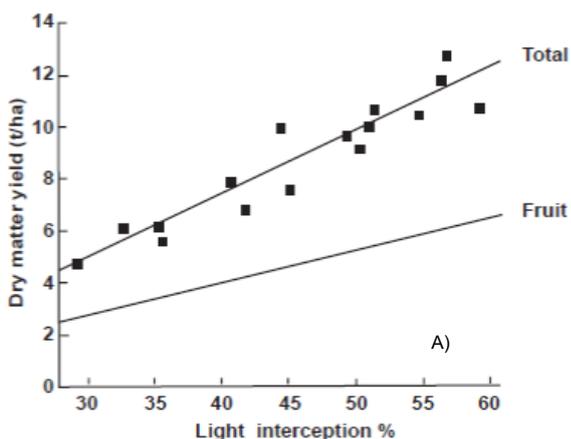


Figure 2.1 A.

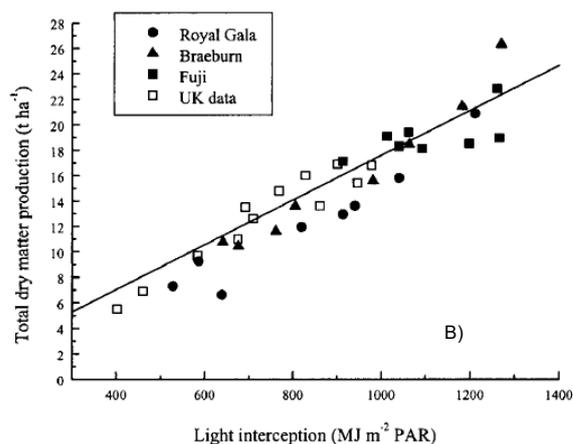


Figure 2.1B.

Figure 2.1 A. Relationship between light interception (%) and total dry matter production and FDM yield (t/ha) of Golden delicious/M9 at East Malling. Modified from Palmer, 1999.

Figure 2.1 B Relationship between seasonal intercepted PAR (MJ/m²) and total dry matter production (t/ha) of Royal Gala, Braeburn, Fuji and averaged seasonal data for the UK.

For apple, new training systems have been developed abroad and have shown promising results with regard to yield and quality. For pear, the different training systems in the AG Thames/EMR CPO have delivered a threefold increase in yield in comparison to commercial orchards, due in part to improved canopy light interception. Reflective covers or mulches can improve the amount of light intercepted by the tree canopy by up to 30% in all types of weather, with corresponding improvements in apple and pear quality and yield ([Iglesias and Alegre 2009](#); [Privé, Russell et al. 2011](#); [Guo 2013](#)).

Work package 3: To determine the impact of thinning strategies on fruit quality and FDM and to develop recommendations to optimise yield of high quality fruit FAST LLP, NRI UoG

UK apple growers have recently expanded their production of Gala from high intensity plantings. To accommodate additional volume, it is estimated that around 30% of this harvest must be aimed at a later market window (FAST LLP, 2016).

Improved availability of consistently high-quality fruit will enable UK growers to compete with Southern hemisphere imports at the start of the new season window. Extending the UK Gala season by 3 to 4 weeks could generate financial returns of £2 to £3 million per year across the industry (FAST LLP, 2016).

Many studies have been undertaken on both thinning and pruning. In terms of thinning, the optimum crop load required to achieve a good yield of fruit with the required fruit size is well established. However, the effect of thinning practices on the accumulation of FDM during the growth and development period is less well understood. No recent work has measured any effects on FDM on Gala in the UK. Manipulating tree architecture through different use of different pruning strategies have been trialled to improve uniformity of fruit size and colour and increase yield by way of increasing light interception throughout the canopy. Currently, the potential to increase yield can be increased without reducing FDM is not yet understood.

To increase FDM it is necessary to understand the controlling factors. There are two periods during fruit development when carbohydrate supply (from photosynthesising leaves) can be limiting; in the first weeks (typically 2 to 4 weeks from full bloom) of fruit development and just before harvest when light levels and temperature decline. Several studies have shown that reducing crop load increases FDM of the remaining fruit (Wünsche 2000, Wünsche 2005, Sharples 1968, Palmer 1997, Kelner et al 2000). However, it would also be helpful to understand how timing of thinning affects fruit cell number (which is determined by early in fruit development) and how this impacts on quality. Photosynthate from leaves tends to be translocated to nearby fruits on the same branch/spur.

It is particularly important to develop knowledge of the impacts of the time of thinning on FDM by understanding the processes, not simply the outcomes and the former enables proposal of practical tree management strategies. Through utilising a commercial orchard with documented high fruit FDM, it will be possible to manipulate crop load based on tree age, precocity of flowering and size of branches, and quantify changes in FDM changes from flowering stages through to fruit development.

From previous studies, changes in % FDM from full bloom have been charted; a decrease after blossom is often seen, associated with high respiration rates of developing fruitlets, and then increases towards the end of the cell division phase before reaching a plateau which remains fairly stable for the remainder of the season (see Figure 3.1).

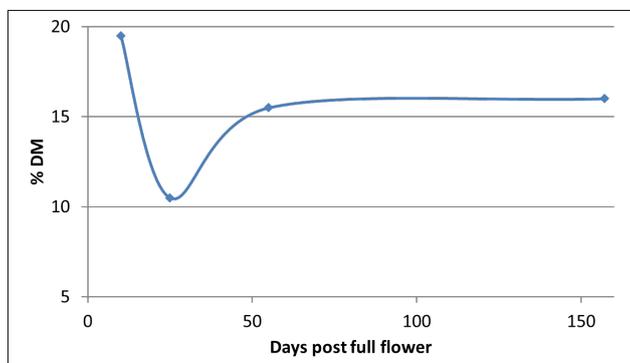


Figure 3.1. Preliminary FDM data from Gala taken during flowering and fruit development

From initial studies in two orchards the timing of thinning is thought to affect the degree to which FDM falls and rises again and potentially influence the final FDM at harvest (FAST LLP data unpublished). Many growers do not achieve the optimum crop load until late in the season - typically mid to late June through to the end of July. This trial has investigated the timing of thinning events and different thinning strategies the optimum crop load at earlier stages in the fruit development cycle compare with typical industry practice in terms of FDM accumulation.

The aim of the trial is to develop practical short, medium and long-term strategies to help UK growers to optimise quality and storability of UK apples, in particular for long term storage beyond April.

This project will provide direct benefits to the growers within the project timescale as it will provide them with strategies to improve FDM.

Gala was used as a model variety to understand the relationship between quality and FDM, how to manipulate this and in order to follow changes in FDM and components during fruit development.

WP 4 The Use of Chlorophyll Fluorescence as a Tool to Determine Fruit Maturity, Landseer Ltd.

Work package 4 focused on developing a non-destructive method to optimise harvest date and identifying the orchards suitable for long term storage. This can be achieved by choosing the right fruit with high dry matter and balanced minerals that are picked at the right time for extended keeping quality during long term storage.

Early work conducted by Landseer tested a chlorophyll fluorescence (CF) meter (Handy Pea, HansaTech) as a non-destructive method to track changes in the decline of fluorescence yield from chloroplasts within the skin of fruit. Changes in fluorescence yield were correlated with maturity assessments (starch clearance patterns, % Brix and fruit firmness) of Gala apples sequentially harvested around commercial harvest dates. A noticeable decline in CF output observed 7 to 10 days prior to changes in starch clearance patterns suggested that this method could provide an early warning of increasing fruit maturity prior to changes observed in starch clearance patterns. If such a tool can be commercialised it can provide growers with a useful tool to allow them to organise their picking schedules around the optimum harvest date for long-term storage. CF profiling of fruit in orchards where fruits have ample mineral content for long-term storage and high FDM then picking fruit at optimal maturity will ensure fruit performed well in long-term storage.

Work Package 1 Meta-analysis of Fruit Dry Matter Years 1-3 (2017-2019) NRI-UoG/FAST

Materials and Methods

Data sets from FAST LLP supplied over 3 consecutive seasons (2015/16, 2016/17 & 2017/2018) were used to conduct a series of multiple correlation and regression analyses to identify links between mineral analysis data of fruits at harvest with the propensity to accumulate dry matter.

Initial correlation tests were performed on the 2015/2016 and 2016/2017 data sets using fruit, leaf and soil mineral analysis data correlated against FDM using a Pearson test ($p < 0.05$). The analysis was performed using GGally package (ggplot2); an extension package “from RStudio” (R Core Team, 2014). Correlation coefficients were determined from Lindley and Scott (1995).

Following on from Pearson’s correlation analysis a linear model using Library Lattice in RStudio was conducted. In the first instance a linear model (lm) including all minerals (lm (DM ~ Ca + N + KCA + Cu + Fe + K + Mg + Mn + P + Zn + B)) was undertaken. In order to assess the contribution of individual mineral elements within combined model, ANOVA (analyses of variance) was used to perform an analysis of the relative contributions from explained and unexplained sources of variance in a continuous response variable. Significant effects were tested with the F statistic, which assumes random sampling of independent replicates, homogeneous within-sample variances, and a normal distribution of the residual error variation around sample means (Doncaster and Davey, 2007). ANOVA was carried out to determine whether there were significant differences between minerals using RStudio (R Core Team, 2014). The mineral elements identified as having a significant effect on FDM were added in different combinations in a second series of restricted linear models. The regression models were tested against the Akaike Information Criterion (AIC) to confirm the best fit.

Results: WP 1: Meta-analysis

Orchard surveys compiled by FAST have been collated on dry matter and mineral analysis from fruitlets, soil and leaves of 56 Gala orchards.

A preliminary multiple regression analysis was used to determine whether mineral content of soils influences fruit development and dry matter accumulation and, moreover, the extent to which mineral content of the soil influences leaflet and fruitlet analysis.

From the two years of available data (2015-2016) FDM variation across all the data sets ranged from 13.6% to 18.9%, a span of 5.3% FDM.

The Gala dataset consisted of 56 measurements with a range from 13.8% to 16.0% FDM and a span of 2.2% FDM. Additional mineral and leaf analysis for a subset of Gala orchards exist.

Pearson's Correlations of FDM against soil, leaf and fruit mineral analysis found a weak positive correlation of Fruit Dry Matter and Fe, K, Mg, P K:Ca ratio and a negative correlation with Zinc and Ca:N ratio.

Calcium, Nitrogen, Copper, Manganese and Boron content of fruit did not influence the rate of FDM content in fruit. See Figures 1.1.1 and 1.1.2.

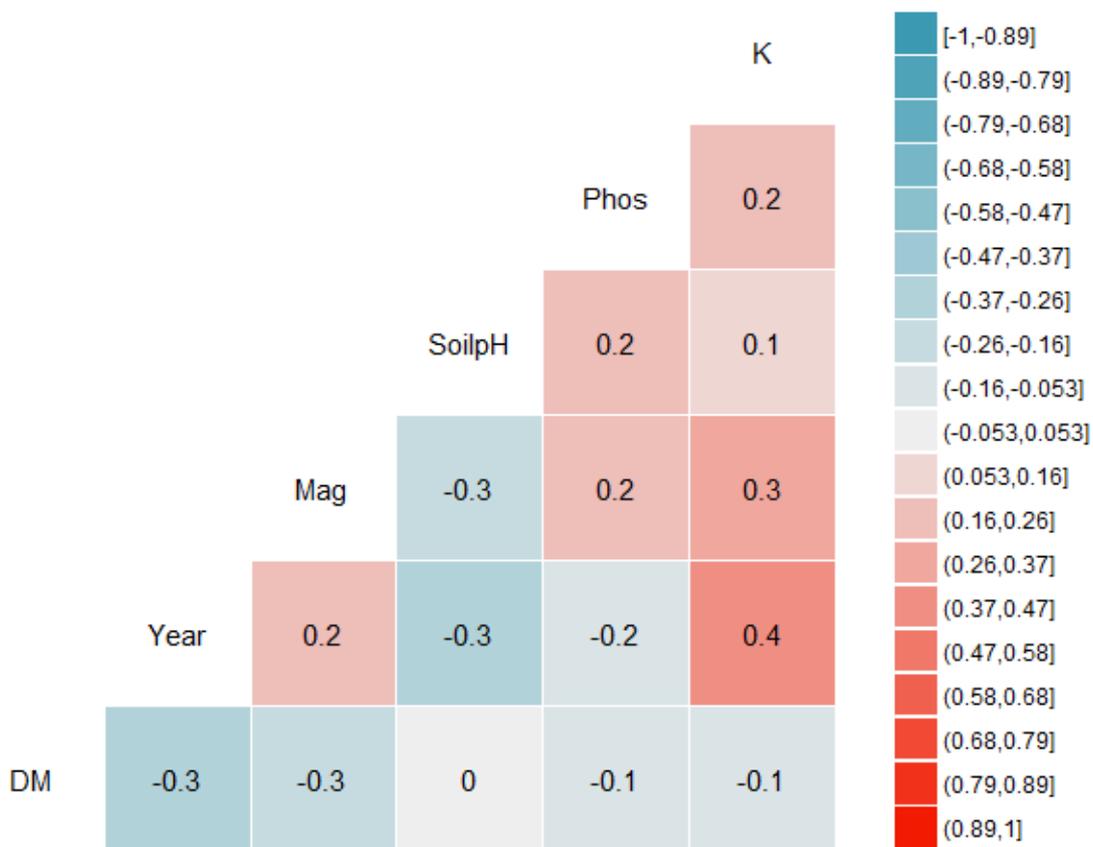


Figure 1.1.3. Pearson's correlation tests ($P < 0.05$) of mineral analysis from soil samples and Fruit Dry Matter (DM) in Gala apples from 56 orchards over two seasons (2015-16, 2016-17).

With soil analysis, far fewer elements are measured and a smaller number of samples were available compared to leaf and fruits analysis. Soil analysis data identified a weak negative correlation of Mg content in the soil and FDM. Soil pH and Phosphorous and Potassium content of soil samples had no bearing on FDM content.

Multiple regression analysis of fruit mineral content and FDM identified that there was a weak positive relationship between higher fruit potassium and magnesium and FDM and a negative relationship with Zinc, with higher fruit zinc content having lower FDM (Figure 1.1.4).

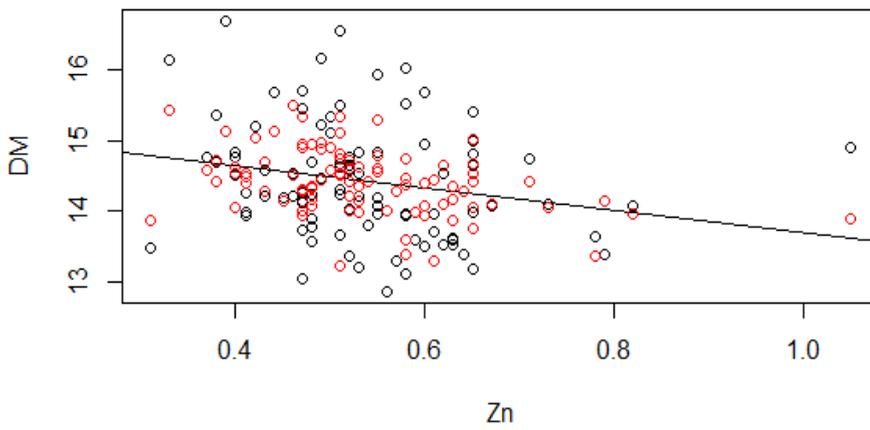
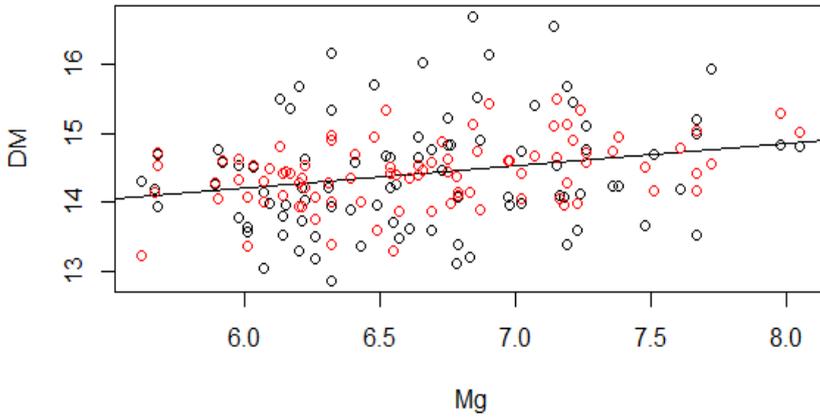
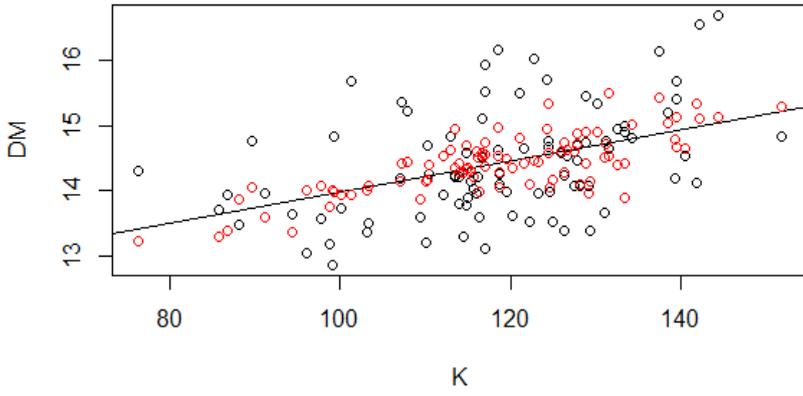


Figure 1.1.4. Multiple regression analysis depicting the positive relationship between K, Mg with FDM and a weak negative relationship with Zn. Concentration of mineral elements are in mg/100g FW.

Work Package 2. Increasing light interception by centrifugal pruning and deployment of reflective mulches covers: 2016 - 2020 Years 1-5 NIAB-EMR/NRI-UoG

Materials and Methods

In the Autumn of 2016, innovative centrifugal pruning and training systems were initiated and compared with a standard tall spindle tree within a 4 year old Gala/M9 orchard at NIAB-EMR (Figures 2.1, 2.2). This resulted in the removal of most of the main fruiting branches, resulting in a decrease in the yield potential for the next two to three years. As a consequence, the results presented for years 2-3 in this report need to be taken with caution.

The impact of pruning systems on tree architecture and canopy development were monitored using LiDAR to estimate tree row volume, porosity, specific leaf area and light levels studied using a AccuPAR. . For selected parts of the trial a more detailed categorisation was undertaken in terms of light interception by the fruit bearing branch.

Within the orchards reflective mulches were laid either side of the rows after flowering to determine the effects of improved light penetration and on Class 1 yields, FDM and components of quality fruit quality (TSS, colour). Size measurements of fruit were taken from the end of July at weekly intervals to monitor fruit expansion rates.

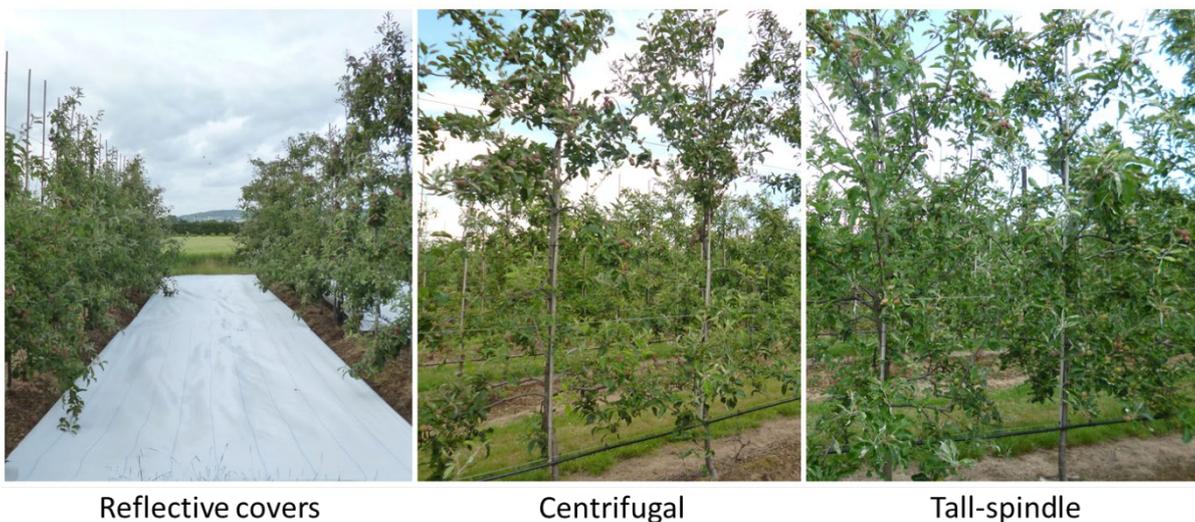
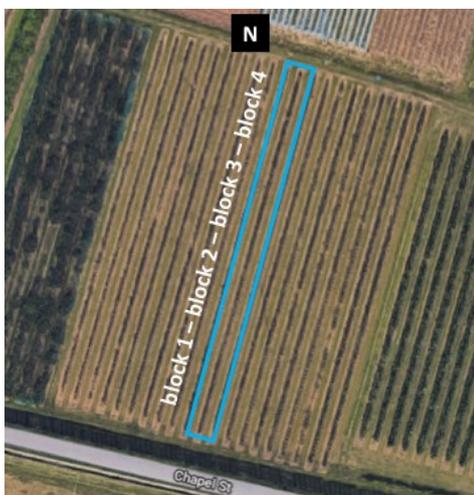


Figure 2.2. Pictures of the treatments applied during the experiment. The training and reflective mulches have been applied to a 4-year-old M9 Gala orchard located at NIAB EMR.



After Grading fruits were transferred to the Produce Quality Centre, where fruit maturity measurements were undertaken along with % Dry Matter estimates. Apples were sampled for dry matter, taking segments from opposite eighths, removing the core. Tissue was chopped into 1 cm pieces, 50 g of tissue was weighed to 2 dcp, dried in an oven for 48 hours and reweighed. Tissue was then placed back into the oven for a further 24 hours and reweighed. Additional samples were frozen and freeze dried for 5 days at -80°C and then reweighed. Freeze dried samples were powdered using a spice grinder.

Powdered tissue samples (0.2g) were used for sugar extraction in 80% ethanol hot ethanol (70°C) for 120 mins in a shaking water bath with periodic vortexing, samples the supernatant was collected following centrifugation (12,000 rpm) and filtered through $0.45\ \mu\text{m}$ syringe filters and followed by analysis of sugars by HPLC using an Agilent Zorbax Carbohydrate(150 mm x 4.6 mm x $5\ \mu\text{m}$ column) maintained at 30°C using 75% acetonitrile running at $2\ \text{mL min}^{-1}$ as the mobile phase. Sugars were detected using a refractive index detector (Agilent 1200 refractive index detector). Data was analysed using the data system EZChrom 3.3 (Agilent). The remaining harvested fruit was randomised within their orchard treatments and stored in 3% CO_2 , 1% CO_2 ($0.5\text{-}1.0^{\circ}\text{C}$) for 5 months, after which fruits were assessed immediately ex-store and again after 7 days shelf-life at 18°C

WP2 Results

Year 2 Increasing light interception by centrifugal pruning and deployment of reflective mulches covers (2018/19)

The primary role of the treatments was to generate a range of light levels intercepted by the canopy and fruits during the growing season. To measure light penetration through the canopy, the tree has been divided in three canopy zones - upper, middle and lower. The AccuPAR readings were taken during the morning and the afternoon of 14, 15, 24 and 25 August, giving sixty-five readings per treatment. The AccuPAR readings are expressed as a % of the external PAR in order to compensate for temporal variations in light intensity.

The pruning system effects the percentage light penetration with the CS system intercepting an average 41.5% of the external light, compared with 34.4% for TS. These results are preliminary and to be confirmed in the next years when the trees will be more comparable between the two systems. The results are, however, consistent with observations in other studies in which the CS pruning system has been reported as beneficial to light interception. Within the tree, there is a gradient of light from the top to the bottom of the tree, with the upper part of the tree intercepting between 49% and 53% of the external light, the bottom between 32 and 25% of the light (Figure 2.2.4).

For all the treatments and location in the tree, the reflective covers had a positive effect on the % of light intercepted by the canopy. This resulted in an increase of the fruit temperature, measured at the same time as light by thermal imaging (data not shown).

Effect of the treatments on yield and grading

The suppression of most of the branches at pruning time resulted in a reduction of yield for the CS treatments with an average yield of 45 kg compared to an average 61 kg for TS (Figure 2.2.5). It is expected that the respective yield will become more comparable in the next three years. The reduction in yield in CS is lower than expected after pruning.

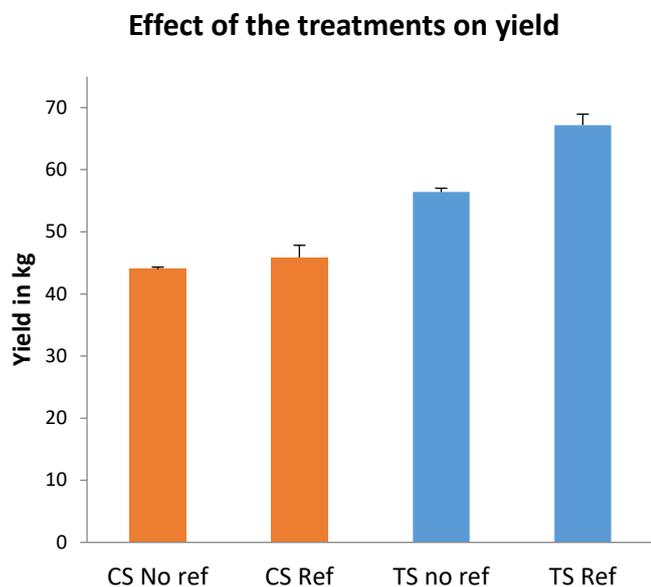


Figure 2.2.4. Effect of the training system (Centrifugal (CS) in orange and Tall spindle (TS) in blue) with or without reflective mulches on total yield.

Effect of the pruning system on the % of light intercepted by the canopy



Figure 2.2.5. Effect of the training system (Centrifugal (CS) in orange and Tall spindle (TS) in blue) with or without reflective mulches on the % of light intercepted by the canopy at three different height of the canopy.

The application of reflective covers at fruit set did increase yield in both treatments by 5% (CS) and 19% (TS). This increase in yield is encouraging regarding the effects of light on apple production and highlights the dramatic effect that light levels can have on apple production under the UK climate. These differences in yield will have an impact on the quality assessments and need to be confirmed in the next years before drawing any definitive conclusion. The treatments also had some effects on the grade of the apple fruits at harvest (Figure 2.2.6).

Effect of the treatments on apple grading at harvest

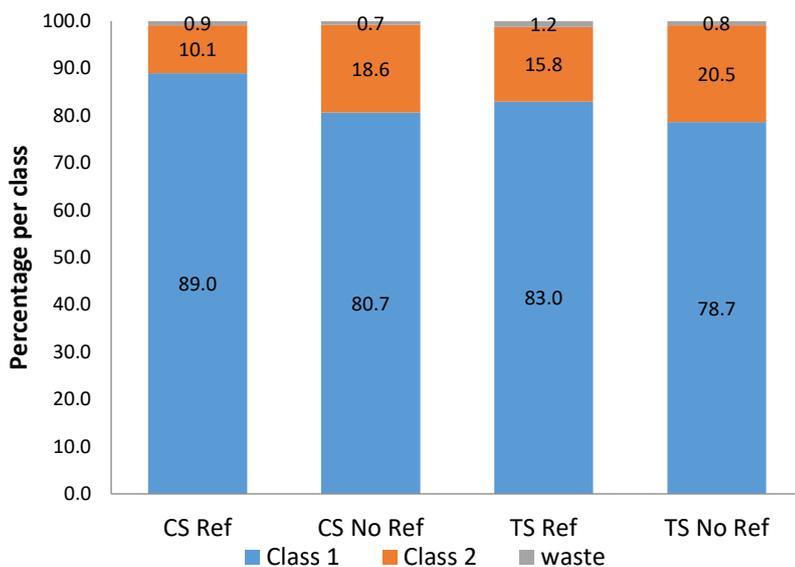


Figure 2.2.6. Effect of the treatments on apple grading, in percentage of total yield for each category.

Waste was low for all treatments, representing between 0.7 and 1.2% of the harvested crop. The Class 1 apples represented on average 84.5% (CS) and 80.9% (TS); Class 2 fruits 14.4% (CS) and 18.2 (TS)%. The reflective covers did increase the proportion of Class 1 fruits in both pruning systems with an increase by 10% for CS and 5% for TS. Whilst the increased Class 1 to Class 2 ratio observed with CS could be explained by a lower yield in comparison to TS, the reflective mulch had a positive effect on both yield and grading of the crop for both pruning systems.

Harvest Analysis- NRI

Apples from the top and bottom of the trees under reflective covers and pruning regimes were harvested on 20 September and transferred to the PQC where fruits were sampled for dry matter content and assessed non-destructively for DM content using a Felix 750.

The apples harvested from the higher canopy (>1.5 m) of TS trees were on average 0.6-0.8% higher in FDM, but just failed to reach significance. However, the % BRIX^o of juice samples was significantly higher in fruits from the upper canopy. Tall Spindle tree apples harvested in the absence of reflective mulches fruits from the upper canopy averaged 12 % BRIX^o compared to 11.4% Brix^o from fruits from the lower canopy (<0.6M). In CS pruned trees, the % FDM in fruits from the upper canopy averaged 13.5% compared to 12.7% in the lower canopy. With centrifugally pruned trees, % FDM was highest in the upper canopy fruit (12.9% FDM) compared to 12.4% in the lower canopy.

The incorporation of reflective covers failed to elevate % FDM in conventional Tall Spindle trees. In the centrifugal trees reflective covers yielded fruit with 13.5% FDM in the upper canopy compared to 12.9% FDM where no reflective covers were used, but the difference was not statistically significant. No increase in % Brix^o was observed in apples grown under reflective covers in centrifugally pruned trees (Figures 2.2.7 and 2.2.8).

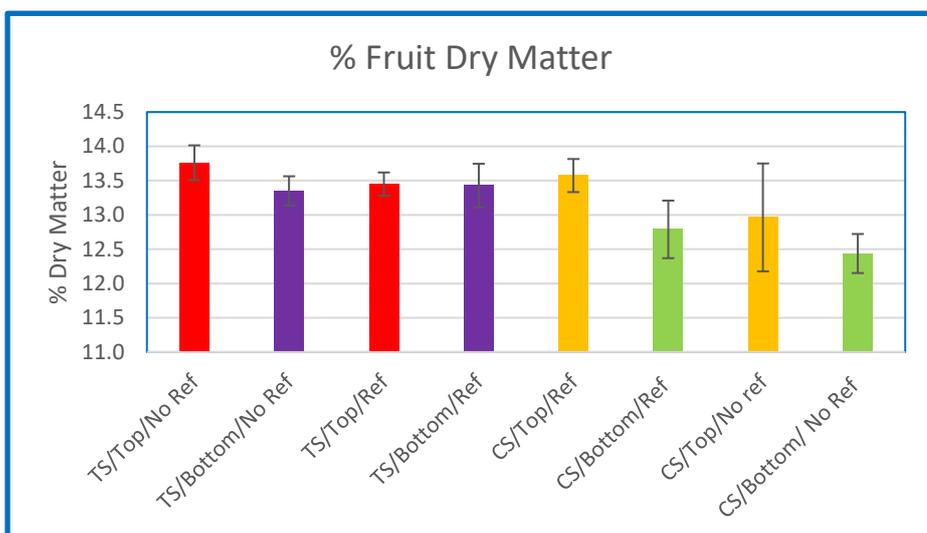


Figure 2.2.7. The % FDM of Gala apples grown under conventional Tall Spindle or Centrifugal Pruning systems harvested from the top (1.5 m) or bottom (0.6 m) canopy. Trees were grown under the presence or absence of reflective covers.

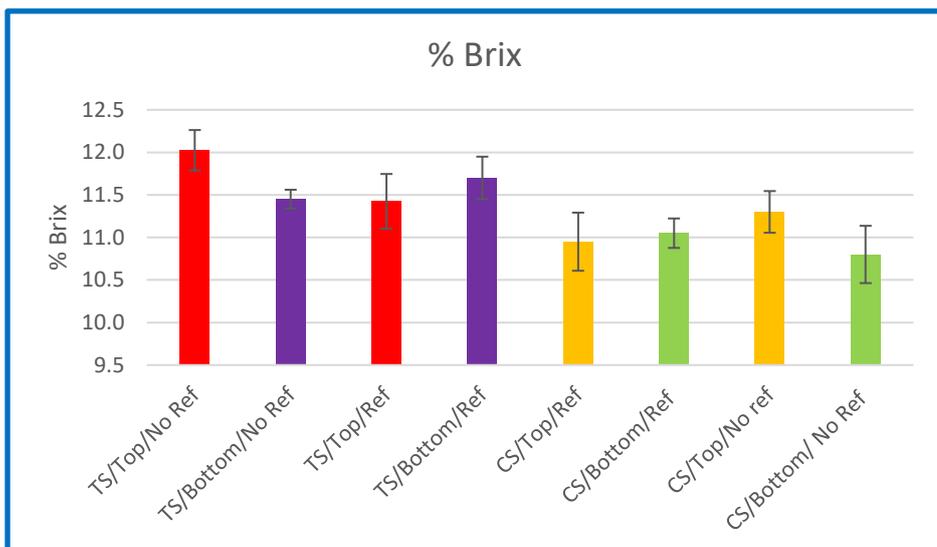


Figure 2.2.8. The % Brix of Gala apples grown under conventional Tall Spindle or Centrifugal Pruning systems harvested from the top (1.5 m) or bottom (0.6 m) canopy. Trees were grown under the presence or absence of reflective covers.

The bulk of the remaining fruit was randomised within their orchard treatments, while damage, diseased and misshapen fruits were discarded. Fruits were stored in 3% CO₂, 1% O₂ (0.5-1.0°C) for 5 months, after which fruits were assessed immediately ex-store and again after 7 days at 18°C. The full interaction of pruning treatments, reflective mulches and tree position of harvested fruits were not significant (P<0.05) and are not reported on. However, a number of differences between overall means and individual two way interactions were significant (P<0.05). Fruits located on the top of the tree produced apples firmer than at the bottom and in particular (see Table 2.2.1) this effect was most pronounced on centrifugally pruned trees. As expected, ex-store fruit was firmer than fruit assessed after 7 days shelf-life (18°C) and fruit from conventional tall spindle trees were firmest. Fruit under reflective covers were slightly softer.

Table 2.2.1. The impact of pruning systems (Tall spindle vs Centrifugal training) and the deployment of reflective covers on the firmness (N) of Gala stored for 5 months at 3% CO₂, 1% O₂ at 0.5-1.0°C. Overall means.

Fruit Position	Top (>1.5M)	Bottom (0.6 M)	P value
	63.7	62.2	0.018
	LSD _{0.05} 1.151		
Assessment	Ex-store	Shelf-life	
	64.5	61.4	<0.01

	LSD _{0.05} 1.151		
Fruit Position	Bottom (0.6 M)	Top (>1.5 M)	
Centrifugal	61.7	64.3	0.049
Tall Spindle	62.8	63.1	
	LSD _{0.05} 1.627		
Assessment	Ex-store	Shelf-life	
Centrifugal	63.8	62.1	0.022
Tall Spindle	65.1	60.7	
	LSD _{0.05} 1.627		
Assessment	Ex-store	Shelf-life	
No covers	65.5	61.1	0.027
Reflective covers	63.5	61.7	
	LSD _{0.05} 1.627		

Figures in bold are significantly different (P<0.05) from data in opposing column.

The overall effect of fruit grown under different pruning and reflective mulches on the retention of % BRIX^o in fruit found apples sampled from the top of the tree canopy higher in % BRIX^o than fruit sampled lower down the tree. Tall spindle trees were higher in year 1 of the centrifugal tree conversion (Table 2.2.2).

Table 2.2.2. The impact of pruning systems (Tall spindle versus Centrifugal training) and the presence of reflective covers on the % BRIX^o of Gala stored for 5 months at 3% CO₂, 1% O₂ at 0.5-1.0°C. Overall means.

Pruning	Tall Spindle	Centrifugal	LSD _{0.05}
	11.7	11.10	0.271 (P<0.01)
Fruit Position	Top	Bottom	
	11.6	11.2	0.383 (P=0.016)
Assessment	Ex-store	Shelf-life	
	11.6	11.3	0.271 (P=0.043)

Figures in bold are significantly different (P<0.05) from data in opposing column.

Interestingly, the use of reflective covers reduced the incidence of rotting in stored fruit. This effect was most pronounced in fruit harvested from the upper canopy where fruit grown under reflective covers recorded an incidence of 1.2% rots, compared to 10% rots in fruits from the upper canopy where no covers were in place (Table 2.2.3). This may be a result of reducing the amount of inoculum on the orchard floor that is redirected into the canopy during heavy rain. However, the highest incidence of rots were in the upper canopy suggesting other temperature/UV effects may be influencing the incidence of rotting.

Table 2.2.3. The impact of pruning systems (Tall spindle versus Centrifugal training) and the presence of reflective mulches on the % rotting of Gala stored for 5 months at 3% CO₂, 1% O₂ at 0.5-1.0°C. Overall means.

% Rotting	Reflective Covers	No Covers
Overall	3.1	8.1
LSD _{0.05} (P=0.048)	4.96	
Position		
Top	1.2	10.0
Bottom	5.0	6.2
LSD _{0.05} (P=0.048)	7.10	

Figures in bold are significantly different (P<0.05) from data in opposing column.

Year 3: WP 2: Increasing light interception by centrifugal pruning and deployment of reflective mulches covers (2018/19)

Five Decagon loggers with 2 VP4 sensors per logger installed on the 22^d of August, provided data on air temperature and humidity. The temperature recorded was transformed into growing degree days (GDD) accumulated during a period of 4 weeks. The treatments with reflective covers showed an average accumulation of an extra 5.4 degrees than the treatments without reflective covers at the time of the harvest, on 14 September (Figure 3.2.4).

