

**Project title:** Developing Practical Strategies to Improve Quality and Storability of UK Apples

**Project number:** TF225

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

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**Previous report:** None

**Key staff:** Debbie Rees, Chris Atkinson NRI - University of Greenwich  
Julien LeCourt - NIAB-EMR  
Abi Dalton - FAST LLP  
Mehrdad Mirzaee, Mark Tully, Colin Carter - Landseer

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

**Industry Representative:** Nigel Jenner, Paul Smith and Nigel Stewart

**Date project commenced:** 1 April 2016

**Date project completed** 31 March 2021

**(or expected completion date):**

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# AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Dr Richard Colgan

Reader in Post-harvest Physiology

Natural Resources Institute, University of Greenwich

Signature .....  ..... Date ...2/11/2017.....

Dr Julien Lecourt

Post Doctoral Research Scientist

NIAB-EMR

Signature .....  ..... Date .....

Dr Mehrdad Mirzaee

Technical Manager

Landseer Ltd]

.....  .....

Signature .....Date .....2/11/2017.....

Abi Dalton

Trials Manager

FAST LLP

Signature *AF Dalton* .....Date .....04/01/18.....

**Report authorised by:**

Name

[Position

Organisation

Signature ..... Date .....

Name

Position

Organisation

Signature ..... Date .....

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## **GROWER SUMMARY**

### **Headline**

Position of fruit within the tree canopy influences the accumulation of fruit dry matter content.

Fruit with higher dry matter entering storage maintained higher % Brix throughout CA (3% CO<sub>2</sub> 2% O<sub>2</sub>) storage.

Chlorophyll fluorescence may have the potential to track changes in harvest maturity

### **Background**

Fruit dry matter (FDM) content is considered a good indicator of high sugar and acid content (% Brix<sup>0</sup>) and eating quality of apples at harvest. Also, 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 for UK apple industry and may afford the opportunity to identify orchard consignments that can be stored for longer.

Several research groups, including work of Palmer (1999) in New Zealand have linked high dry matter content (FDM) of fruit 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.

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 published and unpublished 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.

### **Summary**

Meta-analysis (UoG/FAST LLP/NIAB-EMR) for the two years of FDM data for commercial Gala and Braeburn orchards identified 56 Gala orchards where mineral analysis (soil or leaf) existed to allow some correlative analysis FDM against soil and leaf quality attributes. However, the FDM data spread for Gala was only 2.2%, suggesting that identifying a strong

driver for increased FDM would be difficult to determine. Further FDM data from 2016-2017 season may help to strengthen the data set.

Most of WP2 (NIAB-EMR) outputs started in year 2 of the project and fall outside of this reporting period. Conversion of tall spindle trees to a centrifugal growth habit was undertaken in the winter of 2016. The first crop was harvested in September 2017 (year 2). Additional assessment of tree architecture using LIDAR and light interception and thermal imaging assessment of fruit temperature will be reported in year 2 of the project.

In the first year of WP3 (FAST LLP/UoG) identifying difference in fruit position within the canopy found that fruit harvested above 1.5 M were higher in FDM than fruit harvested at ~ 0.6 M in the lower canopy of the tree. There was insufficient difference in FDM between fruits harvested on the North and South aspect of the tree to suggest a difference in light interception between the hours of sunshine captured between morning and afternoon.

Analysis of the different components of FDM (UoG) from post-flowering through to harvest found a rise in the amount of soluble sugars as fruitlets increased and a decrease in the proportion of alcohol insoluble fibre. This suggests that much of the increase in sugar during fruit development was associated with a decrease in cell wall carbohydrate. The proportion of starch content remained relatively constant up to the point of harvest.

Advanced warning of the onset of starch clearance would allow growers more time to organise harvest and increase the likelihood of a greater proportion of the first pick Gala crop being harvested within the short window necessary to ensure fruit are suitable for long term storage. Chlorophyll Fluorescence (CF) (Landseer Ltd) affords an opportunity to provide information to growers regarding changes in fruit maturity in advance of changes in starch clearance patterns. Analysis of chlorophyll fluorescence outputs from six commercial orchards found that on average CF outputs could predict the decrease in starch to 75% content 7-10 days before the event. Further work is ongoing to determine the impact of early warning and potentially more precise harvesting forecasting on the storage quality of fruit.

## **Financial Benefits**

None to date.

## **Action Points**

Harvesting fruits high in the canopy separately will provide consignments with higher DM.

## **SCIENCE SECTION**

### **Introduction**

#### **Background**

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. Further research in this area is required to determine how environmental conditions and management practices employed during growth affect FDM at harvest and during storage and to determine the relationship between FDM and fruit ex-store quality for UK fruit.

Several research groups have linked high FDM of fruit at harvest to good quality and good storability. 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, with little or weak scientific evidence of causal relationships.

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 published and unpublished 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

#### **Existing information on factors controlling fruit quality**

An extensive body of literature exists where aspects of fruit growth, development and quality have been linked to FDM. The inclusion of FDM data in such studies is very much an integral metric measured routinely and there are numerous studies which have attempted to link changes in FDM with genetics, environment and crop management. Despite this apparent wealth of information its useful interpretation is limited to correlative analysis of apparent relationships between and across factors that vary, such as cultivar, rootstock, tree age or



seasonal factors. The recent desk study commissioned by AHDB (Biddlecombe 2014, TF 222) outlines some of these more recent studies and covers much of what we think influences FDM. However, the document demonstrates clearly that outcomes are derived from assumptions using correlative analysis with variable levels of statistical relevance. Strategies to optimise fruit quality, for consumers, require that we have the capacity to understand how quality attributes are determined during the production process and how these are maintained throughout the supply chain. If these strategies are to be developed and optimised using our current assumptions of the processes involved, derived from this correlative analysis, then innovation will be restricted in scope to evaluation of a 'trial and error' basis. It is also true that without a more generic understanding of the processes which determine FDM, derived from this correlative approach, experimental evidence will have limited impact and application beyond the regime from which the correlative analysis has been derived, e.g. cultivar and geographical location.

### **Recent work on fruit quality commissioned by AHDB**

Prior to the publication of this tender, AHDB commissioned a series of reviews on the relationship between FDM and fruit quality (as mentioned above), 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 on the basis of 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<sup>0</sup> at harvest and to a better % Brix<sup>0</sup> coming out of store. Fruit with % Brix<sup>0</sup> in excess of 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<sup>0</sup> 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. A number of alternative CA regimes such as 3% CO<sub>2</sub> 2% O<sub>2</sub> (+ SF) and 3% CO<sub>2</sub> (0.6-0.4%

O<sub>2</sub>) scored more highly than conventional regimes in taste panel assessments, despite having similar firmness, %Brix<sup>0</sup> and acid ratios. Storage in oxygen <1% retained selected volatiles compared to conventional storage in 5% CO<sub>2</sub> and 1% O<sub>2</sub> where high CO<sub>2</sub> 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, are investigating the potential to use precision irrigation and targeted fertigation to improve marketable yields, consistency of cropping and fruit quality of Gala and Braeburn.

## **Work Packages**

### **Work package 1**

**To carry out a meta-analysis to provide an evidenced-based understanding how fruit FDM can be manipulated to optimise fruit quality.**

**WP1 2016-2017 Year 1 NRI Chris Atkinson, Stephen Young with support from Richard Colgan and Debbie Rees, Julien LeCourt NIAB-EMR.**

Meta-analysis can be described simply as “carrying out research about previous research” in a systematic manner of review. The concept is based on combining the outputs of a number of diverse studies which have measured similar factors, and aggregating these outputs provides more reliable and precise statistical descriptors that can help to inform appropriate outputs, e.g. crop management strategies. Meta-analysis can also help to identify causes of inconsistency between results from various studies, e.g. due to different sampling approaches, to develop new hypotheses from patterns that were not previously apparent, to find sources of disagreement in results from diverse sources and to identify potential modes of action. The latter can be particularly important in determining future route

### **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.**

**WP 2 2016- 2020 Years 1-5 EMR (Julien Lecourt)**

#### **Background**

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 (eg Lakso, 1996, Figure 1A) and increased light interception promotes dry matter accumulation (eg Palmer et al. 1992, Figure 1B), TSS, fruit colouration and profitability (Jackson 1970; Robinson and Lakso 1988; Kappel and Neilsen 1994; Wunsche, Lakso et al. 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.

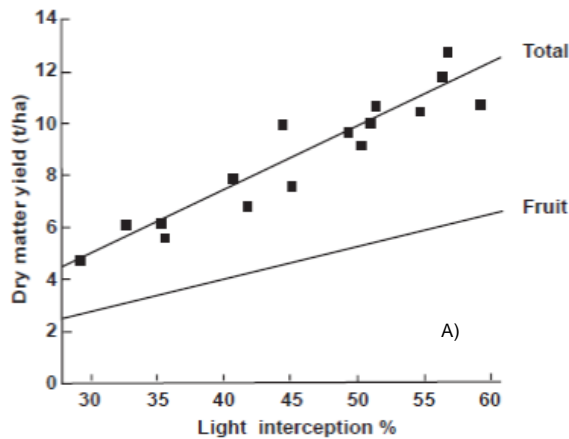


Figure 1. A.

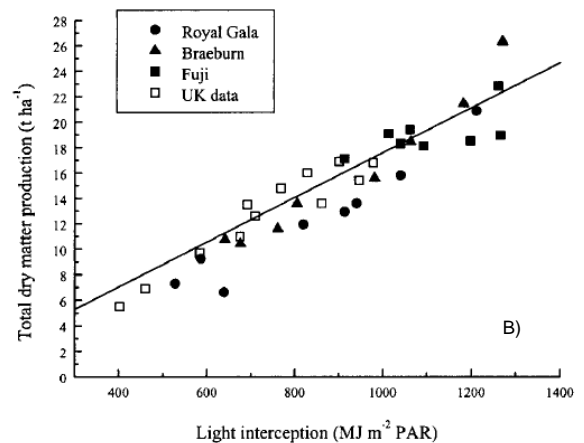


Figure 1. B.

