Project title:	The effect of ethylene control strategies on the development of rotting in Bramley's Seedling apples
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Project leader:	Dr. Angela M. Berrie
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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.

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GROWER SUMMARY

Headlines

- The use of either SmartFresh™ or ethylene scrubbing reduces the incidence of scald in long-term CA stored Bramley's seedling apples.
- In situations where the incidence of rotting by *Nectria* are high, ethylene scrubbing is a good strategy for maintaining good fruit quality and helping to reduce rot development.

Background and expected deliverables

Losses due to fungal rots in controlled atmosphere (CA)-stored Bramley can be a significant problem particularly in fruit stored beyond June where losses of 10% or more are not uncommon. Much of the rotting in the later stored fruit is caused by *Nectria galligena*. However, as recent rot surveys have shown, *Fusarium* is also of increasing importance, mainly arising from core rots that result from infection in late blossom.

The introduction of low oxygen storage conditions (5% CO_2 + 1% O_2) have resulted in major improvements in the storage quality of Bramley apples particularly in the control of bitter pit and superficial scald. Using this storage regime, it is not necessary to use the chemical antioxidant diphenylamine (DPA) for control of scald for storage up to six months duration. However, studies have shown that this storage regime also results in an increase in the development of *Nectria* fruit rots, so it is not recommended for orchards with a significant canker problem. Changing the CA conditions to 6% CO_2 + 2% O_2 reduces the rot problem, but increases the risk of scald without DPA treatment.

As the use of DPA is no longer permitted in the UK, storage regimescapable of controlling ethylene and hence scald, become even more important. Two technologies offering ethylene and hence scald control include the use of SmartFresh™ and ethylene scrubbing. Both of these technologies are effective in reducing scald by controlling ethylene but differ in the way in which ethylene is controlled; SmartFresh™ acts by retarding ripening changes such as fruit softening by blocking the ethylene receptor, thereby preventing the auto-stimulation of ethylene production. Ethylene scrubbing removes ethylene from stores once it has been produced. The effect of these two methods of ethylene control on rot development has not previously been studied simultaneously in a commercial environment. Both the technologies have previously 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).

The overall aim of this project was 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 by assessing fruit from a range of commercial stores. (Note: Originally this project planned to test the use of ozone treatment of stores, but this technology is no longer being used with apples). In addition, a common set of test apples was distributed within the same stores. A parallel set of small-scale trials was also set up within experimental CA chambers at East Malling Research. In both sets of trials fruit inoculated with *Nectria* was included so the effects of the treatments on rot development could be assessed.

Summary of the project and main conclusions

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 50 ppb or below. Ethylene concentrations were lower in the Bi-On stores than the catalytic scrubbed stores. The quality of fruit was assessed when the stores were opened and after a subsequent seven days at 18°C. From these assessments and assessments from the small-scale trials that were opened after nine months, the following conclusions are drawn.

For both the commercial store trials and the small scale CA chamber trials, the increase in the rate of rot development in the fruit inoculated with Nectria was significantly greater for SmartFresh^{TM}-treated fruit (Figure 1). This was expected because as well as triggering ripening, ethylene is involved in the defence mechanisms of fruit against pathogens such as Nectria. As SmartFresh^{TM} acts by blocking ethylene production, it will inhibit fruit defence. However, this effect was not accompanied by any observable increase in natural rates of rotting in the commercial stores, so that losses due to rots were the same in stores using ethylene scrubbing and SmartFresh^{TM} .

The treatments had beneficial effects on development of physiological disorders. Although in the seasons studied, the incidence of scald in commercial samples were low, the small-scale trial confirmed that both ethylene scrubbing and SmartFresh^{TM} significantly reduce this problem. Senescent breakdown and low temperature breakdown were reduced slightly by scrubbing but significantly by SmartFresh^{TM}. No treatment differences were observed for the incidence of CO_2 injury in the commercial trials, but there were indications in the small-scale trial that SmartFresh™ can exacerbate this problem.