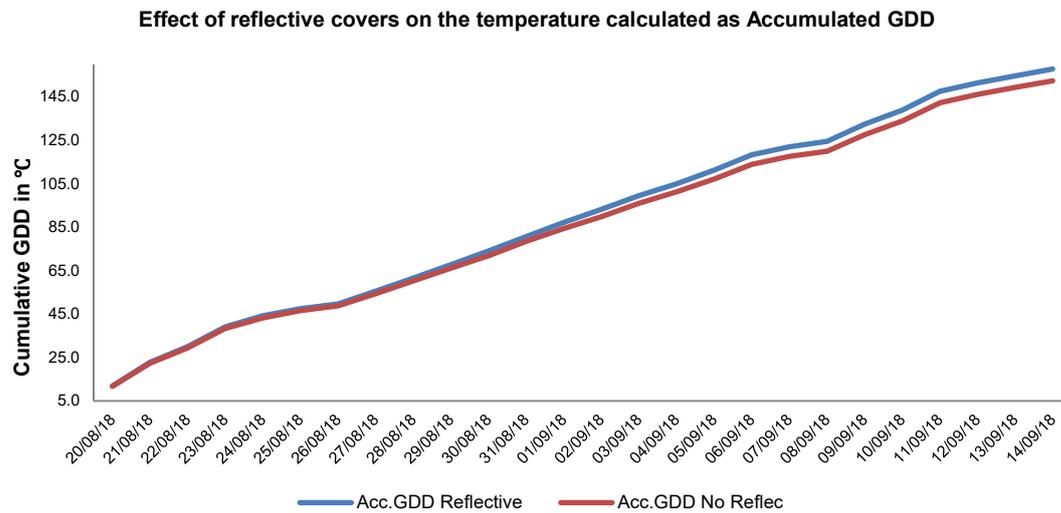


Figure 3.2.4 Growing day degrees with or without the presence of reflective covers

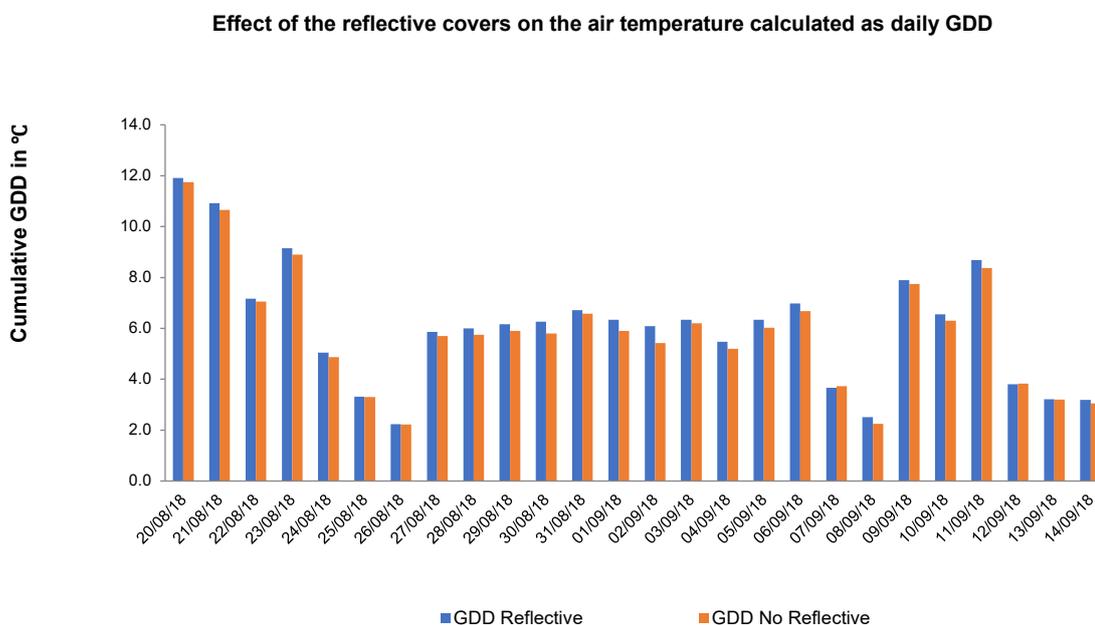


Figure 3.2.5. Cumulative growing day degrees (GDD)

Daily calculation of GDD (Figure 3.2.5) showed higher GDD values on the areas with reflective covers. Differences range from 0.1 degrees on 22 and 31 August, to 0.7 degrees on 7 September. No statistically significant difference were shown.

The use of reflective covers showed a tendency to decrease the percentage of humidity in the air in comparison with the areas without reflective covers. Out of the four weeks, only five days from 27 August to 31 August showed a higher humidity in the zones with reflective covers.

Effect of the treatments on light interception

The reflective covers resulted in 29.45 % of the incident light to be reflected to the canopy against 4.27 % in the zones without (Figure 3.2.6). The results as shown below represent the mean of twenty measurements per treatment showing statistically significant differences between the treatments, LSD = 1.

The Centrifugate System (CS) shows a greater amount of light penetrating the canopy in the with 19.8 % against 12.5 % in Tall Spindle (TS) (Figure 3.2.7). However, this difference was not statistically significant due to the large inter-tree variability.

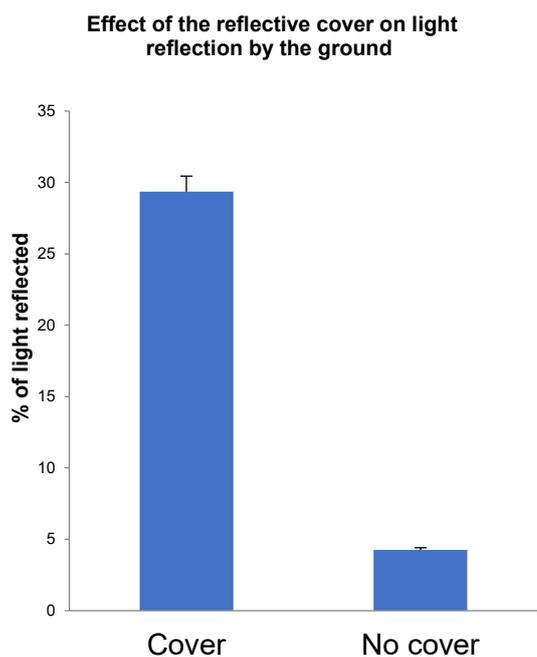


Figure 3.2.6. % light reflectance from the alleyway

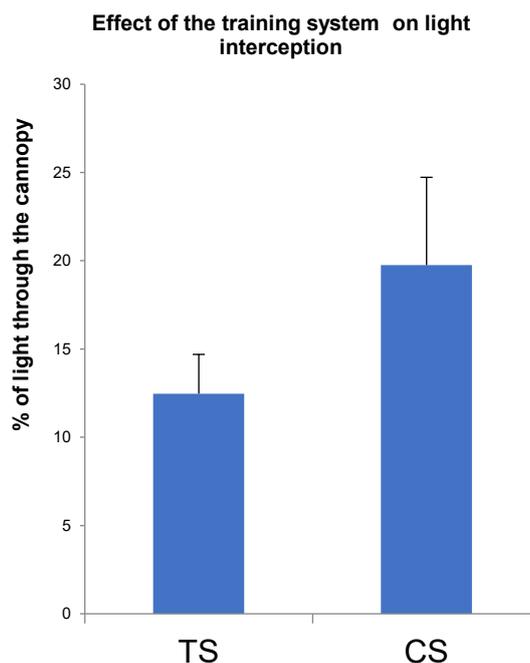


Figure 3.2.7 Light penetration through the canopy

Data of the temperature of the apples was recorded with a thermal camera FLIR ThermoCam P25 at two different days, on the 7th (cloudy day) and 13th (sunny day) of September just before the harvest (Table 3.21). For both days, reflective covers resulted in higher temperature in the canopy. On the 7th September, the apple temperature of the CS and TS training system was 0.28 °C and 2.66 °C higher with reflective covers,

respectively. On the 13th of September, the apple temperature of the CS and TS training system was 1.15 °C and 0.41 °C higher with reflective covers, respectively. Results are means of 9 fruits per treatment. The training systems treatments did not significantly affect fruit temperature (Table 3.2.1).

Effects of the training systems and reflective covers on the rate of fruit expansion.

Fruit height and diameter were measured weekly and the Fruit Expansion Rate (FER) between two successive measurements was calculated to determine whether these parameters were affected by the treatments (Figure 3.2.8 a/b). Fruit size was similar between the two training systems. However, reflective covers induced a slightly higher average fruit height and diameter for the two training systems. Differences were however not statistically significant.

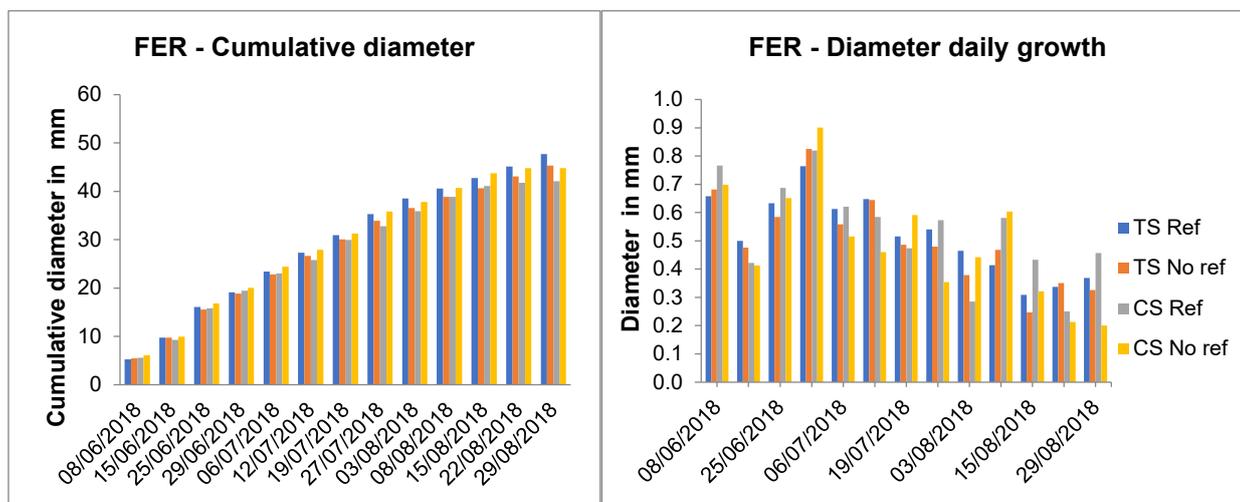


Figure 3.2.8a/b. Fruit expansion rate (FER) expressed as a cumulative diameter (a) and daily diameter growth rate (b).

Fruit size and yield at harvest

Yield was higher for the tall spindle trees but CS trees are recovering from their conversion and now produce yield close to TS. Average fruit fresh weight from the CS treatments was 10.1 kg with reflective covers and 8.74 kg without covers (Table 3.2.2). From the TS treatments, fruit fresh weight was 11.75 kg with reflective covers and 10.88 kg without. The detailed yield records from the selected experimental trees showed that

the total percentage of marketable fruit from each tree in CS was 90.03% with reflective covers and 88.43% without. Total percentage of marketable fruit from the TS treatments, was 91.79% with reflective covers and 89.58% without (Table 3.2.3). These differences were not statistically significant. The treatments did not result in significant changes in fruit size either.

Table 3.2.1. Effects of the two training systems and reflective covers on 'Gala/M9' fruit temperature.

Treatment	07/09/2018	13/09/2018
Centrifugal No Covers	25.4	25.8
Centrifugal Reflective Covers	25.7	26.9
Tall Spindle No Covers	23.1	26.1
Tall Spindle Reflective Covers	25.7	26.5
F-prob	n.s.	n.s.
LSD	2.61	1.72

Table 3.2.2. Effects of the two training systems and reflective covers on 'Gala/M9' fruit weight. From experimental selected trees.

Treatment	Yield per tree (kg)	Marketable fruit (%)
Centrifugal No Covers	8.7	88.4
Centrifugal Reflective Covers	10.1	90.0
Tall Spindle No Covers	10.9	89.6
Tall Spindle Reflective Covers	11.8	91.8
F-prob	n.s.	n.s.
LSD	5.09	6.04

Harvest Analysis- NRI

Apples were harvested on 28th September 2018 and transferred to the PQC where fruits were sampled for firmness, starch and % Brix. Analysis of harvest data found that fruit from centrifugal pruned trees were generally higher in starch content (Table 3.2.4) than those in standard tall spindle trees and were 4 N firmer ($p < 0.05$). Centrifugal pruned trees had lower yields (9.42 kg/tree) compared to tall spindle (11.3 kg/tree) and crop loads are still recovering from conversion pruning in 2017. With the resultant lower fruit numbers, it is likely that fruit maturity would have been retarded, leading to firmer fruit. Overall, centrifugal pruning failed to increase % Brix, %FDM or sugars (Table 3.2.4).

Table 3.2.4 Overall effect of implementation of reflective covers from fruit set to harvest on the harvest maturity, Fruit Dry Matter and sugar content of Gala apples at harvest

	Pruning		fprob	LSD _{0.05}
	Tall Spindle	Centrifugal		
% Starch	40.9	52.5	0.042	11.03
% Brix	13.5	13.8	0.161	0.44
Firmness (N)	71.5	75.2	0.047	3.65
% FDM (Oven)	17.1	17.5	0.120	0.56
% FDM (FD)	17.4	17.9	0.284	1.23
Fructose ($\mu\text{L}/\mu\text{L}$)	31.9	32.5	0.642	3.18
Sucrose ($\mu\text{L}/\mu\text{L}$)	22.3	22.2	0.913	1.19

Table 3.2.5 Overall effect of reflective covers on the harvest maturity, Fruit Dry Matter and sugar content of Gala apples at harvest

	Covers		fprob	LSD _{0.05}
	Reflective	None		
% Starch	41.4	52.0	0.056	11.03
% Brix	13.6	13.7	0.336	0.444
Firmness (N)	70.5	76.2	0.013	3.65
% FDM (Oven)	17.1	17.4	0.286	0.564
% FDM (F.D.)	17.5	17.8	0.604	1.234
Fructose (μL/μL)	32.52	31.8	0.561	3.181
Sucrose (μL/μL)	22.3	22.2	0.827	1.19

Overall, the use of reflective covers increased fruit maturity at harvest (Table 3.2.5) and fruit were generally lower in starch coverage (41.4 %) although this just failed to reach significance ($p < 0.05$). Moreover, increased fruit maturity led to lower fruit firmness (70.5 N). In the absence of reflective covers average fruit starches at harvest were 52.5% and firmness values of 75.2 N. There was no effect of reflective covers on the % Brix, % FDM or fructose or sucrose content.

Analysis of the interaction between reflective covers and pruning regimes showed that reflective covers had the biggest impact on advancing maturity in tall spindle trees where starch content averaged 33.5% compared to 48.2% in tall spindle trees in the absence of covers (Table 3.2.6). The contrast is even greater when centrifugally pruned trees grown in the absence of covers (55.8%) are compared with fruit grown on tall spindle trees with reflective covers (33.5%). The effect of centrifugal pruning retarding fruit maturity and

delaying softening may in part be due to lower fruit numbers per tree affecting fruit maturity at this stage in the conversion process.

Similarly, fruit firmness followed the same pattern with fruit grown on centrifugally pruned trees under reflective covers were softer than fruit cultivated without covers. However, there was no sign that the interaction of pruning with the addition of reflective covers has increased fruit dry matter or % Brix or fructose and sucrose content at harvest.

Table 3.2.6 The interaction of pruning systems on 6-year-old Gala (M9) and reflective covers in alleyways on fruit maturity, fruit dry matter and sugar content at harvest

	Pruning	Covers		fprob	LSD _{0.05}
		Reflective	None		
% Starch	Tall Spindle	33.5	48.2	0.363	15.6
	Centrifugal	49.2	55.8		
% Brix	Tall Spindle	13.5	13.5	0.336	0.63
	Centrifugal	13.6	14.0		
Firmness (N)	Tall Spindle	67.6	75.4	0.174	5.17
	Centrifugal	73.5	77.0		
% FDM (Oven)	Tall Spindle	17.0	17.1	0.501	0.797
	Centrifugal	17.3	17.7		
% FDM (FD)	Tall Spindle	17.4	17.4	0.604	1.745
	Centrifugal	17.7	18.2		
Fructose (µl/µl)	Tall Spindle	32.1	31.7	0.76	4.499
	Centrifugal	33.0	31.9		
Sucrose (µl/µl)	Tall Spindle	22.6	22.0	0.308	1.383
	Centrifugal	22.0	22.4		

N.B % FDM= Fruit Dry Matter estimated either using standard oven drying or by Freeze Drying (FD)

Storage

Interestingly, at harvest fruit from centrifugally pruned trees were of less mature and marginally firmer at harvest firmness than those trained on tall spindle trees (Figure 3.2.7). However, during storage Gala harvested from centrifugal trees softened more quickly at the final inspection after coming out of CA storage (0.5-1.0°C) in March and this was irrespective of whether fruit had been grown under reflective covers. There was no effect of pruning or covers on the Ex-store % Brix or the incidence of rots. Internal quality of fruits deteriorated by the final inspection mostly due to the over maturity of fruit entering store (Figure 3.2.7). Fruits from centrifugally pruned trees had an increased incidence of senescent breakdown and where this was combined with reflective covers the incidence of breakdown increased.

Table 3.2.7 The storage quality of Gala apple trained under tall spindle and centrifugal systems with or without addition of reflective cover placed in the alleyways post-fruit set. Fruits were stored in 3%CO₂, 1% O₂ (0.5-1.0°C) for 6 months.

	Pruning	No Cover			Reflective Cover			f.prob	LSD _{0.05}
		Dec	Feb	March	Dec	Feb	March		
Firmness	Tall Spindle	66.1	70.8	70.5	65.4	62.9	63.2	0.074	4.76
(N)	Centrifugal	68.8	65.5	63.2	72.4	67.7	60.8		
% Brix	Tall Spindle	13.9	15.1	12.9	14.2	14.2	14.3	0.571	1.755
	Centrifugal	14.9	14.1	13.6	14.9	14.3	13.1		
% Rots	Tall Spindle	0.0	5.0	10.0	0.0	0.0	10.0	0.619	10.89
	Centrifugal	0.0	0.0	5.0	0.0	0.0	10.0		
%Sen Bdn	Tall Spindle	0.0	0.0	20.0	0.0	0.0	60.0	<0.001	8.89
	Centrifugal	0.0	0.0	60.0	0.0	0.0	90.0		
Sen Bdn	Tall Spindle	0.0	0.0	5.0	0.0	0.0	14.0	<0.001	2.87
Index	Centrifugal	0.0	0.0	17.0	0.0	0.0	24.9		

N.B Senescent Breakdown Index (Sen Bdn Index) has a maximum value of 60

Year 4: WP 2 Increasing light interception by centrifugal pruning and deployment of reflective mulches covers 2019/2020

Effect of the treatments on fruit growth

Fruit size measurements were taken at twice weekly intervals from fruit tagged either side of the row and width and height measurements were recorded (Figure 4.2.1). Fruit size of Gala trained in a centrifugal manner with reflective covers positioned in the alleyways led to a small (1-2 mm) increase in fruit size over the course of fruit development (Figure 4.2.1). Due to a high incidence of apple scab (*Venturia inaequalis*) yield and class 1 data was not collected as the incidence of disease was sufficiently high to affect data.

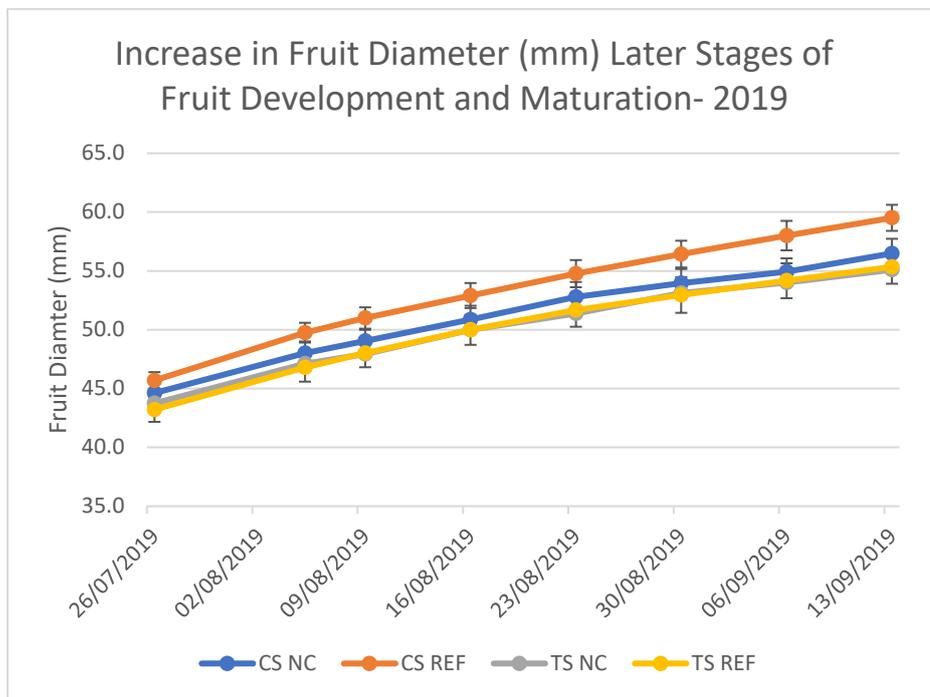


Figure 4.2.1 Growth rate (mm) of Gala apples subject to centrifugal training and positioning of reflective covers in alleyways. CS NC: Centrifugal No Covers; CS REF: Centrifugal Reflective Covers,

Harvest Analysis- NRI

Apples were harvested on 19th September 2019 and transferred to the PQC where fruits were sampled for firmness, starch and % Brix, followed by FDM assessments. Trees under the centrifugal system produced fruit with 0.4% higher FDM with apples grown under the centrifugal system accumulating 15% FDM compared to 14.6% in apples harvested from TS trees (Table 4.2.1). Moreover, fruit grown under CS were less mature based on I.E.C. and CTIFL starch patterns than fruit grown under conventional tall spindle trees (Table 4.2.1). The effect is most likely a result of fewer fruit numbers on the tree. Reducing crop load has been reported to inhibit fruit maturity (Johnson 1993).

The presence of reflective covers raised %FDM to 14.9% where covers were placed in alleyways compared to 14.6% in their absence. When averaged across the whole tree the presence of reflective covers did not alter fruit maturity characteristics (I.E.C., CTIFL Starch), however, the sucrose content of fruits was higher in apples cultivated under reflective covers. During fruit development and maturation the concentration of sucrose declines as it is converted into fructose and glucose. When averaged across pruning systems fruits from the upper canopy were marginally less mature based on I.E.C values but this

was not backed up by starch patterns. Delayed maturity in the top of the tree is most likely the result of fewer fruits. Fruits at the top of the canopy were on average 0.3% higher in %FDM with fruits yielding 14.9% FDM compared to 14.6% in those harvest in the lower canopy.

Table 4.2.1. Overall effects of training system, sampling position and the presence of reflective covers on %FDM and fruit Maturity

<i>Pruning</i>	<i>Tall Spindle</i>	<i>Centrifugal</i>	<i>F.prob</i>	<i>LSD_{0.05}</i>
<i>I.E.C (ppb)</i>	369	216	<.001	42.5
% DM	14.6	15.0	0.001	0.08
CTIFL starch	6.9	6.3	0.014	0.41
Fructose	130.6	122.7	0.082	12.92
Glucose	18.8	19.3	0.689	2.78
Sucrose	90.3	85.7	0.18	7.12
<i>Covers</i>	<i>Reflective Covers</i>	<i>No Covers</i>	<i>F.prob</i>	<i>LSD_{0.05}</i>
<i>I.E.C (ppb)</i>	277	308	0.147	42.5
% DM	14.9	14.6	0.006	0.08
CTIFL starch	6.7	6.5	0.419	0.41
Fructose	128.4	124.9	0.393	12.92
Glucose	17.8	20.3	0.069	2.78
Sucrose	96.2	79.8	<.001	7.12
<i>Position</i>	<i>Top</i>	<i>Bottom</i>	<i>F.prob</i>	<i>LSD_{0.05}</i>
<i>I.E.C (ppb)</i>	266	319	0.016	42.5
%DM	14.9	14.6	0.014	0.08
CTIFL starch	6.9	6.4	0.027	0.41
Fructose	130.6	122.7	0.084	12.92
Glucose	18.8	19.3	0.688	2.78
Sucrose	88.7	87.3	0.681	7.12

N.B numbers in bold represent values significantly different (P<0.05) in the same row

Maturity.

When the full interaction between pruning systems, reflective covers and fruit position within the tree was considered (Table 4.2.2) the impact of the centrifugal system was to delay maturity in fruit from the top of the canopy and this may be a direct result of fewer apples in the top canopy. Within the full interaction, the correlation between I.E.C and fruit starch staining patterns (CTIFL) was not well correlated (Table 4.2.2)

%FDM was raised in the lower canopy of centrifugally pruned trees where the positioning of reflective covers was practiced (Table 4.2.2). However, in tall spindle systems no benefit of reflective covers was observed. The % Brix of fruit was not increased even in trees where the combination of centrifugal system and reflective covers raised %FDM. Moreover, a higher fruit firmness (N) was recorded in fruit that were higher in %FDM

Table 4.2.2 The interaction between Training Systems, Reflective Covers and Fruit Position on Fruit Maturity attributes at Harvest

	Reflective Covers	Int. Eth. Conc. ppb		Starch CTIFL		% FDM		% Brix		Firmness (N)	
		Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
Tall Spindle	Yes	385	366	6.8	8.1	14.8	14.6	11.9	11.8	79.6	77
	No	348	377	6.2	6.5	14.4	14.6	12.1	11.8	83	81.3
Centrifugal	Yes	153	204	5.8	6.1	14.8	15.6	11.7	12	83.6	86.6
	No	179	330	6.7	6.8	14.6	14.9	12	12.1	82.5	82.7
F.prob		0.545		0.349		6.42		0.419		0.327	
LSD _{0.05}											
P x C x Pos.		80.5		0.35		0.3867		0.54		3.97	

N.B. Values in bold are significantly different ($p < 0.05$) from fruit harvested from the control (Tall Spindle, No Covers) in the same column. To convert fruit firmness from Newtons (N) to kg divide values by 9.8

Reflective covers had a general effect of raising sucrose content but this was only significant in some treatment combinations while the pool of fructose and glucose in fruit at harvest was unaffected by training system or reflective covers. Red colour intensity was not effected by consistently by reflective cover or pruning system.

Table 4.2.3. The effect of pruning system, positioning of reflective covers in alleyways and sampling position within trees of Fruit quality of Gala

<i>Pruning</i>	<i>Reflective Covers</i>	Fructose		Glucose		Sucrose		<i>Yellow (b*)</i>		<i>Red (a*)</i>	
		<i>Top</i>	<i>Bottom</i>	<i>Top</i>	<i>Bottom</i>	<i>Top</i>	<i>Bottom</i>	<i>Top</i>	<i>Bottom</i>	<i>Top</i>	<i>Bottom</i>
<i>Tall Spindle</i>	<i>Yes</i>	146.3	122.1	17.7	18.1	109.5	92	18.6	20.8	41.2	41
	<i>No</i>	127.4	126.6	18.7	20.6	81.2	78.4	22.1	24.5	40	31.9
<i>Centrifugal</i>	<i>Yes</i>	121.6	123.8	17.7	17.5	84	99.4	20.9	23.8	40.1	36.5
	<i>No</i>	126.9	118.6	21	20.9	80	79.6	18.8	18.7	39.9	41.7
f.prob		0.065		0.782		0.039		0.131		0.002	
LSD _{0.05} pruning x covers x position		18.27		5.552		14.24		1.58		3.42	

N.B. Values in bold are significantly different ($p < 0.05$) from fruit harvested from the control (Tall Spindle, No Covers) in the same column

Year 5, WP 2: Increasing light interception by centrifugal pruning and deployment of reflective mulches covers (2020/2021)

Data was analysed for the impact of pruning, the presence of reflective covers and combined effects of pruning techniques and reflective covers.

The positioning of reflective covers increased the amount of light intercepted in the lower canopy by 25% while centrifugal training systems where trees are trained to allow a column of light to penetrate down the trunk

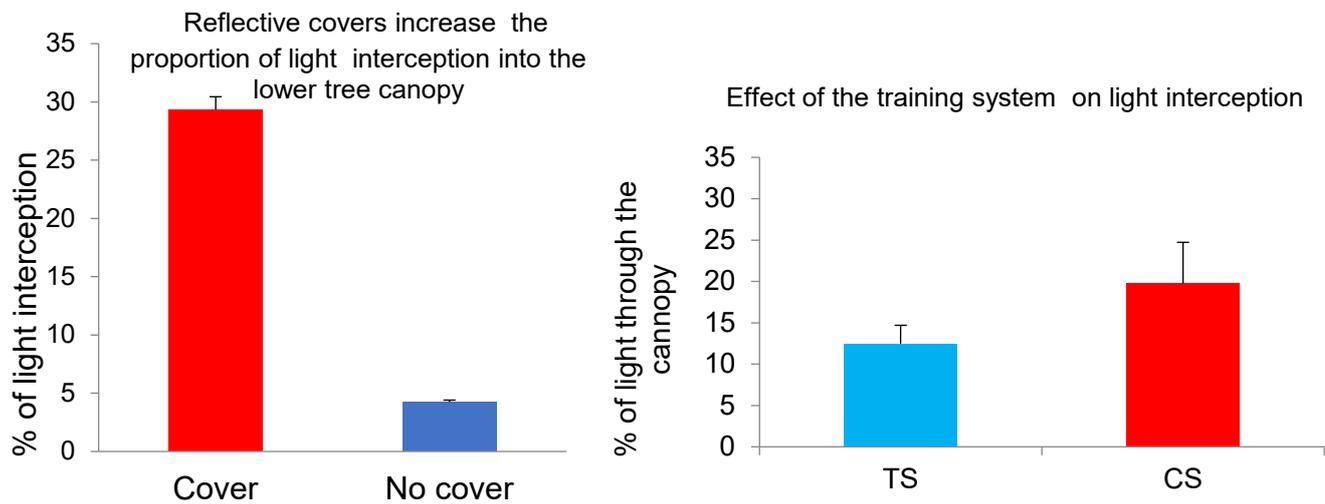


Figure 5.2.1 The impact of positioning reflective covers and centrifugal pruning on improving light penetration and light interception into the lower canopy of Gala apple trees

With high levels of sunshine hours during the summer of 2020 Gala apples returned high %DM content (16.8-17.8 %). Significant differences in treatments was not observed due to high variability between plots, however, in general, the presence of reflective covers raised %DM content of apples harvested from branches below <1.5 M, by 0.6%. Centrifugal pruning led to a small (0.3%) and non-significant increase in %DM in lower canopy fruit. As expected, fruits in the upper canopy with access to ample light levels were not effected by pruning or the presence of reflective covers.

% Brix was not effected by pruning and only a small non-significant ($p>0.05$) 0.3% increase in % Brix was observed in low hanging fruit where covers were positioned under standard tall spindle trees. Trees subject centrifugally pruning

Table 5.2.1 Harvest maturity of Gala apples grown under centrifugal pruning system with or without placement of reflective covers within alleyways

Pruning	Tall Spindle	Centrifugal	F.pr.	LSD
Colour A (Red)	39.9	41.4	0.2	2.1
Colour B (Yellow)	17.2	18.0	0.4	2.1
% Brix	14.9	15.2	0.1	0.4
% Starch	34.8	43.3	0.02	7.0
Firmness (N)	73.6	74.5	0.5	3.2
% DM	17.4	17.5	0.6	0.6
Reflective Covers	Covers	No Covers	F.pr.	LSD
Colour A (Red)	41.3	40.0	0.2	2.1
Colour B (Yellow)	18.2	17.0	0.2	2.1
% Brix	15.1	15.0	0.9	0.4
% Starch	40.4	37.7	0.4	7.0
Firmness (N)	73.2	74.9	0.3	3.2
% DM	17.6	17.3	0.4	0.6
Position	Top	Bottom	F.pr.	LSD
Colour A (Red)	40.6	40.7	0.9	2.1
Colour B (Yellow)	17.3	17.9	0.5	2.1
% Brix	15.0	15.1	0.8	0.4
% Starch	40.4	37.7	0.6	7.0
Firmness (N)	75.2	72.9	0.1	3.2
% DM	17.7	17.3	0.2	0.6

Table 5.2.2. The interaction of pruning techniques and the positioning of reflective covers in alleyways on % Dry matter and % Brix of Gala apples at harvest

% DM		Position		F.pr.	LSD
Pruning	Covers	Bottom	Top		
Tall Spindle	Ref. Covers	17.4	17.7	0.823	1.273
	No Covers	16.8	17.7		
Centrifugal	Ref. Covers	17.7	17.6		
	No Covers	17.1	17.8		
% Brix		Bottom	Top	0.82	0.736
Tall Spindle	Ref. Covers	15.0	14.9		
	No Covers	14.7	15.0		
Centrifugal	Ref. Covers	15.3	15.1		
	No Covers	15.3	15.2		

Fruit quality was assessed in March, April and May, when averaged across all storage assessment dates. Interestingly tall spindle trees overall yielded fruit that retained slightly better firmness 65.7 N (6.6 kg) versus 64.2 N (6.4 kg). Moreover, in the absence of reflective covers fruit in store over the 8 months of storage was marginally firmer 65.6 N (6.6 kg) versus 64.3 N (6.4 kg).

The incidence of rotting in fruit caused mainly by *Neonectria* infections increased with time of storage. Overall fruit under reflective covers had a higher incidence of rotting- although from the harvest maturity data this is not caused by a reflective covers impacting on harvest maturity, nevertheless fruits in general were over mature when harvested.

In late-stored Gala in assessments made in April (7 months) and May (8 months) fruits from lower branches where exposure to increased light interception through reflective covers led to 0.5-0.7% % Brix increase

There were no perceived benefits of centrifugal pruning or the placement of reflective covers in terms of improving other storage quality attributes such as firmness- in this trial that had been

Table 5.2.3 Ex-Store assessments of Gala stored under 5% CO₂, 1% O₂ at 0.5°C for 8 months, fruits were assessed after 6, 7 and 8 months storage

Pruning	Centrifugal	Tall Spindle		F.pr.	LSD_{0.05}
% Brix	15.1	15.1		0.66	0.27
Firmness (N)	64.2	65.7		0.01	1.16
% Rots	17.1	19.8		0.30	2.53
Covers	Reflective Covers	No Covers		F.pr.	LSD_{0.05}
% Brix	15.1	15.1		0.80	0.27
Firmness (N)	64.3	65.6		0.03	1.16
% Rots	21.2	15.6		0.04	2.53
Position	Top	Bottom		F.pr.	LSD_{0.05}
% Brix	15.2	15.0		0.10	0.27
Firmness (N)	65.1	64.8		0.53	1.16
% Rots	16.0	20.8		0.07	2.53
Inspection	March	April	May	F.pr.	LSD_{0.05}
% Brix	15.3	15.1	15.0	0.17	0.33
Firmness (N)	65.9	64.4	64.6	0.10	1.43
% Rots	11.2	14.4	29.7	<.001	3.10

Work Package 3: Thinning Strategies to Raise Fruit Dry Matter and Increase Fruit Quality

Materials and Methods

Year 1 Fruit Dry Matter Accumulation During Fruitlet and Fruit Development 2016-2017

The initial phase in year 1 quantified changes in DMC accumulation from flowering through to fruit harvest from flowers and fruitlets positioned high in the canopy above 1.5 M and those in the shaded parts of the canopy (0.75-1 M), harvested from north and south facing aspects of each tree (Figure 1.3.1) Initial samples were first weighed (Fresh weight FW) before freezing whole in liquid nitrogen while fruitlets greater than 35 mm were sectioned and opposite eighths of cortex were frozen and stored at -80°C. Samples were then subject to freeze drying (-80°C) for 48 hours, after which samples were reweighed and a % FMD was calculated. Thereafter freeze dried material was ground in either with a pestle and mortar or larger samples were powdered in a spice grinder. Samples were then subject to separation and analysis of sugars, fibre and %starch following the method of Tsuchida et al. (2015). Additional samples were collected at harvest and stored at 0.5-1.0°C in 3% CO₂ 2%O₂ until May 2017.



Figure 1.3.1 – Aerial photograph of trial orchard.

The orchard was approximately 2.25ha (300m long rows x 75m wide) split into 3 sections (blocks). Row spacing of 2.25m and tree spacing of 1.25m within a row with 1m between twin rows. There were 16 twin bed rows with between 31 and 50 trees per row approximately.

Sampling Strategy

Gala was harvested in year 1 (2016) on 14 occasions (Table 1.3.1) :

1. Upper well lit (UL) – un shaded, stake height (approximately 2m high), on leader or ends of branches at top of tree.

- Lower shaded (LS) – shaded, lowest branches, approximately 1m high, midway between tip and trunk at bottom of tree.

Table 1.3.1. Sample event number and dates with crop growth stage and description.

Event	CGS (BBCH)	Stage / weeks after petal fall	Fruit size	Date (actual or w/c)
1	67/68	Flowers fading / petal fall (NRI only)		20 May
2	69	Petal fall	Up to 5mm	27 May
3	70	1 week post petal fall		3 June
4	71	2 weeks after petal fall approx	Up to 10mm	8 June
5		3 weeks after petal fall		15 June
6		4 weeks after petal fall	Up to 20mm	22 June
7		5 weeks after petal fall		29 June
8		6 weeks after petal fall / second fruit fall		6 July
9		7 weeks after petal fall		13 July
10		8 weeks after petal fall		19 July
11		9 weeks after petal fall		27 July
12		10 weeks after petal fall		3 August
13	74	11 weeks after petal fall (T stage)	Up to 40mm	10 August
14	99	Harvest		12 September

The trees for each sampling point used were marked with flash tape for the week 1 event. In subsequent events, trees were counted from the flash tape according to the corresponding week number of the trial. If any trees were either pollinators or dead/not representative, these trees were missed, not counted and replacements found.

Collection of fruit samples were from two areas of the trees (sampling plan Figure 1.3.2).

- Upper well lit (UL) – sample numbers 1 – 12,
- Lower shaded (LS) – samples numbers 13-24

12	7	6	1	BLOCK 1
24	19	18	13	
11	8	5	2	BLOCK 2
23	20	17	14	
10	9	4	3	BLOCK 3
22	21	16	15	

Figure 1.3.2. Sampling plan.

Opposite trees within the alley were used enabling quick collection of fruit. 40 fruits were collected per sample at the earliest stages (from 2 pairs of trees), decreasing to 30/20 fruit as size developed (from 2 pairs of trees) and 10 at harvest (from 1 pair of trees). Fruit was selected from the alley aspect of trees (ie not the backs within the twin bed). Different clusters but with similar numbers of fruit in each cluster from half way along the branch (Treatment B lower shaded). The King fruit were discarded. Guard trees surrounded each plot and plots started 5 trees in from the end of a row.

Year 2: WP 3 Bud, flower and fruitlet thinning strategies (2017-2020)

The second year (2017) of the trial used an established Gala orchard at FAST LLP, Brogdale Farm, Faversham - Latitude 51.294933, Longitude 0.882898, Reservoir Field, Block 1B (Figure 2.3.1).



Figure 2.3.1. Aerial photograph of FAST trial orchard, Faversham.

The orchard section was approximately 0.07ha. There were four 50m long rows spaced at 3.5m with trees at 1.0m apart within the row.

Treatments: There were 7 treatments comprising 1 novel, 1 mechanical, 2 chemical and 3 hand thinning methods and 1 control (Table 2.3.1)

1. **Control** - no thinning
2. **Bud** thinning
BBCH 52-54 (end of bud swelling to mouse ear) via bud extinction using MAFCOT Equilifruit tool ratios
3. **Mechanical** thinning
BBCH 65-66 - 60% first open flower using hand held device
4. **Exilis** - chemical thinning

BBCH 70-72 - Fine Exilis 6-Benzyladenine + Fixor (PGR) (funded by Fine)

5. **Brevis** - chemical thinning

BBCH 70-71 & 71-72 - Adama Brevis 150 SG metamitron 15% (PGR) (funded by Adama)

6. **Standard** Hand Thinning

BBCH 71-72 - Fruit size 15mm to 25mm, pre/up to second fruit fall

7. **Size** Hand Thinning

BBCH 73 - Event 1 fruit size from 25mm to 30mm, event 2 fruit size 40mm (BBCH 74)

8. **Late** Hand Thinning

BBCH 73-74 - Fruit size 30mm to 40mm, after second fruit fall

Trial design

The trial was made up of 1 area in 4 rows. The trial was arranged in a randomised complete block design. Each row represented a replicate block and there were 4 replicates per treatment. Each replicate treatment plot had 3 trees. There were 12 trees per treatment and 96 treatment trees in total. Guard trees were situated between replicate plots and at the ends of each row making 132 trees total (Figure 2.3.2).

