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Project number:	TF 191
Project leader:	Dr Angela M Berrie
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Project coordinator:	TBA
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Key words:	Apple, Bramley's Seedling, Storage ethylene, CA storage, Smartfresh^{TM}, Nectria

DISCLAIMER:

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.

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Signature .... Date ....

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GROWER SUMMARY

Headline

- The use of either SmartFresh™ or ethylene scrubbing reduces the incidence of scald in long-term CA stored Bramley's Seedling apples.

Background

The development of fungal rots in controlled atmosphere (CA)-stored Bramley's Seedling apples continues to be a major problem for growers. It is not uncommon for losses to reach 10% of the stored crop late in the storage season and exceptionally losses as high as 20% have been reported. Much of the rotting is caused by *Nectria galligena*, the fungus that causes apple canker in the orchard.

The introduction of scrubbed low oxygen storage conditions (5% CO_2 + 1% O_2) resulted in major improvements in the storage quality of Bramley apples particularly in the control of bitter pit and superficial scald. The use of the chemical antioxidant, diphenylamine (DPA) was not necessary for the first 6 months of storage. However, it was known that this treatment would promote the development of *n*ectria fruit rots and was not advised for orchards with a significant canker problem. In reality it has proved difficult to grow Bramley trees without significant orchard infections of the fungus. Changing the CA conditions to 6% CO_2 + 2% O_2 reduces the rot problem, but without DPA treatment, scald becomes a problem.

As the use of DPA is no longer permitted, the use of storage protocols capable of controlling scald becomes even more important. Two such technologies are the use of SmartFresh™ and ethylene scrubbing. Both of these technologies control the effects of ethylene and are therefore effective in reducing scald. However, it is also important to assess these technologies in terms of their effects on rot development.

SmartFresh™

The use of SmartFresh™ (1-MCP) is now a well-established pre-storage treatment for Bramley, with the main benefit of controlling scald without the need for post-harvest DPA treatment (scald-free period dependent on CA condition used). Since the effect of SmartFresh™ is to retard ripening changes such as fruit softening by blocking the ethylene receptor, thereby preventing the auto-stimulation of ethylene production, a beneficial effect on control of rotting may be anticipated. On the other hand, as ethylene is an important signaling compound in the induction of defence against pathogens, blocking its action could have some adverse effects (McDowell and Dangl, 2000)

Ethylene removal

Ethylene removal from CA stores is an effective means of achieving benefits similar to those described for SmartFresh^{TM} but effects on rot development have not been studied extensively.

Both the technologies described above have been tested in commercial stores. However, a rigorous comparison of these technologies is confounded by differences between stores and differences between the apples stored within them (orchard characteristics, management, growing season etc).

This project seeks to make a rigorous comparison of the technologies by assessing fruit from a range of commercial stores, and in addition distributing a common set of test apples within the same stores. These commercial trials are supported by a parallel set of small-scale trials set up within experimental CA chambers (80 kg capacity).

The overall aim of the project is to compare the effects of SmartFresh^{TM} (1-MCP) and ethylene scrubbing (Bi-On (ethylene absorbant) and catalytic scrubbing) on the development of fungal rots in CA-stored Bramley apples under commercial conditions.

Several growers are already testing these three storage strategies. However, there are very few instances where more than one technology is being tested under comparable conditions, or where a technology is being compared to a “control” store with no treatment. The approach of this project is to exploit the existing commercial trials and to maximise the information that can be obtained. The project outcomes will allow growers to optimize their storage strategies and minimise losses

Summary of the project and main conclusions

Bins of apples were obtained from three orchards and one bin from each orchard distributed among commercial 5:1 stores using the different technologies. In addition, netted samples were artificially inoculated with *Nectria* to determine the effects of the technologies on rate of disease development.

Table 1 shows the concentrations of ethylene measured within the six commercial stores in November 2009 and in February/March 2010. Ethylene scrubbing both by catalytic methods and by potassium permanganate absorption (Bi-On) appeared to be very effective and capable of keeping ethylene levels down to 100 ppb or below.

Ethylene concentrations were lower in the Bi-On stores than the catalytic scrubbed stores.

Table 1. Ethylene concentrations within commercial stores. Samples were taken through the sampling port, stored in air sampling bags and analysed by GC with an FID

	Ethylene concentration within store (ppb)	
	Nov-09	**Feb/Mar-10**
Untreated B*	10,268	
Catalytic scrubbed A*	12.8	
Catalytic scrubbed B	100.3	284
Bi-On scrubbed A	2.7	75
Bi-On scrubbed B	9.4	83.4
SmartFresh™ A	770	2520

* stores opened before second ethylene measurement

The fruit quality wasassessed when the stores were opened and after a subsequent 7 days at ambient conditions. From these assessments, and assessments from the small-scale trials that were opened after 9 months, the following conclusions are drawn. Note that these are from a single season of data, and will need to be tested for a second season.

- Ethylene scrubbing during storage and SmartFresh™ treatment on Bramley's Seedling apples were compared for their effects on the rate of rotting of *n*ectria inoculated fruit. For both trials reported here; the commercial store trial and the small scale CA chamber trial, the rate of rotting was significantly greater for SmartFresh™ treated fruit (see Figure 1). This is consistent with the hypothesis stating that ethylene is an important signalling compound in the induction of defence against pathogens, and that blocking its action by the use of SmartFresh™ could have some adverse effects.
- This effect was not accompanied by any observable increase in natural rates of rotting in the commercial stores. Therefore it will be an important objective in the remaining part of this project to establish the practical implications of this finding.
- Although no significant levels of scald were observed in the commercial trial, the small-scale trial confirmed that both ethylene scrubbing and SmartFresh™ significantly reduce this problem.