Figure 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 1. B. Relationship between seasonal intercepted PAR (MJ/m<sup>2</sup>) 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 Concept Pear Orchard (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**

**WP 3 2016-2020 Years 1-5 Abi Dalton & Tim Biddlecombe FAST LLP, Debbie Rees & Richard Colgan 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 3-4 weeks would generate financial returns of £2-3 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 for good yield of the required fruit size is known, but not the effect on FDM of achieving this optimum crop load at different times in the season. No recent work has measured any effects on FDM on Gala in the UK. Although considerable work has been undertaken to try out different pruning strategies, mainly to increase light interception and therefore yield, the effect of different tree architectures on fruit FDM and whether fruit load can be increased without reducing FDM is not yet understood.

In order 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 2-4 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 (Wunsche 2000, 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 stage through to fruit development.

From previous studies, changes in % FDM from full bloom have been charted and 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 2).

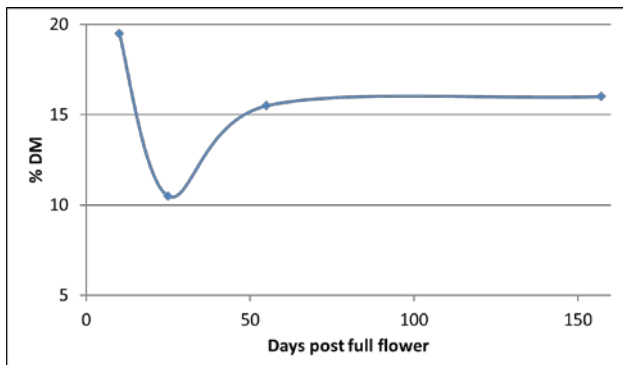


Figure 2. Preliminary FDM data from Gala taken during flowering and fruit development

From initial studies in two orchards the timing of thinning is thought to affect the degree to which FDM falls and rises again and thus 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 will in subsequent years investigate the effect of achieving the optimum crop load at much earlier stages via different thinning strategies and 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.

The initial phase (Year 1) was used to chart the changes in FDM content during the growing season and to quantify how early FDM is determined in the fruit development cycle; specifically at what point cell division ceases and starch accumulation becomes the main factor controlling FDM increase. This was achieved from detailed measurements of FDM and the components (structural carbohydrates, non-structural carbohydrates) through the cell division period. How this varies with fruit position within the tree canopy was investigated. Fruit from the 'upper' well-lit and 'lower' shaded portions of the canopy were collected from the selected orchard and how FDM varied with these different fruit positions in the tree was monitored to give insights into how the FDM progression may change. Further samples were collected at harvest time to determine how FDM may have progressed and the implications on storage potential and quality. This will provide important information, not only on the reasons for initial difference in FDM, but also how it may change over the season and the

implications for storage and consumer perception. It will also provide an informed basis for the applications of tree management strategies, such as approaches to thinning which could be applied in subsequent years.

Outputs of a meta-analysis to improve the understanding of factors influencing FDM will inform work on manipulating resource allocation and FDM by a range of thinning strategies to manipulate crop load at different timings. Treatment effects on the variability in FDM, its chemical components and related quality parameters within orchards and within the tree will also be identified.

The review conducted on thinning technologies comes to some conclusions very relevant to this programme. For example, it is concluded that the resulting crop load is more important than the method used. It is likely that chemicals such as Brevis that inhibit photosynthesis could have effects that have not yet been identified. The review points out that thinning studies have tended to focus on crop yield rather than quality. Another key observation is that there is a need to determine long-term effects on the trees. A five year trial as planned in this programme is clearly vital.

Following the outcome of the experiment in Year 1, strategies to manipulate crop load at different timings in the selected Gala orchard will be investigated and related to FDM to allow targeting of optimum crop load at much earlier stages. The timing and intensity of thinning treatments most likely to influence FDM will be determined via different hand, mechanical and chemical thinning methods and timings of events related to days after full bloom. The timings and intensity will be selected with the aim of proving a positive or negative effect on FDM. The treatments will be applied to replicated blocks and designed in consultation with a statistician.

If after 2 or 3 years it is found that a particular thinning timing or crop load intensity has a major influence on FDM then the work package may be adapted to investigate variations in crop load compared to the typical orchard practice.

To achieve a more enlightened process orientated approach, a commercial orchard was utilised in order to follow changes in FDM and components of FDM during fruit development in 2016 (Year 1). During Years 2 to 5 of the project different thinning treatments will be applied and it is envisaged this may increase FDM in terms of cell number and in terms of starch accumulation. This approach will enable orchard management strategies to be linked with differential changes in FDM within and between orchards.

## **Work package 4**

**Identification of the optimal harvest date of high dry matter Gala apples to deliver optimal consumer experience after extended storage under modern storage regimes.**

**WP 4 2016 -2019 Years 1 -3 Mehrdad Mirzaee Landseer Ltd**

This work package focused on developing a non-destructive method to optimise harvest date prediction for improving the long term storage quality of Gala.

While FDM content of fruit provides a good metric for determining the sweetness and overall eating quality of fruit, in order to maximise fruit quality at the end of storage fruits need to be picked within a narrow harvest maturity window to ensure that the benefits of CA storage are fully maximised.

The current best practice for harvesting Gala for long term storage is to pick when starch coverage declines to 90-80% of coverage (CTIFL 3-4). Often this window in starch clearance is narrow and decline in starch occurs by 3% per day once fruits get to around 90% starch coverage.

This does not allow growers enough time to organise picking before starch levels decline further. Analysis of starch coverage is difficult to determine accurately, and while there are some tablet based image analysis software available these require calibration before harvest. Non- destructive methods such as chlorophyll fluorescence may be used as a tool for fruit maturity testing and provide an early prediction of optimum harvest date. Initial studies (Landseer) indicate that the system may provide up to 7 to 10 days prior warning for growers to pick their fruit for long-term stored fruit.



## **Materials and methods**

### **Work package 1.**

**To carry out a meta-analysis to provide an evidenced-based understanding how fruit FDM can be manipulated to optimise fruit quality.**

**WP1 2016-2017 Year 1 NRI Chris Atkinson, Stephen Young with support from Richard Colgan and Debbie Rees, Julien LeCourt NIAB-EMR.**

Published and unpublished data sets (see below) where measurements of FDM have been made will be re-examined. The analysis will include an initial phase in which information is collected from many sources and screened for relevant methods and outputs, with respect to FDM. Appropriate choices as to how and where to search for studies in an objective way will be made to avoid bias. A second phase will include critical reading of those sources and data sets which include relevant interpretive analysis of factors influencing fruit FDM. A selective screening process will then be applied to enable an appropriate statistical analysis approach to be developed which 'best suits' the available data. The specific skills required to refine this statistical approach will be achieved through working with an existing statistical biometrician consultancy at NRI UoG. This technical input is required to ensure that sources of bias are avoided and that only studies which are methodologically sound are included in the meta-analysis i.e. the 'best evidence syntheses'.

## Materials and methods

### Work package 2.

**To determine the impact of increasing light interception in vertically trained high-density orchards by pruning and/or using reflective mulches**

#### WP 2 2016- 2020 Years 1-5 EMR (Julien Lecourt)

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 EMR (Figure 3). Within the orchards reflective mulches were laid either side of the rows between at the cell division stage (April/May) and two weeks before harvest to determine the effects of improved light penetration and on Class 1 yields, FDM and components of fruit quality (TSS, colour). The impact of pruning systems on tree architecture and canopy development are being monitored using LiDAR which can estimate tree row volume, porosity, specific leaf area) and light levels studied using AccuPAR.

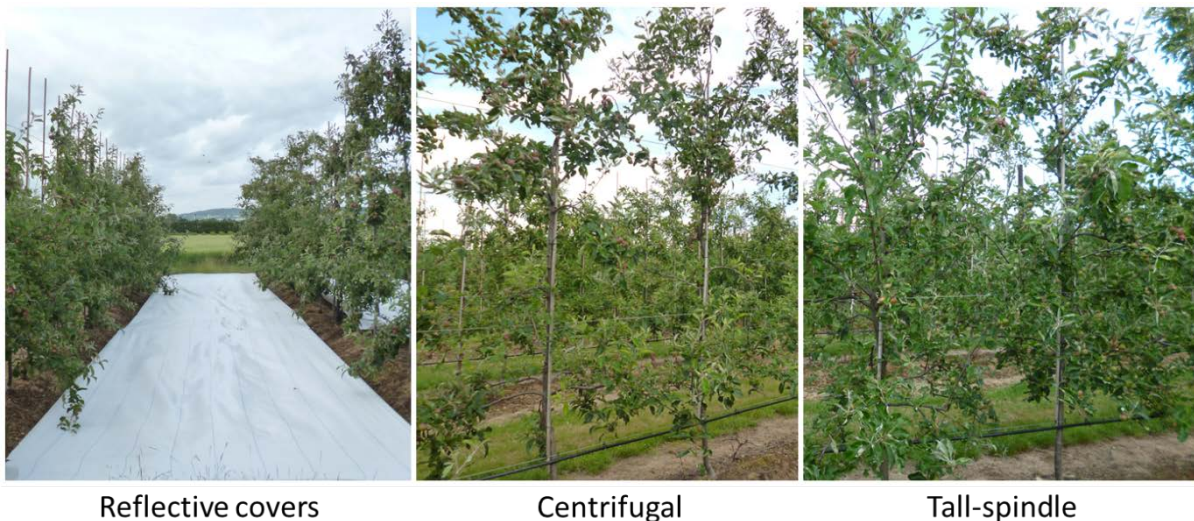


Figure 3. Training and reflective mulches in a 4 year old M9 Gala orchard

The first harvest for this trial will take place in September 2017. Fruit will be sampled from each experimental tree and categorised into 3-4 sub samples by position within the tree. Analysis of fruit quality attributes will be carried out to quantify the effects of manipulating light interception on fruit FDM and quality attributes at harvest and following storage. For selected parts of the experiment in Project Years 1 & 2, a more detailed categorisation will be undertaken in terms of light interception by the fruit bearing branch. Analysis of fruit quality attributes is described in WP4. Where applicable, high throughput phenotyping tools currently being developed in other IUK projects will be used to quantify treatment effects on aspects of fruit quality.

## Materials and methods

### 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

WP 3 2016-2020 Years 1-5 Abi Dalton & Tim Biddlecombe FAST LLP, Debbie Rees & Richard Colgan NRI-UoG

### Materials and methods

#### Location

The first year of the trial used an established twin bed Gala orchard in Hernehill, Kent, Latitude 51.308656, Longitude 0.968422 (see Figure 4).



Figure 4. 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.

