Project title:	Strategies for broccoli management to improve quality and extend storage life
Project number:	FV 395
Project leader:	Dr Richard J. Colgan
Report:	Final report
Previous report:	None
Key staff:	Dr Richard Colgan (NRI) - Technical lead on implementation of storage trials and vegetable physiology Dr Debbie Rees (NRI) - Plant Physiologist and technical specialist in chlorophyll fluorescence Dr Marcin Glowacz (NRI): Biochemist Mr Dudley Farnham (NRI) Analytical Chemist
Location of project:	Produce Quality Centre East Malling Research New Road East Malling, Kent ME19 6BJ
Project coordinator:	Gavin Willerton
Date project commenced:	1 April 2011
Date project completed (or expected completion date):	30 November 2013
Key words:	Broccoli, Storage, Shelf-life extension, Ethylene, 1-MCP, Azoxystrobin, Cypermethrin, Ethylene scrubbing.

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

AUTHENTICATION

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

Dr Richard Colgan

Research Fellow

Natural Resources Institute, University of Greenwich

Signature .... Date ....

Report authorised by:

Dr John Orchard

Director of Research

Natural Resources Institute, University of Greenwich

Signature .... Date ....

CONTENTS

GROWER SUMMARY

Headline

- Storage life and shelf-life quality of broccoli cv. Ironman may be improved by:
  - Removing background ethylene via ethylene scrubbing;
  - In-field application of azoxystrobin or post-harvest application of 1-MCP, or a combination of azoxystrobin followed by 1-MCP.
- Chlorophyll fluorescence may be more useful than visual assessments or colour meter readings in detecting changes in product quality.

Background

The aim of this project is to define strategies for pre- and post-harvest management of broccoli to improve quality and extend storage life. The outputs of the project will enable the industry to reduce waste and crop losses both in the field and post-harvest. The strategies tested include: technologies to reduce the concentrations of ethylene in pack-houses and store rooms, particularly evaluating the suitability of ethylene scrubbing technology; the use of the ethylene antagonist SmartFresh™ (1-MCP); and the use of pre-harvest chemical treatments to manipulate ethylene production and response by broccoli. In addition, the project is investigating the use of chlorophyll fluorescence to assess maturity and shelf-life of broccoli at harvest and thereby to improve the consistency of the harvested crop.

Summary of project and main conclusions

Background ethylene sampled in store rooms is sufficient to accelerate the rate of broccoli senescence during shelf-life.

Implementation of ethylene scrubbing technology on a commercial broccoli store was shown to reduce background ethylene from 400 ppb to ~200 ppb. The benefit in lower ethylene was to maintain the background green colour of broccoli during storage and shelf-life and to reduce (1–2%) weight loss.

The impact on of pre-harvest sprays of cypermethrin and azoxystrobin over a 3 seasons, used as part of standard crop protection programme on broccoli quality was assessed. Results from trials confirm that azoxystrobin has secondary beneficial effects on improving the retention of green background colour during shelf-life.

A split application of 1-MCP 312 ppb applied at harvest, and a second application after three weeks storage, reduced the onset of senescence during shelf-life (18°C). Moreover, combining the split application of 1-MCP to broccoli treated with azoxystrobin three weeks before harvest was the most effective treatment retarding the onset of senescence in broccoli in one out of the two years this protocol was tested. A split dose of 1-MCP reduced the decline in vitamin C (ascorbic acid) content in broccoli stored over a 21 day period. The effectiveness of treatments in reducing senescence is affected by pre-harvest stresses encountered by broccoli during the growing season.

Chlorophyll fluorescence has been used as a tool to study the process of photosynthesis for many decades. Models of the functioning of the photosynthetic system have been used to relate fluorescence characteristics to specific physiological aspects of chloroplasts. The characteristic that appeared to be most sensitive to broccoli senescence was reaction centres/cross-section (RC/CS), which relates to the concentration of functional photosynthetic reaction centres.

Chlorophyll fluorescence was able to track changes in head quality with storage and there is limited evidence that the technology can identify changes in head maturity at harvest.

Financial Benefits

In-field application of azoxystrobin, minimising the build-up of ethylene in the storage environment, post-harvest treatment using 1-MCP, and a combined treatment of pre-harvest azoxystrobin application followed by post-harvest 1-MCP application have all been shown to improve the storage and shelf-life of broccoli. Management of broccoli in this way can keep product quality higher for longer, thereby reducing the need to import broccoli during low UK production times, as well as reducing waste and crop losses.

Action Points

- Reducing background ethylene in stores can improve storage and shelf-life of broccoli. Use of electric-powered fork-lift trucks in confined storage spaces will lower the the build-up of ethylene.
- Broccoli product quality can be extended by using a split application of 1-MCP at harvest and prior to removal from storage. On crops previously treated with azoxystrobin as a crop protection measure, subsequent application of 1-MCP can have secondary additive effects on improving shelf-life quality.

SCIENCE SECTION

Introduction

In order to meet the fluctuating demand for broccoli by the retail sector and to ensure a year-round supply of broccoli for UK consumers, broccoli may need to be stored for periods of up to 2–3 weeks. Moreover, the year-round demand for broccoli in the UK necessitates the import of produce overland from Spain and other Mediterranean countries between December and May. Maintaining the quality of UK stored product and that of imported broccoli requires intervention in the supply chain to ensure broccoli maintains a fresh, green appearance with minimal water loss.

Overall aim of project

To define strategies for broccoli pre- and post-harvest management to improve quality and extend storage life.

Specific objectives

To evaluate the potential to improve quality and to extend storage/shelf-life through:

a) The management of ethylene concentrations in pack-houses and store rooms.
b) The use of post-harvest treatments of broccoli heads with SmartFresh^{TM} (1-methylcyclopropene; 1-MCP).
c) The use of pre-harvest chemical treatments to manipulate ethylene production and response.

To define a protocol for use of chlorophyll fluorescence to assess maturity and shelf-life of broccoli at harvest and thereby to improve the consistency of the harvested crop.

Materials and methods

Harvesting and initial inspection

In 2011, broccoli (*Brassica oleracea* var. *italica*) cvs. Ironman and Parthenon were harvested from Lincolnshire and Fife, respectively. Produce was transported by cool chain to the Produce Quality Centre at East Malling Research, Kent, UK. For the subsequent two field seasons the *cv*. Ironman, harvested from Lincolnshire was selected for storage trials.

On arrival, broccoli was placed in a 3°C jacketed air store to facilitate sorting and randomisation of samples. Thereafter, broccoli was transferred to a holding temperature of 1°C. Unless otherwise stated ten heads of broccoli were placed in plastic boxes, each box represented a replicate, and three replicates per variety were used. All boxes were initially weighed using a tarred balance before loading into controlled atmosphere storage cabinets; each chamber was 360 L in volume. Data-loggers (Easylog) recording humidity and temperature were placed inside each cabinet. Cabinets were flushed (3 L min^{-1}) with humidified air. Additional removal of CO_2 was achieved via automatic flushing of the storage environment controlled by an ICA66 CA system.

Quantification of ethylene

Ethylene concentrations were monitored 3-4 times a week during the ethylene treatment phase of the project. Replicate 1 mL samples of the cabinet head space were analysed by a gas chromatograph (GC) with a flame ionisation detector (FID). Samples were injected into an injection port heated to 130°C on a GC (ATI-Unicam 610 series) fitted with an FID set at 250°C and a 1 m long, 6 mm OD glass column packed with 100/120 mesh alumunia maintained at 130°C and flushed with nitrogen at 43 mL min^{-1} at 10 psi. Eluted peaks were integrated using a delta integrator (Delta Data Systems). A standard ethylene gas mixture (840 nL L^{-1}) supplied by BOC was used to calibrate the GC at the beginning of each run. The limit of detection was 4 nL L^{-1}.

Assessments of quality

Assessments of product quality were made after 0, 7, 14 and 21 days of storage at 1°C, 100% RH. Boxes were removed individually from cold store and a colour measurement on the greenest part of each broccoli head was made using a Minolta colour meter. Subsequently, chlorophyll fluorescence of selected heads was measured using a Handy PEA chlorophyll fluorescence meter. Each box was weighed and photographed and heads were scored for visual appearance before samples were returned to store. After 21 days at 1°C, broccoli was transferred to a shelf-life room at 18°C where samples were reassessed after 2, 3 and 6 days.

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).

In addition, the transformation of colour data to generate Chroma $(*a^2 + *b^2)^{0.5}$ and Hue angle: (arctangent(*b/*a)/2π)x 360° values were used to measure the overall change in visual appearance with time. Data were analysed by ANOVA using Genstat version 11.

Chlorophyll fluorescence

Deterioration/senescence of broccoli is associated with a progressive loss of chlorophyll from the heads, and a loss in the ability of buds to photosynthesise. Whereas the loss of chlorophyll can be assessed by measuring colour changes, characteristics of chlorophyll fluorescence (CF) can be used to assess not only chlorophyll concentrations, but also loss of photosynthetic function. Potentially, therefore, CF could provide a more accurate measure of senescence/deterioration than colour. Measurements of chlorophyll fluorescence were made on four broccoli heads selected per replicate

Fig 1. Handy Pea Chlorophyll fluorescence meter (Hansatech Instruments Ltd)

The figure below shows a typical fluorescence trace obtained using a fluorimeter such as the Handy PEA. Several characteristics of the trace can be measured: Fo, Fv, Fm, Tfm and Area above the curve are indicated in the figure. Models of the functioning of the photosynthetic system have been used to relate the fluorescence characteristics to specific physiological aspects of chloroplasts (www.hansatech-instruments.com).

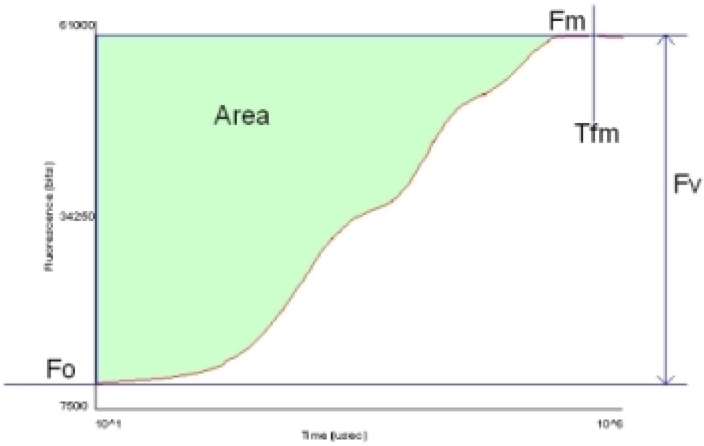


Fig 2.A typical fluorescence trace obtained using a non-modulated fluorimeter such as the Handy PEA (Hansatech Instruments Ltd, UK).

Total ascorbic acid extraction and determination

Ascorbic acid (AsA) was extracted and analysed using a method described by Bergquist et al. (2006) with some modifications. Freeze dried samples (2.5 g) were extracted with 10 ml of cold (4 °C) 3.0% (30 g/l w/v in H_2O) *meta*-phosphoric acid (HPO_3) (Sigma-Aldrich, UK). Samples were homogenized with a Janke & Kunkel IKA Labortechnik ultraturrax T25 homogenizer (IKA, Germany). The extracts were centrifuged at 4,000 x g for 30 min at 4 °C. Supernatants were filtered through 0.45 µl syringe filters (Chromacol Ltd, UK) and 1.0 ml was collected in Eppendorf tubes. Following filtration, extracts were microfuged at 9,300 x g for 5 min. 500 ml was transferred into new Eppendorf tubes and mixed thoroughly, with an equal volume of 1% (11 mg/ml w/v in 1 M K_2HPO_4/H_2O (1/4, v/v)) DTT solution (DL-Dithiothreitol) (Sigma-Aldrich, UK). These samples were left for 40 min at room temperature (20±1.0 °C), and then microfuged at 9,300 x g for 5 min. Samples were transferred into HPLC vials for total AsA (vitamin C) determination. Samples were analysed using an Agilent 1100 HPLC (Agilent, UK). The concentration of AsA was determined according to external AsA standards (Sigma-Aldrich, UK). The volume of 20 ml of each sample was analysed in this process.

Environmental monitoring of ethylene in pack-houses store rooms and packing rigs

On visits to Lincolnshire replicated samples of air taken using a vacuum pump to fill 1 L sample bags (Teflar) from a series of pack houses, stores and harvesting rigs. Similar measurements were taken by East of Scotland Growers. Air samples were then analysed for ethylene by GC-FID.

Assessing the impact of background ethylene concentrations on storage quality of broccoli

Year 1

Broccoli cv Ironman was placed into storage cabinets on the 21St June 2011. Six replicates of 10 heads per replicate were used. Cabinets were attached to a flow (3 L min^{-1}) of humidified air or air amended with 100, 250 or 500 ppb ethylene (BOC). Measurement of ethylene concentrations were made 3-4 times per week.

Improving storage quality of broccoli with post-harvest application of 1-MCP- Year 1

After loading into chambers, 1-MCP was placed in a sealed 100 mL Duran bottle. Containing deionized water (10 ml) at room temperature (20°C), after rapid mixing bottles were placed inside each cabinet and opened followed by immediate sealing of the air tight cabinet lids. Each CA chamber contained a motorised fan allowing circulation of air around the boxes during the 24 hour treatment phase. All automatic ventilation/CA correction controls were inactivated and external ports sealed during 1-MCP -treatment. Control cabinets were treated in a similar manner. Treatments consisted of 625 ppb 1-MCP on day 0, a half dose of 312 ppb 1-MCP on Day 0 repeated on day 8, or untreated controls.

After 24 hour treatment, cabinet lids were opened and 1-MCP exhausted, cabinets were resealed and a flow of humidified air at 3 L min^{-1} was passed through the chamber. In addition, the maximum tolerance for CO_2 build up for each cabinet was set at 0.2% and controlled by automatic injection of compressed air, oxygen and carbon dioxide concentrations were monitored and regulated using an ICA66 controlled atmosphere system.

Year 2 and 3

A split dose (312 ppb) of 1-MCP was applied to *cv*. Ironman treated previously pre-harvest with azoxystrobin or cypermethrin; the first application 312 ppb was made immediately after harvest and a second following 3 weeks storage at 1°C . Treatments were carried for 24 hours at 1°C.

Measurements of colour, chlorophyll fluorescence and weight and were made at harvest and at weekly intervals during three weeks storage at 1°C followed by assessments during shelf-life after 2, 3 and 6 days at 18°C.

Pre-harvest management factors affecting post-harvest quality of broccoli: Impact of pre-harvest sprays of cypermethrin and azoxystrobin

Year 1

A trial site for Iron Man in Lincolnshire was kindly supplied by Produce World of Butterwick and a field site in Fife for cv Parthenon was supplied by the East of Scotland Growers. Spray trials were designed by Allium and Brassica Agronomy ltd. The trial area consisted of a 24 m x 69 m site within which 3 replicated randomised plots were assigned to each treatment. Each plot consisted of 8 rows x 23 m in length; plant spacing was 40-41 cm per row and approximately 56 plants per row with a total of 448 plants per plot.

