Project title:	Apples & Pears: Developing strategies for using chlorophyll fluorescence to assess maturity and physiological stress
Project number:	TF 142
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Key words:	Chlorophyll fluorescence, apple, harvest maturity, storage disorders, chilling injury, low temperature damage, non-destructive techniques

‘The results and conclusions in this report are based on an investigation conducted over one year. The conditions under which the experiment was carried out and the results obtained have been reported with detail and 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.’

Contents

Grower Summary

1. Headline

Can chlorophyll fluorescence provide a non-destructive method to assess harvest maturity and storage quality of apples and pears? First trial season now complete.

2. Background and expected deliverables

Chlorophyll fluorescence

We all know that leaves are generally green. If our eyes were more sensitive, we would also be able to see that they emit a low level of red light or fluorescence. Leaves contain chlorophyll which absorbs sunlight to drive the process of photosynthesis. Not all the absorbed energy can be used, and so, to prevent the excess energy destroying components of the photosynthetic apparatus, it is reemitted as fluorescence and heat. Over the past few decades scientists have learnt how to use the pattern of emitted fluorescence to obtain information about the mechanism of photosynthesis and the components that drive the process within the cells of the leaf.

Many fruits also contain chlorophyll, and are able to carry out photosynthesis often even until fully ripe. Over the last decade there has been growing interest in using chlorophyll fluorescence to assess postharvest quality of fruit and vegetables. For this project we are examining two aspects of apple and pear fruit quality in order to determine whether chlorophyll fluorescence can provide a non-destructive method of assessment. These two aspects are: measurement of fruit maturity to predict and optimise harvest date, and assessment of tissue stress/damage during long-term storage.

Chlorophyll fluorescence to assess fruit maturity.

Ripening of apples and pears is associated with a loss of starch, an increase in sugars and a softening of the tissues due to breakdown of cell walls. In addition, most varieties lose their green colour during ripening. This is a result of the progressive loss of chlorophyll, which is associated with a loss in the ability to photosynthesise. Whereas the loss of chlorophyll can be, and is, measured by scoring for colour changes, patterns of chlorophyll fluorescence can

be used to assess not only chlorophyll levels, but also other components and processes of the photosynthetic system which are influenced by the levels of metabolites such as sugars and starch. Potentially, therefore, fluorescence could provide a much more accurate measure of maturity than other non-destructive methods such as colour.

Chlorophyll fluorescence to assess fruit damage during long-term storage

One of the key components of the photosynthetic system is called photosystem 2, or PS2. This is a particularly important component as it is able to use sunlight to split water, releasing oxygen. However, the component is also very delicate and sensitive to many stresses. To maintain photosynthetic function the cell must be able to continually repair and rebuild PS2. Indeed, it has been estimated that on average each PS2 is repaired every 30 minutes in a photosynthesising leaf. Several researchers have used chlorophyll fluorescence to measure PS2 function and have tried to use this to assess tissue health. In this project we are taking a slightly difference approach which we believe is more reliable. We deliberately damage PS2 with bright light and then measure the ability of cells to repair PS2. PS2 function does not directly affect fruit quality – we are merely using the ability to repair this component as a measure of tissue health.

This project is a collaboration between NRI, EMR, FAST and Hansatech Instruments Ltd (HI). Funding is being provided jointly by HDC and HI. HI is a UK company that produces chlorophyll fluorimeters. If either part of this project is successful, the longer-term objective is to develop equipment appropriate for standard use by growers. In the case of prediction of harvest time, a grower would be better able to allocate resources if he could follow the maturation of the fruit over the weeks prior to harvest. In the case of long-term storage, advanced warning of the onset of visible damage would allow the grower to market the fruit before any such damage became too serious.

3. Summary of the project and main conclusions

Chlorophyll fluorescence to assess fruit maturity.

A trial was conducted in collaboration with the Quality Fruit Group (QFG). For a selection of the orchards used by QFG a sample of fruit was assessed for fluorescence characteristics as well as normal quality characteristics. The specific aim was to see if maturity could be predicted by (modelled by) fluorescence characteristics alone. At least two seasons of data will be needed to check the validity of any models, but initial results are summarised in Figures 1-3. For Gala and Cox, we have assumed that starch levels are the most important factor used to determine harvest time, while for Conference we have assumed that the Streif index would be used. The figures show the fruit maturity (measured and predicted) averaged over all orchards considered for seven assessment dates. The results are encouraging, in that close relationships between the maturity and models are obtained.

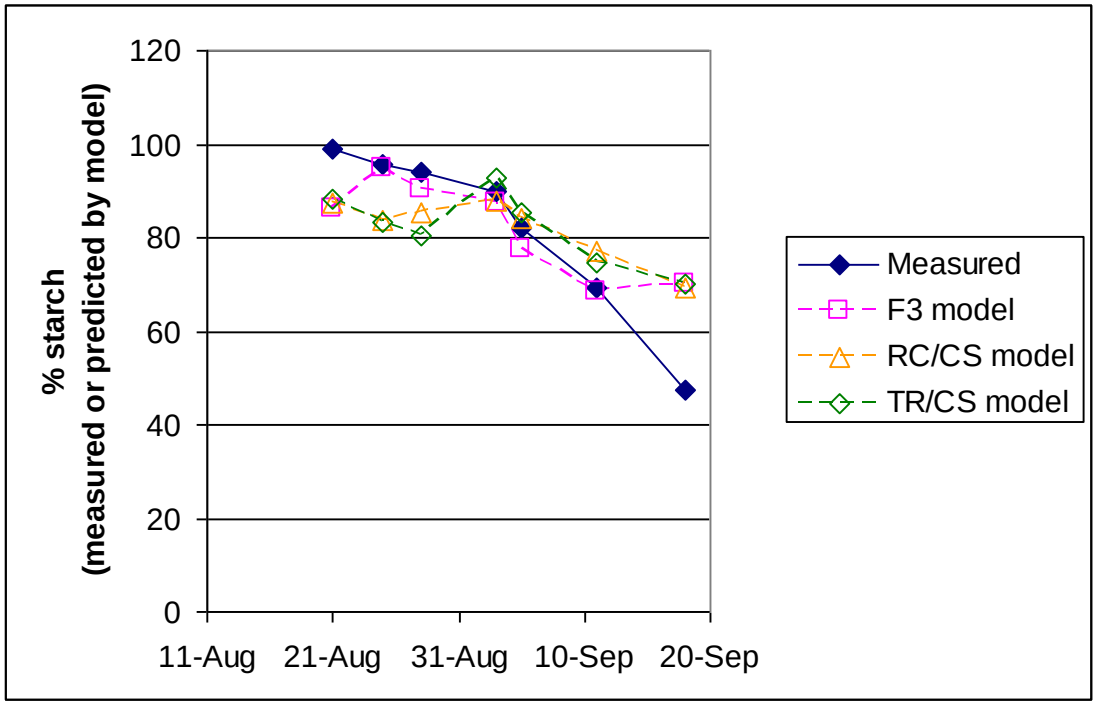


Figure 1: Cox apples. Average starch levels measured over 4 weeks, and levels predicted by models.

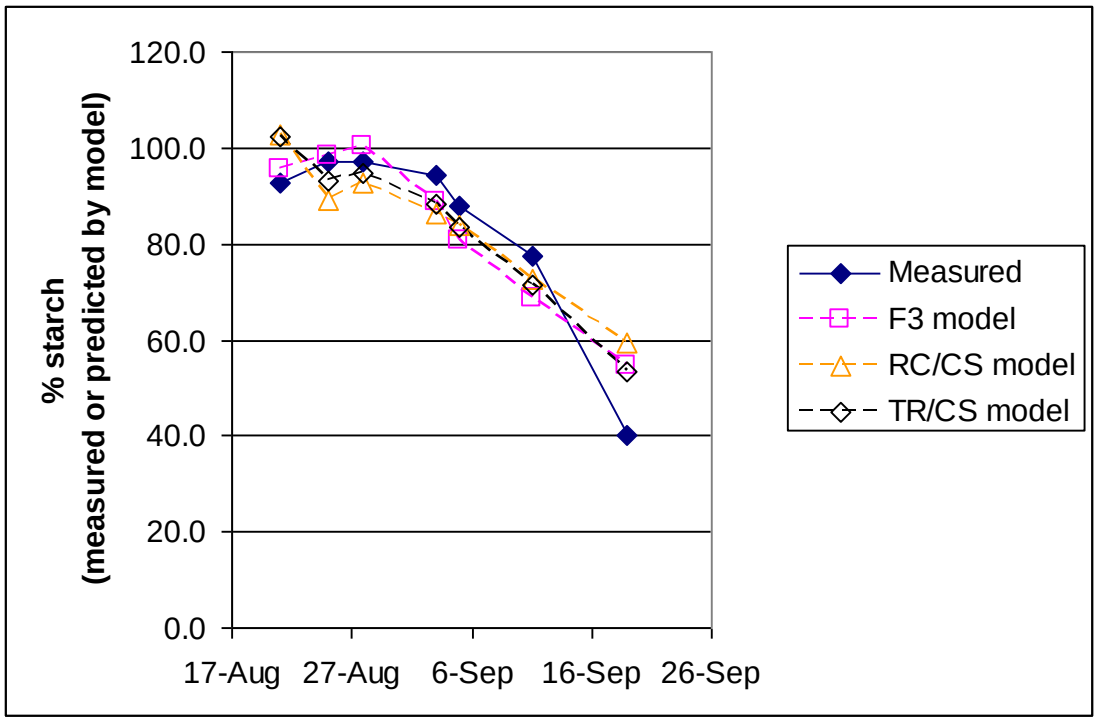


Figure 2: Gala apples. Average starch levels measured over 4 weeks, and levels predicted by models.