- Breakdown (senescent and low temperature were considered together) was reduced slightly by scrubbing but significantly by SmartFresh™.
- Although no treatment differences were observed for the incidence of CO_2 injury in the commercial trial there were indications that SmartFresh™ can exacerbate this problem in the small-scale trial.

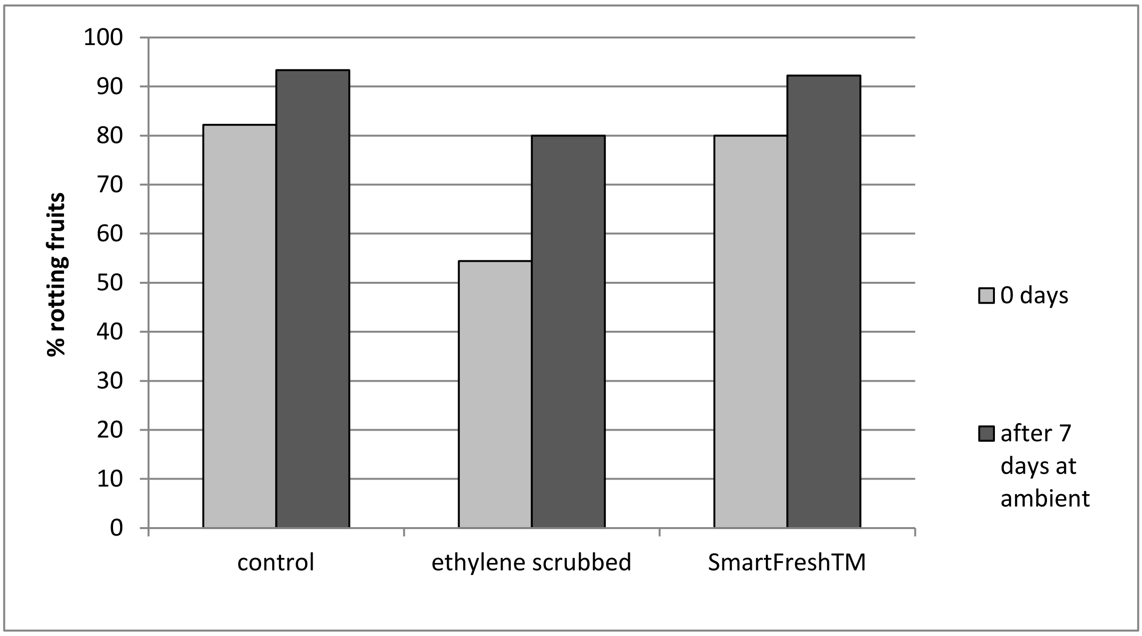

Figure 1. Average % rotting fruits in inoculated nets immediately on removal from storage chambers and after 7 days at ambient. Treatment effects p = 0.002, 0.013 LSD 12.5, 8.6 for 0 and 7 days respectively

Financial benefits

No financial benefits are evident at this stage of the project.

Action points for growers

No action points have been identified at this stage of the project.

SCIENCE SECTION

Introduction

The development of fungal rots in controlled atmosphere (CA)-stored Bramley's Seedling apples continues to be a major problem for growers. It is not uncommon for losses to reach 10% of the stored crop late in the storage season and exceptionally losses as high as 20% have been reported. Much of the rotting is caused by *Nectria galligena*, the fungus that causes apple canker in the orchard.

The introduction of scrubbed low oxygen storage conditions (5% CO_2 + 1% O_2) resulted in major improvements in the storage quality of Bramley apples particularly in the control of bitter pit and superficial scald. The use of the chemical antioxidant, diphenylamine (DPA) was not necessary for the first 6 months of storage. However, it was known that this condition would promote the development of *n*ectria fruit rots and was not advised for fruit from orchards with a significant canker problem. In reality it has proved difficult to grow Bramley trees without significant orchard infections of the fungus. Changing the CA conditions to 6% CO_2 + 2% O_2 reduces the rot problem, but without DPA treatment scald became a problem.

As the use of DPA is no longer permitted, the use of storage protocols capable of controlling scald becomes even more important. Two such technologies are the use of SmartFresh^{TM} and ethylene scrubbing. Both of these technologies control the effects of ethylene and are therefore effective in reducing scald. However, it is also important to assess these technologies in terms of their effects on rot development.

SmartFresh^{TM}

SmartFresh^{TM} (1-Methylcyclopropene (1-MCP)) has been used successfully during storage of several apple varieties in the UK since 2003. As an ethylene antagonist it blocks the ethylene binding site and slows down ethylene stimulated physiological changes, including those associated with ripening such as fruit softening. It is considered to be especially effective in controlling ethylene mediated processes during storage of Bramley apples that, unlike most eating varieties, are picked several weeks before the climacteric phase. In Bramley it has been shown to control scald and obviated the need for DPA drenching (Johnson, 2008). However there have been reports that SmartFresh^{TM} increases sensitivity to CO_2 injury (Lafer, 2003), so that a delay in imposing CA conditions is necessary (Colgan *et al.,* 1999).

