

Project title: Application of chlorophyll fluorescence for prediction of harvest maturity in broccoli

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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.

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CONTENTS

Headline.....	1
Background.....	1
Summary	2
Financial Benefits	4
Action Points.....	4
Introduction	5
Materials and methods	8
Season 1 field trials: To relate chlorophyll fluorescence to broccoli maturity for two broccoli varieties.....	8
Season 2 field trials: To test the measurement protocol, and to test/ refine the CF predictive model for broccoli harvested over a wider range of conditions.....	8
Season 3 field trials: To validate the prediction model, to test a specially developed sensor head and to test the effect of mineral nutrition on head keeping qualities.....	9
Chlorophyll fluorescence (CF) measurements.....	9
Chlorophyll Fluorescence measurement protocol.....	11
Testing double pulse method.....	12
Trial A: To optimise double pulse protocol.....	12
Maturity assessment.....	14
Colour measurements	14
Results.....	16
The development and testing of algorithms using of Chlorophyll Fluorescence (CF) characteristics to predict broccoli storage life.....	16
Season 1 field trials: To relate chlorophyll fluorescence to broccoli maturity for two broccoli varieties.....	16
Season 2 field trials: To test the measurement protocol, and to test/ refine the CF predictive model for broccoli harvested over a wider range of conditions.....	18
Season 3 field trials: To validate the prediction model and to test a specially developed sensor head and to test the effect of mineral nutrition on head keeping qualities.....	22
Testing a specialised sensor head.....	26

Field nutrition and broccoli head quality.....	27
Practical aspects of CF measurement	28
In season 1 it was shown that measurement in sunlight is impractical.	28
Discussion	29
Conclusions	30
Knowledge and Technology Transfer	30
Acknowledgements.....	30
Glossary.....	31
References	31
Appendix 1	33

GROWER SUMMARY

Headline

A non-destructive measurement of harvested broccoli heads able to predict storage potential using chlorophyll fluorescence has been identified and tested over three seasons. A practical and portable instrument to use by the broccoli industry is being developed.

Background

It is difficult to pinpoint exactly when broccoli is at the right harvest maturity for good storage behaviour and shelf-life. Areas of a crop with seemingly identical heads, harvested at the same time, can show widely differing keeping qualities. This creates an obvious problem for scheduling a crop that, with variability in weather and consumer demand through the season, may need to be stored for up to three weeks to balance supply and demand.

The technology of chlorophyll fluorescence could potentially be used to monitor the maturity and health of broccoli heads. This project investigated two key questions: can chlorophyll fluorescence be used to assess heads at harvest for their subsequent keeping quality?; and can the technology be used to inform crop management decisions in the field and after harvest?

Chlorophyll fluorescence

Green plant tissues contain chloroplasts, the microscopic organs within the cells where photosynthesis takes place. The chlorophyll molecules in the chloroplasts absorb sunlight. Most of the energy received is used to drive photosynthesis which in turn supplies energy to the plant, but a portion is unused and re-emitted by the chlorophyll as fluorescence. The more active the chloroplasts the more energy is released as fluorescence.

For decades scientists have used this as a tool to study some fundamental aspects of photosynthesis. For example, it can indicate both the concentration and the activity or health of chloroplasts within plant tissue. Chloroplasts are very sensitive, rapidly losing activity if the tissues become stressed, so that measuring chlorophyll fluorescence has been used to assess crop health in the field and, in particular, disease load for arable crops. Changes in fruit and vegetable maturity are also associated with changes in chloroplast function and concentration. The ripening of most fruit involves very significant loss of green colour and this is mostly due to a loss of chloroplasts. It is already known from work in project TF 142, that chlorophyll fluorescence can be a valuable tool to assess maturity of tree fruit.

Summary

As a technique that can measure both the concentration and the activity/health of chloroplasts within plant tissues, chlorophyll fluorescence has been used to assess maturity and health for a wide range of crops. Specifically chlorophyll fluorescence has been used to map changes in the health of broccoli during storage and shelf-life (FV395) where a decline in the number of active chloroplasts is correlated with a reduction in head quality leading to senescence.

Within this project we have demonstrated the following: We have demonstrated that a measurement of chlorophyll fluorescence characteristics of broccoli heads taken immediately after harvest can be used to predict storage quality of broccoli consignments. This has been confirmed over three seasons. The following three figures show actual quality of broccoli consignments following 2 weeks of storage (expressed in terms of a maturity index) plotted against the quality predicted from a measurement taken immediately after harvest.

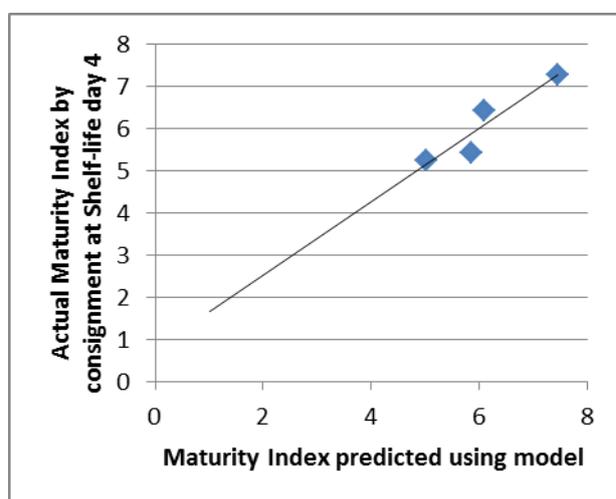


Figure A. 2014 Actual v predicted Maturity Index after 4 days shelf-life using the model developed using head diameter and chlorophyll fluorescence

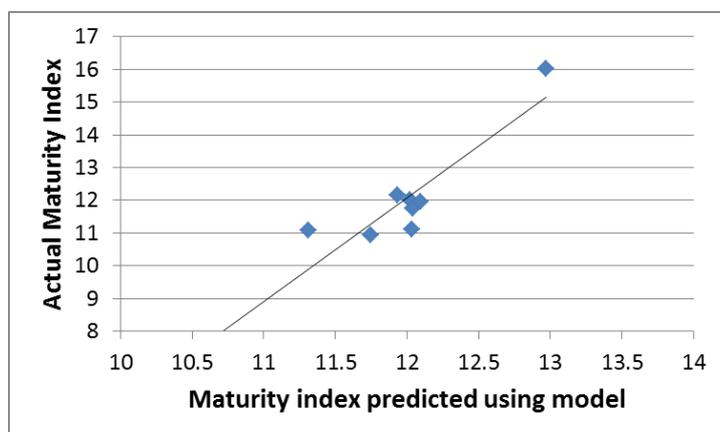


Figure B. 2015 Actual v predicted Maturity Index after 4 days shelf-life for 8 consignments of broccoli using the model developed using head diameter and chlorophyll fluorescence

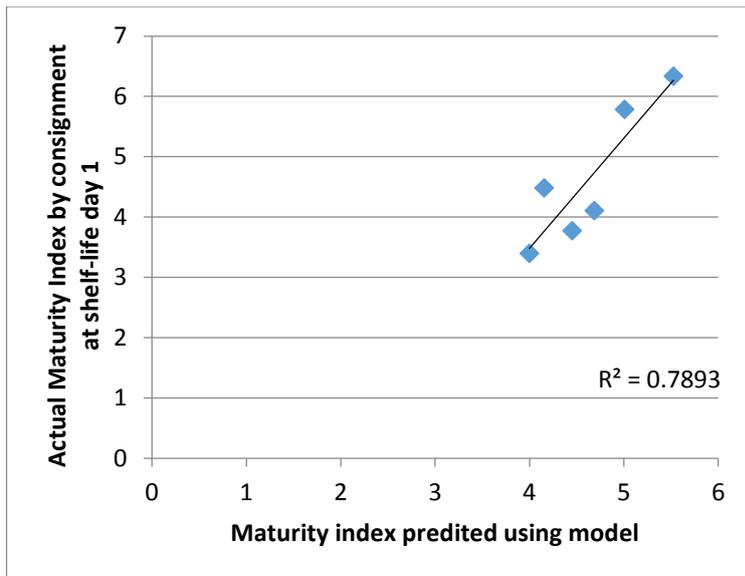


Figure C. 2016 Actual v predicted Maturity Index after 1 day shelf-life for 6 consignments of broccoli (with different field nutrition treatments) using the model developed using head diameter and chlorophyll fluorescence

In order to translate the investigative trials carried out within this project into the development of a practical tool for the broccoli industry the following issues are important.

Speed of measurement: At the moment the measurement depends on four individual point measurements across a broccoli head. In order to achieve a rapid assessment, it will be necessary to design a sensor head that can carry out multiple measurements simultaneously. A prototype produced by Hansatech Instruments Ltd, was tested in the final year of this project, but will require further development.

Field or packhouse measurement: Tests have so far indicated that measurements in the field are difficult to interpret due to interference from sunlight, even when measurements are made close to dawn. As a result, assessment of heads will need to be carried out after harvest, and away from bright sunlight. Furthermore, the results suggest that the measurement is sensitive to temperature. This needs further investigation so that a temperature compensation algorithm can be added to improve accuracy.

The use of CF to optimise field management: One original objective of this project was to test this technology for both storage life prediction and for optimising field management. In practice a measurement that depends on the broccoli head, is less practical as a field management tool, due to the rapid development of the head near to harvest time, so that any information provided would only be available towards the end of the growing season. However, if applied to leaves, this technology might provide a valuable tool for following mineral nutrition. Given the interference of sunlight, this would probably involve harvesting leaves. This application will be investigated in more detail.

Financial Benefits

The potential financial benefits from this project will arise as a result of growers being able to improve field management and also to be able to predict the storage potential of consignments, so that they can optimise scheduling of harvesting and the order of distribution of consignments.

