Project title:	Sustaining UK fresh onion supply by improving consumer acceptability, quality and availability
Project number:	FV 308 (HortLink HL0182)
Project Leader:	Dr Leon A. Terry, Cranfield University
Annual Report:	2008 - 2009
Previous reports:	Annual report 2007/2008
Key workers:	Dr Gemma Chope, Cranfield University, Dr John Hammond, Warwick HRI, Dr Andrew Thompson, Warwick HRI.
Location of project:	Cranfield University, Bedfordshire, MK43 0AL
Project co-ordinator:	David E. O'Connor, Allium and Brassica Centre Wash Road, Kirton Boston Lincs PE20 1QQ
Date project commenced:	1 April 2007
Final report date:	30th June 2010
Consortium members:	Cranfield University (CU); Allium and Brassica Centre (ABC); University of Warwick (HRI); Horticultural Development Council (HDC); G's Marketing Ltd (G's); Moulton Bulb Co. Ltd. (MBC); PG Rix Farms Ltd (PGR); Bedfordshire Growers Ltd (BG); Rustler Produce Ltd (RP); Suffolk Onion Growers Ltd (SOG); Elveden farms Ltd (EF); Sainsbury's Supermarkets Ltd (SS); Restrain Company Ltd (RC); Syngenta Seeds Ltd (S+G).
Key words:	Onions, Storage, curing.

Table of Contents

Grower Summary

Headline

Curing at lower temperatures of 20 or 24ºC rather than 28ºC had only marginal effects on the visual appearance of onion bulbs and did not affect primary quality traits such as dry weight, pungency, disease incidence or sprout growth. This potential means that growers could spend less on energy inputs during curing whilst still delivering the same onion quality.

Background and expected deliverables

This project will use temporal and spatial profiling of identified biochemical and molecular markers to allow factors influencing dormancy/sprout suppression to be identified. This will lead to possibilities in developing microarray techniques for screening cultivars and breeding for better storability and flavour. Knowledge of effects of endogenous ABA and ethylene in non-climacteric systems may be transferable to other non-climacteric UK fresh produce types.

Grower uptake and customer acceptance

- The bulb onion industry is highly co-ordinated and the uptake of new technology would be phased in over 2-3 years. Beneficial technology will be taken up universally as the industry now consists of mostly large professional enterprises.
- Improved consumer acceptability of product as it would be free from all pesticide residues at harvest.
- Improved maintenance of flavour and quality through better storage and shelf-life techniques.

Grower capital investment and cost recovery

- It is unlikely that significant investment will be needed as most onion producers have modern efficient storage facilities in place. Indeed, curing onions at lower temperature whilst maintaining quality will reduce costs.

- Reduction of waste. Currently 17.5% of bulb onions are below retail quality and are used for processing. The target is a 30% reduction, leading to a 50% value increase, saving £1.3 m p.a. A further 10% unsaleable waste is mostly composted / ploughed in. The target is a 30% reduction, with 3% more saleable = £1.5 m saving (total £2.8 m) p.a.

The project has the following objectives:

1. Develop a chemometric model based on temporal and spatial profiles of biochemical markers linked to onion bulb dormancy induction and sprout suppression.

2. Produce the first microarray for onion to identify genes that are up or down regulated during dormancy and sprout suppression.

3. Elucidate mechanisms related to dormancy and sprout suppression, as influenced by applied treatments, for maximum storage and shelf life.

4. Prolong storage and shelf life whilst reducing energy inputs by optimisation of postharvest treatments as monitored by biochemical and gene expression markers.

The milestones for Year 2 are as follows:

P1.2 - Construct chemometric model for onion dormancy and sprout suppression (CU; 24 months). - **Completed**

P2.2 - Complete design and fabrication of onion oligonucleotide microarray (WHRI; 24 months). - **Completed**

P1.3 - Identify markers (chemometric and molecular) for dormancy induction, sprout suppression and attributes related to bulb quality (CU and WHRI; 24 months) - **Completed**

P3.2 - Complete first ethylene and 1-MCP experiment (CU; 20 months). - **Completed**

P4.2 - Establish the optimum curing regime with lowest energy input, which is still capable of providing sufficient control of postharvest neck rot (CU and ABC, 20 months). – **Completed**

Summary of the project and main conclusions

In addition to the laboratory trials and biochemical analysis, bulk storage trials were carried out alongside to test commercial storage conditions. The main findings of both the laboratory and bulk trials are detailed below.