SOUTH							
R1	R2	R3	R4	Number	Treatment		
G	G	G	G	1	Control		
2	4	6	3	2	Bud		
G	G	G	G	3	Mechanical		
3	1	4	2	4	Exilis		
G	G	G	G	5	Brevis		
8	3	5	8	6	Standard		
G	G	G	G	7	Size		
1	2	7	4	8	Late		
G	G	G	G	G	Guard tree		
4	5	3	7				
G	G	G	G				
6	6	2	6				
G	G	G	G				
5	7	1	1				
G	G	G	G				
7	8	8	5				
G	G	G	G				
R1	R2	R3	R4				
NORTH							

Figure 2.3.2 Trial Plan.

Table 2.3.1a Thinning Treatments :Applications, timing and descriptions-2017-2018 (years 2-3)

NO	TREATMENT	RATE WATER VOLUME	EVENTS	BBCH STAGE	DESCRIPTION, FRUITLET SIZE & CONDITIONS
1.	Control	Na	Na	Na	Na
2.	Bud	Na	1	52-54	MAFCOT Equilifruit tool used to extinguish excess buds and gain optimum per branch diameter of 5 fruits/cm ² of trunk 160 fruits per tree
3.	Mechanical	6-7 km/ha at around 270 rpm (depending on orchard flower density)	1	65-66	60% first open flower
4.	Chemical Exilis & Fixor*	Exilis 3.5 to 7.5 L/ha Fixor 100ml/ha 100 L water	1 per year maximum application	70 -72	8 to 10mm Exilis + Fixor (no treatment above 10mm) (7 to 15mm Exilis alone) Above 15°C with increasing temperatures for 3 to 4 days after
5.	Chemical Brevis*	1.1kg/ha to 1.65g/ha (2.2kg/ha max) 1000 L water	2 NB minimum 5 days between applications	70-71 71-72	Application 1 8-10mm Application 2 12-14mm (not made in 2017) 9-11mm optimum (8-14mm max window) lower water volumes (min 350L/ha) & no tank mixing

6.	Standard Hand Thinning	Na	1	72-73	15mm to 25mm pre/up to 2nd fruit fall (50 days post full bloom)
7.	Size Hand Thinning	Na	2	71-72 & 74	Event 1 from 25mm-30mm (BBCH 73), event 2 at 40mm (BBCH 74)
8.	Late Hand Thinning	Na	1	74	30mm to 40mm after 2nd fruit fall

* Chemical thinners were applied using manufacturers' recommendations and adapted according to conditions before, during and after applications (see product label, SDS and guidelines (Appendix 1)).

Table 2.3.1b Thinning Treatments :Applications, timing and descriptions-2019 (year 4)

NO	DESCRIPTION	RATE & WATER VOLUME	EVENTS per APPLICATIONS	BBCH STAGE	DETAILS
1	Control	Na	Na	Na	Na
2	Singles	Na	1	71-72	Fruit size 10-20mm before fruit fall
3	Single (>.1.5 M) Doubles (< 1.5 M)	Na	1	71-72	Fruit size 10-20mm before fruit fall
4	Chemical Exilis & Fixor*	Exilis 3.5 L/ha to 7.5 L/ha in 100 L water Fixor 100ml/ha	1 per year maximum application	70 -72	8 to 10mm Exilis + Fixor (no treatment > 10mm) 7 to 15mm Exilis alone KING FRUIT SIZE >15°C & increasing temperatures 3 to 4 days after
5	Chemical Brevis*	1.1kg/ha to 1.65g/ha (2.2kg/ha max) in 1000L water	2 NB minimum 5 days between applications	1 = 70-71 2 = 71-72	Application 1 8-10mm Application 2 12-14mm KING FRUIT SIZE 9-11mm (8-14mm max window) lower water volumes (min 350L/ha no tank mix
6	Hand Thinning Standard	Na	1	71-73	15mm to 25mm Pre/up to 2nd fruit fall (50 days post full bloom)
7	Hand Thinning Size	Na	2	1 = 73 2 = 74	Event 1 from 25mm-30mm (at fruit fall) Event 2 at 40mm (late, after fruit fall)
8	Doubles		1	71-72	Fruit size 10-20mm before fruit all

* Chemical thinners were applied using manufacturers' recommendations and adapted according to conditions before, during and after applications (see product label, SDS and guidelines)

Table 2.3.1c Thinning Treatments :Applications, timing and descriptions-2020 (year 5)

NO	DESCRIPTION	RATE & WATER VOLUME	EVENTS / APPLICATIONS	BBCH STAGE	DETAILS
1	Control	Na	Na	Na	Na
2	Singles	Na	1	71-72	Fruit size 10-20mm before fruit fall
3	Single (>.1.5 M) Doubles (< 1.5 M)	Na	1	71-72	Fruit size 10-20mm before fruit fall
4	Chemical Exilis & Fixor*	Exilis 3.5 L/ha to 7.5 L/ha in 100 L water Fixor 100ml/ha	1 per year maximum application	70 -72	8 to 10mm Exilis + Fixor (no treatment > 10mm) 7 to 15mm Exilis alone KING FRUIT SIZE >15°C & increasing temperatures 3 to 4 days after
5	Chemical Brevis*	1.1kg/ha to 1.65g/ha (2.2kg/ha max) in 1000L water	2 NB minimum 5 days between applications	1 = 70-71 2 = 71-72	Application 1 8-10mm Application 2 12-14mm KING FRUIT SIZE 9-11mm (8-14mm max window) lower water volumes (min 350L/ha) no tank mix
8	Doubles	Na	1	71-72	Fruit size 10-20mm before fruit fall

* Chemical thinners were applied using manufacturers' recommendations and adapted according to conditions before, during and after applications (see product label, SDS and guidelines)

Bud thinning 2017-2018

Treatment 2 Bud Thinning was achieved after pruning using a MAFCOT Equilifruit tool to gain optimum buds per branch diameter. The diameter was measured at the base of each branch nearest to the trunk and the values associated with the branch size used to reduce bud numbers. See Figure 2.3.3.



Figure 2.3.3. Mafcot Equilifruit Tool.

Mechanical thinning 2017-2018

An Electroflor machine was used (Ins 9534 BT telescopic pole 1.3-1.8m with battery & mains charger, control & box (Agricare)). Practice was undertaken on similar Gala trees prior to thinning treatment trees to ensure consistent results. See Figure 2.3.4



Figure 2.3.4. Electroflor machine.

Bud extinction (Treatment 2) and mechanical flower thinning using the Electroflor (Treatment 3) and late thinning were discontinued after the first two years (2017-2018). Thereafter (2019-2020) these treatments were replaced with early thinning (BBCH71-72; fruitlet size 10-20 mm) of fruit cluster to singles (T2) across the canopy, singles > 1.5 M/doubles < 1.5 M (T3) or doubles (T8) across the tree.

Hand thinning

Treatment 7 Hand Thinning Size (2017-2019) involved removal of all fruit below the size required and predicted to reach optimum at harvest (63mm). This was predicted using weekly size curves from the FAST members' Top Fruit Advisory. Each of the two events involved removing fruits of different sizes from clusters which resulted in varying numbers of fruit per cluster remaining in all portions of the tree.

Treatments 6 and 8 Hand Thinning Standard (2017-2019) and Late (2017-2018) were carried out by removal of fruit from clusters leaving doubles below 1.5m and singles above 1.5m.

Thinning per treatment was carried out by the same FAST Trials Team member. No quality thinning for any treatment was carried out since it was deemed to be too subjective and there was a variable and light crop load; based on the Gala Standard of 5 fruits/cm² of trunk there were fewer than the optimum of approximately 160 fruits per tree (at 1m apart for 60 t/ha).

Treatment 2 (2019-2020) : Singles was carried out by removal of fruitlets from clusters leaving single fruitlets per cluster. Treatment 3 Singles/Doubles (2019-2020) clusters were thinned to singles in the canopy above 1.5 M and doubles below 1.5 M. Treatment. Treatment 8 Doubles (2019-2020) treatment thinning each flower cluster to 2 fruitlets per cluster. Treatments 2, 3 and 8 were implemented at BBCH stage 71-72 Fruit size 10-20mm before fruit fall.

Treatments were applied to 4 replicate blocks of 3 tree/treatment in years 2017-2019. In the final year 2020 Standard thinning and thinning to size were dropped and extra replicates (five per thinning treatment) were applied to the orchard. In year 5 (2019-2020) standard thinning, late thinning and thinning to size were dropped in favour of increasing the number of replicates from 4 to 5 reps.

The unthinned trees (Treatment 1 Control), Exilis (Treatment 4) and Brevis (Treatment 5) trees remained constant treatments throughout the trial.

Crop Care

The trees/plants were grown according to Good Agricultural Practice following IPM protocols. Regular crop monitoring was carried out by a BASIS qualified FAST advisor for

pest and disease. Standard commercial spray programmes were applied as necessary or if thresholds were exceeded and according to IPM Best Practice. Biological control was introduced as appropriate. A standard commercial nutrition programme was followed as recommended by FAST advisors and based on previous soil samples. Standard hand pruning was carried out in spring and summer pruning of the tops as required in July. See Appendix 2 Chronology.

Assessments

Physiological and monitoring

- Weekly observations of the trial area was made throughout season.
- Weekly monitoring of BBCH CGS (Crop Growth Stage) on Control plots was commenced approximately 1 month prior to BBCH 53 (bud burst) and recorded continuing up to BBCH 74 (fruit up to 40mm T stage).
- Temperature, RH and PAR was monitored via SMS remote sensing equipment.

Visual

- Photographs were taken of the middle tree in each treatment plot after each fruit drop event, of fruit dropped under trees, plus prior to and after each thinning event and at harvest (relevant photographs Appendix 3).

Fruit counts

- A membrane was installed under the middle tree in each treatment plot (from wheeling to wheeling and adjacent trunks) before BBCH stage 55 (bud thinning) and removed after BBCH stage 74 (hand thinning late).
- Numbers of fruit naturally shed and fallen onto the membrane at each fruit drop event were counted from the middle tree in each treatment plot.
- Counts of fruit removed from the middle tree in each treatment plot at each thinning event (treatments 6, 7 and 8 only) were recorded. Comparisons of fruit dropped naturally and deliberately thinned were made.

Dry matter – pre harvest (years 2-3 only)

Samples were collected at 2 events prior to harvest:

- 1 week post full bloom – BBCH 70

- 11 weeks post full bloom – BBCH 74, fruits 40mm after second fruit fall (T stage) and after all thinning events

12 fruits per plot were removed and FDM assessed. 4 fruit from each treatment tree were taken, 2 from each side, high and low in the canopy and from 2 year old wood. In subsequent years FDM was assessed on fruit at harvest only

Harvest

Starch progression was monitored weekly at 3 events commencing 3 weeks prior to the predicted harvest date (as per the FAST advisory) to accurately estimate the optimum harvest date. 10 fruit from guard trees in the trial area were selected at random at each event and processed.

Samples from each side of each treatment tree from 2 year old wood within the top, middle and bottom of canopy were collected prior to harvest for:

- Maturity - 30 fruits per treatment plot total (10 per tree, 5 from each side): Starch, % Brix, Fruit firmness
- Dry Matter & Fruit Mineral Analysis - 12 fruit total (4 per tree, 2 from each side)
- Quality - 60 fruit per treatment plot were assessed (20 per tree, 10 from each side): Fruit was sorted into C1 and waste. The waste was categorised, counted & weighed (under/over size <55mm / >80mm, disease, russet, pest, misshape, physical damage). The C1 fruit was graded according to 5 size classes (55- 60mm, 60-65mm, 65-70mm, 70-75mm, 75-80mm), counted & weighed. The percentage was calculated for waste & C1 fruit plus numbers in each size class
- Storage and quality (NRI) - 12 fruit total per plot were sampled (4 fruit per tree, 2 from each side)

Fruit was picked from the 3 tree plot and weighed and estimates of yield/ha was estimated from the average of 4 replicate plots per treatment.

The average total yield kg and T/H per treatment was calculated plus Class 1 T/Ha, % Class 1 and Waste, average Waste categories, Fruit Weight, Size Distribution and Starch %, % BRIX^o and Pressure (kg/mm).

Structural Carbohydrate, Starch and Sugar analysis NRI (Year 1: 2016)

In 2016 FDM of each sample was assessed by two methods – oven drying (FAST) and by freeze drying (NRI).

Rainfall, sunshine hours and Accumulated Day Degrees data was collated and plotted against the dry matter %. Samples for structural and non-structural carbohydrate assay were collected by NRI and measured at NRI facilities. NRI samples were collected from flowering through to fruit harvest from flowers and fruitlets positioned high in the canopy above 1.5 M and those in the shaded parts of the canopy (0.75-1 M), harvested from north and south facing aspects of each tree. Initial samples were first weighed (Fresh weight, FW) before freezing whole in liquid nitrogen while fruitlets greater than 35 mm were sectioned and opposite eighths of cortex were frozen and stored at -80°C. Samples were then subject to freeze drying (-80°C) for 48 hours, after which samples were reweighed and a % FDM was calculated. Thereafter freeze dried material was ground in either with a pestle and mortar or larger samples were powdered in a spice grinder. Samples were then subject to separation and analysis of sugars, fibre and %starch following the method of Tsuchida et al. (2015). Additional samples were collected at harvest and stored at 0.5-1.0°C in 3% CO₂ 2%O₂ until May 2017.

Sampling, Dry Matter and Sugar analysis NRI (2017-2020)

Samples for sugar analysis were collected by NRI at three time-points, after petal fall on at the beginning of May, at fruitlet stage mid-July, after the final thinning treatment had been applied and at harvest. Initial samples were first weighed (Fresh weight, FW) before freezing whole in liquid nitrogen while fruitlets greater than 35 mm were sectioned and processed as described above. Samples were then subject to an analysis of sugars by extraction of 0.2g of powdered tissue in 80% ethanol, 20% dH₂O for 120 mins with periodic vortexing; following incubation, the supernatant was collected following centrifugation (12,000 rpm) and filtered through 0.45 µM syringe filters and followed by analysis of sugars by HPLC.

Storage 2016-2020

Replicated 20 fruit samples of Gala from thinning treatments were placed in nets and loaded into storage cabinets at the JMB, fruits were cooled overnight to 0.5-1.0 °C, thereafter Controlled Atmosphere conditions of 3% CO₂, 1% O₂ were established through nitrogen flushing and the addition of CO₂, cabinet atmospheres were maintained using an ICA 6000 system with atmospheres sampled every 2 hours, with adjustments made through automatic injection of N₂ and CO₂ and venting with air. Nets of fruit were removed in March, April, May and June where available for immediate ex-store assessment with additional 20 fruit

samples subject to 7 days at 18°C. All fruit samples were subject to firmness (11 mm) probe on peeled equatorial regions of the fruit measuring the firmness (N) at 8 mm depth and maximum load (N) using a Llyod LRX. Composite juice samples were tested for % Brix using a ATAGO refractometer. Internal quality of fruits was assessed by cutting fruits three places across the equatorial region, at the calyx end and across the shoulder of the fruit.

Data Analysis

Data was subject to ANOVA using Genstat (version 20)-overall means and significant interactions of treatment x position or treatment x time are presented in the tables with appropriate $LSD_{0.05}$.

WP3 Results

Year 1: WP 3. Thinning and Crop Load (2016/2017)

Dry matter data was collected by FAST and NRI separately showed that dry weight from oven drying was 1-2% lower than when freeze drying was used. FDM accumulation followed similar patterns across the two sampling strategies.

Fruit harvested from the upper canopy (Top) maintained a higher FMD during the early stages of fruit development before FMD in top and bottom fruit samples declining to 13.1-13.4% FMD around 4 weeks post petal fall (Figure 1.3.3). Thereafter, FMD increased until harvest, where FMD content in Gala harvested from the upper canopy was approximately 0.7% higher in FMD (16.5%) compared to fruit from the lower canopy (15.8%). It has been reported that high respiration during cell division is responsible for the initial decline in FDM as sugars are respired. Fruits sampled initially from high in the canopy resulted in a higher FDM than fruits sampled from the shaded part of the canopy development. This demonstrates the importance of improving light penetration and light interception during fruit development .

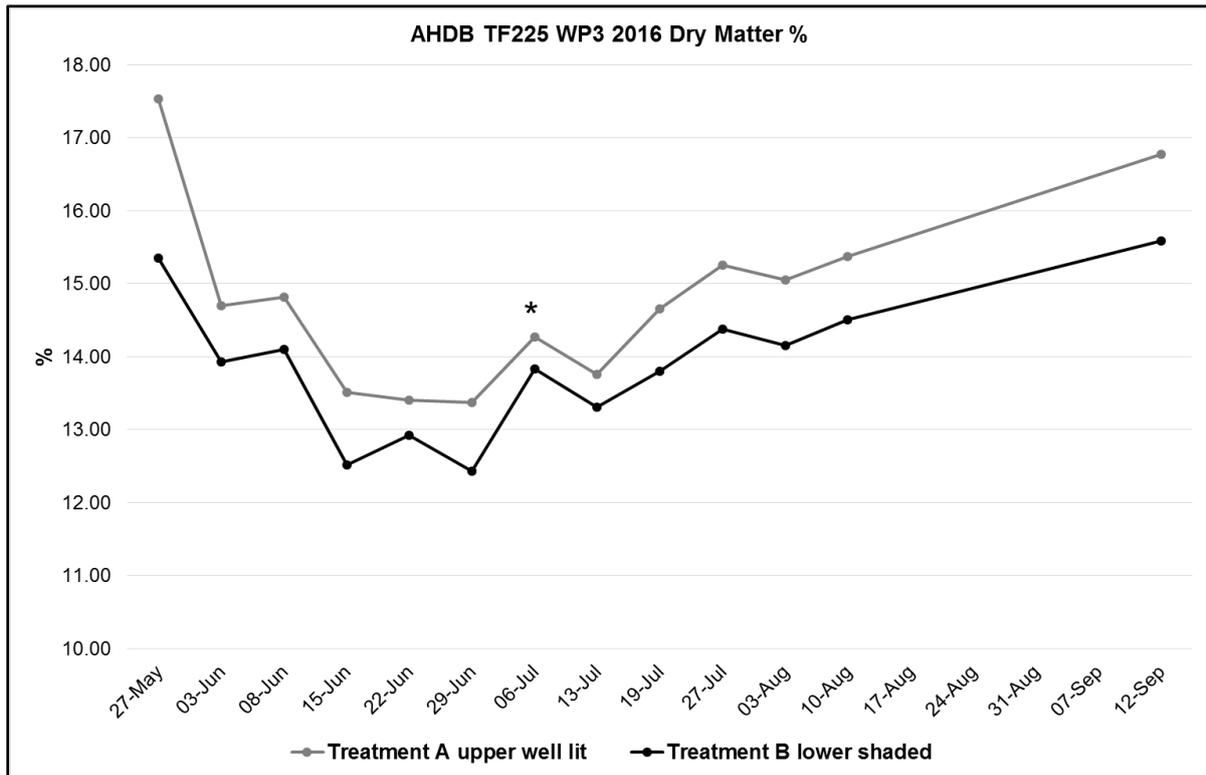


Figure 1.3.3. % dry matter content progression from BBCH CGS 69 (petal fall) to harvest (BBCH 99). * denotes non-significant ($p < 0.05$) event

Additional samples were harvested by NRI which also analysed structural carbohydrate. A similar pattern of FDM accumulation was reported via the freeze drying method with a drop in dry matter content around petal fall where it is assumed that increased respiration of fruitlets is proportionally burn off more sugars reducing the overall FDM content, before increasing over the summer months to harvest. The FDM content of fruit from the upper canopy was 1-1.5% more than shaded fruit in the lower canopy at harvest (Figure 1.3.4).

The freeze drying method albeit the values were 1-2% higher with initial sample dates with fruitlets. A certain amount of molecular water can be attached sugars and are hard to remove through freeze drying, however, oven drying runs the risk of losing carbon through over drying especially when drying very fragile fruitlets. However, by harvest FDM from both methods were very similar (Figure 1.3.4) .

Additional samples were collected at harvest and stored at 0.5-1.0°C in 3% CO₂ 2%O₂ until May 2017 (Table 4).

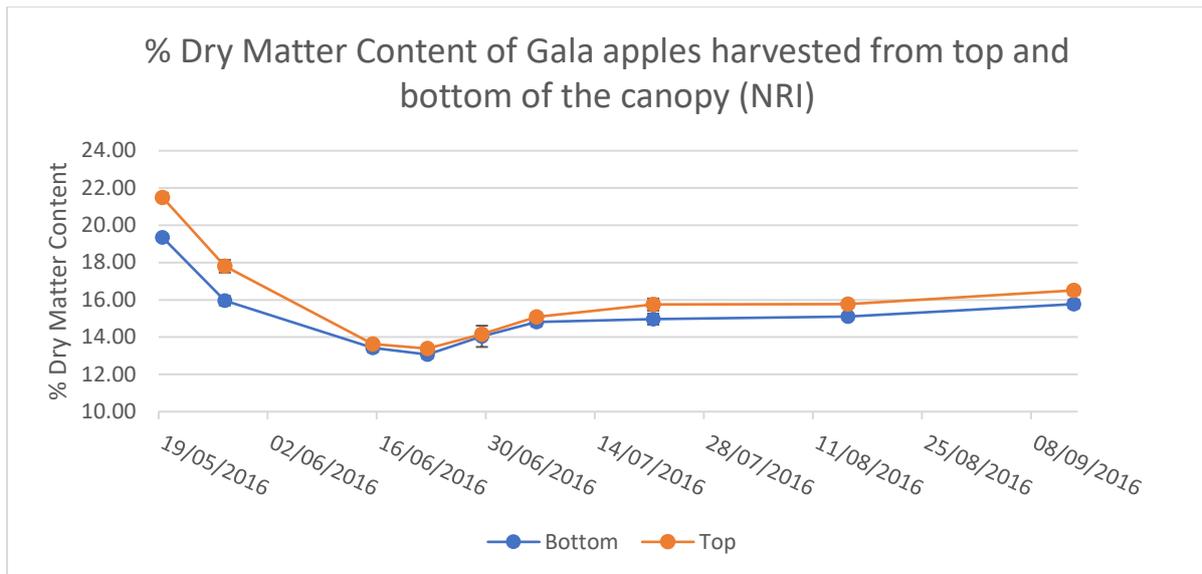


Figure 1.3.4.. Dry matter content (%) of Gala fruit picked from (Top) exposed upper branches, and (Bottom) shaded lower branches of a Gala orchard. Each data point is the mean of 24 +/- SE. Each sample consisted of 5-10 fruit (number of fruit per sample decreased through the season).

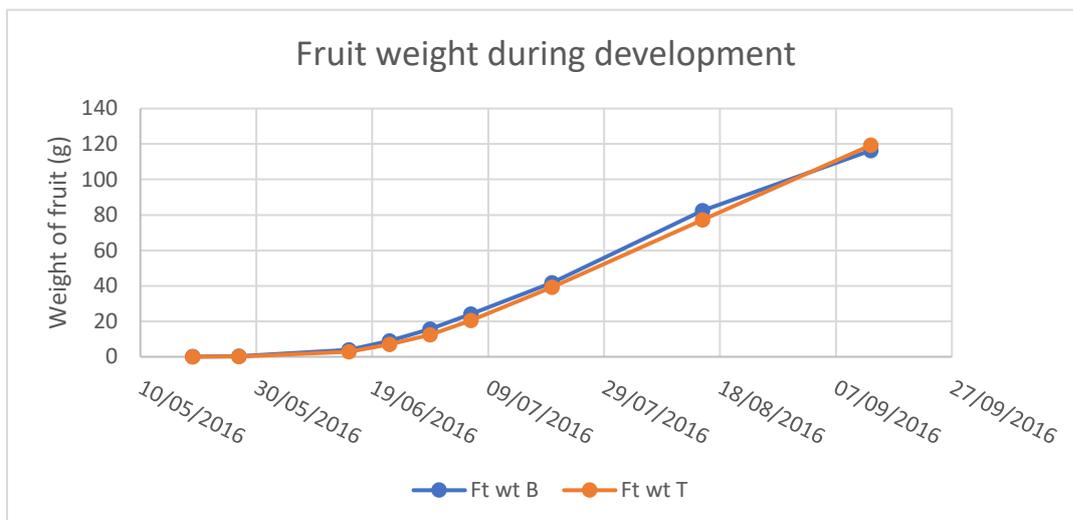


Figure 1.3.5 . Fruit weight during development of Gala fruit picked from the top (T) exposed upper branches, and bottom (B) shaded lower branches of a Gala orchard. Each data point is the mean of 24 samples of 5-10 fruit (number of fruit per sample decreased through the season).

Fruit weight from the top of the canopy was initially smaller than fruit taken from the bottom, however, during fruit development positional effects on fruit weight declined (Fig 1.3.5). Early stages of fruit development are influenced by the reserves of carbohydrate in the adjacent branches, therefore, branch size and age influences early fruit development.

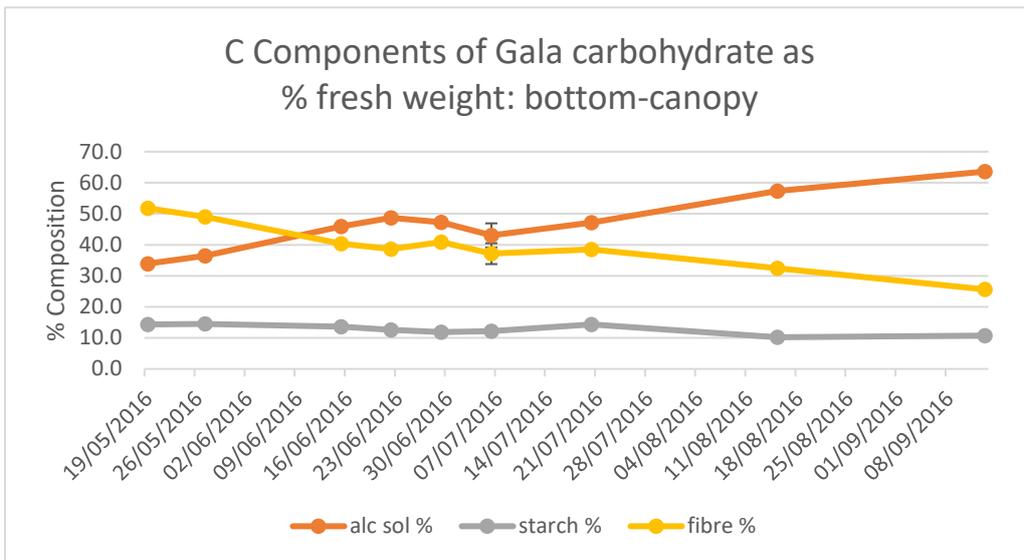
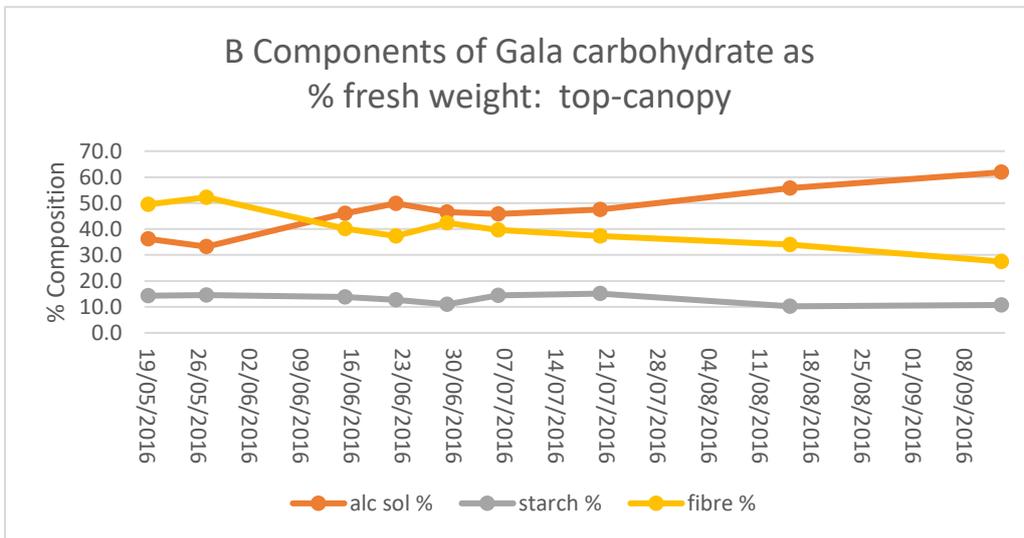
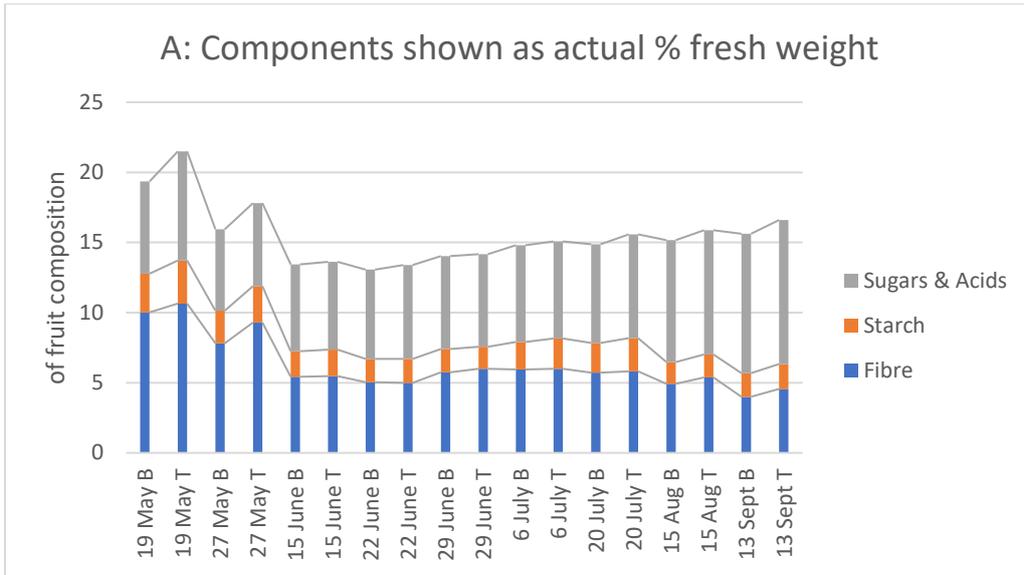


Figure 1.3.6. Changes in components of dry matter during fruit development; A; overall comparison of changes in starch, alcoholic soluble sugars and fibre, B; comparison of starch, alcoholic soluble sugars and fibre in fruit harvested from the top of the tree canopy and C; bottom of the tree canopy. Means (n=12) +/- SE.

Analysis of individual components of dry matter in samples collected over the season were fractionated into alcohol soluble (sugars and acids), fibre (cell wall component) and starch (1.3.6.A) show the alcohol soluble (sugars/acids) component of DM increased during fruit development from 34% of fruit weight soon after flowering rising to 64% at harvest (***) $P < 0.05$, while fruit fibre content (cell walls) which constitutes structural carbohydrates decreased as a proportion of the fresh weight of fruit from 52% soon after flowering to 25% at harvest (***, $p < 0.05$). No difference in the proportion alcohol soluble fraction (sugars) was observed between fruit sampled between the top and bottom of the tree canopy (1.3.6 B, 1.3.6.C).

In general, the fibre content of fruits harvested from the top and bottom of the canopy were similar during fruit development, with the exception of fruitlets harvested in late May (27/5/2016), where those from the top of the canopy were higher ($p < 0.05$) in fibre (52.2%) than equivalent fruitlets harvested from the lower canopy (49% fibre). Moreover, at commercial harvest (13/9/2016) mature fruits harvested from the top of the canopy were 2% higher in fibre content than fruits from the bottom, but just failed to reach significance ($p < 0.05$). Fibre accumulation calculated on the basis of g fibre/ fruit weight g/day reveals fruit from the lower canopy accumulating less fibre than fruit from the top of the canopy (Figure 1.3.7)

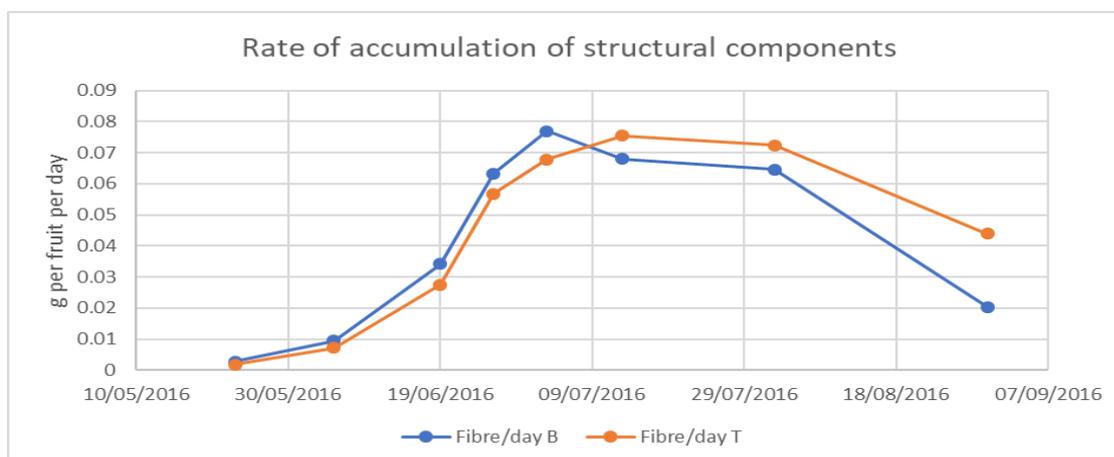


Figure 1.3.7. Accumulation of structural carbohydrates (fibre) in Gala apples harvested from petal fall through to commercial harvest.

Starch content (Figure 1.3.6 A) of Gala was highest (14.3%) soon after flowering (19/5/2016) and decreased (***, $p < 0.05$) for the first 6 weeks post-petal fall to 11 % before rising briefly to 15% starch 8 weeks post petal fall followed by a decline to 10.7% (%FW) starch present at harvest. There was no significant difference between Gala harvested from the top or bottom canopy in terms of the distribution of starch or alcohol soluble sugars.

The compositional changes with a reduction in the proportion of structural carbohydrate based on a FW basis during development is accounted for by cell expansion and the increase in cellular solutes in the form of sorbitol and sucrose (alcohol soluble sugars) entering the fruit initially through symplastic connections from the phloem while during the latter stages of fruit development apoplastic routes account for a significant proportion of solute movement into fruit cells.

Storage-trials

Samples of Gala were taken from top and bottom of the canopy and stored in 90 kg storage chambers under CA conditions of 3% CO₂, 1% O₂ (0.5-1.0°C) and stored for 8 months. Fruits were assessed after 5, 6.5 and 8 months, at each occasion fruits were subject to a shelf-life assessment of 7 days at 18°C. In general, firmness of fruit was maintained throughout the storage period, with a loss of 4-10 N (4.1-1.0 kg) over the 8 month storage period. No internal defects were found and the incidence of post-harvest disease was very low.

Positional effects of fruits within the canopy were found in the post-harvest quality of Gala. Fruits harvested from the upper canopy were higher in % Brix and delayed in maturity based on starch clearance, although no difference in internal ethylene concentration was observed. During storage and shelf-life assessments, Gala harvested from the top of the canopy were generally 2-3 N firmer (Table 1.3.2) and with a % Brix on average 1% higher compared to fruit from the lower canopy and this effect was seen throughout the storage period (Table 1.3.3).

Table 1.3.2. Harvest Maturity of Gala harvested from the upper canopy “Top” (>1.5 M) and the lower canopy – “Bottom” (<1.5 M)

Sample date	Position	Firmness (kg)	% Brix	Starch (CTIFL)	Ethylene (ppb)
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Harvest	Top	8.8	11.7	5.35	370
	SE	0.085	0.00	0.21	148.49
	Bottom	8.3	10.6	6.7	322.5
	SE	0.227	0.57	0.14	207.18

Table 1.3.3. Storage quality of Gala harvested from the upper canopy “Top” (>1.5 M) and the lower canopy – “Bottom” (<1.5 M)

		Firmness (kg)		% Brix	
Position		Ex-store	Shelf-life	Ex-store	Shelf-life
5 Months	Top	8	8.4	13.2	14
	SE	0.12	0.007	0.28	0
	Bottom	7.8	8.1	12.7	12.6
	SE	0.014	0.149	0.42	0.35
6.5 Months	Top	8	8	12.9	13.8
	SE	0.432	0.44	0.07	0.42
	Bottom	7.8	7.8	12.4	12.5
	SE	0.035	0.036	0.21	0.071
8 Months	Top	8.1	8.5	13	13.7
	SE	0.021	0.05	0.14	0.21
	Bottom	7.7	8.2	13	12.5
	SE	0.021	0.007	0.14	0.14

Samples of Gala were taken from top and bottom of the canopy and stored in 90kg storage chambers under CA conditions of 3% CO₂, 1% O₂ (0.5-1.0°C). In general, fruits harvested from the upper canopy were higher in % Brix⁰ and delayed in maturity based on starch clearance, although no difference in internal ethylene concentration was observed. Fruits were generally 2-3 N firmer (Table 4) and the % Brix⁰ content of fruit remained on average 1% higher in fruit from the top of the canopy compared to fruit from the lower canopy.

Year 2: WP 3. Bud and Fruitlet Thinning (2017/2018).

Bud stage monitoring for treatment events commenced in March 2017. Treatment 2 Bud Extinction was carried out on 24 March (BBCH 54, mouse ear). Treatment 3 Mechanical Thinning was carried out on 19 April at full bloom (BBCH 65, 60% flowers open and first petals falling). See Appendix 2 WP 3 Chronology.

Fruit size assessments for chemical and hand thinning treatment events were commenced in May 2017. Treatments 4 and 5 (chemicals Exilis and Brevis) were applied on 23 May (BBCH 71, fruit size 12mm). Day and night temperatures plus light were monitored and forecasts consulted to ensure optimum conditions before, at and after application (Table 2.3.2)

Table 2.3.2. Day and night temperature and light before, at and after chemical application events (23 May 2018).

	AIR TEMPERATURE °C				LIGHT w/m2
	DAY		NIGHT		TOTAL
	MAX	MIN	MAX	MIN	DAILY
18/05/2017	17.2	9.4	10.8	7.8	8597
19/05/2017	16.1	8.9	10.2	5.6	10950
20/05/2017	17.5	6.5	11.6	6.5	19599
21/05/2017	21.5	7.5	13.6	7.2	24603
22/05/2017	25.2	10	15.7	9.8	20704
23/05/2017	24.6	12	18.1	11.5	17726
24/05/2017	27.7	13.2	18.6	8.6	23629
25/05/2017	25.9	11.9	15.8	6.9	26091
26/05/2017	26.4	9.8	18.6	9.8	26789
27/05/2017	26	17.4	16.8	9.3	22379
28/05/2017	25.9	11.2	17.7	11.9	18094

Treatment 6 Hand Thinning Standard was carried out on 3 June (BBCH 72, fruit size 21mm). Treatment 7 Hand Thinning Size was carried out on 15 June (BBCH 73, fruit size 30mm) and 3 July (BBCH 74, fruit size 40mm). Treatment 8 was carried out on 30 June (BBCH 74, fruit size 40mm).

Second fruit fall commenced around 2 June and continued until around 29 June. Leaves for mineral analysis were collected on 26 April when N levels were found to be within normal limits. Temperature monitoring in the orchard commenced in spring. Below zero values were recorded on 18, 19, 20, 25 and 27 April plus on 10 May during full bloom and at early fruitlet. Many flowers were seen to have fallen and there was significant damage to fruitlets in the lower half of the tree canopy below 1.5m noted on 27 April. Fallen fruit was collected for counting on 2, 6 and 29 June. The number, average and total amount of fruit fallen and removed was calculated (Table 2.3.2 and Figure 2.3.5.)