Action Points

No specific change in practices is recommended until a field instrument is available. However, in order to ensure that the technology development is focused as effectively as possible to industry needs, the researchers welcome input from growers on the way in which they would envisage using the technology.

SCIENCE SECTION

Introduction

Broccoli is a hardy cool season Brassica that is grown predominantly in East Anglia/Lincolnshire and the East of Scotland. UK production figures for broccoli and cauliflower combined in 2013 estimated production of 155,000 tonnes with a total value greater than £100 M although a decrease in production was recorded in 2014 with a total value of only £79 M. In 2015 broccoli production increased by 6% to 72,000 tonnes and a value of £31 M. To ensure continuity in the supply of broccoli to the retail sector it is valuable to be able to predict the time required for broccoli heads to reach the required market size, and also to be able to predict the storage life following harvest. Unpredictable climate conditions during the growing season have meant that both time of head initiation and rate of head growth can be variable. While recent studies on improving the storage life of Brassicas (FV 395) have yielded some promising results in improving the quality of stored broccoli, allowing for peaks and troughs in demand and supply to be smoothed out, such benefits are strongly dependent on the quality of the harvested crop. Models, such as the “Wellesbourne Cauliflower Model” predict the time taken to reach the required head size (7-20 cm) incorporating the effect of solar radiation and temperature to estimate the effective day-degrees during the growing season (Wurr *et al* 1991, 1992). While these models help to manage crop productivity it has been observed that a range of physiological maturities exist between commercially harvested heads leading to variability in the storage and shelf-life characteristics (AHDB Field Crops Technical Panel, personal communication). Variation in temperature or excessive rainfall during the growing season often translates into poor storage and shelf-life potential of the crop.

The overall objective of the work described in this report is the development of sensors adapted to field or postharvest use that will enable the assessment of broccoli head maturity, health and storage potential. This would afford the opportunity for field operatives to make an assessment of optimum harvest date for particular field sites and to predict storage and shelf-life potential of heads once harvested.

Chlorophyll Fluorescence (CF) analysis, is a technique that can measure both the concentration and the activity/health of chloroplasts within plant tissue. The technique has been used to assess health for a wide range of crops and specifically to map changes in the health of broccoli during storage and shelf-life (FV 395) where a decline in the number of active chloroplasts is correlated with a reduction in head quality leading to senescence.

As plant tissues such as broccoli age, the aging process includes loss of photosynthetic function and the shrinkage and breakdown of chloroplasts (Krupinska 2006). As broccoli

heads age this is clearly seen as loss in green colour. Previous studies have correlated changes in colour of broccoli with the quantity of chlorophyll and carotenoid pigments using colour meter data (L^*, a^*, b^*) (Fernández-León *et al* 2012). However, while a relationship between chlorophyll content and green colour clearly exists, CF can assess chlorophyll concentration more accurately than colour (Gitelson *et al* 1999), and moreover is an indication of chloroplast function, therefore providing a stronger, more robust relationship with maturity. CF has the potential to correlate the health of tissues with storage and shelf-life. Importantly CF can provide an earlier indication of the onset of senescence than visual or colour meter assessment.

The importance of broccoli over other green vegetables is in part due to its phytonutrient content, as it is an abundant source of vitamin C, antioxidants and other phytonutrients such as isothiocyanates. Any assessment of harvest maturity and shelf-life should therefore ideally consider the nutrient content. Broccoli is an excellent source of phytonutrients made up of ascorbic acid, phenolic acids, flavonoids (quercetin and kaempferol). Quercetin and kaempferol are reported to accumulate with developmental stage of broccoli, peaking just after commercial harvest maturity (Krumbein *et al* 2007) and may provide a biochemical indicator of physiological maturity that can be correlated with chlorophyll fluorescence signals.

In addition, broccoli is an important source of isothiocyanates that are derived from the hydrolysis of glucosinolates (GLS) which show protective effects against cancer (Keck and Finely 2004). In general the complement of intact glucosinolates (glucoraphanin, sinigrin, and glucobrassicin) peak approximately 40 days after transplant followed by a decline as broccoli heads reach maturity giving rise to corresponding isothiocyanates (sulforaphane, allyl isothiocyanate and idole-3-carbinol) that peak in over-mature heads prior to a decline with the onset of senescence (Botero-O'mary *et al* 2003).

On the basis of the issues described above, the original objectives of this project were as follows:

- 1) To optimise an existing chlorophyll fluorimeter for use on broccoli heads in collaboration with the manufacturer (Hansatech Instruments Limited)
- 2) To relate chlorophyll fluorescence profiles of broccoli to maturation in the field as estimated by the effective day degrees after transplant and morphological characteristics
- 3) To Identify biochemical changes (antioxidants and isothiocyanates) during broccoli head maturation
- 4) To develop strategies for predicting the shelf-life of broccoli consignments at harvest in order to improve scheduling of broccoli marketing
- 5) To model broccoli head maturity, including biochemical and morphological changes in terms of chlorophyll fluorescence profile.

However, the project has progressed to primarily address objectives 1, 4 and 5, with less emphasis on objectives 2 and 3. The project had a very practical focus, aiming to produce a tool that will provide financial benefit to UK broccoli growers. As understanding of the technology; its strengths and its limitations developed, the focus moved to an exploration of the use of CF to predict the storage life of harvested heads, rather than to predict optimal harvest date. Likewise resources were allocated to ensure that a sufficient range of data were collected to validate the algorithm developed to predict shelf-life, so that less emphasis was placed on biochemical changes in broccoli heads, and how these could be used as markers of maturation. Nevertheless, beyond the end of this project, the project team intend to complete a set of chemical analyses of heads of different maturities and will update the final AHDB report. The project has started to investigate the effect of mineral nutrition on storage life, which was not originally a major focus.

The programme of work was conducted over three seasons with the following aims

Season 1:

- To identify a measurement (probably using CF) at harvest to predict the subsequent keeping qualities of broccoli heads.

Season 2:

- To test and refine the CF predictive model identified in season 1 for broccoli harvested over a wider range of conditions.
- To test the CF measuring protocol identified on season 1 in order to optimise the design of a specialised probe.

Season 3

- To further validate the CF predictive model for heads harvested over a wider range of conditions, including a range of mineral nutrition treatments.
- To design and test a specialised CF sensor head, with multiple sensors and shaped to reduce external light interference.

Materials and methods

Season 1 field trials: To relate chlorophyll fluorescence to broccoli maturity for two broccoli varieties.

Field trials were grown in clay soils on land belonging to Boundary Farm, owned by T.E. and S.W. Bradley, near Preston, Kent CT3. For most trials, plants of Iron Man and Steel were provided by the Allium and Brassica Centre (ABC) at planting, and subsequent trial management was undertaken by T.E. and S.W. Bradley. For the last trials of the season Steel plants were provided by T.E. and S.W. Bradley. The broccoli heads used in the main trials reported here were harvested from field trials as follows:

Table 1. Summary of field/storage trials conducted during 2014

Date harvested	Variety	No. heads and head size range
11 July 2014	Iron Man	29 heads 9 - 13 cm
16 July 2014		20 heads >9 cm
11 July 2014	Steel	19 heads 9 - 13 cm
16 July 2014		26 heads >9 cm
22 Sept 2014	Iron Man	13 heads 9 - 13 cm
30 Sept 2014	Steel	26 heads 10 - 18 cm
23 Oct 2014	Steel	52 heads 8 - 15 cm

Season 2 field trials: To test the measurement protocol, and to test/ refine the CF predictive model for broccoli harvested over a wider range of conditions.

Five trials were carried out in 2015 using field sites in Kent and Lincolnshire as summarised in Table 2.

Table 2. Summary of field/storage trials conducted during 2015

Trial	Harvest date	Varieties	Growing location
A	Purchased 14 July 2015	Unknown	Purchased from local supermarket
B	30 July 2015	Steel	Kent
C	14 and 21 Sept 2015	Parthenon, Iron Man, Steel	Kent
D	19 Oct 2015	Parthenon, Iron Man	Lincolnshire
E	16 Nov 2015	Iron Man	Kent

Broccoli was grown in Kent on clay soils near Preston, Kent CT3, and in Lincolnshire on Weston Marsh, at Holbeach Hurn, and at Sandholme. The commercial varieties; Iron Man, Steel and Parthenon were used, grown as commercial crops using standard practices.

Season 3 field trials: To validate the prediction model, to test a specially developed sensor head and to test the effect of mineral nutrition on head keeping qualities.

Broccoli (variety Iron Man) was grown in Lincolnshire at the Elsoms Trial site (PE11 3UL) in a trial managed by ABC, with six treatments (1= control, 2= 1 L/ha Inca, 3= 150 KgN/ha, 4= 1 L/ha Inca + 150 KgN/ha, 5= 300 KgN/ha, 6= 1 L/ha Inca + 150 KgN/ha) and 4 plots per treatment arranged in a randomised complete block.

Further details on trial management are provided in Appendix 1.

Chlorophyll fluorescence (CF) measurements

Chlorophyll fluorescence (CF) measurements were made using a Handy Pea Chlorophyll Fluorimeter (Hansatech Instruments Ltd, King's Lynn, UK). Chlorophyll fluorimeters can be built with a modulated excitation light so that the effects of external light can be filtered out electronically. However, for these trials in order to be able to measure the full range of chlorophyll fluorescence characteristics it was necessary to use a non-modulated fluorimeter, which means that any external light entering the measuring head will interfere with the measurement. The measuring head was fitted with a plate to restrict the measuring area to 4 mm diameter so that the area measured is exposed to a constant excitation light intensity from the light emitting diodes in the head (the plate can be observed as a white disc in Figure 1 (right hand photograph)).