#### Treatments

The two treatments in this first year were:

1. Upper well lit (UL) – un-shaded, stake height (approximately 2m high), on leader or ends of branches at top of tree.
2. Lower shaded (LS) – shaded, lowest branches, approximately 1m high, midway between tip and trunk at bottom of tree.

## Trial design

The trial was made up of 3 blocks that crossed 4 alleys (Figure 5).

Each block was made up of 4 replicates per treatment.

A total of 24 trees were used per treatment.

| ROW/ALLEY | 4 | 3 | 2 | 1 |         |
|-----------|---|---|---|---|---------|
|           |   |   |   |   |         |
|           | 1 | 1 | 1 | 1 | BLOCK 1 |
|           | 2 | 2 | 2 | 2 |         |
|           |   |   |   |   |         |
|           | 1 | 1 | 1 | 1 | BLOCK 2 |
|           | 2 | 2 | 2 | 2 |         |
|           |   |   |   |   |         |
|           | 1 | 1 | 1 | 1 | BLOCK 3 |
|           | 2 | 2 | 2 | 2 |         |

Figure 5. Trial Plan.

## Husbandry

A standard commercial programme for management of pest and disease, foliar feed sprays plus herbicides etc. was applied as per growers' routine.

Husbandry was also carried out as usual by the grower.

Assessments

## Sampling and events

Fruit samples were taken on 15 occasions, weekly commencing at BBCH CGS 67 (flowers fading) continuing through the cell division stage and then at harvest. See Table 1 for events and description of crop growth stage.

Table 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 2017          |
| 2     | 69         | Petal fall                                   | Up to 5mm  | 27 May 2017          |
| 3     | 70         | 1 week post petal fall                       |            | 3 June 2017          |
| 4     | 71         | 2 weeks after petal fall approx.             | Up to 10mm | 8 June 2017          |
| 5     |            | 3 weeks after petal fall                     |            | 15 June 2017         |
| 6     |            | 4 weeks after petal fall                     | Up to 20mm | 22 June 2017         |
| 7     |            | 5 weeks after petal fall                     |            | 29 June 2017         |
| 8     |            | 6 weeks after petal fall / second fruit fall |            | 6 July 2017          |
| 9     |            | 7 weeks after petal fall                     |            | 13 July 2017         |
| 10    |            | 8 weeks after petal fall                     |            | 19 July 2017         |
| 11    |            | 9 weeks after petal fall                     |            | 27 July 2017         |
| 12    |            | 10 weeks after petal fall                    |            | 3 August 2017        |
| 13    | 74         | 11 weeks after petal fall (T stage)          | Up to 40mm | 10 August 2017       |
| 14    | 99         | Harvest                                      |            | 12 Sept. 2017        |

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, treatments:

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

See sampling plan Figure 6.

|                        |                       |                       |                       |                |
|------------------------|-----------------------|-----------------------|-----------------------|----------------|
| <b>12</b><br><b>24</b> | <b>7</b><br><b>19</b> | <b>6</b><br><b>18</b> | <b>1</b><br><b>13</b> | <b>BLOCK 1</b> |
|                        |                       |                       |                       |                |
| <b>11</b><br><b>23</b> | <b>8</b><br><b>20</b> | <b>5</b><br><b>17</b> | <b>2</b><br><b>14</b> | <b>BLOCK 2</b> |
|                        |                       |                       |                       |                |
| <b>10</b><br><b>22</b> | <b>9</b><br><b>21</b> | <b>4</b><br><b>16</b> | <b>3</b><br><b>15</b> | <b>BLOCK 3</b> |

Figure 6. 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 (i.e. not the backs within the twin bed).

Different clusters but with similar numbers of fruit in each cluster.

Half way along the branch (Treatment B lower shaded).

The King fruit was always avoided. Fruit was not picked from Row 1, 2, 15 or 16 or the first 5-10 trees of each row.

### **Laboratory analysis**

FDM of each sample was assessed in the FAST laboratory and the percentage FDM calculated.

Rainfall, sunshine hours and Accumulated Day Degrees data was collated and plotted against the FDM (%).

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 (see Figure 4). 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% CO<sub>2</sub>, 2% O<sub>2</sub> until May 2017.

### **Statistical Analysis**

Statistical analysis was carried out using Analysis of Variance (ANOVA) and multiple range tests (MRTs) used to determine whether the differences between individual treatments were statistically significant. Charts/tables are shown with standard error bars (where applicable). The results of the MRTs where statistically significant effects (P value < 0.05) were evident are detailed in charts/tables with P-values and LSDs indicated.

## **Materials and methods**

### **Work package 4**

#### **Identification of the optimal harvest date of high dry matter Gala apples to deliver optimal consumer experience after extended storage under modern storage regimes**

##### **WP 4 2016 – 2019 Years 1 - 3 Mehrdad Mirzaee Landseer Ltd**

The pocket PEA chlorophyll fluorescence (CF) meter (Hansatech Ltd) was used as a method to measure changes in fruit maturity. The device has been used extensively to chart changes in plant health. CF profiles and mineral analysis and FDM of different clones of Gala as fruitlet (25-30mm) were measured in 17 selected orchards in Kent in Mid July 2016. Samples were taken from each compass point on a tree, (4 fruitlets per tree, all samples picked from middle height of trees). Samples were taken as a “W” pattern across the orchard taking samples at appropriate points.

In Mid-August, 9 of these orchards were selected which had the highest FDM and fruit (55-60 mm) were picked for the second assessment. After analysing CF, mineral profiles and FDM of fruit, a subset of 6 orchards (including clones: Mondial, Galaxy and Schniga) were selected for fruit monitoring 2-3 weeks before commercial harvest time.

Orchards were selected for their suitability for long-term storage based on fruit having high FDM and good mineral profiles. Chlorophyll fluorescence monitoring was restricted to fruit that was suitable for long term-storage. A comparison of CF outputs, starch-iodine test, firmness and % Brix<sup>0</sup> readings were made for each pick date.



## Results

### Work Package 1: Meta-analysis

Currently unpublished data from orchard surveys compiled by FAST have been collated on dry matter and mineral analysis from fruitlets, soil and leaves consisting of 134 orchard x sampling date observations, over two seasons (2015/16; 2016/17) for 12 Braeburn, 2 Conference Pear, 6 Cox, 45 Gala and 2 Kanzi 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. Other parameters such as tree age, rootstock and orchard aspect may provide additional granularity to the analysis.

From the two years of available data (2015-2016) FDM variation across all the data sets ranged from 13.6-18.9%, a span of 5.3% FDM. The data set was restricted to Braeburn and Gala, eliminating varieties with minimal number of samples. For Braeburn the dataset consists of 11 orchards with FDM ranging from 13.6-14.8%, a span of 1.2%. The Gala dataset consisted of 58 measurements with a range from 13.8-16.0% FDM and a span of 2.2% FDM. Additional mineral and leaf analysis for a subset of Gala orchards exist. With this current data set, it is likely that future data analysis will be restricted to comparing the interaction of leaf/soil analysis and mineral content of fruit on the influence of FDM on Gala. However, with a 2.2% spread in FDM the challenge will be to link other metrics such as leaf N to tangible differences in FDM.

## **Work Package 2. Pruning and reflective mulches**

Harvest maturity data and tree architecture data will be discussed in the next report as the harvest data for the first trial season (September 2017) falls outside the current reporting period.

## **Work package 3. Thinning and fruit position**

FAST sampled on 13 occasions removing between 40 and 20 fruits (depending on crop stage) per tree per treatment with 12 replicates. FDM content of fruit was charted (Figure 7) and related to the amount of rainfall (Figure 8), sunshine hours (Figure 9) and Accumulated Day Degrees (ADD) (Figure 10) during fruit development.

The % FDM of both treatments decreased 2 weeks post petal fall until 9 weeks post petal fall and then increased until harvest.

Values of % FDM were distinct between treatments with the upper well-lit fruit having higher values than the lower shaded fruit.

There were statistically significant differences in % FDM between treatments at all sampling events except on 6 July. See Table 2 for P values and LSDs and Table 3 for correlation data.

There were visual differences in fruit appearance (Figures 11 and 12).

Table 2. % FDM per treatment per sample date

| TREATMENT | DESCRIPTION              | 27-May  | 03-Jun  | 08-Jun  | 15-Jun  | 22-Jun  | 29-Jun  | 06-Jul | 13-Jul  | 19-Jul  | 27-Jul  | 03-Aug  | 10-Aug  | 12-Sep  |
|-----------|--------------------------|---------|---------|---------|---------|---------|---------|--------|---------|---------|---------|---------|---------|---------|
| 1         | Mean                     | 17.53   | 14.70   | 14.81   | 13.51   | 13.40   | 13.37   | 14.27  | 13.75   | 14.65   | 15.26   | 15.05   | 15.38   | 16.78   |
| 2         | Mean                     | 15.35   | 13.93   | 14.10   | 12.51   | 12.92   | 12.43   | 13.83  | 13.31   | 13.80   | 14.38   | 14.16   | 14.51   | 15.59   |
| 1         | Minimum                  | 16.75   | 13.47   | 13.91   | 12.67   | 12.71   | 12.14   | 13.60  | 13.40   | 13.94   | 14.42   | 14.29   | 14.32   | 15.56   |
| 1         | Maximum                  | 18.01   | 15.26   | 15.29   | 13.90   | 14.32   | 14.08   | 15.00  | 14.25   | 15.68   | 16.18   | 15.80   | 16.05   | 18.06   |
| 2         | Minimum                  | 14.51   | 13.22   | 12.80   | 11.73   | 12.34   | 11.26   | 12.75  | 12.81   | 13.12   | 13.25   | 13.24   | 13.44   | 13.42   |
| 2         | Maximum                  | 16.59   | 14.35   | 14.78   | 12.98   | 13.51   | 13.34   | 17.51  | 13.81   | 14.70   | 15.01   | 14.89   | 15.53   | 17.65   |
| 1         | Median                   | 17.62   | 14.79   | 14.93   | 13.61   | 13.38   | 13.50   | 14.34  | 13.78   | 14.55   | 15.25   | 15.04   | 15.64   | 16.87   |
| 2         | Median                   | 15.28   | 13.96   | 14.22   | 12.58   | 12.96   | 12.54   | 13.65  | 13.30   | 13.71   | 14.57   | 14.07   | 14.64   | 15.56   |
|           | Statistical significance | *       | *       | *       | *       | *       | *       |        | *       | *       | *       | *       | *       | *       |
|           | P value                  | <0.0000 | 0.0002  | 0.0014  | <0.0000 | 0.0061  | 0.0003  | 0.2901 | 0.0021  | 0.0003  | 0.001   | 0.0001  | 0.0013  | 0.0101  |
|           | lsd                      | 0.00574 | 0.00492 | 0.00553 | 0.00372 | 0.00434 | 0.00592 |        | 0.00351 | 0.00558 | 0.00658 | 0.00545 | 0.00676 | 0.01241 |