Due to second fruit fall coinciding with fruit falling due to chemical treatments it was not possible to separately determine the amounts for different methods/causes. However, the percentage of total fruit fallen/removed per treatment was calculated (Figure 2.3.6).

There were no significant effects of treatment on natural fruit fall or total fruit fall/removal except for hand thinning Treatments 6, 7 and 8.

There were statistically significant differences between treatments 6, 7 and 8 of fruit naturally dropped and fruit removed by hand thinning. See Figure 2.3.7. Treatment 7 Hand Thinning Size had significantly fewer fruit removed by hand thinning than 6 or 8. Treatments 6 and 8 had significantly fewer fruit fallen naturally compared to Treatment 7.

Table 2.3.3. Average and total number of Fruit Fallen and Removed per Treatment (via fruit drop and thinning combined).

Treatment	Mean fruit number	Total fruit number
1	115.0	460.0
2	103.8	415.0
3	100.3	401.0
4	161.5	646.0
5	237.0	948.0
6	187.3	749.0
7	158.0	632.0
8	155.0	620.0

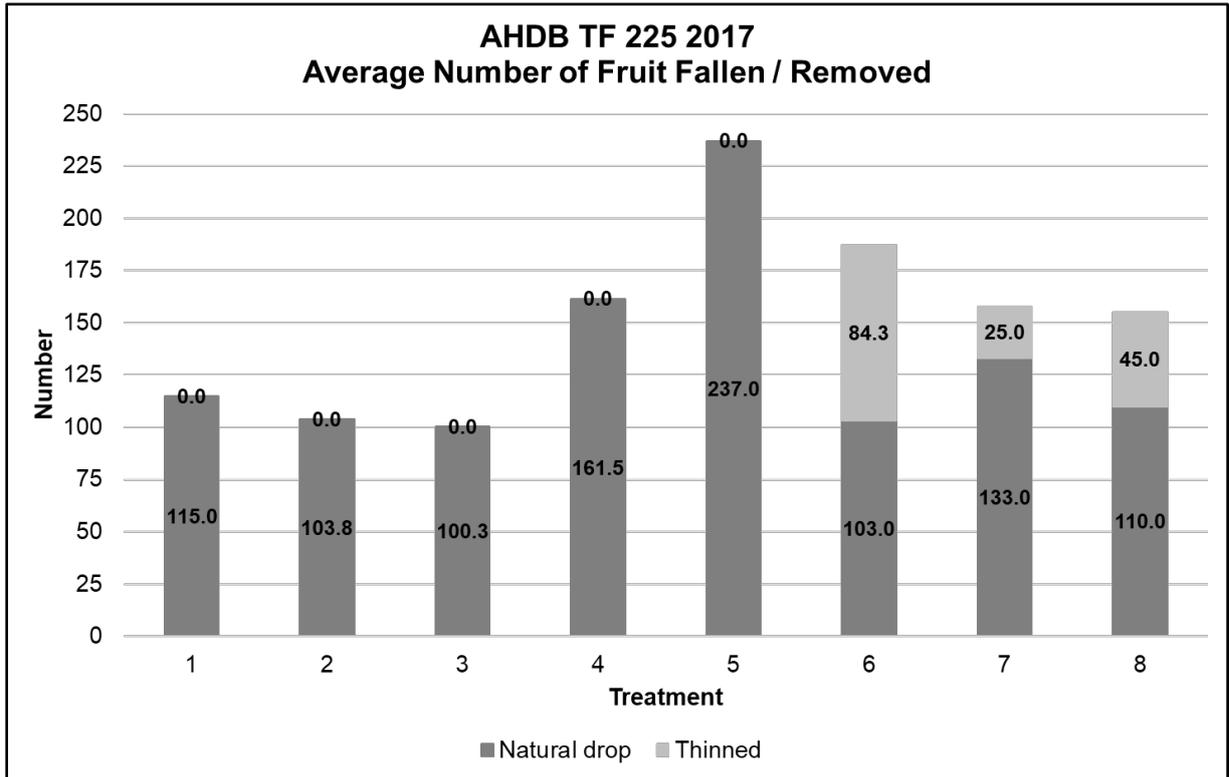


Figure 2.3.5. Average Number of Fruit Fallen and Removed.

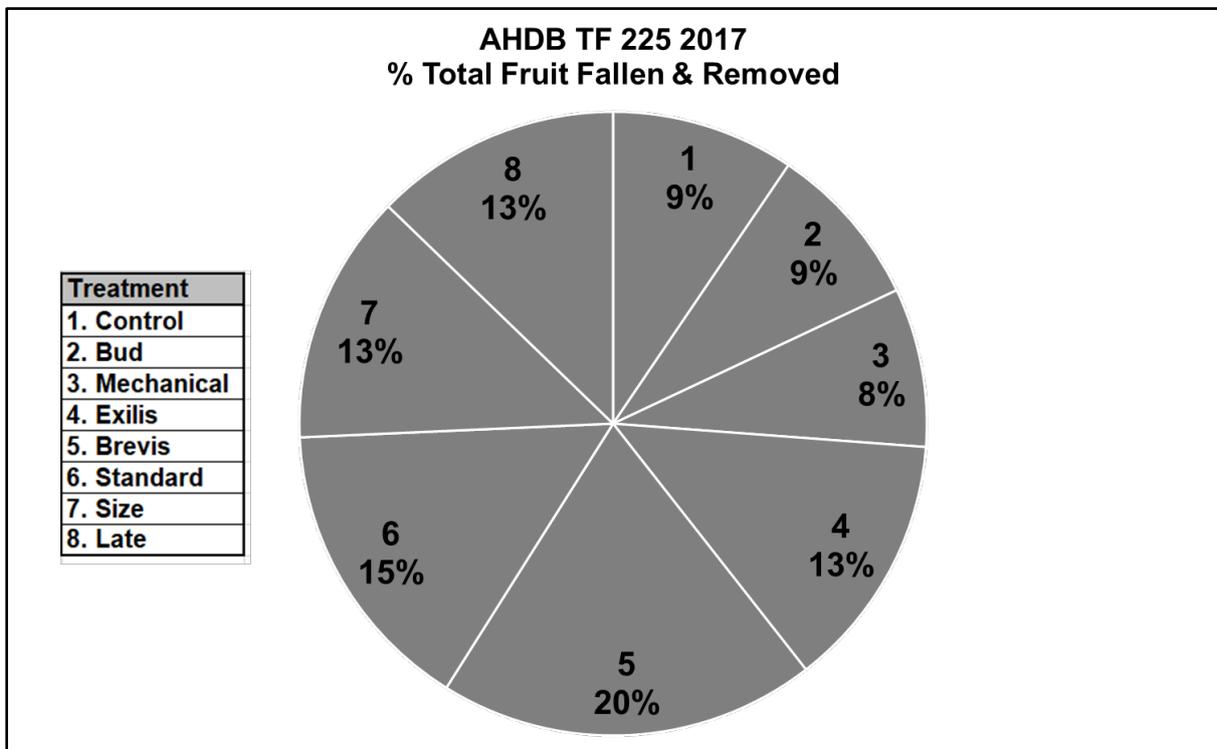


Figure 2.3.6. Percentage Total Fruit Fallen and Removed.

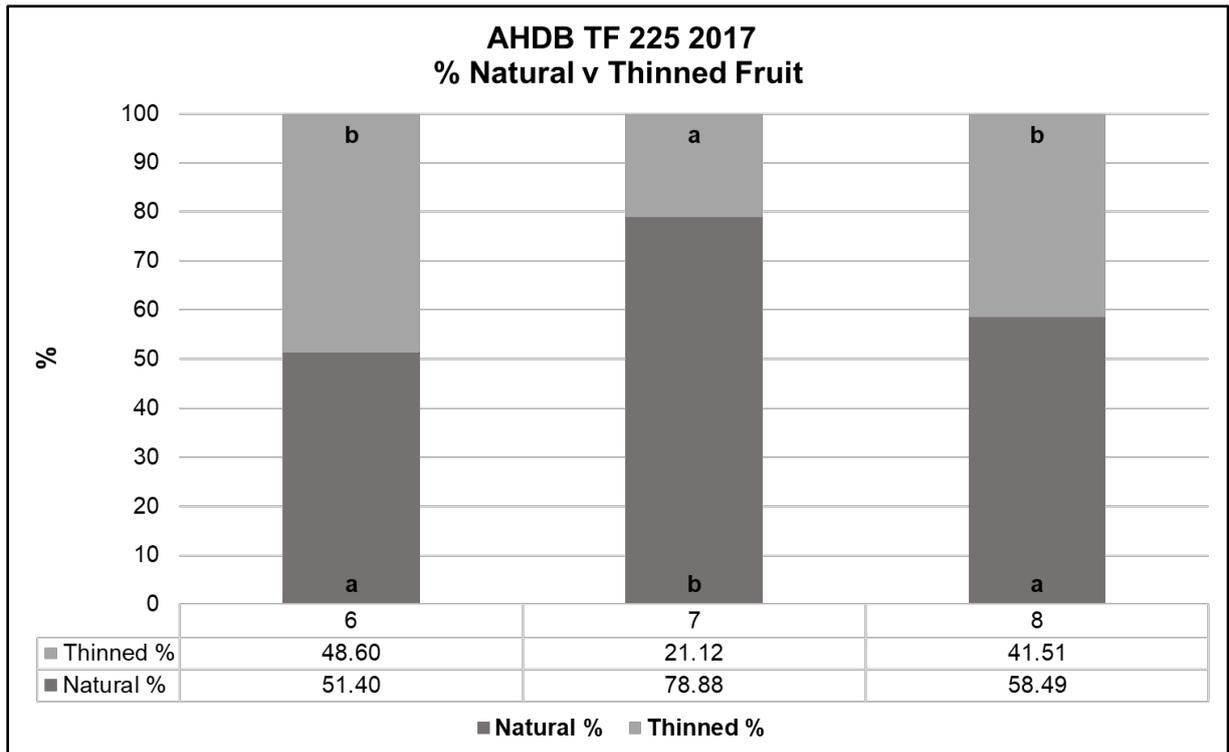


Figure 2.3.7. Percentage Natural v Thinned Fruit (hand thinned treatments only). Results with different letters are significantly different from one another. P value < 0.0001.

Starch tests for harvest prediction were carried out on 4, 11 and 14 September when values were 89.7%, 88.6% and 86% respectively. Pressure was also carried out on 11 and 14 September when results were 8.8kg and 8.6kg, respectively. % BRIX^o on 14 September was 12%. Fruit mineral analysis was carried out on 12 September and a pick date of 18 September recommended based on laboratory storage predictions. Fruit was harvested on 20 September. Total yields per treatment and average per tree in kg were calculated plus average total yield T/ha and Class 1 yields T/ha (Table 2.3.4).

There were no significant effects of treatment upon yield. Treatments 1, 7 and 5 had the highest average total yield T/ha and Treatments 8, 6 and 2 the lowest. However, Treatments 7 4 and 3 had the highest average Class 1 yield T/ha and Treatments 6, 5 and 8 the lowest (Figures 2.3.8, 2.3.9 and 2.3.10).

Table 2.3.4. Yields summary – total yield per treatment kg, average total per tree kg, average total T/Ha and average Class 1 T/Ha.

TREATMENT	TOTAL YIELD PER TREATMENT KG (C1 & Waste)	AVERAGE TOTAL YIELD PER TREE KG (C1 & Waste)	AVERAGE TOTAL YIELD T/HA (C1 & Waste)	AVERAGE CLASS 1 T/HA
1	261.3	21.8	62.2	33.5
2	233.1	19.4	55.5	33.6
3	235.0	19.6	55.9	34.7
4	222.9	20.3	58.0	35.3
5	255.9	21.3	60.9	29.6
6	230.8	19.2	55.0	28.8
7	260.4	21.7	62.0	36.2
8	215.3	17.9	51.3	29.7

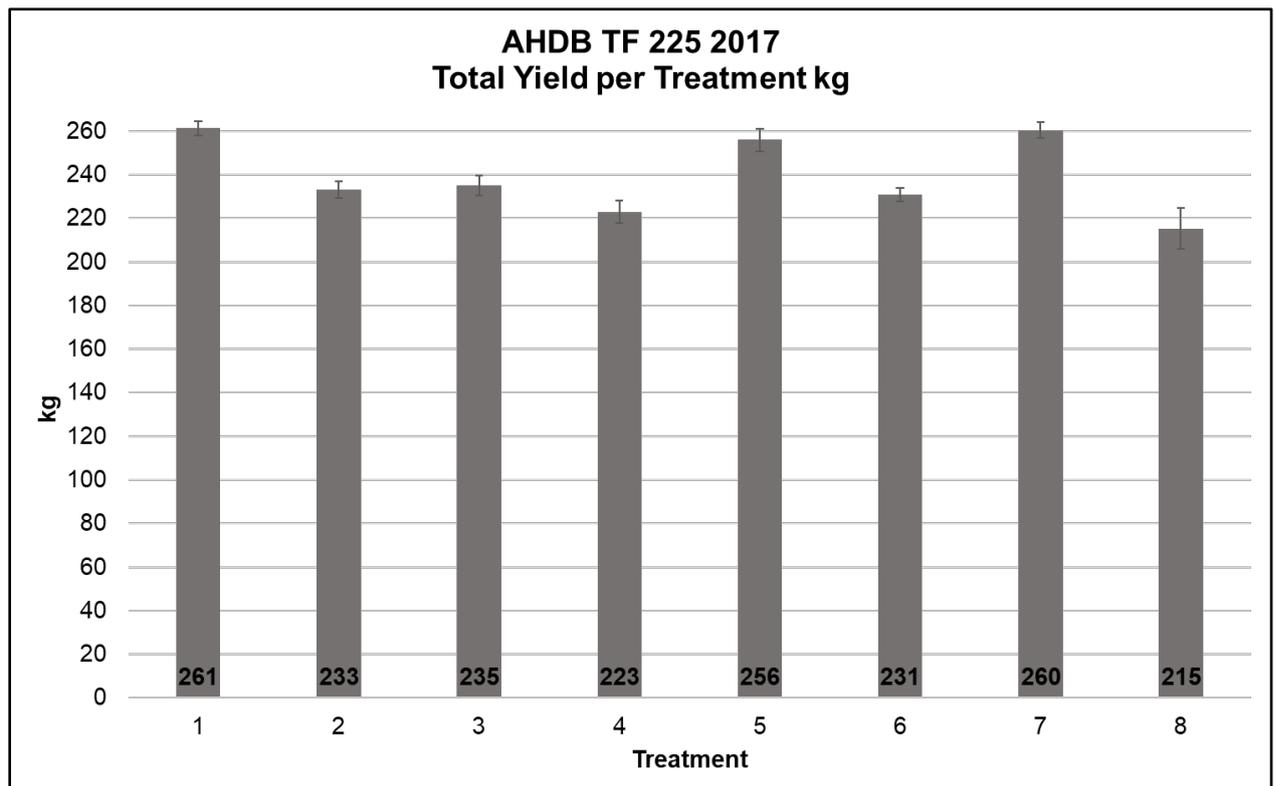


Figure 2.3.8. Total yield per treatment kg. No significant effects of treatments (P value 0.6400).

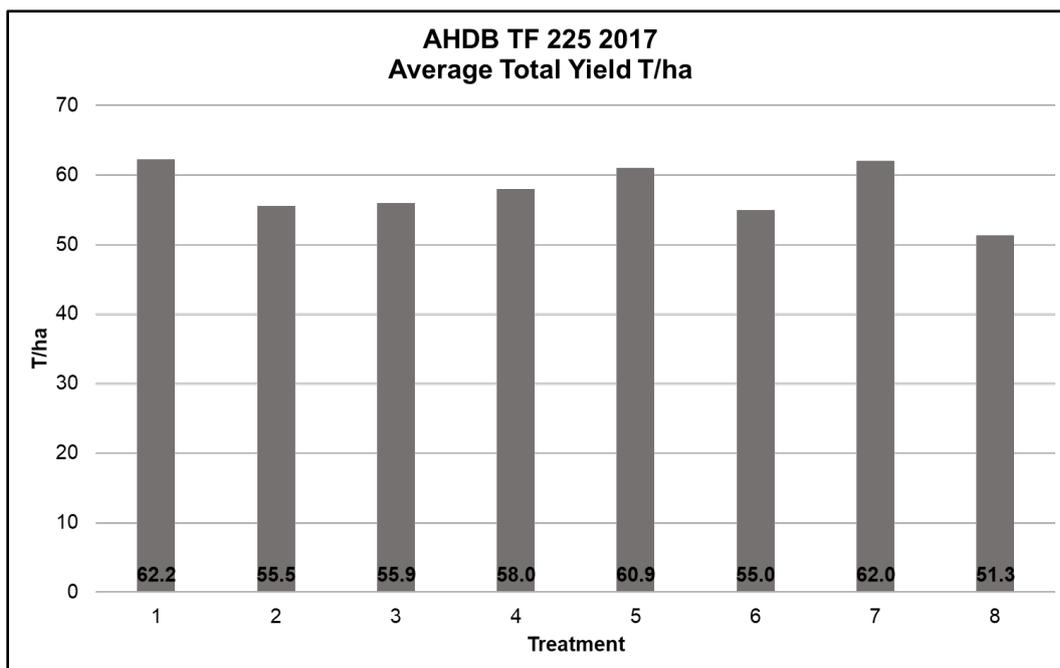


Figure 2.3.9. Average Total Yield per Treatment T/Ha (includes Class 1 and Waste). No significant differences (P value 0.6588).

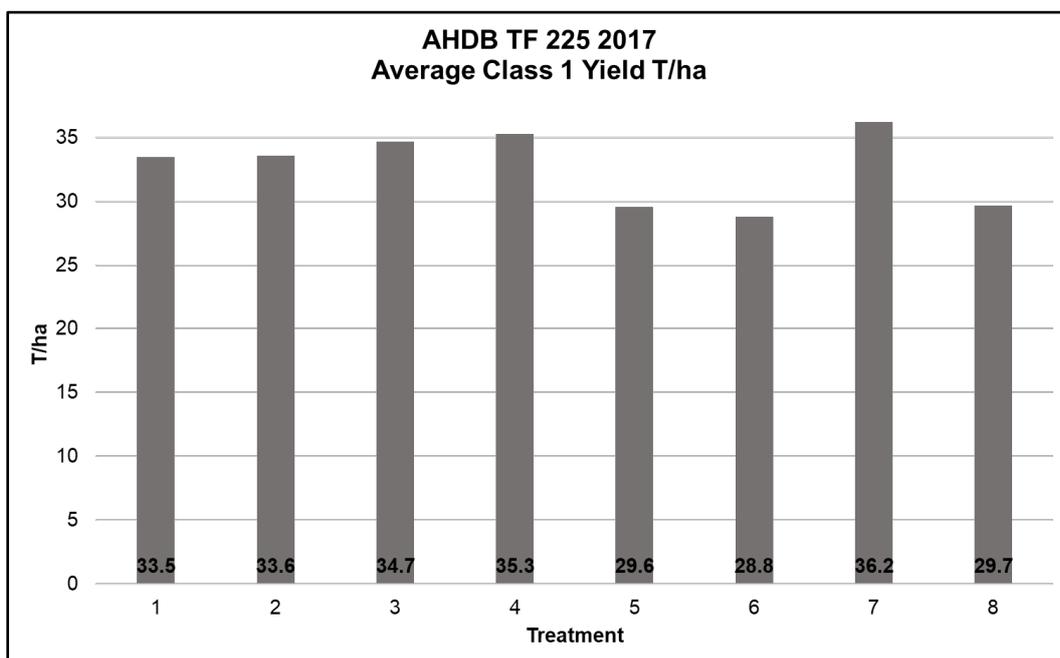


Figure 2.3.10. Average Class 1 Yield per Treatment T/ha. No significant differences (P value 0.3306).

Fruit sampling for maturity assessments was carried out on 19 September and fruit processed on 20 September. Fruit quality assessments commenced in October.

There were no significant effects of treatment on fruit quality (grade out) when the 60 fruit per treatment plot sampled at harvest were assessed. Treatment 2, 3, and 4 had the highest Class 1 percentages and 5, 6 and 8 the lowest (Figure 2.3.11).

There were no significant effects of treatment on waste categories except for lack of % Red category. Treatment 8 had significantly fewer fruit lacking colour compared to Treatments 3, 4, 5 and 6. Treatment 3 had the highest numbers of fruit with poor colour. Frost damage and small fruit accounted for most of the other Waste in 2017. Treatment 8 had the most damaged fruit and 6 the least. However, Treatment 6 had the most misshaped fruit and 3 the least. Treatment 1 had the most small fruit and Treatment 3 the least. But Treatment 3 had the most diseased fruit and treatment 6, 7 and 8 no disease was present. Treatment 6 had the most fruit with other defects (mostly oversize fruit > 80mm) and Treatments 4 and 5 had none (Figure 2.3.12).

There were no significant effects of treatment upon average fruit weight. Average fruit weight was between 110g and 130g and all treatments' fruit weight reached the minimum industry standard (110g). Treatments 8, 7 and 2 had the highest average fruit weight and 1, 4 and 5 the lowest (Figure 2.3.13).

There were no significant effects of treatment upon size distribution. Over 50% of the Class 1 fruit assessed were between 60mm and 70mm except for Treatment 7. Treatment 1 had the most fruit 55-60mm and Treatment 8 the least. Treatment 5 had the most fruit 60-65mm and Treatment 8 the least. Treatment 8 had the most fruit 65-70mm and Treatment 5 the least. Treatment 7 had the most fruit 70-75mm and Treatment 1 the least. Treatment 6 had the most fruit 75-80mm and Treatment 1 had none (Figure 2.3.14).

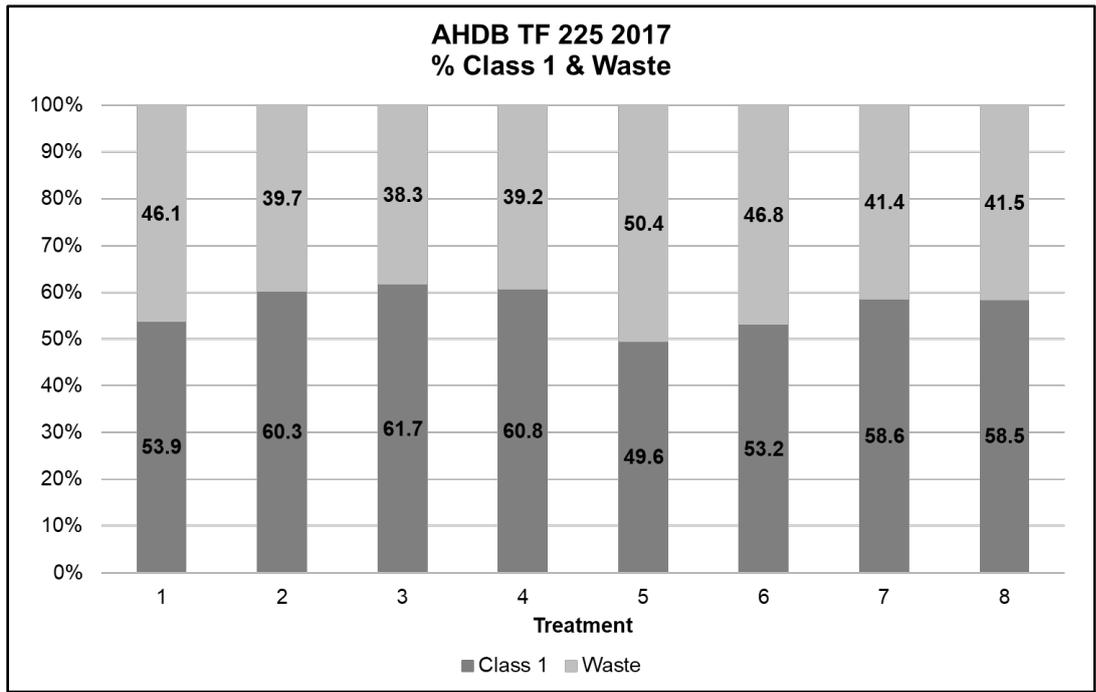


Figure 2.3.11. Percentage class by weight. No significant effects (Class 1 P value = 0.1599, Waste P value = 0.1603).

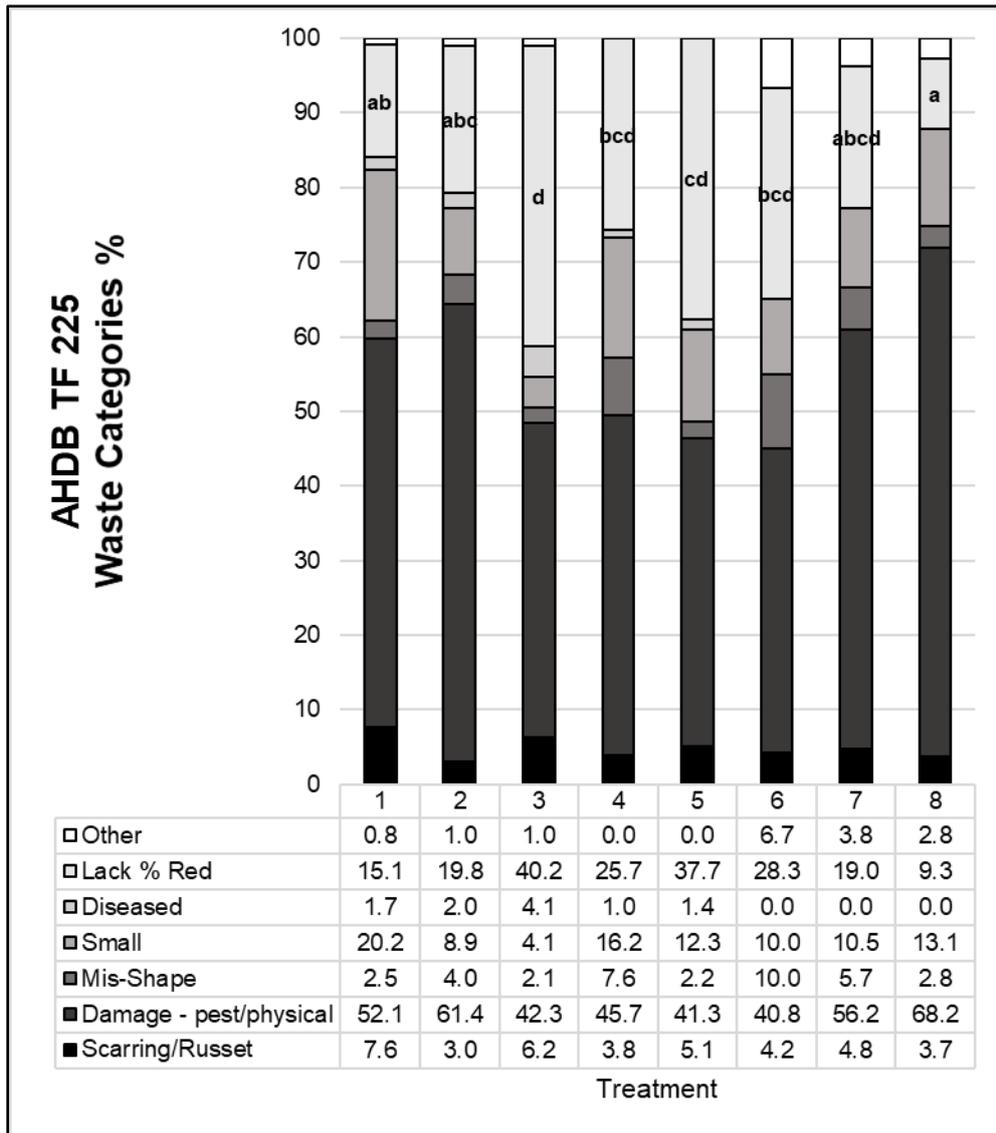


Figure 2.3.12. Waste categories %. Significant effects of treatment were noted for Lack of % Red (P value 0.0167). There were no significant effects of treatment for Scarring/Russet (P value 0.9962), Damage (P value 0.0855), Mis-Shape (P value 0.2083), Small (P value 0.8211), Diseased (P value 0.1191) or Other (P value 0.631).

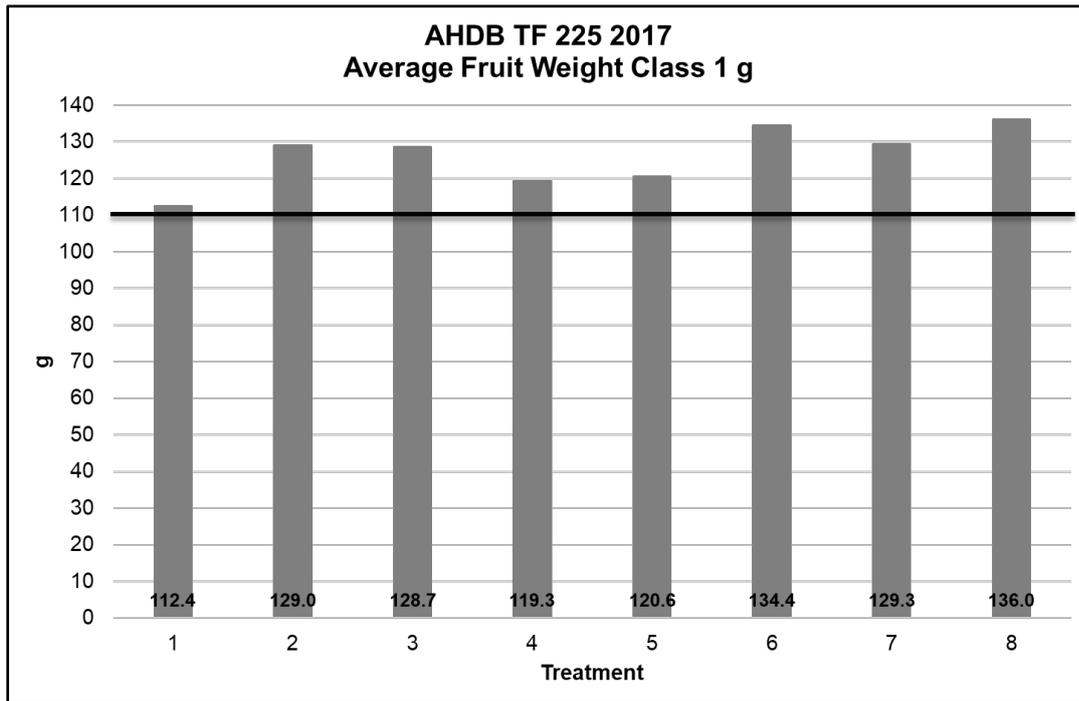


Figure 2.3.13. Average fruit weight Class 1. No significant effects (P value 0.2071). Black line denotes minimum industry standard weight required.

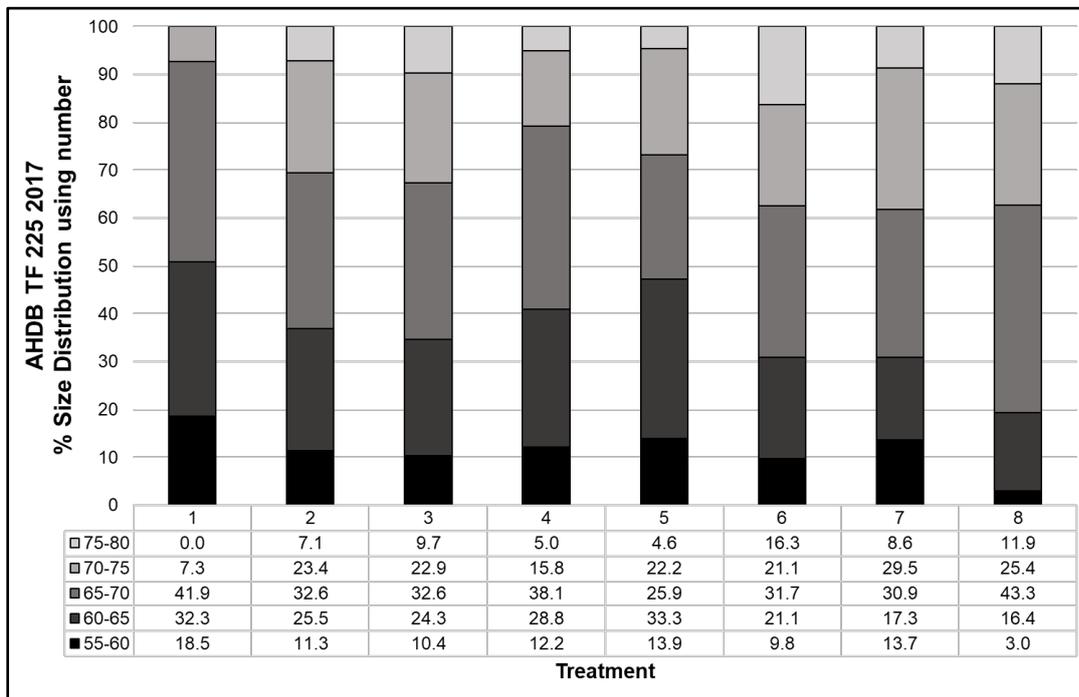


Figure 2.3.14. Percentage size distribution using number of fruit. No significant effects (P values = 55-60mm 0.2022, 60-65mm 0.5408, 65-70mm 0.0717, 70-75mm 0.2098, 75-80mm 0.4413).

There were no statistically significant effects of treatments on starch. The optimum starch of 80% at harvest was reached for all treatments except 6 (Figure 2.3.15).

There were statistical effects of treatments on % BRIX^o where Treatment 8 Hand Thinning Late had significantly higher % BRIX^o than all other treatments. Only treatments 5, 7 and 8 reached the optimum % BRIX^o of 12 at harvest (Figure 2.3.16).

There were statistical effects of treatments on fruit firmness where Treatment 3 (Mechanical Thinning) had significantly lower pressure than all other treatments except 2 (Bud Thinning). The optimum pressure of 8kg/m² at harvest was reached by all treatments (Figure 2.3.17).

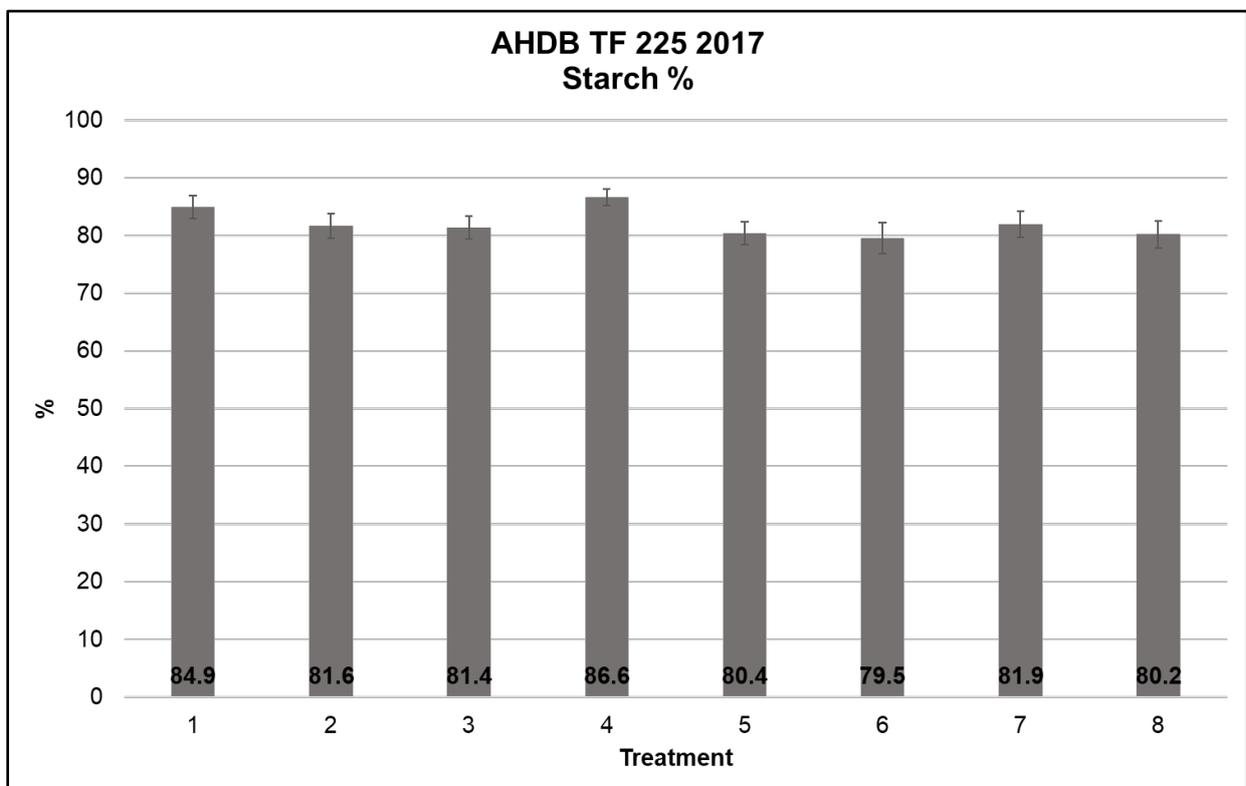


Figure 2.3.15. Percentage starch from fruit sampled at harvest. No significant effects (P value 0.2509).

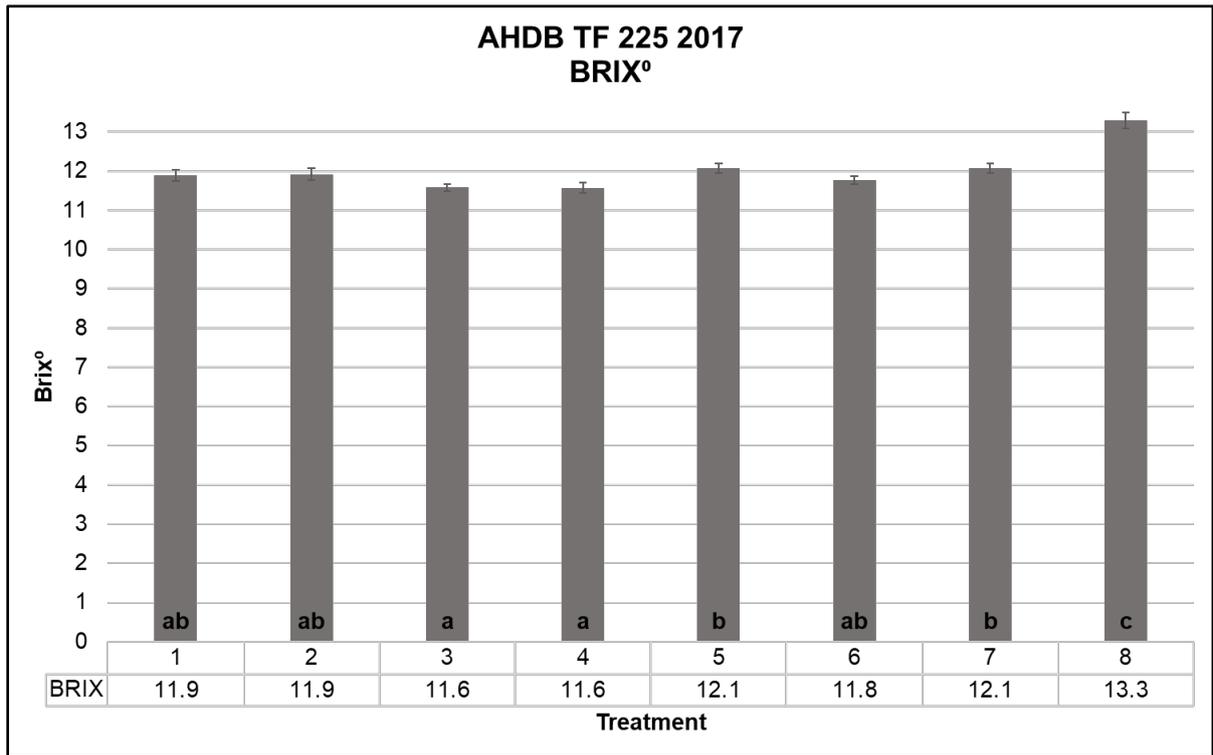


Figure 2.3.16. BRIX. There were significant effects (P value <0.0001).