Amistar 250 WC (azoxystrobin 22.9% [w/w]) was applied at a rate of 1L ha^{-1} in a volume of 300 L ha^{-1} and Permasect C (cypermethrin 11 % EC [w/w]) at 0.25 L ha^{-1} in a volume of 300 L ha^{-1} using a Berthoud Electric Sprayer with 02 – F110 VP nozzles spaced at 50 cm intervals. Sprays were applied 3 weeks before harvest, by AB Agronomy ltd on the Lincolnshire field site and by East of Scotland Growers on the site situated in Fife.

Year 2 and 3

Spray trials were repeated on the *cv*. Ironman in a field site in Lincolnshire, spray trials were managed by Allium and Brassica Agronomy Ltd and the sites were maintained by Produce World of Butterwick.

Amistar 250 WC (azoxystrobin 22.9% [w/w]) was applied at a rate of 1L ha^{-1} in a volume of 300 L ha^{-1} and Permasect C (cypermethrin 11 % EC [w/w]) at 0.25 L ha^{-1} in a volume of 300 L ha^{-1} using a Berthoud Electric Sprayer with 02 – F110 VP nozzles spaced at 50 cm intervals. Sprays were applied three weeks before harvest, by AB Agronomy Ltd. on the Lincolnshire field site.

Broccoli heads were harvested to commercial specifications and after harvesting, produce was blast cooled immediately before being sent via cool chain to the Produce Quality Centre at East Malling Research. Broccoli heads were randomised between treatments before being placed into storage chambers at 1°C, with a flow (3 l kg^{-1} h^{-1}) of humidified air (95-100 % RH). 1-MCP application was applied as a split dose 312 ppb at harvest and after 21 days in store to broccoli treated previously with azoxystrobin or cypermethrin.

Ethylene scrubbing of storage atmospheres to extend the storage life of broccoli

An ethylene scrubber (model 1CE400F; Absorbger SAS, France) was installed in a commercial broccoli store at Produce World of Butterwick and allowed to run for several weeks before the trial commenced. Freshly harvested broccoli *cv.* Ironman from the same field site was divided into two pallets; one was placed in the ethylene-scrubbed store, the other in an adjacent store of the same dimensions and temperature (0–1°C, 100% RH) and management regime. The trial pallets were kept in store for two weeks before being transported via cool chain (3°C) to the Produce Quality Centre at East Malling. Broccoli was randomised within treatments and three trays (ten broccoli heads per tray) of scrubbed and non-scrubbed broccoli was placed in an 18°C shelf-life room, while another three trays were placed at 10°C shelf-life. Broccoli was inspected at day 0, 1, 2, 3 and 6 for treatments stored at 18°C and day 0, 1, 2, 3, 6, 7, 8, 10 and 13 for treatments stored at 10°C.

The impact of broccoli maturity at harvest on storage quality

Broccoli *cv.* Ironman, were harvested at two stages of maturity: Harvest Mature and crop that was estimated to be 7 days away from the commercial harvest date. Heads were blast cooled and shipped via cool chain to the Produce Quality Centre. Broccoli was treated with 1-MCP at 312 ppb for 24 hours at harvest and repeat treatment was applied after 21 days of storage at 1°C. Broccoli was then transferred to shelf-life conditions of 18°C. Assessments of produce quality were made at intervals as described earlier.

Results

Ethylene monitoring

The background readings for ethylene sampled from various points in the broccoli supply chain were measured for four handling facilities and were variable (Table 1.1 and 1.2). In general, store-rooms and chillers had higher ethylene concentrations than pack-houses. In the latter, the larger areas and greater air-flow reduced ethylene to 44 ppb or lower, with one packhouse recording ethylene below the threshold of detection (2 ppb). Ethylene concentrations in storerooms and chillers ranged from 210-386 ppb at one site, while at others background ethylene in storerooms was measured between 4-47 ppb. The range in concentrations may be a consequence of the intensity of fork-lift activity

Table 1.1 *Ethylene mapping of stores, packhouses and harvesting rigs (facility 1). Air samples were collected using a Vac-U-Sampler (SKC) filling 1 Litre Teflon coated sample bags (SKC). Air samples (1 mL) were analysed using GC-FID (Agilient 6890N).*

Location	Ethylene ppb	SE
Broccoli store, 6°C, misted, stocks up to 3 days from harvest	325.0	12.6
Broccoli store, not misted, stocks up to 5 days from harvest	210.4	16.3
Blast chiller	386.5	13.5
Cauliflower storeroom	294.5	83.5
Pack-house	44.0	1.0
Harvesting rig	1.0	0.0

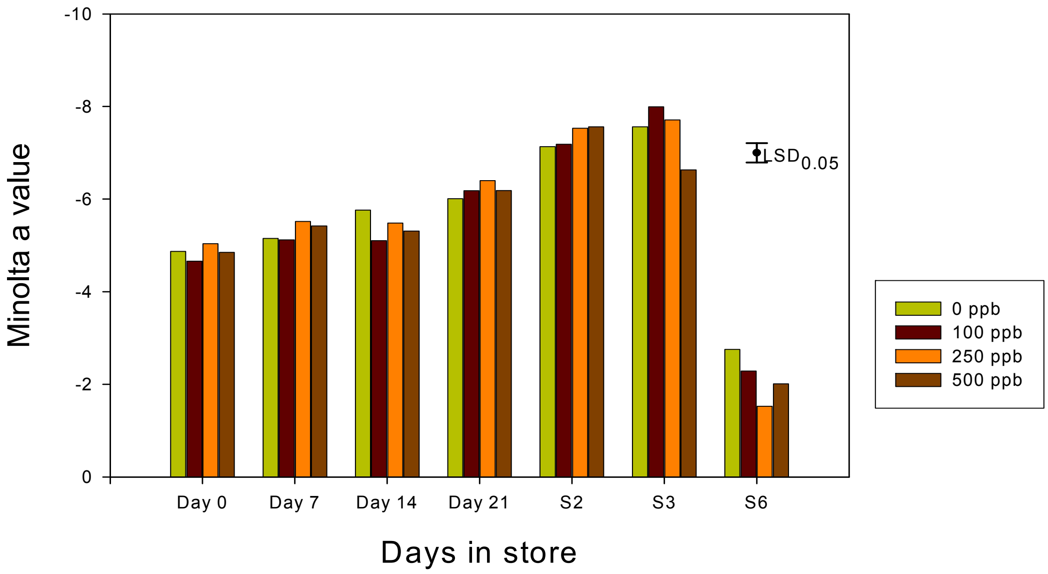
Table 1.2 *Ethylene mapping of stores packhouses and harvesting rigs at facilities 1-3. Air samples were collected using a Vac-U-Sampler (SKC) filling 1 Litre Teflon coated sample bags (SKC). Air samples (1 mL) were analysed using GC-FID (Agilient 6890N).*

Location	Ethylene ppb	SE
Site 2		
Pack-house	0.4	0.4
Chiller	10.8	1.5
Field	0.0	
Site 3		
Chiller	4.1	1.5
Site 4		
Chiller	46.8	1.3
Pack-house	43.5	1.6

Assessing the impact of background ethylene concentrations on storage quality of broccoli

A gradual colour change in broccoli from blue/green to green was observed during 21 days of storage at 1°C (Fig. 1.1) leading to an increase in Minolta '-a values'. During this storage phase no significant differences were observed between treatments. Transfer to shelf-life conditions accelerated changes in blue-green to dark green colour with a further initial increase in Minolta '-a values'. After 3 days of shelf-life heads exposed previously to 500 ppb ethylene showed a significant ($P<0.05$) reduction in background green colour as the first signs of senescence was observed. After 6 days broccoli from all treatments had deteriorated and with heads in an advanced state of senescence. Those exposed to 250-500 ppb ethylene exhibited the greatest deterioration. An increase in background yellow colour (b-values) was observed earlier than changes in green colour during shelf-life (Fig. 1.1). Broccoli exposed to 250 and 500 ppb ethylene during storage led to an increase in yellow colour development after 2 days and 100 ppb treatment after 3 days shelf-life.

The shape of the fluorescence rise measured using the chlorophyll fluorimeter can be used to calculate a range of chloroplast characteristics using models that have been developed of chloroplast function. Several characteristics were tested for their sensitivity to broccoli senescence. The characteristic that appeared to be most sensitive was RC/CS (reaction centres/cross-section), which relates to the concentration of functional photosynthetic reaction centres. Changes in RC/CS were in advance of those detected by the colour meter, with a decline in ethylene-treated broccoli seen after 2 days of shelf-life, and a significant difference between treatments (Fig. 1.2).



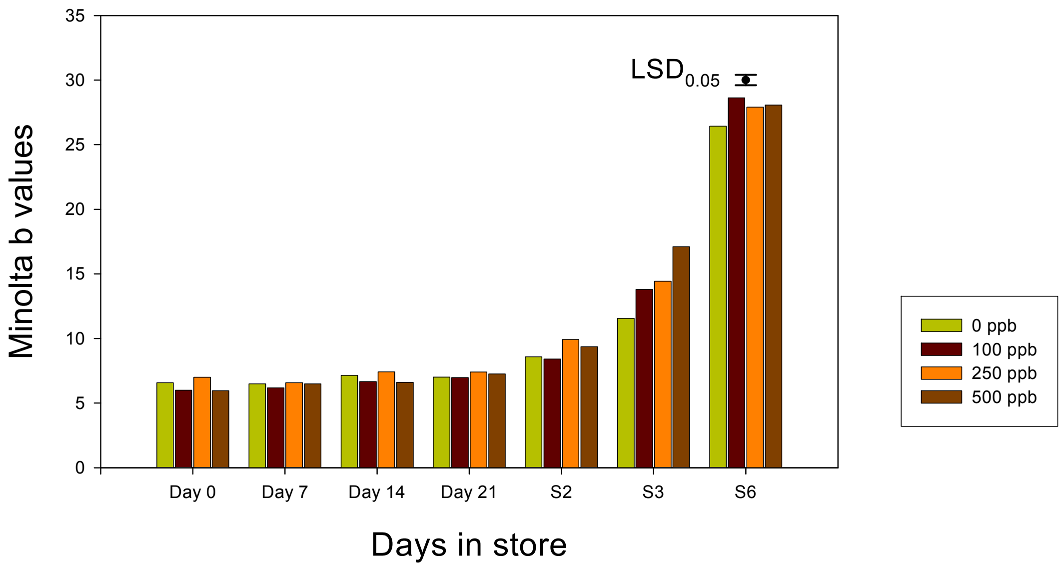


Figure 1.1 *The effect of ethylene on background green (Minolta '-a value') and yellow colour (Minolta b values) of broccoli cv Ironman during storage at 1°C (100% RH) followed by 6 days shelf-life at 18°C (S2…S6)*

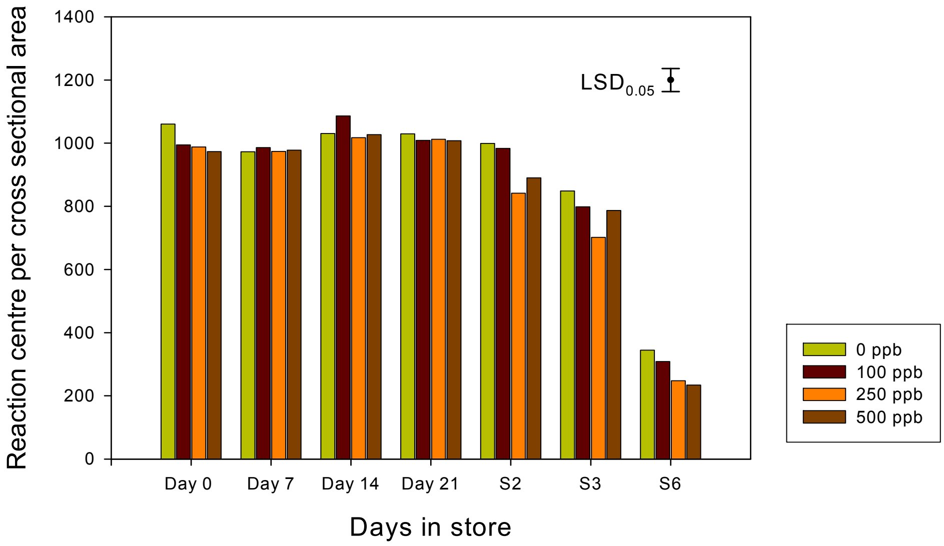


Figure 1.2 *The effect of ethylene on chlorophyll fluorescence profiles of broccoli cv Ironman during storage at 1°C (100% RH) followed by 6 days shelf-life at 18°C (S2…S6)*

Visual assessment

A visual assessment of broccoli during storage recorded colour changes (Table 1.3. & Fig 1.4) from blue/dark-green (score A) to dark-green (score B). Observed colour changes mirrored Minolta 'a values' where background green intensity (more negative a values) increased during storage. Broccoli exposed to 250-500 ppb ethylene changed from blue/green to green more rapidly. By the end of 21 days storage broccoli retained its green background colour in all treatments (Fig 1.3) however, those exposed to ethylene were generally more flaccid and outer florets showing signs of drooping (score C), but was less evident where ethylene was removed from the storage environment. During shelf-life broccoli that had been exposed to 250-500 ppb ethylene during storage senesced earlier than untreated controls.

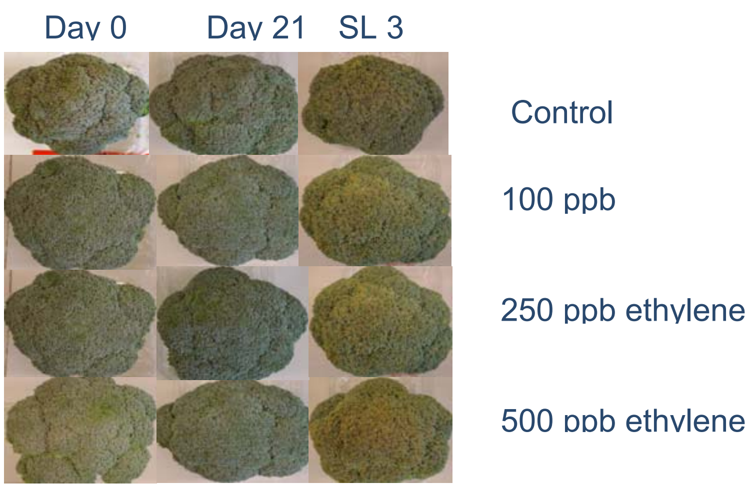


Figure 1.3. The effect of low concentrations of background ethylene on the storage quality and shelf-life (SL 3) of broccoli cv Ironman.