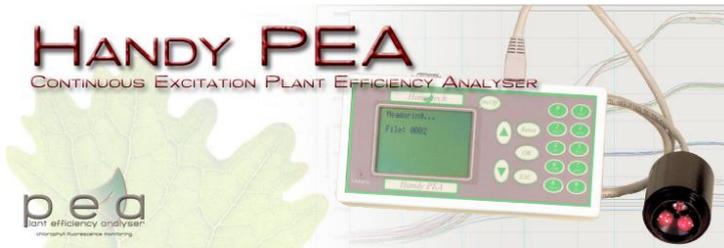


Figure 1. Handy Pea Chlorophyll fluorimeter (Hansatech Instruments Ltd, King's Lynn, UK). RH picture: Measurement from a broccoli head using an adapted leaf clip.



The fluorescence transient was measured immediately following the first and second pulse of a double pulse sequence (2 s pulse $2000 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, 3 s delay, 2 s pulse $2000 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Models to interpret fluorescence transients assume that plant material is dark adapted (usually for at least 15 minutes), so for practical measurements, this double pulse protocol was developed during earlier trials to standardise the state of the chloroplasts at the start of the transient and therefore allow measurements without prior dark adaptation. The rationale for using this pulse sequence with a 3 s delay was tested in trial A (see below).

Figure 2 shows a typical fluorescence trace (fluorescence transient) obtained from photosynthetic tissue. The fluorescence yield at several points on the trace are measured: F_0 (minimum fluorescence yield), F_m (maximum fluorescence yield), F_v (variable fluorescence = $F_m - F_0$), F_1 , F_2 , F_3 , F_4 , F_5 (fluorescence yield after 10, 30, 100 μs , 1, 3, ms respectively). In addition T_{fm} (time to reach F_m) and Area above the curve, indicated in the figure are calculated. Models of the functioning of the photosynthetic system have been used to relate the fluorescence characteristics to specific physiological aspects of chloroplasts. These are described in detail at (www.hansatech-instruments.com) and in Strasser et al. 2004.

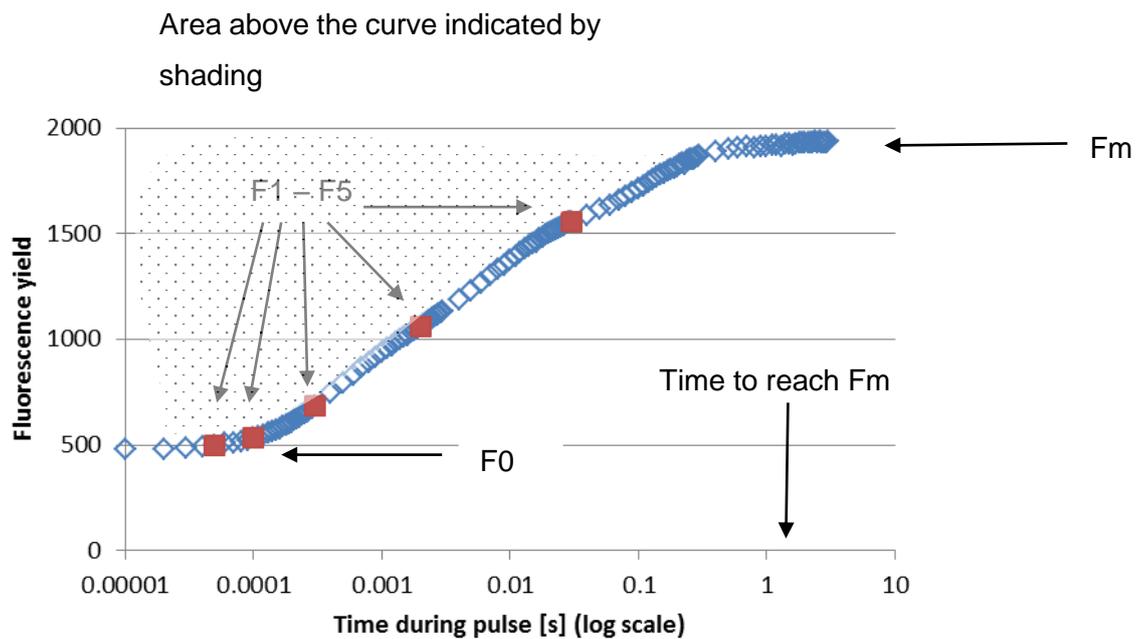


Figure 2. A typical trace of fluorescence yield from a broccoli head exposed to a 3 s light pulse obtained using a non-modulated fluorimeter such as the Handy PEA (Hansatech Instruments Ltd, UK). Some of the parameters used to calculate the fluorescence characteristics are indicated on the figure including F_0 (initial fluorescence yield), $F_1 - F_5$ (Fluorescence yield at 50 μ s, 100 μ s, 300 μ s, 2 ms and 30 ms respectively), F_m (maximum fluorescence yield), time to reach F_m .

Chlorophyll Fluorescence measurement protocol

Figure 3 shows CF characteristics were measured using the Hansatech Handy Pea, in four positions across the head positioned on the centre of a whorl wherever possible (outer, inner, inner, outer whorl). Initially a “double pulse” protocol (5 s pulses, 3 s delay, 5 s pulse) was used, with the CF parameters recorded from the second pulse. This strategy was followed to ensure that the photosynthetic tissues were in a consistent state for transient analysis. The trials described in the following section were designed to test the rationale for this approach.

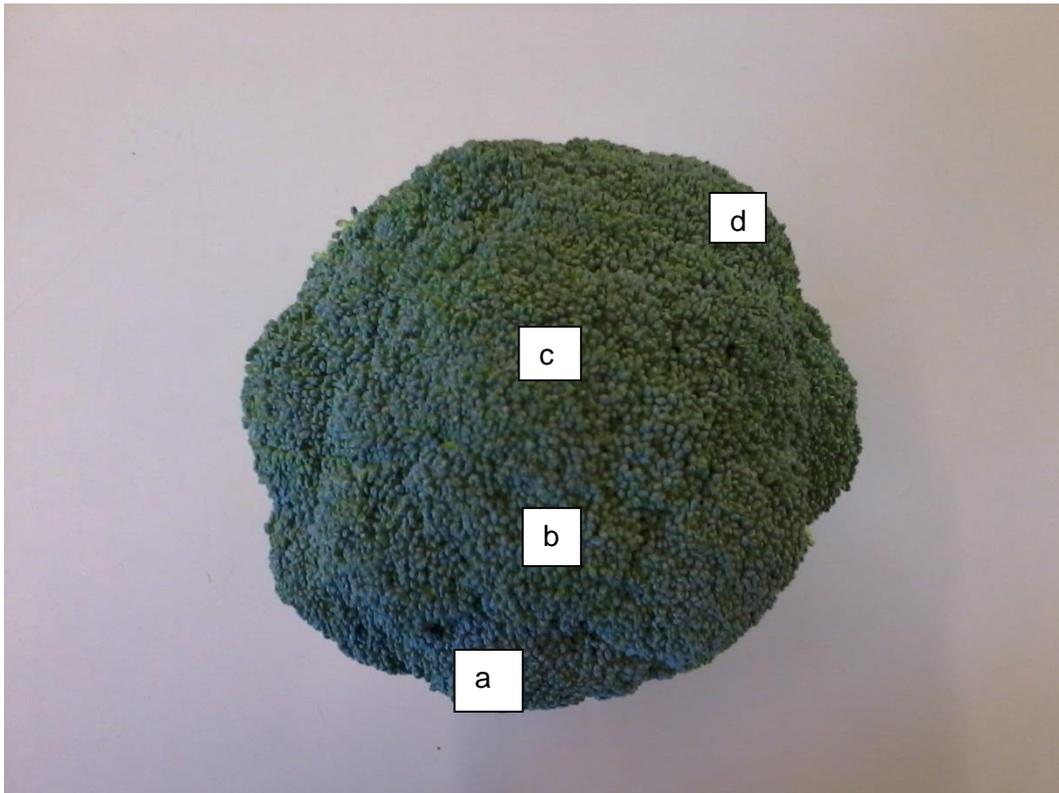


Figure 3. A broccoli head showing the position of the four measurements (a, b, c, d) of CF characteristics.

Testing double pulse method

Trial A: To optimise double pulse protocol

In July 2015, a series of measurements was carried out on 10 broccoli heads over 4 days (Figure 4) at ambient in order to test aspects of the methodology. Three protocols for collecting CF data were compared; measurements on dark adapted heads, and double pulse protocols with 1, 3 or 5 s delay between pulses. This allowed two aspects of the methodology to be investigated.

Firstly a double pulse was used on the assumption that by using the fluorescence profile from the second pulse the state of the chloroplasts would be more uniform and less affected by differences in the ambient light conditions. To test this a simple comparison of data from the different protocols was carried out by calculating the correlation between specific characteristics for each head. Table 3 shows the correlation coefficients between characteristics measured using the dark adapted protocol and the other protocols for selected CF characteristics. There was no evidence that the second pulse gave more reliable data (i.e. more strongly correlated with the data from the dark adapted protocol) than the first. The correlations are much stronger for F_v and F_v/F_m than for F_0 , F_2 and F_3 .

Secondly a comparison of data from repeat measurements from the same heads gave an indication of the variability introduced each time the CF probe was repositioned on the broccoli head. Interestingly the data is much more stable for Fv and Fv/Fm than for F0, F2 and F3. Possible contributing factors to the variability are interference of background light and variability of chloroplast content across the broccoli head. Background light would cause an erroneous increase in detect fluorescence which would introduce errors that would be larger for F0, F1-F5 than for Fv (=Fm – F0).

As a result of these findings, the use of the double pulse was stopped.