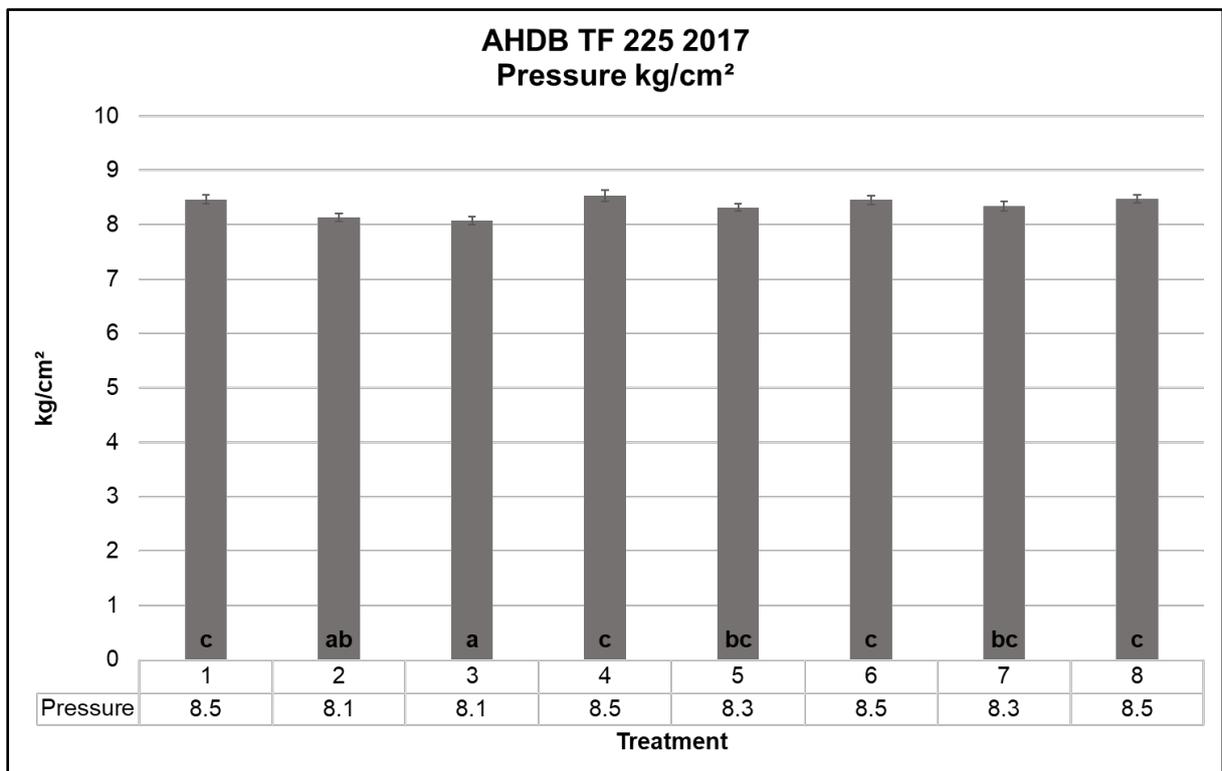


Figure 2.3.17. Fruit Firmness (kg cm²). There were significant effects (P value <0.0001).

Fruit Dry Matter and Sugar Analysis (NRI)

Fruitlet samples were harvested on 9 May, 13 July and 27 September 2017 and FDM analysis shows that application of Brevis had a transitory effect on raising FDM in fruitlets sampled in May (22% FDM) but failed to reach significance ($p < 0.05$) compared to 19.5-20.6% FDM in other treatments (Figure 2.3.18).

As fruits developed and increased in fruit size FDM decreased to ~16.7-17.1% % FDM in samples sampled in July and 16.4-17.3% FDM in apples sampled at harvest.

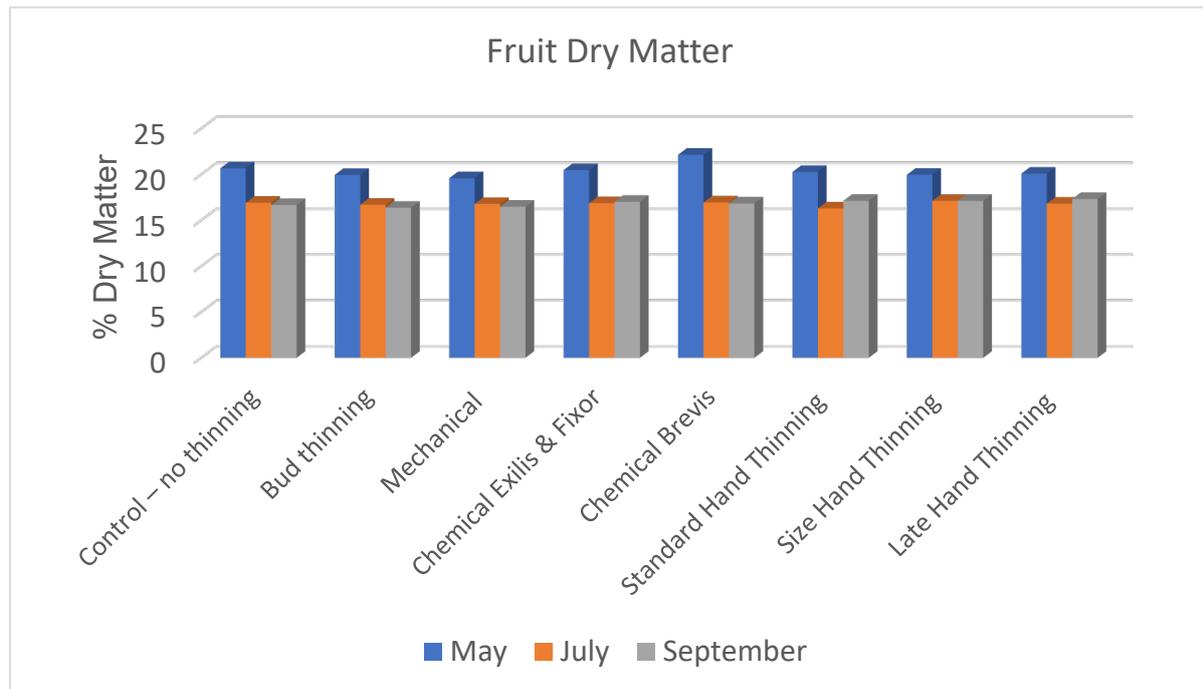


Figure 2.3.18. Dry matter content (%) of Gala fruit picked. Each data point is the mean of 24 $LSD_{0.05} = 1.67$ on 70 df. Each sample consisted of 5-10 fruit (number of fruits per sample decreased through the season).

At harvest the FDM was between 0.54% and 1.5 % higher than the fruit from the lower canopy (Figure 2.3.19). Fruit harvested from the upper canopy of tree subject to late thinning accumulated the highest amount of FDM (17.6%) (Figure 2.3.19).

Maturity of Gala at harvest showed a significant amount of starch clearance suggesting fruits were only suitable for short-term CA storage. The soluble solid content % BRIX^o of fruit in general was low (~11.5%) with the exception of fruit from Late Hand Thinning (treatment 7) where % BRIX^o at harvest averaged (13.3%).

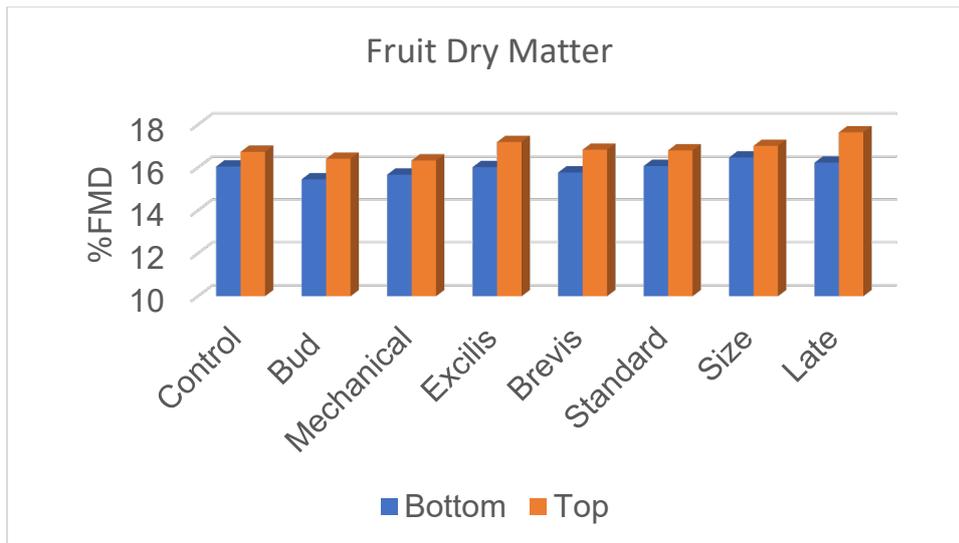


Figure 2.3.19 Positional effects of FDM accumulation between Gala sampled from the top of the canopy (>1.5 m) and bottom of the tree canopy (0.6 m) at harvest (27 September 2017). $LSD_{0.05}$ fruit position x thinning treatment = 1.08 on 44 df.

Sugar analysis of freeze dried material of apple samples taken at harvest has shown that fructose content was higher ($p < 0.05$) in fruit harvested from the lower canopy, while sucrose was highest ($p < 0.05$) in fruit harvested from the upper canopy (Table 3.5). Treatment differences in fructose (Figure 3.21) and sucrose (Figure 3.22) content were not significant ($P < 0.05$), however it was interesting to note that Brevis treated trees yielded fruit with the highest fructose content.

Table 2.3.5. Overall effect of tree position on the accumulation of Fructose and Sucrose ($\mu\text{l}/\mu\text{l}$) in Gala apples.

<i>Tree position</i>	<i>Bottom</i>	<i>Top</i>	<i>P-value</i>	<i>LSD_{0.05}</i> <i>45 df</i>
Fructose	45.29	42.48	<0.01	1.06
Sucrose	24.84	27.18	0.02	1.435

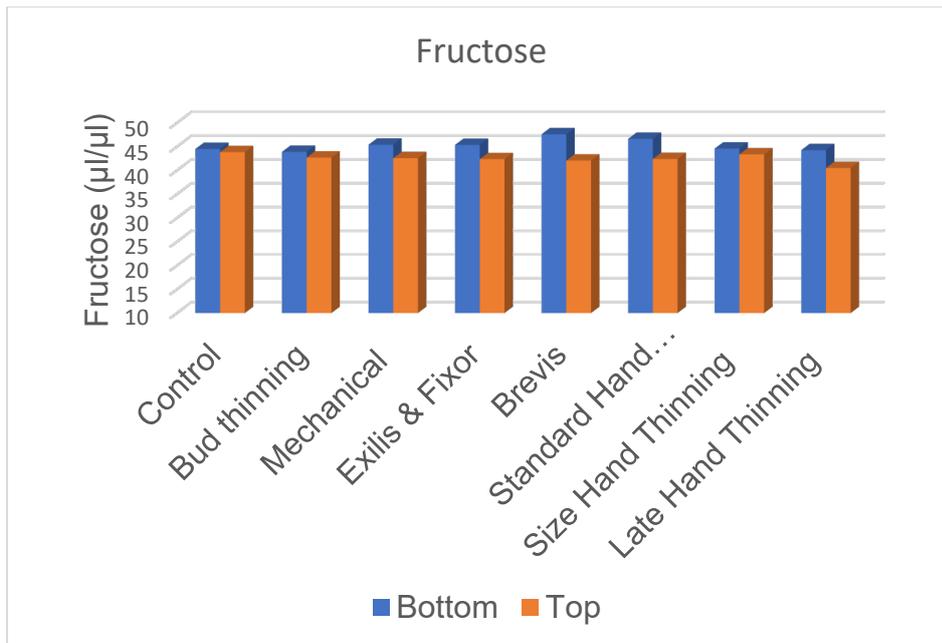


Figure 2.3.20. The effect of fruit position within the canopy and fruit and thinning practices on fructose content of Gala at harvest (27 September 2017); $LSD_{0.05}$ fruit position x treatment = 4.56 on 45 df.

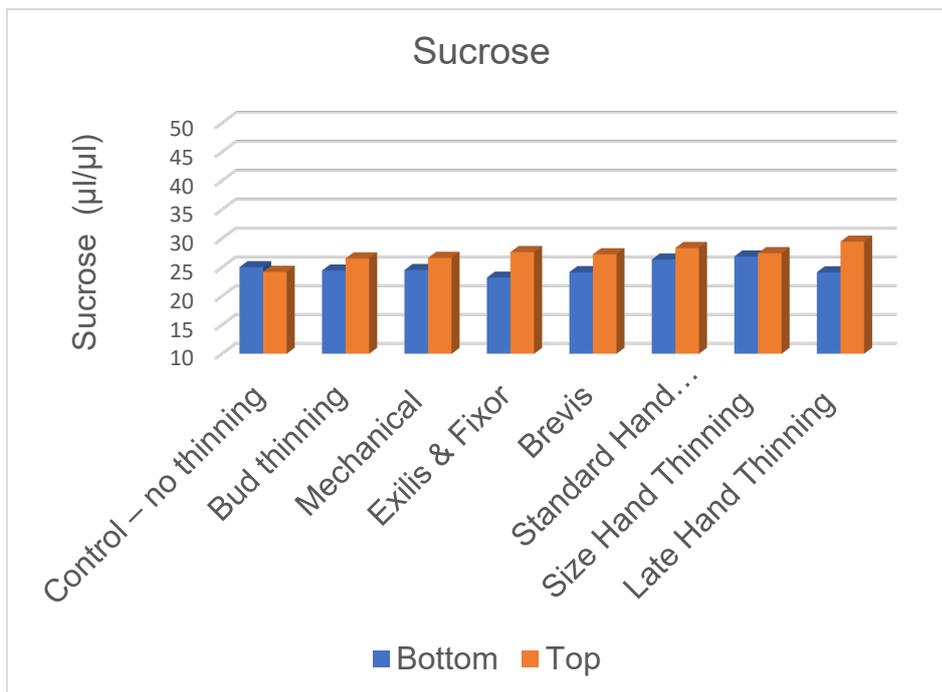


Figure 2.3.21. The effect of fruit position within the canopy and fruit and thinning practices on sucrose content of Gala at harvest (27 September 2017). $LSD_{0.05}$ fruit position x treatment = 4.059 on 45 df.

Late hand-thinning produced fruit with the highest (13.3%) sugar content (% BRIX°) at harvest (Figure 2.3.22).

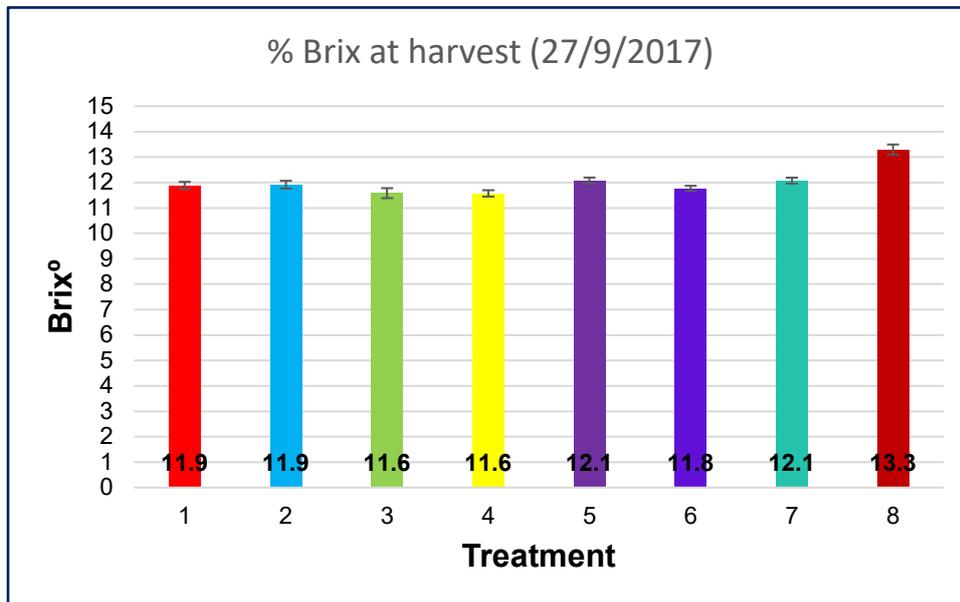


Figure 2.3.22. Soluble solids content (%Brix) of Gala apples subjects a range of bud and fruitlet thinning treatments.

Analysis of FDM content using a Felix-750 NIR dry matter analyser between top and bottom harvested fruit. Variability in FDM distribution was influenced by thinning technique, but no one technique increased FDM significantly. Fruit harvested from the lower canopy were more variable in FDM content than fruit from the top of the canopy. See Figure 2.3.23.

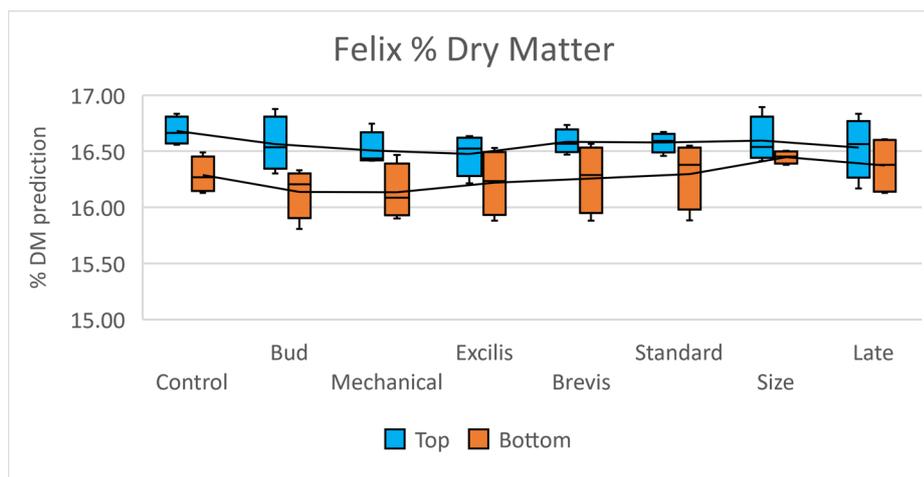


Figure 2.3.23. The Felix-750 NIR-Gala Model (NRI-UoG) predicting FDM content of apples at harvest subject to different thinning strategies (Fast LLP).

After removal from storage in 3% CO₂; 1% O₂, 0.5-1.0°C after 5 months, fruits were analysed for quality attributes. No significant difference was observed in fruit firmness (71.8-73.3N; 7.2-7.3 kg) across treatments, while sugar content was highest in late thinned fruit (13.9%) compared to mechanical thinned fruit where % BRIX^o measured 12.2%. The incidence of rotting varied across the treatments with the highest recorded in hand thinned (Table 2.3.6).

Table 2.3.6. Fruit Quality Analysis after 5 months Storage (3% CO₂; 1% O₂), 0.5-1.0°C.

	Control	Bud Thinning	Mechanical	Exilis Fixor	Brevis	Std Hand Thinning	Size hand thinning	Late thinning	LSD _{0.05}
Firmness(N)	73.1	72.3	73.3	72.6	72.7	71.8	72.1	72.2	3.29
% Brix	13.5	13.4	12.2	13.7	13.6	13.7	13.8	13.9	1.35
% Rots	1.3	1.6	1.3	2.5	1.3	2.8	6.25	0	5.30

Conversion of Firmness (N) to kg divided by 9.61.

The compositional changes with a reduction in the proportion of structural carbohydrate based on a FW basis during development is accounted for by cell expansion and the increase in cellular solutes in the form of sorbitol and sucrose (alcohol soluble sugars) entering the fruit initially through symplastic connections from the phloem while during the latter stages of fruit development apoplastic routes account for a significant proportion of solute movement into fruit cells.

Summary and Key Findings- Year 2

Significant damage occurs to fruit flowers at full bloom and early fruitlet during sub zero temperatures (10% kill at -2.2°C and 90% kill at -4°C). Frost damage caused significant losses causing excessive flower fall and lower than optimum yields (> 40 T/Ha) due to damaged fruit. Class 1 percentages were also affected and lower than commercial optimum (<85% Class 1)

The significant differences in % fruit fallen naturally and hand thinned between Treatments 6, 7 and 8 indicates that the early hand thinning may have been too early and fruit which may have fallen naturally later was picked off.

Despite the lack of significant effects of treatment upon yield and class in 2017, Mechanical (T3) Thinning and Exilis (T4) may offer the grower the most cost effective methods of thinning. Treatments 7 and 2 are also worth considering. All treatments had optimum fruit size between 120g and 130g.

There was very low disease pressure in 2017 and skin finish was good. Based on Waste Category analysis, the hand thinning methods may have removed any diseased fruit even though the operative aimed not to.

The Mechanical Thinning method may have delayed initial growth. The later leaf emergence and consequently later growth cessation may have shaded the fruit hence the poorer colour. Colour was generally poor in 2017.

FDM at harvest were similar across all treatments. Fructose was highest in fruits from the lower canopy, while sucrose was most abundant in fruit from the upper canopy. Trees subject to late thinning (fruit size 30mm to 40mm: BBCH 73-74), after second fruit fall produced fruit with the highest % BRIX^o at harvest, but this effect was lost in storage.

Year 3. WP3: Bud and Fruitlet Thinning (2018/2019).

Bud thinning was completed on the 24th March using the MAFCOT Equilifruit tool to estimate the number of buds to be retained. Fruit size assessments for chemical and hand thinning treatment events were commenced in May 2018. Treatments 4 and 5 (chemicals Exilis and Brevis) were applied on 23 May (BBCH 71, fruit size 12mm). Maximum and minimum temperatures along with the sum of daily light radiation 5 days before and after the application date on the 23/5/2018 (Table 3.3.1).

Table 3.3.1. Maximum and minimum temperatures and light before, 5 days before and after chemical application events (23 May 2018).

	Max Temp °C	Min Temp °C	Light W/M ² (Sum)

18/05/2018	18.2	3.1	47797.2
19/05/2018	20.2	3.7	49819.3
20/05/2018	20.1	6.1	40623.6
21/05/2018	22	7.6	27540.2
22/05/2018	24	10.3	38479.5
23/05/2018	19	10	35412.4
24/05/2018	18.8	11.3	14770.3
25/05/2018	22.1	13.1	18887.7
26/05/2018	23.7	13.3	41778
27/05/2018	27.4	13.9	38872.6
28/05/2018	25.4	12.6	43706.2

Treatment 6 Hand Thinning Standard was carried out on 3 June (BBCH 72, fruit size 21mm). Treatment 7 Hand Thinning Size was carried out on 14 June (BBCH 73, fruit size 30mm) and 3 July (BBCH 74, fruit size 40mm). Treatment 8 was carried out on 3rd July (BBCH 74, fruit size 40mm).

The proportion of fruit remaining on the tree at harvest after different thinning practices implemented during the growing season (Table 3.3.2 and Fig. 3.3.6) Over half (52.1%) the initial fruitlets/fruits aborted in non-thinned trees by September and this was a similar proportion to trees subject to mechanical flower thinning (54.3%). Bud thinning did not reduce the number of fruitlets on the tree, but more fruits were lost in June drop with 42.7% of total fruitlets developing into fully mature fruits, representing a 10% reduction in fruit number over non-thinned trees. Treatment with Exilis or Brevis led to a similar reduction in final fruit number with 30.4 and 33.8% remaining at harvest, respectively. Similar numbers were achieved through standard hand thinning practices (34.3%) or where thinning was delayed (late thinning treatment) yielded 31.3% of fruitlets remaining on the tree till harvest. The techniques that reduced fruit numbers the most was thinning to size where only 22.6 % of fruit remained at harvest.

Yield analysis from the trees revealed no significant effect of thinning practices on harvest weight per tree.

Table 3.3.2. Fruit numbers per plot prior to June drop and at Harvest.

Treatment	Total fruits before fruit fall	Total fruit at harvest	% Remaining
1	509	265	52.1
2	553	236	42.7
3	409	222	54.3
4	553	168	30.4
5	518	175	33.8
6	557	191	34.3
7	552	125	22.6
8	489	153	31.3

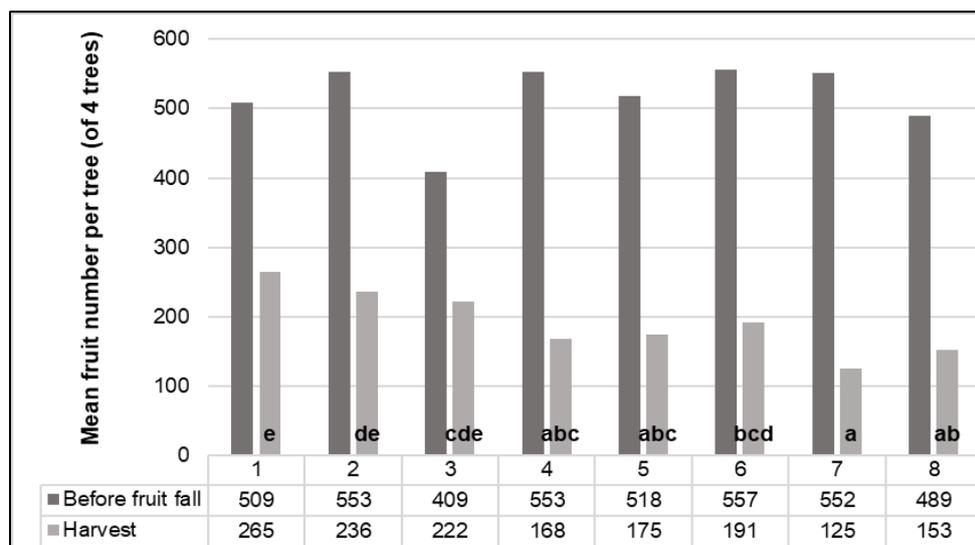


Figure 3.3.6. The effect of thinning practices on the Average Number of Fruits Counted on the Tree before fruit fall and at harvest. Treatment 1: unthinned control, 2: bud removal, 3:

mechanical flower removal, 4: Exilis, 5: Brevis, 6: standard thinning, 7: thinning to size, 8: late thinning.

Less hand thinning was required when fruitlets were thinned to size (26.2%) or late season thinning (15.2%) compared to 49.8% of fruit requiring thinning using the standard method prior to June drop (Fig. 3.7). However, there was a 10% reduction in fruit at harvest where thinning to size was implemented compared to standard thinning.

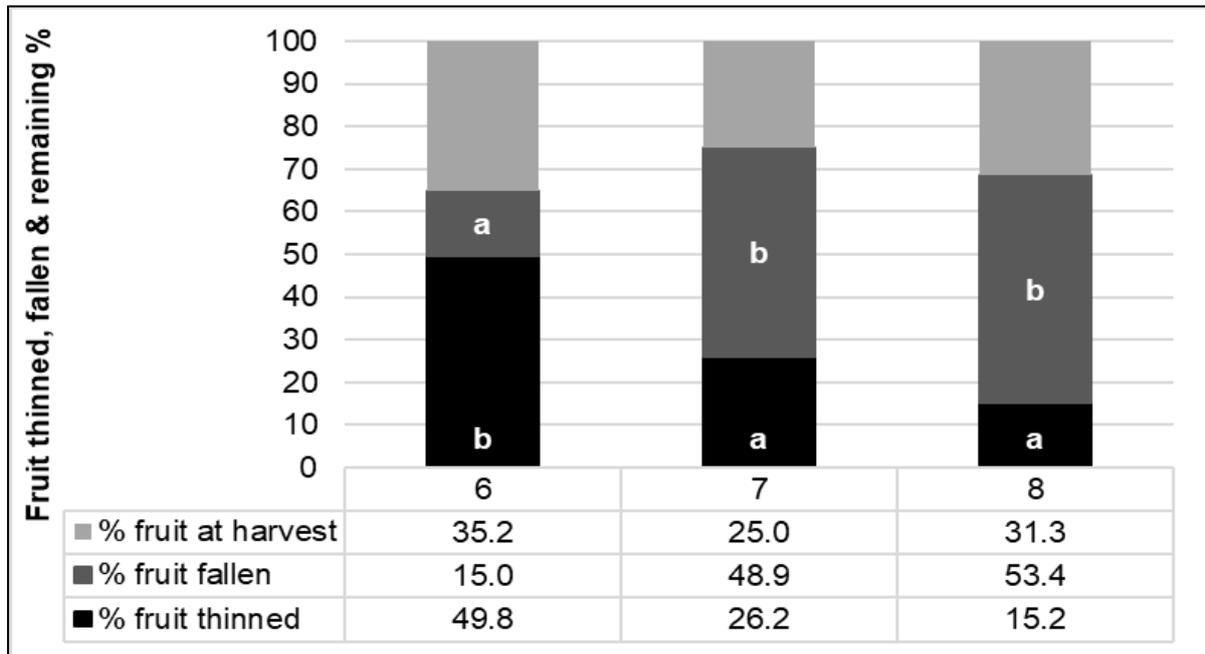


Figure 3.3.7. The effect of standard thinning (6): thinning to size (7) and late thinning (8) practices on the Percentage Natural v Thinned Fruit

Fruit maturity at harvest

Starch tests for harvest prediction were carried out on 4, 11 and 14 September when values were 89.7%, 88.6% and 86% respectively. Pressure was also carried out on 11 and 14 September when results were 8.8kg and 8.6kg. % BRIX^o on 14 September was 12%.

Fruit mineral analysis was carried out on 12 September and a pick date of 18 September recommended based on laboratory storage predictions. Fruit was harvested on 20 September. Total yields per treatment and average per tree in kg were calculated plus average total yield T/ha and Class 1 yields T/ha. See Table 3.3.4.

There were no significant effects of treatment upon yield. Treatments 1, 7 and 5 had the highest average total yield T/ha and treatments 8, 6 and 2 the lowest. However, treatments 7

4 and 3 had the highest average Class 1 yield T/ha and Treatments 6, 5 and 8 the lowest (Figures 3.3.8, 3.3.9 and 3.3.10)

Yields of fruit per tree (Fig 3.3.9) were not significantly different ($p < 0.05$) across treatments, with unthinned trees averaging 24 kg per tree while those subject to bud thinning and mechanical flower thinning producing 25.3 and 25.0 kg per tree, respectively. Yields from Exilis (20.1 kg/tree) and Brevis (20.8 kg) were slightly lower but comparable to standard thinning (23.9 kg), thinning to size (21.6 kg) and late thinning (21.6)

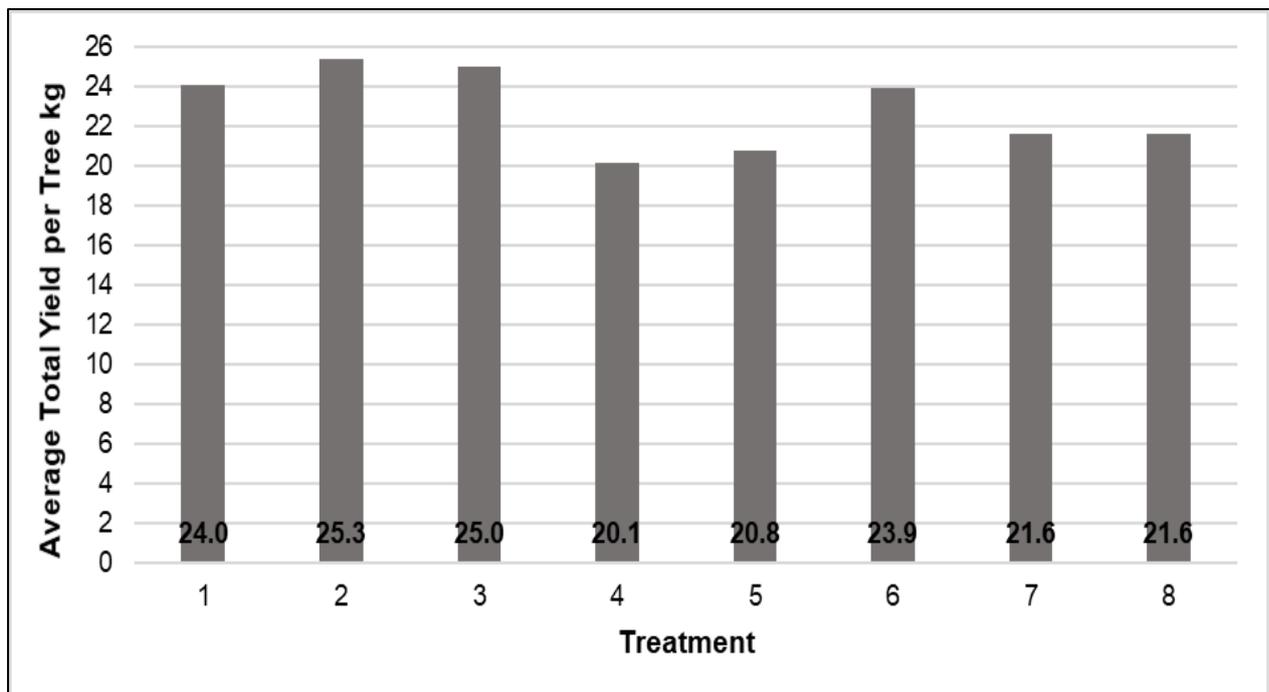


Figure 3.3.9. The effect of thinning practices on the Total Yield per Treatment (kg) of apples at harvest. Treatment 1: unthinned control, 2: bud removal, 3: mechanical flower removal, 4: Exilis, 5: Brevis, 6: standard thinning, 7: thinning to size, 8: late thinning.

After grading the proportion of class 1 fruit in unthinned plots averaged 78.3% of the total yield compared to 85.3 % where standard thinning practices were implemented (Fig. 3.3.10), similar levels of class 1 fruit were achieved using thinning to size and late thinning with 84 and 87.8% of fruit reaching class 1, respectively. Exilis significantly lowered ($p < 0.05$) the proportion of class 1 fruit with 10% lower class 1 fruit compared to the unthinned control and this was associated with a higher proportion of misshapped and diseased fruit (Fig 3.3.11). The biggest proportion of undersized (<55 mm) fruit was found in unthinned and trees subject to bud thinning suggesting the timing of early thinning practices has an important bearing on fruit

size. Thinning to size produce the least undersized and misshapen fruit. Recalculating the amount of class 1 fruit as tonnes per hectare (Figure 3.3.12) shows considerable variation in yields across treatments, with untreated plots producing 53.7 t/ha compared standard thinning practices of 58.2 t/ha, lower class 1 fruit were observed in trees treated with Exilis or Brevis but differences failed to reach significance ($p < 0.05$).

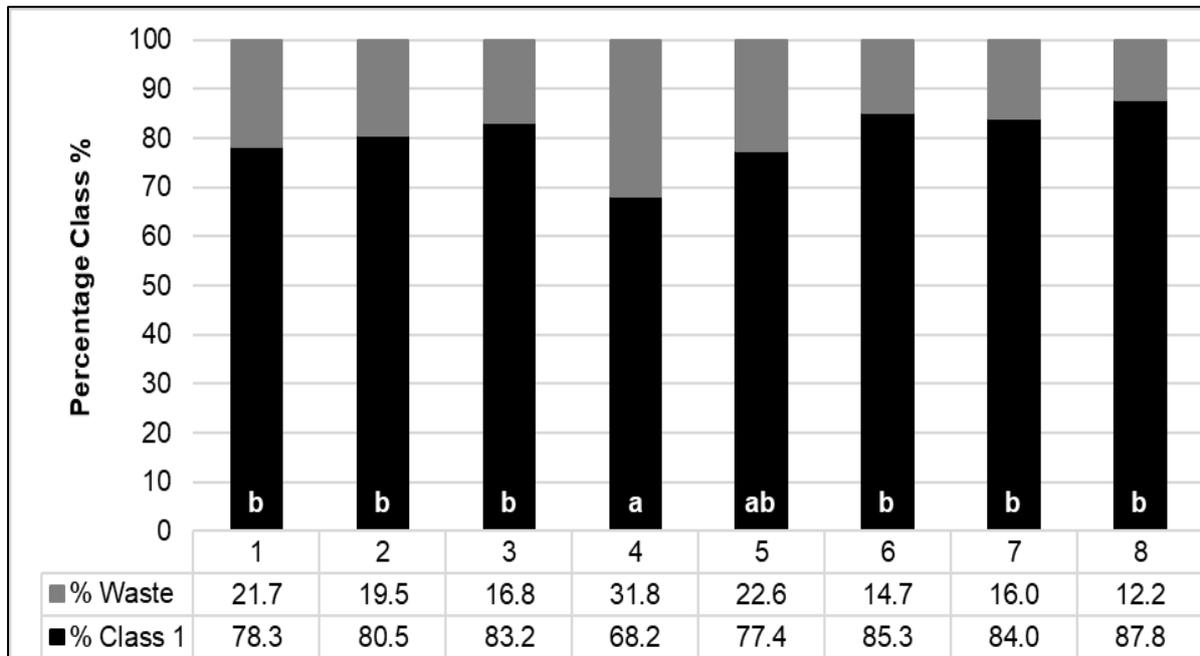


Figure 3.3.10. The effect of thinning practices on the Proportion of Class 1 apples at harvest. Treatment 1: unthinned control, 2: bud removal, 3: mechanical flower removal, 4: Exilis, 5: Brevis, 6: standard thinning, 7: thinning to size, 8: late thinning.