Table 1.3. *Visual assessment scores of (Brassica oleracea var. italica) cv. Ironman exposed to 0,100, 250 or 500 ppb ethylene during 21 days storage at 1°C (100% RH) followed by 6 days shelf-life (SL). Heads were assessed on arrival (Day 0) and after 7, 14 and 21 days. Shelf-life assessments were carried out after 2, 3 and 6 days (SL2..SL6).*

		Ironman		
Inspection	Control	100 ppb	250 ppb	500 bbp
Blue-green				
Day 0	100	100	97.5	100
Day 7	92.5	87.5	95	92.5
Day 14	82.5	80	**65**	**75**
Day 21	15	**5**	**0**	**2.5**
SL 2	0	0	0	0
SL 3	0	0	0	0
SL 6	0	0	0	0
$LSD_{0.05}$ 10.1 on 84 df				
Green				
Day 0	0	0	0	0
Day 7	7.5	12.5	2.5	7.5
Day 14	17.5	20	30	25
Day 21	72.5	65	**55**	67.5
SL 2	50	40	22.5	25
SL 3	97.5	87.5	27.5	60
SL 6	0	0	0	0
$LSD_{0.05}$ 14.4 on 84 df				
Green-florets starting to loosen				
Day 0	0	0	2.5	0
Day 7	0	0	2.5	0
Day 14	0	0	5	0
Day 21	12.5	**30**	**37.5**	**30**
SL 2	50	57.5	50	57.5
SL 3	2.5	12.5	**72.5**	40
SL 6	0	0	0	0
$LSD_{0.05}$ 11.9 0n 84 df				
Senescent				
Day 0	0	0	0	0
Day 7	0	0	0	0
Day 14	0	0	0	0
Day 21	0	0	**7.5**	0
SL 2	0	2.5	**27.5**	**17.5**
SL 3	0	0	0	0
SL 6	100	100	100	100
$LSD_{0.05}$ 6.3 on 84 df				

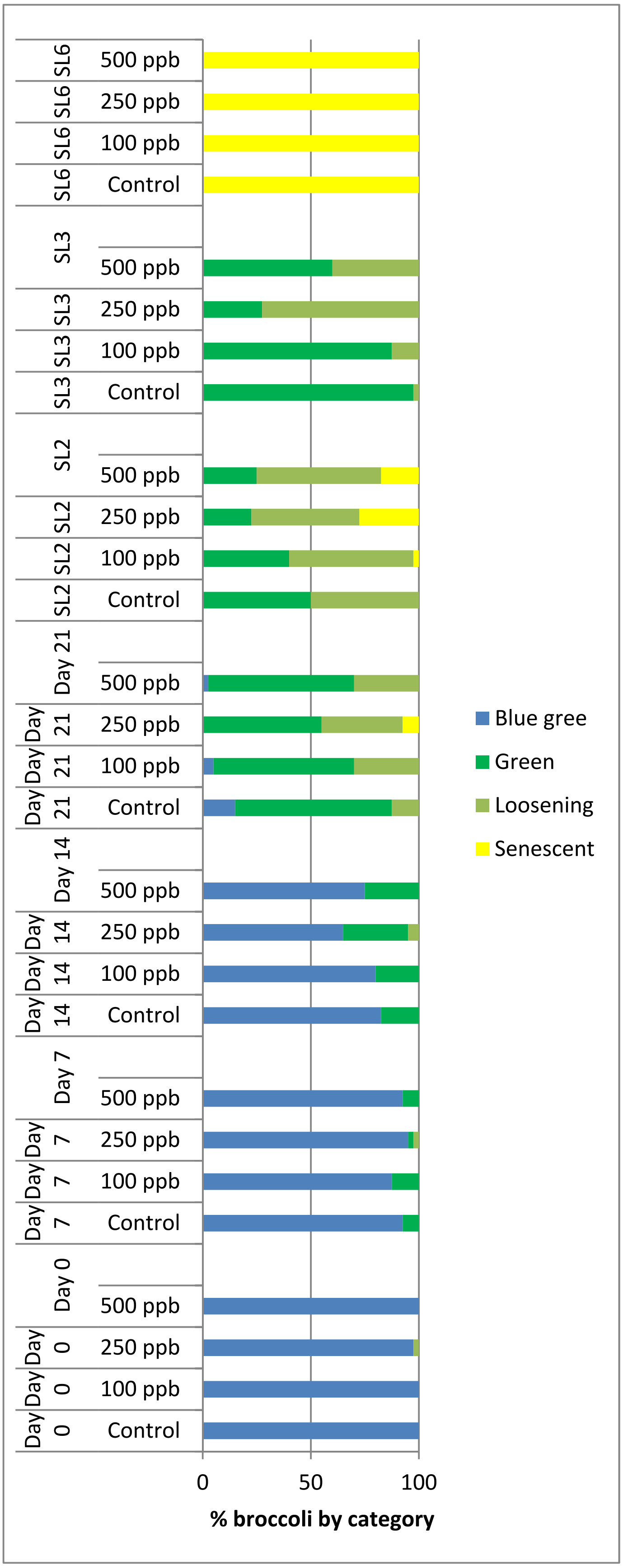


Figure 1.4 *Graphical presentation of data in Table 1.3.*

Improving storage quality of broccoli with post-harvest application of 1-MCP

In 2011, the effects of 1-MCP on the storage and shelf-life of broccoli *cv's* Ironman and Parthenon were assessed. The background green colour of Parthenon did not change during storage, and declined rapidly after 6 days of shelf-life due to chlorophyll degradation (Fig 2.1, 2.2). The lack of effects of 1-MCP was attributed partly of the favourable storage environment of low-temperature (1°C) and a constant movement of saturated air over the heads that maintained the quality of broccoli during storage. Measurements of background yellow development (Minolta b values) of florets found no treatment effects during storage and a small decrease in yellowing associated with 1-MCP-treated heads after 2 days shelf-life. However, no difference between a single dose and split dose treatments on background yellow development was observed (Fig 2.1).

No change in green background colour of Ironman was observed during 21 days of storage at 1°C (100 % RH). On transfer to shelf-life conditions a change from blue/green to green colour was observed in untreated broccoli during the first 3 days of shelf-life followed by a large decline background green colour by day 6 of shelf-life. The rate of senescence of 1-MCP treated broccoli was lower than other treatments during shelf-life (Fig 2.2) A corresponding increase in yellow background was observed during shelf-life but no treatment effects of 1-MCP were observed (Fig 2.2).

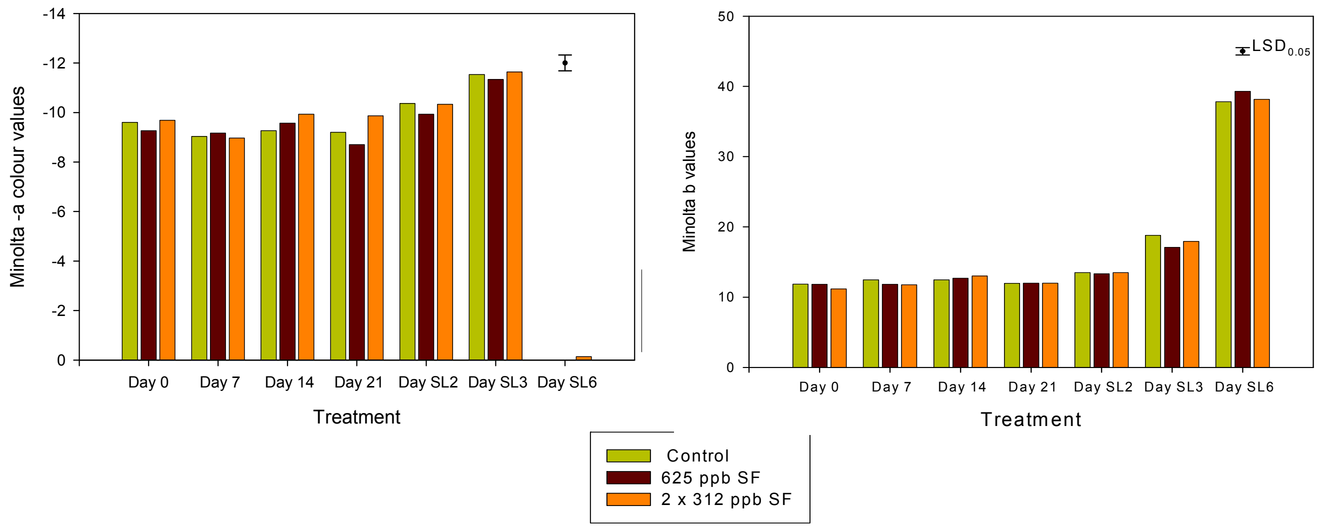


Figure 2.1. *The effect of 1-MCP™ on background green (Minolta '–a values') and background yellow (Minolta 'b values') colour of broccoli cv Parthenon during 21 days storage at 1°C and 6 days shelf-life at 18°C (SL, 2…6).*

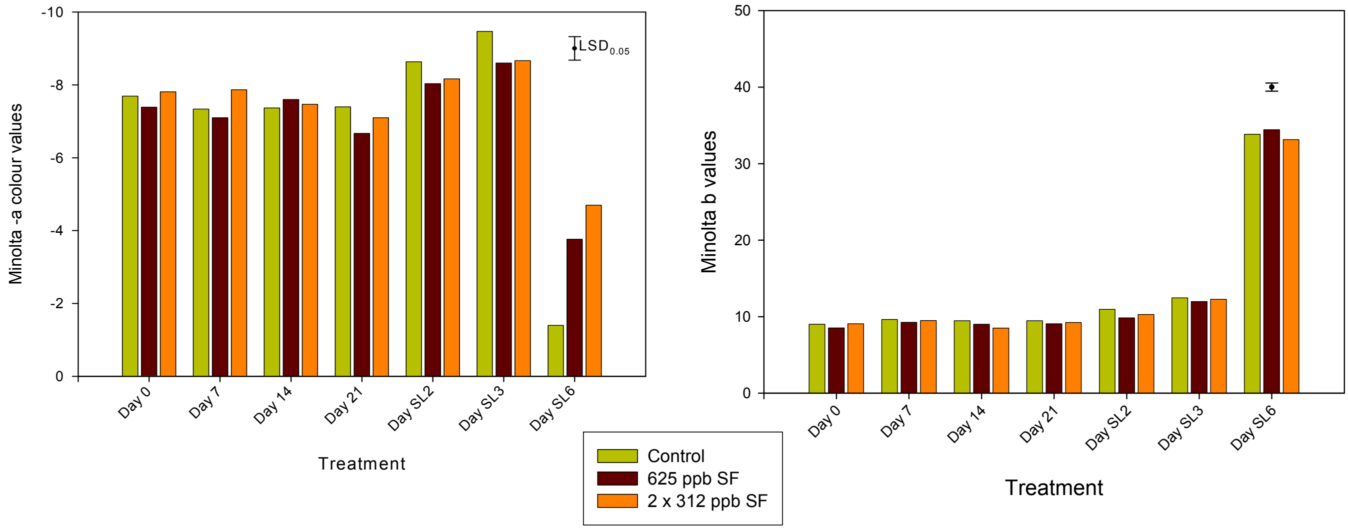


Figure 2.2. *The effect of 1-MCP™ on background green (Minolta '–a values') and yellow colour (Minolta 'b' values) of broccoli cv Ironman during 21 days storage at 1°C and 6 days shelf-life at 18°C (SL, 2…6).*

Visual Assessment

Treatment effects in loss of background green colour were not observed using a Minolta colour meter until broccoli was subject to shelf-life conditions. Visual assessment scores which take into account head colour, bud quality, and tightness and turgidity of heads appears to have been more sensitive in detecting changes in product quality. 1-MCP treatments generally improved the visual quality of Ironman (Table 2.1), larger numbers of broccoli heads were categorised as maintaining blue/green appearance after 14 days of storage than untreated broccoli. 1-MCP treatments maintained the compactness of the inflorescence and reduced the number of flaccid outer florets after 2-3 weeks storage at 1°C. While 1-MCP improved the visual quality of Parthenon compared to untreated controls on days 14 and 21.

Table 2.1 *Visual assessment scores of (Brassica oleracea var. italica) cv. Ironman treated with 625 ppb 1-MCP (1-methylcylopropene) on day 0 or a split application of 312 ppb 1-MCP on day 0 repeated on day 8. Treatment lasted 24 hours at 1°C. Broccoli was stored for 21 days storage at 1°C (100% RH) followed by 6 days shelf-life (SL). Heads were assessed on arrival (Day 0) and after 7, 14 and 21 days. Shelf-life assessments were carried out after 2, 3 and 6 days (SL2..SL6).*

	Ironman			Parthenon		
Inspection	1-MCP 312ppb	1-MCP 625ppb	Control	1-MCP 312ppb	1-MCP 625ppb	Control
Blue-green				**Dark-green**		
Day 0	100.0	100.0	100.0	100	100	100
Day 7	100.0	100.0	100.0	86.7	96.7	86.7
Day 14	**30.0**	**50.0**	0.0	46.7	43.3	33.3
Day 21	0.0	0.0	0.0	0.0	0.0	0.0
SL 2	0.0	0.0	0.0	0.0	0.0	0.0
SL 3	0.0	0.0	0.0	0.0	0.0	0.0
SL 6	0.0	0.0	0.0	0.0	0.0	0.0
LSD $_{0.05}$ 6.2 on 81 df						
Green						
Day 0	0.0	0.0	0.0	0.0	0.0	0.0
Day 7	0.0	0.0	0.0	3.3	3.3	10.0
Day 14	36.7	40.0	40.0	43.3	43.3	46.7
Day 21	10.0	**36.7**	10.0	**76.7**	**70.0**	46.7
SL 2	53.3	43.3	40.0	16.7	20.0	15.0
SL 3	46.7	43.3	46.7	0.0	0.0	13.3
SL 6	0.0	0.0	0.0	0.0	0.0	0.0
LSD $_{0.05}$ 14.8 on 81 df						
Green-florets starting to loosen						
Day 0	0.0	0.0	0.0	0.0	0.0	0.0
Day 7	0.0	0.0	0.0	6.7	0.0	3.3
Day 14	16.7	10	50	10.0	10.0	20.0
Day 21	66.7	53.3	63.3	**23.3**	**30.0**	53.3
SL 2	46.7	56.7	60.0	33.3	**46.7**	25.0
SL 3	53.3	56.7	53.3	23.3	33.3	26.7
SL 6	0.0	0.0	0.0	0.0	0.0	0.0
LSD $_{0.05}$ 15.4 0n 81 df						
Senescent						
Day 7	0.0	0.0	0.0	0.0	0.0	0.0
Day 14	0.0	0.0	0.0	3.3	0.0	0.0
Day 21	16.7	**0.0**	10.0	0.0	3.3	0.0
SL 2	**6.7**	**0.0**	26.7	0.0	0.0	0.0
SL 3	0.0	0.0	0.0	50.0	**16.7**	55.0
SL 6	0.0	0.0	0.0	76.7	63.3	60.0
LSD$_{0.05}$ 10.96 on 81 df	100.0	100.0	100.0	100	100.0	100.0