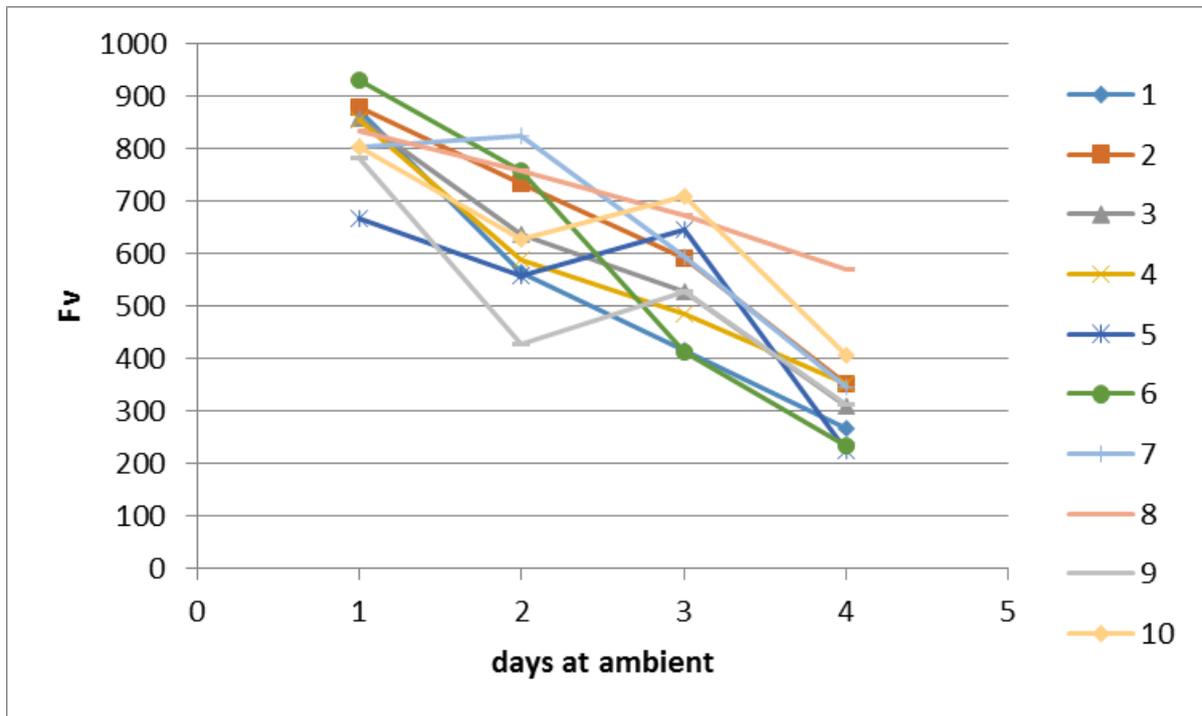


Figure 4. Fv measured on 10 individual heads (labelled 1-10) over 4 days storage at ambient.

Table 3. Correlations between CF characteristics measured by different protocols. Comparison of repeat measurements considered the first pulse for different pulse protocols.

	Correlation coefficient r						
	Dark adapted v 1s delay		Dark adapted v 3s delay		Dark adapted v 5s delay		Comparison of repeat measurements
	Pulse 1	Pulse 2	Pulse 1	Pulse 2	Pulse 1	Pulse 2	
Fo	0.593	0.528	0.642	0.574	0.619	0.623	0.700
F2	0.565	0.508	0.647	0.566	0.621	0.600	0.694
F3	0.544	0.499	0.644	0.565	0.634	0.590	0.701
Fv	0.843	0.844	0.861	0.844	0.883	0.889	0.933
Fv/Fm	0.824	0.820	0.818	0.769	0.878	0.874	0.899

Maturity assessment

Each head was assessed visually using a scoring system adapted from Wurr *et al* 1991.

- Stem turgor (Turgid – slightly flaccid, very flaccid) 0, 1, 2
- Head colour: blue-green 1, green 2, light green 3, 10% yellowing 4, 20% yellowing 5, 30% yellowing 6, 40% yellowing 7, 50% yellowing 8, 60% yellowing 9, 70% yellowing 10
- Bud compactness (closed – open + yellow petals – open + green and white sepals) 0, 1, 2
- Bud elongation (Flat head with no elongation – increasing unevenness as buds elongate – individual buds extending) 0, 1, 2
- Floret loosening (firm – florets beginning to loosen – florets wide apart) 0, 1, 2

Maturity Index (MI) = head colour score + stem turgor score + bud compactness score + bud elongation score + floret loosening score.

Colour measurements

Colour measurement using a Minolta colour meter set to measure in L *a *b* mode provided a measure of loss of green background (*a scale) and the increase in yellowing (*b scale) (Figure 5).

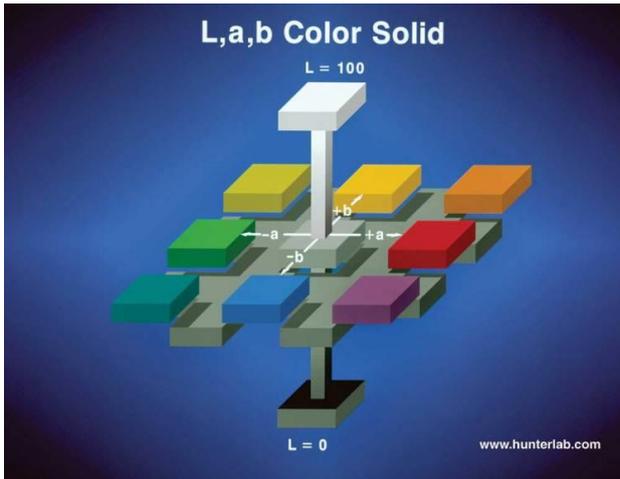


Figure 5. The $L^* a^* b^*$, colour space and Minolta colour meter used to measure machine colour values.

Results

The development and testing of algorithms using of Chlorophyll Fluorescence (CF) characteristics to predict broccoli storage life.

The research described in this report involved trials conducted over three seasons. The full set of trials is summarised here, but further details for season 1 & 2 are given in Annual Reports 2015 and 2016.

Season 1 field trials: To relate chlorophyll fluorescence to broccoli maturity for two broccoli varieties.

In season 1 broccoli heads with a range of maturity were harvested from five field trials (three with variety Steel, and two with variety Iron Man) between July and October 2014. The quality of the heads presented as Maturity Index (see Materials and Methods), assessed over two weeks low temperature storage, followed by shelf-life determination at 18°C are illustrated in Figure 6.

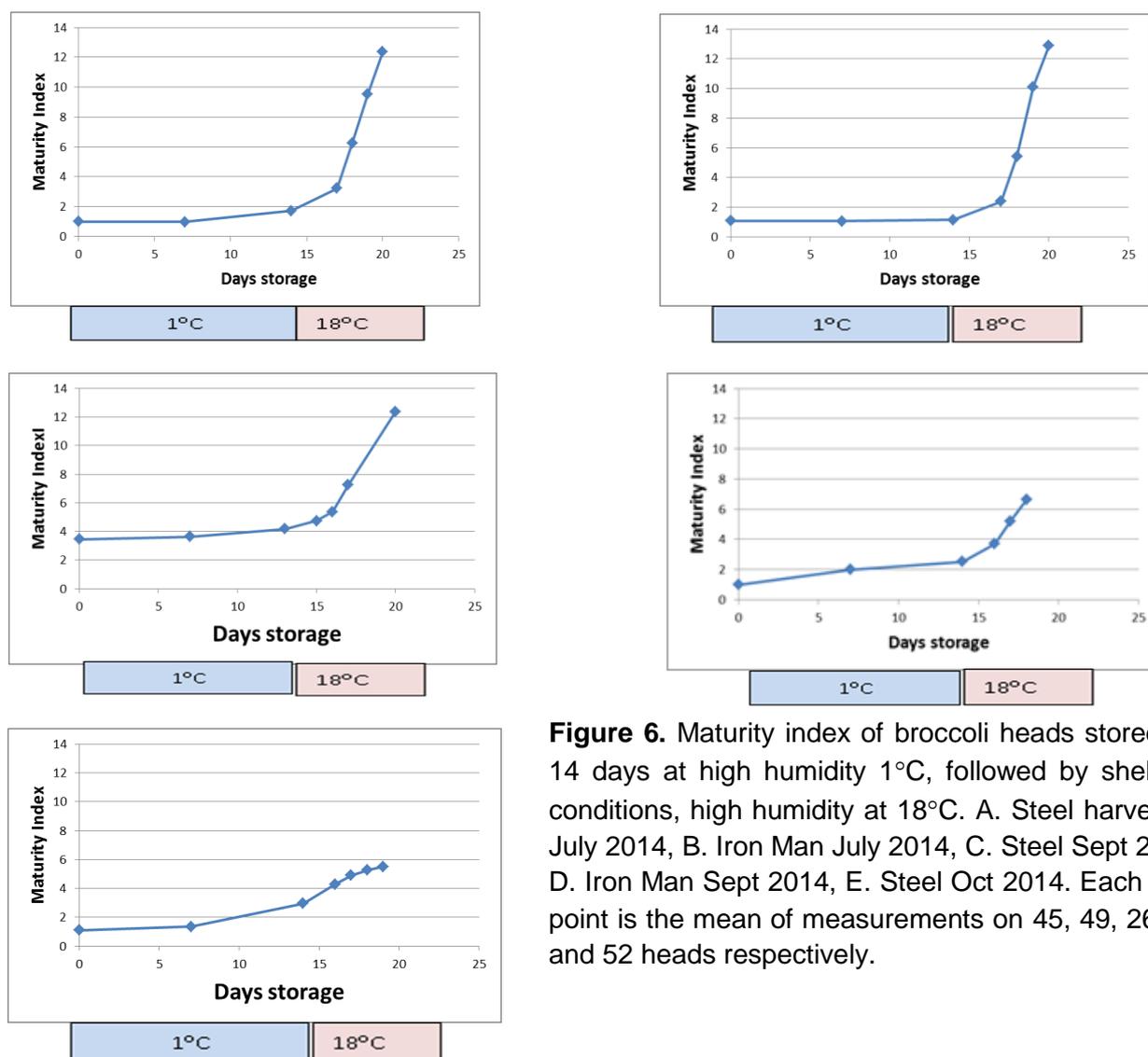


Figure 6. Maturity index of broccoli heads stored for 14 days at high humidity 1°C, followed by shelf-life conditions, high humidity at 18°C. A. Steel harvested July 2014, B. Iron Man July 2014, C. Steel Sept 2014, D. Iron Man Sept 2014, E. Steel Oct 2014. Each data point is the mean of measurements on 45, 49, 26, 13 and 52 heads respectively.