1. Grower Bulk Demonstration Trial (ABC)

Alongside the main scientific trial a bulk demonstration trial was carried out by the Allium and Brassica Centre (ABC), to illustrate to the grower partners the potential effects of the different curing regimes on bulk samples. This trial was not replicated; therefore caution is necessary in interpreting any results presented.

In summary, bulbs of the same varieties (Red Baron, Sherpa, Wellington) from the same sites (Elveden, Findlays) were harvested at the same time as bulbs for the main scientific work (Year 1 Ann Report). At each site approximately 0.5 tonne of each variety was harvested into bulk bins and dried/cured at Sutton Bridge at three temperatures 20, 24 & 28ºC (Plate 1). Until this point their treatment mirrored that of the bulbs used for the scientific experiments.

Plate 1. Onions cv. Sherpa cured at 20ºC(left) or 24ºC (right) in the bulk storage trial.

After curing the bins were transported to the Allium and Brassica Centre for long term storage in their experimental box store. The storage temperature was set at 3ºC with continuous commercial *Restrain* ethylene treatment. Before and after curing, and 4 months into storage, 3 x 50 (+60 mm) bulb samples from each bin were weighed to establish any difference in weight loss from the different curing temperatures. The bulbs were removed from storage on 6th May 2008 to enable further recording and grading prior to the project meeting on 15th May 2008. On 13/14th May 2008 the bulk samples were graded over a commercial line (courtesy Moulton Bulb Co.) into <60, 60-80, >80 mm sizes. The proportion of rots and rejects were also recorded as weights.

Very little external visible sprouting was observed at this stage (Plate 2). Therefore 50 bulbs from each bulk bin were sampled for internal sprouting and then the bulks were kept to estimate sprouting/shelf life on 15 June 2008.

Plate 2. Onions cv. Red Baron cured at 28ºC in the bulk storage box and cut open to asses internal sprouting.

On 15th May 2008 all partners viewed the bulk samples (Plate 3). The samples were first displayed blind and partners were asked to estimate the likely curing temperature, based on their experience, and then score the overall quality of the samples on a simple 1-9 scale (9=highest quality)

Plate 3. Grower assessment of the bulk storage trials on 15th May 2008.

Grower Bulk Demonstration Trial - Results and Discussion

Overall from the data there was very little difference between curing temperatures for any trait with no obvious difference in weight loss. Essentially this mirrored the findings in the main science section (Part 1). Similarly at grade out it might be expected that more neck rot disease would occur at the lower curing temperatures but there was no evidence in this trial. The grade out summary is shown in Table 1.

Table 1: Bulk storage grading and sprouting summary

Curing Temp	%60-80mm	%<60mm	%rots	%length internal sprouting
20°C	57.5	33.5	8.2	21.0
24°C	63.0	27.5	8.7	16.2
28°C	57.8	30.0	11.0	18.3

Internal growth and subsequent sprouting is shown in Figure 1 averaged over the 3 varieties and again no consistent differences were detected between different curing temperatures