Parthenon

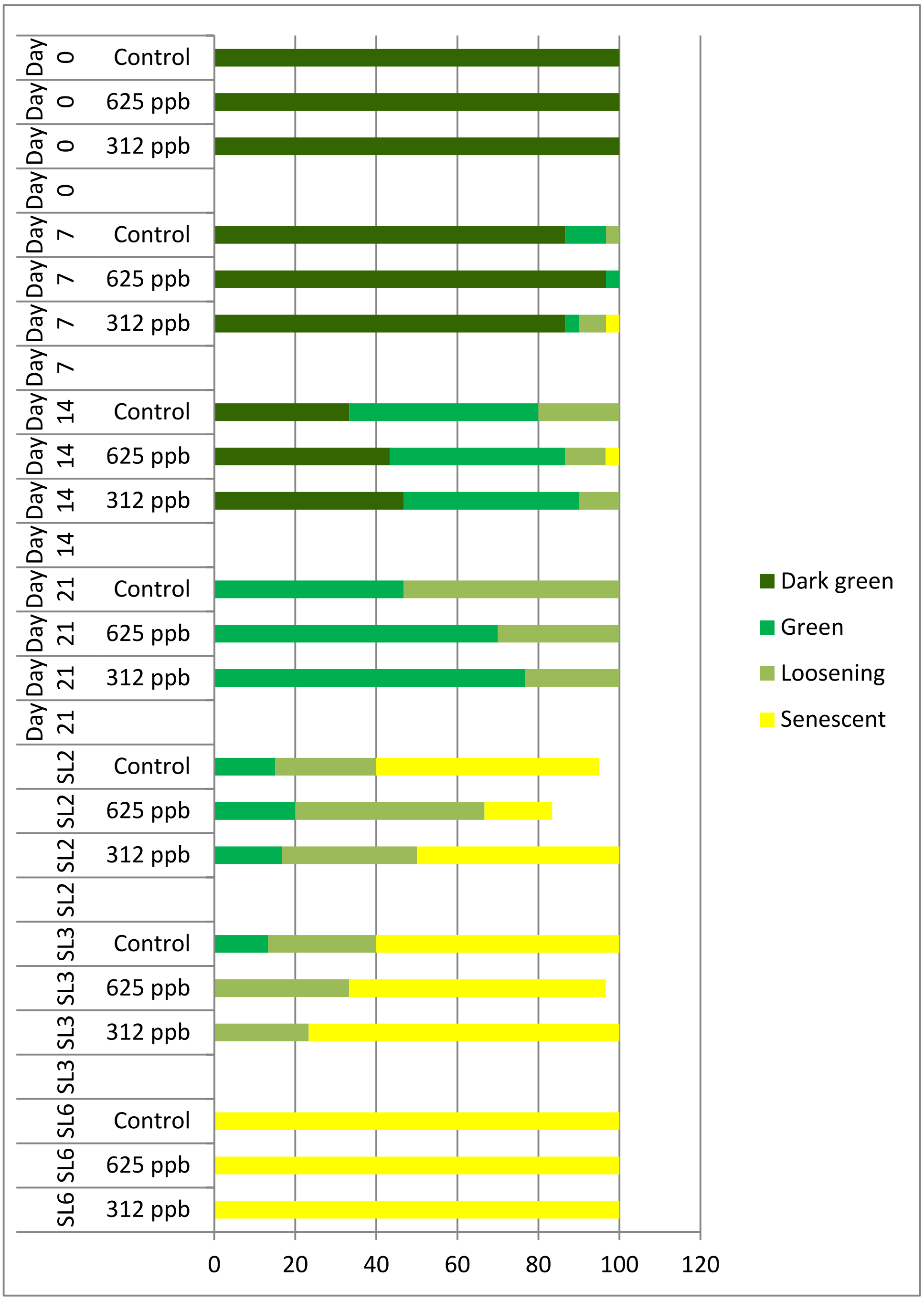


Figure 2.3 *Graphical presentation of data on Parthenon from Table 2.1*

Iron Man

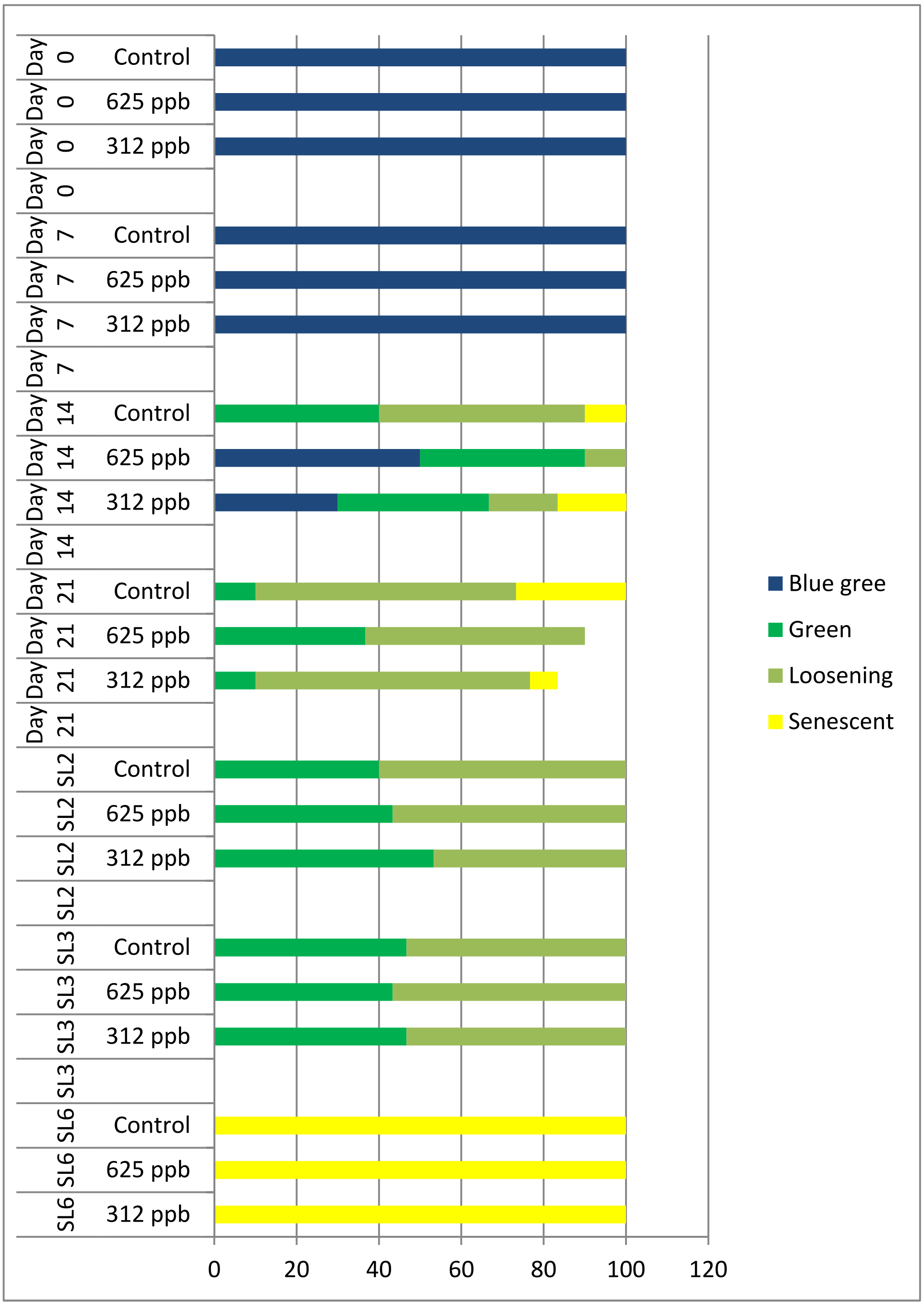


Figure 2.4 *Graphical presentation of data on Iron Man from Table 2.1*

Pre-harvest management factors affecting post-harvest quality of broccoli: Impact of pre-harvest sprays of Permasect C (cypermethrin) and Amistar (Azoxystrobin)

The background green colour (Minolta -a values) of cv. Ironman rose slowly during storage (Fig. 3.1), while the yellow (Minolta b values) colour values remained constant. Transfer to shelf-life conditions resulted in broccoli changing from blue/green to green leading to a small increase in back-ground green colour.

No significant trend in treatment effects was observed in background green colour assessments. However, azoxystrobin and permasect-C-treated Ironman were less prone to senescence (lower b values) during shelf-life than untreated controls (Figure 3.1).

The effect of azoxystrobin and cypermethrin treatment on Parthenon was less apparent. Few changes in background green or yellow colour were observed over the 21 day storage period (Fig 3.2). Under shelf-life conditions a slight rise in the background green colour was observed during the first 3 days of shelf-life followed by a decline by day 6. Cypermethrin treated Parthenon appeared to be more resilient to loss of green colour than other treatments.

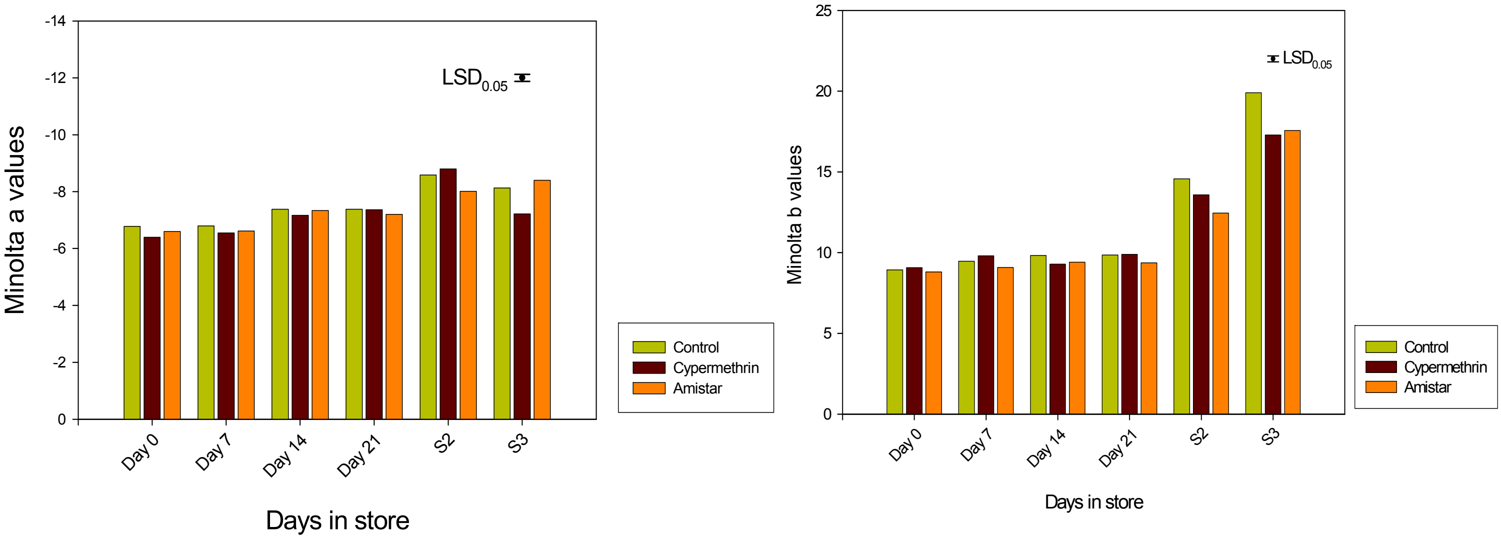


Figure 3.1 *Effect of pre-harvest application of cypermethrin and azoxystrobin on green colour retention and yellowing in cv. Ironman. Sprays were applied 3 weeks before harvest. Broccoli was stored at 1°C (100% RH) for 21 days followed by 6 days shelf-life at 18°C*

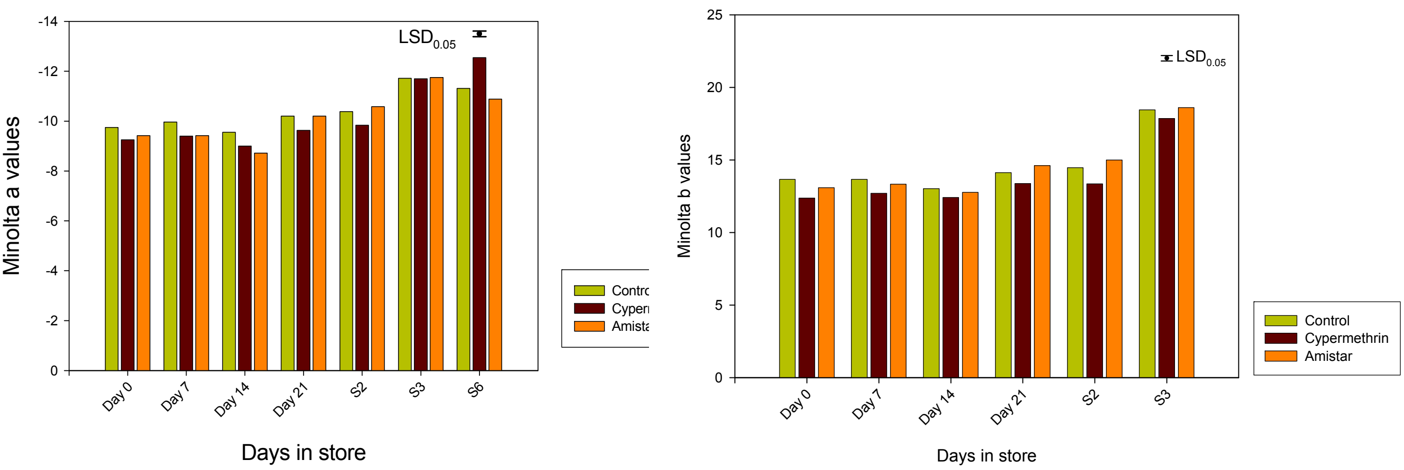


Figure 3.2 *Effect of pre-harvest application of cypermethrin and azoxystrobin on green colour retention and yellowing in cv..Parthenon. Sprays were applied 3 weeks before harvest. Broccoli was stored at 1°C (100% RH) for 21 days followed by 6 days shelf-life at 18°C*

Amistar-treated Ironman had higher fluorescence readings at harvest and after 7 days storage compared to other treatments (Fig 3.3), but thereafter, a steep decline in fluorescence was recorded and no further treatment differences were observed. The drop in chlorophyll activity was not mirrored in colour-meter readings and suggests the technique may be able to discriminate biochemical changes in florets before visual signs of deterioration are observed. Chlorophyll fluorescence in Parthenon remained stable over the storage period but declined during shelf-life.