The specific objective of these trials was to identify a measurement at harvest that could predict the subsequent keeping qualities of broccoli heads. For practical reasons we chose to relate characteristics measured at harvest to the Maturity Index (MI) after four days of shelf-life (MI-SL4). In order to identify the characteristics with most potential to predict broccoli quality, the correlation for the full range of CF characteristics with MI-SL4 was calculated for each trial. The most consistent relationship was found with $F\beta$, the fluorescence yield at a specific time point in the fluorescence transient (a full explanation is provided in Materials and Methods). The correlation coefficients for $F\beta$ are shown in Table 4, together with correlation coefficients for machine measured colour score b^* (indicates “yellowness”).

Table 4. Correlation coefficients (r) for individual field trials between head characteristics measured at harvest and the MI after four days of shelf-life (MI-SL4). For the Iron Man trial harvested in September CF measurements were not collected immediately after harvest.

Trial date/variety	d.f.	Head diameter	(L*a*b* colour) b value	$F\beta$	RC/CS
July/Steel	34	0.252	-0.283	-0.368*	-0.417*
Sept/Steel	24	0.413*	-0.570**	-0.391*	-0.425*
Oct/Steel	47	0.682***	-0.037	-0.436**	-0.156
July/Iron Man	47	0.717***	-0.307*	-0.780***	-0.704***
Sept/Iron Man	48	0.456**	-0.611***	--	--

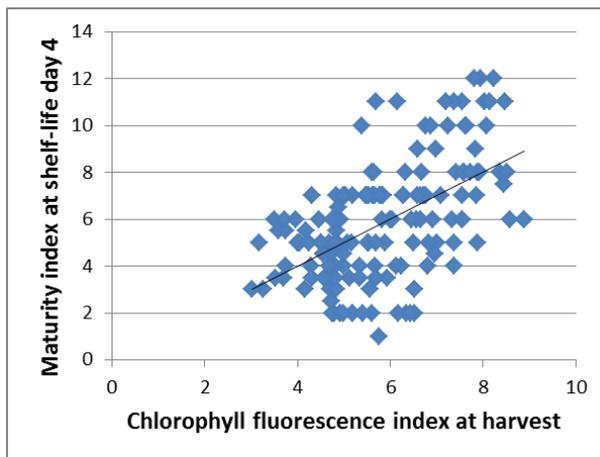
*, **, *** significant to 5, 1, 0.1%

The correlation coefficients were then calculated between harvest characteristics and MI-SL4 for all heads across all trials (Iron Man in September was omitted as the CF characteristics were not measured at harvest). For colour b value the correlation was not significant ($r = 0.03$), but for $F\beta$ value it was very significant $r = -0.54$.

An algorithm to predict MI-SL4 from $F\beta$ at harvest was therefore calculated as $MI-SL4 = -0.0121F\beta + 17.131$. This can be improved by including head size, in which case the algorithm becomes $MI-SL4 = 0.286 \cdot \text{diameter} - 0.00605F\beta + 8.301$.

Figure 7 shows these algorithms fitted to each individual head in four trials and the average for each consignment. There was no indication from the data that the two varieties should have different algorithms.

a)



b)

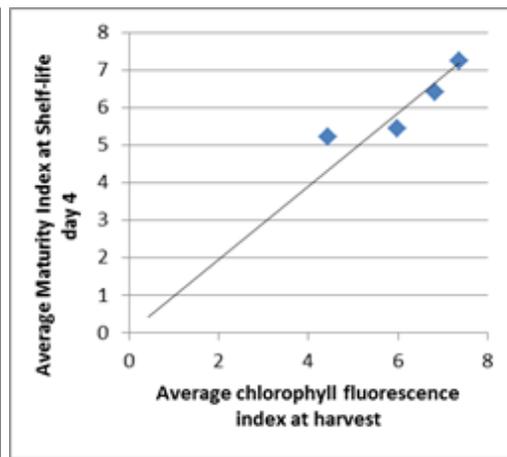
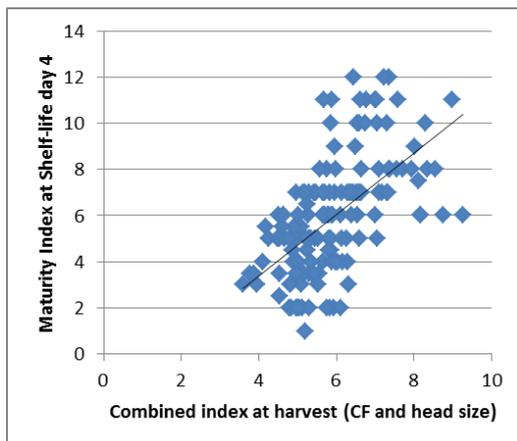


Figure 7. Actual v predicted Maturity Index after 4 days shelf-life using the algorithm developed using $F\beta$. a) Each point relates to a single broccoli head. $MI-SL4 = -0.0121F\beta + 17.131$ $R = 0.54$ b) Each point is an average over the whole broccoli harvest.

a)



b)

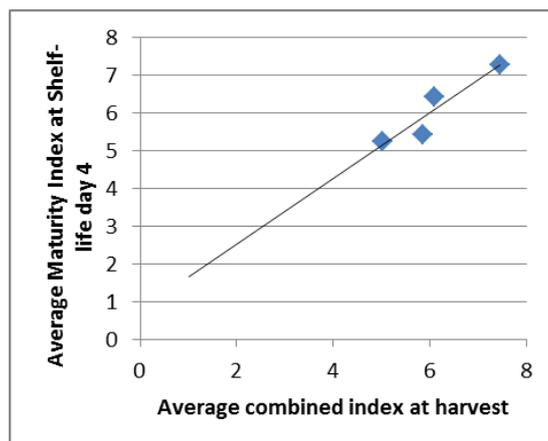


Figure 7. Actual v predicted Maturity Index after 4 days shelf-life using the algorithm developed using head diameter and $F\beta$. a) Each point relates to a single broccoli head. $MI-SL4 = 0.286 * \text{diameter} - 0.00605F\beta + 8.301$ $R = 0.59$ b) Each point is an average over the whole broccoli harvest.

Season 2 field trials: To test the measurement protocol, and to test/ refine the CF predictive model for broccoli harvested over a wider range of conditions.

In season 2, three trials were conducted covering three varieties grown in Kent and Lincolnshire and harvested on four dates through September, October and November 2015 in order to provide consignments of broccoli with a range of keeping qualities to enable us to test again which characteristics measured at harvest could predict storability most accurately.

The quality changes of the consignments are shown in Figure 8 in terms of maturity index (MI = head colour score + stem turgor score + bud compactness score + bud elongation score + floret loosening score). The range in rates of quality loss was not very great between whole consignments except for consignment E that exhibited a notably rapid loss in quality.

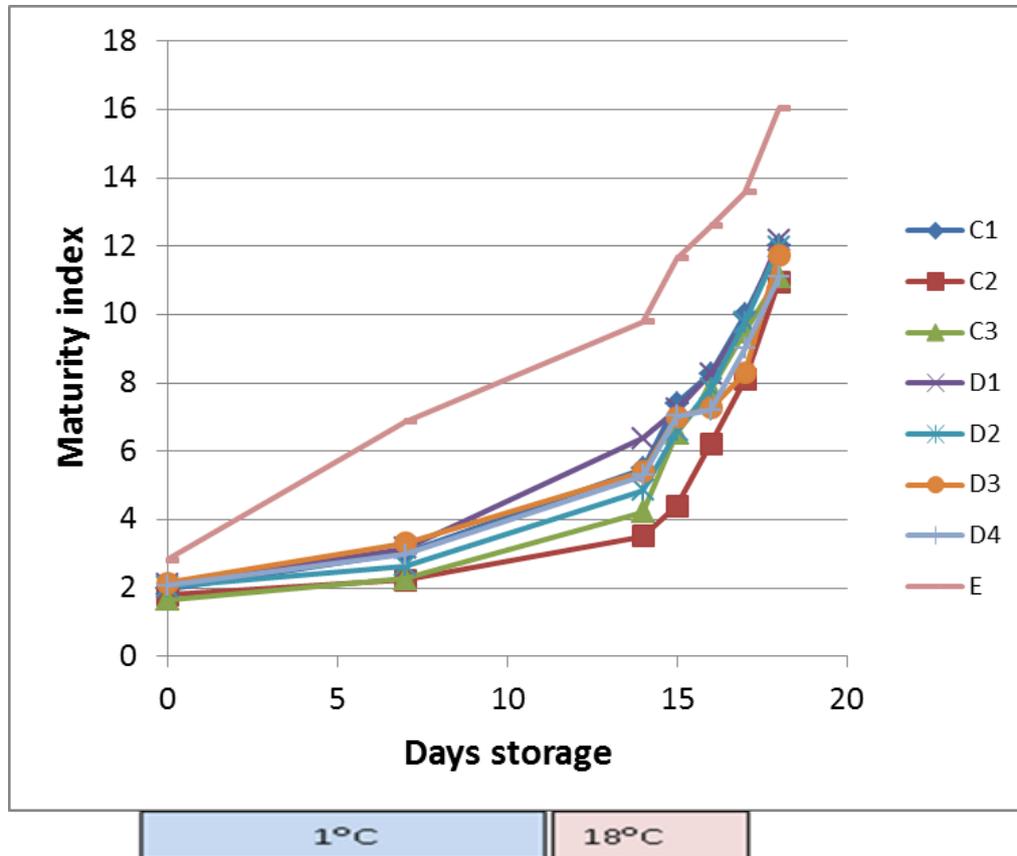


Figure 8. Quality of broccoli consignments in terms of Maturity Index during storage for 14 days at high humidity 1°C, followed by shelf-life conditions under high humidity at 18°C. The consignments are defined in Table 2.