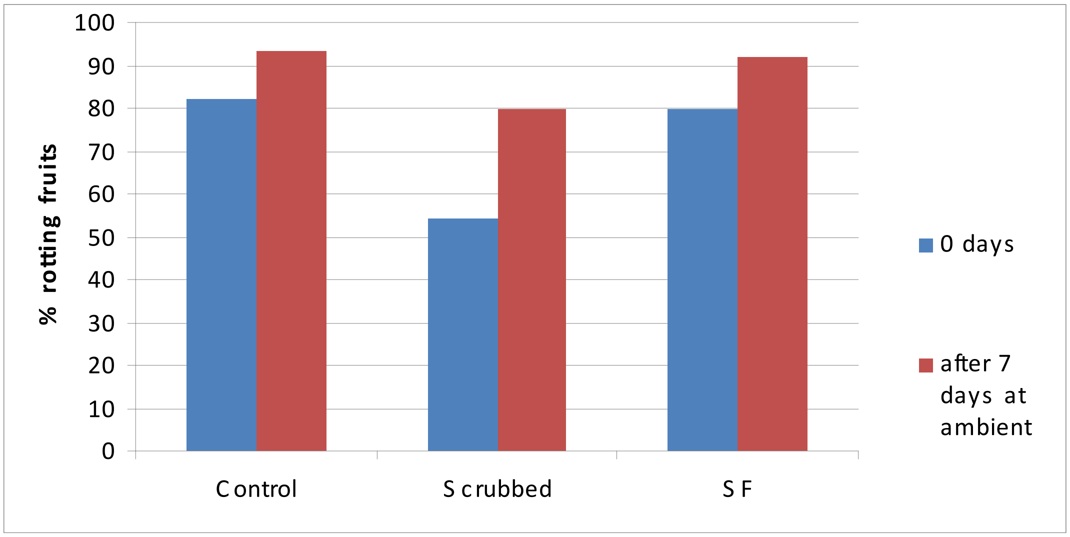


Figure 1: Average % rotting fruits in inoculated nets immediately on removal from storage chambers and after 7 days at 18°C showing that blocking ethylene action by SmartFresh™ can increase rates of rotting, even though other results indicate that this is unlikely to be commercially significant. Treatment effects $p = 0.002$, 0.013 $LSD_{0.05}$ 12.5, 8.6 for 0 and 7 days respectively.

Financial benefits

This project confirms the financial benefits of the use of either SmartFresh™ or ethylene scrubbing to reduce the incidence of scald in long-term CA stored Bramley's seedling apples.

Action points for growers

- Where growers have a significant problem with *Nectria* infection in stored Bramley apples, the use of ethylene scrubbing during storage should be considered as a strategy for longer-term storage.

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 six months of storage. However, it was known that this storage strategy would promote the development of nectria 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 can become an issue.

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, which is promoted by ethylene. However, it is also important to assess these technologies in terms of their effects on rot development. (Note: originally this project was also to test the use of ozone treatment of stores, but this technology is no longer being used for long-term apple storage).

1-MCP effects on apple fruit quality and susceptibility to rots

SmartFresh™ (1-Methyl cyclopropene (1-MCP)) has been used successfully during the storage of apple varieties in the UK since 2003. As an ethylene antagonist it 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 dessert cultivars, are picked several weeks before the climacteric. In Bramley 1-MCP has been shown to control scald and obviate the need for DPA drenching (Johnson, 2008). However, there have been reports that SmartFresh™ increases sensitivity to CO_2-injury (Lafer, 2003), so that a delay in imposing CA conditions is recommended where 1-MCP is used (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 defence mechanisms, and hence tends to reduce storage rots. However, ethylene is also directly involved in the signalling system responsible for stimulating tissue defences against invading pathogens, 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 HRI-EM (now renamed EMR) on Bramley apples in 2002, the application of 'SmartFresh™' reduced the overall incidence 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. This includes the use of ethylene scrubbing within retail packs as well as of whole CA stores. 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 concentrations 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). Bishop and Manning report an unreplicated trial to compare the effects of ethylene scrubbing with 1-MCP treatment. They report that 1-MCP reduced ethylene to 0.5-1.6 ppm. Internal fruit ethylene concentrations were approximately 30 ppb for both treatments.

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 *Nectria* or *Penicillium* in un-inoculated fruit. However, a higher incidence of *Nectria* 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).

Effective trial strategies for assessing the effect of storage strategies on development of rots

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). It is a challenge to carry out scientific studies to compare storage strategies in terms of their effects on rot development; high fruit variability means that trials must be large to obtain meaningful data on natural levels of rot. An alternative strategy is to use artificial inoculation. However, although this is useful in providing information about biological mechanisms, it does not always provide an accurate indication of the commercial significance of results. The large numbers of fruit available in commercial scale experiments are helpful in providing reliable data on rot incidence but it is important that such experiments are conducted in a scientific manner so that data is statistically valid. In this project the commercial trials have been supported by a parallel set of small-scale trials set up within experimental CA chambers (80Kg capacity).

Overall aim of project

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

(Note: originally this project was also to test the use of ozone treatment of stores, but this technology is no longer being used for long-term apple storage).

Specific objectives

Several growers are already testing these 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 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.

   Introducing fruits artificially inoculated with *Nectria* to enable a direct assessment of the effects of ethylene removal on fungal growth

Materials and Method.

In each season (2009 and 2010) Bramley apples from each of three orchards (A, B, C in 2009 and D, E, F in 2010) were harvested by a commercial producer and delivered to EMR on the following day. The harvest date was 31 August in 2009 and 6 September in 2010.

Table 1: Bramley orchards that fruit sourced from

2009	
Orchard A	M26 rootstock, planted in 1990
Orchard B	M26 rootstock, planted in 1991
Orchard C	M26 rootstock planted in 1999
2010	
Orchard D	M9 rootstock planted in 2004
Orchard E	M9 rootstock planted in 1983
Orchard F	M9 rootstock planted in 1985

20 fruits per orchard (two-three 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 a remaining bin from each orchard used to set up a parallel small scale trial within the Produce Quality Centre facilities (Jim Mount Building) 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 one 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 and six commercial stores were selected in 2009 and 2010 respectively. These were distributed across several growers, but all used CA at 5% CO_2 : 1% O_2 . In 2009 the stores included two each with SmartFresh^{TM} treatment, ethylene scrubbing using Bi-On, ethylene scrubbing using catalytic scrubbers, and two controls with no ethylene control treatments. In 2010, as it was harder to locate commercial stores with ethylene scrubbing, only one store using Bi-On and one store using a catalytic scrubber was included in the trial.

a)

b)

Figure 2: a) A bin prepared for delivery to a commercial store in 2009. Two nets of inoculated fruit were placed at the top at opposite corners of the bins.

b) Bins of fruit from three orchards prepared for delivery to a commercial store in 2010.