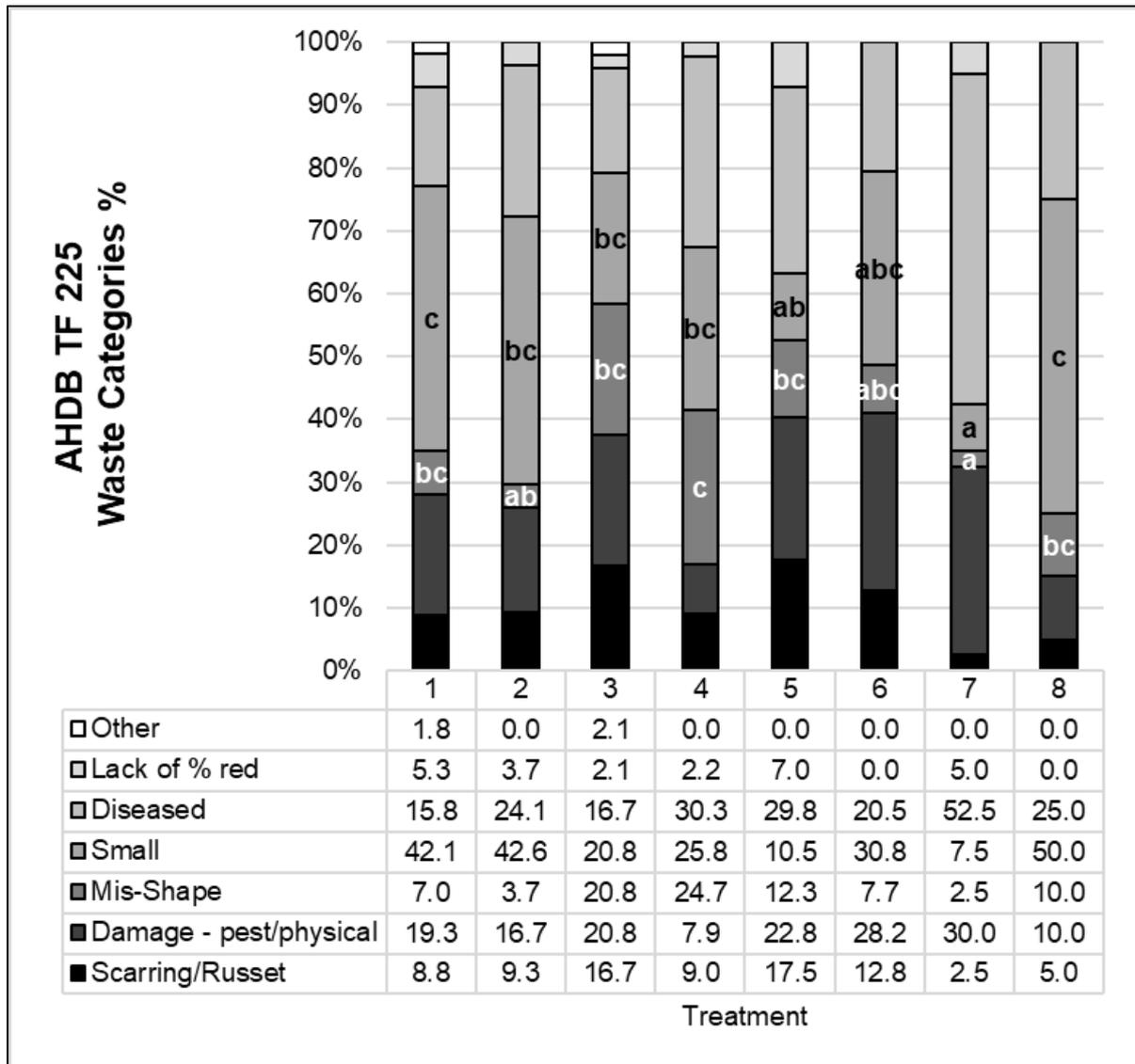


Figure 3.3.11. The effect of thinning practices on the Proportion of Waste categories of apples at harvest. Treatment 1: unthinned control, 2: bud removal, 3: mechanical flower removal, 4: Exilis, 5: Brevis, 6: standard thinning, 7: thinning to size, 8: late thinning.

Analysis of fruit weights of class 1 fruit found Exilis (treatment 4) produced significantly larger fruit ($p < 0.05$) than unthinned trees, with an average weight of 131.0 g/fruit standard thinning averaged class 1 fruits of 122.9 g, thinning to size averaged 128.9 g/fruit and late thinning 117.0g/fruit (Fig. 3.3.12). Bud thinning produced average fruit weight very similar to fruit from unthinned trees

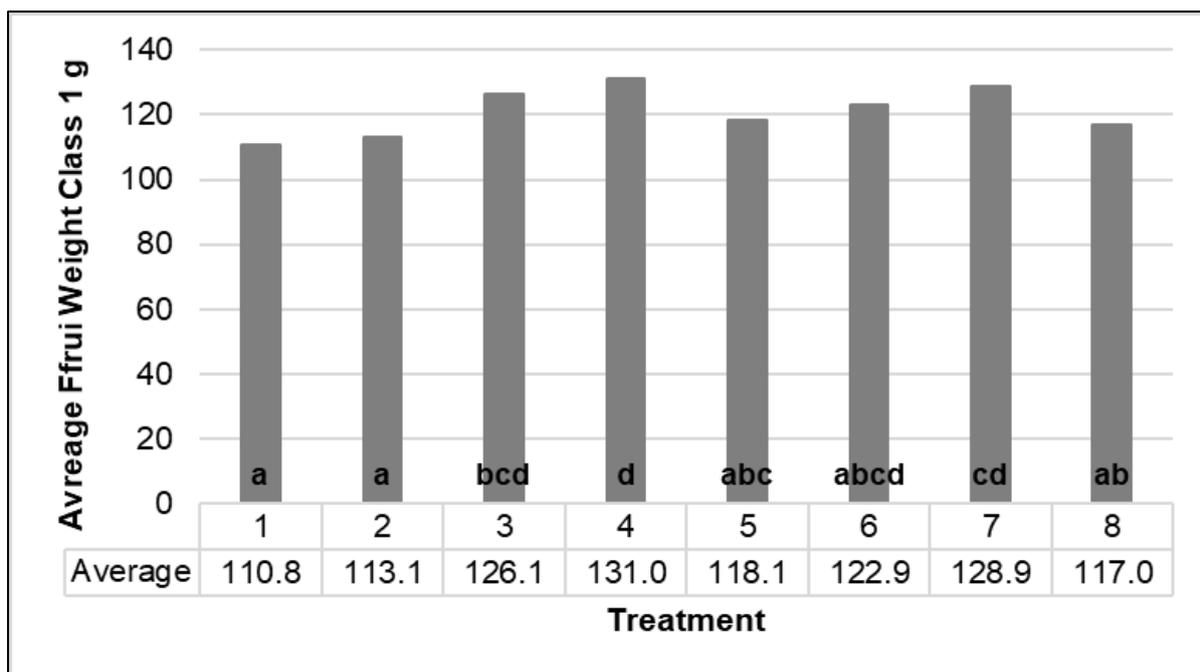


Figure 3.3.12. The effect of thinning practices on the Average Fruit Weight Class 1 of apples at harvest. Treatment 1: unthinned control, 2: bud removal, 3: mechanical flower removal, 4: Exilis, 5: Brevis, 6: standard thinning, 7: thinning to size, 8: late thinning.

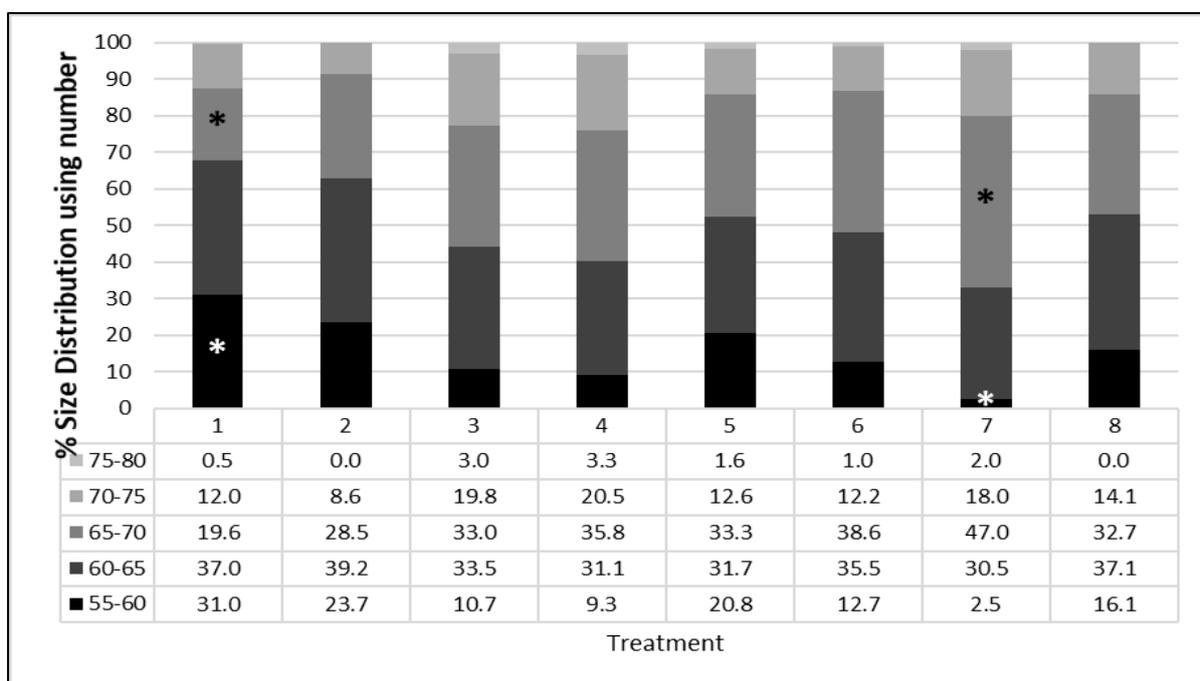


Figure 3.3.13. The effect of thinning practices on the Size Categories of apples at harvest. Treatment 1: unthinned control, 2: bud removal, 3: mechanical flower removal, 4: Exilis, 5: Brevis, 6: standard thinning, 7: thinning to size, 8: late thinning.

The size classes of fruit were affected by thinning practices and treatments, fewer 55-60 mm sized fruit were observed in mechanically thinned trees and those that received Exilis, with the least number in trees where thinning to size was practiced, where fruit size was increased with significantly more 65-70 mm fruit recorded (Fig 3.3.13).

Harvest maturity measurements by the FAST team found no effect of thinning treatments on starch patterns and fruit maturity, with all trees harvested between 70-80% fruit starch (Figure 3.3.14) and % Brix average 12-12.5% (Figure 3.3.15) with fruit firmness ranging from 9.2-9.5 kg (Figure 3.3.16).

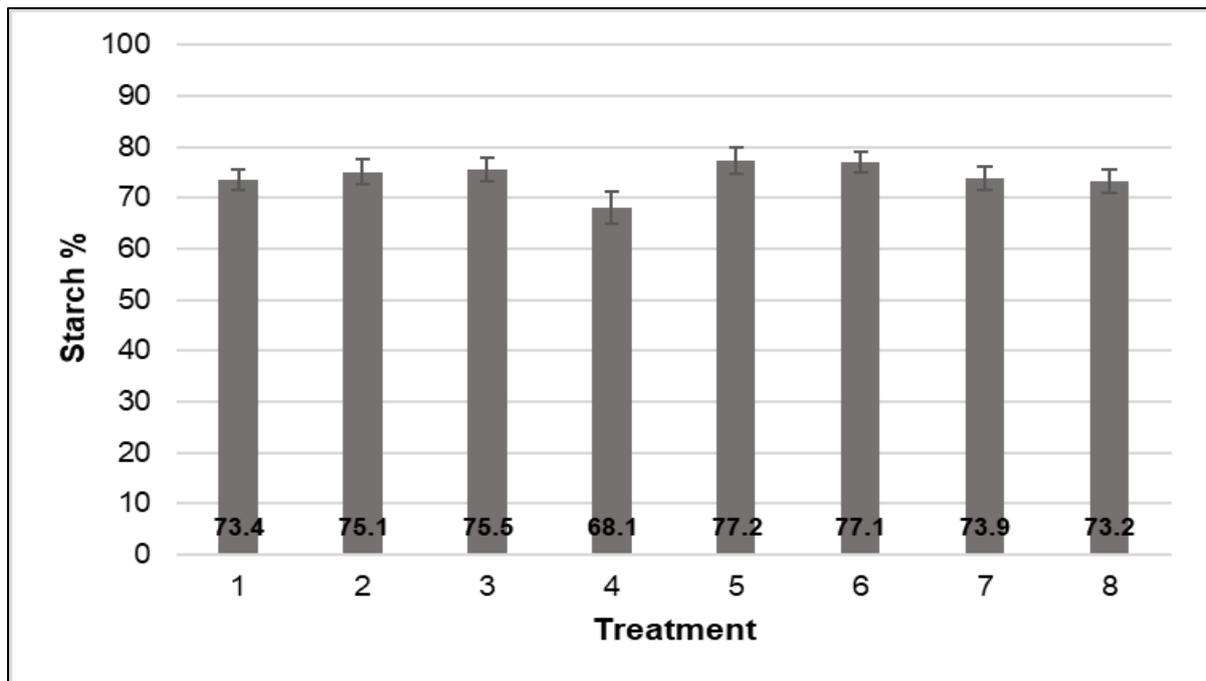


Figure 3.3.14. The effect of thinning practices on Percentage Starch content from fruit sampled at harvest. Treatment 1: unthinned control, 2: bud removal, 3: mechanical flower removal, 4: Exilis, 5: Brevis, 6: standard thinning, 7: thinning to size, 8: late thinning.

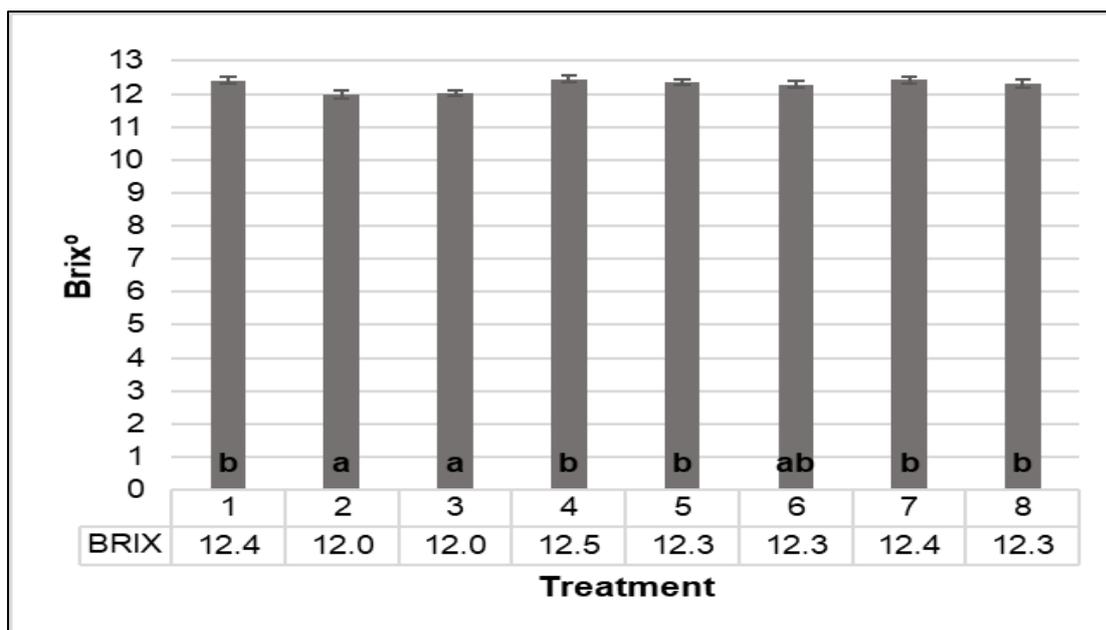


Figure 3.3.15. The effect of thinning practices on %BRIX of fruit at harvest. Treatment 1: unthinned control, 2: bud removal, 3: mechanical flower removal, 4: Exilis, 5: Brevis, 6: standard thinning, 7: thinning to size, 8: late thinning.

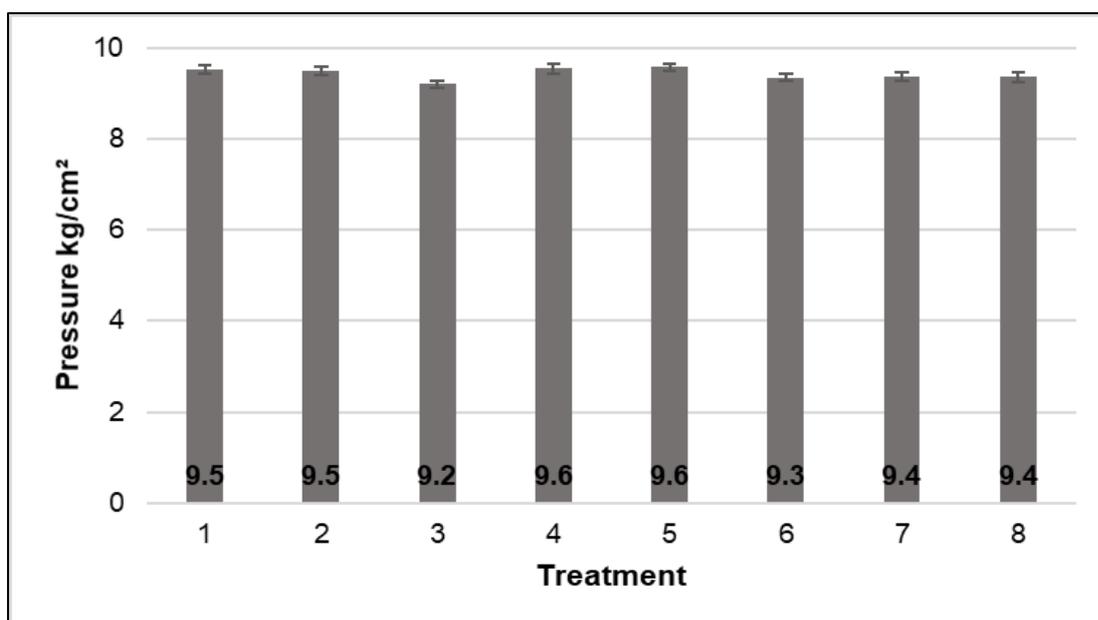


Figure 3.3.16. The effect of thinning practices on Fruit Firmness (kg cm²) of fruit at harvest. Treatment 1: unthinned control, 2: bud removal, 3: mechanical flower removal, 4: Exilis, 5: Brevis, 6: standard thinning, 7: thinning to size, 8: late thinning.

Fruit Dry Matter and Sugar Analysis

Apples sampled from each plot were taken to the Produce Quality Centre for harvest maturity analysis and for CA storage. Fruit firmness data measured using a Lloyd penetrometer showed firmness ranged from 83.7-86.9 N equivalent to 8.6-8.9 kg/cm in pressure across treatments (Table 3.3.3). No difference in harvest firmness between treatments was observed. Harvest maturity based on starch content indicated fruit sampled from trees treated with Brevis or where trees were 'thinned to size' led to fruit with slightly more advanced fruit maturity and less starch ~74.9-75.9% compared to 83% in fruit from un-thinned trees. % Brix content of fruit ranged from 11.4-12.0 % across treatments and no treatment effects of thinning were observed. Equally, no effect on Fruit Dry Matter was recorded across treatments with %FDM ranging between 15.5-16.9% when estimated using oven drying and 15.9-16.4% when freeze drying was applied. Brevis treated fruit recorded the highest %FDM under both techniques but failed to reach significance ($p < 0.05$). Analysis of freeze-dried samples for sugars revealed that all thinning treatments raised fructose and sucrose content in apples compared to un-thinned fruit, however, no difference in sugar profiles between thinning treatments were observed on the content of individual sugars.

Table 3.3.3 Harvest maturity and %FDM and sugar content of Gala apples subject to different thinning practices during fruit development.

Treatment	Control	Bud	Mechanical	Exilis	Brevis	Standard	Size	Late	F.prob	LSD _{0.05}
Firmness (N)	83.7	86.5	83.8	86.9	86.5	86.6	85.5	83.9	0.55	4.8
% Brix	11.7	11.4	11.4	12	11.9	11.7	12	11.8	0.18	0.56
% Starch	83	79.8	83.7	77.8	74.9	80.9	75.9	77	0.04	5.38
% FDM (OD)	15.7	15.5	16.4	15.7	16.9	16.9	15.9	15.8	0.86	2.82
%FDM (FD)	15.9	15.9	15.9	16.1	16.4	15.9	15.9	16.1	0.68	0.68
Fructose(µl/µl)	25.1	32.8	33.3	32.9	32.7	34	32.8	34.7	0.18	6.96
Sucrose (µl/µl)	14.3	19.3	20	20.9	21.4	20.4	20.5	20	0.12	4.66

OD: Oven Drying, FD: Freeze Drying

Samples of apples from each thinning treatment were subject to CA storage (3% CO₂, 1% O₂) at 0.5-1.0°C and sampled after 3,5,6,7 and 8 months. Overall means, averaged over each sampling point show that application of Exilis, Brevis, standard hand thinning or where fruit were thinned to size led to fruit that were marginally firmer (1.2-1.8 N equivalent to 0.12-0.18 kg/cm), Fruit treated with Brevis and those subject to standard hand thinning had slightly higher %Brix readings (13.0%) compared to un-thinned fruit (12.6%). No effect of thinning on rot development was observed.

Table 3.3.4 Overall means of ex-store quality measurements of Gala apples subject to different thinning practices and stored in 3% CO₂, 1% O₂ (0.5-1.0°C)

	Control	Bud	Mechanical	Exilis	Brevis	Standard	Size	Late	LSD _{0.05}	F.prob
Firm. (N)	80.4	79.9	80.6	81.8	82.8	81.7	81.6	80.5	1.282	<0.001
% Brix	12.6	12.7	12.6	12.8	13.0	13.0	12.8	12.6	0.278	0.013
% Rots	1.4	0.7	2.9	0.7	0.7	2.1	2.1	0.7	8.465	0.776

N.B to convert fruit firmness (N) to kg divide by 9.61

Analysis of Firmness data for changes in quality over time found no appreciable loss of fruit firmness over 8 months of storage of Gala when assessed immediately ex-store (Table 3.3.5) Final samples were also subject to 7 days at 18°C where no loss in firmness was observed. The benefits of retaining good fruit firmness throughout the storage season were achieved treated without SmartFreshSM, however, CA conditions were established immediately using N₂ and CO₂ flushing after fruits had reached storage temperature.

Table 3.3.5 Firmness (N) of fruit sampled ex-store and after shelf-life (SL) during CA storage

Firmness (N)	September	December	February	March	April	April SL	May

Control	84	79	79	76	82	82	81
Bud	84	78	77	78	81	81	81
Mechanical	87	78	77	80	80	82	81
Exilis	86	78	80	81	80	86	81
Brevis	87	82	81	80	83	83	83
Standard	86	80	81	81	82	83	80
Size	87	82	80	77	81	82	82
Late	84	77	79	80	82	83	79
LSD _{0.05}	3.39		F.prob	0.297			

N.B to convert Firmness in newtons (N) to kg divide by 9.81.

Apple juice taken from samples at harvest and across storage showed a small rise in Brix from 11.4-12% Brix at harvest rising to 12.9-13.5% at the end of storage in May 2019 (Table 3.3.6). No treatment differences were observed across individual sampling points.

Table 3.3.6 Changes in % Brix during storage

	Harvest	December	February	March	April	April SL	May
Control	11.4	12.7	12.7	12.7	13.1	12.9	13.0

Bud	11.7	12.3	13.2	12.9	13.1	12.5	13.3
Mechanical	11.4	12.6	13.2	12.8	12.8	12.6	12.9
Exilis	11.9	13.1	13.4	13.2	12.2	13.1	13.0
Brevis	12.0	13.5	13.2	12.3	13.7	12.7	13.5
Standard	12.0	13.5	13.7	13.0	13.7	12.4	13.2
Size	11.7	13.1	13.5	12.4	12.8	13.0	13.4
Late	11.8	13.0	13.1	12.5	12.1	13.0	13.2
LSD _{0.05}	0.736		F.prob	0.024			

No signs of senescent breakdown or other internal disorders were observed in fruit cut equatorially or across the stem bowl when inspected at each sample point during storage.

Summary and Key Findings WP3

- Thinning techniques did not impact on overall yield or the % of Class 1 fruit.
- Application of Exilis led to an increase in misshapen fruits and lowered the amount of Class 1 fruit at harvest.
- Average fruit weight was higher in trees receiving mechanical thinning, Exilis, thinning to size compared to fruit from unthinned trees.
- Size classes were affected by thinning. Unthinned fruit produced higher numbers fruit in the 55-60 mm size category. Thinning to size led to fruit with the highest fruit size having the most apples in the 60-70 mm category
- 2018 was exceptionally hot with prolonged periods without rain
- Chemical thinners applied to whole of canopy and were at times too effective.
- Differences in yield between treatments and year likely due to variability of trees
- Fruit weight averages in 2018 were comparable to weights measured 2017 (between 110g and 131g)

Year 4: WP3 Bud and Fruitlet Thinning (Year 2019/20)

As expected fruit thinning reduced the yield per tree but in most cases was this compensated by increasing the proportion of class 1 fruit. The overall impact of implementing thinning was to increase the proportion of class 1 fruit – however only ~60% of the final yield was considered to be suitable for class 1 market in those trees subject to hand thinning (Singles, Singles/Doubles, Doubles, Standard or thinning to size). Exilis was the only spray treatment that produced the same proportion of class 1 fruit, while Brevis treatments yielded only 48.1% Class 1 fruit which was in-line with unthinned trees

Fruit Thinning

Fruit thinning practices reduced overall yield per tree but increased the percentage of Class I fruit (Table 4.3.1). While no single thinning treatment stood out as a preferred treatment in terms of increasing class I yield, there were differences associated with size distribution of fruit and the sources of rejection (Fig 4.3.1, Table 4.3.2, Table 4.3.3). In general, the earlier thinning takes place after full bloom, the greater the chance on increasing fruit size and weight at harvest. Hand thinning to singles on the upper canopy and doubles on clusters in the lower canopy implemented while fruitlets were at 10-20 mm stage produced a larger proportion of fruit in the 65-70 mm category (48.6%) compared to standard thinning (37.2%) where the same procedure is conducted when fruitlets are between 15-25 mm in size. Implementing thinning earlier appeared to have a stronger influence on fruit size at harvest than the number of fruitlets left per cluster (singles, doubles or singles/doubles). Thinning to size was equally effective as early thinning in increasing fruit size at harvest. The effect of thinning strategies on weight of fruit from each size class (Table 4.3.1); thinning to singles, singles/doubles or doubles when fruitlets were between 10-20 mm had the effect of increasing the yield of fruits in the 65-70 mm category and was equal to the thinning to size strategy. Early thinning (10-20 mm) increased the yield of class1 as did thinning to size (Table 4.3.1).

Table 4.3.1 Yield and Class 1 grade outs for Gala subject to different fruitlet thinning treatments.

	<i>Control</i>	<i>Singles</i>	<i>Singles/ Doubles</i>	<i>Exilis</i>	<i>Brevis</i>	<i>Standard</i>	<i>Size</i>	<i>Doubles</i>	<i>F.prob</i>	<i>LSD_{0.05}</i>
%Class1	50.6	63.1	59.5	61.8	48.1	61.9	61.6	58	0.525	17.38

Yield/Tree (kg)	37.1	25.0	30.8	30.3	23.0	28.3	29.1	25.4	0.002	5.93
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N.B. Values in bold are significantly different ($p < 0.05$) from fruit harvested from the control (Unthinned) in the same row.

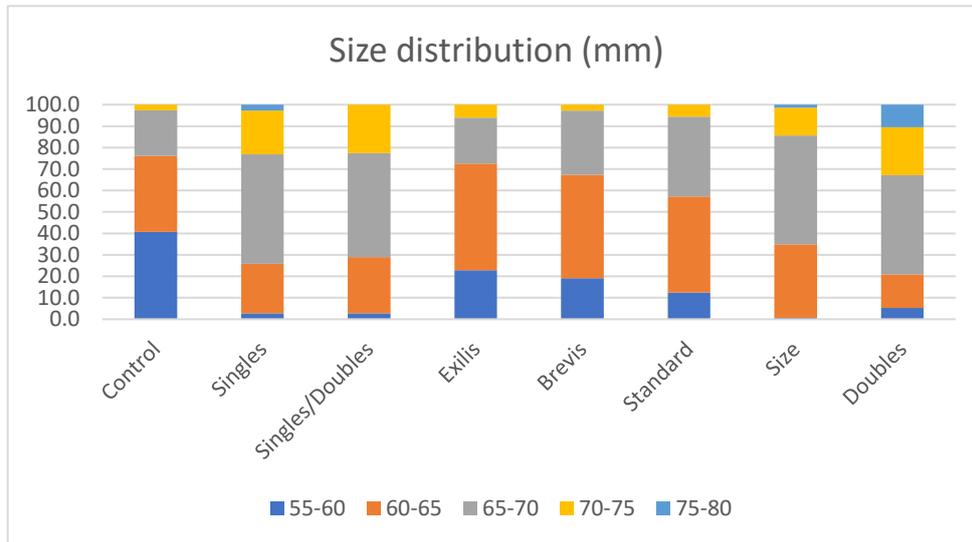


Figure 4.3.1 The size distribution of Gala apples at harvest subject to different thinning practices at fruitlet stage.

Table 4.3.2. The size distribution of Gala apples at harvest subject to different thinning practices at fruitlet stage.

Size Class (mm)	Control	Singles	Singles/Doubles	Exilis	Brevis	Standard	Size	Doubles
55-60	40.7	2.7	2.8	22.9	19.1	12.4	0.7	5.2

60-65	35.4	23.1	26.1	49.6	48.2	44.8	34.2	15.7
65-70	21.2	51.0	48.6	21.4	30.0	37.2	50.7	46.3
70-75	2.7	20.4	22.5	6.1	2.7	5.5	13.0	22.4
75-80	0.0	2.7	0.0	0.0	0.0	0.0	1.4	10.4

Table 4.3.3 The weight (g) of Class 1 Gala apples in each size category as a result of fruitlet thinning strategies. Figures in parenthesis represent average fruit numbers making up the yield in each size category averaged across 4 replicate plots.

Treatment	55-60 mm	60-65 mm	65-70 mm	70-75 mm	75-80 mm	Total wt
Unthinned	908 (11.5)	1032 (10)	751 (6)	110 (0.8)	0	560
Singles	40 (1)	933 (8.5)	2464 (18.8)	1207 (7.5)	187 (1.0)	966
Singles/Doubles	62 (1.0)	960 (9.3)	2264 (17.3)	1259 (8)	0	909
Exilis	568 (7.5)	1716 (16.3)	908 (7)	306 (2)	0	699
Brevis	402 (5.3)	1377 (13.3)	1052 (8.3)	108 (0.8)	0	588
Standard	346 (4.5)	1734 (16.3)	1747 (13.5)	297 (2.0)	0	825
Size	12 (0.3)	1344 (12.5)	2402 (18.5)	746 (4.8)	86 (0.5)	918
Doubles	94 (1.8)	559 (5.3)	2000 (15.5)	1190 (7.5)	654 (3.5)	899
LSD _{0.05} 663.2 Treatment x Size class			F.prob <0.001			
LSD _{0.05} 296.6 Treatment (Total weight)			F.prob <0.035			

N.B. Values in bold are significantly different ($p < 0.05$) from fruit harvested from the control (unthinned trees) in the same column

Treatment with Exilis and Brevis shifted the majority of fruit to the 60-65 mm category (Table 4.3.2). This was also observed in trees subject to standard thinning practices, while as expected unthinned trees produced the largest proportion of 55-60 mm. Thinning to: size, singles, singles/doubles or doubles across the tree shifted size category of Gala within in these plots, with a greater proportion of 65-70 mm fruit.. Thinning to Singles, Singles/Doubles, Doubles or to Size minimised fruit size below 60 mm. Thinning to doubles across the tree had the unexpected result of shifting fruit size towards larger fruit with over 10% of the size classes in the 75-80 mm category (Table 4.3.2).

The effect of thinning strategies on weight of fruit from each size class can be seen in Table 4.3.3; thinning to singles, singles/doubles or doubles when fruitlets were between 10-20 mm had the effect of increasing the yield of fruits in the 65-70 mm category and was equal to the thinning to size strategy. Early thinning (10-20 mm) increased the yield of class1 as did thinning to size (Table 4.3.3). A more detailed analysis of grade out data found that lower grade out figures for Gala treated with Brevis was associated with a higher numbers of small fruits <55 mm (Table 4.3.4). Unthinned trees produced a significant number of small undersized fruits.

Table 4.3.4. Grade out losses of Gala at harvest caused by thinning practices at fruitlet stage

Grade out- Numbers of fruit	<i>Control</i>	<i>Singles</i>	<i>Singles/Doubles</i>	<i>Exilis</i>	<i>Brevis</i>	<i>Standard</i>	<i>Size</i>	<i>Doubles</i>
Scarring/Russet	1.5	2.8	2.0	1.5	0.8	0.8	1.0	2.0
Damage - pest/physical	7.5	5.5	8.5	6.5	9.3	8.0	9.0	9.3
Misshapen	1.5	2.0	2.3	2.8	1.8	0.8	1.0	2.3
Small	12.5	1.3	2.3	4.3	7.3	1.0	0.8	1.5
Diseased	9.8	10.5	7.3	7.8	12.3	11.8	11.5	9.5
Lack % Red	0.5	1.0	3.0	0.8	1.5	1.5	0.5	2.0
Unmarketable	33.3	23.0	25.3	23.5	32.8	23.8	23.8	26.5
Marketable	28.3	36.8	35.5	32.8	27.5	36.3	37.0	33.5
Total (n=60)	61.5	59.8	60.8	56.3	60.3	60.0	60.8	60.0

Thinning practices that raised FDM were restricted to trees where fruitlets were thinned to singles across the tree, or subject to standard thinning or thinning to size (Table 4.3.5). This only translated to increased % Brix in fruit where thinning to size had been practiced. In general %FDM in the 2019 season was low partly due to the cooler summer compared to 2018 and previous years. When treatment effects were averaged across the whole tree Brevis and Exilis applied at BBCH 70-71 & 71-72 just failed to significantly increase %FDM (Table 4.3.5).

Thinning generally improved fruit firmness across the treatments raising firmness by 4-5 N (0.4-0.5 kg). Thinning to single fruitlets per cluster across the tree produced the firmest fruit at 92.6 N (9.2 kg) at harvest (Table 4.3.5).

Table 4.3.5 Overall effects (averaged across the tree) of thinning practices at fruitlet stage on Gala maturity and %FDM at harvest

<i>Thinning</i>	<i>Control</i>	<i>Singles</i>	<i>Singles/ Doubles</i>	<i>Exilis</i>	<i>Brevis</i>	<i>Standard</i>	<i>Size</i>	<i>Doubles</i>	<i>F.prob</i>	<i>LSD_{0.05}</i>
<i>I.E.C ppb</i>	193.2	289.8	341.3	282.3	328.3	384.1	401.1	342.7	<.001	52.57
Starch	5.3	4.1	4.1	4.0	4.2	4.6	4.5	4.9	0.051	0.98
% Brix	12.0	12.4	11.4	11.8	12.1	12.2	12.8	12.6	<.001	0.61
% DM	15.4	16.4	15.7	15.7	16.1	16.5	16.5	16.1	0.127	0.87
Fructose	123.7	103.0	105.8	114.8	119.0	110.5	123.9	111.1	<.001	9.23
Glucose	14.0	9.8	9.7	12.0	12.2	12.0	12.3	11.3	0.004	2.11
Sucrose	75.8	81.2	81.3	74.7	83.1	82.7	85.0	82.3	0.041	6.77
Firm (N)	84.5	92.6	88.4	88.3	87.2	89.6	88.9	89.3	0.003	3.40

N.B. Values in bold are significantly different ($p < 0.05$) from fruit harvested from the control (unthinned trees) in the same row. To convert fruit firmness from Newtons (N) to kg divide values by 9.8

Harvest maturity

Harvest maturity of Gala assessed on I.E.C. found that thinned fruit were more mature than unthinned fruit (Table 4.3.4) but significant variation was found between fruit harvested from the top and bottom of the canopy (Table 4.3.5). Interestingly, the lower internal ethylene concentrations found in fruit at harvest did not correlate well with starch staining patterns. It has been previously reported (Johnson 1995) that starch and soluble solids patterns are poor indicators of harvest maturity in crop load trials where fruitlet number have been manipulated through flower and fruitlet thinning. In general, sucrose concentrations were higher in fruit with higher internal ethylene. Fruitlets subject to thinning to either singles, singles/doubles or

treated with Exilis-treated fruit were less ripe than fruit from many of the other thinning treatments.

Overall effects of fruit position on the trees averaged across thinning treatments

Averaged across treatments fruits at the top of the canopy were more mature based on the Internal ethylene concentration but this was not reflected in starch clearance patterns (Table 4.3.6). FDM content was higher (0.6%) in fruits harvested from the top of the canopy (> 1.5 M). Fruit maturity was more advanced based on I.E.C in fruit sampled higher in the canopy but no corresponding change in starch clearance patterns or sucrose content was observed.

There was no effect of fruit position on sucrose content when averaged across all thinning treatments however, there was a strong interaction between, fruit position and individual thinning treatments on sucrose.

Table 4.3.6 The overall effect of sampling position of apples (>1.5 M, <1.5M) on the tree averaged across thinning treatments

<i>Position</i>	<i>Top</i>	<i>Bottom</i>	<i>F.Prob</i>	<i>LSD_{0.05}</i>
<i>I.E.C ppb</i>	362.9	277.7	<.001	26.29
Starch CTIFL	4.4	4.5	0.72	0.45
% DM	16.4	15.8	0.01	0.43
Fructose µg/µl	111.4	116.5	0.03	4.61
Glucose µg/µl	11.4	11.9	0.38	1.06
Sucrose µg/µl	81.2	80.3	0.58	3.38

N.B. Values in bold are significantly different ($p < 0.05$) from fruit harvested from the control (unthinned trees) in the same row

Fruit Dry Matter, % Brix and Sugars

When observing the range of harvest maturity, sugar and carbohydrate accumulation across the tree additional patterns in treatment effects become apparent. Thinning treatments applied to fruitlets >1.5 M raised %FDM between 0.2-1.7% above that of unthinned trees (Table 4.3.6). Brevis treated fruit from the upper canopy recorded 17% FDM, while only 15.3% FDM in the lower canopy which was no higher than apples from unthinned controls. Exilis showed a similar pattern of effect with 16.4 %FDM in fruit from the upper canopy compared to 15.1% in the lower canopy. Thinning to singles across the tree, standard thinning, thinning to size

and retaining double fruit per cluster across the tree both increased and improved uniformity of FDM across the canopy (Table 4.3.6). No impact of thinning treatments on %Brix were observed at harvest, however, in general, fruits from the top of the canopy had marginally higher % Brix (not significant $p < 0.05$).

All hand thinned treatments led to a rise in sucrose content at harvest (Table 4.3.7), interestingly Brevis and Exilis failed to increase sucrose in apples harvested from higher in the canopy despite having higher %FDM. Fructose and glucose content remained lower in Gala from thinned trees which may correlate with fruits having an increased harvest maturity and higher respiration rates utilising amounts of reducing sugars.

Fruit Maturity

In general, fruits from the higher part (1.5 M) of the canopy were more advanced in maturity than trees from the lower canopy (Table 4.3.7). Certain thinning treatments advanced fruit maturity based on higher I.E.C at harvest. Trees that were thinned to singles fruitlets in the higher canopy with double fruitlets (Singles/Doubles) in the lower canopy, Brevis-treated trees and those where fruitlets have been thinned to size all resulted higher I.E.C. Interestingly, where trees had been thinned to singles across the tree, no such increase in maturity was observed.

A disparity between I.E.C values and starch readings based on iodine staining was recorded and this difference was greatest in fruits harvested from the lower canopy of unthinned trees where the lowest internal ethylene concentration (ppb) but recorded but fruits had the most advanced starch clearance patterns (CTIFL score of 5.4 and I.E.C of 130 ppm). Apples harvested from the top of the canopy had higher I.E.C values.

Table 4.3.7 The interaction between Thinning Treatments and sampling position of apples (>1.5 M, <1.5M) on the tree.

Thinning	Internal Eth Conc. ppb		CTIFL Starch score		% DM		Fructose		Glucose		Sucrose	
	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
Unthinned	255.6	130.8	5.3	5.4	15.5	15.3	122.7	124.7	13.8	14.1	76.5	75.0
Singles	282.8	296.9	3.7	4.5	16.5	16.3	99.8	106.3	9.0	10.5	80.1	82.3
Sing/Dou	434.7	247.9	4.2	4.0	15.9	15.5	100.2	111.4	8.9	10.4	78.3	84.2

Exilis	299.4	265.3	4	3.9	16.4	15.1	112.8	116.7	12.1	11.8	74.0	75.5
Brevis	393.7	262.9	4.3	4.1	17.0	15.3	112.7	125.3	11.8	12.5	83.4	82.9
Standard	380.8	387.3	4.3	4.9	16.7	16.3	108.5	112.5	11.3	12.7	84.8	80.5
Size	480.0	322.2	4.8	4.2	16.7	16.3	122	125.7	12.6	12.0	86.8	83.3
Doubles	376.6	308.8	4.7	5.1	16.3	16.0	112.7	109.4	11.7	11.0	86.0	78.6
F.prob	<.001		0.813		0.127		0.739		0.883		0.62	
LSD_{0.05}	74.35		1.262				4.61		1.056			
Treat x Pos.					0.433						3.384	

N.B Figures in bold are significantly different ($p < 0.05$) from the control (unthinned) trees in the same column of data.