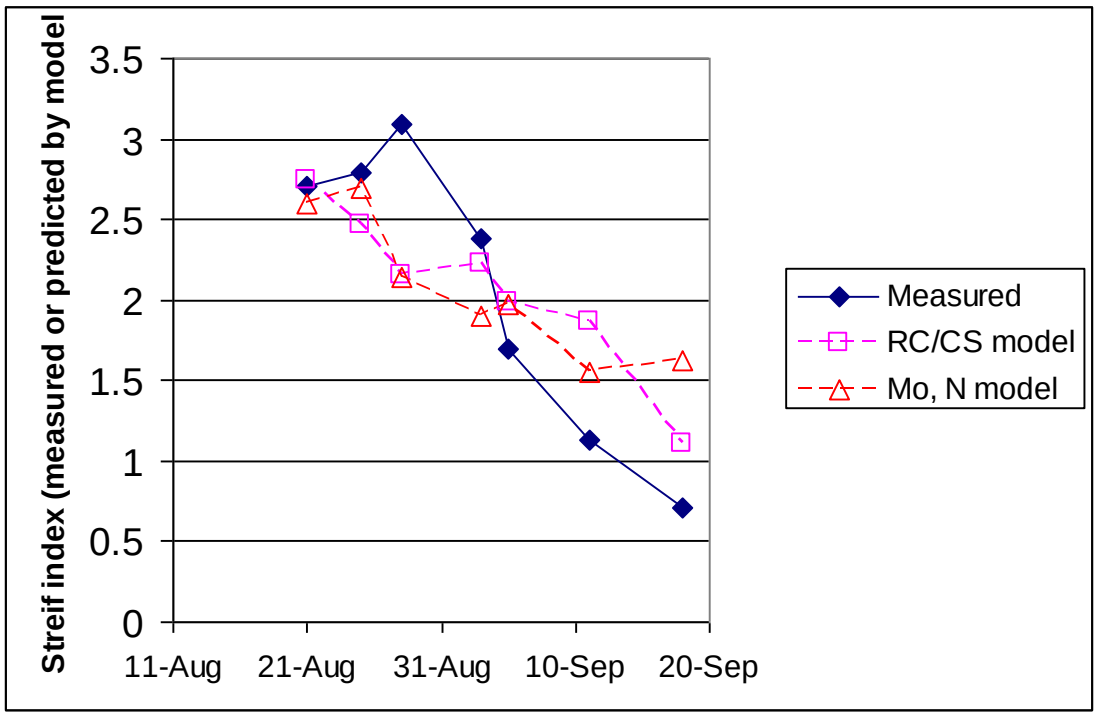


Figure 3: Conference pears. Streif index measured over 4 weeks and index predicted by models.

Chlorophyll fluorescence to assess fruit damage during long-term storage

Cox apples were stored at East Malling Research Station at normal CA (1.2% O_2) over a range of temperatures (4, 2, and 0ºC). At the lowest storage temperature we would normally expect to see low temperature damage, and the objective of the trial was to determine if we could see a loss of tissue health in advance of visible damage. Unfortunately, significant low temperature damage was not seen in these trials. This is probably due to two factors; firstly, the hot growing season would have produced more resistant apples, and secondly due to the time taken to refine the methodology, the apples were not placed at low temperature until January. Nevertheless, the data obtained illustrates the feasibility of the methodology, and will also provide baseline data for a second season of trials.

In the course of this project we have learnt that in apples maintained at low temperature PS2 repair can only just keep up with normal rates of damage, so that little or no recovery of normal function is observed. However, in stressed tissue, it appears that the slowing of repair rates results in a progressive decrease in PS2 function. Figure 4 shows PS2 levels measured before and after deliberate damage with high light, and after 24 hours of recovery for the three storage temperatures. At 0ºC repair rates become slower after 8 weeks of storage so that a decrease in PS2 function is seen. We would predict that this would be associated with visible tissue damage after a certain time, probably 1-2 months – but a second season of data will be needed to observe this. Tissue stress was also observed in these apples using other fluorescence techniques (data shown in main report only).

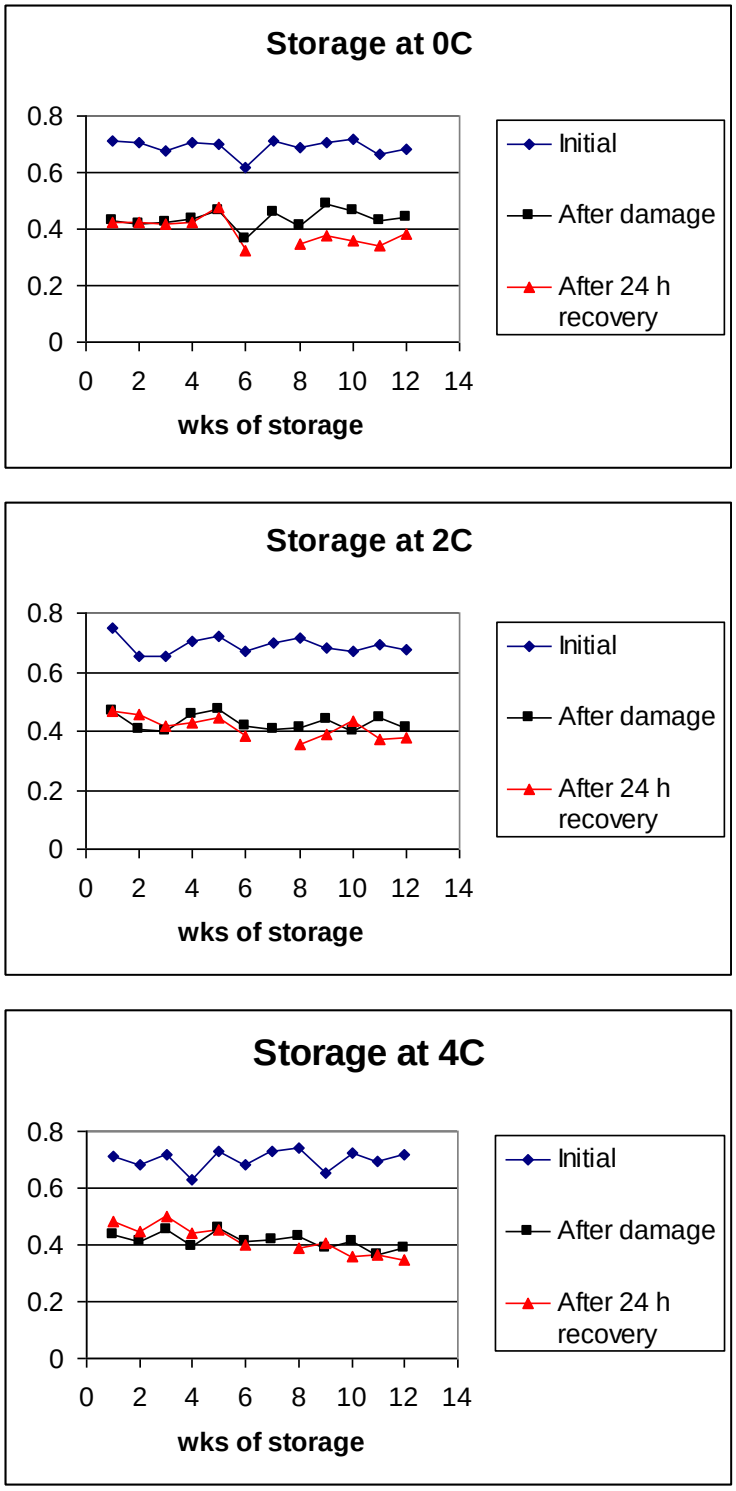


Figure 4: PS2 function in apples before and after high light damage, and after 24 hours recovery. Apples were stored at 0,2,and 4 ºC. The units of PS2 function relate to fluorescence characteristics, and not PS2 activity.

4. Financial benefits

Not applicable at this time.

5. Action points for growers

None at this time.

Science Section

Introduction

This project relates to the use of chlorophyll fluorescence as a non-invasive method for assessing two aspects of fruit quality: maturity (for optimisation of harvest time), and early detection of tissue damage during storage. The proposal builds on research work undertaken at NRI over the past five years (Ross 2002).

The problems to be addressed

The present method for assessing optimum harvest time for an apple orchard involves taking a sample of fruit and making destructive measurements of starch, sugar, acids, colour and texture. This is time consuming, and, being destructive, limits the size of sample that can be used, and therefore the accuracy. The development of rapid, cheap non-destructive methods for assessing fruit on the tree would potentially benefit all apple growers in the UK.

During long-term storage, fruit are stored under conditions near the limit of their tolerance (e.g. in terms of low temperature and atmospheric composition). As consignments vary in their sensitivity to stresses, storage conditions are generally modified to provide a margin of safety, hence limiting storage potential. Even so, unexpected physiological disorders often develop, and by the time the disorders are visually apparent, consignments can have suffered significant reduction in quality, leading to substantial economic loss. Methods for detection of tissue damage at an early stage, especially non-destructive methods, would provide clear advantages to all apple storers.

Chlorophyll fluorescence

Techniques for measuring chlorophyll fluorescence have been developed as a tool for investigating photoynthesis. (Briantais *et al.* 1986, Schreiber and Bilger 1993). Recently there has been growing interest in the use of chlorophyll fluorescence for the assessment of quality of harvested plant products. This is possible, because by

assessing the health of the photosynthetic processes, chlorophyll fluorescence measurement can also give an indication of the overall health of the tissues.

Other non-invasive methods for assessing fruit quality

A number of other non-invasive techniques are presently being investigated for their post-harvest applications, and might be particularly valuable if used in conjunction with chlorophyll fluorescence. Among these methods are electronic volatile sensors (electronic noses) and acoustic resonance. Electronic volatile sensors can be used to determine the volatiles released by foodstuffs. NRI has recently been investigating the sensitivity of individual sensors to fruit quality changes during storage and ripening, with the view to developing a cheap handheld machine for use in the horticulture industry. Acoustic resonance is being investigated by commercial companies (e.g. Aweta and Sinclair) as a method for assessing textural changes during ripening.

Commercial objectives

The objectives of this project are firstly to determine if chlorophyll fluorescence can be used as a practical non-invasive method (either on its own, or in conjunction with other available methods) for assessment of fruit maturity on-the-tree and fruit physiological damage during storage by the UK apple and pear industry.

If the technique is found to be useful, then plans for the production of appropriate instruments (cheap, rapid, handheld) to be used by the industry will be developed in collaboration with Hansatech Instruments Limited.