Ethylene concentrations within the stores were measured during the storage period 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. A vacuu-chamber was used during the sampling to ensure that there was no contamination of the sample by the sampling pump. The pump is used to suck air out of the vacuu-chamber, so that in turn air is sucked into the sampling bag, which is placed inside the chamber with a sampling tube directed to the sampling point.

Figure 3: Sampling of the store atmosphere during the storage period, using Teflon air sampling bags and a vacuu-chamber.

As each commercial store was opened, the sample bins were assessed for overall levels of rot. The inoculated nets were assessed for *Nectria* development, and fruit samples from each orchard were removed for quality assessment, both immediately and after seven 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 (Llyod-LRX). Colours were determined using either a Hunter-lab or a Minolta 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.

Smaller scale trial conducted in CA chambers at the Produce Quality Centre

Six boxes of fruit and six 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 3.5ºC. The next day all netted fruit were removed from bags. Two boxes and two inoculated nets from each orchard were treated with SmartFresh™ at

3.5ºC for 24 hours.

The following day boxes of apples were weighed and placed in six CA chambers, two for each treatment; SmartFresh™ 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 three weeks. Ethylene concentrations were measured at regular intervals. Fruit quality was assessed after nine months storage

Results

Commercial store trial

In season 1 (2009-2010), 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, so that bins from six stores were assessed for the trial.

In season 2 (2010-2011), bins were distributed to six commercial stores; two each of SmartFresh™ treated and untreated, one Bi-On scrubbed and one catalytic scrubbed. One SmartFresh™ and two untreated stores were opened too early for assessments to be useful, so that bins from three stores were assessed for the trial.

Table 2 shows the concentrations of ethylene measured within the six commercial stores in season 1, and the three commercial stores in season 2.

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.

Store	Ethylene concentration within store (ppb)	
	Nov 2009	Feb/Mar 2010
Untreated B*	10268	
Catalytic scrub A*	12.8	
Catalytic scrub B	100.3	284
Bion scrub A	2.7	75
Bion scrub B	9.4	83.4
Smartfresh A	770	2520
		Mar 2011
Catalytic scrub		0
Bion scrub		12
Smartfresh		12

* stores opened before second ethylene measurement

The stores were opened at various times from early January through to late May in season 1 and July for season 2. In season 1 two stores were only assessed for ethylene concentration early in the storage season as they were opened before the end of January. The untreated store (B) was opened before the second ethylene assessment but already had a high ethylene level (>10,000 ppb) 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 100 ppb or below, especially in season 2, in which 12 ppb was the highest concentration recorded. Ethylene concentrations were lower in the Bi-On stores than the catalytic scrubbed stores in season 1, but both methods maintained very low ethylene concentrations in season 2. A potential disadvantage of the Bi-On method often mentioned is that if the potassium permanganate becomes saturated with ethylene later in the season, scrubbing may become less efficient. The ethylene measurements do not indicate that saturation occurred by the February/March assessment in either season.

The fruit quality and the incidence 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 for each season.

On removal from store and after seven days at ambient, the softest fruit were from the untreated store in season 1. SmartFresh™ treatment and ethylene scrubbing both improved retention of firmness. There was no clear difference between scrubbing and SmartFresh™ treatment, although there was a relatively high rate of softening in the catalytic scrubbed A store in season 1 and the Bi-On store in season 2.

There were only small differences in colour and in %Brix on removal from store, and no obvious treatment effects. Interestingly, in season 1 colour change (increase in yellow, indicating loss of chlorophyll) during the week at ambient seemed to be greater for the SmartFresh™ store, and loss of sugar (% Brix) was also greater for this store. Colour change was less apparent in season 2.

The % rotting observed in *Nectria* inoculated samples and for uninoculated fruit are shown in Table 4 (Results are not available for untreated stores in season 1 due to loss of inoculated nets). For inoculated fruit there is a natural tendency for rotting to increase with time. However, rotting was significantly ($p<0.001$) greater for the SmartFresh™ treated fruit than for scrubbed fruit in season 1, and the same trend was observed for season 2. This was not reflected as higher natural amounts of either *Nectria* or total rot in the whole bins. In fact the level of *Nectria* rot for all orchards in both seasons was low (< 1%). However, it should be noted that each bin is generally filled from about two-four 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 bins from each of the orchards 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™-treated bins and other fruit been more obvious.

Both carbon dioxide injury and bitter pit increased with storage time. There were no clear treatment differences in season 1, but carbon dioxide injury was not observed in the SmartFresh™ store in season 2. No significant scald was seen in any bins in either season (data not shown).