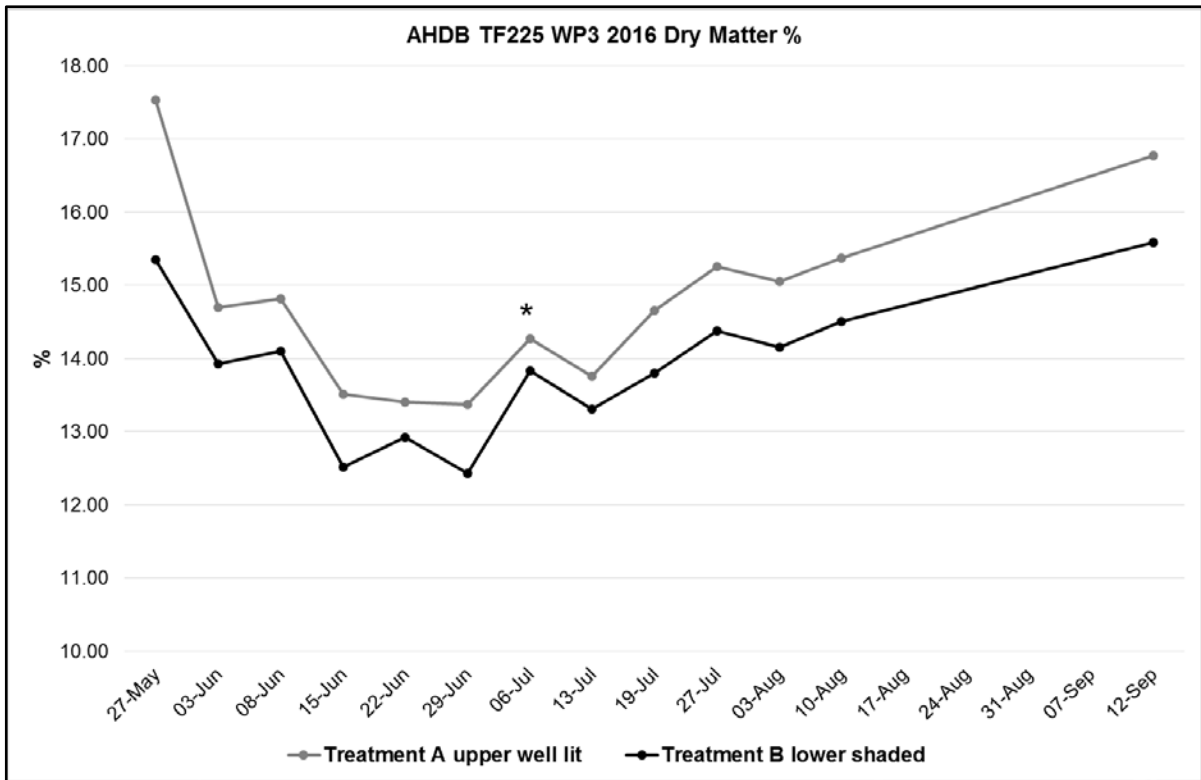


Figure 7. % FDM content progression from BBCH CGS 69 (petal fall) to harvest (BBCH 99). \* denotes results without statistical significance.

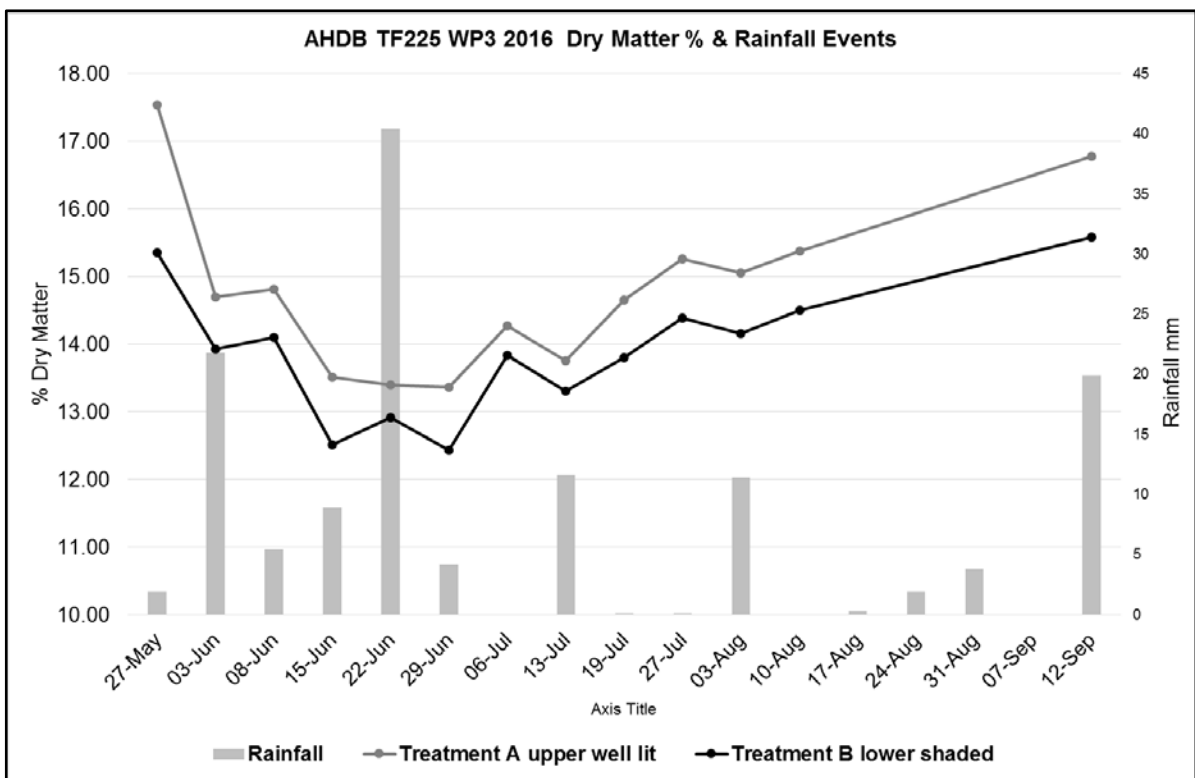


Figure 8. Dry matter progression with rainfall events (mm per week) plotted.

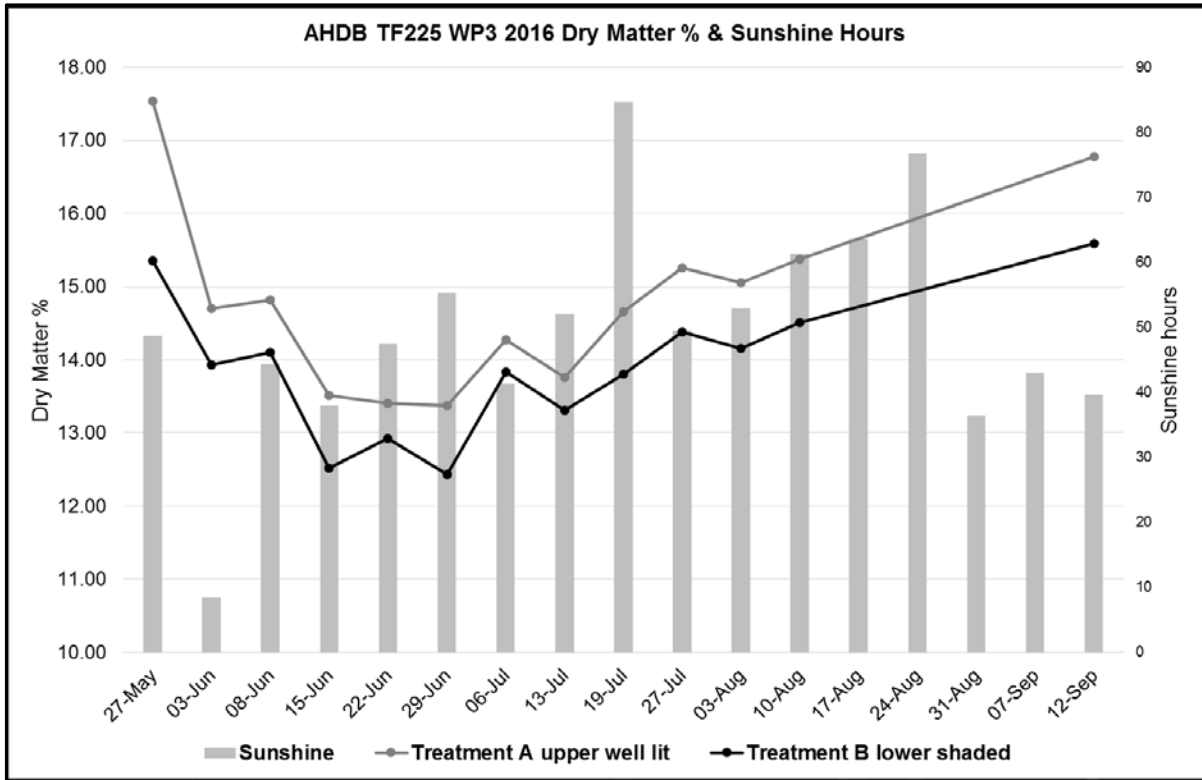


Figure 9. Dry matter progression with total sunshine hours (per week) plotted.

