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Project leader:	Dr Richard J. Colgan
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Location of project:	Produce Quality Centre East Malling Research New Road East Malling, Kent ME19 6BJ
Project coordinator:	Gavin Willerton
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DISCLAIMER

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.

Signature ....Richard Colgan........................ Date . 31st March 2013.

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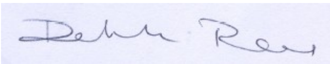
Signature .....  .... Date 31st March 2013

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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 Amistar or Permasect C, or post-harvest application of SmartFresh^{TM}, or a combination of Amistar followed by SmartFresh^{TM}.
- 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 SmartFreshTM (1-methylcyclopropene); and the use of pre-harvest chemical treatments to manipulate ethylene production and response by broccoli heads. 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 the project and main conclusions

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 weight loss by 1–2%.

In these second year trials, a repeat of Year 1 trials was carried out on the impact on broccoli quality of pre-harvest sprays of Permasect C and Amistar, both used as part of standard crop protection programmes. Results from trials in Year 2 confirm that Amistar has secondary beneficial effects on improving shelf-life quality of broccoli. SmartFresh^{TM} applied at harvest, and a second application after three weeks storage, reduced the onset of senescence during shelf-life (at 18°C), while combining the split application of SmartFresh^{TM}

to broccoli treated with Amistar three weeks before harvest was the most effective treatment to reduce the onset of senescence in broccoli. The effectiveness of treatments on 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.

Financial benefits

In-field application of Amistar and Permasect C, minimising the build-up of ethylene in the storage environment, post-harvest treatment using SmartFresh™, and a combined treatment of pre-harvest Amistar application followed by post-harvest SmartFresh™ 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 for growers

- Reducing background ethylene in stores can improve storage and shelf-life of broccoli. Use of electric-powered fork-lift trucks in confined storage spaces can help to prevent the build-up of ethylene.
- Broccoli product quality can be extended by using a split application of SmartFresh™ at harvest and prior to removal from storage. On crops previously treated with Amistar as a crop protection measure, subsequent application of SmartFresh™ has a secondary additive effect on shelf-life and 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™ (1-methylcyclopropene; 1-MCP).

c) The use of pre-harvest chemical treatments to manipulate ethylene production and response.

d) To define a protocol to use 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

Broccoli (*Brassica oleracea* var. Italica) cv. Ironman was harvested from Lincolnshire on two occasions (14 August and 9 October 2012). Produce was transported by cool chain to the Produce Quality Centre at East Malling Research, Kent, UK.

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 360L in volume. Data-loggers (Easylog) recording humidity and temperature were placed inside each cabinet.

Quantification of ethylene

Ethylene concentrations were monitored regularly during the ethylene treatment phase of the project. Replicate 1mL 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 1m long, 6mm OD glass column packed with 100/120 mesh alumunia maintained at 130°C and flushed with nitrogen at 43mL min^{-1} at 10psi. Eluted peaks were integrated using a delta integrator (Delta Data Systems). A standard ethylene gas mixture (840nL L^{-1}) supplied by BOC was used to calibrate the GC at the beginning of each run. The limit of detection was 4nL 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 (see below) 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).

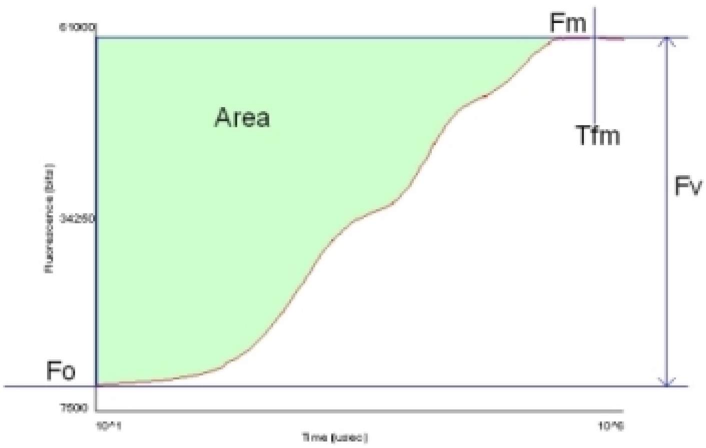


Fig 2.A typical fluorescence trace obtained using a non-modulated fluorimeter such as the Handy PEA (Hansatech Instruments Ltd, UK).