Table 3: Fruit quality on removal from store and after seven days at ambient (Shelf-life)

Store	Date opened	Orchard	Post-storage				Post shelf-life			
			Colour a	Colour b	% Brix	Firm-ness Kgf	Colour a	Colour b	% Brix	Firm-ness kgf
5:1 scrubbed (A)	11 January 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 January 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 Bion (B)	23 March 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 Bion (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 SmartFresh (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**

Store	Date opened	Orchard	Post-storage				Post shelf-life			
			Colour a*	Colour b*	% Brix	Firm-ness KgF	Colour a*	Colour b*	% Brix	Firm-ness
5:1 Bi-On	Last week March 2011	D	-19.34	47.00	10.1	7.86	-17.91	49.29	10.2	6.00
		E	-16.88	48.50	11.4	7.38	-17.43	50.54	11.0	5.12
		F	-17.85	47.82	11.6	9.16	-17.77	48.97	11.5	5.86
		Mean	**-18.02**	**47.77**	**11.0**	**8.13**	**-17.70**	**49.60**	**10.9**	**5.66**
5:1 SmartFresh	Last week April 2011	D	-17.44	48.72	9.6	8.02	-17.48	50.89	10.4	6.32
		E	-17.86	48.20	11.0	8.16	-15.62	51.40	10.9	6.62
		F	-18.88	47.88	12.5	8.92	-18.38	50.17	12.0	7.78
		Mean	**-18.06**	**48.27**	**11.0**	**8.37**	**-17.16**	**50.82**	**11.1**	**6.91**
5:1 scrubbed	July 2011	D	-19.74	47.95	10.2	8.34	-18.43	49.73	9.6	6.40
		E	-19.03	47.68	10.6	7.99	-17.82	50.87	10.4	6.68
		F	-18.74	45.65	12.6	8.76	-18.18	48.91	12.4	8.00
		Mean	**-19.17**	**47.09**	**11.1**	**8.36**	**-18.14**	**49.84**	**10.8**	**7.03**

*In Season 1 colour was measured using a Hunter Lab with D=type light source and in season 2 with a Minolta colour meter with C-type light source, so that the a and b values were higher in season 2 than those measured in the previous season.

Table 4: Levels of rot for inoculated fruit and whole bins, and disorders within sampled fruit

Store type	Date opened	Orchard	inoculated nets	Whole Bin assessment		Sample fruit removed for quality assessment	
			% *Nectria*	% *Nectria*	% total rots	% CO2 injury	% bitter pit
5:1 scrubbed (A)	11 January 2010	A	33	0.4	1.8	0	0
		B	23	0.00	1.5	0	0
		C	10	0.00	0.5	0	0
		Mean	**22**	**0.1**	**1.2**	**0**	**0**
5:1 untreated (B)	End January 2010	A	-	0.2	1.7	0	0
		B	-	1.7	3.4	0	0
		C	-	0.1	2.6	0	0
		Mean		**0.7**	**2.6**	**0**	**0**
5:1 Bion (B)	23 March 2010	A	29	0.7	3.8	0	0
		B	35	0.00	5.6	0	0
		C	17	0.00	2.6	0	7
		Mean	**45**	**0.2**	**4.0**	**0**	**2**
5:1 Bion (A)	10 May 2010	A	50	0.3	2.6	2	2
		B	53	0.6	4.0	0	5
		C	33	0	6.5	0	0
		Mean	**26**	**0.3**	**3.6**	**1**	**2**
5:1 SmartFresh (A)	10 May 2010	A	97	03	4.4	10	5
		B	97	0.7	2.5	0	5
		C	90	0.3	0.8	2	0
		Mean	**94**	**0.4**	**2.6**	**4**	**3**
5:1 scrubbed (B)	End May 2010	A	82	0.5	3.4	15	5
		B	73	1.5	8.2	15	8
		C	47	0.4	2.6	15	0
		Mean	**66**	**0.8**	**4.7**	**15**	**4**
Treatment effect (p)			<0.001				
$LSD_{0.05}$ (Treatment means)			19				