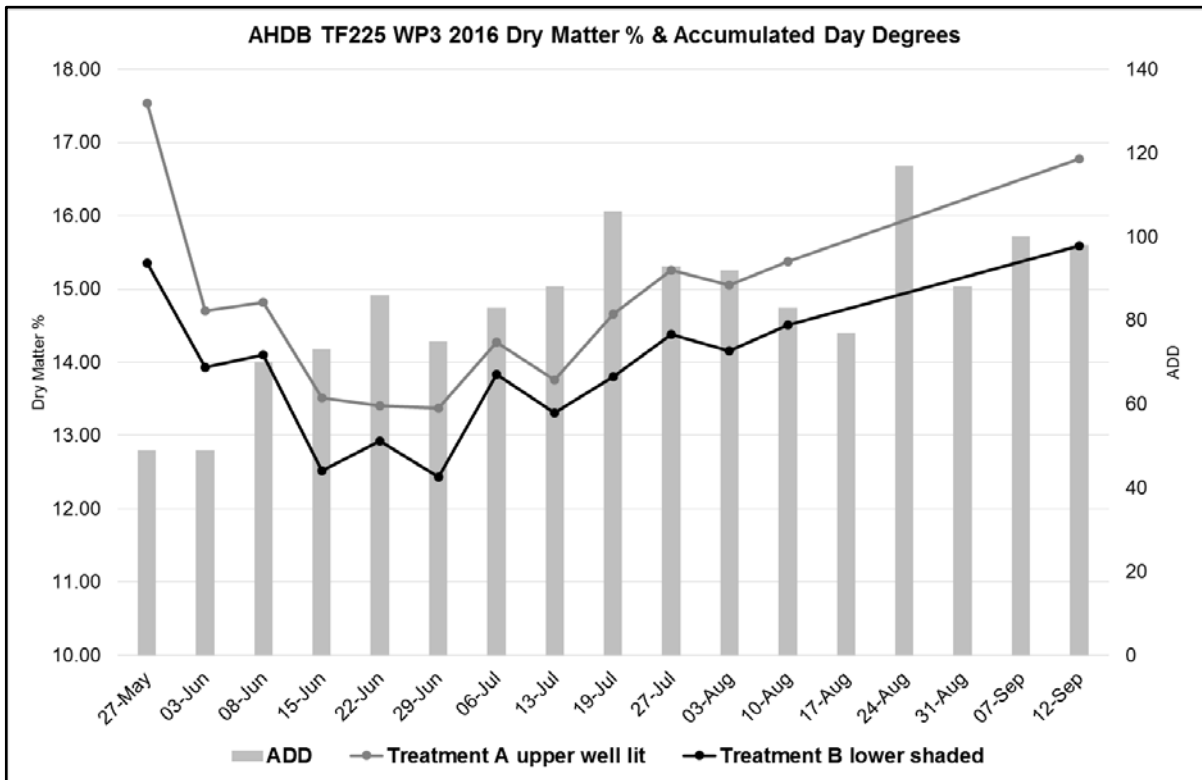


Figure 10. Dry matter progression with Accumulated Day Degrees (per week) plotted.

Table 3. Correlation values of treatments with physiological parameters (by week analysis)

| Treatment & parameter | Correlation value | Description                           |
|-----------------------|-------------------|---------------------------------------|
| Upper & Rain          | -0.416            | medium degree of negative correlation |
| Lower & Rain          | -0.389            | medium degree of negative correlation |
| Upper & Sunshine      | 0.063             | weak positive correlation             |
| Lower & Sunshine      | 0.051             | weak positive correlation             |
| Upper & ADD           | -0.363            | low degree of negative correlation    |
| Lower & ADD           | -0.217            | low degree of negative correlation    |



Figure 11. The 2016 Trials site at Herne Hill.



Figure 12. Sample examples - block, area of tree and sample number recorded.

Additional samples were harvested by NRI which also analysed structural carbohydrate. The initial phase in Year 1 quantified changes in FDM accumulation from flowering through to fruit harvest from flowers and fruitlets positioned high in the canopy and those in the shaded parts of the canopy, harvested from north and south facing aspects of each tree (Figure 13). Additional samples were collected at harvest and stored at 0.5-1.0°C in 3% CO<sub>2</sub>, 2%O<sub>2</sub> until May 2017 (Table 4).

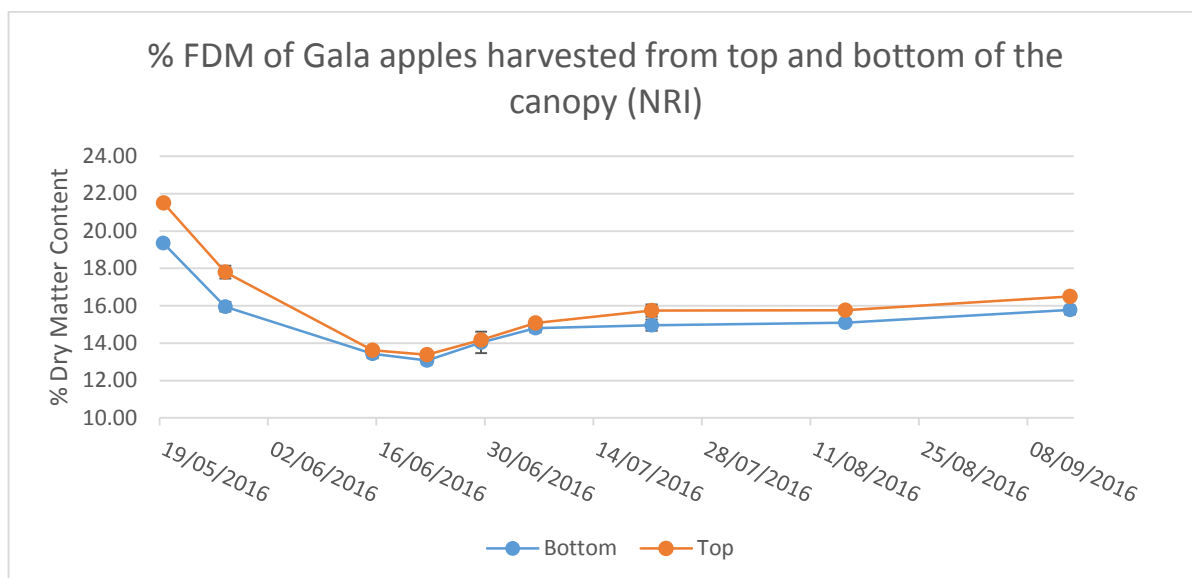


Figure 13. % FDM % of Gala fruit picked from (Top) exposed upper branches, and (Bottom) shaded lower branches of a Gala orchard. Each data point is the mean of 24 +/- SE. Each sample consisted of 5-10 fruit (number of fruit per sample decreased through the season).

Fruit harvested from the upper canopy (Top) maintained a higher FDM during the early stages of fruit development before FDM in top and bottom fruit samples declining to 13.1-13.4% FMD around 4 weeks post petal fall. Thereafter, FDM increased until harvest, where FDM content in Gala harvested from the upper canopy was approximately 0.7% higher in FDM (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.

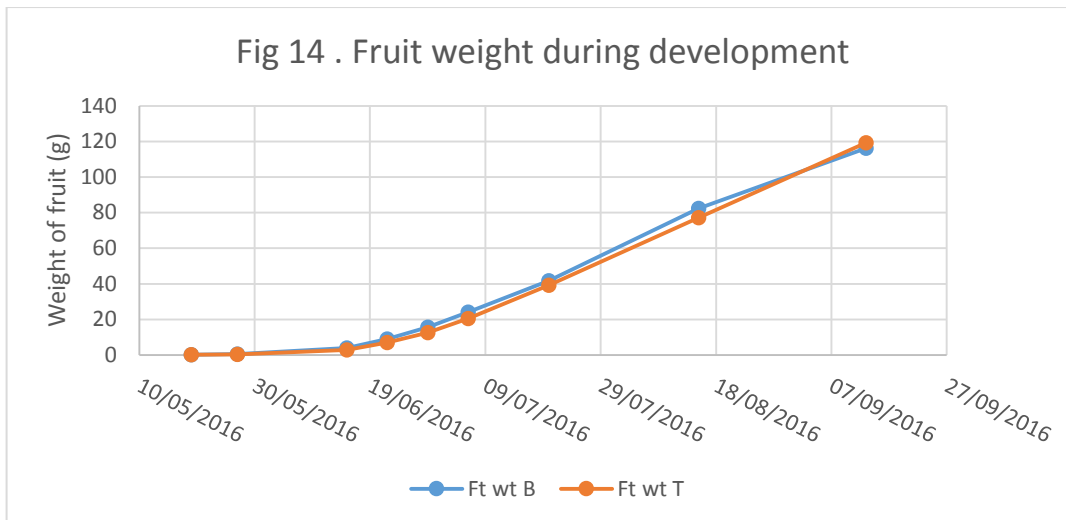


Figure 14. Fruit weight (Ft wt) during development of Gala fruit picked from the top (T) exposed upper branches, and bottom (B) shaded lower branches of a Gala orchard. Each data point is the mean of 24 samples of 5-10 fruit (number of fruit per sample decreased through the season).

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 (Figure 14). 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.