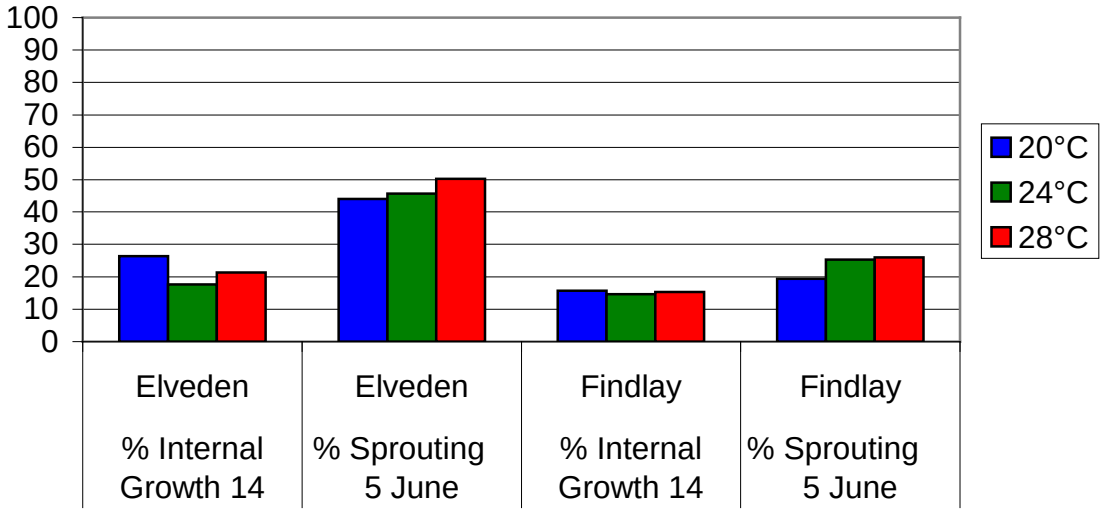


Figure 1. Internal growth and sprouting – mean of the three varieties

During the grower demonstration the results from the coded display were collected and collated. On average growers were only about 50% accurate at estimating the correct curing temperature. The summary of the quality scoring is shown in Table 2. Despite very little previous evidence of measured differences between curing temperatures, grower preferences, based on final bulb appearance/quality seem to favour the lower temperatures as producing better bulbs. For example - 3 out of the top 4 ranked samples were cured at 20^{o}C and 4 out of 5 worst ranked samples were cured at 28^{o}C.

Overall the bulk trial seemed to suggest little overall difference between curing temperatures for weight loss, sprouting and disease incidence, with larger difference occurring between varieties and sites. However grower preferences on bulb quality seemed to favour lower curing temperatures especially for the variety Red Baron.

Table 2. Summary of grower scoring of blind display of bulk bulbs 15th May 2008 (9=highest overall quality)

Rank	Site	Variety	Curing Temp	Av Score
1	Elv	Well	20	7.91
2	Elv	RBaron	20	7.55
3	Elv	Sherpa	24	7.50
4	Elv	Sherpa	20	7.45
5	Elv	Sherpa	28	7.27
6	Fin/Fin/Fin	W/Sh/Sh	20/24/20	7.09

11	Fin	Sherpa	28	6.64
12	Fin	Well	24	6.33
13	Fin	Well	28	6.18
14	Elv	RBaron	28	5.91
15	Fin	RBaron	28	5.80

2. Variety Trial

Fourteen varieties were grown in UK (by NIAB in Suffolk) and seven grown in Lelystad in Holland from seed supplied by Syngenta (Table 3). The range represented both traditional and more modern new and experimental varieties. The initial aim was to demonstrate potential differences from the two different extreme curing temperatures 20 & 28^{o}C and potential differences from growing under traditional UK and Dutch growing practices. The primary difference being that Dutch growers leave bulbs to dry in the field longer than in UK, where they are normally harvested relatively green. Unfortunately due to difficulty in acquiring seed in time only two varieties were common to both trials so any comparison between growing systems was difficult.

After harvest the bulbs from both sites were collected and for each variety 3 x 50 bulb nets were cured at the two temperatures alongside the main experimental bulbs. Subsequently they were stored at ABC in a separate bin but in the same store as the bulk storage trial described above. On 13 May, for each variety at each curing temperature, the total number and number of rotten bulbs was counted. Because there was no visible sprouting at this stage a sample of 10 bulbs was cut vertically and the length of internal sprouting measured along with bulb height and the relative shoot height calculated.

Table 3 Summary of Syngenta variety trial

Variety ID	Variety name	Production site	% rotting after curing at 20°C	% rotting after curing at 28°C	Relative shoot length after curing at 20°C	Relative shoot length after curing at 28°C
KA	Hyfort	NIAB	60.0	2.0	0.29	0.34
KJ	Wellington	NIAB	12.0	0.0	0.34	0.33
DK	Sherpa	NIAB	6.0	6.0	0.46	0.49
KP	Centro	NIAB	66.0	0.0	0.29	0.28
KV	Vision	NIAB	22.0	4.0	0.20	0.20
SA	SG 8295	NIAB	8.0	6.0	0.31	0.35
SB	SG 8301	NIAB	8.0	10.0	0.26	0.27
SC	SG 8353	NIAB	16.0	2.0	0.22	0.23
KM	Hybing	NIAB	8.0	4.0	0.33	0.34
LA	Red Baron	NIAB	24.6	12.0	0.51	0.58
LB	Red Spark	NIAB	18.4	3.2	0.46	0.48
DP	Romy	NIAB	1.6	8.2	0.54	0.60
LC	Kamal	NIAB	4.9	4.0	0.57	0.55
KD	Samira	NIAB	2.0	4.0	0.49	0.46
1628	Mission	Holland	0.0	0.0	0.36	0.46
1626	Sunskin	Holland	4.0	6.0	0.44	0.35
1625	Napoleon	Holland	0.0	0.0	0.46	0.66
1624	ONL 301	Holland	10.0	10.0	0.52	0.48
1623	ONL 295	Holland	26.0	36.0	0.39	0.34
1622	Vision	Holland	6.0	12.0	0.39	0.49
1621	Wellington	Holland	12.0	12.0	0.40	0.49
Means over all values			15.0	6.7	0.39	0.42