The full range of CF characteristics was tested again during this season for its correlation with quality indicators at each day of shelf-life assessment. As a result of this analysis an updated algorithm that was improved compared to season 1, was developed to predict MI on days 17 (Shelf-life day 3) and 18 (Shelf-life day 4) in terms of $F\alpha$ and Head size (note $F\alpha$ and $F\beta$ are both fluorescence yields during the fluorescence transient but at different time points). Figure 9 shows actual quality scores on day 18 plotted against predicted quality scores for all 252 heads used in this set of trials.



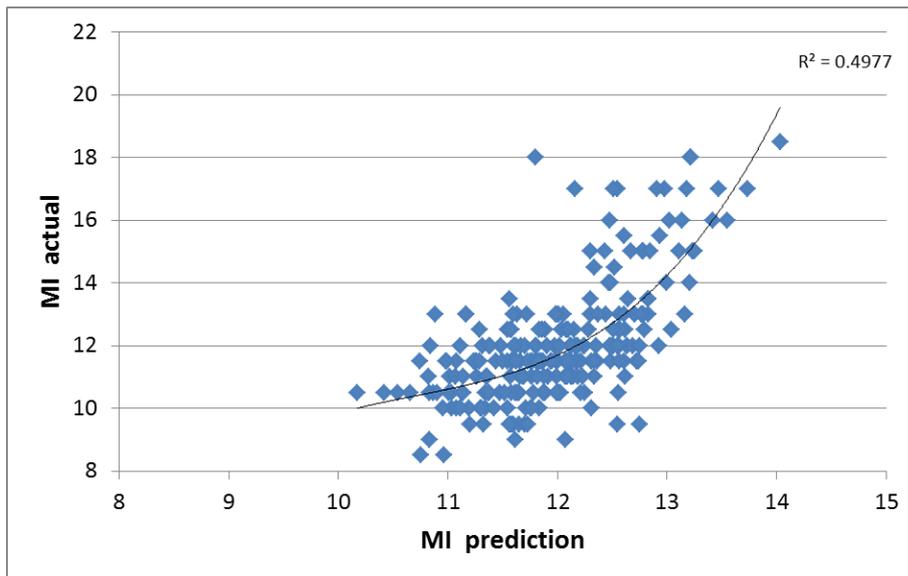
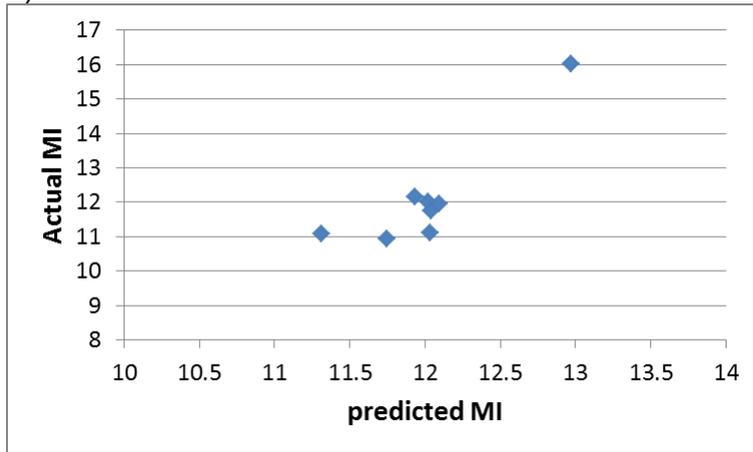


Figure 9. Actual MI on day 18 plotted against the value predicted from measurement of CF characteristics and head size at harvest. Prediction models are $0.43 \text{ size} - 0.008 F_{\alpha} + 5$ and $0.46 \text{ size} - 0.009 F_{\alpha} + 10.6$. The r value for line fit is 0.71 in both cases.

The same algorithm was tested for its ability to predict the quality of whole consignments in terms MI on day 18 (Figure 10a).

The relationship between F_{α} at harvest and average shelf-life of each consignment was also tested (Figure 10 b). Head shelf-life was calculated on the basis of the day at which any of the key quality attributes (stem turgor, bud compactness/elongation, floret tightness and head colour) became unacceptable.

a)



b)

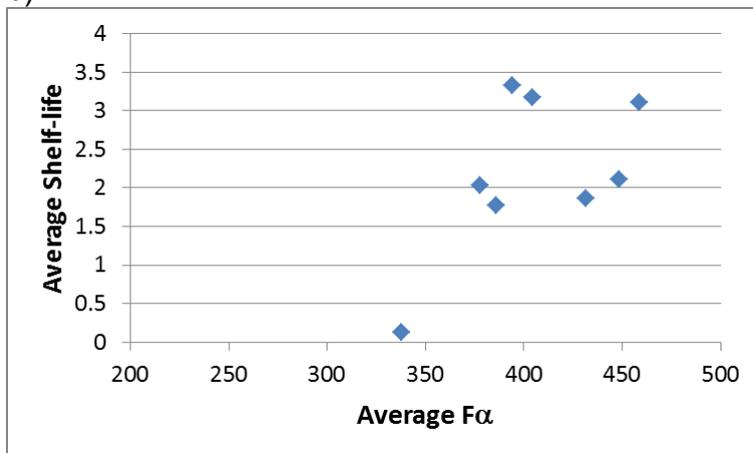


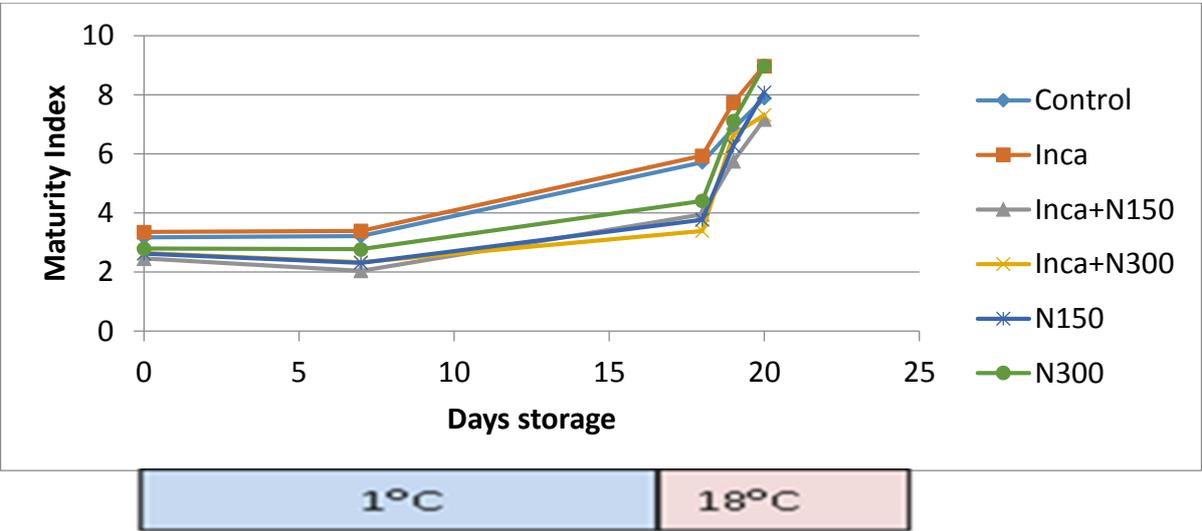
Figure 10. a) The relationship between actual and predicted MI at 18 days for broccoli consignments. Each data point is the mean of 30 or 36 heads. b) Shows the relationship between average shelf-life of each consignment and the average $F\alpha$ measured.

Season 3 field trials: To validate the prediction model and to test a specially developed sensor head and to test the effect of mineral nutrition on head keeping qualities

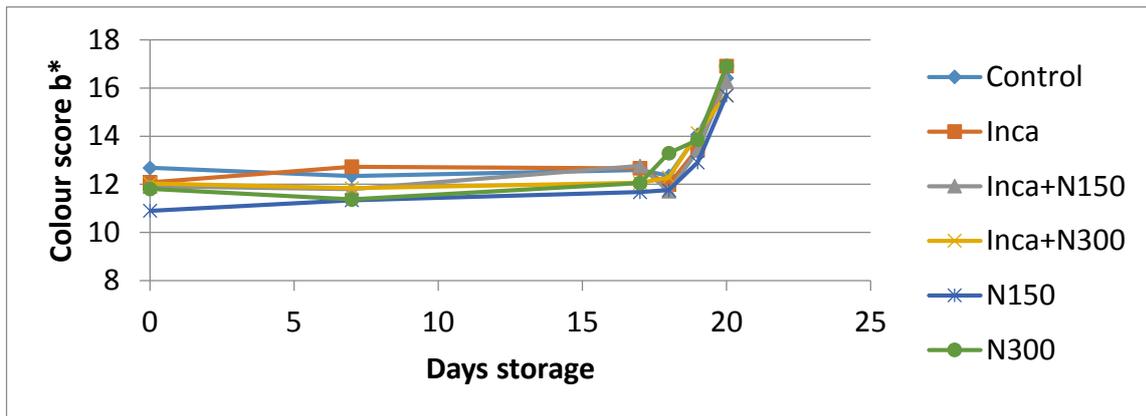
During season 3, in order to validate the predictive algorithm, a field trial was set up specifically to produce heads with varying storage characteristics. This was achieved by using a range of nitrogen applications with and without a foliar calcium treatment (InCa), as this was also expected to provide information useful to the broccoli industry on the benefits of field nutrition treatments. This same trial was used to test a multiple sensor head produced by Hansatech Instruments Ltd.