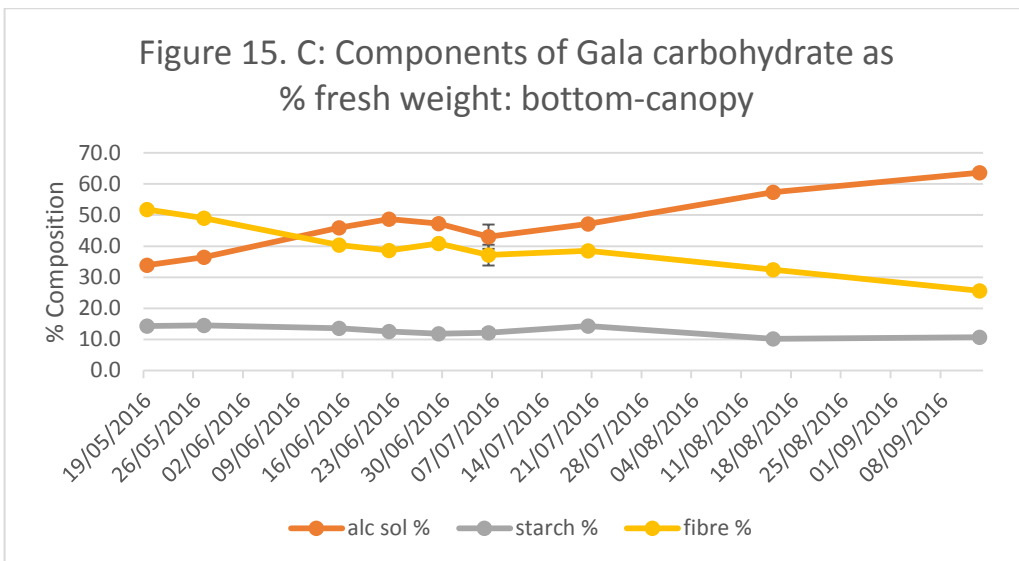
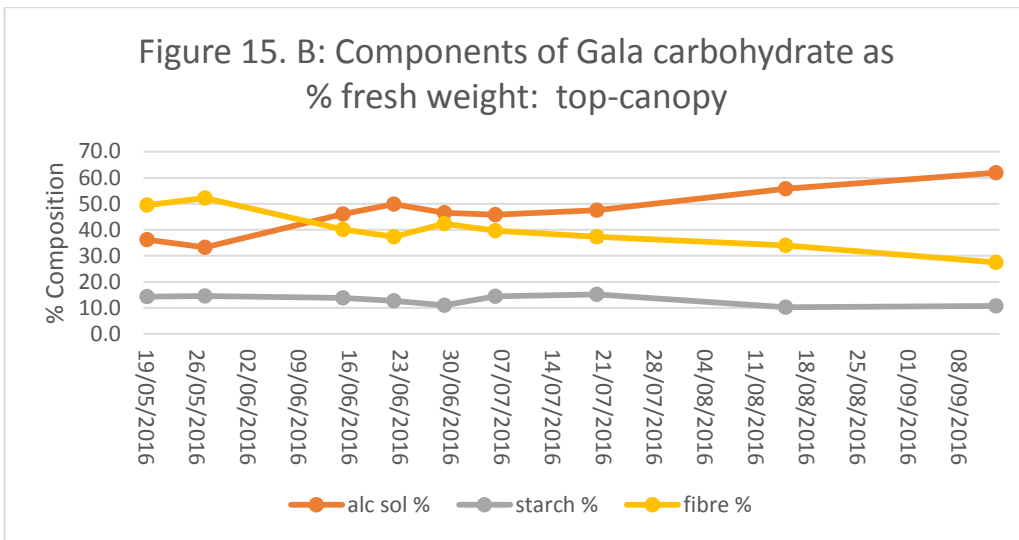
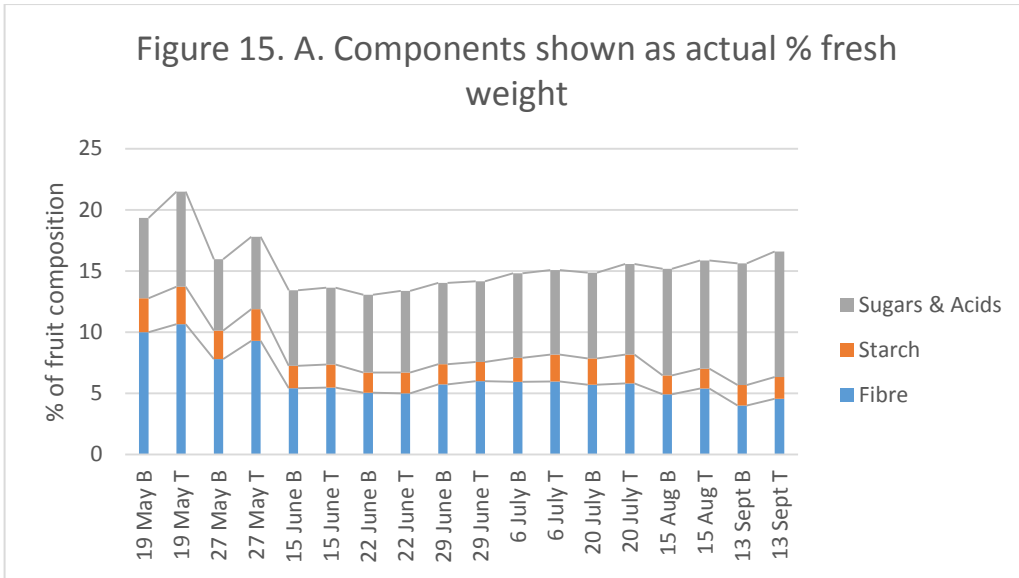


Figure 15. Changes in components of FDM during fruit development; Figure 15.A. Overall comparison between top and bottom canopy, Figure 15.B. Comparison of starch, alcoholic

soluble sugars and starch in fruit harvested from the top of the tree canopy and Figure 15.C., Bottom of the tree canopy. Means (n=12) +/- SE.

Analysis of individual components of FDM in samples collected over the season were fractionated into alcohol soluble (sugars and acids), fibre (cell wall component) and starch.

The alcohol soluble (sugars/acids) component of FDM 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.

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 FDM than fruits from the bottom, however, this difference was not statistically significant (*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 16).

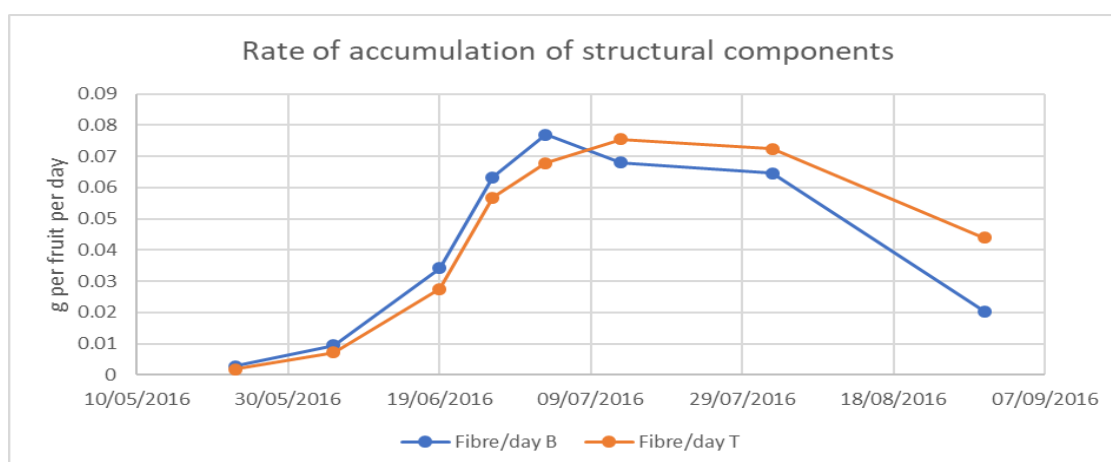


Figure 16. Accumulation of structural carbohydrates (fibre) in Gala apples harvested from petal fall through to commercial harvest.

Starch content (Figure 15) of Gala was highest (14.3%) soon after flowering (19/5/2016) and decreased (\*\**P*<0.05) for the first six weeks post-petal fall to 11 % before rising briefly to 15% starch eight 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 fresh weight (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% CO<sub>2</sub>, 1% O<sub>2</sub> (0.5-1.0°C) and stored for 8 months. Fruits were assessed after 5, 6.5 and 8 months and 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 4) 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 4. Harvest Maturity and storage quality of Gala harvested from the upper canopy: Top (>1.5 M) and the lower canopy: Bottom (<1.0-0.5 M)

| Sample date     | Position |    | Firmness (kg) | % Brix°     | Starch ctfI | Ethylene (ppb) |
|-----------------|----------|----|---------------|-------------|-------------|----------------|
| 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         |
| 5 Months        | Top      |    | 8.0           | 13.2        |             |                |
|                 |          | SE | 0.120         | 0.28        |             |                |
|                 | Bottom   |    | 7.8           | 12.7        |             |                |
|                 |          | SE | 0.014         | 0.42        |             |                |
| 5 Month + SL    | Top      |    | 8.4           | 14          |             |                |
|                 |          | SE | 0.007         | 0.00        |             |                |
|                 | Bottom   |    | 8.1           | 12.6        |             |                |
|                 |          | SE | 0.149         | 0.35        |             |                |
| 6.5 Months + SL | Top      |    | 8.0           | 12.9        |             |                |
|                 |          | SE | 0.432         | 0.07        |             |                |
|                 | Bottom   |    | 7.8           | 12.4        |             |                |
|                 |          | SE | 0.035         | 0.21        |             |                |
| 6.5 months + SL | Top      |    | <b>8.0</b>    | <b>13.8</b> |             |                |
|                 |          | SE | 0.44          | 0.42        |             |                |
|                 | Bottom   |    | 7.8           | 12.5        |             |                |
|                 |          | SE | 0.036         | 0.071       |             |                |
| 8 Months        | Top      |    | 8.1           | 13.0        |             |                |
|                 |          | SE | 0.021         | 0.14        |             |                |
|                 | Bottom   |    | 7.7           | 13.0        |             |                |
|                 |          | SE | 0.021         | 0.14        |             |                |
| 8 Months + SL   | Top      |    | 8.5           | 13.7        |             |                |
|                 |          | SE | 0.050         | 0.21        |             |                |
|                 | Bottom   |    | 8.2           | 12.5        |             |                |
|                 |          | SE | 0.007         | 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% CO<sub>2</sub>, 1% O<sub>2</sub> (0.5-1.0°C). In general, fruits harvested from the upper canopy were higher in % Brix<sup>0</sup> 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 % Brix<sup>0</sup> content of fruit remained on average 1% higher in fruit from the top of the canopy compared to fruit from the lower canopy.

### **Key findings 2016 FAST**

- The % FDM of both treatments decreased 2 weeks post petal fall until 9 weeks post petal fall and then increased until harvest.
- Values of % FDM were distinct between treatments with the upper well-lit fruit having higher values than the lower shaded fruit.
- There were statistically significant differences in % FDM between treatments at all sampling events except on 6 July – Treatment 2 had significantly lower % FDM compared to Treatment 1.
- Medium degree of negative correlation between upper/lower fruit sampled and rainfall.
- Weak positive correlation between upper/lower fruit sampled and sunshine hours.
- Low degree of negative correlation between upper/lower fruit sampled and accumulated day degrees.

### **Key findings 2016 NRI**

- Fruit weight increases were similar between fruit sampled between the top and bottom of the canopy
- % FDM of fruitlets at petal fall peaked at 21.5% and then declined over the next four week period to 13.4% during the period of cell division before increasing at the final harvest to 16.5% FDM.
- Small increase (0.7 %) in FDM in fruit sampled from the top of the canopy was observed.
- The proportion of alcohol soluble fraction (sugars) increased from petal fall through to commercial harvest
- The proportion of fruit fibre (structural carbohydrate) decreased from a high at petal fall declining until harvest

- Starch content declined from 14.3 % at petal fall to 11% six weeks post-petal fall, rising to 15% starch eight weeks post petal fall before declining to 11% (%FW) starch at commercial harvest.
- During CA (3% CO<sub>2</sub>, 2% O<sub>2</sub>) storage fruit harvested from the top of the canopy maintained 0.5-1.0 % higher Brix content than fruit harvested from the lower canopy.