Chlorophyll fluorescence to assess fruit maturity on-the-tree

Most, but not all fruit lose chlorophyll during ripening. However, whether or not they lose chlorophyll, the capacity for photosynthesis, and therefore the fluorescence characteristics change. Several studies have considered changes in fluorescence characteristics of fruit after harvest, and have developed models relating fluorescence to shelf-life (e.g. Ahmed *et al.* 1998;. Toivonen, 1992) Ross considered fluorescence changes prior to harvest and related these to changes in quality characteristics for apples and pears. Working with the Quality Fruit Group he developed models of fruit maturity (as determined by the Streif index) in terms of fluorescence characteristics

alone. These measurements, unlike surface colour measurements are not affected by the presence of masking pigments such as found in many apple varieties.

Chlorophyll fluorescence to assess development of physiological damage during storage.

There are several studies reported in the scientific literature where scientists have tried to relate chlorophyll fluorescence characteristics of harvested horticultural produce to quality. Most of these studies have focused on fluorescence characteristics directly associated with functioning of photosystem 2 (PS2). PS2 is a vital part of the photosynthetic system in the chloroplast, which is involved in the oxidation of water to produce oxygen. As it is extremely sensitive to damage, there are generally very efficient mechanisms for repair, but these can be inhibited when the tissues are stressed. (e.g. Smillie *et al.* 1987, Tian *et al* 1996, van Kooten *et al.* 1992). The state of PS2 can be assessed by a fluorescence parameter termed Fv/Fm which can be measured in 1-2 seconds The validity of using Fv/Fm as a quality index depends on the assumption that damage to the photosynthetic apparatus, can give a good indication of the overall health of the tissue. Ross (2002) found that direct measurement of Fv/Fm, as used by many investigators is not a reliable method for assessing tissue damage, whereas the rate of repair of PS2 after removal from stress provides a much more robust strategy. For this project techniques to follow PS2 repair are used.

Related work

As far as we are aware, no work has yet been carried out on the use of chlorophyll fluorescence for quality assessment of apples and pears in the UK. Scientists in the USA have investigated the use of chlorophyll fluorescence to assess fruit stress in stores, but using a different approach to the method described here (www.optisci.com). Scientists at Michigan State University of the USA are also investigating the use of fluorescence to assess shelf-life (but not storage stress) of apple fruit (ADC Newsletter, June 2002).

Hansatech Instruments Ltd, a collaborator and funder of this project have been involved in a collaborative Hortlink project using chlorophyll fluorescence as a potential predictor and quality assurance tool for the likely robustness of pot plants (Poinsettia and Begonia) in home life after distribution and marketing via the multiple retailers (project ended October 2002).

Materials and Methods

Assessing fruit maturity to optimise harvest time

For this study we collaborated with the Quality Fruit Group (QFG) during their 2003 survey of fruit maturity. Fruits were collected from a range of orchards, chosen to have a range of maturity dates in the South East of England. Although the QFG survey included twelve cox orchards, seven Gala orchards and five Conference pear orchards, for this study Cox apples were used from five orchards, Gala from five orchards and Conference pears from four orchards. Thirty fruits were harvested from each orchard either once or twice a week through August and September 2003 (see actual dates below). Immediately after harvest, the fruits were placed in thick black plastic bags and transported to a central location (Gaskains in Selling).

Dates of Quality Fruit Group measurements

Monday 18th August (incomplete set of orchards – no fluorescence measurements taken)

Thursday 21st August

Tuesday 26th August

Thursday 28th August

Monday 1st September

Wednesday 3th September

Thursday 11th September

Thursday 18th September

Assessment of fluorescence characteristics

The fruits from each orchard in turn were transferred without being exposed to light into a darkened box specially designed to allow measurement using a PEA fluorimeter while keeping the fruit dark adapted. 10 medium sized fruit were selected and a chlorophyll fluorescence transient recorded from each. The measurement was taken on the equator and the fluorimeter parameters were set as follows: Pulse light intensity 2000 μE.m-2.s-1, Pulse length 5 s. Each fruit was numbered so that the

fluorescence characteristics could be matched with the quality measurements from that specific fruit.

Quality measurements

Of the original 30 fruits from each orchard, 20 (including the 10 selected for fluorescence measurements) were selected to give an indication of size and weight. Size was measured as total summed diameter and weight as total weight of 20 fruit. The 10 fruit used for the fluorescence measurements were then assessed for background colour (by colour chart) (pears not assessed due to russetting), hardness (by penetrometer, 8mm probe for apples, 11mm probe for pears), total soluble solids (by handheld refractometer), starch conversion (by iodine staining).

Assessment of photosynthetic capacity

A second experiment was conducted using the FMS fluorimeter. The discarded apples from 5 cox orchards were transported to NRI for testing on the next day. These apples were light adapted under a desk lamp. Subsequently the FMS was used to measure ΦPSII (a measure of steady state photosynthetic rate) at light intensity 1000 $\mu E.m^{-2}.s^{-1}$). Pulses were applied at one minute intervals until a steady state was reached. Due to the light adaptation, steady state was achieved within 2 minutes. A far red light was used to measure Fo'. Each apple was then assessed for quality characteristics as described for the quality fruit group.

Modelling

For this season, the relationship between starch level and fluorescence characteristics was determined by creating linear regression models of % starch in terms of fluorescence characteristics using Genstat (Version 6.1). For Conference pears linear regression models of the Streif index were also created. The data used were those for each sample of 10 fruit (One such sample from each orchard on each sampling day) rather than the individual fruits. For each variety, correlation matrices were constructed between % starch (and Streif index for Conference) and fluorescence characteristics. Those characteristics that had the highest correlation with % starch (or Streif index) were tested in the linear regression models. Correlations coefficients

are given in appendix 2. The best models were considered to be those that accounted for the highest % variance.

Detecting fruit stress during long-term storage.

Cox apples were harvested from orchards at East Malling Research. The fruits were graded, and randomised, and then stored under standard conditions (3.5-4.0°C, 1.2% oxygen) from September 2003 until January 2004 when the trial was initiated.

Three storage treatments were used: Control: (3.5-4.0°C, 1.2% oxygen), T1: (1.5 – 2.0°C, 1.2% oxygen), T2: (0 – 0.5°C, 1.2% oxygen). Within this report, for simplicity, these treatments will be referred to as 4°C, 2°C and 0°C respectively.
One controlled atmosphere chamber was maintained under each set of conditions. Each chamber contained 4 boxes of apples (capacity approximately 120 apples) and one "recovery box". The "recovery box" was used for the experiment on recovery of photoinhibition, it had a strip light over the top, and was lined with black material to prevent light affecting apples in the other boxes.

In situ measurement of fluorescence characteristics
For i*n situ* measurement of fluorescence characteristics, probes that interfaced with the Handy Pea were designed and built specifically for this project by Hansatech Instruments Ltd, and were positioned in a box on the lower level of the chamber. Boxes were positioned so that there was space above this box. These probes could be clamped so that they were pressed securely against the surface of an apple. A ring of foam around the detector ensured that the portion of apple being measured was shielded from external light. Two probes were placed in each chamber. The probes were moved onto fresh apples after each measurement. Measurements were carried out on two days each week, such that four measurements were taken for each treatment for each week. Probes were always in position for at least 22 hours before any measurements were recorded.

During the first seven weeks of storage, a standard fluorescence transient of dark adapted tissue was recorded (pulse intensity 2000 $\mu E.m^{-2}.s^{-1}$, pulse length 5s). From

seven weeks onwards, measurements were also taken over a range of increasing light intensities, to allow calculation of photosynthetic rates.

Recovery of photoinhibition.

Initial tests indicated that photoinhibition of PS2 required higher light intensities than originally envisaged. Due to time constraints, during this season of trials, it was not technically feasible to introduce such intensities into the CA chambers. Thus, the photoinhibitory treatment was carried out outside the chambers, but while the apples were still cold..

The procedure used was as follows:

- Open chamber for one treatment and remove 12 apples. (4 apples from each of 3 boxes).
- Label apples, and mark position on apple for subsequent fluorescence measurements (by sticking on a paper ring re enforcer).
- Photoinhibit by placing the light guide of the FMS directly against the surface of the apple, and using 50% actinic light for 4 minutes (approximately 5500 $\mu E.m^{-2}.s^{-1}$).
- Position a closable clip on apple using elasticated fitting.
- Close clip, and dark adapt for one hour at low temperature in appropriate low temperature store (but not under CA conditions).
- Measure fluorescence transient using Handy PEA (pulse intensity 2000 $\mu E.m^{-2}.s^{-1}$, pulse length 5s)..
- Open clip and replace apple in appropriate store in the designated "recovery box" under low level light.
- Repeat this procedure until all 3 storage treatments have been done.

After 24 hours, open store, remove apples, close clip, and dark adapt for one hour at low temperature as before. Measure fluorescence transient using Handy PEA.

Place apples at room temperature under light for a further 24 hours. Dark adapt for one hour at low temperature as before. Measure fluorescence transient using Handy PEA.

Once the measurement had been completed apples maintained under ambient conditions for quality assessment after a further week.

Results and Discussion

Assessing fruit maturity to optimise harvest time

Although both firmness and starch levels are important for assessing harvest maturity, and for some varieties the sugar levels are also considered (e.g. by using the Streif index) starch conversion is probably the single most important factor. This was particularly apparent in 2003 which was an unusual year due to very high sunshine, and therefore very high assimilation. The discussions within the Quality Fruit Group that were observed, suggested that recommendations were given to growers primarily on the basis of starch conversion. Picking was recommended at the point that starch breakdown became obvious as observed by iodine staining. For this reason the initial analysis of the data obtained for Cox and Gala has concentrated on looking at the relationship between starch levels and fluorescence characteristics. For Conference pears we have also considered the Streif index.

To be useable the relationship must be consistent between seasons. For this reason in order to assess the potential of this technique, we need a minimum of two seasons of data. However, in this report we start to examine the relationships that emerge for the first season of work. We are particularly interested where we find similar relationships between varieties.

Initially we have used linear regression analysis, although once we have two seasons of data, we will revisit the analysis and use more sophisticated techniques. Appendix 2 lists the characteristics of the fluorescence transient, and the correlation coefficients between these characteristics and starch %, and with Streif index. Those characteristics with the highest correlations were used where constructing models.