Pre-harvest and post-harvest management of broccoli: Impact of pre-harvest sprays of Permasect C and Amistar and post-harvest application of SmartFresh™

A trial site for Ironman in Lincolnshire was kindly supplied by Marshalls (Produce World). Spray trials were designed by Allium and Brassica Agronomy Ltd. The trial area consisted of a 24 m x 69 m site within which three 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 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 cooled immediately and sent via cool chain to the Produce Quality Centre at East

Malling Research. Here, broccoli cv. Ironman was randomised between treatments before being placed into storage chambers at 1°C, with a flow (1 l kg^{-1} h^{-1}) of humidified air (100 % RH).

SmartFresh™ was applied as a split application; broccoli was treated with 312 ppb immediately after harvest and again following three weeks storage. Treatment was carried for 24 hours at 1°C. SmartFresh™ was exhausted from the cabinet holding broccoli after treatment.

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.

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

An ethylene scrubber model 1CE400F (ABSOGER SAS, France) was installed in a commercial broccoli store at Marshalls (Produce World) and was 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 being 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.

Results and discussion

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 Amistar retained green background colour for longer compared to untreated or broccoli treated with Permasect C (Fig 3.A). Broccoli treated with Amistar were slower to develop a yellow background colour and the combination of Amistar followed by a post-harvest split treatment with SmartFresh^{TM} provided an additive effect and was the most effective treatment combination to reducing yellowing during shelf-life (Fig 3.B). Post-harvest treatment with SmartFresh^{TM} helped to reduce loss of green background colour, however, no additive effect of SmartFresh^{TM} and Permasect C was observed (Fig 3.A). 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.C).

Broccoli is known to exhibit a high respiration rate even at 0–1°C (19–21 mg CO_2 kg^{-1} h^{-1}) resulting in high water loss and a reduction in saleable commodity. Broccoli treated with Permasect C prior to harvest resulted in less water loss during storage than untreated controls (Fig 3.D). Moreover, treatment with SmartFresh^{TM} also led to lower water loss during storage, although no additive effect of combining the treatment was observed. Amistar treatment reduced weight loss of broccoli compared to the controls but was less effective than Permasect C or SmartFresh^{TM}.

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 Amistar or Permasect C had a higher dry matter content suggesting that less carbohydrate was metabolised during storage (Table 1). Interestingly, broccoli treated with SmartFresh^{TM} had similar dry matter content to that of freshly harvested produce.

Ethylene production and respiration rates of broccoli at harvest identified that the untreated control broccoli had the highest ethylene production rate while heads treated with Permasect C had a lower rate of ethylene production at harvest (Table 2). However, during storage an increase in respiration rates was observed but no treatment effects were noted (Table 3). The ethylene production of broccoli was variable and there were no large treatment effects (Table 4).