## April 2017 onwards (Season 2)

Information gained from the first year of study provided an informed basis for the applications of tree management strategies, such as approaches to thinning which could be applied in subsequent years.

Following the outcome of the experiment in Year 1, strategies to manipulate crop load at different timings in a high FDM Gala orchard (FAST: Brogdale) are currently under investigation and thinning strategies related to fruit quality.

Different thinning strategies (timing, method, material) will be undertaken and effects on FDM, yield, quality, fruit size and maturity ascertained via physiological assessments, sampling and laboratory analysis. Fruit counts will be undertaken following first and second fruit falls (combined), fruit removed by hand counted and the % of fruit lost to natural processes determined to ascertain differences between treatments. Other assessments included final yield, maturity and quality (colour and % BRIX°) and FDM (collected and analysed by NRI).

### Treatments 2017

1. No thinning (**Control**)
2. **Bud** thinning – BBCH 52-54 (end of bud swelling to mouse ear) via bud extinction using MAFCOT Equilifruit tool ratios, completed 24 March
3. **Mechanical** thinning – 60% first open flower (BBCH 65-66) (hand held device), completed 19 May
4. Chemical thinning – Fine **Exilis** 6-Benzyladenine + Fixor (funded by Fine) (BBCH 70-72) (PGR), completed 23 May
5. Chemical thinning – Adama **Brevis** 150 SG metamitron 15% (funded by Adama) (BBCH 70-71 & 71-72) (PGR), completed 23 May
6. Hand Thinning **Standard** – fruit size 15mm to 25mm (BBCH 71-72), pre/up to second fruit fall, completed 3 June
7. Hand Thinning **Size** – event 1 fruit size from 25mm to 30mm (BBCH 73), event 2 fruit size 40mm (BBCH 74), completed 14 June

8. Hand Thinning **Late** – fruit size 30mm to 40mm (BBCH 73-74), after second fruit fall-completed 3 July

### **Sampling 2017**

- Post petal fall 9 May
- After second fruit fall (post Treatment 8) – from 27 June.
- Harvest prediction mid-September.

### **Key findings 2017 FAST**

- Frost events in mid-April caused significant damage and losses to lower canopy of all treatment trees.
- Chemicals applied only to tops of trees.
- Very little fruit lost at first fruit fall.
- Second fruit fall started 2 June.
- Fruit size at Treatment 6 = 25mm.
- Fruit counts for second fruit fall for all treatments including chemical were undertaken after 27 June.

## Work Package 4. Chlorophyll Fluorescence prediction of harvest maturity

Changes in CF readings provided on average 7-10 days warning of imminent changes in fruit starch clearance and thus was able to help predict harvest date (Table 5).

Fruit Chlorophyll fluorescence decreases during fruit growth and maturity. Up to 57 fluorescence parameters are generated during a fluorescence reading by the PEA software. Each output parameter was evaluated and changes in individual outputs were correlated with changes in starch clearance patterns. From this earlier analysis (Landseer) the following formula was constructed. The degradation formula was based on constructing a baseline CF measurement at fruitlet (25-30 mm) stage and continuing measuring fruits with the PEA fluorimeter until the reduction is less than 50% of the baseline CF:

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

This provided an early CF warning for changes in starch degradation during increasing maturity (Table 5), providing 7- 10 days advanced warning than relying on changes in the starch index (less than 80-75%).

Table 5. Comparison of changes in internal ethylene and starch and CF ratio between samples picked on two dates from four orchards

| 12/09/2016                      |         |                       |                       |                 |                                 |              |                      |  |  |
|---------------------------------|---------|-----------------------|-----------------------|-----------------|---------------------------------|--------------|----------------------|--|--|
| Orchard/ clone                  |         | Average of 10 samples | Average of 30 samples | starch Landseer | Internal ethylene Concentration | % Starch PQC | AvF1 July (Fruitlat) | (10 samples) CF degradation: (AvFn-σAvFn)/(AvF1-σAvF1)<50% | (30 samples) CF degradation: (AvFn-σAvFn)/(AvF1-σAvF1)<50% |
| Dodges (Galaxy)                 | AvF     | 2062                  | 2351                  |                 |                                 |              | 5822                 |  |  |
|                                 | AvF-STD | 1329                  | 1293                  | 80%             | 864 ppb                         | 71%          | 4359                 | 0.30   | 0.30   |
| Stallance (Galaxy) Griffen Farm | AvF     | 1371                  | 2408                  |                 |                                 |              | 4792                 |  |  |
|                                 | AvF-STD | 581                   | 1115                  | 80%             | 96 ppb                          | 86%          | 3718                 | 0.16   | 0.30   |
| Mystole (Schniga)               | AvF     | 2365                  | 2518                  |                 |                                 |              | 5849                 |  |  |
|                                 | AvF-STD | 1324                  | 1394                  | 85%             | 32 ppb                          | 95%          | 4443                 | 0.30   | 0.31   |
| Barnyard (Mondial)              | AvF     | 4773                  | 4441                  |                 |                                 |              | 6212                 |  |  |
|                                 | AvF-STD | 2524                  | 2610                  | 75%             | 172 ppb                         | 72%          | 4995                 | 0.51   | 0.52   |
| 19/09/2016                      |         |                       |                       |                 |                                 |              |                      |  |  |
| Orchard/ clone                  |         | Average of 10 samples | Average of 30 samples | starch Landseer | Internal ethylene               | Starch PQC   | AvF1 July (Fruitlat) | (10 samples) CF degradation: (AvFn-σAvFn)/(AvF1-σAvF1)<50% | (30 samples) CF degradation: (AvFn-σAvFn)/(AvF1-σAvF1)<50% |
| Dodges (Galaxy)                 | AvF     | 2806                  | 2886                  |                 |                                 |              | 5822                 |  |  |
|                                 | AvF-STD | 1329                  | 1124                  | 78%             | 92 ppb                          | 85%          | 4359                 | 0.30   | 0.26   |
| Stallance (Galaxy)              | AvF     | 1454                  | 1457                  |                 |                                 |              | 4792                 |  |  |
|                                 | AvF-STD | 829                   | 832                   | 75%             | 453 ppb                         | 75%          | 3718                 | 0.22   | 0.22   |
| Mystole (Schniga)               | AvF     | 1998                  | 1881                  |                 |                                 |              | 5849                 |  |  |
|                                 | AvF-STD | 1090                  | 1141                  | 75%             | 704 ppb                         | 88%          | 4443                 | 0.25   | 0.26   |
| Barnyard                        | AvF     | 2990                  | 2725                  |                 |                                 |              | 6212                 |  |  |
|                                 | AvF-STD | 2075                  | 1193                  | 65%             | 981 ppb                         | 49%          | 4995                 | 0.42   | 0.24   |



This provided an early CF warning for changes in starch degradation during increasing maturity, providing seven to ten days advanced warning than relying on changes in the starch index (less than 80-75%). Two harvest assessments from each orchard, after seven to ten days of CF warning fruit were picked (CF-pick) and after three days when starch was less than 80-75%, fruit were picked (starch-pick). Internal ethylene production of samples from four orchards after CF-pick and starch-pick were measured at the Produce Quality Centre (NRI/NIAB-EMR) by GC-FID (Table 5).

In general, CF-picked samples were harvested around between 71-95% starch content (PQC) but more importantly they were at the beginning of the start of the climacteric rise in ethylene production, whereas the later picked fruit targeted for 75% starch content ranged between 49-88% starch, but were generally much higher in ethylene. Additional analysis of fruit for IEC in samples harvested between CF-alert and 75% starch maturity would strengthen the accuracy of the prediction linking fruit maturity currently based on starch clearance patterns with changes in the ethylene production and the climacteric rise in fruit respiration.

Concurrently, CF-measurements, were compared with a DA-meter (Turani/Italy) which measures chlorophyll content at two wavelengths of 670 and 720 nm and provides a maturity index. The results in different orchards were inconsistent sometimes predicting earlier, sometimes predicting later than starch index (Table 6).

Samples as CF-pick and starch-pick collected from each orchard and stored in three different storage regimes: DCA, 5%CO<sub>2</sub>: 1%O<sub>2</sub> and 3%CO<sub>2</sub>:2%O<sub>2</sub>. Samples were all treated with SmartFresh<sup>SM</sup>, only one group of samples kept as untreated for DCA store.

Samples kept in commercial stores for long term storage (9 months). Extra samples were collected to enable two monitoring assessments prior to end of storage.

The first monitoring was held at the end of January 2017, second monitoring was Mid-March 2017 and up to this stage all fruit were stored in their predefined atmospheres. A third monitoring was conducted at the end of May 2017.

Samples were stored in commercial stores, targeted atmospheres were reliant on the time taken for commercial stores being filled with fruit. It took a couple of weeks to establish regimes for different treatments. During the critical period of six to nine months storage some stores like 5:1 and 2% regimes were opened earlier than DCA store and fruit was moved to different atmospheres making direct comparisons between different regimes difficult.

The monitoring fruit during storage and shelf life in different regimes did not show significant difference in quality of fruit; however, as a long-term storage application of SmartFresh-treated fruit maintained fruit quality during shelf life regardless of regime adopted.

% Brix<sup>0</sup> increased by 0.5 % during the seven days before the CF pick (designed to predict 80-85% Starch degradation). Although % Brix<sup>0</sup>, at harvest was lower than specifications set out for long-term storage (% Brix<sup>0</sup>>12), it increased significantly during storage. Since the aim of the project is long term storage it is important to have the maximum Brix<sup>0</sup> at the end of long term storage.

Maintenance of flavour during long term storage is associated with retention of ample acidity, since acidity decreases with later harvesting this can affect fruit taste in long term storage. While current best practice for long-term storage of Gala is to pick fruit at 85-90% starch (Defra Best Practice Guide/AHDB TF221), having sufficient warning when fruits start maturing and clearing starch provides an additional tool for growers

There was a correlation between high FDM and higher acidity of fruit after long term storage (9 months). Also, samples treated with SmartFresh<sup>sm</sup> maintained significantly more acid than DCA alone during storage and CF-pick samples had slightly higher acidity.