During apple storage it is advantageous to slow down most ethylene stimulated processes either by ethylene scrubbers or antagonists such as 1-MCP. A slow down in the rate of ripening leaves fruit firmer and with more active defense mechanisms and hence tends to reduce storage rots. However, ethylene is also directly involved in the signaling system responsible for stimulating tissue defenses against invading pathogens (McDowell and Dangl, 2000), so that under some circumstances there may be increased susceptibility to rots. The scientific literature on the effects of 1-MCP treatment of fruit on disorders and susceptibility to rotting is inconsistent and varies by species (Blankenship and Dole, 2003).

In experimental scale trials carried out at East Malling Research (EMR) on Bramley apples in 2002, the application of SmartFresh™ reduced the overall level of rotting in fruit stored for 273 days by about 50%. Two years earlier the application of 1-MCP in the form of EthylBloc generally reduced rotting particularly in air-stored fruit (Johnson, 2001; 2002 Confidential reports to Rohm and Haas Italia srl).

Ethylene scrubbing

Ethylene scrubbing is now being used more widely within the fresh produce industry with beneficial effects on shelf-life and quality in most cases. It has been reported that the use of ethylene scrubbers, specifically Bi-On (a system based on potassium permanganate absorption of ethylene marketed by ICA Ltd) can reduce the ethylene levels in a Bramley store from the normal range of 15 – 30 ppm down to 0.5 ppm, reducing softening and extending shelf-life (Bishop and Manning, 2008).

In experiments carried out on Cox at EMR the establishment of low ethylene conditions by the use of catalytic ethylene converters had no effect on the incidence of rotting caused by *n*ectria or penicillium in un-inoculated fruit. However, a higher incidence of *n*ectria was recorded in inoculated fruit kept in a low ethylene environment (Johnson, *et al*., 1993). On the other hand, ethylene removal from a 9 tonne semi-commercial store of Bramley apples resulted in 2% less rot than in a similar store containing DPA-drenched fruit. It was also noted that in bins of ethylene-scrubbed fruit rots were confined to individual fruits, whereas in un-scrubbed bins some rotting had spread to adjacent fruits (Dover, 1985).

Both the technologies described above have been tested out in commercial stores. However, a rigorous comparison of these technologies is confounded by differences between stores and differences between the apples stored within them (orchard characteristics, management, growing season, etc.). This project seeks to make a rigorous scientific comparison of the technologies by assessing fruit from a range of commercial stores, and in addition distributing a common set of test apples within the same stores. The large numbers of fruit available for commercial scale trials are helpful in providing reliable data on rot incidence but it is important that such trials are conducted in scientific manner so that data is statistically valid. The commercial trials are supported by a parallel set of small-scale replicated trials set up within experimental CA chambers (80 kg capacity).

Overall aim of project

The overall aim of the project is to compare the effects of SmartFresh^{TM} (1-MCP) and ethylene scrubbing (Bi-On (ethylene absorbant) and catalytic scrubbing) on the development of fungal rots in CA-stored Bramley apples under commercial conditions.

Specific Objectives

Several growers are already testing these three storage strategies. However, there are very few instances where more than one technology is being tested under comparable conditions, or where a technology is being compared to a 'control' store with no treatment. The approach of this project is to exploit the existing trials and to maximise the information that can be obtained by:

1. providing technical support and manpower for any existing trials, for assessment of rotting in terms of quantification of wastage due to rots and correct identification of the causal pathogens
2. increasing the opportunities for direct treatment comparisons within commercial stores by distributing bins of fruit from selected growers to stores undergoing a range of treatments
3. introducing fruits artificially inoculated with *n*ectria to enable a direct assessment of the effects of ethylene removal on fungal growth

Materials and Methods

Nine bins of Bramley apples from each of three orchards (A, B and C) were harvested on Monday 31st August by a commercial producer and delivered to EMR on Tuesday 1st September. These were from trees on M.26, planted in 1990, 1991 and 1999 respectively. Twenty fruits per orchard (2-3 per bin) were selected for quality assessment, and a further 20 for mineral analysis. Eight bins from each orchard were distributed to commercial stores, and the remaining bin used to set up a parallel small scale trial within the Jim Mount Centre facilities at EMR.

Fruit treatment for commercial store trial

Two lots of 30 fruits were selected from each bin and artificially inoculated with *Nectria galligena.* Six isolates of *N. galligena* previously obtained from wood cankers or fruit rots were grown on Snay medium under UV lights for 1 week at ambient temperature to encourage spore (conidia) production. Fungal cultures were then scraped and rinsed with distilled water into a flask to prepare a spore suspension which was made up to 5 litres. Spore concentration (conidia) was checked on a haemocytometer. Fruit were dipped in a fungal spore solution containing 3.7 $x10^{-3}$ nectria spores per ml for 1 min. The samples of 30 fruit were placed in nets in plastic bags and the fruit left overnight to incubate at ambient temperature and high humidity to allow the nectria conidia to germinate and infect fruit. Meanwhile the bins were kept in a cold store at 3.5ºC. The next day fruit were placed back in the bins, for distribution to the commercial stores. Eight commercial stores were used, all CA at 5% CO_2, 1% O_2; two each with SmartFresh^{TM} treatment, ethylene scrubbing using Bi-On, ethylene scrubbing using catalytic scrubbers, and two controls with no ethylene control treatments.