Although several linear regression models can be constructed, those shown in Table 1.1 and illustrated in Figures 1.1-1.3 have been selected in particular, as the same characteristics provide reasonable models for all varieties. The % variance accounted for gives an indication as to how good each model is (a better fit gives a higher %).

Several of the characteristics considered are expressed as actual measures of the photosynthetic apparatus. These have been calculated from the fluorescence measurements using a theoretical framework developed by Strasser *et al.* (references need to be included) For example RC/CS (reaction centres/cross section) is a measure of photosystem 2 reaction centres per surface area, while TR/CS (transfer/cross section) is a measure of the rate of photosynthetic reaction per surface area. F3 is the fluorescence yield after 300 μs (Check this).

Table 1.1: Linear regression models of starch levels (and Streif index for Conference) using fluorescence characteristics

Fruit/variety	Models	% variance accounted for
Apple	-22.5+0.1001 F3	43.4
Cox	-0.7 + 0.1854 RC/CS	24.7
	-14.2 + 0.3290 TR/CS	26.1
	161.2 – 52.39 Colour score	48.3
Apple	19.67 + 0.07729 F3	75.2
Gala	22.28 + 0.2162 RC/CS	56.3
	11.17 + 0.3341 TR/CS	72.2
	145.5 – 26.57 Colour	27.2
Pear	-72.8 + 0.1768 RC/CS	44.5
Conference	-43.4 + 0.305 TR/CS	14.4
Pear	6.93 – 13.88 Mo – 0.0811 N	30.9
Conference	-3.93 + 0.00715 RC/CS	33.2
Streif index		

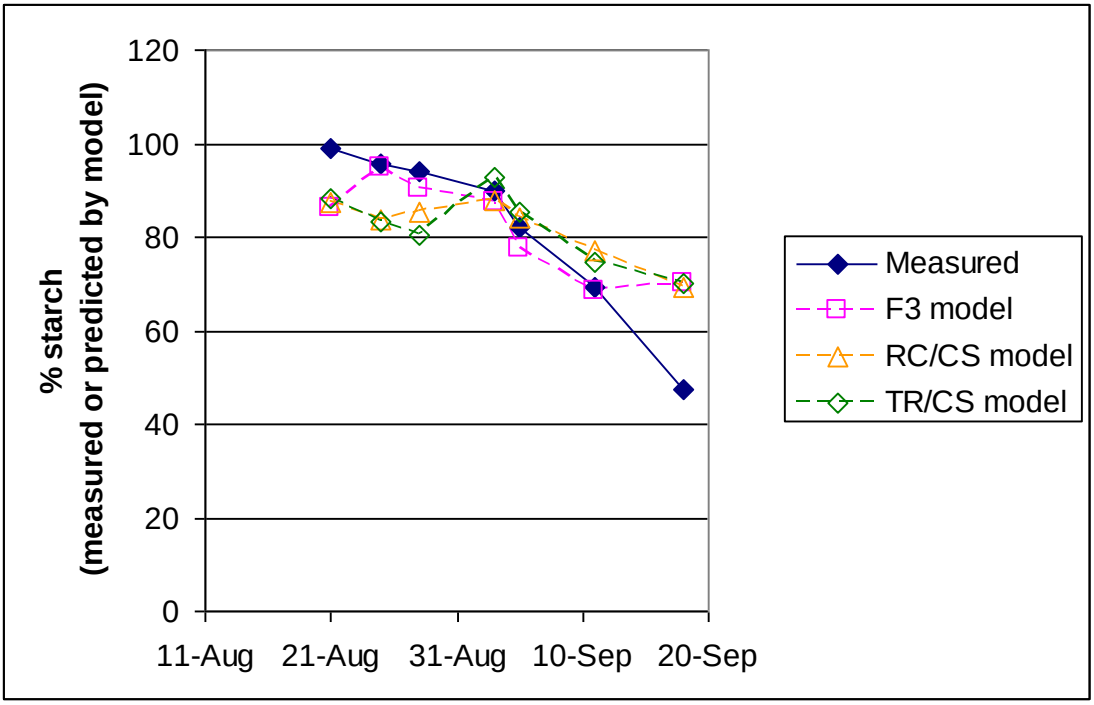


Figure 1.1: Cox apples. Average starch levels measured over 4 weeks, and levels predicted by models.

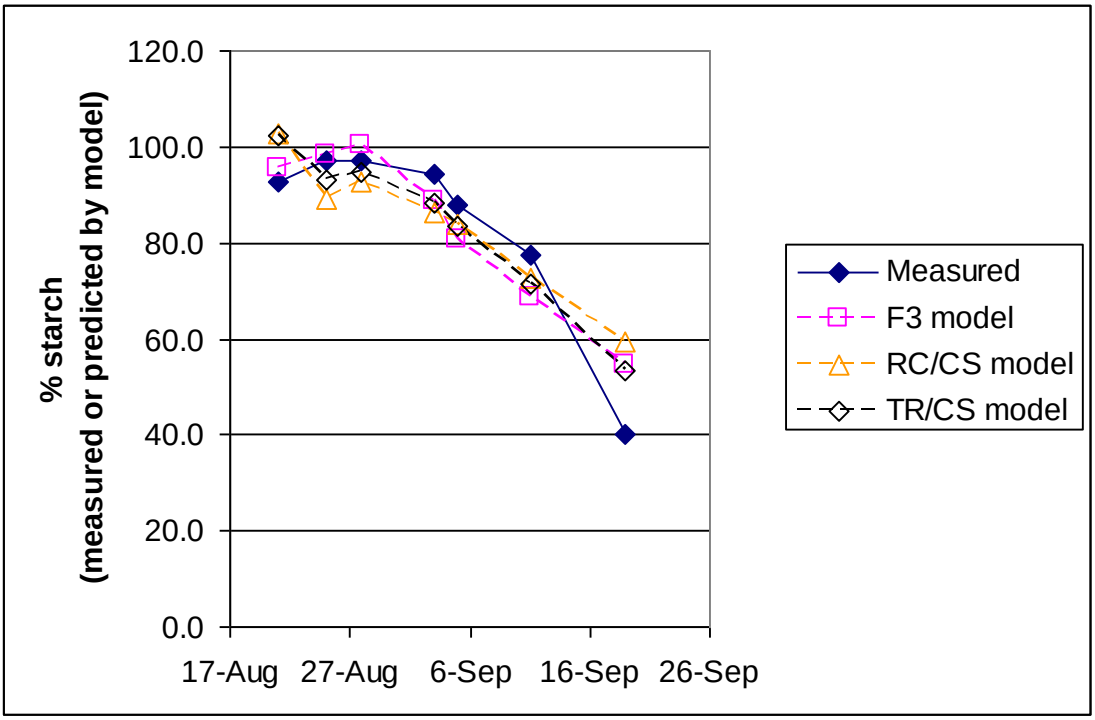
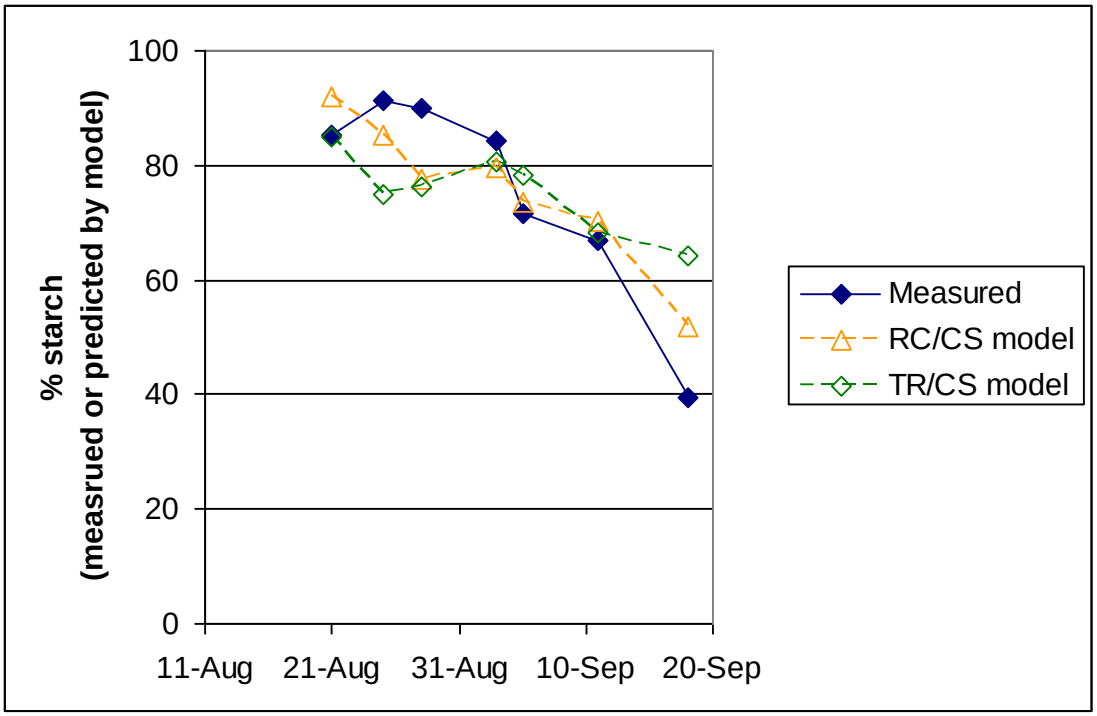


Figure 1.2: Gala apples. Average starch levels measured over 4 weeks, and levels predicted by models.