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

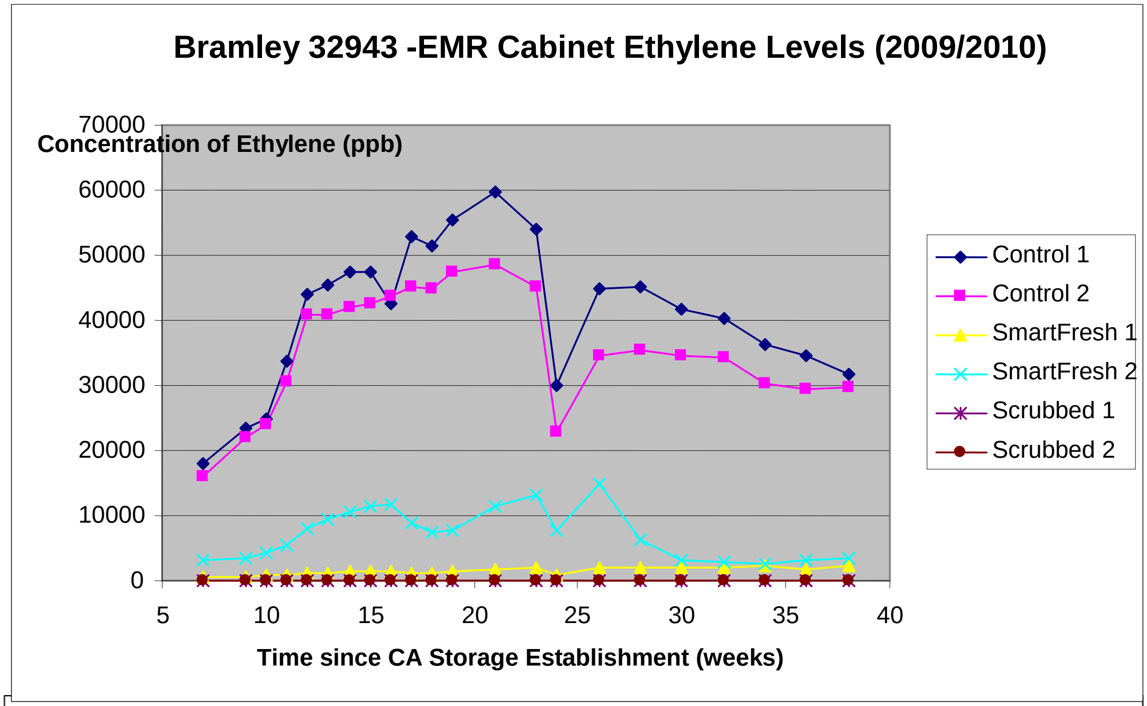
Store type	Date opened	Orchard	inoculated nets	Whole Bin assessment		Sample fruit removed for quality assessment			
			% *Nectria*	% *Nectria*	% total rots	% CO2 injury	% bitter pit	LTB	Core Flush
5:1 Bi-On	Last week March 2011	D	58	1.6	5.2	45	0	45	0
		E	85	0.1	3.7	15	2	68	0
		F	67	0.0	0.6	10	0	2	0
		Mean	**70**	**0.6**	**3.2**	**23**	**1**	**38**	**0**
5:1 SmartFresh	Last week April 2011	D	100	1.7	5.7	0	15	55	0
		E	98	0.4	4.9	0	15	45	0
		F	95	0.0	0.6	0	2	5	0
		Mean	**98**	**0.7**	**3.7**	**0**	**11**	**35**	**0**
5:1 scrubbed	July 2011	D	97	1.6	6.1	38	8	0	38
		E	98	1.3	9.1	35	10	0	38
		F	83	0.1	1.1	0	25	0	0
		Mean	**93**	**1.0**	**5.4**	**24**	**14**	**0**	**25**
Treatment effect (p)			0.035						
$LSD_{0.05}$ (Treatment means)			20						

% Nectria in inoculated nets was analysed by Anova. For other data the number of samples was not sufficient for Kruskall Wallace analysis. LTB = Low temperature browning.

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 potassium permanganate to fruit was much higher than would be the case in commercial stores.

Figure 3 shows the ethylene concentrations within the six cabinets for both seasons. Scrubbed cabinets had very low ethylene concentrations throughout (only above 50 ppb on two occasions). Ethylene in cabinets of SmartFresh^{TM}-treated fruit were intermediate. In season 1, SmartFresh^{TM} replicate 2 ethylene levels were consistently higher than those for replicate 1, suggesting that there were rotten or riper ethylene producing fruit within that cabinet. This would not affect the efficacy of SmartFresh^{TM}. For the untreated controls, ethylene concentrations peaked above 40,000 ppb in season 1, but only reached 8,000 and 12,000 ppb in season 2. This suggests that the fruit had a tendency for lower ethylene production in season 2, which would be consistent with the very low concentrations of ethylene measured in the scrubbed and SmartFresh^{TM}-treated commercial stores.