Figure 2. A bin prepared for delivery to a commercial store. Two nets of inoculated fruit were placed at the top at opposite corners of the bins

Stores were assessed for ethylene concentration by taking samples from the sampling port, and storing this in a gas sampling bag prior to analysis using a gas chromatograph with a flame ionization detector.

When each store was opened, the sample bins were assessed for overall levels of rot. The inoculated nets were assessed for nectria development, and samples from each orchard were removed for quality assessment, both immediately and after 7 days at ambient.

Fruit quality assessment

Fruit quality assessments were carried out using two samples of 10 fruit in each case. Firmness was measured using a motorised penetrometer (LRX). Colours were determined using a hunter-lab colourmeter (LAB), soluble solids (° Brix) were measured using a digital refractometer. Fruits were cut at the equator and the calyx end to assess for internal disorders.

Internal ethylene concentrations were measured at harvest by taking a sample from the internal cavity and measuring ethylene by gas chromatography using a flame ionization detector.

Small-scale trial conducted in CA chambers at East Malling Research

Six boxes of fruit and 6 nets of 30 fruits each were selected from each of the three orchards used for the commercial trials. The netted fruits were inoculated with *Nectria galligena* as described above, and left in bags overnight at ambient temperature. The boxes were stored at 3.5 ºC. The next day all netted fruit were removed from bags. Two boxes and 2 inoculated nets were treated with SmartFresh^{TM} at 3.5 ºC for 24 hours.

The following day boxes of apples were weighed and placed in 6 CA chambers, two for each treatment; SmartFresh^{TM} treated, ethylene scrubbed and untreated controls. Ethylene scrubbing consisted of two “tubes” of potassium permanganate impregnated material (Bi-On) placed across the top of the lower boxes.

Inoculated fruit were placed in the lower layer of the chamber (orchards in random order) and uninoculated fruit in the upper layer.

CA conditions; 1% O_2, 5% CO_2 were established after 3 weeks. Ethylene concentrations were measured at regular intervals. Fruit quality was assessed after 9 months storage.

Results

Commercial store trial

Bins of test fruit were distributed to eight commercial stores; two each of SmartFresh™ treated, Bi-On scrubbed, catalytic scrubbed and untreated. Two stores (one SmartFresh™ and one untreated) were opened too early for assessments to be useful. Table 1 shows the concentrations of ethylene measured within the six remaining commercial stores in November 2009 and in February/March 2010. The stores were opened at various times from early January through to late May. For this reason two stores were only assessed for ethylene concentration on one occasion. The untreated store (B) was opened before the second ethylene assessment but already had a high ethylene level (>10 ppm) at the November assessment indicating that the fruit had entered the climacteric phase. Ethylene scrubbing both by catalytic methods and by potassium permanganate absorption (Bi-On) appeared to be very effective and capable of keeping ethylene levels down to 0.1 ppm or below. Ethylene concentrations were lower in the Bi-On stores than the catalytic scrubbed stores. A disadvantage of the Bi-On method often mentioned is that once the potassium permanganate has been saturated with ethylene, scrubbing becomes less efficient. The ethylene measurements do not indicate that saturation occurred by the February/March assessment.

Table 2. Ethylene concentrations within commercial stores. Samples were taken through the sampling port, stored in air sampling bags and analysed by GC with an FID

	Ethylene concentration within store (ppm)	
	Nov-09	**Feb/Mar-10**
Untreated B*	10.268	
Catalytic scrubbed A*	0.013	
Catalytic scrubbed B	0.100	0.284
Bi-On scrubbed A	0.003	0.075
Bi-On scrubbed B	0.009	0.083
Smartfresh™ A	0.770	2.520

* stores opened before second ethylene measurement

The fruit quality and the level of rots, both for inoculated and uninoculated fruit assessed when the stores were opened are summarized in Tables 3 and 4, in chronological order of store opening.

On removal from store and after 7 days at ambient, the softest fruit were from the untreated store. SmartFresh^{TM} treatment and ethylene scrubbing both improved retention of firmness. There was no clear difference between scrubbing and SmartFresh^{TM} treatment, although there was a relatively high rate of softening in the Catalytic scrubbed A store.

There were only small differences in colour and in ° Brix on removal from store, and no obvious treatment effects. Interestingly colour change (increase in 'a' indicating loss of chlorophyll) during the week at ambient seemed to be greater for the SmartFresh^{TM} store, and loss of sugar (° Brix) was also greater for this store.

The percent rotting observed in *n*ectria inoculated samples and for uninoculated fruit are given in Table 3, (results are not available for untreated stores due to loss of inoculated nets). For inoculated fruit, rotting was significantly ($p<0.001$) greater for the SmartFresh^{TM} treated fruit than for scrubbed fruit. This was not reflected as higher levels of rot for uninoculated fruit in the whole bins. However, each bin is generally filled from about 2-4 trees at one location in the orchard. The incidence of nectria canker and other rots varies considerably from tree to tree across an orchard so the potential for rots in an individual bin will vary considerably. In this study it was not possible to randomize the fruit across the eight bins from each of the orchards A, B and C, nor was it possible to store more than one bin from each of the orchards in the commercial stores. Had this been possible then the bin to bin variation may have been reduced and differences in natural rotting between SmartFresh^{TM}-treated bins and other fruit been more obvious.