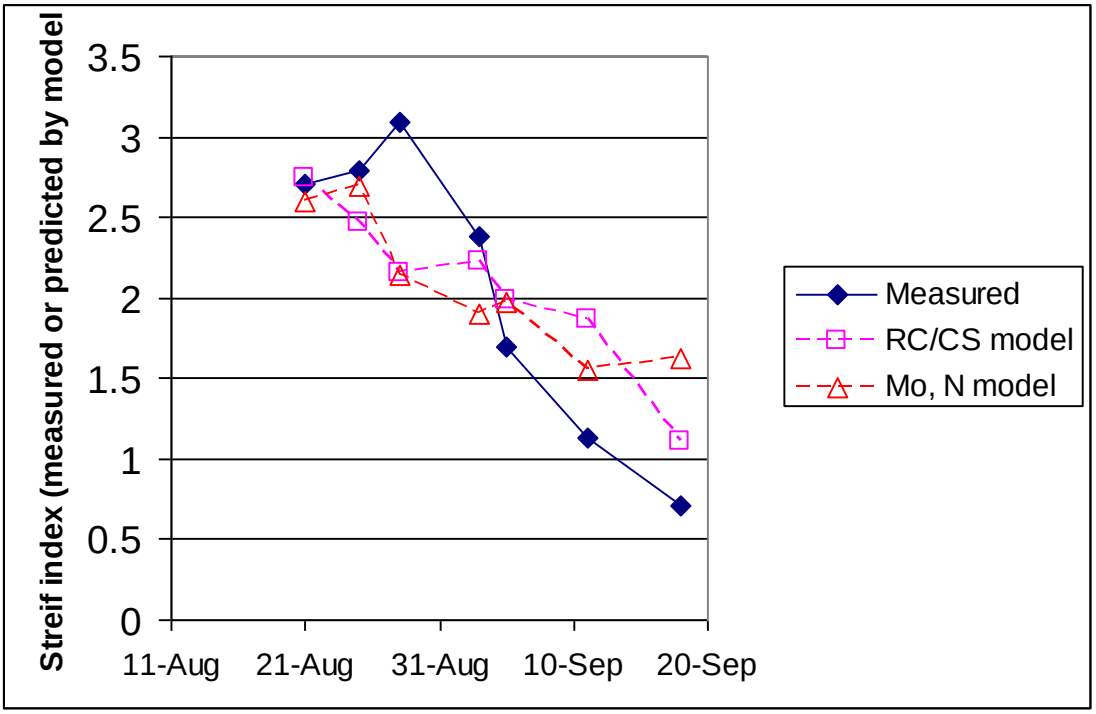


Figure 1.3: Conference pears. A) Average starch levels measured over 4 weeks, and levels predicted by models. B) Streif index measured over 4 weeks and index predicted by models.

For Cox and Gala it was possible to compare the fluorescence models with colour scoring. Although for Cox, colour scoring was as accurate as the fluorescence models, In the case of Gala the fluorescence models were considerably better. Conference is not easy to assess for background colour due to russetting.

The individual values measured and predicted for each 10 fruit sample are given in appendix 3.

Assessment of photosynthetic capacity of Cox apples

A separate trial was carried out to see if harvest maturity might be related to the photosynthetic capacity of apples. This can be assessed by measuring a characteristics known as ΦPS2. Figure 1.4 shows the Photosynthetic capacity (ΦPS2 x light intensity) and starch for Cox samples over the measurement period. While the Starch decreases at a fairly constant rate as seen in the other trial, the photosynthetic capacity shows a distinct jump at the start of September. This is possibly an indication that levels of sunlight increased at this time. This suggests that if this characteristic is sensitive to short-term weather fluctuations, it would not be useful for assessing harvest maturity.

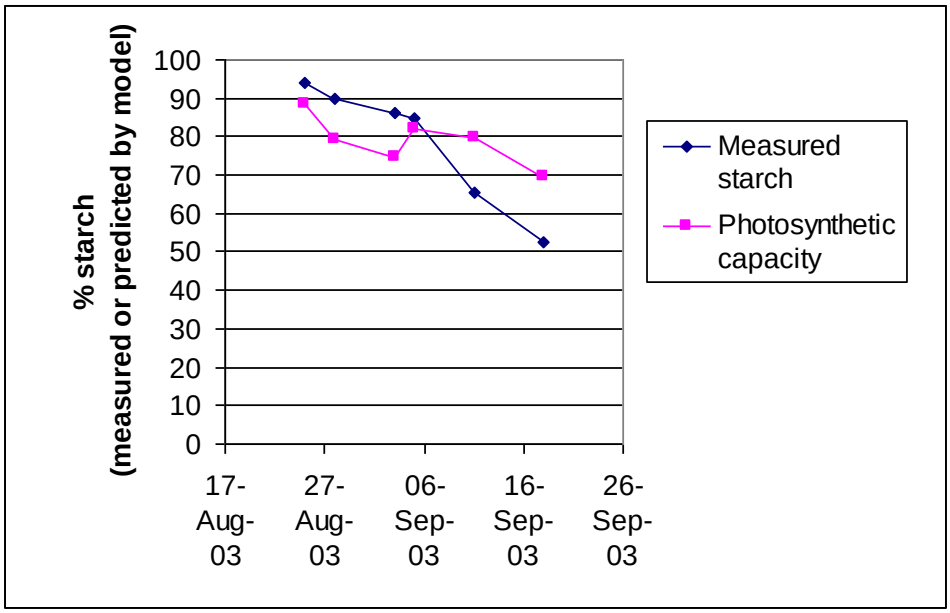


Figure 1.4 % starch, and photosynthetic capacity of Cox apples sampled over four weeks. For photosynthetic capacity, random units are used to allow direct comparison between the two factors.

Detecting fruit stress during long-term storage.

Apple quality during storage

Apples removed from storage were cut and assessed for quality after a week of storage under ambient conditions to allow physiological disorders to develop visible symptoms. Unfortunately, for the purposes of this trial, very few apples developed physiological damage (Figures 2.1 a-c). We believe that during this season of trials the apples were particularly resistant to low temperature damage due to the high temperatures to which they were exposed during the growing season. This would have been exacerbated as they were not removed to low temperatures until after 3 months of storage.

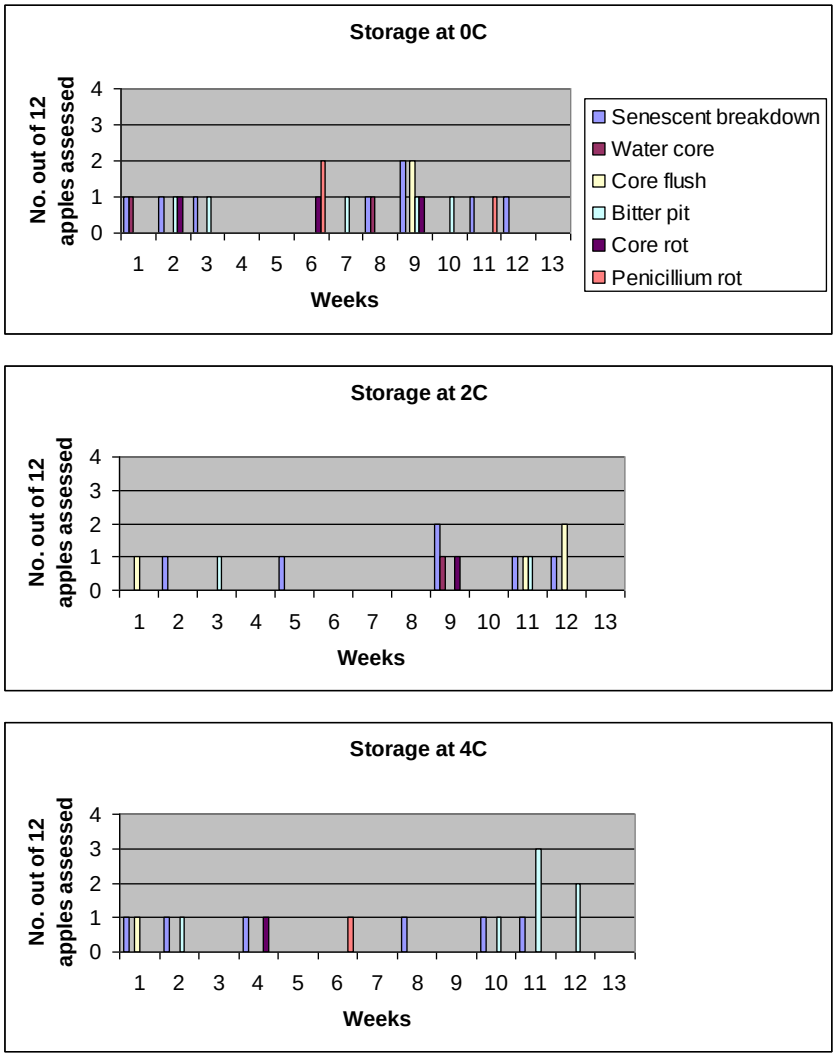


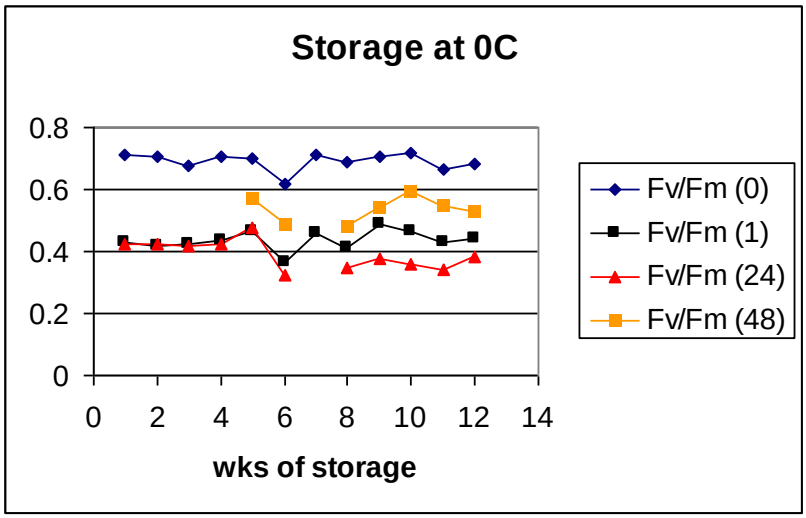
Figure 2.1. Physiological disorders, and rots observed in apple samples removed from storage and after a further 7 days at ambient. 12 apples were sampled from each treatment each week.