Firmness and Colour

Hand thinned treatments led to firmer fruit in the upper canopy but these treatments did not impact on fruit firmness in the lower canopy, apart from where trees had been thinned to single fruitlets per cluster across the tree (Table 4.3.8). Fruit colour was not effected by thinning treatments (Table 4.3.8).

Table 4.3.8 The effect of tree position on the accumulation of %FDM, % Brix, Firmness and Fruit colour

	% DM		Red Colour		Yellow Colour		% Brix		Firmness	
	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
Control	15.5	15.3	42.6	38.4	18.6	19.9	12.2	11.8	82.5	86.4
Singles	16.5	16.3	43.1	40.1	17.5	20.8	12.5	12.2	93.2	92.0
Singles/Doubles	15.9	15.5	42.9	38.4	18.2	21.2	11.9	10.9	90.4	86.4
Exilis	16.4	15.1	42.4	41.6	17.6	19.2	11.8	11.8	87.9	88.7
Brevis	17.0	15.3	40.5	35.8	19.9	20.9	12.3	11.8	87.3	87.0
Standard	16.7	16.3	42.5	39.4	19.0	20.9	12.1	12.4	89.9	89.3
Size	16.7	16.3	44.5	41.3	17.0	20.4	12.9	12.6	89.1	88.7

Doubles	16.3	16.0	43.3	38.5	20.1	22.1	12.8	12.5	89.8	88.8
F.prob	0.585		0.801		0.835		0.665		0.571	
LSD _{0.05}	1.22		3.61		2.62		0.86		4.81	
Treat x Pos.										

N.B. Values in bold are significantly different ($p < 0.05$) from fruit harvested from unthinned trees in the same column. To convert Newtons (N) to kg divide values by 9.86.

Storage Year 4 (2019/2020)

Fruits were stored in 3% CO₂/ 1% O₂ at 0.5-1.0°C for 8-9 months. Fruits were removed and a single ex-store assessment was undertaken. Gala from the thinning trial- demonstrated that fruits that had undergone thinning retained their increased Brix content throughout storage. Those fruit subject to thinning to singles, standard thinning, thinning to size or where trees treated with Brevis had % brix content 0.7-0.9% over unthinned fruit. Moreover, Brevis was as effective as hand-thinning in raising Brix content of fruit. Overall fruit retained firmness throughout storage losing less than 10 N (~1 kg) in pressure throughout 9 months storage- these fruit received no additional post-harvest treatments prior to sealing the cabinets (Table 4.3.9) however, no shelf-life assessments were taken for this particular trial due to Covid-9.

Table 4.3.9 Overall fruit firmness (N) and % brix values of Gala apples subject to fruitlet thinning treatments, stored for 9 months in (3% CO₂/ 1% O₂ at 0.5-1.0°C)

<i>Thinning</i>	<i>Control</i>	<i>Singles</i>	<i>Singles/ Doubles</i>	<i>Exilis</i>	<i>Brevis</i>	<i>Standard</i>	<i>Size</i>	<i>Doubles</i>	<i>F.prob</i>	<i>LSD_{0.05}</i>
% Brix	13.0	13.7	13.4	13.5	13.8	13.9	13.9	13.5	0.033	0.572
Firmness (N)	80.7	81.0	80.9	81.5	82.0	82.0	80.8	82.0	0.904	2.690

The quality of fruit coming out of store after 9 months was excellent- with a minimal decline in firmness. There was no difference in fruit firmness readings between fruit selected from the top and bottom fruit of the canopy. % Brix values were generally higher for upper canopy fruit with fruit hand-thinned to singles, singles/doubles, doubles, standard thinning and thinning to size, Brevis-treated (Table 4.4.2), but differences failed to reach significance ($p < 0.05$).

Table 4.4.2 Fruit firmness (N) and % Brix values of Gala apples from the top (> 1.5 M) and bottom (<1.5 M) subject to fruitlet thinning treatments, stored for 9 months in (and)

	Firmness (N)		% Brix	
Firmness (N)	Top	Bottom	Top	Bottom
Control	80.8	80.5	13.0	13.0
Singles	84.0	77.9	13.8	13.5
Singles/Doubles	82.4	79.5	13.7	13.0
Exilis	82.7	80.3	13.7	13.3
Brevis	82.6	81.4	14.0	13.6
Standard	83.2	80.8	14.4	13.3
Size	81.3	80.3	14.1	13.7
Doubles	82.8	81.2	13.8	13.2
F.prob	0.57		0.75	
LSD _{0.05}	3.8		0.81	

N.B. Values in bold are significantly different ($p < 0.05$) from fruit harvested from the control (unthinned trees) in the same column. To convert fruit firmness from Newtons (N) to kg divide values by 9.86.

Year 5: WP 3, Fruitlet Thinning Trial.

Harvest maturity assessments of Gala apples were undertaken at the PQC-NRI (Figure 5.3.1) and at FAST (Figure 5.3.2) from trees subject to different thinning treatments at FAST LLP experimental orchards.

Fruit sampled by NRI staff segregated fruit from the top (>1.5 M) and bottom (<1.5 M) of the tree while the FAST sampling took a representative sample across the whole tree. Harvest maturity data from NRI was analysed for treatment and position and for interactions between thinning treatments x fruit position on the tree.

When harvest maturity data (NRI) was averaged across sampling positions fruit maturity results for NRI and FAST assessments followed similar patterns in the 2020 season for starch content. Starch maturity testing found no significant ($p > 0.05$) difference between treatments Brevis treated fruit (T5) were less red and more yellow (B) than fruit from other treatments

Harvest maturity measurements were taken by NRI (18/9/2021) and later in the week by FAST (22/9/21). Higher starch values for NRI reflect earlier pick date and no significant effect of thinning practices on maturity was observed in the NRI or Fast 2020 harvest maturity data. Interestingly, % Brix content was lowest in Gala apples where fruits had been thinned to singles (T2) and was consistently the lowest in % Brix across NRI & FAST measures of fruit maturity. Fruits from this thinning treatment were significantly larger (65.7 mm) and heavier (130.2 g) than fruits where a more standard thinning practice (T2) of leaving singles fruitlets per cluster above 1.5 M and double fruitlets per cluster in branches growing below 1.5 M here fruits weight averaged 118 g and fruit size was 63.7 mm.

Dry Matter content was generally higher in 2020 averaging 16.6% using freeze drying compared to 16.0% where oven drying was used (Figure 5.3.1). The high DM values reflect high sunshine hours (1174) during the 2020 growing season compared to 938 in 2019 (Figure 5.3.2).

Exilis treated fruit had higher in fruit firmness at harvest due to lower yield 49.8 tonnes /ha compared to unthinned control fruit 60.9 tonnes/ha. Thinning treatments did not significantly increase the amount of class I fruit, the highest yield 31.9 tonnes/ha was generated through thinning trees early to either singles, equally thinning to singles/double or doubles or treatment with Brevis yielded similar levels of class I fruit.

Table 5.3.1. Harvest Maturity of Gala apples subject to different thinning protocols. Data is averaged across samples taken from the top (>1.5 M) and bottom (<1.5 M) of the tree (NRI).

Treatment	Singles/						F.pr.	LSD
	Control	Singles	Doubles	Exilis	Brevis	Doubles		
Colour L	43.0	43.9	44.2	42.9	46.6	43.5	0.044	2.46
Colour A	40.4	39.4	40.1	39.9	36.4	39.1	0.054	2.71
Colour B	18.6	19.0	19.6	18.2	20.6	18.6	0.022	1.46
% Starch	81.9	81.5	83.9	79.3	82.6	80.4	0.243	3.91
% Brix	12.3	11.9	11.4	12.6	12.1	12.2	0.022	0.67
Firmness (N)	85.2	87.1	84.6	89.5	84.2	86.1	0.038	3.47
% DM	16.6	16.1	16.4	17.1	16.8	16.7	0.033	0.62
Fructose $\mu\text{L}/\mu\text{L}$	19.2	18.0	18.0	18.0	18.5	18.4	0.041	1.093

Glucose $\mu\text{L}/\mu\text{L}$	2.5	2.1	1.9	2.1	2.1	2.1	0.101	0.401
Sucrose $\mu\text{L}/\mu\text{L}$	9.2	9.1	9.9	10.0	9.7	9.0	0.018	0.819

Figures in bold are values significantly different ($p < 0.05$) from the un-thinned control trees in the same row

Table 5.3.2 Harvest Maturity Firmness, Starch, % Brix, and yield data (FAST)

Treatment	Control	Singles	Singles/ Doubles	Exilis	Brevis	Doubles	F.pr	LSD
Starch	67.8	75.2	72.3	69	67.7	68	0.31	7.86
% Brix	12.6	12.1	12.0	12.8	12.3	12.9	<.001	0.26
% DM	16.1	15.8	15.5	16.7	15.7	16.1	0.317	1.07
Firmness (kg)	9.8	9.5	9.3	10.0	9.5	9.8	<0.001	0.26
Yield (kg) /tree	21.3	19.8	23.9	17.4	21.9	21.7	0.130	4.60
Yield (T) per ha	60.9	56.5	68.3	49.8	62.7	62	0.130	13.22
Yield (T) class 1/ha	24.4	28.8	28.7	22.1	29.3	31.9	0.608	12.27

Mineral analysis of fruits at harvest found that fruit were subject to Exilis treatments were higher in phosphorous and potassium content, leading to a higher K:Ca ratio (Figure 5.3.3). Thinning to singles/doubles and doubles raised calcium content. Fruits treated with Exilis had a lower crop load per tree and increase in P & K may be an effect in part of crop load

Table 5.3.3 mineral analysis and dry matter data

	Control	Singles	Singles/ Doubles	Exilis	Brevis	Doubles	F pr.	LSD
Avg Weight	97.3	130.2	118.0	115.0	106.2	102.0	0.002	15.50
Avg diameter	50.2	65.7	63.6	61.9	61.3	60.4	0.105	10.93
N	30.3	27.2	29.3	35.7	33.5	30.9	0.140	6.49
P	10.4	10.8	10.4	12.1	10.5	10.6	0.002	0.82
K	91.3	100.1	97.0	105.3	95.3	98.7	0.149	10.28
Mg	5.1	6.0	5.5	5.5	5.3	5.3	0.134	0.68
Ca	10.6	12.1	12.9	10.4	11.8	13.2	0.073	2.18
KCA	8.8	8.4	7.6	10.4	8.4	7.7	0.088	2.01
Cu	0.4	0.4	0.4	0.4	0.4	0.4	0.844	0.07
Fe	1.6	1.7	1.7	1.7	1.6	1.6	0.822	0.31
Mn	0.5	0.5	0.5	0.5	0.5	0.5	0.510	0.08
B	2.3	2.2	2.3	3.0	2.4	2.6	0.513	0.87
% DM	16.1	15.8	15.5	16.7	15.7	16.1	0.317	1.066

Numbers of fruit suffering from scaring and physical/pest damage were similar across treatments (Table 5.3.4). Exilis treated trees (T4) produced more Mis-shaped fruits 5.4% compared to untreated control trees. Small fruit size was highest in unthinned (T1) trees and where trees were subject to thinning to doubles across the tree (T8).

Table 5.3.4 Grade out data types of losses

Treatment	Singles/							LSD
	Control	Singles	Doubles	Exilis	Brevis	Doubles	F.pr	
Scaring/damage	2.8	4.9	4.3	2.9	2.3	3.9	0.871	4.9
Damage - pest/physical	17.7	11.1	18.7	17.7	20.9	19.0	0.704	12.7
Mis-Shape	1.1	2.2	0.0	5.4	0.8	0.0	0.017	3.2
Small	43.1	21.8	21.1	26.3	27.0	38.0	0.036	15.5
Diseased	25.0	47.7	46.9	37.4	29.4	30.2	0.082	18.6
Lack of % red	10.3	12.3	9.1	10.3	19.6	8.8	0.354	10.9

Table 5.3.5 Size distribution of fruit subject to different thinning practices

Treatment	55-60	60-65	65-70	70-75	75-80
Control	36.7	42.1	16.8	4.4	0.0
Singles	18.2	33.6	27.3	17.8	3.2

Singles/Doubles	24.8	38.6	22.6	11.3	2.8
Exilis	15.8	33.0	35.7	15.5	0.0
Brevis	23.8	36.5	26.1	10.6	0.6
Doubles	35.4	34.6	23.9	3.4	2.7
F pr.	0.028	0.874	0.165	0.15	0.29
LSD	14.4	19.1	14.4	13.2	4.0

Unthinned Gala had the highest proportion of small 55-60 mm fruit, interestingly trees thinned to doubles also led to a large proportion of small (55-60 mm) fruit (Table 5.3.5). None of the thinning treatments significantly shifted fruit into the 60-65 mm or 65-70 mm size category, while fruit thinned to singles across the tree had higher proportion of 70-75 mm fruit.

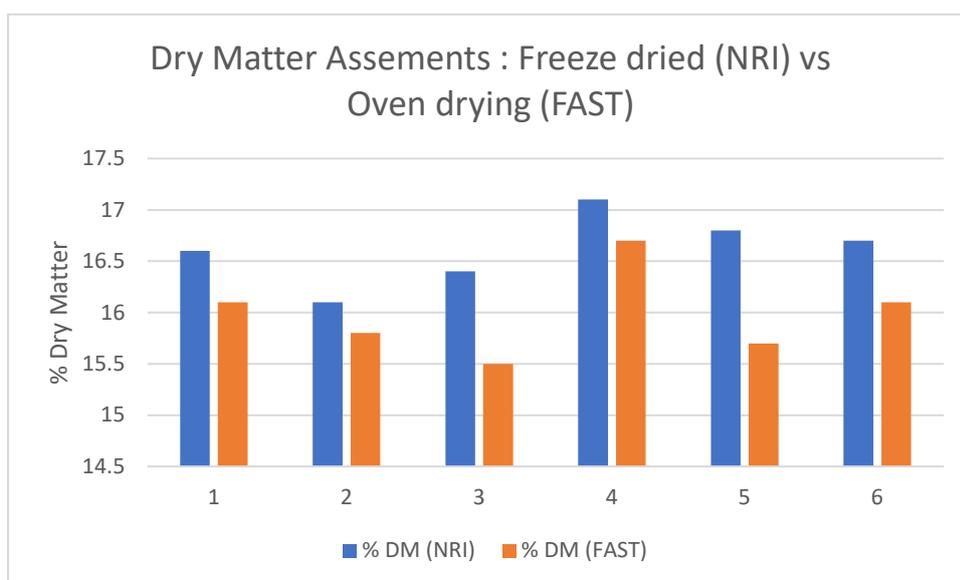


Figure 5.3.1 Comparison of thinning treatments on dry matter content of Gala apple: 1: Control, 2: Singles, 3: Singles/Doubles, 4: Exilis, 5: Brevis, 6: Doubles.

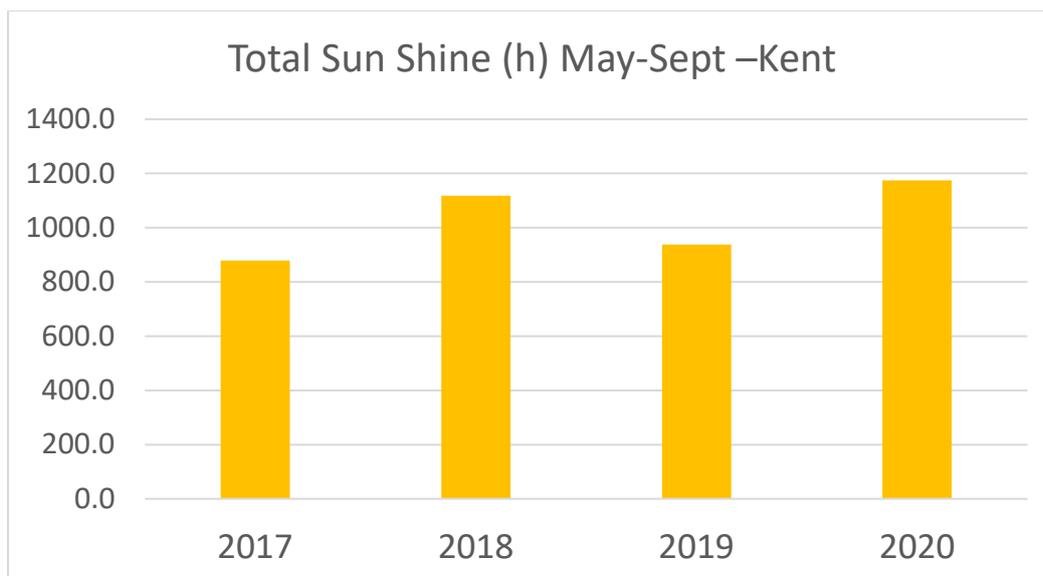


Figure 5.3.2 Comparison of sunshine hours over the 2017-2020 period

Sampling fruit across the tree found apples in the upper canopy had a higher red colour intensity and less background yellow. Fruits from the higher canopy were firmer and had 0.4% increase in dry matter Table (5.3.6)

Table 5.3.6 Harvest Maturity of Gala apples subject to harvesting from either the top (>1.5 M) or the bottom of the canopy (<1.5 M) averaged across all 6 thinning treatments.

Position	Top	Bottom	F.pr.	LSD _{0.05}
Colour L	42.6	45.4	<.001	1.42
Colour A (Red)	40.7	37.8	<.001	1.57
Colour B (Yellow)	18.4	19.8	0.001	0.84
% Starch	82.3	80.9	0.206	2.26
% Brix	12.2	12.0	0.492	0.39
Firmness	87.7	84.5	0.002	2.00
% DM	16.8	16.4	0.02	0.36
Fructose $\mu\text{L}/\mu\text{L}$	18.3	18.3	0.149	0.232
Glucose $\mu\text{L}/\mu\text{L}$	2.2	2.1	0.044	0.631
Sucrose $\mu\text{L}/\mu\text{L}$	9.6	9.4	0.106	0.473

Work Package 4. Prediction of harvest maturity of Gala using Chlorophyll fluorescence

Work package 4 focused on developing a non-destructive method to optimise harvest date and identifying the orchards suitable for long term storage. This can be achieved by choosing the right fruit with high dry matter and balanced minerals that are picked at the right time for extended keeping quality during long term storage. If this process is carried out correctly then UK Gala should compete effectively with Southern hemisphere fruit both on fruit firmness and, more crucially, taste.

Current practice for harvesting of Gala for long term storage is to pick fruit when starch coverage has dropped to 85-80% coverage and where background red colour has developed sufficiently to satisfy the marketing desks. However, this narrow window does not afford growers sufficient time to organise harvesting crews in time to pick orchards before maturity advances observed through declining starch content. The results gathered between 2016-2018 confirmed that monitoring fruit using a chlorophyll fluorescence meter provided a non-destructive tool for fruit maturity and that changes in CF output were observed 7 to 10 days prior to changes in starch clearance patterns providing an early warning to growers to organise their picking schedules around the optimum harvest date for long-term storage. Where CF profiles was used on orchards where fruits had ample mineral content for long-term storage and high FDM then picking fruit at optimal maturity meant fruit performed well in long-term storage.

Sampling of Gala orchards for CF analysis was standardised to reduce variability, fruitlets (25-30mm) were picked in 9 selected orchards in Kent in the first week of July 2017.

Samples were taken from each compass point on a tree, North, East, South and West (4 fruitlets per tree, all samples picked from middle height of trees). Samples were taken in a “W” pattern across the orchard taking samples at appropriate points.

In the first week of August sample collection fruit (55-60 mm) from 9 orchards were repeated. After analysing CF, mineral profiles and FDM of fruit, according to the flow chart (see below) five of orchards were selected based on their suitability for long-term storage and were monitored with CF for a period of 2-3 weeks prior to commercial harvest. CF prediction model was restricted to fruit intended for long term storage (Table 4.1).

Table 4.1: Comparison of dry matter and mineral analysis in 9 orchard and selecting 5 orchards for the long-term storage (season 2017-18).

FIELDREF	Test	Clone	DMC	CF (AvF)	WT	Interpretation	N	Interpretation	P	Interpretation	K	Interpretation	Mg	Interpretation	Ca	Interpretation
Orchard 1	Fruitlet (July 2017)	Schneiga	15.6	4664	36.34	Normal	87.36	Normal	13.62	Low	147.78	High	11.02	High	16.59	High
	Fruit (August 2017)		16	4703	97.71	Normal	35.2	Low	9.4	VeryLow	94.28	Sli Low	7.45	Normal	10.49	High
Orchard 2	Fruitlet (July 2017)	Mondial	13.2	5665	40.22	Normal	83.16	Normal	13.21	Low	135.24	Normal	10.04	Normal	14.42	Normal
	Fruit (August 2017)		14.6	4752	103.06	Normal	39.42	Sli low	7.55	VeryLow	87.9	Low	6.92	Normal	10.24	High
Orchard 3	Fruitlet (July 2017)	Galaxy	14.2	4989	48.28	Normal	55.38	Sli low	14.73	Sli Low	129.68	Normal	9.41	Normal	14.86	High
	Fruit (August 2017)		13.6	4291	111.31	Normal	36.72	Sli low	11.08	Sli Low	93.09	Sli Low	6.55	Normal	10.71	High
Orchard 4	Fruitlet (July 2017)	Galaxy	13.2	4775	43.51	Normal	80.52	Normal	13.02	Low	145.7	High	9.83	Normal	16.34	High
	Fruit (August 2017)		13.6	4553	101.74	Normal	38.08	Sli low	8.39	VeryLow	86.75	Low	6.95	Normal	10.06	High
Orchard 5	Fruitlet (July 2017)	Schneiga	14	5914	47.64	Normal	81.2	High	13.45	Low	125.9	Normal	8.78	Normal	14.15	Normal
	Fruit (August 2017)		13.6	6109	85.96	Normal	48.96	Normal	9.36	VeryLow	69.26	Very Low	7.11	Normal	10.69	High
Orchard 6	Fruitlet (July 2017)	Mondial	12.8	5983	46.57	Normal	67.84	Normal	13.1	Low	103.43	Sli Low	8.08	Normal	15.43	High
	Fruit (August 2017)		13	5363	98.36	Normal	46.8	Normal	9.7	Low	85.76	Low	7.03	Normal	13.9	High
Orchard 7	Fruitlet (July 2017)	Mondial	14.2	4660	46.66	Normal	65.32	Normal	14.3	Sli Low	118.25	Normal	9.28	Normal	16.21	High
	Fruit (August 2017)		12.8	4726	114.86	Normal	42.24	Normal	10.45	Low	64.28	Very Low	6.04	Normal	10.17	High
Orchard 8	Fruitlet (July 2017)	Galaxy	13.6	6441	49.59	Normal	72.08	Normal	11.16	VeryLow	124.99	Normal	9.3	Normal	14.59	High
	Fruit (August 2017)		13.2	5163	88.01	Normal	36.96	Low	6.48	VeryLow	71.29	Very Low	6.16	Normal	10.85	High
Orchard 9	Fruitlet (July 2017)	Mondial	13.4	6602	58.42	Normal	73.7	High	15.01	Normal	144.37	High	8.82	Normal	14.53	High
	Fruit (August 2017)		14	5403	135.49	High	36.4	Sli low	8.7	Low	84.04	Low	6.04	Normal	8.99	High

A comparison of CF outputs based on the formula below. The formula charting CF decline with advancing maturity requires construction of a baseline CF measurement at fruitlet (25-30 mm) stage and continuing measuring fruits with the PEA fluorimeter until the reduction passes 50% of the baseline CF level taken at fruitlet stage:

$$\text{CF degradation} = \frac{(Fn - \sigma Fn)}{(F1 - \sigma F1)} < 50\%$$

Standard starch, firmness and % Brix readings were made for each pick date. Fruits were harvested from each orchard samples based either on the prediction from the decline in chlorophyll fluorescence termed “CF pick” and or harvested when fruits had reached 80% Starch content based on standard iodine staining of an equatorial section of fruit termed “Starch pick”. The full decision tree process of selecting orchards and subsequent sampling is detailed in Figure 4.1.

Nets of 20 fruit were selected for storage, after cooling, half of the samples were treated with SmartFresh prior to moving them their final storage conditions. Samples were stored in two regimes and locations for 9 months:

5%CO₂: 1%O₂ (Control & +SF) at (Howt Green) (only CF pick samples).

5%CO₂: 1%O₂ (Control & +SF) at PQC (East Malling) (CF pick and starch pick samples).

Initial monitoring of fruit removed from commercial stores (5%CO₂: 1%O₂) was limited to two time points: mid-April and mid-May 2018.

Samples stored in the PQC were stored until mid-June 2018 at this point all samples removed allowed to warm to ambient before measurements of CF, % dry matter, mineral analysis and quality assessments were undertaken. Samples in May were sent for mineral analysis and FDM assessment to YARA analytic (Table 4.3). Fruits were subject to CF measurements, fruit firmness, Brix^o and acidity analysis at Landseer.

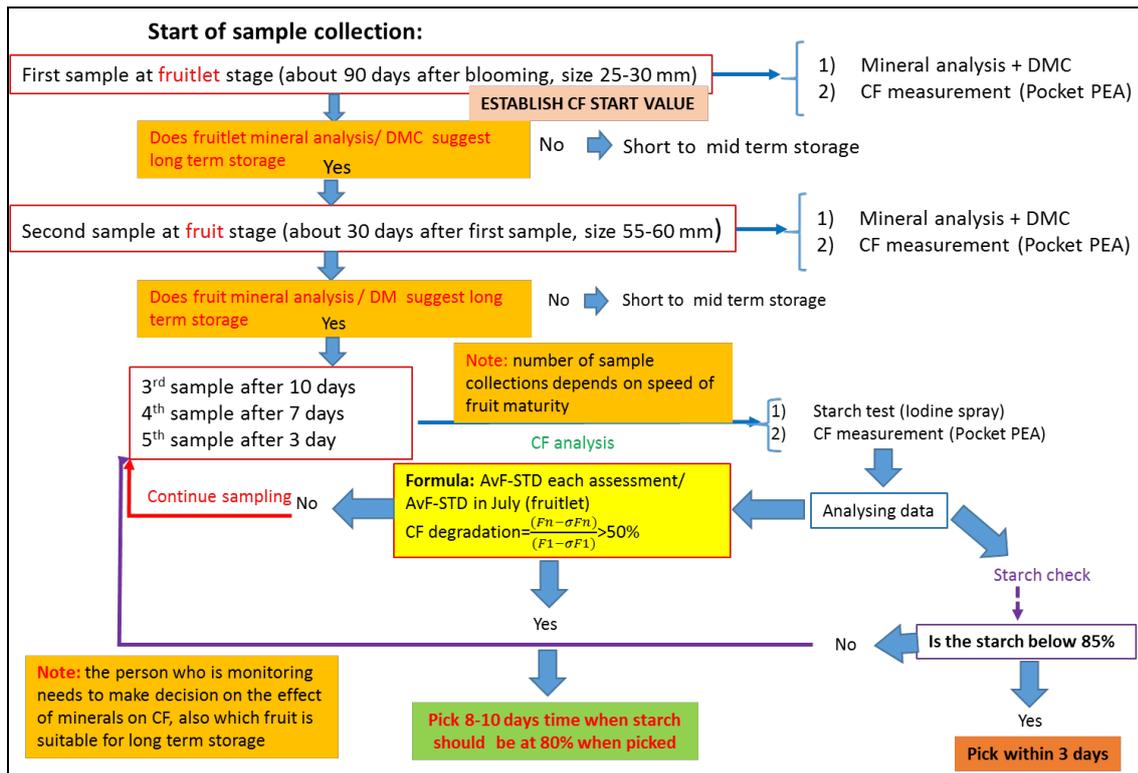


Figure 4.1. Decision tree flowchart for the process of sampling and analysing data for selecting the best orchards for long term storage and early warning for the best picking date.

Table 4.2: Comparison of fruit maturity warning by chlorophyll fluorescence Pocket PEA(CF) and starch staining patterns (2018).

2018										
	AvF-STD (11 July)	AvF-STD (14 Aug)	AvF-STD (17 Aug)	AvF-STD (20 Aug)	AvF-STD (23 Aug)	AvF-STD (27 Aug)	AvF-STD (31 Aug)	AvF-STD (3 Sept)	AvF-STD (7 Sept)	AvF-STD (10 Sept)
Barnyard	4761	3685	3145	2911	3041	1838	1492	1634	797	696
Ratio to July		0.77	0.66	0.61	0.64	0.39	0.31	0.34	0.17	0.15
	DMC:15	DMC:13.4				CF alert		CF pick	DMC:12.6	
Starch		92%	90%	92%	90%	85%	82%	80%	75%	70%
Monks	4566	3180		3097	3054	2034	1198	1227	644	334
Ratio to July		0.70		0.68	0.67	0.45	0.26	0.27	0.14	0.07
	DMC:14.8	DMC:15				CF alert		CF pick	DMC:14.2	
Starch		94%		94%	92%	90%	86%	80%	75%	70%
Gibbens	4959	3773	3805	3348	3194	2639	1905	1738	1214	897
Ratio to July		0.76	0.77	0.68	0.64	0.53	0.38	0.35	0.24	0.18
	DMC:16.6	DMC:15.4				CF alert			DMC:14.6	CF pick
Starch		95%	94%	92%	92%	92%	92%	90%	85%	82%
Hill Top	4549	3038	2842	2642	2584	1585	1042	1069	416	304
Ratio to July		0.67	0.62	0.58	0.57	0.35	0.23	0.23	0.09	0.07
	DMC:15.6	DMC:13.6				CF alert		CF pick	DMC:13.4	
Starch		93%	91%	91%	90%	87%	86%	80%	75%	70%
Mystole	4665	3410		3040	3158	2412	2069	1652	1292	696
Ratio to July		0.73		0.65	0.68	0.52	0.44	0.35	0.28	0.15
	DMC:16.2	DMC:15.6				CF alert			DMC:14	CF pick
Starch		94%		94%	92%	92%	91%	86%	87%	82%

CF analysis predicted that Gala orchards from Barnyard, Monks and Hill Top were mature and should be harvested in advance of orchards Gibbens and Mystole. Apples samples from these orchard were analysed for Internal Ethylene Concentration (IEC) at the Produce Quality Centre. Based of IEC values the maturity of apples from selected orchards was ranked Monks>Barnyard>Hill top> Gibbens>Mystole, with Gala from Monks being the most mature and Mystole the least (Figure 4.3)

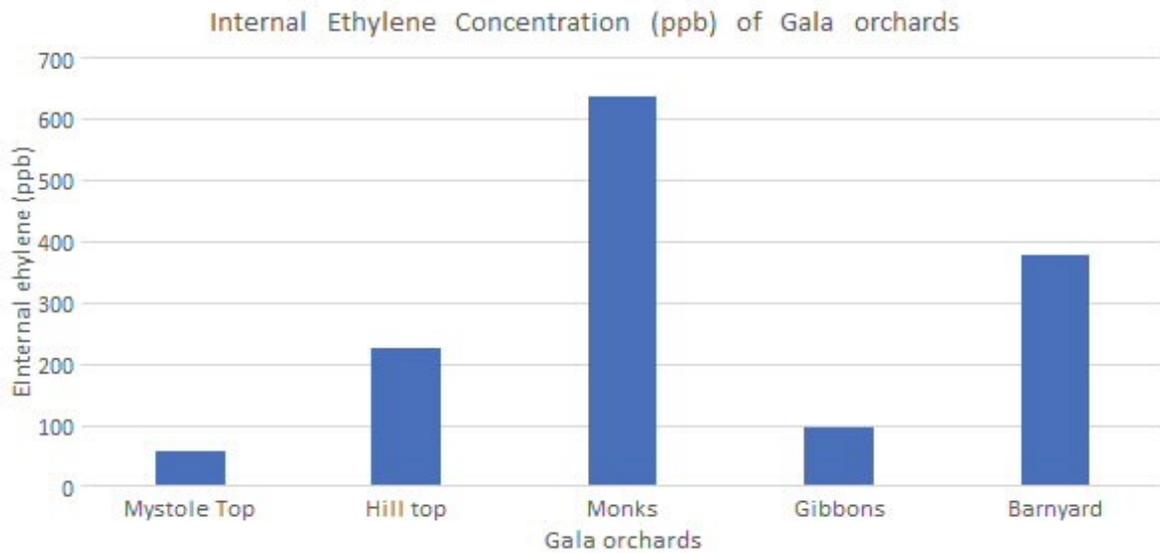


Figure 4.3 : comparison of internal ethylene production in samples from each orchard 10 days before harvest.

Table 4.3: Mineral analysis in fruitlets during fruit development and apples sampled after 9 months storage from selected orchards.

Comparison of changes in minerals and CF in fruitlet and fruit between July 2018 to June 2019														
FIELDREF	Test	Clone	DMC	CF (AvF-STD)	N	Interpretation	P	Interpretation	K	Interpretation	Mg	Interpretation	Ca	Interpretation
MYSTOLE TOP (NEWMMA FRUIT)	Fruitlet (July 2018)	Schneiga	16.2	4665	64.04	Normal	13.28	Sli Low	133.8	Normal	8.59	Normal	12.96	Normal
	Fruit (August 2018)		15.6	3410	45.28	Normal	10.34	Normal	114.3	Normal	6.45	High	9.47	High
	Fruit (September 2018)		14	1652	40.6	Normal	9.59	Sli Low	101.7	Normal	5.59	High	7.9	Normal
	Fruit (June 2019) Untreated		13.6	635	34	Sli Low	8.8	Low	90.45	Sli Low	5.05	High	7.04	Normal
	Fruit (June 2019) SmartFresh		15.2	1100	33.4	Sli Low	8.94	Low	110.6	Normal	5.47	High	6.98	Normal
MONKS FARM/ANTONY (SIMON BRAY)	Fruitlet (July 2018)	Mondial	14.8	4566	69.56	Normal	12.9	Sli Low	144	High	9.25	Normal	12.72	Normal
	Fruit (August 2018)		15	3180	46.5	Normal	11.14	Normal	127.9	High	6.51	High	9.88	High
	Fruit (September 2018)		14.2	1227	29.82	Low	10.84	Normal	118.4	Normal	5.93	High	9.72	High
	Fruit (June 2019) Untreated		14.2	173	39.76	Sli Low	11.59	Sli Low	135.2	High	5.96	High	6.53	Normal
	Fruit (June 2019) SmartFresh		12.8	1785	43.52	Normal	10.65	Sli Low	107.6	Normal	5.79	High	7.86	Normal
GIBBENS FARM/COTTAGE GALA (GOATHAM)	Fruitlet (July 2018)	Mondial	16.6	4959	66.4	Sli Low	14.26	Sli Low	144	Normal	9.07	Normal	14.5	Normal
	Fruit (August 2018)		15.4	3180	46.2	Normal	11.27	Normal	112.9	Normal	6.19	High	11.11	High
	Fruit (September 2018)		14.6	1227	35.04	Sli Low	10.39	Normal	99.82	Sli Low	5.79	High	10.59	High
	Fruit (June 2019) Untreated		13.8	2792	33.12	Sli Low	8.93	Low	97.4	Sli Low	4.84	Normal	7.33	Normal
	Fruit (June 2019) SmartFresh		13.8	1484	33.12	Sli Low	9.03	Low	86.6	Sli Low	4.5	Normal	6.94	Normal
HILL TOP GORE (GOATHAM)	Fruitlet (July 2018)	Galaxy	15.6	4549	51.48	Low	9.22	Very Low	140.2	Normal	8.3	Normal	12.56	Normal
	Fruit (August 2018)		13.6	3038	39.44	Sli Low	8.57	Low	105.9	Normal	5.68	High	7.81	Normal
	Fruit (September 2018)		13.4	1069	32.16	Sli Low	6.57	Very Low	110.3	Normal	5.12	High	8.4	High
	Fruit (June 2019) Untreated		12.6	999	25.2	Low	6.07	Very Low	102.1	Normal	4.98	Normal	6.99	Normal
	Fruit (June 2019) SmartFresh		12.4	1729	33.48	Sli Low	6.86	Very Low	106.6	Normal	5.17	High	7.03	Normal
BARNYARD (GOATHAM)	Fruitlet (July 2018)	Mondial	15	4761	57	Sli Low	12.34	Sli Low	128.5	Normal	8.23	Normal	15.68	High
	Fruit (August 2018)		13.4	3685	40.2	Normal	10.01	Normal	101.2	Normal	5.45	High	9.28	High
	Fruit (September 2018)		12.6	1634	31.5	Sli Low	8.08	Low	93.62	Sli Low	4.93	Normal	8.46	High
	Fruit (June 2019) Untreated		11.4	2498	23.94	Low	8.96	Low	89.21	Sli Low	4.47	Normal	6.75	Normal
	Fruit (June 2019) SmartFresh		12	2962	36	Sli Low	7.58	Very Low	87.89	Sli Low	4.63	Normal	7.94	Normal

Measurements of CF, firmness (kg/cm²), % Brix and acidity were undertaken at Landseer. In addition, a subset of apples was taste tested by a panel of 22 industry and trade representatives as growers, researchers (AHDB & NIAB EMR), marketers and journalists on 1st of July 2019 at Jim Mount (East Malling).

The UK apple samples were selected as high or low dry matter in different storage regimes; (5:1 and DCA) and samples were either untreated or treated with SmartFresh. Gala apples

from the Southern hemisphere (Chili and South Africa) were obtained from 3 different supermarket chains and tested for firmness, % brix and acidity.

UK Gala samples used in taste panels they were kept for 7 days at ambient and compared to Southern hemisphere fruit stored for 6 days in chilled conditions (4°C). Fruits were segmented into quarters just prior to sampling for taste tests. The panel scored each slice as Poor (1 point) Acceptable (2 points) and Good (3 points) for overall taste. Pairs of samples providing comparisons of fruit with high or low dry matter stored in different storage were compared with a slice of apple from the Southern hemisphere.

The results showed a significant preference in favour of the UK Gala specially samples with high dry matter (Figure 4.4). All samples with high dry matter were preferred in taste to samples with low dry matter and the samples from the southern hemisphere.