Both CO_2 injury and bitter pit increased with storage time, but there were no clear treatment differences. No significant scald was seen in any bins (data not shown).

Table 3. Quality of Bramley's Seedling apples after storage at 5% O_2, 1% CO_2 (5:1) with a range of ethylene management strategies. Apples were assessed on removal from store and after 7 days subsequent storage at 20°C (shelf-life). For each orchard 20 apples were assessed at each time. (SmartF^{TM} = SmartFresh^{TM})

Store type	Date opened	Orchard and overall mean	Post-storage				Post shelf-life			
			Colour a	Colour b	° Brix	Firm-ness	Colour a	Colour b	° Brix	Firm-ness
5:1 scrubbed (A)	11 Jan 2010	A	-9.89	44.36	10.1	9.19	-7.86	47.60	10.2	5.80
		B	-10.46	42.22	9.9	9.34	-7.84	46.46	10.3	6.10
		C	-10.07	44.17	11.6	8.80	-7.94	46.48	11.4	5.91
		Mean	**-10.14**	**43.58**	**10.5**	**9.11**	**-7.88**	**46.85**	**10.6**	**5.94**
5:1 untreated (B)	End Jan 2010	A	-9.56	42.62	10.8	7.37	-7.11	46.12	11.0	4.68
		B	-9.59	41.83	10.5	7.85	-7.64	44.51	10.0	5.94
		C	-9.14	42.13	11.4	7.73	-7.23	46.42	11.4	5.86
		Mean	**-9.43**	**42.19**	**10.9**	**7.65**	**-7.33**	**45.68**	**10.8**	**5.49**
5:1 Bi-On (B)	23 Mar 2010	A	-9.50	43.89	11.0	9.20	-7.13	45.63	9.8	8.01
		B	-9.30	44.66	11.3	9.18	-7.90	45.31	11.7	8.58
		C	-9.54	43.77	10.1	8.95	-7.59	45.24	10.4	8.14
		Mean	**-9.45**	**44.11**	**10.8**	**9.11**	**-7.54**	**45.39**	**10.6**	**8.24**
5:1 Bi-On (A)	10 May 2010	A	-9.82	41.49	10.6	8.57	-8.55	44.18	10.4	8.35
		B	-10.19	40.99	10.3	8.99	-8.10	44.82	10.6	8.48
		C	-10.33	41.01	11.0	9.06	-7.63	43.79	10.7	8.52
		Mean	**-10.11**	**41.16**	**10.6**	**8.87**	**-8.09**	**44.26**	**10.6**	**8.45**
5:1 SmartF.^{TM} (A)	10 May 2010	A	-9.23	47.28	11.8	8.76	-6.67	46.17	10.8	6.81
		B	-9.53	45.73	10.6	9.48	-6.54	45.99	10.6	7.32
		C	-9.44	45.84	11.4	9.03	-6.65	45.60	10.9	7.01
		Mean	**-9.40**	**46.28**	**11.3**	**9.09**	**-6.62**	**45.92**	**10.8**	**7.05**
5:1 scrubbed (B)	End May 2010	A	-9.85	44.36	11.2	7.63	-7.52	46.89	10.6	6.73
		B	-9.56	43.57	10.3	7.64	-7.17	46.83	10.0	6.33
		C	-9.94	43.99	11.3	8.34	-7.49	46.73	11.2	7.90
		Mean	**-9.78**	**43.97**	**10.9**	**7.87**	**-7.39**	**46.82**	**10.6**	**6.99**

Table 4. Levels of rot (for inoculated nets of fruit and whole bins), and disorders within sampled fruit for Bramley's Seedling apples after storage at 5% O_2, 1% CO_2 (5:1) with a range of ethylene management strategies. Two nets of 30 fruit inoculated by dipping in a suspension of *n*ectria spores were placed in each bin at the start of storage. Disorders were assessed for 40 apples from each bin. (SmartF™ = SmartFresh™)

Store type	Date opened	Orchard and overall mean	Inoculated nets	Whole bin assessment		40 sample fruit removed for quality assessment	
			% *n*ectria	% *n*ectria	% total rots	% CO2 injury	% bitter pit
5:1 scrubbed (A)	11 Jan 2010	A	33.3	0.44	1.77	0	0
		B	23.3	0.00	1.52	0	0
		C	10.0	0.00	0.47	0	0
		Mean	**21.8**	**0.15**	**1.25**	**0**	**0**
5:1 untreated (B)	End Jan 2010	A	-	0.23	1.72	0	0
		B	-	1.72	3.35	0	0
		C	-	0.07	2.59	0	0
		Mean		**0.67**	**2.55**	**0**	**0**
5:1 Bi-On (B)	23 Mar 2010	A	29.3	0.67	3.78	0	0
		B	34.6	0.00	5.60	0	0
		C	16.7	0.00	2.63	0	7.5
		Mean	**45.1**	**0.22**	**4.00**	**0**	**0.25**
5:1 Bi-On (A)	10 May 2010	A	50.0	0.33	2.6	2.5	2.5
		B	53.3	0.63	4.0	0	5.0
		C	33.3	0	6.5	0	0
		Mean	**26.5**	**0.32**	**3.6**	**0.8**	**2.5**
		A	96.7	0.27	4.42	10	5.0
		B	96.7	0.68	2.46	0	5.0
		C	90.2	0.26	0.78	2.5	0
		Mean	**94.4**	**0.40**	**2.55**	**4.2**	**3.3**
5:1 SmartF.™ (A)	10 May 2010	A	81.7	0.51	3.44	15.0	5.0
		B	73.3	1.52	8.16	15.0	7.5
		C	46.7	0.37	2.61	15.0	0
		Mean	**66.5**	**0.80**	**4.74**	**15.0**	**4.2**
Treatment effect (p)			<0.001				
LSD (Treatment means)			18.9				