Fig. 3.A

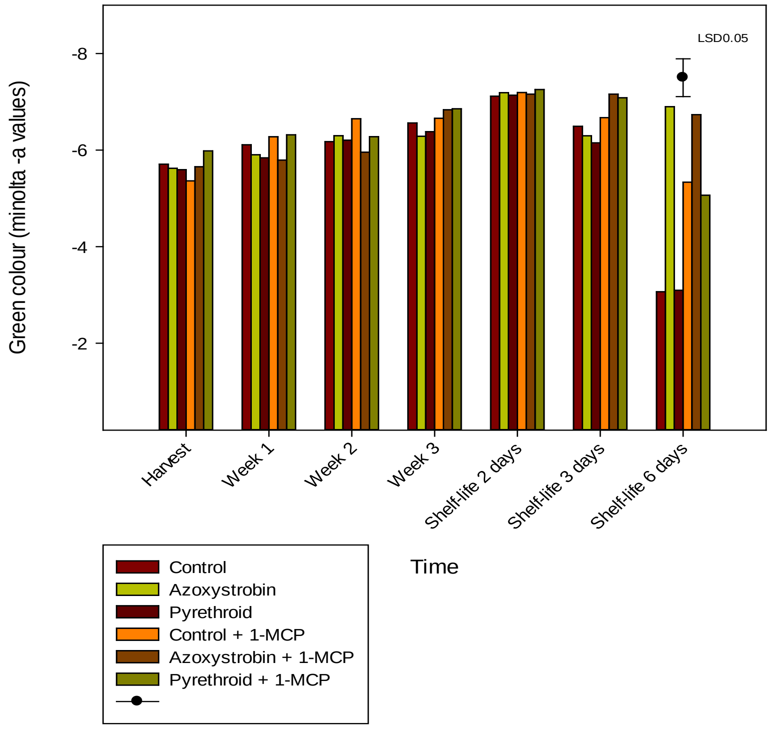


Fig. 3.B

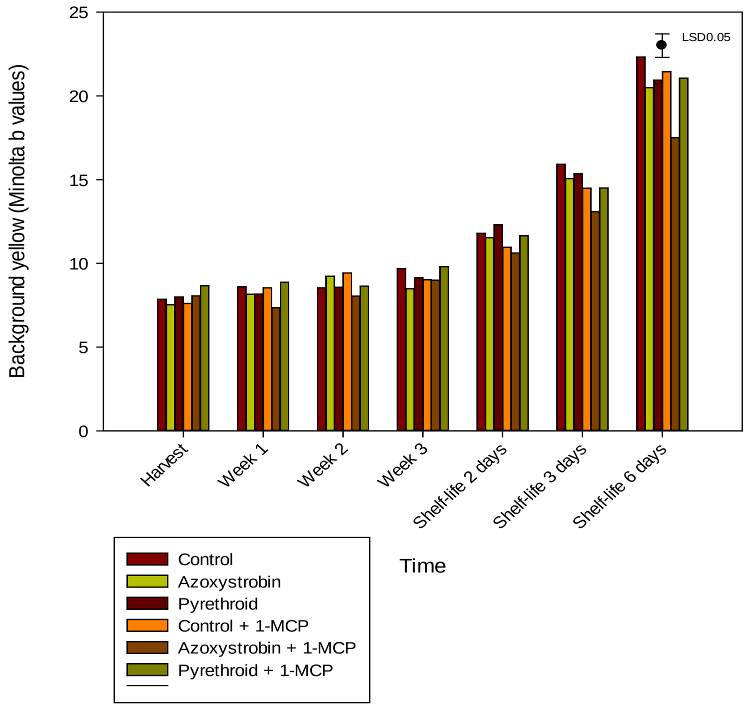


Fig.3.A & 3.B. The impact of pre- and post-harvest treatments on the green (A) and yellow (B) back ground colour of broccoli cv. Ironman subject to three weeks storage at 1°C (100% RH) followed by six days shelf-life at 18°C (85% RH)