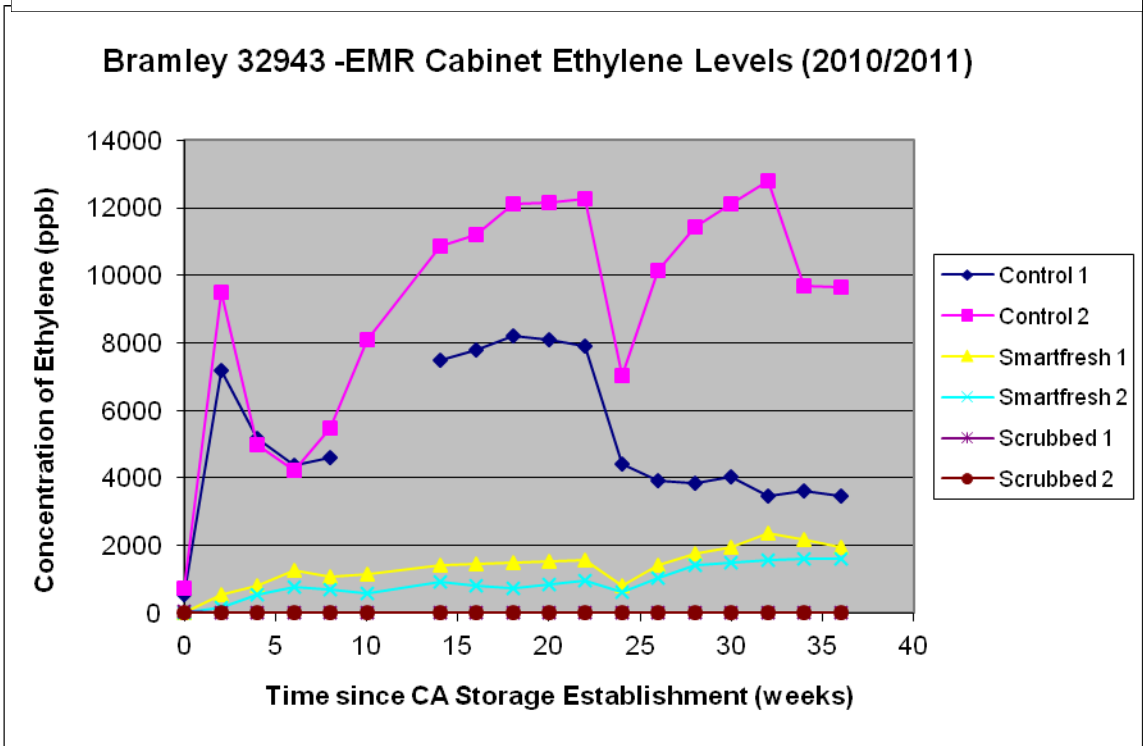


Figure 3: Ethylene concentrations within CA chambers in season 1 (2009/2010) and season 2 (2010/2011).

The quality of fruit on removal from the chambers after nine months storage and after a subsequent seven 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 for both seasons. 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 fruit following storage in CA chambers

Season 1									
		On removal from storage				After seven days at ambient			
Treatment	Orchard	Colour a	Colour b	Firmness KgF	% Brix	Colour a	Colour b	Firmness KgF	% Brix
Untreated	A	-9.33	41.60	6.14	10.4	-7.33	46.09	4.35	10.0
	B	-9.52	41.54	6.02	9.9	-7.53	45.45	4.28	9.6
	C	-9.21	41.01	6.87	10.7	-7.02	46.53	5.29	10.8
Control mean		**-9.35**	**41.38**	**6.35**	**10.3**	**-7.29**	**46.02**	**4.64**	**10.1**
Scrubbed	A	-9.60	43.60	6.66	10.1	-8.01	45.25	5.58	10.4
	B	-9.97	43.07	6.54	10.0	-7.65	45.63	5.18	10.0
	C	-9.53	43.12	7.35	10.8	-7.11	46.15	6.46	10.8
Scrubbed mean		**-9.70**	**43.26**	**6.85**	**10.3**	**-7.59**	**45.68**	**5.74**	**10.4**
SmartFresh	A	-9.77	42.56	8.34	10.3	-8.18	44.86	7.70	10.0
	B	-10.12	42.33	9.00	10.1	-8.54	44.62	8.22	9.7
	C	-9.72	42.68	9.25	10.8	-8.25	44.59	9.04	10.9
SmartFresh mean		**-9.87**	**42.52**	**8.86**	**10.4**	**-8.32**	**44.69**	**8.32**	**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**	**0.31**		**0.38**	**0.67**	**0.25**	
Orchard effect (p)		**0.043**	**n.s.**	**<0.001**	**<0.001**	**n.s.**	**n.s.**	**<0.001**	**<0.001**
Season 2									
		On removal from storage				After seven days at ambient			
Treatment	Orchard	Colour a	Colour b	Firmness KgF	% Brix	Colour a	Colour b	Firmness KgF	% Brix
Untreated	D	-19.58	44.45	5.32	10.43	-17.76	49.23	4.68	10.88
	E	-19.85	43.17	6.06	10.58	-18.46	48.41	4.76	9.78
	F	-19.49	44.06	7.62	12.08	-18.25	49.32	6.15	12.25
Control mean		**-19.64**	**43.90**	**6.33**	**11.03**	**-18.16**	**48.99**	**5.20**	**10.97**
Scrubbed	D	-20.07	43.81	7.03	10.10	-18.27	48.43	5.88	10.23
	E	-20.06	43.87	6.74	10.53	-18.14	48.53	5.94	10.33
	F	-19.81	45.00	8.70	12.68	-18.56	48.89	7.29	12.23
Scrubbed mean		**-19.98**	**44.23**	**7.49**	**11.10**	**-18.32**	**48.61**	**6.37**	**10.93**
SmartFresh	D	-19.60	43.45	7.83	10.33	-19.12	46.96	7.45	10.18
	E	-19.85	43.63	7.38	10.40	-18.29	47.03	7.30	11.00
	F	-20.03	44.88	8.88	12.23	-19.28	46.47	9.00	12.43
SmartFresh mean		**-19.82**	**43.99**	**8.03**	**10.98**	**-18.90**	**46.82**	**7.92**	**11.20**
Treatment effect (p)		**0.096**	**n.s.**	**n.s.**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**n.s.**
Treatment LSD (5%)		**0.31**	**0.64**	**0.30**	**0.25**	**0.46**	**0.82**	**0.44**	**0.33**
Orchard effect (p)		**n.s.**	**0.002**	**<0.001**	**<0.001**	**n.s.**	**n.s.**	**<0.001**	**<0.001**

Data were analysed by 2 way anova using Genstat.

Tables 6 and 7 show internal and external disorders observed in the fruit for season 1 and 2, respectively. Scald is eliminated by both scrubbing and SmartFresh^{TM} in season 1, but was observed on three fruit in the SmartFresh^{TM} treatment in season 2. Although SmartFresh^{TM} tends to prevent scald, where over mature fruit is stored scald can still be observed. No distinction was made between internal breakdown due to senescence and low temperature stress in season 1, but they were separated in season 2. Breakdown was reduced slightly by scrubbing but significantly by SmartFresh^{TM} in season 1. Similarly, SmartFresh^{TM} was more effective in season 2. In this case it was only significant for senescent breakdown after seven days.