Parthenon Iron Man

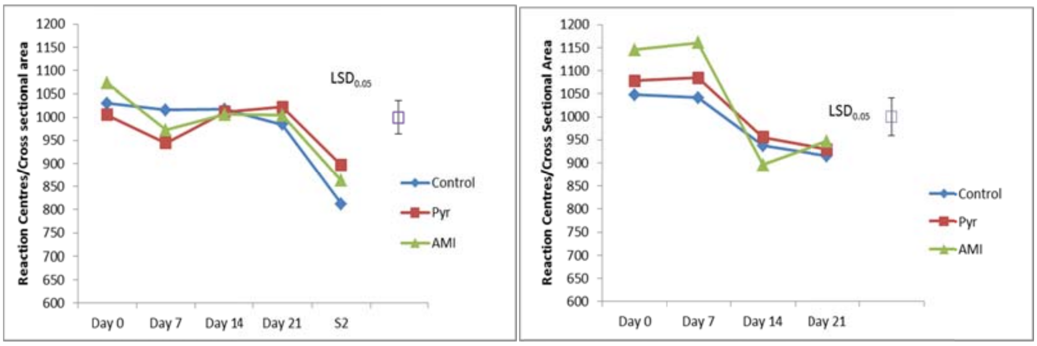


Figure 3.3. *Chlorophyll fluorescence profiles (reaction centres per cross sectional area) for cv's Parthenon and Ironman treated pre-harvest sprays of cypermethrin and azoxystrobin. Sprays were applied 3 weeks before harvest. Broccoli was stored at 1°C (100% RH) for 21 days followed by shelf-life at 18°C.*

Visual assessment

The broccoli cv Ironman was subject to heat stress prior to harvesting and the quality of produce was inferior to earlier consignments. A greater proportion of cypermethrin and azoxystrobin treated broccoli heads were down-graded at the initial assessment compared to untreated controls. During storage azoxystrobin-treated broccoli retained the blue/green background for longer during storage and both cypermethrin and azoxystrobin treated broccoli had fewer heads of broccoli subject to senescence during the initial stages of shelf-life (Table 1.5).

During storage the colour of broccoli florets changed from a blue-green to a dark green background colour and this was reflected in an increase in the –a Minolta values. No treatment differences were observed during the initial three week cold storage period, however, during shelf-life, broccoli treated with azoxystrobin retained green background colour for longer compared to untreated or broccoli treated with cyperrmethrin (Fig 3.4).

Broccoli treated with azoxystrobin were slower to develop a yellow background colour and the combination of azoxystrobin followed by a post-harvest split treatment with 1-MCP provided an additive effect and was the most effective treatment combination to reducing yellowing during shelf-life (Fig 3.5). Post-harvest treatment with 1-MCP helped to reduce loss of green background colour, however, no additive effect of 1-MCP and cyperrmethrin was observed (Fig 3.4). Chlorophyll fluorescent profiles of broccoli during storage declined between 14 and 21 days of storage before significant changes were observed in conventional colour meter readings (Fig 3.6).

Dry matter assessment of broccoli heads and stalks at harvest showed no significant difference in dry matter content, but by the third week of storage broccoli treated with azoxystrobin or cypermethrin had a higher dry matter content suggesting that less carbohydrate was metabolised during storage (Table 1.6). Interestingly, broccoli treated with 1-MCP™ had similar dry matter content to that of freshly harvested produce.

Ethylene production of broccoli heads treated with cypermethrin and azoxystrobin were lower, than the controls, however, only cypermethrin treated broccoli reached significance ($P<0.05$) (Table 1.7). During storage ethylene production rates were variable and no treatment effects were observed (Table 1.9).nDuring storage, respiration rates decreased over the three week period and by week 3 1-MCP treated broccoli had higher respiration rates than where 1-MCP had not been applied.

Broccoli exhibits a high respiration rate after harvest (19–21 mg CO_2 kg^{-1} h^{-1} at 0–1°C) resulting in high water loss and a reduction in saleable commodity. At harvest broccoli treated pre-harvest (Table 1.7) with cypermethrin or azoxystrobin had similar respiration rates (1.4-1.6 mL CO_2 kg^{-1} h^{-1} at 1°C). During storage, respiration rates increased by the first week 7.3-12.4 mL CO_2 kg^{-1} h^{-1} at 1°C but then declined over the subsequent two week period and no effect of pre-harvest treatments on respiration were observed. After 3 weeks storage, 1-MCP treated broccoli had higher respiration rates (Table 1.8).

Broccoli treated with cypermethrin prior to harvest resulted in less water loss during storage (Fig 3.7). Moreover, treatment with 1-MCP led to lower water loss during storage, although no additive effect of combining the cypermethrin and 1-MCP. Azoxystrobin treatment reduced weight loss of broccoli but was less effective than cypermethrin or 1-MCP.

Table 1.5. *Visual assessment scores of (Brassica oleracea var. italica) cvs. Ironman and Parthenon treated pre-harvest with sprays of cypermethrin (Permasect C) and azoxystrobin (Amistar). Sprays were applied 3 weeks before harvest. Broccoli was stored for 21 days storage at 1°C (100% RH) followed by 6 days of shelf-life (SL). Heads were assessed on arrival (Day 0) and after 7, 14 and 21 days. Shelf-life assessments were carried out after 2, 3 and 6 days (SL2…SL6).*

Variety	Ironman			Parthenon		
Inspection	Control	Cypermethrin	Azoxystrobin	Control	Cypermethrin	Azoxystrobin
Blue-green						
Day 0	41.68	**12.5**	**34.73**	100	100	**81**
Day 7	0	0	0	16.7	**0**	**0**
Day 14	0	0	0	0	0	0
Day 21	0	0	0	0	13.9	0
SL 2	0	0	0	0	0	0
SL 3	0	0	0	0	0	0
SL 6	0	0	0			
$LSD_{0.05}$ 4.2 on 105 df					$LSD_{0.05}$ 15.6 on 90 df	
Green						
Day 0	54.2	**87.5**	**65.3**	0	0	8.3
Day 7	27.8	27.8	**44.4**	38.9	**60.9**	45.9
Day 14	19.4	**26.4**	**33.3**	97.2	97.2	94.9
Day 21	30.5	**19.5**	38.9	100	**83.3**	**75.4**
SL 2	0.0	0.0	0.0	16.7	0	0
SL 3	0.0	0.0	0.0	0	0	0
SL 6	0.0	0.0	0.0			
$LSD_{0.05}$ 10.1 on 105 df					$LSD_{0.05}$ 17.5 on 90 df	
Green-florets starting to loosen						
Day 0	4.2	0.0	0.0	0.0	0.0	8.3
Day 7	68.0	70.8	**55.6**	44.4	39.1	54.2
Day 14	58.3	63.9	61.1	1.4	0.0	0.0
Day 21	40.3	**61.1**	**58.3**	0.0	0.0	3.1
SL 2	22.5	**38.9**	31.7	1.4	4.2	5.8
SL 3	1.7	6.1	11.5	0.0	0.0	0.0
SL 6	0.0	0.0	0.0			
$LSD_{0.05}$ 11.7 on 105 df					$LSD_{0.05}$ 9.4 on 90 df	
Senescent						
Day 0	0.0	0.0	0.0	0.0	0.0	0.0
Day 7	4.2	1.4	0.0	0.0	0.0	0.0
Day 14	20.9	**7.0**	**4.2**	1.4	2.8	2.8
Day 21	29.2	**19.4**	**2.8**	0.0	0.0	4.2
SL 2	77.5	**56.9**	**68.3**	94.7	90.3	91.4
SL 3	98.3	93.9	88.5	100.0	97.2	100.0
SL 6	100.0	100.0	100.0			
$LSD_{0.05}$ 8.7 on 105 df					$LSD_{0.05}$ 5.9 on 90 df	

Fig. 3.4

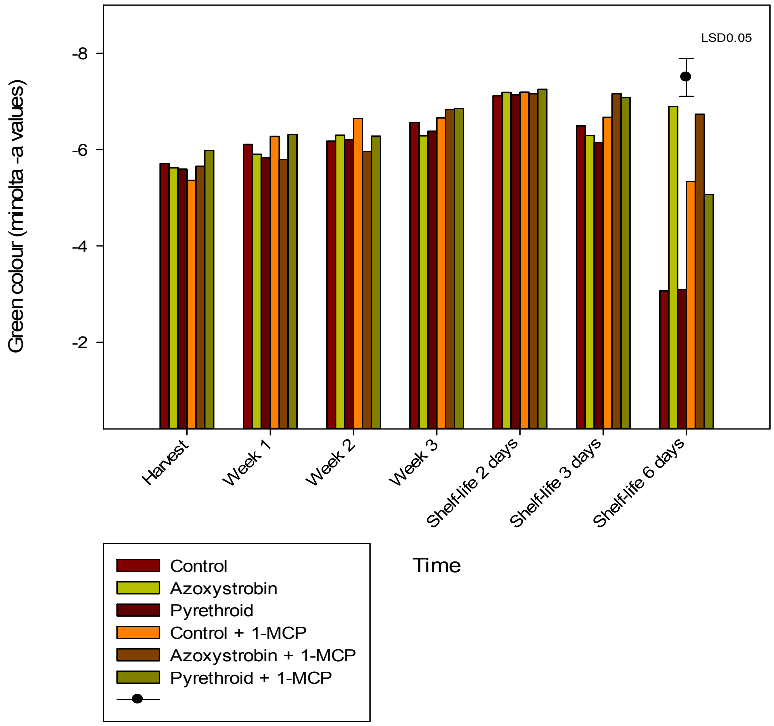


Fig. 3.5

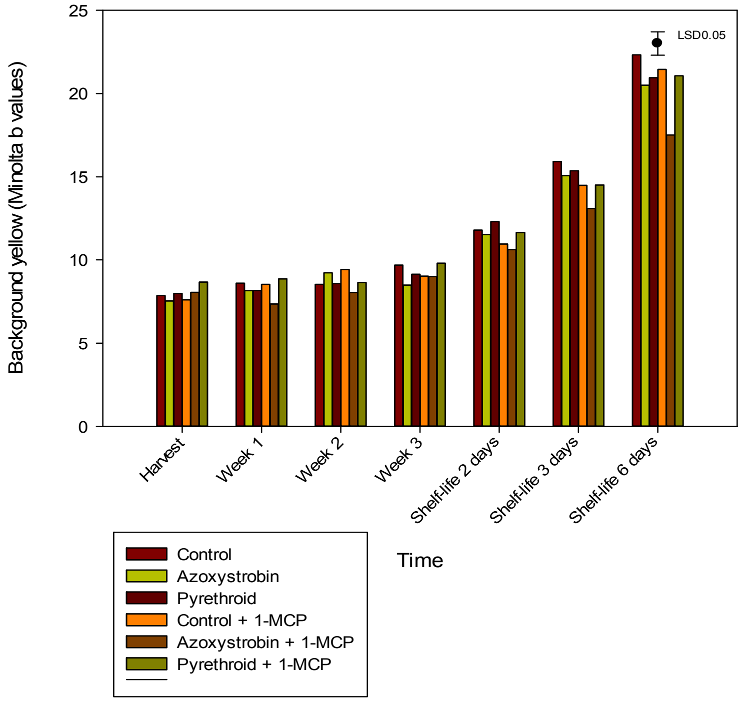


Figure 3.4 & 3.5: *The impact of pre- and post-harvest treatments on the green (A) and yellow (B) back ground colour of broccoli cv. Ironman subject to three weeks storage at 1°C (100% RH) followed by six days shelf-life at 18°C (85% RH)*

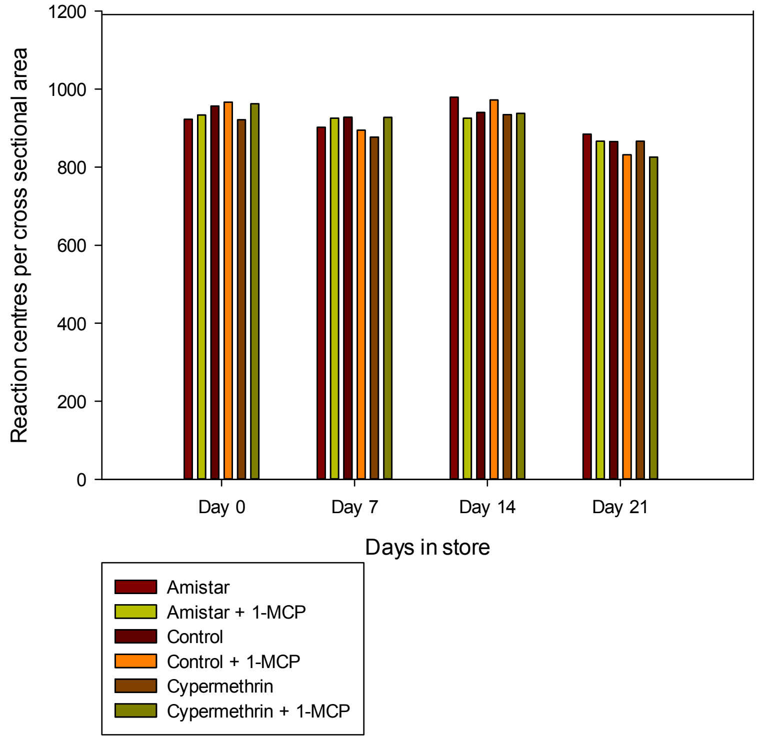


Fig. 3.6: *Chlorophyll fluorescence profiles of broccoli cv. Ironman during storage at 1°C (100% RH)*

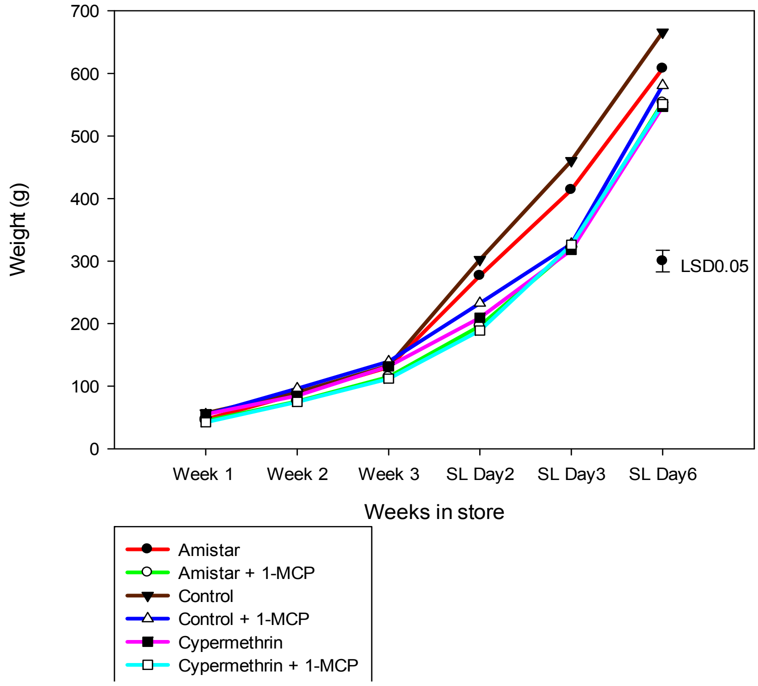


Fig. 3.7: *The impact of pre- and post-harvest treatments on the weight loss of broccoli cv. Ironman subject to three weeks storage at 1°C (100% RH) followed by six days shelf-life at 18°C (85% RH).*