Inoculated nets were mislaid for Untreated (B). % *n*ectria in inoculated nets was analysed by Anova. For other data the number of samples was not sufficient for Kruskall Wallace analysis

Cabinet trial

A small-scale trial using CA chambers (80 kg capacity) was conducted in parallel to the commercial store trial. This had three treatments: untreated control, SmartFresh^{TM} and ethylene scrubbed. Ethylene scrubbing was carried out using potassium permanganate. The objective was to consider the effect of very efficient scrubbing rather than to mimic the commercial situation, so that the ratio of amount of potassium permanganate per fruit was much higher than would be the case in commercial stores. Figure 2 shows the ethylene concentrations within the six cabinets. For the untreated controls, ethylene concentrations peaked above 45,000 ppb. Scrubbed cabinets had very low ethylene concentrations throughout (only above 50 ppb on two occasions). SmartFresh^{TM} levels were intermediate. SmartFresh^{TM} replicate 2 was consistently higher than replicate 1. Given that the same analysis system was used, this suggests that there were rotten or riper ethylene producing fruit within that cabinet. This would not affect the efficacy of SmartFresh^{TM}.

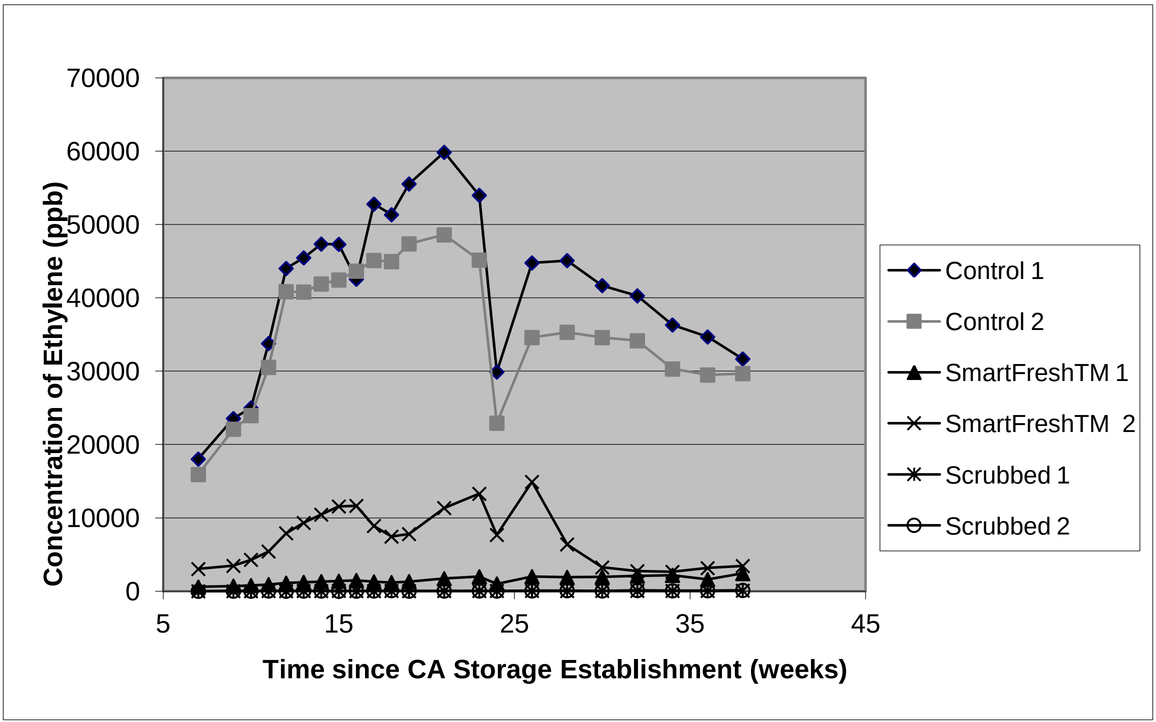

Figure 3. Ethylene concentrations measured within 80 kg CA chambers containing Bramley's Seedling apples stored under 5%O2, 1% CO2 with no ethylene control or treated with SmartFresh TM or with ethylene scrubbing.

The quality of fruit on removal from the chambers after 9 months storage and after a subsequent 7 days at ambient are summarized in Table 5. Consistent with the commercial trials, both SmartFresh™ and ethylene scrubbing led to better maintenance of firmness, although the effect was more marked with SmartFresh™ in this case. Unlike the observation from the commercial store, there is no indication of a more rapid softening, nor a more rapid reduction in sugars during subsequent storage at ambient of SmartFresh™ treated fruit.