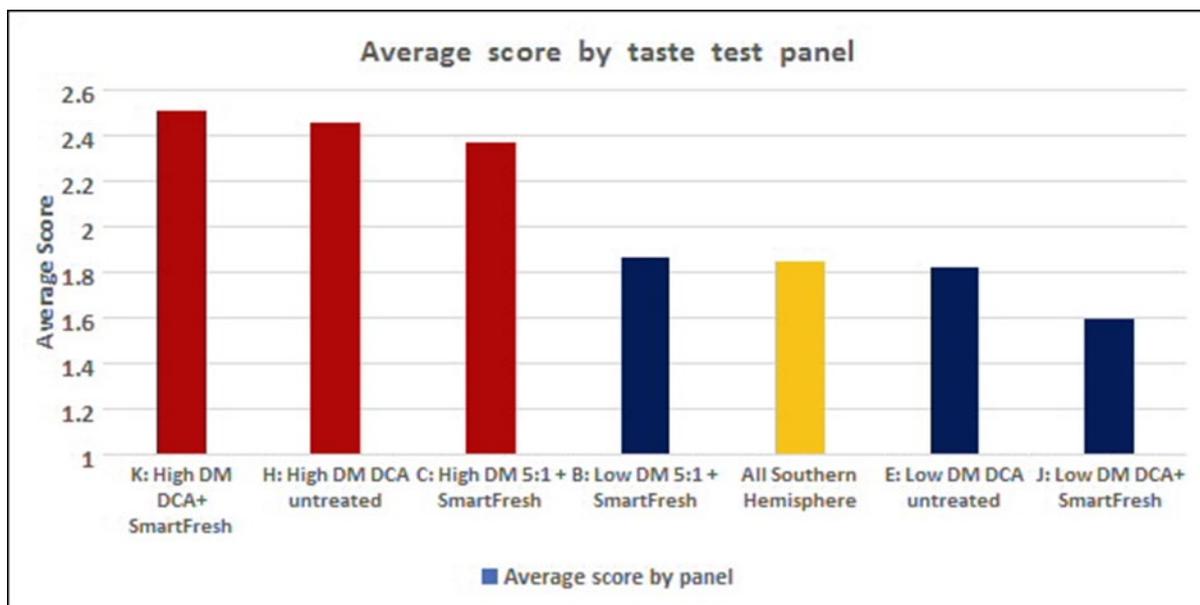


Figure 4.4: Comparison of quality and taste of long term stored UK gala (picked September 2018) with imported gala from Southern hemisphere (picked May 2019).

Fruit firmness of UK apples stored for 10 months under CA conditions, followed by 7 days shelf-life and irrespective of FDM content or SmartFresh application were significantly firmer than new season apples from Southern Hemisphere (Figure 4.5). Samples treated with SmartFreshSM, were firmer than other samples.

Firmness of English gala (7 days S -Life), Southern Hemisphere gala 1 day after buying from retailer

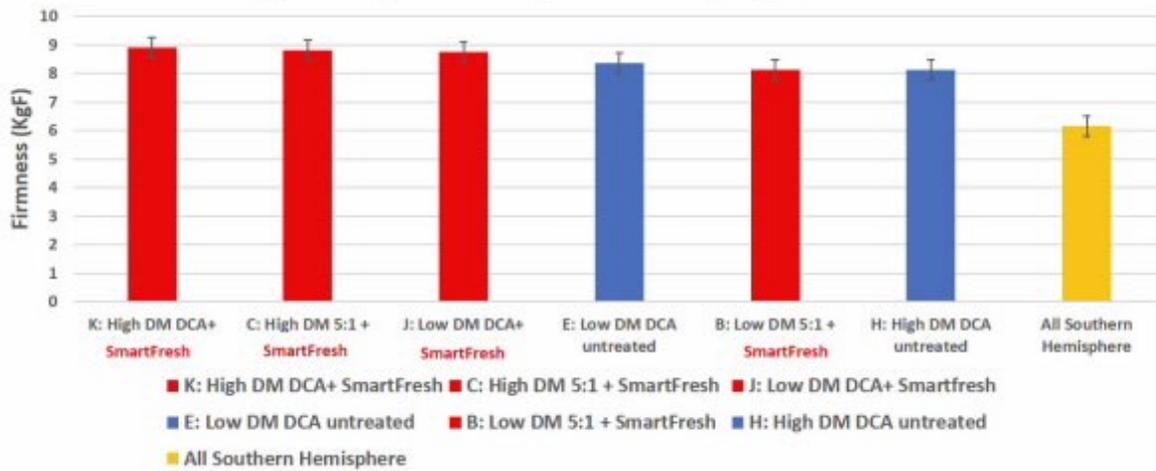


Figure 4.5: Comparison of fruit firmness in different treatments.

Although generally fruit dry matter decreases during storage, the reduction rate in untreated samples was more rapid than those treated with SmartFreshSM between harvest in September 2018 to end of storage in last week of June 2019 (Figure 4.6).

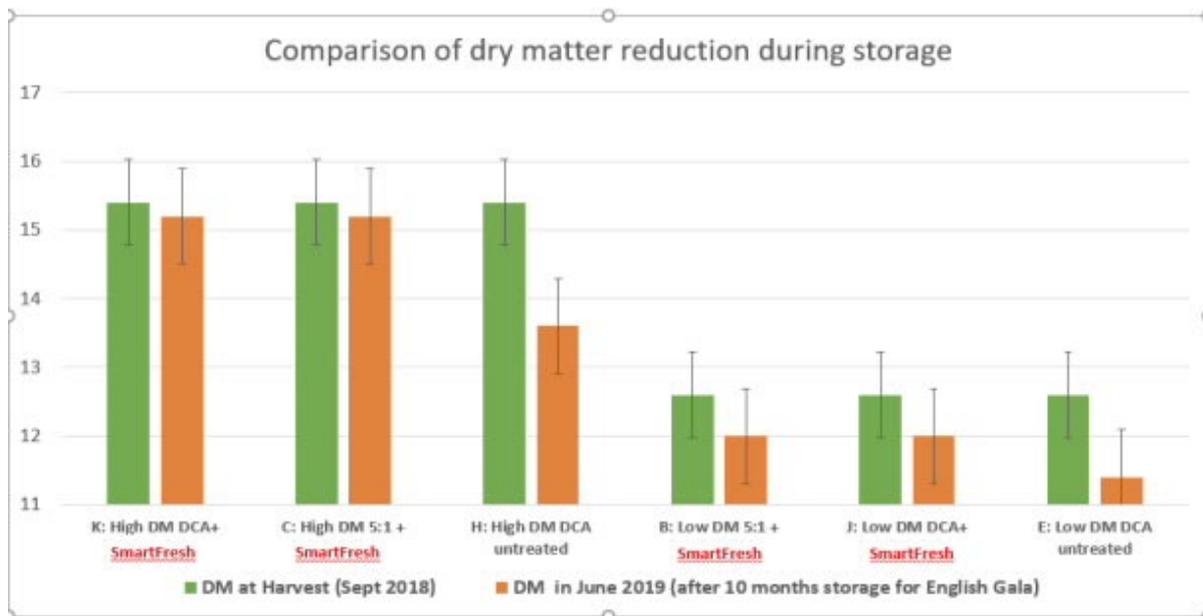


Figure 4.6: Comparison of reduction in dry matter during long term storage

There was a positive relation between dry matter and Brix, samples with higher dry matter, had higher Brix (Figure 4.7).

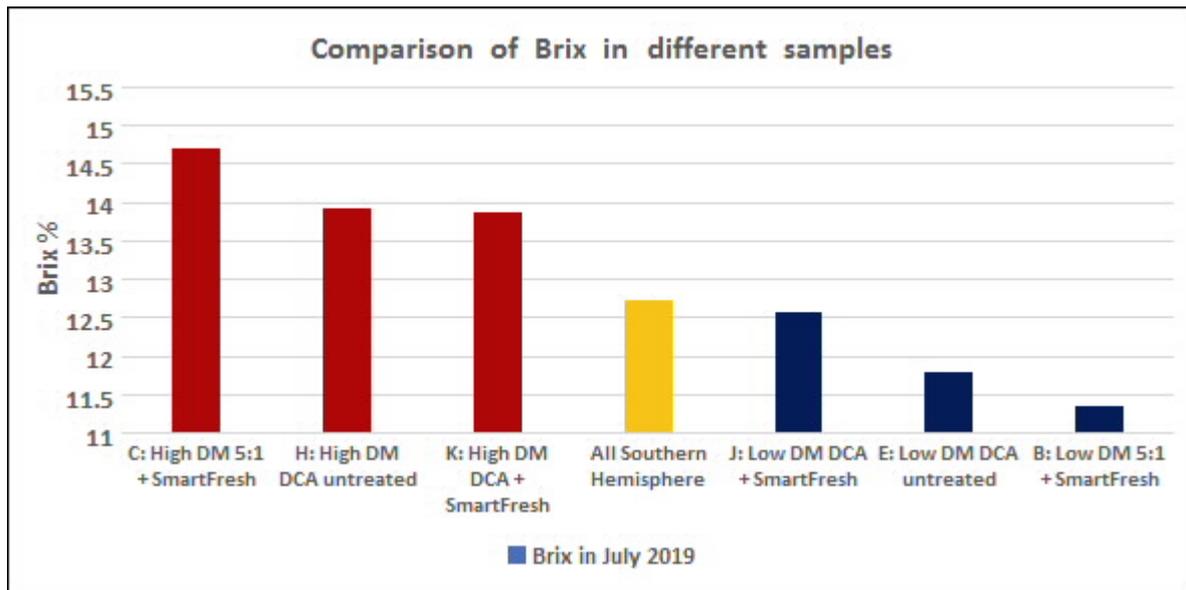


Figure 4.7: Comparison of Brix in different samples. Samples with higher dry matter had significantly higher Brix.

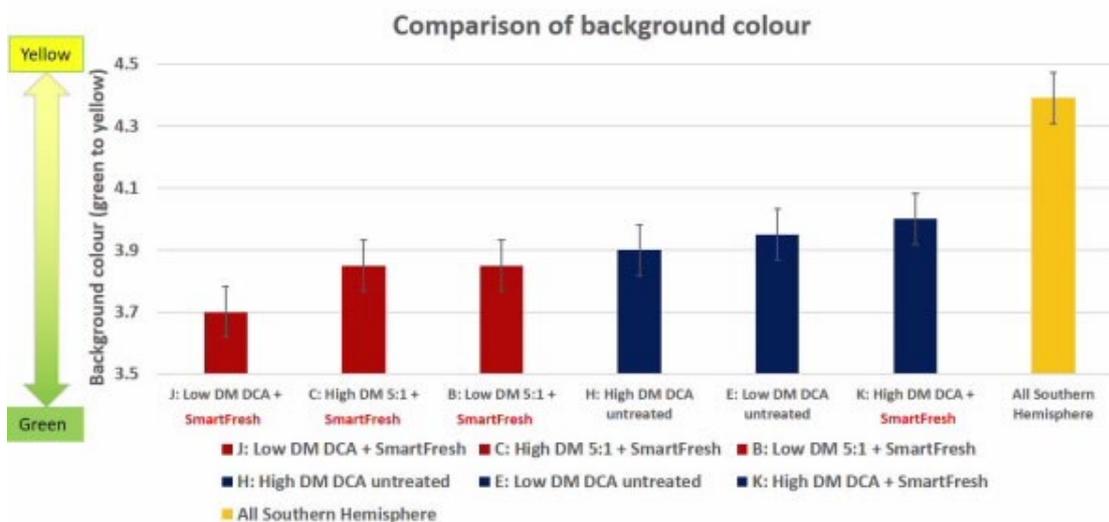


Figure 4.8: Comparison of treatments for maintaining green background colour (Measured by colour cards)

In June after 9 months CA storage all of the UK Gala tested had greener background compared with imported Southern hemisphere Gala (Figure 4.8), which may reflect the longer time period fruits were in transit in refrigerated air-storage. In addition samples treated with SmartFresh retained a greener background than untreated fruit.

Summary results:

Fruit firmness has effect on mouth feel and consumer preference; all UK samples whether treated and untreated retained good firmness above 8 kg/cm during 9 months of CA storage, picking fruit at optimum quality helps to maintain good ex-store texture. Fruit Dry Matter has an important role in maintaining the organoleptic properties; fruits with high dry matter tend to retain sweetness during long-term storage. SmartFresh reduces degradation of chlorophyll and helps to keep background greener during storage.

Results obtained this season confirmed data from the 2 previous years that the prediction model gave 7-10 days early warning for picking samples this could provide a tool to help with the harvest decision making process. Storage monitoring showed that it is possible to store UK Gala for 9 months and retain good eating quality if fruit is picked within the optimum harvest window and that fruits contain the correct complement of minerals and a high FDM. Establishing CA conditions as soon as fruits have reach store temperature and application of SmartFresh can help to maintain quality of fruit. This shows that supermarkets can be supplied by UK Gala after April, and in some cases until late June or early July. The fruit quality and flavour of high dry matter gala which was picked on time and stored for 10 months is similar or better than imported fruit.

Discussion

Year 1 (2016/2017)

Dry matter accumulation of fruit is dependent on the position of fruits within the canopy. Fruits from the high (>1.5 M) canopy were approximately 1% higher (16.5% FDM) than fruits picked from the lower regions of the canopy (<0.6 M) where fruit averaged 15.5% FDM.

High sunlight interception throughout fruit development and possibly as early as bud development in the previous season will impact of subsequent fruit quality. Increasing the amount of light interception by centrifugal pruning techniques affords the opportunity to improve tree performance above existing standard spindle tree architecture systems supported on post and wire structures. Laying down reflective mulches at key developmental stages in the life cycle of fruit buds and developing fruits demonstrates the importance of improving light interception within the orchard on fruit quality at harvest.

Previous reports highlight a strong relationship between overall FDM and the amount of sugar (%Brix) in the crop at harvest, and that this relationship carries on during the early stages of storage (3 months). Analysis of fruits from the first year's trial suggest Gala with high FDM retain elevated sugar content throughout storage (8 months) and during shelf-life.

Factors that impact on dry matter such as crop load are being investigated in the second year of the trial, but others such as soil, tree age and rootstock will clearly affect tree architecture, resource allocation and precocity of flowering and fruit set. Therefore a complex interaction between many agronomic factors plays a part in influencing partitioning of carbohydrate into fruits. Some of these factors are amenable to manipulation than others.

Looking into more detail of the components of FDM, through analysis of starch fibre and sugars, it was found that the proportion of sugars increase with fruit development, but interestingly, the proportion of fibre rather than starch decreased over the fruit development. Most of the starch would be mobilised at harvest and later picked fruit may have shown a lowering of starch content in favour of higher sugars.

Analysis of existing data sets to garner information regarding major influences on FMD accumulation requires sufficient variability within the FMD dataset to enables a correlation with other metrics. The more robust of these parameters are in terms of reliability and uniformity of measurements the more chance for identifying durable influences on FMD. Currently existing farm based data collected to date, restricts analysis to Gala orchards. Leaf and soil nitrogen data may provide some inference to variability in FMD, but with just over a 2% span in FMD, it may prove difficult to tease out differences. Additional data from the 2016/17 season may help to strengthen the analysis.

Being able to predict the onset of changes in starch clearance patterns before such changes in maturity happen offers some interesting options for the future management of harvest maturity prediction. Chloroplast fluorescence is an indirect measure of plant health, when tissues age the amount of energy released in the form of fluorescence increases because energy escapes through the photosynthetic II (PSII) pathway, as the efficiency of the pathway is lost.

While an increase in ethylene synthesis charts the start of the respiratory climacteric, the magnitude and duration of the rise is variety specific. Additional studies on the relationship between internal ethylene and starch clearance patterns has found a tight correlations exists when IEC's <100 ppb and starch content are high (80-95 %), once starch clearance patterns drop below 75% significant variability in the corresponding IEC's exist. With this in mind CF might provide an additional insight into changes in starch clearance. However, it is important to consider that as the relationship between ethylene and starch clearance is not tightly linked as maturity proceeds, any measure attempting to correlate maturity may encounter inherent problems.

Year 2 (2017/2018)

Dry matter accumulation of fruit is dependent on the position of fruits within the canopy. Fruits from the high (>1.5 m) canopy from the thinning trial were approximately 0.5% to 1.0% higher in FDM (16.4% - 17.6%) than fruits picked from the lower regions of the canopy (<0.6 m) where fruit averaged 15.45% to 16.47% FDM. This split was similar to data collected in year 1 where fruits were sampled from a commercial orchard.

High sunlight interception throughout fruit development and possibly as early as bud development in the previous season will impact subsequent fruit quality. Increasing the amount of light interception by centrifugal pruning techniques affords the opportunity to improve tree performance above existing standard spindle tree architecture systems supported on post and wire structures. Laying down reflective mulches at key developmental stages in the life cycle of fruit buds and developing fruits demonstrates the importance of improving light interception within the orchard on fruit quality at harvest.

Previous reports (Palmer 2010; McGlone 2003) highlight a strong relationship between overall FDM and the amount of sugar (% BRIX^o) in the crop at harvest and that this relationship carries on during the early stages of storage (3 months).

Analysis of fruits from the second year's trial (2017/18) suggests Gala from the upper canopy with high FDM retained elevated sugar content throughout 5 months of CA (3% CO₂, 1%O₂, 0.5-1.0°C) storage. While fruit from the upper canopy intercepts more sunlight, increasing light penetration with centrifugal pruning failed to increase FDM; moreover, the positioning of reflective covers within the alleyways did not increase FDM. However, the 2016/17 season was the first year of conversion where significant pruning had been undertaken and the difference between training systems is likely to be greater than in subsequent years.

In this first year of implementing bud and flower thinning strategies no significant impact on FMD content was observed. Early frosts during flowering at the FAST LLP site impacted on crop load and may have influenced the source sink relationships of the different treatments.

Other factors such as soil, tree age and rootstock will clearly affect tree architecture, resource allocation and precocity of flowering and fruit set. Therefore, a complex interaction between many agronomic factors plays a part in influencing partitioning of carbohydrate into fruits. Some of these factors are more amenable to manipulation than others.

Brevis removed over twice the number of fruitlets compared to natural drop in the control trees but did not reduce the overall yield of trees compared to the control; while yield per tree was similar to the control the yield of Class 1 fruit was slightly lower than the control. That the removal of twice the number of fruit per tree failed to increase FDM portioning into fruit suggests there is a certain degree of plasticity within the tree. While thinning strategies to remove fruit numbers based on branch thickness using the Mafcot Equilifruit tool goes some way in manipulating crop load related to tree architecture a greater understanding of maximising crop load for tree canopy using Lidar may be a way forward.

Interestingly, sugar analysis of tissue samples taken at harvest while not showing significant difference between treatments found that fruit taken from the lower canopy were higher in fructose and lower in sucrose than fruit taken higher up the canopy. Considering apples were lower in FDM in the lower canopy it might suggest fruits are maturing earlier in the lower canopy as more of the starch is hydrolysed into sucrose and then into fructose and glucose.

Analysis of existing FMD data sets of 56 orchards provided by FAST LLP using Pearsons Correlations and multiple regression analysis of over 3 years' data of FDM against leaf and fruit mineral analysis found weak positive correlation with Fe, K, Mg, P K:Ca ratio. Leaves under Mg and K deficiency hold on to their photosynthates and are less likely to partition carbohydrates to roots (Cakmak et al 1994) or other sink organs such as fruits. Zhoa (2001) reported that K deficiencies in cotton plants led to lower chlorophyll content, poor chloroplast ultrastructure and reduced translocation of sugars due to reduced entry of sucrose in the transport pool or lower phloem loading. Increasing leaf Mg and K may help to encourage greater translocation of photosynthates into fruits increasing FDM. Importantly, Mg and K act as antagonists to calcium binding to pectin in the middle lamella and pectins within the cell wall; increasing fruit Mg and K excessively could have implications for the long term storage capacity of fruit unless fruit calcium concentrations can be increased at the same time.

Being able to predict the onset of changes in starch clearance patterns before such changes in maturity happen offers some interesting options for the future management of harvest maturity prediction. Chloroplast fluorescence is an indirect measure of plant health; when tissues age the amount of energy released in the form of fluorescence increases because energy escapes through the photosynthetic II (PSII) pathway, as the efficiency of the pathway is lost.

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Year 3 (2018/19)

High sunlight interception throughout fruit development and possibly as early as bud development in the previous season will impact subsequent fruit quality. Increasing the amount of light interception by centrifugal pruning techniques affords the opportunity to improve tree performance above existing standard spindle tree architecture systems supported on post and wire structures. Laying down reflective mulches at key developmental stages in the life cycle of fruit buds and developing fruits demonstrates the importance of improving light interception within the orchard on fruit quality at harvest.

In previous year's trial higher FDM was observed in fruit samples segregated for position on the tree with higher FDM found at the top of the canopy. In the 2018 trial an amalgamated sample taken from across the tree canopy was used for FDM analysis. Centrifugally pruned trees had a 0.4-0.5% increase in FDM compared to fruit cultivated on standard tall spindle trees, however the sugar content predominately made up of fructose and sucrose failed to show any increase in fruits from either training system. Interestingly, in the 2018 trial reflective covers had no additional benefit in increasing FDM or sugars. Overall, reflective covers advanced the maturity of Gala in fruit harvested from tall spindle trees, leading to more rapid loss of starch in fruit at harvest across the canopy. This was most probably a result of increasing light and heat into the lower part of the canopy.

In contrast, apples from centrifugally pruned trees fruit maturity was less advanced most probably a result of lower fruit numbers per tree.

The 2018 growing season was particularly warm with higher than average sunshine hours and this is reflected in the high FDM (~17% FDM) content of fruit irrespective of training system or presence of reflective covers. In years with weather patterns more consistent with the Long Term Averages (LTA) treatment differences may be expected to be more pronounced.

Previous reports (Palmer 2010; McGlone 2003) highlight a strong relationship between overall FDM and the amount of sugar (% BRIX^o) in the crop at harvest and that this relationship carries on during the early stages of storage (3 months).

While fruit from the upper canopy intercepts more sunlight, increasing light penetration with centrifugal pruning or reflecting light back up into the canopy failed to increase FDM, however, the high % FDM observed across treatments suggests that the relationship between light interception and FDM is not linear and that in 2018 season fruits were at the higher end of FDM spectrum.

In this second year of implementing bud and flower thinning strategies no significant impact on FMD content was observed, however, Brevis and Exilis treated trees were as effective as standard hand thinning in reducing fruit numbers at harvest but Exilis increased the number of misshapen fruits. In two years of the trials application of Brevis was as effective as standard thinning in increasing FDM, increasing the plot size of treatments will help to reduce the variability of treatments. Weather events around application time are critical to the effectiveness of chemical thinning agents and caution should be taken when considering a second application, if the first failed due to poor uptake.

Bud thinning and mechanical removal of flowers were the least effective at reducing fruit numbers at harvest, and bud thinning had no effect on raising %FDM, however, all of the thinning treatments raised sucrose and fructose content in fruit at harvest to a similar concentration, suggesting there is a certain degree of plasticity in the way fruits accumulate sugars. In trees where bud/flowers were removed, a higher retention of fruits retained similar amounts of sugars to treatments where fewer fruits remained on the tree at harvest but where thinning events happened later in the fruits development cycle. Targeting thinning strategies around cell division may help to increase FDM- this is the focus of the 2019 trial.

Brevis not only lowers fruit numbers but also reduces the photosynthetic capacity of shoots and the growth rate of water shoots which act as a sink for carbohydrate. Understanding the source sink relationship between shoots and fruits under different thinning treatments may provide a greater understanding of how best to manipulate tree architecture to increase FDM.

Other factors such as soil, tree age and rootstock will clearly affect tree architecture, resource allocation and precocity of flowering and fruit set. Therefore, a complex interaction between many agronomic factors plays a part in influencing portioning of carbohydrate into fruits. Some of these factors are more amenable to manipulation than others.

Thinning strategies to remove fruit numbers based on branch thickness, using the Mafcot Equilifruit tool, goes some way in manipulating crop load in relation to tree architecture. However, a greater understanding of the optimum crop load and fruit number in relation to the size of the tree canopy and how this impacts on raising %FDM is required; using LIDAR to generate architectural profiles of tree canopies may take this forward.

Analysis of existing FMD data sets of 56 orchards provided by FAST LLP using Pearson's Correlations and multiple regression analysis of over 3 years' data of FDM against leaf and fruit mineral analysis found weak positive correlation with Fe, K, Mg, P, K:Ca ratio. Leaves under Mg and K deficiency hold on to their photosynthates and are less likely to partition carbohydrates to roots (Cakmak et al 1994) or other sink organs such as fruits. Zhou (2001) reported that K deficiencies in cotton plants led to lower chlorophyll content, poor chloroplast ultrastructure and reduced translocation of sugars due to reduced entry of sucrose in the transport pool or lower phloem loading. Increasing leaf Mg and K may help to encourage greater translocation of photosynthates into fruits increasing FDM. Importantly, Mg and K act as antagonists to calcium binding to pectin in the middle lamella and pectins within the cell wall; increasing fruit Mg and K excessively could have implications for the long-term storage capacity of fruit unless fruit calcium concentrations can be increased at the same time.

Being able to predict the onset of changes in starch clearance patterns before such changes in maturity happen offers some interesting options for the future management of harvest maturity prediction. Chloroplast fluorescence is an indirect measure of plant health; when tissues age the amount of energy released in the form of fluorescence increases because energy escapes through the photosynthetic II (PSII) pathway, as the efficiency of the pathway is lost.

While an increase in ethylene synthesis charts the start of the respiratory climacteric, the magnitude and duration of the rise is variety specific. Additional studies on the relationship between internal ethylene and starch clearance patterns has found a tight correlation exists when IEC's <100 ppb and starch content are high (80-95 %); once starch clearance patterns drop below 75% significant variability in the corresponding IEC's exist. With this in mind CF might provide an additional insight into changes in starch clearance. However, it is important to consider that as the relationship between ethylene and starch clearance is not tightly linked as maturity proceeds, any measure attempting to correlate maturity may encounter inherent problems.

Year 4 (2019/2020)

Dry matter accumulation of fruit is dependent on the position of fruits within the canopy. Fruits from the high (>1.5 m) canopy were approximately 0.6% higher in FDM (16.4%) than fruits picked from the lower regions of the canopy (<0.6 m) where fruit averaged 15.8% FDM.

Increasing the amount of light interception by centrifugal pruning techniques affords the opportunity to improve tree performance above existing standard spindle tree architecture systems.

Centrifugal pruning changes the proportion vegetative shoots in favour of fruit bearing shoots (Willamue *et al.*, 2004, Stephen *et al.*, 2008) and improves light penetration through the canopy. Over time this system should help to improve yields, increase FDM and reduce the need for pruning. In current trials at NIAB-EMR, trees are recovering from their initial re-training and in 2019 a severe scab infection caused a decline in yields and tree health, however year on year yields are recovering to match those of tall spindle trees. Centrifugal growing systems have increased light penetration and used in conjunction with reflective covers the system is able to increase FDM in the lower canopy.

Previous reports (Palmer 2010; McGlone 2003) highlight a strong relationship between overall FDM and the amount of sugar (% Brix^o) in the crop at harvest and that this relationship carries on during the early stages of storage (3 months). In this current trial higher %FDM have not translated into higher % brix in fruit at harvest. The difference in %FDM between treatments was relatively small (0.6%) and thus may not have resulted in significant increase in %Brix within apples. The presence of reflective covers increased light interception within the canopy and raised fruit sucrose and fructose content in fruit from the upper canopy of Tall Spindle trees, and increased sucrose content in fruit from Centrifugally pruned trees reflective covers.

Interpreting changes in sugar content in fruit is difficult. Fructose is the predominant sugar resulting of sorbitol loading into fruit breaking down to fructose, with only 20% of the photosynthate entering fruit as sucrose. Fructose and glucose are in continual flux through utilisation in respiration and replenishment through the breakdown of sucrose by the action of invertases. Fruit maturity and crop load also play a role in influencing sucrose content as there is a general trend of decreasing sucrose content as fruits reach maturation. In the thinning trial, fructose, glucose and sucrose were lower in fruit where thinning had been practiced. Crop load influences the source sink relationship between leaves/shoots and fruits

It is difficult to relate % Brix and starch profiles to maturity where different thinning treatments have been imposed that impact on crop load (Johnson, 1992, 1994, 1995). The I.E.C is a more

accurate measure of fruit maturity and from the results all thinning treatments raised I.E.C values. It has been reported previously that thinning practices can advance fruit maturity (Johnson 1995). Fruit from the upper canopy was more advanced in maturity than fruit from the lower canopy. Interestingly the most significant contrast in I.E.C's was observed where trees were thinned to singles in the higher canopy and doubles in the lower canopy when fruitlets were 10-20 mm in diameter, when this process was repeated a few weeks later as part of the standard thinning practice the resultant effects on maturity at harvest of low hanging was an equaling out of fruit maturity across the tree. A similar large difference between fruit from the top and bottom of the canopy were seen where apples had been thinned to size. While fruit maturity tended to be higher in fruit from the higher canopy based on I.E.C, a similar difference in individual sugars was not observed.

Other factors such as soil, tree age and rootstock will clearly affect tree architecture, resource allocation and precocity of flowering and fruit set. Therefore, a complex interaction between many agronomic factors plays a part in influencing partitioning of carbohydrate into fruits. Some of these factors are more amenable to manipulation than others.

Increased firmness was more closely correlated with FMD rather than fruit maturity at harvest with the least firm fruit recorded in unthinned fruit most likely the result of less structural carbohydrate being laid down in cell walls in fruit during development.

In terms of achieving manipulation of FDM by chemical application of thinning agents, Brevis and Exilis raised %FDM above unthinned controls and achieved a similar increase to hand thinned trees. Brevis treated Gala yielded 17% FDM in fruit from the upper canopy. However, Brevis treated-trees exhibited a higher grade out due to undersized fruits reducing the overall % of class 1 fruit. The lack of effect of Exilis and Brevis in the lower canopy may be due to poorer chemical uptake by more densely packed canopy in the lower part of the tree.

Fruit quality was retained during storage in 3% CO₂/ 1% O₂ at 0.5-1.0°C and this was helped by rapid establishment of CA conditions at the start of storage using nitrogen flushing once fruit had reached store temperature. The higher %Brix in fruit in thinned fruit and in particular fruit harvested in the upper canopy was retained through out storage.

Year 5 (2020/2021)

The impact of Reflective covers and Centrifugal pruning on harvest data was less in year 5 compared to the previous year. High sunshine hours during the growing season may have contributed to the lack of difference.

However, in the lower branches <1.5 M of the canopy reflective covers raised %DM content of apples by 0.6% where increasing %FDM is most needed (Table 5.2.1). Centrifugal pruning led to a small (0.3%) and non-significant increase in %DM in lower canopy. Fruit from the higher canopy tend to have greater access to sunlight and with fewer fruit per spur leads to greater accumulation of %FDM. At harvest, there was little difference in the %Brix between treatments and this was also reflected in the fructose, glucose and sucrose content of Gala. In addition to structural carbohydrates and simple sugars, FDM content also includes small amounts of lipids, proteins and phytonutrients that can help protect fruit against premature senescence during storage. In the samples observed during storage little internal damage was observed but raising FMD in varieties such as Cox and Bramely where a higher incidence of external and internal damage occurs during storage.

Fruit thinning practices did not have significant impact on raising dry matter content and again high sunshine hours may have raised %FDM across the treatments. Instigating earlier fruit thinning practices (10-20 mm) at the point of cell division increased fruit size rather than raise %FDM. Treatments with Brevis produced similar yields (62.7 T/ha) to hand thinned crop however, with Exilis yields were down to 49.8 t/ha compared to the best-hand thinned treatment 68.3 t/ha in fruit subject to early thinning to singles and doubles. Yields are based on 5 replicates of 3 tree plots so may not represent a true yield per orchard. Fruit from Exilis treated trees had slightly better texture raised and %FDM and higher P & K fruit content, this may be a consequence of reduced yield per tree. There was a lack of treatment differences this year which may have been a result of high sunshine hours reducing treatment effects.

Conclusions

Year 1

WP 1 Changes occur with FDM during fruit development- significant drop during cell division stage followed by a slow rise until harvest.

WP 1 Initial analysis of historical FDM data (FAST LLP) has found only a 2 % variation in FDM across 56 Gala orchards.

WP 2/3 Higher FDM found in the top of the canopy.

WP 2 Centrifugal Pruning techniques can increase light capture for fruits and reduce the need for subsequent pruning.

WP 4 Chlorophyll fluorescence has shown it is able to predict changes in starch clearance patterns 7-10 days before starch clearance was observed.

Year 2

WP1 Meta analysis of existing data sets showed a weak positive correlation with higher K and Mg content in fruit with higher FDM while excess zinc was considered detrimental to FDM accumulation.

WP 2 Centrifugal Pruning increased light penetration and interception (41.5%) throughout the canopy compared to Tall Spindle trees (34.4%). Positioning of reflective covers increased yield by 5% in CS trees and 19% in TS trees.

WP3 No one technique increased FDM significantly and FDM at harvest were similar across all treatments. 2017 was a difficult year to predict how much to thin and the yield and grade out were affected by frost but size, quality and maturity parameters were acceptable for all treatments. Late thinning increased % BRIX^o and hand thinning may encourage larger fruit to develop. Treatments 3 and 4 (mechanical and chemical Exilis) performed most optimally in 2017 and Treatment 7 (Size) appears to be the most optimum of the hand thinning treatments. Overall higher fructose content was found in fruit from the lower canopy while higher sucrose content was found in fruit in the upper canopy which may be a reflection on rate of fruit maturation. Effects of increased % BRIX^o at harvest for later thinning treatments were lost in storage.

WP4 Confirmation of the first two seasons of the prediction model gave 7 to 10 days' early warning for picking samples which could be a valuable logistical and planning tool to help in the harvest strategy decision making process. Also, storage monitoring results showed the possibility of storage of English Gala with a good quality for more than 9 months if fruit has been picked at the right time with good balance of minerals and high dry matter and being stored in the right condition with application of SmartFresh to maintain quality of fruit.

Year 3

WP 2 Centrifugally pruned trees had a 0.4-0.5% increase in FDM, but no significant increase in sugars (glucose, fructose and sucrose) was observed.

Reflective covers had no additional benefit in increasing FDM or sugars.

2018 had higher than average sunshine hours, reflected in the high FDM (~17% FDM) irrespective of training system or presence of reflective covers.

Bud and flower thinning strategies had no significant impact on FMD content.

Brevis and Exilis treated trees were as effective as standard hand thinning in reducing fruit numbers at harvest, however, Exilis increased the number of misshapen fruits.

Year 4

WP 2 Centrifugally pruned trees combined with reflective covers raised the %FDM in fruit from the lower canopy. The Combination of centrifugal system and reflective covers delayed fruit maturity -however this may be influenced by crop load. In 2019 significant incidence of apple scab was recorded in certain plots and this will have skewed the data.

WP 3- Thinning in general increased %FDM, but changing the timing of hand thinning did not increase FDM. Some thinning strategies had a stronger effect on manipulating size of fruit and different practices may be useful for delivering fruit profiles for particular customers. Apples from the upper canopy had higher %FDM and %Brix.

Year 5

WP 2. The presence of reflective covers raised %DM content of apples harvested from branches below <1.5 M, by 0.6%.Centrifugal pruning led to a small (0.3%) and non-significant increase in %DM in lower canopy fruit.Trees subject centrifugally pruning were less mature based on starch-iodine staining protocols.

The impact of Reflective covers and centrifugal pruning was less effective in a year of high sunshine.

WP 3. Thinning treatments did not effect %FDM, while thinning to single fruit across the tree led to large fruit with lower % Brix. Exilis treated fruit had higher in fruit firmness at harvest due to lower yield

Overall Conclusion

Manipulation of canopy structure and the insertion of reflective covers in the alleyway appears to have delivered a more consistent effect on raising %Fruit dry matter, particularly in fruit developing in the lower half of the canopy. These effects are more pronounced in years where there is less sunshine, where fruits positioned within the lower canopy struggle to gain sufficient light, resulting in poorer dry matter accumulation, sugar development and a reduced fruit growth rate. Combining centrifugal pruning with reflective covers was the best combination for raising % FDM. There are additional costs associated with converting

existing trees to a centrifugal pruned system and significant yield penalties while trees recover which may take 2 years post-pruning. The cost of covers also needs to be factored in to the economics of production- deployment in this trials were from full petal through to harvest. Clearly there between seasons it is not possible to anticipate the benefits of deployment and so in some years the net return may be small. There is also the need to factor in durability and life span of these covers against to increased financial return of growing a more uniform crop in terms of higher sugars. In 2021 where sunshine hours have been significantly lower particularly through August- % Brix content has been lower and many consignments have struggled to reach minimum levels set by the retail sector.

Manipulation of crop load through bud, flower and fruitlet thinning achieved either through hand thinning, mechanical removal or through chemical application, altered fruit numbers at harvest. The influence of final fruit number on the accumulation of dry matter was not clear. Thinning clearly effected fruit size which in turn may influence fruit dry matter content. Crop load also influences the amount of carbohydrate partitioning to branches, trunk and roots so a certain degree of plasticity was seen when crop load was influenced, the timing of thinning events had a more noticeable effect on fruit size rather than increasing %FDM. Superimposed on thinning practice is position within the tree and yearly variation in sunshine hours that influence final %FDM content. Fruit maturity varied across the canopy with fruit in the upper canopy more mature than fruits in the lower canopy where standard thinning had been implemented Certain thinning practices delayed the advancement of fruit maturity

Plant nutrition has been identified as having a role in the accumulation of sugars. There was a weak association between higher concentrations of magnesium and potassium in the leaves and fruits of Gala trees and higher % Fruit Dry Matter, while fruit higher in zinc tended to have lower Fruit Dry matter. Magnesium plays a central role within the chlorophyll molecules. Plant leaves that are deficient in Mg have been reported to hold on to photosynthates rather than export to other organs such as fruits and roots.

Magnesium (Mg), as a central element of the chlorophyll (Chl) molecule and the activator of more than 300 enzymes. It plays key roles in various physiological and biochemical processes, including Chlorophyll biosynthesis and activating enzymes involved in respiration, photosynthesis and nucleic acid synthesis. Therefore, it is not surprising to see that fruits and leaves with less magnesium may accumulate less carbohydrate in the cell walls or as simple sugars. However, magnesium is antagonistic to the functioning of calcium within the cell so application of magnesium to trees must be done with caution otherwise a calcium/magnesium imbalance within the fruit may occur increasing the risk of fruit softening or the development of senescent disorders.

Future Work

Understanding the relationship between crop load and tree architecture will allow better targeting of thinning treatments. Moreover the partitioning of carbohydrate to roots requires further study. The use of root pruning to reduce above ground vigour has been demonstrated previously, but its effect on accumulation of carbohydrates in fruit requires further study.

Chlorophyll fluorescence as a technique to chart changes in chloroplast activity and advancing maturity has been demonstrated when fruit have been removed from the field. The requirement for dark adaption of fruits in order for measurements to take place in the field requires further study.

Knowledge and Technology Transfer

Colgan, R.J. & Lecourt J. Optimising Fruit Dry Matter for long-term storage of Gala. AHDB-Tree Fruit day, 23 February 2017. (NIAB-EMR)

Lecourt, J and Colgan R.J Agronomist.day Demonstration of pruning and reflective mulches. 13 September 2017. (NIAB-EMR)

Lecourt, J and Colgan, R.J Agronomist day Demonstration of pruning and reflective mulches. September 2018. (NIAB-EMR)

Dalton, A.F. Thinning Effects on Fruit Dry Matter. Fruit Science Live Event (FAST, ICL & BASF). 24 July 2018.

Colgan, R.J. Optimising Fruit Dry Matter for long-term storage of Gala. AHDB Tree Fruit Panel meeting 6 March 2018.

Dalton, A.F. Thinning Effects on Fruit Dry Matter. AHDB Tree Fruit Panel meeting 6 March 2018.

Dalton, A.F. Thinning Effects on Fruit Dry Matter. AHDB Tree Fruit Day 22 February 2018.

Lecourt, J. Centrifugal Pruning. AHDB Tree Fruit Day 22 February 2018.

Merhdad, M. Application of chlorophyll fluorescence to predict fruit maturity in Gala Apples. AHDB Tree Fruit Day 22 February 2018.

Dalton, A.F. Thinning Effects on Fruit Dry Matter. FAST LLP Members Conference 1 February 2018.

Colgan R.J. Manipulating Fruit Dry Matter to Improve Long-term Storage of Gala (TF225) AHDB Tree Fruit Day 25th February 2021.

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