Table 1.6: *Dry matter content of broccoli cv. Ironman*

Treatment	Position	Harvest	3 weeks at 1°C Control	1-MCP
Control	head	30.9	30.1	30.8
Cypermethrin	head	31.5	33.0	29.8
Azoxystrobin	head	31.5	32.5	31.6
Control	stalk	25.0	25.6	26.2
Cypermethrin	stalk	25.3	29.2	24.3
Azoxystrobin	stalk	26.5	27.6	27.8

Table 1.7: *Respiration and ethylene production rates at harvest (1°C) of broccoli cv. Ironman treated three weeks before harvest with azoxystrobin and cypermethrin.*

	Azoxystrobin	Cypermethrin	Control	$LSD_{0.05}$ on 6df
ml CO_2 $g^{-1}h^{-1}$	1.4	1.6	1.4	0.852
Ethylene nL $g^{-1}h^{-1}$	8	6.7	14.8	7.59

Table 1.8: *Respiration rate of broccoli cv. Ironman during storage at 1°C (100% RH).*

Respiration mL CO_2 $g^{-1}h^{-1}$	Week 1	Week 2	Week 3
Azoxystrobin	11.3	7.3	6.5
Cypermethrin	10.8	9.9	6.5
Control	12.0	9.9	6.2
Control+1-MCP	8.9	10.1	9.7
Cypermethrin+1-MCP	11.6	8.6	10.0
Azoxystrobin+1-MCP	12.4	11.0	8.8

$LSD_{0.05}$ for treatment x inspection interaction = 2.28 on 45 df

Table 1.9 *Ethylene production rate of broccoli cv. Ironman during storage at 1°C (100% RH)*

Ethylene production (nL $g^{-1}h^{-1}$)	Week 1	Week 2	Week 3
Azoxystrobin	6.0	4.9	7.6
Cypermethrin	3.8	4.0	6.2
Control	4.4	6.3	5.1
Control+1-MCP	9.0	5.2	7.3
Cypermethrin+1-MCP	11.9	7.0	8.7
Azoxystrobin+1-MCP	10.5	9.8	16.4

$LSD_{0.05}$ for treatment x inspection interaction = 8.35 on 45 df

Ethylene scrubbing trial

The background green colour of ethylene scrubbed broccoli was darker (lower –a minolta values) than un-scrubbed broccoli and this was maintained for the six day period of shelf-life at 18°C and for 13 days at 10°C (Fig. 4.A). At 18°C, the quality of broccoli had declined by day 6 and heads were starting to turn yellow.

Changes in the yellow background of broccoli increased with time for the first two days at 18°C and six days at 10°C with few changes in yellow background colour observed between treatments.

Interestingly by the end of the shelf-life period, broccoli subject to scrubbing was slightly more yellow than that which had been stored in a conventional un-scrubbed store (Fig. 4.B). Chlorophyll fluorescence (CF) profiles followed changes observed in colour changes of broccoli heads during shelf-life and were able to track the increase in broccoli yellowing with time (Fig. 4.C). This data indicates that CF tracked changes in senescence before changes in background green colour were observed.

In addition, ethylene scrubbing reduced weight loss (Table 5). On transfer of ethylene-scrubbed broccoli to shelf-life at 18°C, broccoli lost 1.8% less water than broccoli in the control over a six day period (Fig. 4.D). In comparison at 10°C ethylene-scrubbed broccoli lost 1.1% less water than untreated controls over a 13 day period (Fig.4.D).

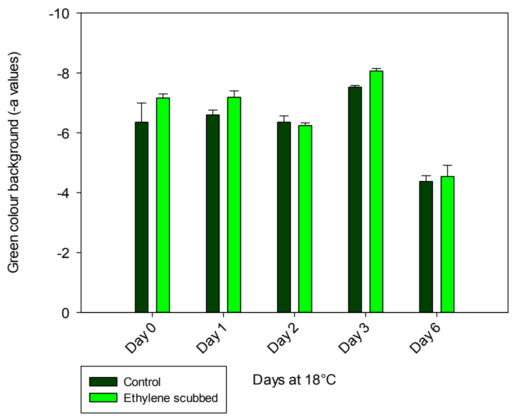


Fig. 4.A

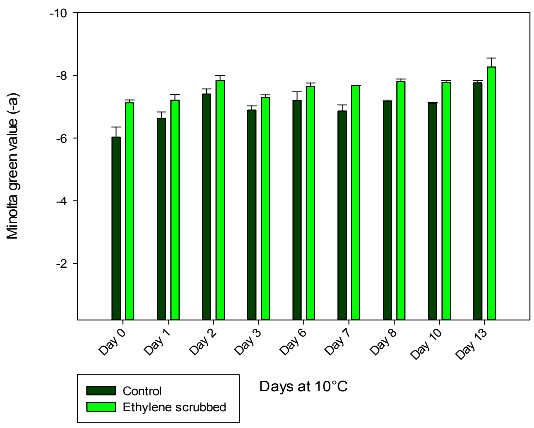
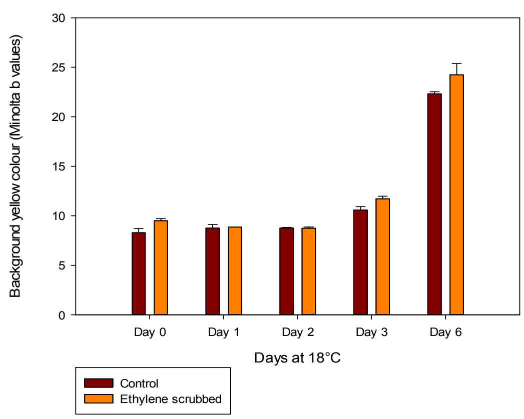


Fig. 4.B

Fig 4.A and 4.B.: *Background green colour (Minolta -a values) in broccoli cv. Ironman stored for two weeks in either an ethylene-scrubbed environment (~200ppb) or conventional cold store (~400 ppb) 1°C (100% RH) followed by shelf-life evaluation at 18°C and 85% RH (Fig 4.A) or 10°C (Fig 4.B).*



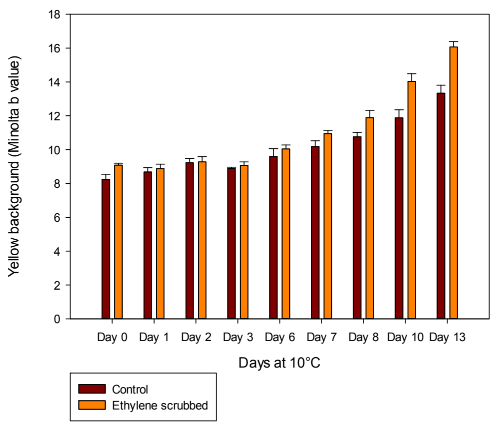


Figure 4 C and 4D: *Background yellow colour (Minolta b values) in broccoli cv. Ironman stored for two weeks in either an ethylene-scrubbed environment (~200ppb) or conventional cold store (~400 ppb) 1°C (100% RH) followed by shelf life evaluation at 18°C or 10°C (85% RH).*

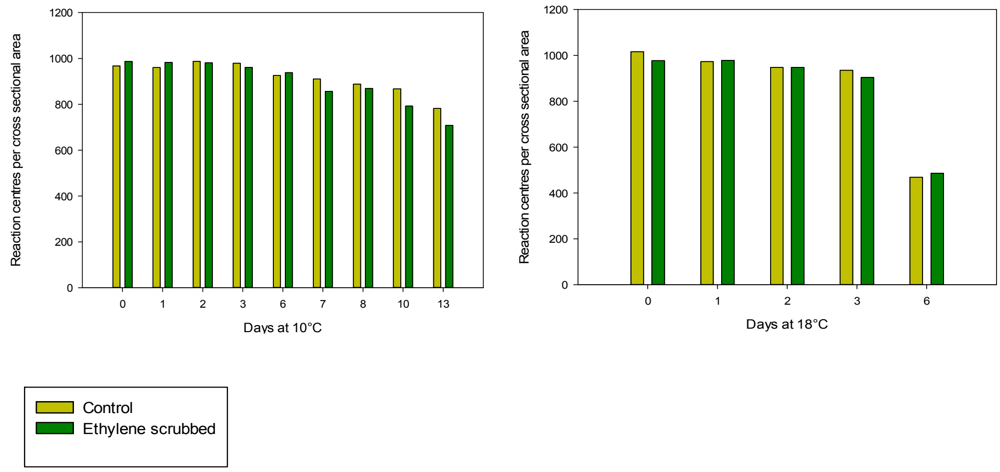


Figure 4.C: *Chlorophyll fluorescence profiles of broccoli cv. Ironman stored in an ethylene-scrubbed store at 1°C (100% RH) prior to shelf-life at either 10°C or 18°C (85% RH)*

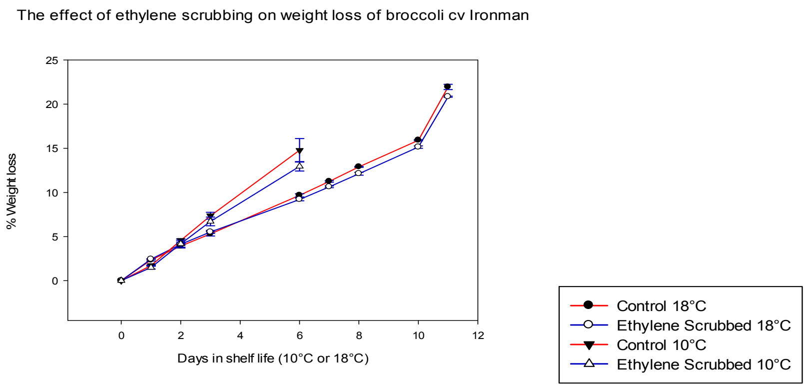


Figure 4.D: *Weight loss profiles of broccoli cv. Ironman stored in an ethylene-scrubbed store at 1°C (100% RH) prior to shelf-life at either 10°C or 18°C (85% RH)*

Table 1.10: *% weight loss of Broccoli cv. Ironman stored for two weeks in an ethylene-scrubbed cold store (1°C 100% RH) prior to transfer to shelf-life (10°C and 18°C) conditions*

Treatment	Shelf-life Temperature	Replicate		% WT loss Day 0	(Data is the mean of three replicates, ten heads per rep) Day 1	Day 2	Day 3	Day 6	Day 7	Day 8	Day 10	Day 13
Control	**10°C**	**1**	**Mean**	**0.00**	**2.36**	**3.93**	**5.31**	**9.67**	**11.25**	**12.90**	**15.91**	**21.95**
			SE	**0.00**	**0.12**	**0.22**	**0.25**	**0.19**	**0.09**	**0.05**	**0.06**	**0.29**
Scrubbed	**10°C**	**1**	**Mean**	**0.00**	**2.45**	**4.13**	**5.53**	**9.21**	**10.63**	**12.12**	**15.14**	**20.84**
			SE	**0.00**	**0.02**	**0.11**	**0.17**	**0.16**	**0.10**	**0.17**	**0.14**	**0.06**
Control	**18°C**	**1**	**Mean**	**0.00**	**1.72**	**4.56**	**7.36**	**14.78**				
			SE	**0.00**	**0.08**	**0.18**	**0.38**	**1.33**				
Scrubbed	**18°C**	**1**	**Mean**	**0.00**	**1.51**	**4.19**	**6.72**	**12.96**				
			SE	**0.00**	**0.19**	**0.39**	**0.48**	**0.54**				

Year 3: Weight loss

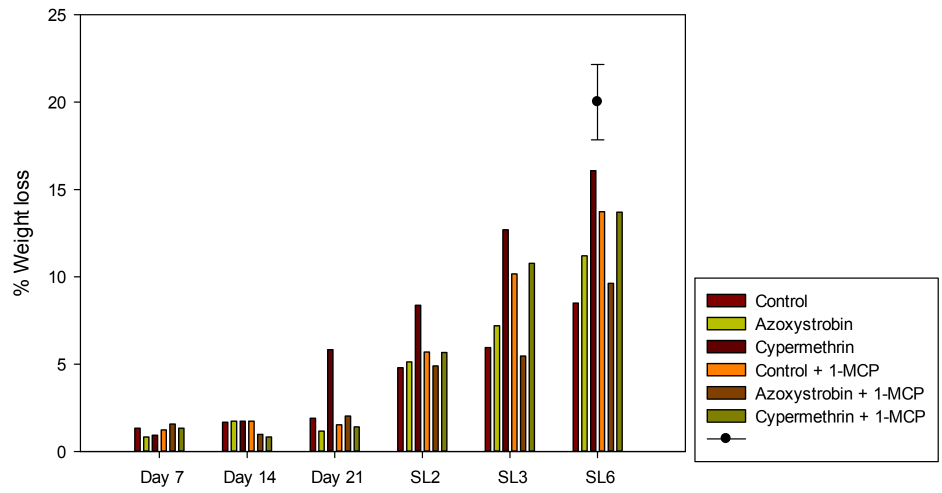


Figure 3.1. *Weight loss of Ironman stored for 21 days at 1°C (95-100% RH) followed by transfer to shelf-life (SL) conditions (18°C) for 6 days.*

Weight loss increased significantly during shelf-life. Broccoli treated with cypermethrin lost more moisture during the later stages of storage and shelf-life than other treatments (Fig 3.1). Cypermethrin treated broccoli appeared to suffer from greater field stress prior to harvest.

Harvest maturity did not influence weight loss during storage in untreated controls. Immature heads treated with 1-MCP lost less moisture after 21 days of air storage (1°C) compared to untreated crop.