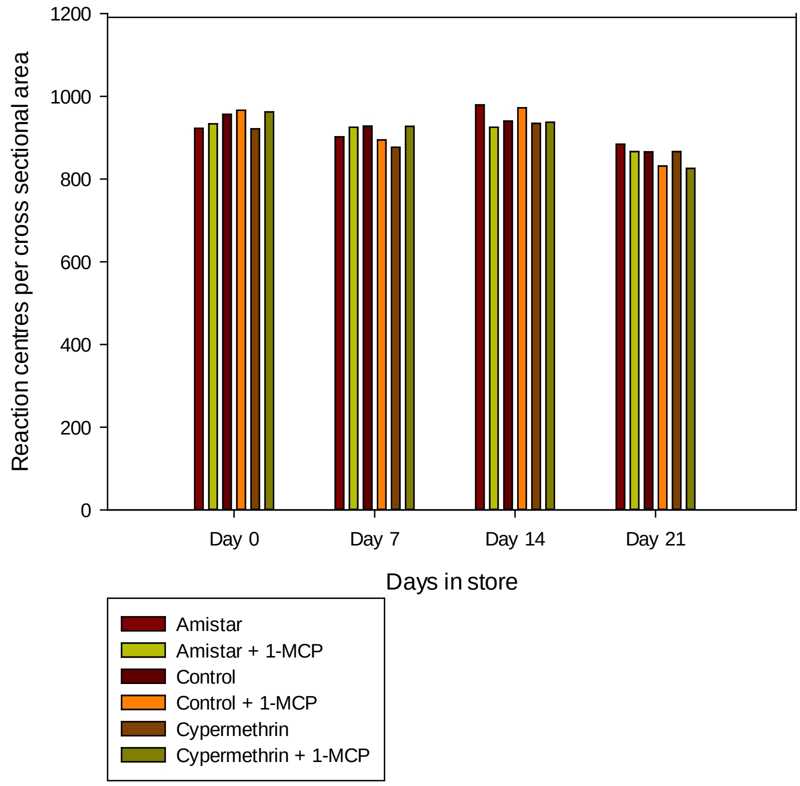


Fig. 3.C. Chlorophyll fluorescence profiles of broccoli cv. Ironman during storage at 1°C (100% RH)

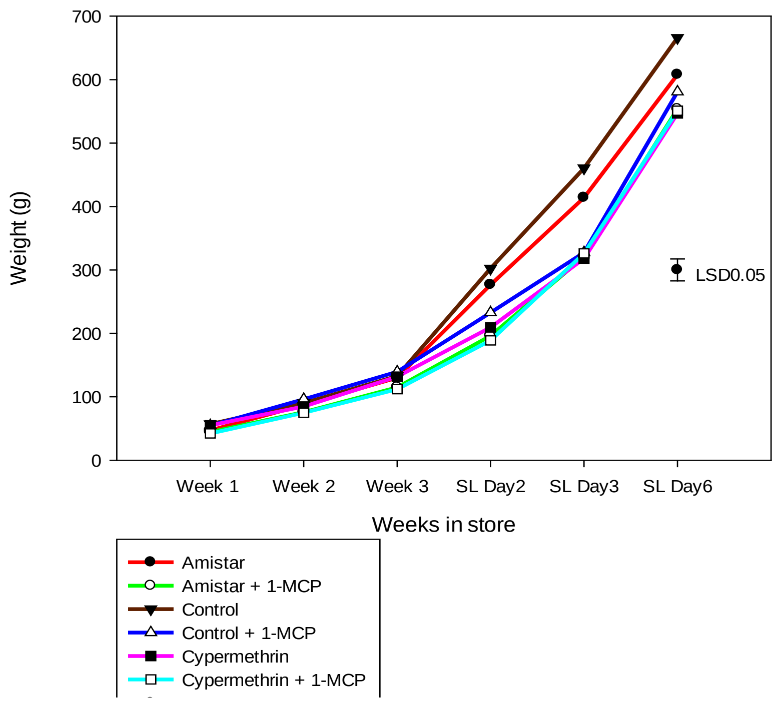


Fig. 3.D. The impact of pre- and post-harvest treatments on the weight loss of broccoli cv. Ironman subject to three weeks storage at 1°C (100% RH) followed by six days shelf-life at 18°C (85% RH)

Table 1. Dry matter content of broccoli cv. Ironman

Treatment	Position	Harvest	3 weeks at 1°C SF	3 weeks at 1°C No SF
Control	head	30.9	30.1	30.8
Permasect C	head	31.5	33.0	29.8
Amistar	head	31.5	32.5	31.6
Control	stalk	25.0	25.6	26.2
Permasect C	stalk	25.3	29.2	24.3
Amistar	stalk	26.5	27.6	27.8

SF= SmartFresh

Table 2. Respiration and ethylene production rates at harvest of broccoli cv. Ironman treated three weeks before harvest with Amistar and Permasect C.

	Amistar	Permasect C	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 3. 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
Amistar	11.3	7.3	6.5
Permasect C	10.8	9.9	6.5
Control	12.0	9.9	6.2
Control+1-MCP	8.9	10.1	9.7
Permasect C+1-MCP	11.6	8.6	10.0
Amistar+1-MCP	12.4	11.0	8.8

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

Table 4. 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
Amistar	6.0	4.9	7.6
Permasect C	3.8	4.0	6.2
Control	4.4	6.3	5.1
Control+1-MCP	9.0	5.2	7.3
Permasect C+1-MCP	11.9	7.0	8.7
Amistar+1-MCP	10.5	9.8	16.4