Table 6. Early maturity warning 7-10 days by PEA Pocket (CF alert) in comparison with starch alert and inconsistent results for DA meter.

| Orchard                             | Fruitlet analysis | Fruit analysis | Monitor Pick 1 | Monitor Pick 2  | Monitor Pick 3              | Monitor Pick 4              | Monitor Pick 5              | Monitor Pick 6  | Monitor Pick 7              | Monitor Pick 8  |
|-------------------------------------|-------------------|----------------|----------------|-----------------|-----------------------------|-----------------------------|-----------------------------|-----------------|-----------------------------|-----------------|
|                                     | 13 July           | 18 Aug         | 25 Aug         | 31 Aug          | 5 Sept                      | 9 Sept                      | 13 Sept                     | 16 Sept         | 19 Sept                     | 23 Sept         |
| MYSTOLE TOP (NEWMARFRUIT) (Schniga) | Yes               | Yes            | Yes            | Yes             | Yes<br>CF alert             | Yes                         | Yes<br>CF store             | Yes<br>ST alert | Yes<br>DA alert<br>ST store |                 |
| MONKS FARM (SIMON BRAY) (Mondial)   | Yes               | Yes            | Yes            | Yes             | Yes<br>DA alert<br>CF alert | Yes                         | Yes<br>CF store             | Yes<br>ST alert | Yes<br>ST store             |                 |
| STALLANCE/18 (GOATHAM) (Galaxy)     | Yes               | Yes            | Yes            | Yes<br>CF alert | Yes                         | Yes<br>DA alert<br>CF store | Yes                         | Yes<br>ST alert | Yes<br>ST store             |                 |
| GIBBENS FARM (GOATHAM) (Mondial)    | Yes               | Yes            | Yes            | Yes             | Yes                         | Yes                         | Yes<br>DA alert<br>CF alert | Yes<br>CF store | Yes<br>ST alert             | Yes<br>ST store |
| BARNYARD (GOATHAM) (Mondial)        | Yes               | Yes            | Yes            | Yes             | Yes<br>CF alert             | Yes                         | Yes<br>ST alert<br>CF store | Yes<br>DA alert | Yes<br>ST store             | Yes             |
| DODGES (Richard Day) (Galaxy)       | Yes               | Yes            | Yes            | Yes             | Yes                         | Yes<br>CF alert             | Yes<br>DA alert<br>CF store | Yes<br>ST alert | Yes<br>ST store             |                 |
| NORTON 3 (CHARLTONS) (Schniga)      | Yes               | Yes            | Yes            | Yes             | Yes                         | Yes                         | Sampling stopped            |                 |                             |                 |
| Gammons (HADLOW) (Mondial)          | Yes               | Yes            | Yes            | Yes             | Yes                         | Yes                         | Sampling stopped            |                 |                             |                 |
| HILL TOP GORE (GOATHAM) (Galaxy)    | Yes               | Yes            | Yes            | Yes             | Yes                         | Yes                         | Sampling stopped            |                 |                             |                 |

Most CF warnings were 7-10 days earlier than Starch alert, Stallance was the longest (16 days)

DA meter alert was not consistent, sometimes before, after or at the same time of Starch alert!

The challenge is to generate a robust model that can incorporate seasonal and orchard variation that will allow this technology to be commercialised. Accuracy of any prediction model depends on sample collection at right time, location and size of sampling.

From 3 years work conducted by Landseer, including this first year as part of the AHDB project, an outline of a decision tree making process is being developed (Figure 17).

Adopting this model may help the UK industry more clearly define that proportion of fruit destined for longest term storage, based on maturity. An early CF warning of 7-10 days could be a valuable logistical and planning tool to help in the harvest strategy decision making process.

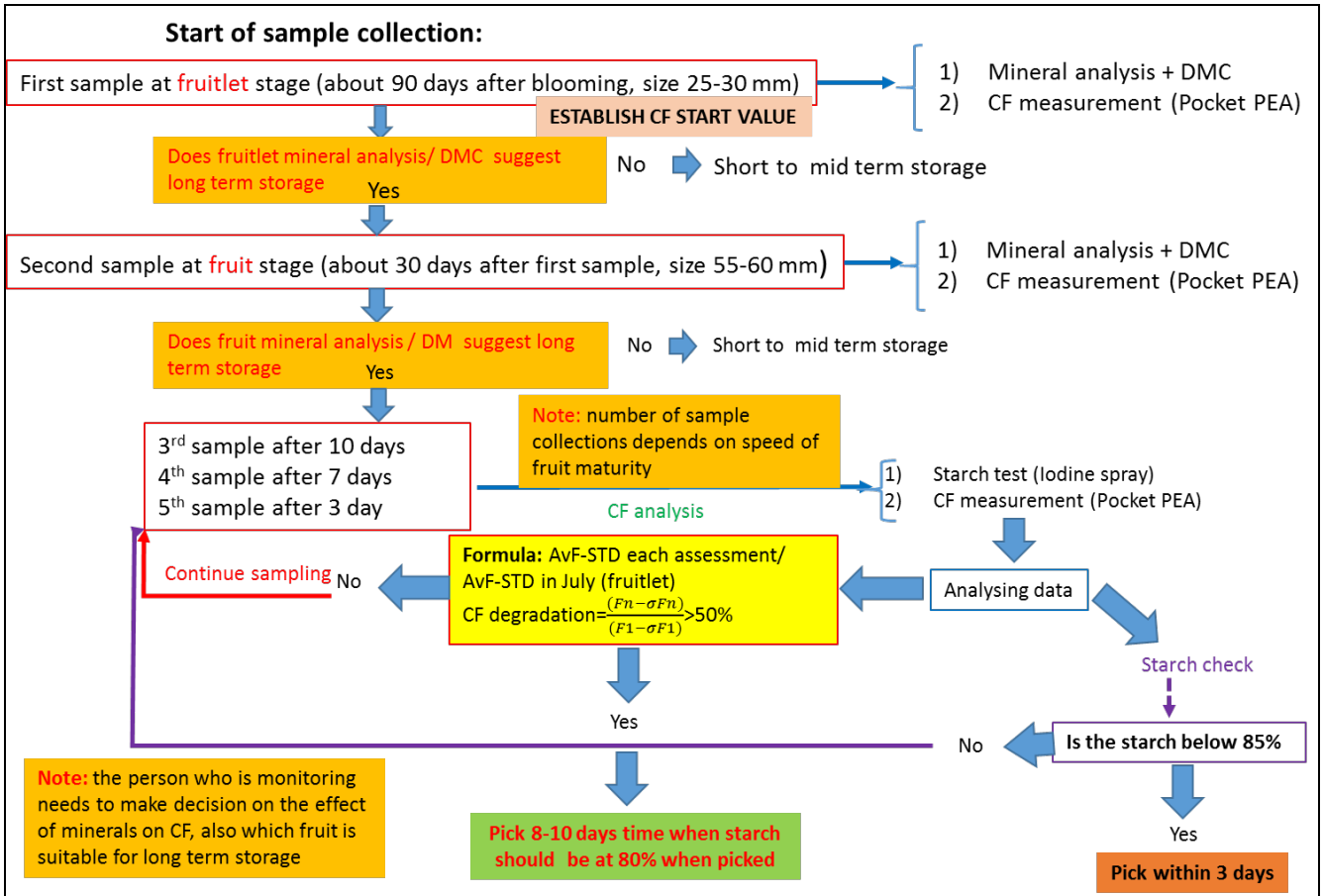


Figure 17. Schematic flow chart for the process of sampling and analysing data for selecting the best orchards for long term storage and best picking date.

## Discussion Work Packages 1-4

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 0.8% higher in FDM (16.5%) than fruits picked from the lower regions of the canopy (<0.6 M) where fruit averaged 15.8% FDM.

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 2002), highlight a strong relationship between overall FDM and the amount of sugar (%Brix<sup>0</sup>) 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 FDM 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.

Considering 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, a significant reduction in the proportion of fibre occurred during fruit development as fruits cells expanded with solutes, in the form of sorbitol, sucrose from phloem and water and nutrients supplied by the xylem. Fruits demand for resources significantly increases around four weeks after full bloom (Lakso 1999) coinciding with a period of cell division and heightened respiration rates associated with cell division have been reported to be responsible for the initial decline in FDM four to six weeks after petal fall.

Smaller changes in starch content were observed during fruit development with a decline from observed during the first six weeks after petal fall at a time where resources are being allocated to cell division. Thereafter, starch reserves increased briefly between six to eight weeks post petal fall before declining again. Most of the later decline in starch is associated with increased fruit size in the latter stages of fruit development.

Analysis of existing data sets to garner information regarding major influences on FDM accumulation requires sufficient variability within the FDM dataset to enable a correlation with other metrics. The more robust these parameters are in terms of reliability and uniformity of measurements the greater the chance for identifying durable influences on FDM. 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 FDM, however the challenge will be to identify the influence of agronomic factors on FMD with a small (2.2%) span in FDM in the current data set. Adding additional data from later seasons and with published data 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 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.

## Conclusions Work Packages 1-4

- Higher FDM is found in the top of the canopy.
- Changes occur with FDM during fruit development: there is a significant drop during cell division stage followed by a slow rise until harvest.
- Centrifugal Pruning techniques can increase light capture for fruits and reduce the need for subsequent pruning.
- Initial analysis of historical FDM data (FAST LLP) has found a 2.2 % variation in FDM across 56 Gala orchards.
- Chlorophyll fluorescence has shown it is able to predict changes in starch clearance patterns seven to ten days before starch clearance was observed.

## Objectives

1. To carry out a meta-analysis to provide an evidenced-based understanding how fruit FDM can be manipulated to optimise fruit quality. Achieved.
2. To determine the impact of increasing light interception by pruning and/or using reflective mulches at different stages of fruit development on fruit quality, FDM, storability and consumer acceptability. Partially achieved.
3. To quantify the impact of thinning treatments on fruit quality, FDM, storability and consumer acceptability and to develop recommendations for thinning strategies to optimise yield of high quality fruit. Partially achieved.
4. To identify the correct timing for harvesting orchards with a high FDM to maximise the eating quality of fruit in the April to June marketing window, and to establish and validate a protocol using chlorophyll fluorescence to predict this timing. Partially achieved.
5. To maximise knowledge exchange and communications interaction with international research groups to enhance this research programme. Partially achieved.
6. To carry out effective project management and mitigation of risks. Partially achieved.

## 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)

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