External CO_2-injury was greatest in SmartFresh^{TM} treated fruit, occurred in controls, but not in scrubbed fruit. No treatment differences for internal CO_2 were observed. In season 2, the differences were much more pronounced, with a higher incidence of CO_2 injury in SmartFresh^{TM} treated fruit.

No significant differences in bitter pit and coreflush were observed.

Overall rotting was least in scrubbed chambers for both seasons, but this was barely statistically significant in season 1(p = 0.05 immediately after storage and n.s. at 7 days) and not significant in season 2.

Figure 4 shows the % rotting for inoculated fruits on removal from storage and after a subsequent seven days at ambient for season 1. A significantly lower rate of rotting was observed for scrubbed fruit. In season 2, rotting was more developed so that all fruit had lesions. However, the same pattern of rotting was observed. Thus controls had the most rot, followed by SmartFresh^{TM} treated fruit and the least lesion development was in the scrubbed treatments.

This is consistent with the observation of inoculated fruit from the commercial store trial.

Table 6: Disorders of fruit following storage in CA chambers in season 1

Treatment	Orchard	% scald		Scald index		BD%		BD index	
		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
Control 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
Scrubbed mean		0.0	0.00	0.0	0.00	28.7	27.51	9.7	8.64
SmartFresh™	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
SmartFresh™ 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 = internal breakdown. No distinction is made between senescent breakdown and low temperature breakdown

Treatment	Orchard	% CO_2 breakdown		CO_2 breakdown index (max 60)		% Int CO_2 breakdown		Int CO2 breakdown index (max 60)	
		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
Control 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
Scrubbed 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
SmartFresh 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	% bitter pit		% core flush		Core flush index (max 60)		% rots	
		0	7	0 d	7	0 d	7	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
Control 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
Scrubbed 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
SmartFresh 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.

Table 7: Disorders of fruit following storage in CA chambers for season 2

Treatment	Orchard	% scald		Scald index		LT B%		LT B index	
		0 d	7 d	0 d	7 d	0 d	7 d	0 d	7 d
Control	D	0.00	0.00	0.00	0.00	95.00	59.44	45.50	22.22
	E	0.00	2.50	0.00	1.00	71.88	89.44	25.88	34.17
	F	0.00	0.00	0.00	0.00	32.50	5.00	12.50	1.00
Control mean		**0.00**	**0.83**	**0.00**	**0.33**	**66.46**	**51.30**	**27.96**	**19.13**
Scrubbed	D	0.00	0.00	0.00	0.00	50.00	82.50	14.00	25.50
	E	0.00	0.00	0.00	0.00	71.67	63.89	21.72	18.33
	F	0.00	0.00	0.00	0.00	25.00	5.00	9.00	1.00
Scrubbed mean		**0.00**	**0.00**	**0.00**	**0.00**	**48.89**	**50.46**	**14.91**	**14.94**
SmartFresh	D	5.56	0.00	4.44	0.00	20.00	50.00	6.00	12.22
	E	2.50	0.00	1.50	0.00	58.61	52.50	18.67	10.50
	F	0.00	0.00	0.00	0.00	15.00	2.50	5.00	0.50
SmartFresh mean		**2.69**	**0.00**	**1.98**	**0.00**	31.20	**35.00**	9.90	**7.74**
Treatment effect (Kruskall Wallis)		0.042	n.s.	0.042	n.s.	n.s.	n.s.	(0.083)	n.s.

LTB = Low temperature breakdown.

Table 7. (continued)

Treatment	Orchard	Sen BD%		Sen BD index	
		0 d	7 d	0 d	7 d
Control	D	10.00	45.28	5.00	12.94
	E	5.00	25.28	3.00	7.11
	F	0.00	0.00	0.00	0.00
Control mean		**5.00**	**23.52**	**2.67**	**6.69**
Scrubbed	D	0.00	2.50	0.00	0.50
	E	0.00	2.50	0.00	0.50
	F	8.06	0.00	1.61	0.00
Scrubbed mean	D	**2.69**	**1.67**	**0.54**	**0.33**
SmartFresh	E	5.56	2.50	2.22	0.50
	F	0.00	2.50	0.00	0.50
	D	2.50	0.00	1.00	0.00
SmartFresh mean		**2.69**	**1.67**	**1.07**	**0.33**
Treatment effect (Kruskall Wallis)		n.s.	0.003	n.s.	0.002