The trial showed no substantive difference for actual storage potential for the two different temperatures. However this was the one trial that pointed to the potential risk from neck rot at the lower curing temperature. Whilst the results varied considerably from variety to variety there were no obvious differences between curing temperatures for the Dutch grown bulbs (Fig. 2).

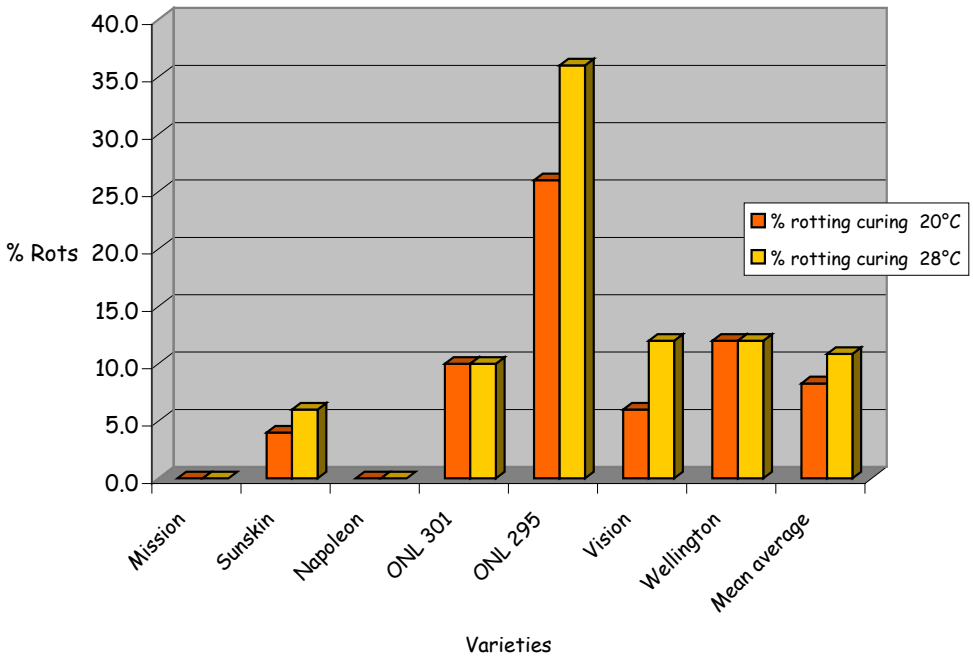


Figure 2. NL-grown variety trial. Percentage rots at 20ºC and 28ºC assessed on 13th May 2008.

From the UK grown bulbs around half the varieties grown showed a substantially higher level of neck rot infection (Fig. 3); levels that would be very serious in a commercial situation. Further more detailed work on the neck rot risk is being carried out in Years 2/3.