Table 5. Quality of Bramley's Seedling apples after storage at 5% O_2, 1% CO_2 (5:1) using a range of ethylene management strategies within 80 kg CA chambers. Apples were assessed on removal from store and after 7 days subsequent storage at 20°C (shelf-life). For each orchard 20 apples were assessed at each time. (SmartF™ = SmartFresh™)

Treatment	Orchard and overall mean	Post storage				Post shelf-life			
		Colour a	**Colour b**	**Firmness**	**° Brix**	**Colour a**	**Colour b**	**Firmness**	**° Brix**
Untreated	A	-9.33	41.60	61.45	10.4	-7.33	46.09	43.50	10.0
	B	-9.52	41.54	60.23	9.9	-7.53	45.45	42.83	9.6
	C	-9.21	41.01	68.73	10.7	-7.02	46.53	52.93	10.8
	mean	**-9.35**	**41.38**	**63.47**	**10.3**	**-7.29**	**46.02**	**46.42**	**10.1**
Ethylene scrubbed	A	-9.60	43.60	66.60	10.1	-8.01	45.25	55.83	10.4
	B	-9.97	43.07	65.38	10.0	-7.65	45.63	51.78	10.0
	C	-9.53	43.12	73.53	10.8	-7.11	46.15	64.55	10.8
	mean	**-9.70**	**43.26**	**68.50**	**10.3**	**-7.59**	**45.68**	**57.38**	**10.4**
SmartF™	A	-9.77	42.56	83.45	10.3	-8.18	44.86	76.98	10.0
	B	-10.12	42.33	89.98	10.1	-8.54	44.62	82.20	9.7
	C	-9.72	42.68	92.50	10.8	-8.25	44.59	90.35	10.9
	mean	**-9.87**	**42.52**	**88.64**	**10.4**	**-8.32**	**44.69**	**83.18**	**10.2**
Treatment effect (p)		**0.008**	**0.002**	**<0.001**	**n.s.**	**<0.001**	**0.001**	**<0.001**	**n.s.**
Treatment LSD (5%)		**0.31**	**0.99**	**3.10**		**0.38**	**0.67**	**2.50**	
Orchard effect (p)		**0.043**	**n.s.**	**<0.001**	**<0.001**	**n.s.**	**n.s.**	**<0.001**	**<0.001**

Data were analysed by 2-way Anova using Genstat.

Table 6 shows internal and external disorders observed in the fruit. Scald was eliminated by both scrubbing and SmartFresh^{TM}. No distinction was made between internal breakdown due to senescence and low temperature stress. Breakdown was reduced slightly by scrubbing but significantly by SmartFresh^{TM}. External CO_2 injury was greatest in SmartFresh^{TM} treated fruit, was present in controls, but not in scrubbed fruit. No treatment differences for internal CO_2 were observed. No significant differences in bitter pit and core flush were observed.

Overall rotting was least in scrubbed chambers, but this was barely statistically significant (p=0.05 immediately after storage and not significant at 7 days).

Figure 4 shows the percent rotting for inoculated fruits on removal from storage and after a subsequent 7 days at ambient. A significantly lower rate of rotting was observed for scrubbed fruit. This is consistent with the observation of inoculated fruit from the commercial store trial.

Table 6. Disorders of Bramley's Seedling apples after storage at 5% O_2, 1% CO_2 (5:1) with a range of ethylene management strategies within 80 kg CA chambers. For each orchard 20 apples were assessed immediately after storage (0 d) and a further 20 apples after 7 days subsequent storage at 20°C (7 d). (SmartF^{TM} = SmartFresh^{TM}**)**

Treatment	**Orchard and overall mean**	**% scald**		**Scald index**		**BD %**		**BD index**	
Time of assessment		**0 d**	**7 d**	**0 d**	**7 d**	**0 d**	**7 d**	**0 d**	**7 d**
Control	A	23.2	29.19	7.5	11.74	53.4	75.08	21.3	29.77
	B	15.0	30.11	5.2	13.37	46.8	64.20	20.5	23.48
	C	17.3	41.65	7.6	22.45	3.6	20.37	1.4	6.08
	mean	**18.5**	**33.65**	**6.8**	**15.85**	**34.6**	**53.22**	**14.4**	**19.78**
Scrubbed	A	0.0	0.00	0.0	0.00	60.0	47.73	21.2	17.14
	B	0.0	0.00	0.0	0.00	23.7	32.08	6.9	8.25
	C	0.0	0.00	0.0	0.00	2.5	2.72	1.0	0.54
	mean	**0.0**	**0.00**	**0.0**	**0.00**	**28.7**	**27.51**	**9.7**	**8.64**
Smart^{F}resh^{TM}	A	0.0	0.00	0.0	0.00	12.8	31.53	3.1	8.06
	B	0.0	0.00	0.0	0.00	1.9	16.09	0.4	4.05
	C	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00
	mean	**0.0**	**0.00**	**0.0**	**0.00**	**4.9**	**15.87**	**1.2**	**4.04**
Treatment effect (Kruskall Wallis)		**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.011**	**0.002**	**0.003**	**0.002**

BD indicates internal breakdown. No distinction is made between senescent breakdown and low temperature breakdown

Table 6 (cont.)