Sen BD = Senescent breakdown

Treatment	Orchard	% CO_2 breakdown		CO_2 breakdown index (max 60)		% Int CO_2 breakdown		Int CO2 breakdown index (max 60)	
		0 d	7 d	0 d	7 d	0 d	7 d	0 d	7 d
Control	D	5.00	2.50	1.00	0.50	25.00	42.78	8.00	14.06
	E	2.50	2.50	0.50	0.50	31.25	34.17	7.38	10.94
	F	0.00	0.00	0.00	0.00	12.50	0.00	3.00	0.00
Control mean		**2.50**	**1.67**	**0.50**	**0.33**	**22.92**	**25.65**	**6.13**	**8.33**
Scrubbed	D	0.00	2.50	0.00	0.50	55.00	25.00	16.00	10.00
	E	10.00	0.00	2.50	0.00	24.72	23.06	7.17	5.11
	F	2.50	0.00	0.50	0.00	2.50	10.28	0.50	4.67
Scrubbed mean		**4.17**	**0.83**	**1.00**	**0.17**	**27.41**	**19.44**	**7.89**	**6.59**
SmartFresh	D	41.67	23.33	14.67	6.72	68.33	60.56	24.00	22.11
	E	17.50	37.50	5.00	14.00	45.56	40.00	16.17	14.50
	F	12.50	0.00	4.00	0.00	20.00	7.50	7.00	2.50
SmartFresh mean		**23.89**	**20.28**	**7.89**	**6.91**	**44.63**	**36.02**	**15.72**	**13.04**
Treatment effect (Kruskall Wallis)		<0.001	<0.001	<0.001	<0.001	n.s.	n.s.	(0.086)	n.s.

Table 7. (cont)

Treatment	Orchard	% bitter pit		% core flush		Core flush index (max 60)		% rots	
		0	7	0 d	7	0 d	7	0 d	7 d
Control	D	0.00	0.00	0.00	68.61	0.00	28.06	30.00	35.00
	E	5.00	0.00	7.50	71.94	3.50	28.89	20.00	12.50
	F	2.50	0.00	5.00	5.00	3.00	1.00	5.00	15.00
Control mean		**2.50**	**0.00**	**4.17**	**48.52**	**2.17**	**19.31**	**18.33**	**20.83**
Scrubbed	D	10.00	5.00	10.00	65.00	3.00	30.50	15.00	17.50
	E	5.00	0.00	24.17	66.39	8.56	26.50	7.50	20.00
	F	0.00	0.00	15.00	5.28	5.00	2.11	5.00	7.50
Scrubbed mean		**5.00**	**1.67**	**16.39**	**45.56**	**5.52**	**19.70**	**9.17**	**15.00**
SmartFresh	D	0.00	0.00	15.56	71.39	5.11	25.28	26.50	27.50
	E	2.50	0.00	31.67	45.00	11.17	15.00	17.50	12.50
	F	0.00	2.50	2.50	2.50	0.50	0.50	2.50	10.00
SmartFresh mean		**0.83**	**0.83**	**16.58**	**39.63**	**5.59**	**13.59**	**15.50**	**16.67**
Treatment effect (Kruskall Wallis)		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

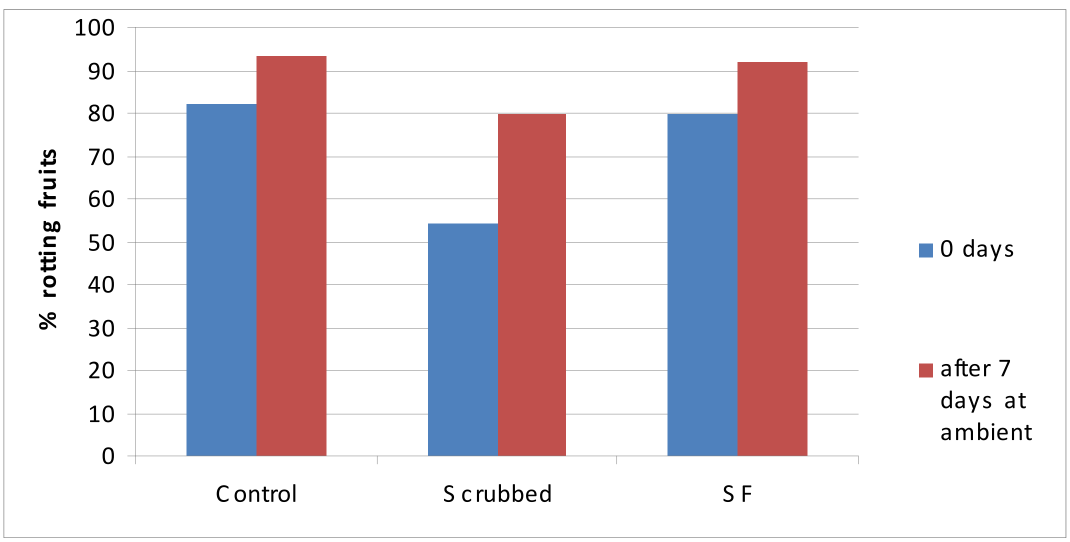


Figure 4: Average % rotting fruits in inoculated nets immediately on removal from storage chambers and after seven days at ambient. Treatment effects p = 0.002, 0.013 $LSD_{0.05}$ 12.5, 8.6 for 0 and 7 days respectively.

Conclusions

Ethylene scrubbing during storage and SmartFresh™ treatment of Bramley's Seedling apples were compared for their effects on the rate of rotting of *Nectria* 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.

This effect was not accompanied by any observable increase in natural rates of rotting in the commercial stores included in this study. The incidence of *Nectria* was low in these stores, and the findings of this project are likely to be more relevant to orchards with high incidence of *Nectria*.

Although no significant superficial scald development was observed in the commercial trial, the small scale trial confirmed that both 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 MFSS and EMRA Days in March 2010 and 2011. The results were presented in an HDC News article in 2012

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