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

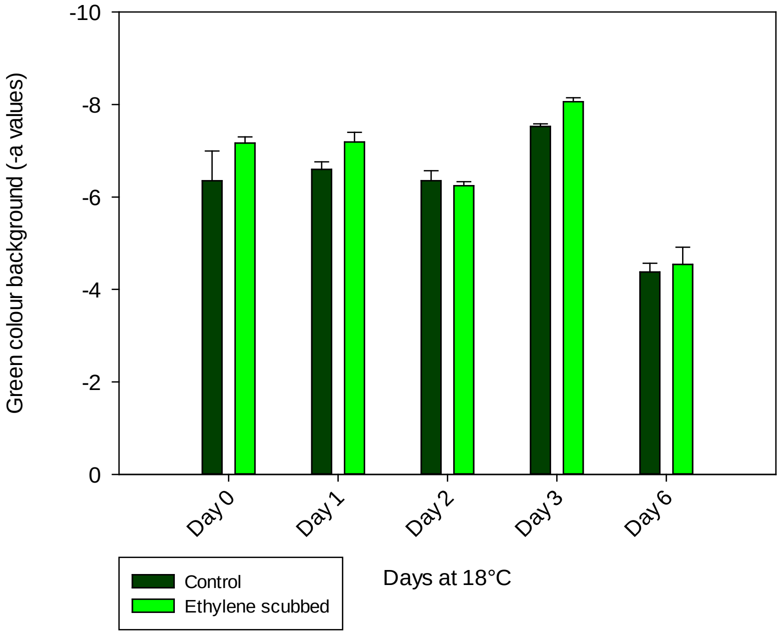
Ethylene scrubbing trial

The background green colour of broccoli from the ethylene-scrubbed store was a darker green 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.

In general, broccoli turns from a blue-green colour to a dark green colour during storage and shelf-life. With the Minolta '-a' readings this change in colour is depicted as a rise in '-a values' with time before the drop in green colour with an increase in senescence. Surprisingly, 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 little difference in yellow background colour observed between treatments. However, 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 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).

In addition to maintaining a better green colour during shelf-life, ethylene scrubbing also helped to reduce 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). Interestingly, ethylene-scrubbed broccoli showed elevated green and yellow background during the later stages of shelf-life.

Fig. 4.A



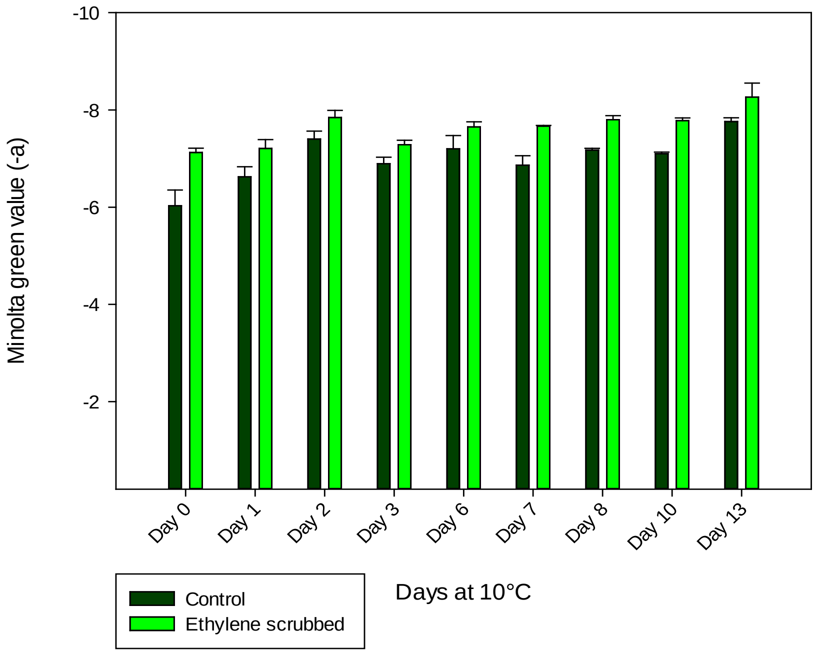


Fig 4.A. Background green colour (Minolta -a values) in broccoli cv. Ironman stored for two weeks in either an ethylene-scrubbed environment (~200ppb) or conventional cold store (~400ppb) 1°C (100% RH) followed by shelf-life evaluation at 18°C and 85% RH (Fig 4.A) or 10°C (Fig 4.B).