Treatment	Orchard and overall mean	% CO_2 injury		CO_2 index (max 60)		% Int CO_2 injury		Int CO_2 Index (max 60)	
Time of assessment		**0 d**	**7 d**	**0 d**	**7 d**	**0 d**	**7 d**	**0 d**	**7 d**
Control	A	2.27	0.00	0.91	0.00	0.00	0.00	0.00	0.00
	B	2.27	0.00	0.45	0.00	0.00	0.00	0.00	0.00
	C	0.00	0.00	0.00	0.00	0.00	3.57	0.00	0.71
	mean	**1.52**	**0.00**	**0.45**	**0.00**	**0.00**	**1.19**	**0.00**	**0.24**
Scrubbed	A	0.00	0.00	0.00	0.00	5.00	0.00	1.00	0.00
	B	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	C	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	mean	**0.00**	**0.00**	**0.00**	**0.00**	**1.67**	**0.00**	**0.33**	**0.00**
SmartFresh™	A	20.00	30.56	4.50	12.08	2.50	0.00	0.50	0.00
	B	4.17	1.92	1.25	0.38	2.08	0.00	0.42	0.00
	C	0.00	1.67	0.00	0.33	0.00	1.67	0.00	0.67
	mean	**8.06**	**11.38**	**1.92**	**4.27**	**1.53**	**0.56**	**0.31**	**0.22**
Treatment effect (Kruskall Wallis)		**0.025**	**<0.001**	**0.025**	**<0.001**	**n.s.**	**n.s.**	**n.s.**	**n.s.**

Table 6 (cont.)

Treatment	Orchard and overall mean	% Bitter pit		% Core flush		Core flush Index (max 60)		% Rots	
Time of assessment		**0 d**	**7 d**	**0 d**	**7 d**	**0 d**	**7 d**	**0 d**	**7 d**
Control	A	0.00	2.50	0.00	13.06	0.00	6.78	6.75	22.50
	B	3.85	2.08	0.00	12.88	0.00	6.44	6.00	9.50
	C	1.79	1.67	0.00	0.00	0.00	0.00	1.75	9.50
	mean	**1.88**	**2.08**	**0.00**	**8.64**	**0.00**	**4.41**	**4.83**	**13.83**
Scrubbed	A	10.00	2.27	4.17	26.59	1.67	9.59	2.00	7.25
	B	4.36	16.25	2.27	14.17	0.91	5.08	0.00	8.75
	C	4.06	0.00	0.00	2.94	0.00	0.88	2.50	2.50
	mean	**6.14**	**6.17**	**2.15**	**14.57**	**0.86**	**5.19**	**1.50**	**6.17**
SmartFresh™	A	5.28	3.13	0.00	30.90	0.00	9.99	10.00	8.75
	B	1.92	2.50	2.08	9.17	0.42	2.25	6.00	8.50
	C	0.00	0.00	0.00	1.67	0.00	0.33	1.75	1.75
	mean	**2.40**	**1.88**	**0.69**	**13.91**	**0.14**	**4.19**	**5.92**	**6.33**
Treatment effect (Kruskall Wallis)		**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**0.05**	**n.s.**

Figure 4. Average % rotting of Bramley's Seedling fruits in inoculated nets immediately on removal from storage chambers and after 7 days at ambient. Treatment effects p = 0.002, 0.013 LSD 12.5, 8.6 for 0 and 7 days respectively

Conclusions

The following conclusions are drawn from a single season of data, and will need to be tested for a second season:

- Ethylene scrubbing during storage and SmartFresh™ treatment on Bramley's Seedling apples were compared for their effects on the rate of rotting of *n*ectria inoculated fruit. For both trials reported here; the commercial store trial and the small scale CA chamber trial, the rate of rotting was significantly greater for SmartFresh™ treated fruit. This is consistent with the hypothesis that since ethylene is an important signalling compound in the induction of defence against pathogens, blocking its action by the use of SmartFresh™ could have some adverse effects
- SmartFresh™ treatment did not induce any observable increase in natural rates of rotting in the commercial stores.
- Although no significant levels of scald were observed in the commercial trial, the small scale trial confirmed that both ethylene scrubbing and SmartFresh™ significantly reduce this problem

- Breakdown was reduced slightly by scrubbing but significantly by SmartFresh™
- Although there were no treatment differences observed for the incidence of CO_2 injury in the commercial store there were indications that SmartFresh™ can exacerbate this problem in the small scale trial

Technology transfer

The project was introduced to growers at the Marden Fruit Show Society and EMRA Day in March 2010.

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References

Bishop, D. and Manning, R. (2008). Ethylene removal from a commercial Bramlely apple store. Technical Bulletin www.icastorage.com/pdfs/Bramley_trial_paper.pdf

Blankenship, S.M. and Dole, J.M. (2003). 1-methylcyclopropene: a review.

Postharvest Biology and Technology 28:1-25.

Colgan, R.J., Dover, C.J., Johnson, D.S. and Pearson, K. (1999). Delayed CA and oxygen at 1 kPa or less control superficial scald without CO_2 injury on 'Bramley's Seedling' apples. Postharvest Biology and Technology 16:223-31.

Dover, C. (1985). Effects of ethylene removal during storage of Bramleys Seedlings. Proceedings of the Fourth National Controlled Atmosphere Conference, USA.

Johnson, D.S. (2003). Improvement in the storage quality of apples in the UK by the use of 1-MCP (SmartFresh™). Acta Horticulturae 599:39-47

Johnson, D.S. (2008). Factors affecting the efficacy of 1-MCP applied to retard apple ripening. Acta Horticulturae 796:59-67.

Lafer, G. (2003). Effects of 1-MCP treatments on fruit quality and storability of different apple varieties. Acta Horticulturae 599:65-69.

McDowell, J.M. and Dangl, J.L. (2000). Signal transduction in the plant immune response. Trends in Biochemical Science 25:79–82.