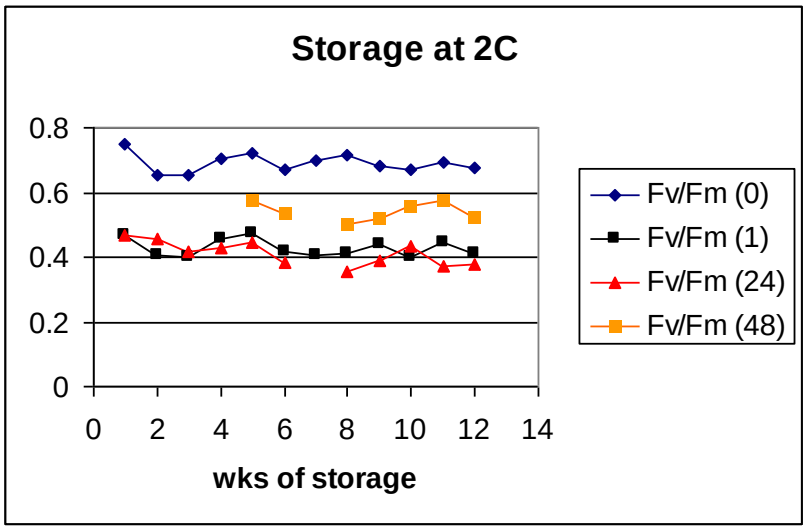
PS2 recovery

The main strategy that we were investigating to assess fruit stress was by considering the rate of resynthesis of PS2 following light induced damage. Damage can be measured by a decrease in the parameter Fv/Fm, and resynthesis by the subsequent increase in this parameter. Initial pilot studies, not presented here, were carried out to determine the light intensity needed to produce sufficient damage of PS2, and also to determine the normal rate of resynthesis. The light intensity needed to produce a sufficient decrease in PS2 was greater than we originally envisaged. For this reason, the damage could not be carried out *in situ,* within the stores, but had to be applied on cold apples immediately after removal from stores. We also discovered that a low level of light increased the rate of resynthesis. For this reason, apples were exposed to a light in the store during the recovery period..

Figures 2.2a-c shows the Fv/Fm before and after photoinhibitory treatment, and during recovery for the three storage treatments over 13 weeks of storage. As well as 24 hours of recovery in the store, apples were left under ambient conditions for a further 24 hours to look at the rate of recovery under these conditions.

The initial Fv/Fm (measured by the FMS) was very similar for the three storage treatments, and showed no trend during the storage period. The extent of photoinhibition also appeared to be very similar for the apples from the three treatments. At low temperature there appeared to be no recovery of Fv/Fm, but once the apples were returned to ambient significant recovery was observed. For the apples stored at 0°C rather than recovering, the Fv/Fm decreased over 24 hours storage at low temperature from week 8 onwards. This is the type of response that we had hoped to see. We would take this as indicating that the tissues were becoming increasingly stressed, and would therefore be less capable of protecting themselves against physiological damage.





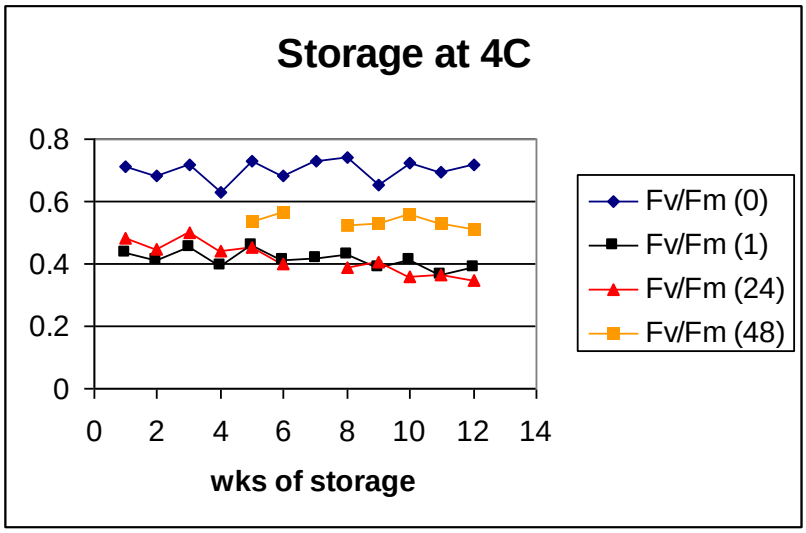


Figure 2.2: Fv/Fm measured before and after photoinhibition, after 24 hours recovery in store and after a further 24 hours recovery at ambient. Each point is the mean for 12 apples (4 from each of 3 boxes). Data for week 7 is incomplete as a different strategy was tested on this day.

Figures 2.3 a and b show Fv/Fm and Fo over 13 weeks of storage for the three storage conditions, while a wider range of characteristics for the three treatments is given in appendix 4.

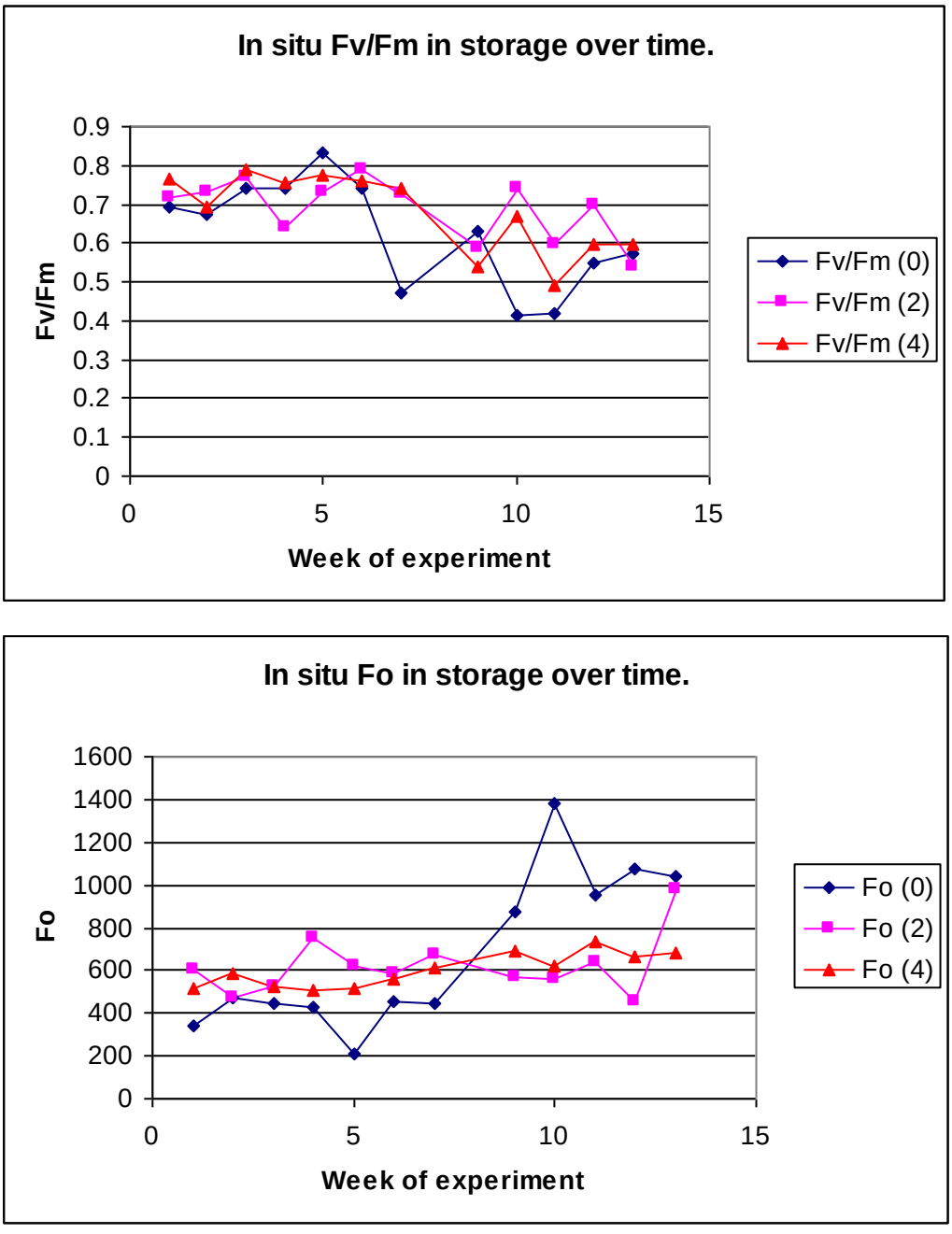


Figure 2.3: Fv/Fm and Fo of Cox apples measured in situ during low temperature storage. Each point is the mean of measurements on 2-4 apples.

A gradual decrease in Fv/Fm is seen at 0°C and 4°C. For 0°C this is particularly apparent from 7 weeks onwards, and this is associated with a rise in Fo from 9 weeks onwards. At 0°C we would expect apples to start to show symptoms of low temperature damage, while at 4°C, we would see fruit senescence more rapidly that at the other storage temperatures.

The FMS and PEA show slight differences here. The FMS does not appear to see the rise in Fo, and therefore the decrease in Fv/Fm. The two machines work on a different basis, and this could suggest a population of PS2 closed rapidly by low light intensities, perhaps disconnected from the rest of the electron transfer chain.

In situ measurements of photosynthetic rates

During these trials, an additional strategy for assessing the fruit was tested from 7 weeks onwards. Apples were exposed to a range of increasing light intensities within the store, and a technique used to measure photosynthetic rate at each light intensity. (Essentially ΦPS2 was measured as for the fruit maturity study.) The objective was to obtain the relationship between light intensity and photosynthetic rate, and thus to assess maximum photosynthetic capacity, and the light intensity at which this was achieved. A typical set of data is shown in Figure 2.4.

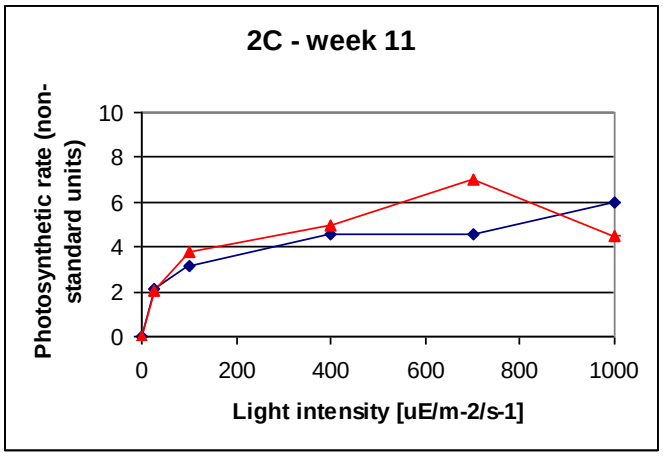


Figure 2.4: Photosynthetic rate over a range of light intensities, calculated from fluorescence characteristics for two apples stored at 2ºC for 11 weeks.