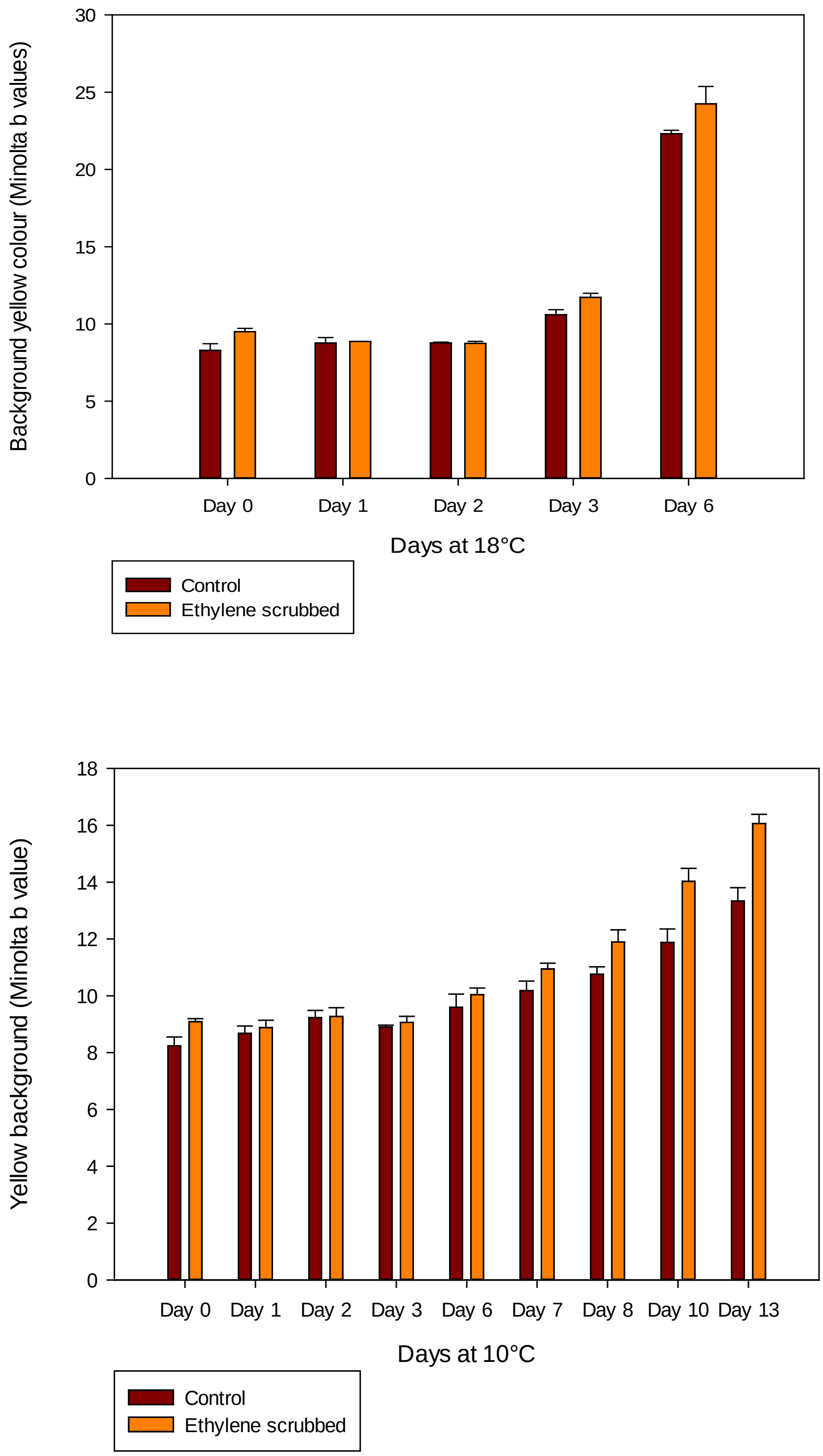
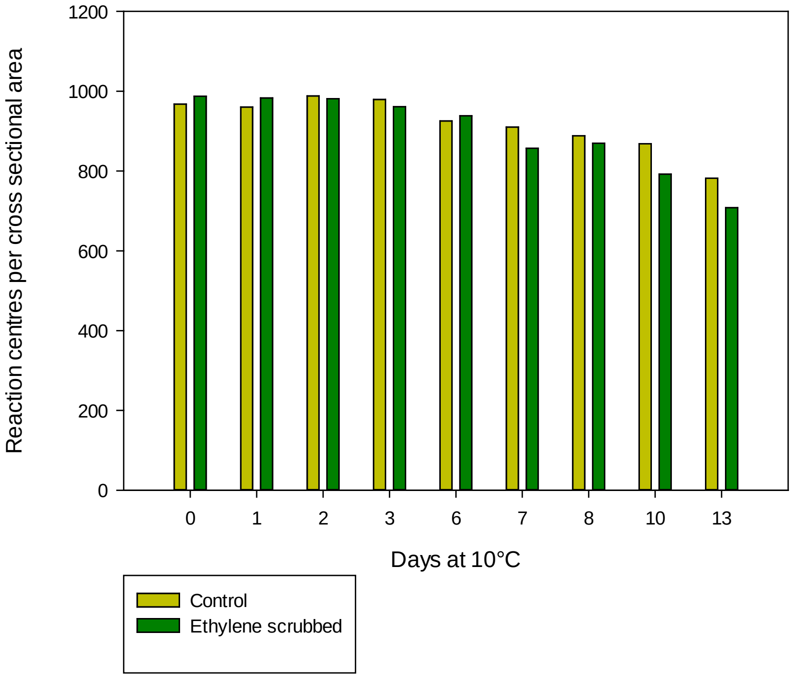