Figures 11 a-d show the storage characteristics by field treatment. In terms of maturity index, the use of InCa with nitrogen appears to prolong storage life relative to Controls.

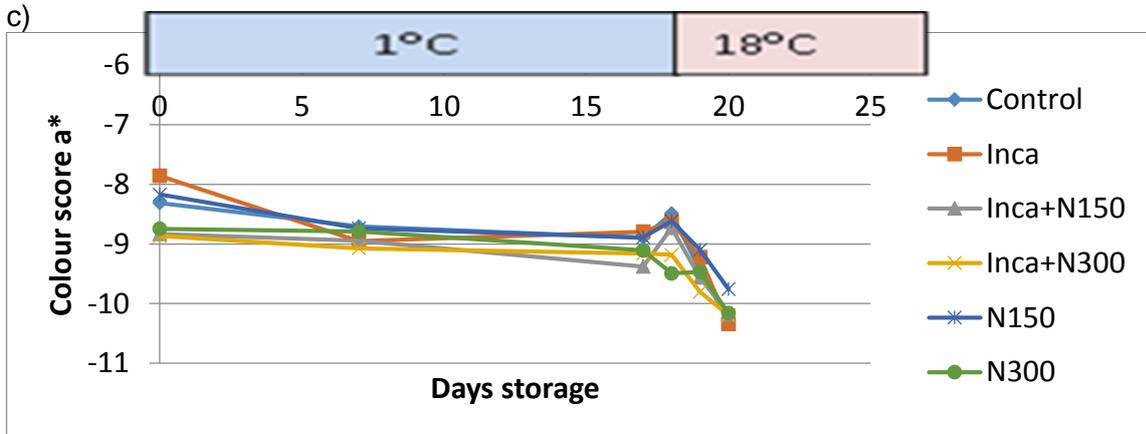
a)



b)



c)



d)

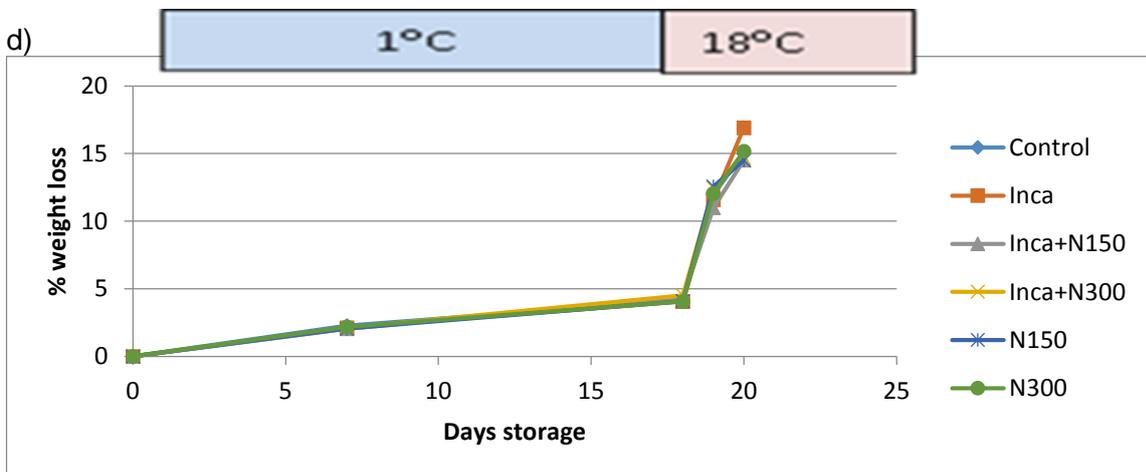
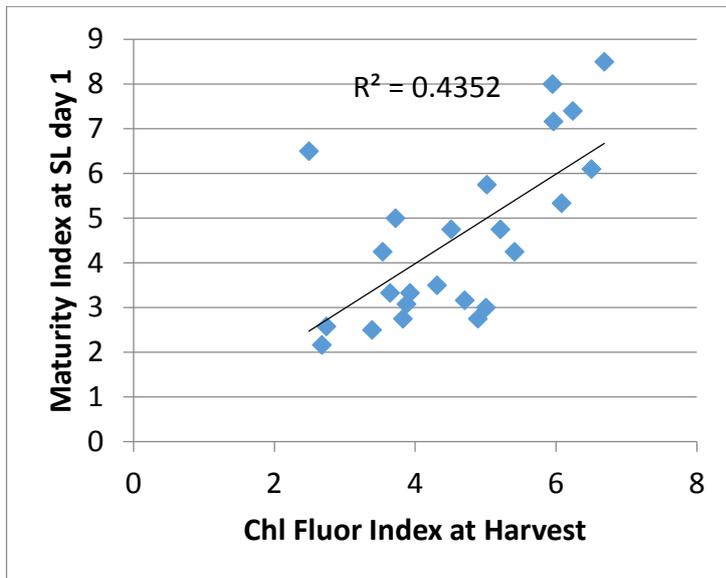


Figure 11. Quality of broccoli consignments in terms of a) Maturity Index b) Colour score b*, c) Colour score a* and d) % weight loss during storage for 17 days at high humidity 1°C, followed by shelf-life conditions under high humidity at 18°C.

In these trials the heads were selected to fit within a specific size range (10 – 12 cm) so that the algorithm tested for MI prediction was used without head size. Probably as a result of sub-optimal head handling (transport by car from Lincolnshire to Kent prior to low temperature storage) the storage quality was notably worse than season 2. For this reason the algorithm was tested for MI at Shelf-life day 1, so that the quality was still within retail range.

Figure 12 (a. data shown by field plot, b. data shown by treatment) shows that the fluorescence characteristic, F_{α} , identified in season 2 was also effective in predicting MI for this third season.

a)



b)

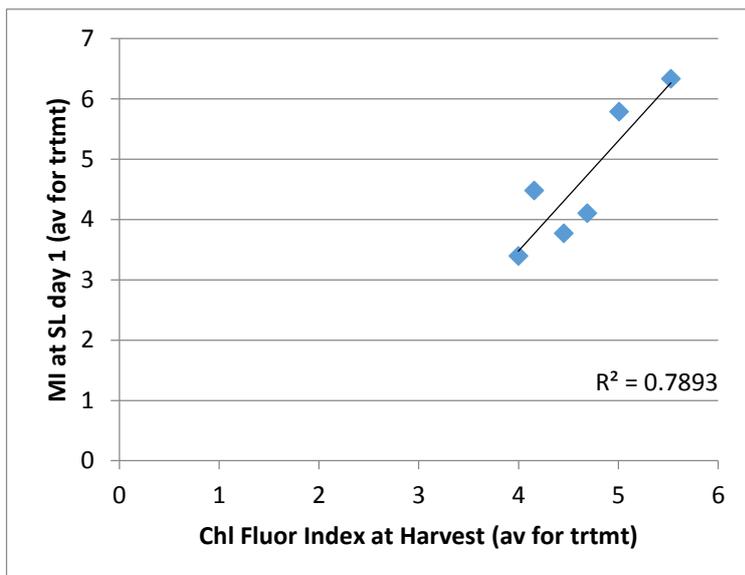


Figure 12. The relationship between actual and predicted MI at 18 days (Shelf-life day 1) for broccoli consignments. Predicted MI is calculated as $-0.0296 F_{\alpha} + 18.362$ a) Each data point relates to a field plot and is the mean of 4 measurements of each of 6 heads. b) Each data point relates to a treatment and is the mean of 6 heads in each of 4 plots.

Testing a specialised sensor head

Figure 13 shows the prototype Pea-lunger multiple sensor head produced by Hansatech Instruments Ltd. This has three LED excitation lights and three sensors capable of measuring the chlorophyll fluorescence transient at different positions across a head. This compares to the single sensor in the Handy PEA that has to be used manually to produce a number of readings. The Pea-lunger was used to measure all the heads within the trial, immediately after the measurements made by the Handy-PEA. The results (not shown) indicated that the data obtained by the Pea-lunger was variable and not able to provide a prediction of MI. The magnitude of F_{α} is very sensitive to the distance of the sensor to the broccoli head, so that we assume the variability is because with the current design it is not possible to position the sensors at a consistent distance from the broccoli head. Further engineering is therefore necessary.



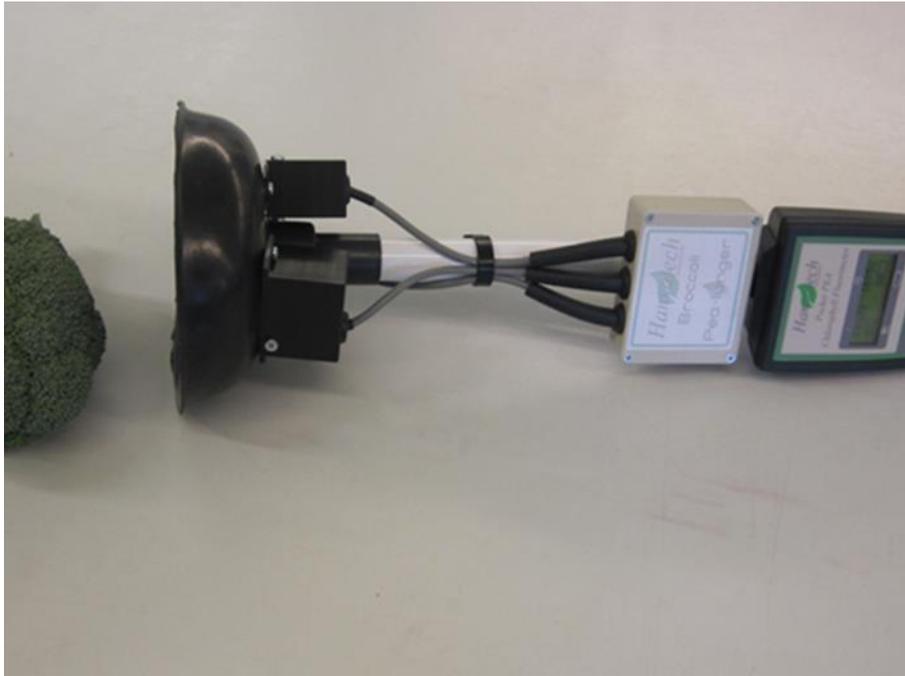


Figure 13. Pea-lunger multiple sensor head produced by Hansatech Instruments Ltd.