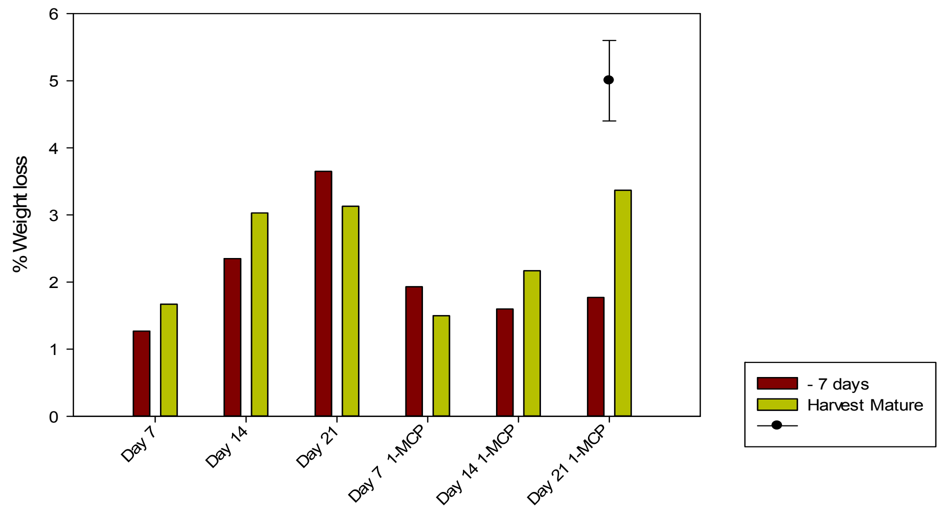


Fig 3.2. *The effect of harvest maturity on moisture loss during storage for 21 days at 1°C followed by transfer to shelf-life conditions (18°C).*

Visual inspection

At harvest no treatment differences in visual quality were observed; all broccoli was blue/dark green in colour with no buds open and florets tightly packed. After 21 days of storage there was little change in visual appearance of Ironman from harvest. Moreover, no treatment effects on visual quality were observed. After transfer to shelf-life conditions (18°C), broccoli lost its blue/green background colour turning a darker green hue. The loss of head integrity and loss of green background colour of buds was first observed after 3 days in shelf-life in control broccoli while the process was retarded in azoxystrobin and cypermethrin treated broccoli.

After 6 days of shelf-life, broccoli began to senesce in all treatments; deterioration in broccoli treated with azoxystrobin or cypermethrin combined with 1-MCP or cypermethrin alone was slower than other treatments.

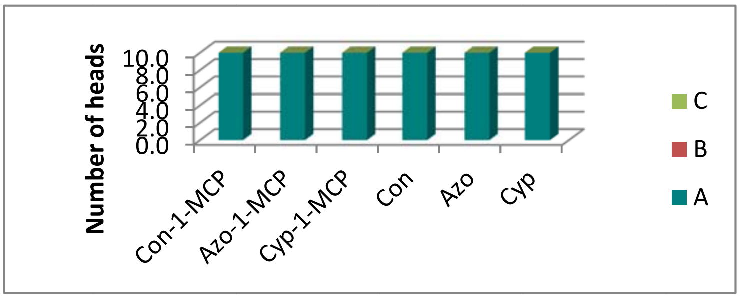


Fig 3.3 Day 0: *visual assessment of broccoli heads at harvest*

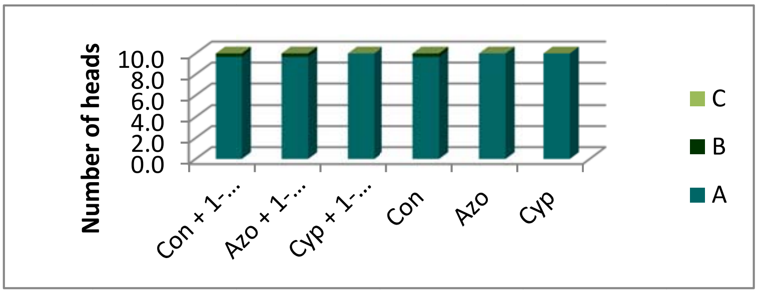


Fig 3.4 Day 21: *visual assessment of Ironman after 21 days storage at 1°C (95-100% RH)*

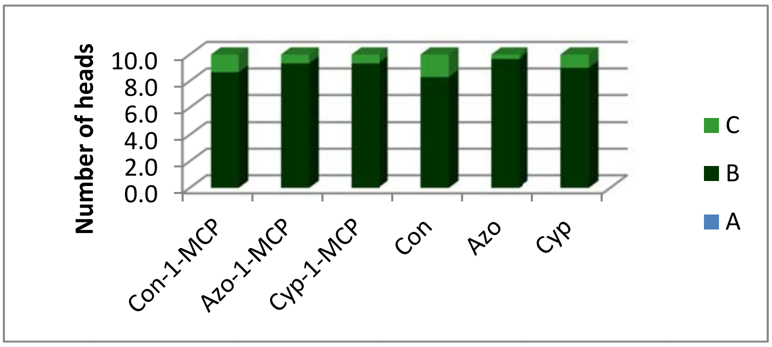


Fig 3.5 Shelf-life day 2: *visual assessment of Ironman after 21 days storage (1°C) + 2 days at 18°C*

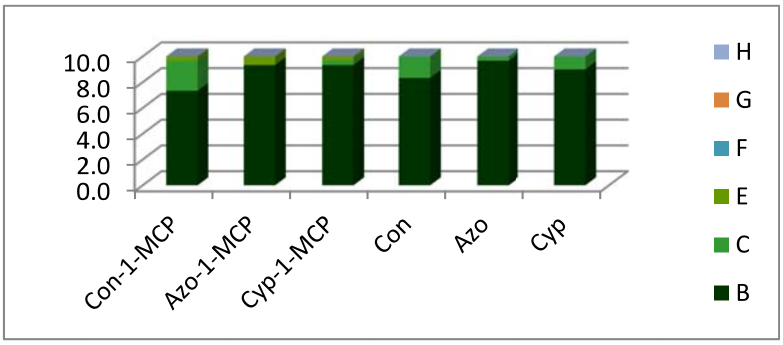


Fig 3.6 Shelf-life day 3: *visual assessment of Ironman after 21 days storage (1°C) + 3 days at 18°C*

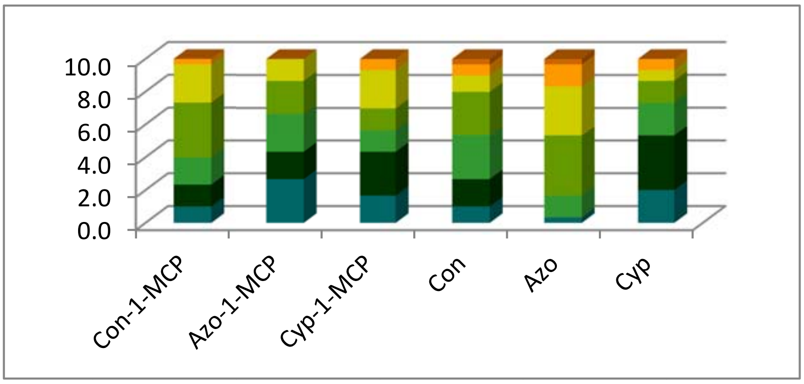


Fig 3.7 Shelf-life day 6: *visual assessment of Ironman after 21 days storage (1°C) + 6 days at 18°C*

Colour Assessments

Changes in green background colour where charted over the 3 week storage period where broccoli changed from blue/green to green leading to a lowering in the Minolta –a colour value, before declining during shelf-life as broccoli turned from green to yellow. Broccoli treated with azoxystrobin remained green after 6 days shelf-life. The application of 1-MCP reduced the decline in green background colour.

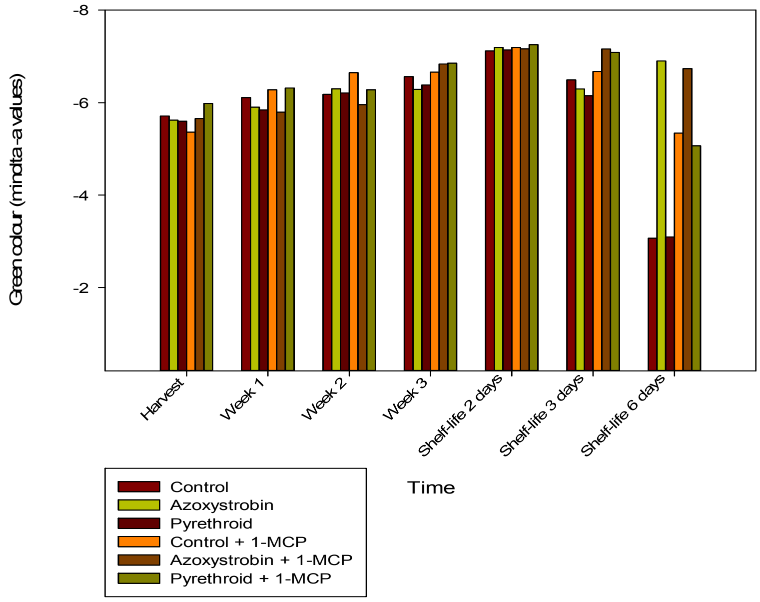


Figure 3.8 *Background green colour of broccoli during 3 weeks storage at 1°C followed by transfer to 18°C*

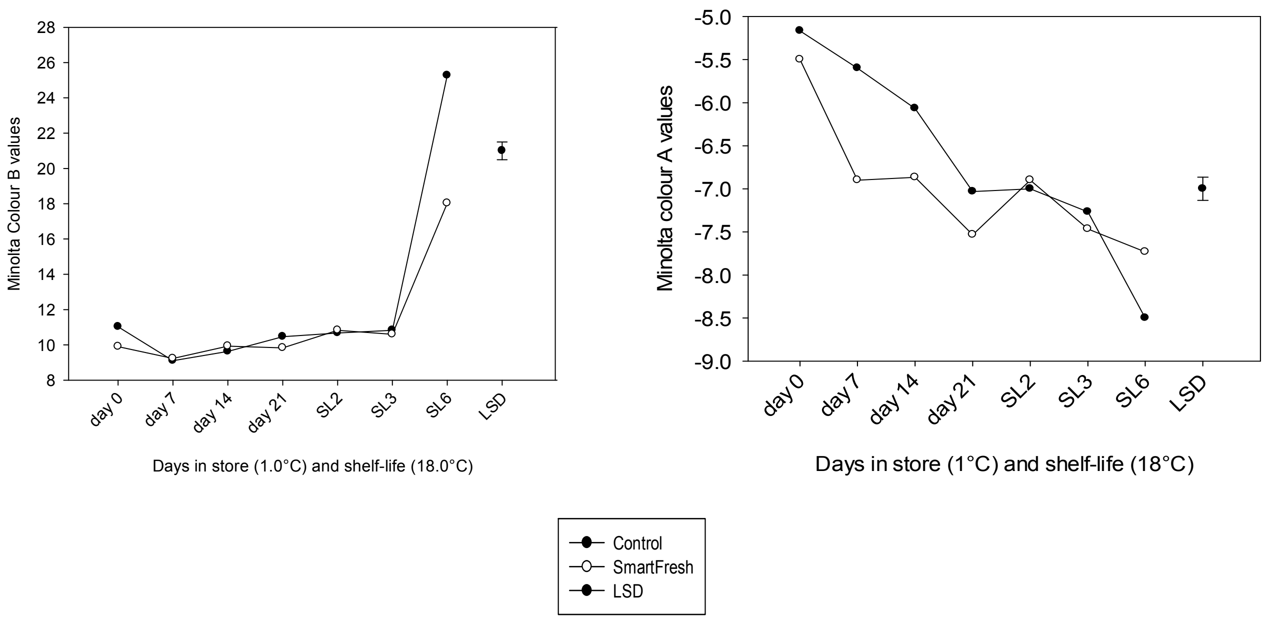


Figure 3.9. *Changes in green and yellow background colour of Ironman stored at 1°C and transferred to shelf-life conditions (18°C). Broccoli was treated pre-harvest with azoxystrobin, cypermethrin and post-harvest with a split dose (312 ppb) of 1-MCP at harvest and after 21 days at 1°C.*

Azoxystrobin retarded the loss of green background colour during storage (Fig 3.8). Application of 1-MCP (SmartFresh) delayed the rate of yellow colour development during shelf-life and retarded the loss of green colour than untreated broccoli (Fig 3.9) during storage and delayed the onset of senescence during the later stages of shelf-life.

Chlorophyll fluorescence activity

There was no significant effect of azoxystrobin or cypermethrin or 1-MCP on chloroplast activity as measured by chlorophyll fluorescence (reaction centres per cross sectional area) (Fig 3.10). Chlorophyll fluorescence was able to determine difference in maturity of broccoli heads at harvest with immature heads having a higher reaction centre per cross sectional area activity than heads harvested a week later (Fig 3.11)

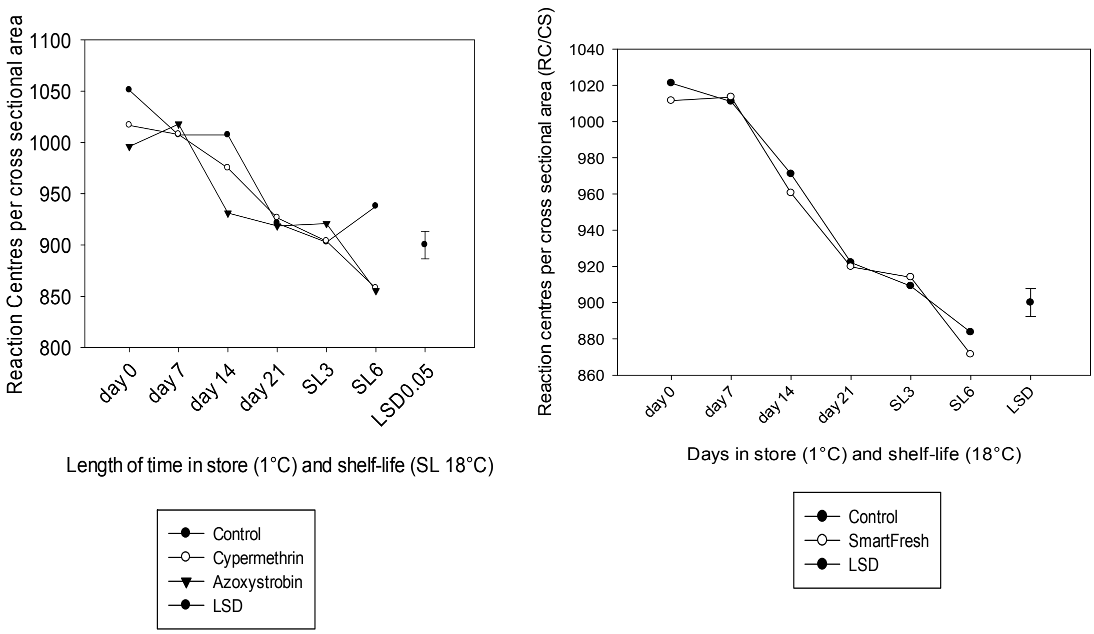


Figure 3.10. *Changes in chloroplast activity (reaction centres per cross sectional area) of Ironman stored at 1°C and transferred to shelf-life conditions (18°C). Broccoli was treated pre-harvest with azoxystrobin, cypermethrin and post-harvest with a split dose (312 ppb) of 1-MCP at harvest and after 21 days at 1°C*

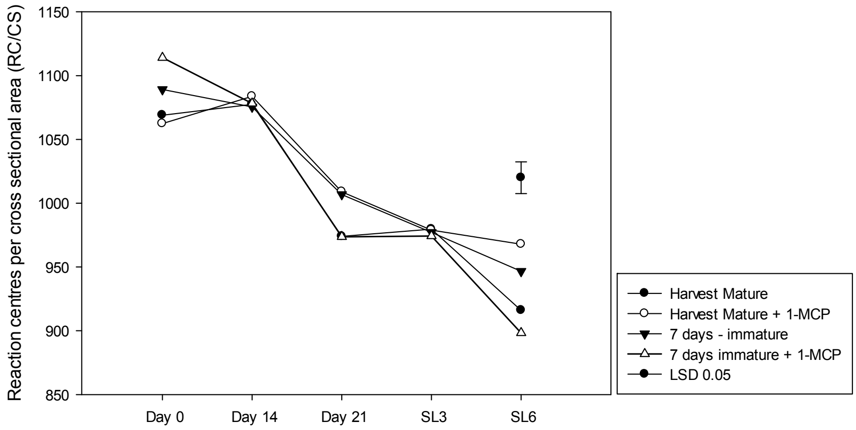


Figure 3.11 *Changes in chloroplast activity (reaction centres per cross sectional area) of Ironman harvested at 2 stages of maturity; harvest maturity and 7 days prior to harvest maturity. Heads were stored at 1°C for 21 days before transfer to shelf-life (SL) conditions (18°C) where heads were assessed after 3 and 6 days. Broccoli was treated with a split dose (312 ppb) of 1-MCP at harvest and after 21 days at 1°C.*

Ascorbic acid content

Azoxystrobin-treated broccoli treated had lower vitamin C content at harvest in 2011 and 2012 trials. During storage there was a general decline in vitamin C content with over the 3 week storage period and treatment difference between pre-harvest field treatments did not improve the retention of vitamin C. In 2012 and 2013 storage seasons the application of 1-MCP after harvest reduced the loss of vitamin C content in longer stored broccoli.