Figure 4 B. Background yellow colour (Minolta b values) in broccoli cv. Ironman stored for two weeks in either an ethylene-scrubbed environment (~200ppb) or conventional cold store (~400 ppb) 1°C (100% RH) followed by shelf life evaluation at 18°C or 10°C (85% RH).



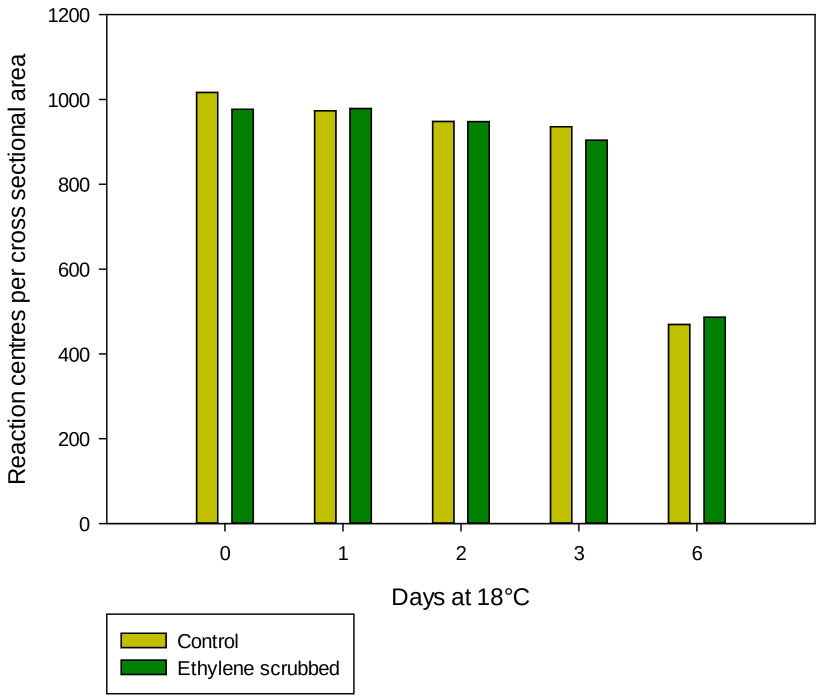


Figure 4.C. Chlorophyll fluorescence profiles of broccoli cv. Ironman stored in an ethylene-scrubbed store at 1°C (100% RH) prior to shelf-life at either 10°C or 18°C (85% RH)