Field nutrition and broccoli head quality

Table 5 shows the mineral content of the heads from each treatment, while Table 6 summarises the keeping qualities of each treatment as indicated by the MI. While nitrogen applications resulted in higher nitrogen content of heads, there is no significant effect of foliar InCa application on the calcium content of heads.

Table 5. Mineral content of broccoli heads grown using a range of nitrogen applications with and without a foliar calcium treatment (InCa). For each treatment 6 heads were sampled from each of four field replicates

	Mg/100g fresh weight					Mg/Kg fresh weight				
	N	Ca	P	K	Mg	Cu	Fe	Mn	Zn	B
Control	605a	60a	96a	478b	21.3a	0.68a	9.6a	3.1a	5.2ab	4.2ab
Inca	578a	67a	93a	474b	21.3a	0.62a	9.9a	2.8a	4.6a	4.0a
Inca+N150	722b	57a	106b	424a	23.8a	0.74a	9.9a	2.9a	6.4b	4.9ab
Inca+N300	768b	54a	110b	412a	24.9a	0.80a	10.3a	2.9a	7.1b	4.7b
N150	699b	57a	106b	414a	22.9a	0.74a	10.2a	2.8a	6.6ab	4.7b
N300	740b	57a	108b	412a	23.6a	0.78a	10.1a	2.8a	6.6b	4.9ab
Treatment effect (p)	<0.001	ns	<0.001	<0.001	0.048	0.05	ns	ns	0.002	0.003

Table 6. Effect of field nutrition on broccoli head quality (Maturity Index on Shelf-life day 1).

Treatment	Treatment description	MI SL day 1
1	Control	5.79
2	1 L/ha InCa	6.33
3	150 Kg N/ha	3.77
4	150 Kg N/ha + 1 L/ha InCa	4.10
5	300 Kg N/ha	4.48
6	300 Kg N/ha + 1 L/ha InCa	3.40

A combination of InCa and N appears to improve quality compared to controls (Low MI indicates good quality), but Analysis of variance indicates that this is not statistically significant.

Practical aspects of CF measurement

For the initial trials reported in seasons 1 and 2, a double pulse protocol was used for the chlorophyll fluorescence measurements on the assumption that by using the fluorescence profile from the second pulse the state of the chloroplasts would be more uniform and less affected by differences in the ambient light conditions. A more detailed investigation in season 2 indicated that there is no advantage of using a double pulse protocol. *This was a very positive result, as it meant that a simpler/cheaper instrument could be developed and that a faster measurement would be possible.*

In season 1 it was shown that measurement in sunlight is impractical. A trial was carried out to compare the use of CF measurements in the field before harvest with those within the laboratory after harvest. It was investigated whether a predawn measurement in the field was feasible, but found that interference with the measurement was apparent as soon as the sun started to rise. The conclusion from these trials was that it is impractical to make measurements in the field for two reasons; the sensitivity of the equipment to bright sunlight, and the variability in response of the broccoli heads when exposed to bright sunlight. Light levels within the laboratory, and in a packhouse, are much lower than in full sunlight, so that the interference with measurements is minimal. For these reasons the final recommendations will be to carry out measurements inside after harvest. Therefore in season 3 we focused on the development of an algorithm for postharvest use.

Discussion

- We have identified a CF measurement for predicting broccoli head quality based on F_0 (fluorescence yield at a specific time-point in the fluorescence transient). Although this clearly provides a prediction of quality, which has been reasonably consistent over 3 seasons, at this point we do not know if this is an intrinsic indicator of the state/health of chloroplasts, or is a measure of the tightness of the broccoli heads (spaces between buds would theoretically reduce the yield of fluorescence returning to the sensor). We believe it is the former, but it would be valuable to establish this more rigorously. A careful comparison of the morphology of heads with varying CF yield should answer this question. The project team will do this when heads are available in the 2017 season.
- The algorithm identified works when broccoli heads are measured immediately after harvest, but not when heads are measured during cold storage. It is assumed that this is a temperature effect; heads coming out of cold storage will warm up over time, providing a range of temperatures. This is an issue that if resolved could increase the value of this technology (e.g. a temperature compensation factor could be included to improve accuracy). The project team will check this when heads are available in the 2017 season.
- The potential of using CF measurements has been tested in the field. However, the measurement is affected by sunlight in two ways; direct interference with the signal (this could be eliminated by engineering) and biological defence mechanisms used by chloroplast against bright light. The latter issue is difficult to solve, and therefore probably means that the technology can only be used on harvested product.
- A sample size of 10 – 15 heads is sufficient to provide a prediction for a broccoli consignment.
- One original objective was to develop this technology as a management tool for broccoli in the field. In retrospect, given that heads develop very rapidly, a management tool based on head assessment is less practical than one based on a leaf measurement. There is potential to use CF assessment of leaves as a measure of plant health for a range of crops, in particular it is a good tool for measurement of chlorophyll concentration and therefore potentially for N management. Given the issue described above about light interference, the easiest strategy would be to harvest leaves and do measurements away from bright light.
- CF was focussed on rather than broccoli head composition, even though this was included as a specific objective in the original project proposal. Beyond the end of this project, the project team intend to complete a set of chemical analyses of heads of different maturities and will share the data with AHDB, providing an updated report.

- Although not an objective within the original project proposal, data collected during the third season of trials indicates InCa treatment (foliar calcium) is worth further testing in combination with nitrogen treatment as a means of improving broccoli quality.

In order to take the results of this project forward and provide a practical tool for UK growers the project team will endeavour to carry out additional work as follows:

- In order to determine the economic value of the technology and the robustness of the method, it should be tested with commercial growers.
- Additional tests should be carried out using the adapted Pea-Lunger sensor head to confirm or not the current conclusions on how the design should be improved.
- Trials should be carried out with commercial growers on the use of the chlorophyll fluorimeter for determining N nutrition and aiding crop management, for broccoli and for other crops.

Conclusions

- An algorithm has been developed to use a simple CF measurement of broccoli heads to predict storage quality. This is consistent over three seasons. The measurement is based on the yield of fluorescence from chlorophylls. Further technical development is necessary to produce a practical head design and to test the practical application of this technology for broccoli growers. The final tool would probably need to include temperature compensation.
- It is recommended that this tool is investigated further for general applicability to crop management, with one specific application to broccoli.

Knowledge and Technology Transfer

“The potential of chlorophyll fluorescence to assess maturity and storage life of broccoli heads”. Debbie Rees, Richard Colgan, Karen Thurston, Lisa Wray-French and Emma Skipper. Talk presented at the Brassica and Leafy Salads Conference 28th January 2015

The project was featured in the Vegetable Farmer, “Freshness Sells”, Professor Geoff Dixon.

Acknowledgements

We would like to thank T.E. and S.W. Bradley who grew plots of broccoli for these trials at their Boundary Farm and allowed us to sample from their own crops. We would also like to thank Lincolnshire Field Products Ltd and T.H. Clements Ltd who allowed us to harvest broccoli heads from their fields in Lincolnshire. Thanks is also due to Hansatech Instruments

Ltd for providing us with a chlorophyll fluorimeter and prototype chlorophyll meter and technical advice.

Glossary

CF Chlorophyll fluorescence

Fv Variable component of the chlorophyll fluorescence transient rise

F α F β , F Ω chlorophyll fluorescence characteristics identified as useful for predicting broccoli shelf-life. These have not been described precisely to maintain commercial confidentiality

MI Maturity index, calculated as = head colour score + floret loosening score+ Stem turgor Bud compactness score +Budd elongation score

SL Shelf-life

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Appendix 1

Season 3 field trial management: To validate the prediction model and to test a specially developed sensor head and to test the effect of mineral nutrition on head keeping qualities

Broccoli (variety Iron Man) was grown in Lincolnshire at the Elsoms Trial site (PE11 3UL) in a trial managed by ABC, with 6 treatments (1= control, 2= 1 L/ha Inca, 3= 150 KgN/ha, 4= 1 L/ha Inca + 150 KgN/ha, 5= 300 KgN/ha, 6= 1 L/ha Inca + 150 KgN/ha) and 4 plots [per treatment arranged in a randomised complete block.

Planting: 14 July 2016, 0.61 x 0.46 spacing. 345 tray size, Verimark drenched.

28 July 2016: nitrogen applied to all required plots as liquid nitrogen 18%. Calculation: plot sized 2.4 x 5.5 m = 13.2 m² (0.00132ha). For 150 Kg/ha, require 833 L 18%/ha = 1.1 L/plot. For 300 Kg/ha require 2.2 L per plot. To ensure even application the Liquid nitrogen was mixed with water up to a total of 5L so that all plots received 5 L. All other plots received 5 L water.

12 August, and 30 August 2016: 1 L/ha InCa applied to required plots.

Trial plan

401 4	402 6	403 5	404 2	405 3	406 1
301 5	302 2	303 3	304 1	305 6	306 4
201 1	202 6	203 2	204 4	205 5	206 3
101 3	102 5	103 4	104 1	105 2	106 6

Base level of Nitrogen according to soil analysis was 66 Kg N/ha