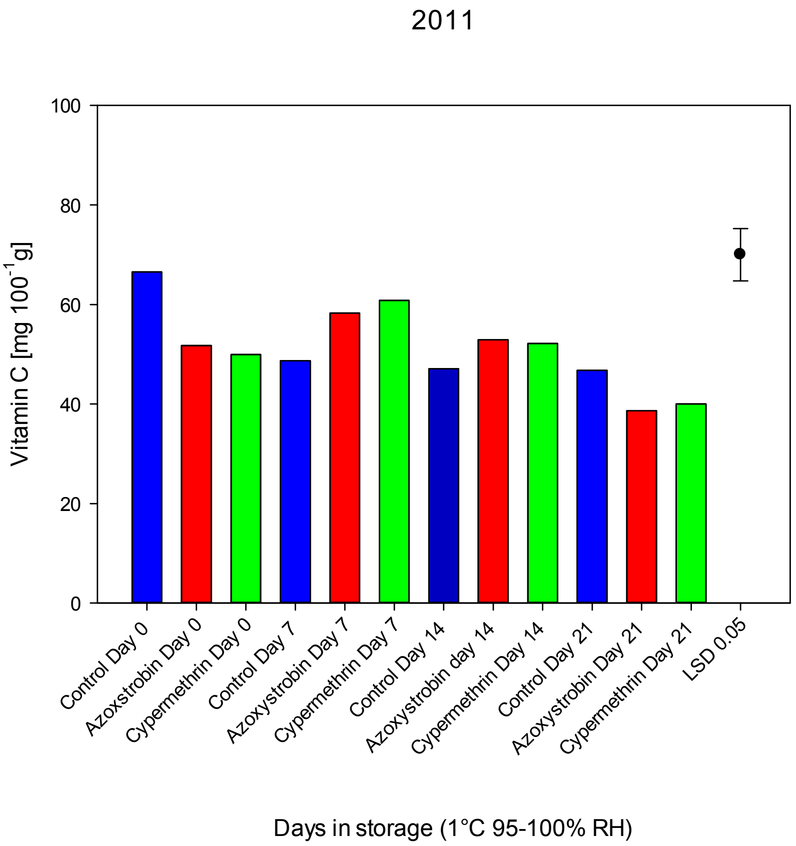


Figure 3.12: *Ascorbic acid content of Ironman treated pre-harvest with azoxystrobin and cypermethrin and stored for 21 days at 1°C (95-100% RH).*

2012

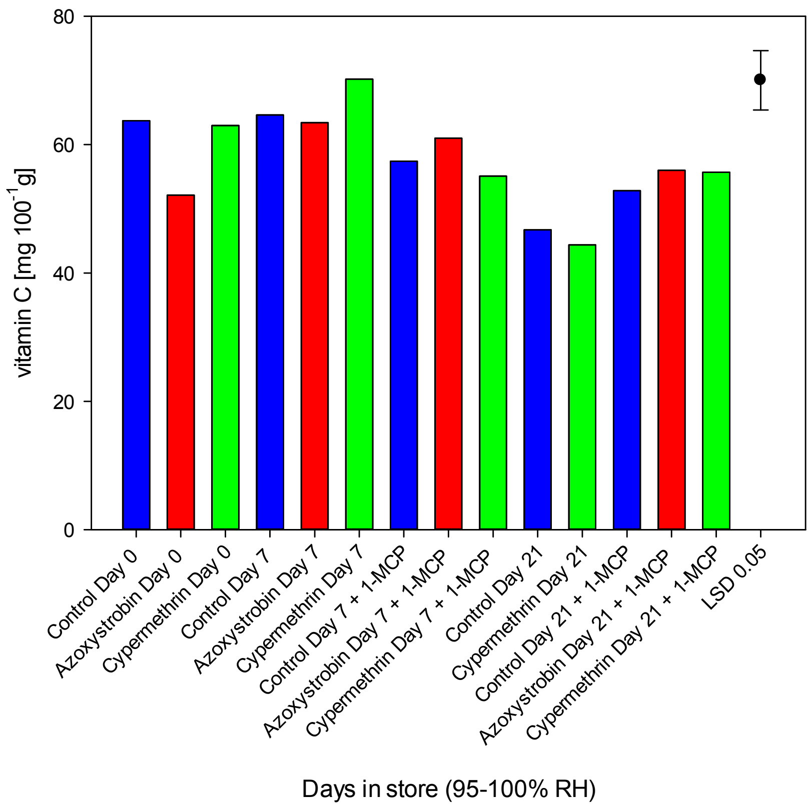


Figure 3.14: *Ascorbic acid content of Ironman treated pre-harvest with azoxystrobin and cypermethrin combined with 1-MCP treatment applied post-harvest. Broccoli was stored for 21 days at 1°C (95-100% RH).*

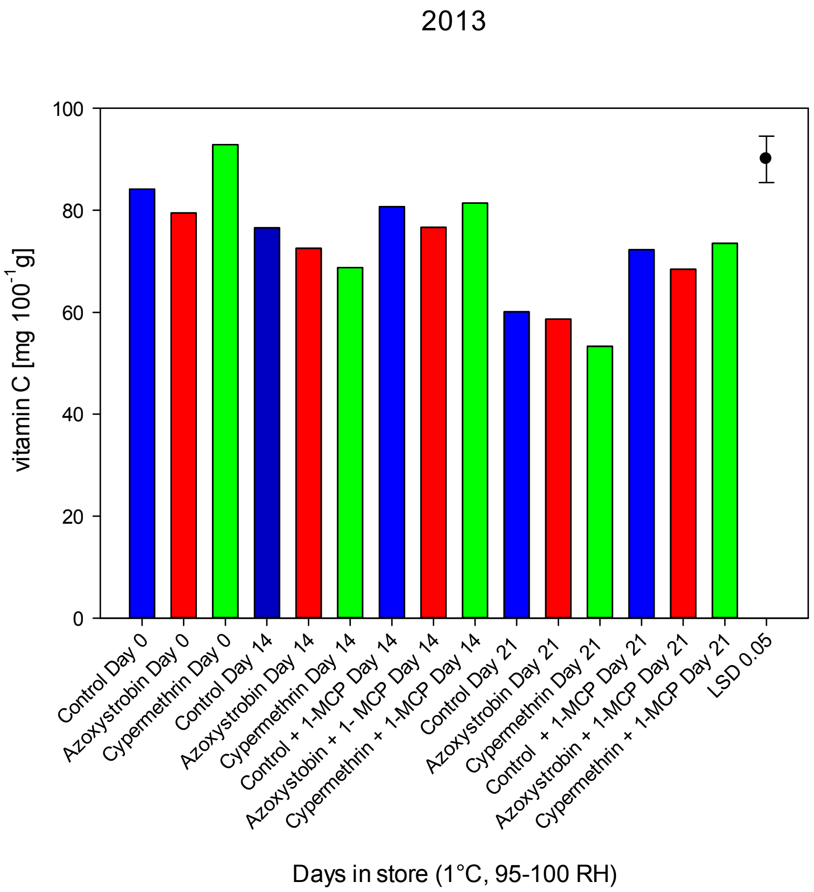


Figure 3.15: *Ascorbic acid content of Ironman treated pre-harvest with azoxystrobin and cypermethrin combined with 1-MCP treatment applied post-harvest. Broccoli was stored for 21 days at 1°C (95-100% RH).*

Discussion

Pre-harvest sprays of azoxystrobin had a significant effect on extending the post-harvest storage life of broccoli, reducing the decline in background green colour and lowering the rate of water loss during shelf-life. Earlier work on cereals has demonstrated azoxystrobin retains green leaf colour (Bertelsen *et al*, 2001) Earlier reports suggested that strobulurlins were capable of inhibiting ACC synthase (Grossman and Retzlaff 1997) and ethylene production, however, later reports suggest inhibition of ACC oxidase was the mode of action (Silverman *et al* 2009). Moreover, strobilurins interference in ROS signalling has been reported to reduce senescence, with azoxystrobin treated wheat leaves showing higher concentrations of H_2O_2 (Wu and von Tiedemann 2001) .While the improvement in overall storage quality of azoxystrobin-treated broccoli may be partly attributed to a reduction in post-harvest disease, the incidence of *Botrytis* was very low across all treatments and only increased by the end of shelf-life. The combination of azoxystrobin and 1-MCP treatment had an additive effect on reducing the decline in senescence. Ethylene exposure is known to increase the degradation of ethylene receptors and increase tissue sensitivity to ethylene (Kevany *et al* 2007); therefore, treatments that are capable of reducing ethylene production in heads prior to harvest may also increase the efficacy of 1-MCP. The reduction in weight loss was most attributable to a lowering in the rate of respiration at harvest and during shelf-life. Respiration rates during cold storage were similar in all treatments, but once at ambient (18°C) water loss in controls exceeded that of other treatments.

While cypermethrin applied pre-harvest reduced ethylene production at harvest, it failed to translate into a lowering in the rate of senescence in shelf-life. However, a reduction in weight loss was observed in cypermethrin-treated heads; the dry matter content of heads and stalk after three weeks storage was greater in cypermethrin-treated broccoli.

Reducing ethylene in the storage atmosphere in brassica stores has proven successful in retarding the rate of decline in broccoli quality during storage and this has translated into an improvement of the quality of broccoli during subsequent shelf-life. Ethylene-scrubbed broccoli had a small improvement in the green background colour coming out of storage after two weeks in a store and this was maintained during a week's shelf-life at 10°C and 18°C. Interestingly, during extended shelf-life studies at 10°C, an increase in the amount of yellow background also increased; an increased opening of the subtended sepals surrounding the buds may have contributed to an increase in the background yellow values.

A lowering of 1–2% in weight loss was also observed in ethylene-scrubbed broccoli and was most likely attributed to a reduced respiration rate at harvest and during shelf-life. Further commercial trials of ethylene-scrubbed broccoli and cabbage in the UK have reported a positive effect on scrubbing extending the storage life of product and reducing wastage.

The success of ethylene scrubbing in improving quality of brassicas during storage and shelf-life is dependent on the effectiveness of the scrubbing technology used. Samples of air taken from ethylene-scrubbed stores where no forklift activity was present showed a halving of ethylene from 140 ppb to 70 ppb. In situations where forklifts were regularly entering stores, ethylene concentrations were approaching 200–250 ppb in scrubbed stores compared to 400 ppb in non-scrubbed stores. Maintaining concentrations below 50–100 ppb can have a significant impact on improving the storage quality of produce. Selection of the most appropriate type of scrubber will depend on the background level of ethylene present in the storage atmosphere.

Conclusions

- Ethylene build up in confined spaces such as store rooms is sufficient to accelerate deterioration during shelf-life.
- Removal of ethylene in store rooms retards the deterioration and lowers moisture loss of broccoli during subsequent shelf-life.
- A split application of 1-MCP™ applied at harvest and prior to removal from storage (1°C 100% RH) can extend product quality.
- 1-MCP application reduced loss of vitamin C content during storage
- Growers should be aware that some azoxystrobin when applied to broccoli for control of disease may have secondary effects on extending shelf-life quality of broccoli

References

Bertelsen, J.R.,. de Neergaard, E. and Smedegaard-Petersen, V. (2001) Fungicidal effects of azoxystrobin and epoxiconazole on phyllosphere fungi, senescence and yield of winter wheat. Plant Pathology, 50, 190-205.

Grossmann, K. and Retzlaff, G. (1997) Bioregulatory effects of the fungicidial strobilurin Kresoxim-methyl in wheat (*Triticum aestivum*)Pesticide Science, 50, 11-20

Kevany, BM, Tieman, DM, Taylor, MG, Dal Cin, V, Klee, HJ (2007). Ethylene receptor degradation controls the timing of ripening in tomato fruit. Plant Journal; 51 (3) 458-467.

Silverman. P F.. Kochan. J.C., Petracek, P. (2009) Strobulins affect ethylene biosynthesis through inhibition of ACC oxidase. (Abstract) The 8th International Symposium on the Plant Hormone Ethylene 21st to 25th June 2009 Cornell University, Ithaca, New York, USA

Wu, Y-W, von Tiedemann, A. (2001) Physiological Effects of Azoxystrobin and Epoxiconazole on Senescence and the Oxidative Status of Wheat. Pesticide Biochemistry and Physiology, 71, (1), 1–10

Knowledge and Technology Transfer

Article for HDC News June 2012. Brassica Grower Association (BGA) conferences in Jan 2012 and Jan 2014, BGA technical event, Lincolnshire, October 2012.

Acknowledgements

The authors would like to acknowledge the Brassica Growers Association, Gavin Willerton (Produce World), Mark Tully (Landseer Ltd.), Ken Hatch (CAUK Ltd.), Absoger SAS (France) and ABC Agronomy Ltd. for their assistance in delivering the project.