The effect of ethylene scrubbing on weight loss of broccoli cv Ironman

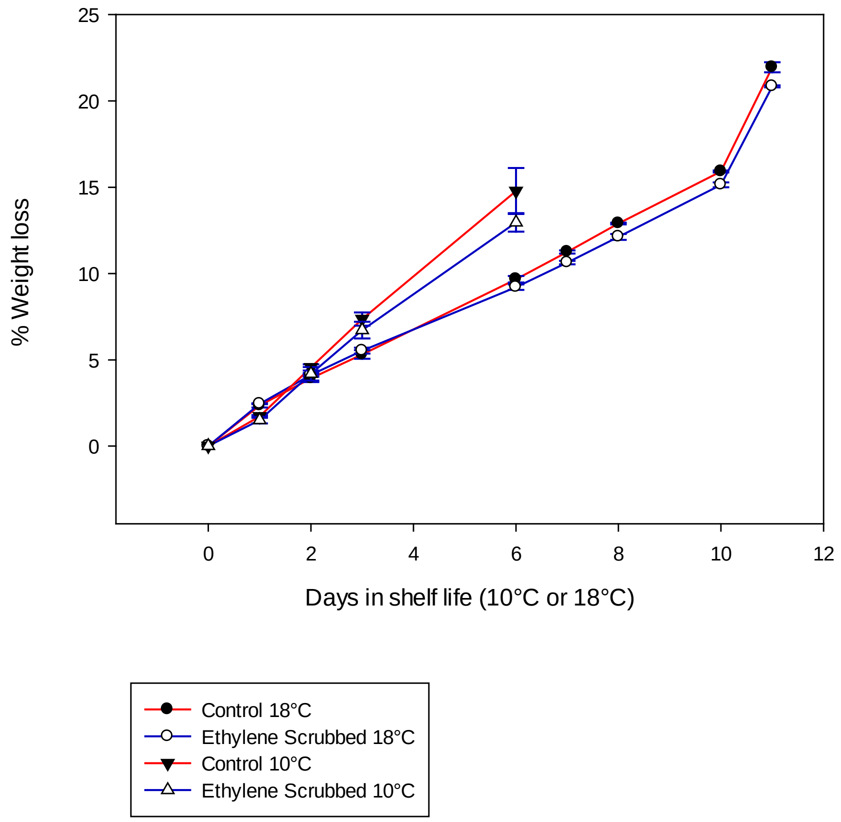


Figure 4.D. Chlorophyll fluorescence profiles of broccoli cv. Ironman stored in an ethylene-scrubbed store at 1°C (100% RH) prior to shelf-life at either 10°C or 18°C (85% RH)

Table 5. % 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**				

Discussion

Pre-harvest sprays of Amistar 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. Earlier work has shown that azoxystrobin inhibits ACC synthase, the penultimate enzyme in the ethylene biosynthesis pathway. The improvement in overall storage quality could be in part attributed to a reduction in post-harvest disease incidence in Amistar-treated broccoli. Although there was less disease incidence recorded during the later stages of shelf-life, no disease heads were observed during storage and the beginning of shelf-life, nevertheless, the presence of cryptic infections cannot be ruled out. The combination of Amistar and SmartFresh^{TM} 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 (Klee 2007); therefore, treatments that are capable of reducing ethylene production in heads prior to harvest may also increase the efficacy of SmartFresh^{TM}. 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 control heads exceeded that of other treatments.

While Permasect C 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 Permasect

C-treated heads; the dry matter content of heads and stalk after three weeks storage was greater in Permasect C-treated broccoli.

Where Amistar and in some cases Permasect C have been used on broccoli for the control of pest and disease outbreaks, there may be the small additional benefit of a delay in the onset of senescence.

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. No difference in respiration rate of broccoli stored at 1°C was observed between treatments. 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.

Due to the poor weather conditions in the 2012 season, not all the scheduled trials for Year 2 of the project could be achieved. Trials to evaluate chlorophyll fluorescence as a means to determine harvest maturity have been delayed to Year 3 of the project.

Conclusions

- Removal of ethylene in store rooms retards the deterioration of broccoli during subsequent shelf-life.
- A split application of SmartFresh™ applied at harvest and prior to removal from storage (1°C 100% RH) can extend product quality.
- Growers should be aware that crop protection agents used for pest and disease control have secondary effects on shelf-life quality of broccoli.

Technology transfer

Article for HDC News June 2012. BGA Technical event, Lincolnshire, October 2012.

Acknowledgements

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