Although light intensities up to 2000 μE.m-2.s-1 were used, only those up to 1000 is presented, as the data obtained at the higher light intensities was very noisy for technical reasons. In most cases the photosynthetic rate appeared to reach a maximum at about 1000 μE.m-2.s-1. No trend in maximum rate during storage was seen, but only 2-4 fruits were assessed per storage treatment per week. If this method is assessed in the last season, only the photosynthetic rate at 1000 need be measured, and more apples will be included.

Conclusions

Assessing fruit maturity to optimise harvest time

For the 2003 season good models of starch levels can be constructed for Gala, Cox and Conference, (and of Streif index for Conference) using chlorophyll fluorescence characteristics alone. The models are superior to other non-destructive methods tested (i.e. colour scoring) except for Cox, where colour provided a slightly better model this year. The true practical value of these models can only be tested once a second year of data has been collected to determine whether the models are consistent between seasons.

Given the importance of starch levels for determining harvest maturity, a second season of trials should include a measurement of starch that can detect the early stages of breakdown which cannot be detected by iodine staining.

Detecting fruit stress during long-term storage.

Methods for assessing fruit stress in storage have now been developed and can be practically applied in a store. However, their value in assessing stress cannot yet be determined, as in the present storage trials very little apple damage was observed. Reasons for this are presented in the results section.

Interestingly two different measurement methods indicated that apples at 0°C are showing stress from about 7 weeks onwards. Unfortunately the trials did not produce sufficient fruit stress to see this translated to visible damage.

Technology Transfer

Feature article scheduled for HDC News, August 2004.

References

Ahmed, N, Perez, R. and Beaudry R.M. (1998) Chlorophyll fluorescence and whole fruit senescence in “Golden Delicious” apple. Acta Horticulturae 464: 121-126

Briantais, J-M, Vernotte, C, Krause, G.H and Weis, E (1986). Chlorophyll a Fluorescence of Higher Plants: Chloroplasts and Leaves. In : Light Emission by Plants and Bacteria, pp 539 - 583.

Ross, M.J. (2002) An investigation into chlorophyll fluorescence as a non-invasive tool for optimising harvesting time and storage conditions for fruit. PhD thesis University of Greenwich, UK

Schreiber, U and Bilger, W (1993). Progress in Chlorophyll Fluorescence Research : Major Developments During the Past Years in Retrospect. In : Progress in Botany, Springer Verlag, Berlin.

Smillie, R.M et al.(1987). Applications of Chlorophyll Fluorescence to the Physiology and Storage of Mango and banana Fruit and the Chilling Tolerance of Mango Cultivars, Asean Food Journal 3: 55 - 59.

Tian, M.S, Woolf, A.B and Ferguson, I.B (1996). Changes in the Colour and Chlorophyll Fluorescence of Broccoli Florets Following How Water Treatment, Journal of the American Society of Horticultural Science 121: 310 - 313.

Toivonen, PMA (1992) Chlorophyll fluorescence as a nondestructive indicator of freshness in harvested broccoli. HortScience. 1992, 27: 1014-1015

van Kooten, O.V et al. (1992). Chilling Damage of Dark Stored Cucumbers Affects the Maximum Quantum Yield of Photosystem II, In : Research in Photosynthesis IV, pp 161 - 164, Kluwer Academic Publishers,

Appendix 1
Quality characteristics used to determine correct maturity for harvest

Cox apples

For Cox apples the recommendations are that harvest time be determined by the Firmness and Starch levels as follows: This assumes CA storage for any storage beyond mid-October (*The best practice guide for UK apple production.).*

Marketing periods	Minimum values at harvest	
	Firmness (Kg) Penetrometer fitted with an 11mm probe	Starch (% cut surface stained black)
Feb/March	8.6	75
Jan/Feb	8.2	70
December	8.0	60
Mid November	7.7	60
Mid October	7.5	50
Immediate	6.5	<50

Gala apples

For Gala apples, fruit should be picked when the starch coverage is 50-90% and firmness in excess of 7 Kg (*The best practice guide for UK apple production.*.

Conference pears

For Conference pears I THINK that the Strief index is used to determine harvest maturity.

Strief index = Firmness (N)/(TSS% x Starch conversion index)

% starch	Starch conversion index
100	1
80	2
75	3
65	4
50	5
30	6
25	7
10	8
5	9
0	10

Appendix 2: Correlation coefficients between fluorescence characteristics and Streif index, and also %Starch for Cox, Gala and Conference

Fluor. parameters	Correlation (r) with Streif index (by sample)			Correlation (r) with Streif index (by individual fruit)		
	Cox	Gala	Conf.	Cox	Gala	Conf.
Colour	-0.803	-0.587		-0.511	-0.445	
F0	(0.215)	0.852	0.264	0.163	0.503	0.111
Fv	0.618	0.811	0.322	0.340	0.526	0.159
Fm	0.665	0.842	0.344	0.380	0.581	0.173
Fv/Fm	0.354	0.573	0.134	0.139	0.205	0.075
F1	0.527	0.873	0.175	0.319	0.615	0.073
F2	0.553	0.891	0.076	0.325	0.639	0.028
F3	0.674	0.872	-0.030	0.399	0.650	-0.005
F4	0.711	0.858	0.213	0.437	0.614	0.112
F5	0.699	0.843	0.441	0.408	0.575	0.211
Area	(-0.225)	0.303	-0.220	-0.100	0.072	-0.091
Tfm	-0.452	-0.449	-0.370	-0.180	-0.177	-0.130
Mo	(-0.147)	0.356	-0.488	-0.050	0.198	-0.180
Vj	(-0.052)	0.186	-0.268	-0.018	0.113	-0.112
Vi	0.503	0.330	0.239	0.239	0.142	0.172
Sm	-0.507	-0.581	-0.340	-0.224	-0.238	-0.145
N	-0.602	-0.592	0.448	-0.262	-0.258	-0.197

Fluor. parameters	Correlation (r) with Streif index (by sample)			Correlation (r) with Streif index (by individual fruit)		
	Cox	Gala	Conf.	Cox	Gala	Conf.
TR/ABS	0.354	0.573	0.134	0.139	0.205	0.075
ET/ABS	(0.194)	0.211	0.304	0.069	0.026	0.124
ET/TR	(0.052)	-0.186	0.268	0.018	-0.113	0.112
ABS/RC	-0.325	-0.335	-0.505	-0.117	-0.090	-0.189
TR/RC	(-0.222)	-0.328	-0.544	-0.078	0.189	-0.195
ET/RC	(-0.228)	-0.013	-0.228	-0.081	-0.028	-0.119
DI/RC	-0.341	-0.530	-0.317	-0.120	-0.181	-0.136
RC/CS	0.551	0.777	0.598	0.339	0.521	0.278
ABS/CS	(0.215)	0.852	0.264	0.163	0.503	0.111
TR/CS	0.484	0.878	0.339	0.297	0.580	0.164
ET/CS	0.378	0.802	0.361	0.241	0.504	0.185
DI/CS	(-0.135)	0.471	0.013	-0.018	0.214	-0.020

Fluor. parameters	Correlation (r) with Starch (by sample)			Correlation (r) with Starch (by individual fruit)		
	Cox	Gala	Conf.	Cox	Gala	Conf.
Colour	-0.706	-0.542		-0.427	-0.432	
F0	0.299	0.835	0.357	0.228	0.509	0.148
Fv	0.519	0.784	0.314	0.290	0.536	0.131
Fm	0.578	0.815	0.349	0.340	0.592	0.150
Fv/Fm	(0.240)	0.592	0.046	0.073	0.239	0.022
F1	0.577	0.873	0.204	0.369	0.626	0.057
F2	0.610	0.891	0.080	0.376	0.641	-0.004
F3	0.671	0.872	-0.094	0.416	0.653	-0.073
F4	0.659	0.835	0.170	0.419	0.623	0.060
F5	0.610	0.809	0.373	0.368	0.582	0.154
Area	(-0.068)	0.421	-0.078	-0.017	0.103	-0.047
Tfm	-0.325	-0.323	-0.374	-0.092	-0.139	-0.124
Mo	(-0.031)	0.394	-0.589	0.020	0.210	-0.241
Vj	(0.062)	0.243	0.381	0.044	0.123	-0.175
Vi	0.430	0.241	0.049	0.212	0.123	0.049
Sm	-0.306	-0.431	-0.191	-0.117	-0.201	-0.093
N	-0.383	-0.411	-0.318	-0.142	-0.205	-0.165

Fluor. parameters	Correlation (r) with Starch (by sample)			Correlation (r) with Starch (by individual fruit)		
	Cox	Gala	Conf.	Cox	Gala	Conf.
TR/ABS	(0.240)	0.592	0.046	0.073	0.239	0.022
ET/ABS	(0.053)	0.174	0.397	-0.002	0.036	0.168
ET/TR	(-0.062)	-0.243	0.381	-0.044	-0.123	0.175
ABS/RC	(-0.207)	-0.361	-0.527	-0.047	-0.127	-0.212
TR/RC	(-0.112)	0.361	-0.605	-0.012	0.212	-0.243
ET/RC	(-0.251)	-0.018	-0.160	-0.085	-0.009	-0.104
DI/RC	(-0.238)	-0.574	-0.253	-0.061	-0.234	-0.104
RC/CS	0.520	0.759	0.683	0.340	0.530	0.327
ABS/CS	0.299	0.835	0.357	0.228	0.509	0.148
TR/CS	0.532	0.854	0.421	0.345	0.587	0.192
ET/CS	0.344	0.779	0.477	0.240	0.524	0.246
DI/CS	(-0.030)	0.479	0.113	0.045	0.215	0.024

Appendix 3: Starch levels measured and predicted for each fruit sample (each orchard on each sampling date). For Conference, measured and predicted Streif index is also given.

Cox

		21-Aug	25-Aug	28-Aug	03-Sep	05-Sep	11-Sep	18-Sep
Anthony	Starch	99.0	99.1	99.8	97.5	98.6	83.0	50.0
	F3 model	89.6	113.4	90.7	98.5	77.3	61.0	70.0
	RC/CS model	85.4	74.4	69.1	85.6	85.0	69.1	60.7
	TR/CS model	87.3	80.0	68.8	99.4	86.0	64.2	64.8
Bardsley	Starch	98.3	90.8	89.0	83.0	72.0	47.5	36.0
	F3 model	95.0	86.0	84.2	71.9	75.5	60.9	56.6
	RC/CS model	88.0	79.1	86.5	76.4	74.0	80.5	63.0
	TR/CS model	101.7	84.8	80.7	79.3	84.0	76.2	62.4
Broadfield	Starch	99.6	96.8	99.3	93.0	73.5	74.0	43.0
	F3 model	97.6	94.5	95.9	91.5	70.3	69.9	82.6
	RC/CS model	85.5	81.5	80.8	87.0	77.9	78.2	65.9
	TR/CS model	89.3	80.8	78.8	90.3	75.0	80.0	67.5
Wakely	Starch		92.3	92.0	79.5	79.0	60.0	39.0
	F3 model		92.4	88.6	83.8	76.9	76.8	67.8
	RC/CS model		84.6	98.7	89.4	87.5	77.1	88.7
	TR/CS model		77.6	86.5	93.6	85.2	76.2	82.8
Wares	Starch	99.2	99.4	89.9	96.3	87.5	82.0	69.0
	F3 model	62.9	88.6	92.4	91.6	88.6	72.7	73.0
	RC/CS model	90.4	99.9	91.0	100.0	97.2	80.1	69.0
	TR/CS model	74.4	92.8	87.7	102.1	97.3	76.3	73.6
Overall	Starch	99.0	95.7	94.0	89.9	82.1	69.3	47.4
	F3 model	86.3	95.0	90.3	87.4	77.7	68.3	70.0
	RC/CS model	87.3	83.9	85.2	87.7	84.3	77.0	69.5
	TR/CS model	88.2	83.2	80.5	92.9	85.5	74.6	70.2

Gala

		21-Aug	25-Aug	28-Aug	1-Sep	3-Sep	11-Sep	18-Sep
Broadfield	Starch	98.2	98.6	97.6	93.5	94.5	85.5	41.0
	F3 model	89.6	95.5	91.6	84.8	82.2	68.4	59.7
	RC/CS model	88.9	87.6	87.7	84.9	84.0	74.1	65.6
	TR/CS model	91.8	91.2	90.8	86.9	86.4	70.1	61.9
Broadwater	Starch	92.3	94.7	94.8	95.3	85.5	69.0	37.0
	F3 model	86.7	99.1	101.3	85.5	82.0	64.4	48.6
	RC/CS model	89.6	89.3	87.3	83.8	93.3	73.3	56.0
	TR/CS model	88.5	91.9	88.8	83.4	90.5	70.3	47.5
Dodges	Starch	98.5	97.8	98.3	97.5	86.5	84.5	49.0
	F3 model	104.6	103.9	108.2	102.9	85.6	82.6	64.8
	RC/CS model	110.1	95.1	93.5	91.6	84.1	78.5	65.8
	TR/CS model	117.5	102.1	96.9	94.5	84.5	82.2	61.9
Honoton	Starch	95.5	97.3	97.4	93.0	86.5	67.5	39.0
	F3 model	97.2	91.7	100.2	89.7	77.8	62.3	55.6
	RC/CS model	100.2	84.4	98.6	88.5	76.1	63.8	58.3
	TR/CS model	104.5	89.2	95.6	94.6	75.9	64.9	52.4
Wakely	Starch	80.0	97.6	97.2	91.5	86.0	81.5	35.0
	F3 model	100.2	101.6	99.8	80.9	75.8	66.1	43.6
	RC/CS model	125.2	89.6	96.8	82.1	82.8	73.4	50.5
	TR/CS model	110.2	91.4	100.5	81.8	79.8	70.3	44.1
Overall	Starch	92.9	97.2	97.1	94.2	87.8	77.6	40.2
	F3 model	95.7	98.4	100.2	88.7	80.7	68.8	54.5
	RC/CS model	102.8	89.2	92.8	86.2	84.1	72.6	59.2
	TR/CS model	102.5	93.2	94.5	88.2	83.4	71.6	53.6

Conference

		21-Aug	25-Aug	28-Aug	03-Sep	05-Sep	11-Sep	18-Sep
Bewely	Starch	94.9	91.5	98.3	92.5	85.0	81.0	77.0
	RC/CS model	94.1	86.2	68.4	91.4	82.5	86.0	60.5
	TR/CS model	75.6	74.3	78.0	79.4	86.6	74.1	67.8
Highland	Starch		95.1	97.0	93.5	80.0	66.5	41.0
	RC/CS model		79.1	72.0	70.3	54.9	56.4	61.2
	TR/CS model		63.7	72.0	72.5	61.9	64.7	67.2
Honoton	Starch	84.5	89.5	80.5	76.0	48.0	63.5	12.0
	RC/CS model	91.9	90.0	91.4	81.6	77.5	76.8	41.3
	TR/CS model	91.9	77.9	79.9	82.4	81.0	69.3	63.7
Redsell	Starch	76.0	88.8	84.0	74.5	73.0	57.0	28.0
	RC/CS model	90.4	86.0	78.5	74.5	79.1	62.1	44.4
	TR/CS model	87.0	83.7	74.6	88.3	83.7	65.1	57.7
Overall	Starch	85.1	91.2	90.0	84.1	71.5	67.0	39.5
	RC/CS model	92.1	85.3	77.6	79.5	73.5	70.3	51.8
	TR/CS model	84.9	74.9	76.1	80.6	78.3	68.3	64.1

		21-Aug	25-Aug	28-Aug	03-Sep	05-Sep	11-Sep	18-Sep
Bewely	Streif	3.65	2.98	3.59	2.88	2.24	1.74	1.25
	RC/CS model	2.82	2.50	1.78	2.71	2.35	2.49	1.46
	Mo,N model	2.73	2.48	1.73	2.38	1.94	2.30	1.60
Highland	Streif		3.13	3.65	2.96	2.03	0.92	0.66
	RC/CS model		2.21	1.93	1.86	1.23	1.30	1.49
	Mo,N model		3.06	2.14	2.24	2.26	1.87	2.48
Honoton	Streif	2.55	2.73	2.33	2.02	0.88	0.96	0.39
	RC/CS model	2.73	2.65	2.71	2.32	2.15	2.12	0.68
	Mo,N model	2.36	2.84	2.35	1.68	1.79	1.56	0.67
Redsell	Streif	1.92	2.32	2.82	1.68	1.64	0.89	0.54
	RC/CS model	2.67	2.49	2.19	2.03	2.21	1.53	0.81
	Mo,N model	2.69	2.38	2.34	1.31	1.89	0.49	1.73
Overall	Measured	2.71	2.79	3.10	2.38	1.70	1.12	0.71
	RC/CS model	2.74	2.46	2.15	2.23	1.99	1.86	1.11
	Mo, N model	2.59	2.69	2.14	1.90	1.97	1.56	1.62

Appendix 4: Fluorescence characteristics measured in situ during storage. Each number is the mean of 2-4 measurements.

Temp	Week	Fo	Fm	Fv/Fm	Tfm ms	Area	F1	F2	F3	F4	F5
0	1	345	1114	0.692	2750	88900	391	436	577	777	917
	2	475	1704	0.675	2600	112800	565	648	915	1183	1345
	3	444	1748	0.743	2325	136350	535	626	915	1176	1356
	4	425	1815	0.740	2567	133333	516	596	900	1287	1403
	5	212	1308	0.831	2750	140550	268	312	524	815	921
	6	458	1788	0.739	3400	203550	570	680	1001	1275	1412
	7	442	1181	0.472	3400	109600	491	543	696	859	923
	9	872	2331	0.631	2788	200500	964	1058	1365	1670	1817
	10	1383	2092	0.412	3000	72667	1445	1514	1679	1772	1875
	11	950	1548	0.419	3275	67300	987	1020	1139	1308	1381
	12	1072	2354	0.550	2675	112900	1147	1218	1487	1834	1972
	13	1044	2413	0.571	2300	132500	1123	1197	1470	1828	1991
2	1	603	2151	0.717	2000	100950	732	866	1221	1529	1785
	2	474	1892	0.730	2250	146950	578	675	985	1301	1486
	3	527	2388	0.771	2175	165350	634	739	1114	1522	1786
	4	751	2080	0.640	2967	117667	877	1016	1360	1663	1747
	5	624	2345	0.734	2400	156800	800	993	1447	1767	1844
	6	589	2796	0.790	2450	212400	756	915	1429	1887	2038
	7	669	2457	0.726	2550	187600	821	985	1396	1714	1846
	9	569	1579	0.588	3250	125450	639	714	934	1139	1240
	10	560	2170	0.741	2100	145200	700	842	1253	1597	1703
	11	635	1671	0.597	2100	124900	698	768	945	1180	1310
	12	455	1577	0.700	2350	107500	503	546	729	1102	1248
	13	978	2199	0.539	1750	80500	1115	1278	1617	1850	1940

Temp	Week	Fo	Fm	Fv/Fm	Tfm ms	Area	F1	F2	F3	F4	F5
4	1	513	2179	0.766	2025	133000	616	712	1048	1465	1712
	2	590	2107	0.694	2050	127800	704	810	1197	1580	1696
	3	529	2505	0.789	1950	158400	686	827	1344	1786	1889
	4	506	2174	0.758	1800	121200	644	773	1216	1604	1706
	5	515	2304	0.773	1925	128350	653	777	1233	1678	1805
	6	558	2397	0.761	1875	146350	710	859	1331	1724	1843
	7	614	2389	0.743	1700	128250	777	934	1414	1789	1894
	9	693	1671	0.539	2475	105975	789	891	1147	1331	1396
	10	623	1910	0.668	1950	77450	760	901	1265	1515	1606
	11	737	1500	0.493	1700	52000	800	872	1037	1185	1276
	12	668	1738	0.599	1700	59650	757	851	1118	1404	1503
	13	682	1846		0.596	1450	55100	796	914	1225	1470