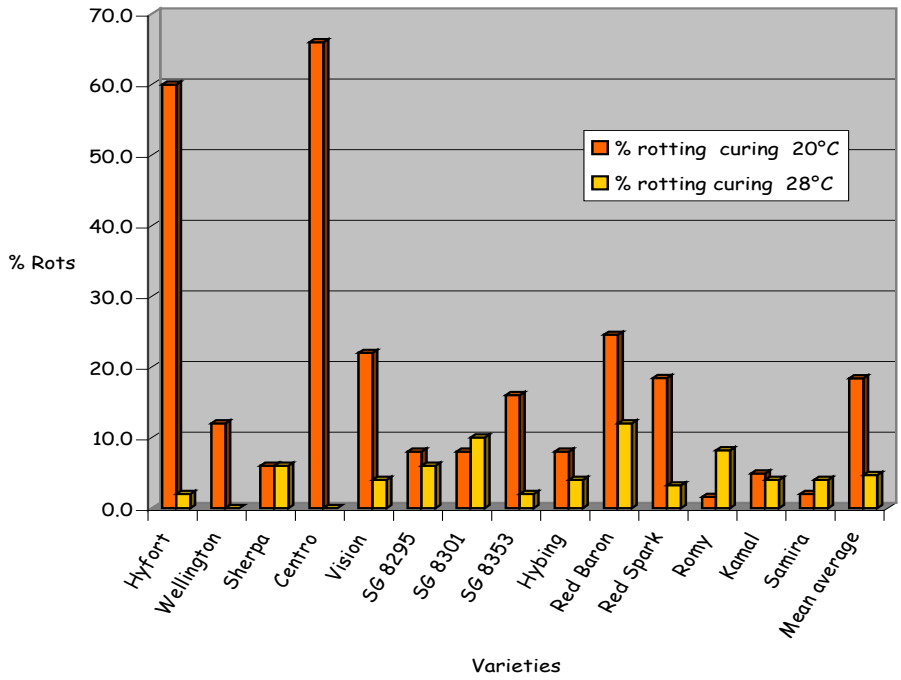


Figure 3. UK-grown variety trial. Percentage rots at 20ºC and 28ºC assessed on 13th May 2008.

3. Main conclusions from biochemical work and how they impact the growers

- Mean abscisic acid (ABA) concentration was significantly affected by treatment. It declined during curing, with the greatest decrease occurring at 28ºC. The onset of sprouting occurred between outturn 3 and 4 (150-200 days), shortly after the increase in ABA concentration.

Markers (such as ABA) that could be used to determine between dormancy and sprout suppression could potentially be used as a predictor of storage life.

- Samples of onion complementary ribonucleic acid (cRNA) have been successfully hybridised to a prototype onion-specific microarray.

The use of a microarray could identify new molecular makers for dormancy and sprout suppression. It will also provide an insight into the physiological mechanisms controlling these processes, which in turn could suggest new targets for manipulation to extend storage life.

- Short treatments of ethylene or 1-Methylcyclopropene (1-MCP) reduced sprout growth of onions cv. Sherpa with no indication of any deleterious effects on bulb quality.

It is possible that ethylene treatment could be used in a different way, such as short treatment periods, which could be potentially as effective as continuous treatment, but with reduced associated costs.

Financial benefits

- Annual value in area of impact: 400,000 tonnes annual production with a farm gate value £130 per tonne = £52 m p.a.
- Expected annual added value: Present drying / curing costs are £9.84 per tonne plus longer term refrigeration at £11.00 per tonne (energy cost only).
- 50% saving anticipated = £10.42 per tonne, with a total crop = £4.17 m gross margin improvement per annum.
- Approximately 1 month additional supply 40,000 tonnes of UK onions = £5.2m addition farm gate sales per annum.
- Substitution of MH-treated imports 20% (90,000 tonnes imported from Holland in 2006). Hence 18,000 tonnes = £2.3 m additional farm gate sales per annum.

Action points for growers

- There are no recommended changes to current grower practice at this stage.

Science Section

Aims of Project

- A more competitive and expanded UK onion industry based on energy efficient and residue-free long-term storage.

- Uptake of new guidelines ensured through HDC publications/events, BOPA Conference and on-farm workshops with established consultants within a well co-ordinated industry.

Parts 1, 2 and 3.

Part 1: Gemma Chope, Leon Terry – CU

Objective 1. Develop a chemometric model based on temporal and spatial profiles of biochemical markers linked to onion bulb dormancy induction and sprout suppression. (CU). [Years 1-2; Tasks T1.1 and T1.2]
Objective 4. Prolong storage and shelf life whilst reducing energy inputs by optimisation of postharvest treatments as monitored by biochemical and gene expression markers (All partners) [Year 1-3 Milestones T4.1, T4.2 and T4.3].

Part 2: Gemma Chope – CU, Andrew Thompson, John Hammond – WHRI

Objective 2. Produce the first microarray for onion to identify genes that are up- or down-regulated during dormancy and sprout suppression. (WHRI). [Years 1-2; Tasks T2.1, T2.2 and T2.3)

Part 3: Katherine Downes, Leon Terry – CU

Objective 3. Elucidate mechanisms related to dormancy and sprout suppression, as influenced by applied treatments, for maximum storage and shelf life (ABC, CU) [Years 1-3; Tasks T3.1, T3.2 and T3.3]

Part 1 - Gemma Chope, Leon Terry – CU

Executive Summary

Onion cvs. Red Baron, Wellington and Shepra were grown at two sites (Elveden and Findlays), and cured at three different temperatures; 20, 24 or 28°C for six weeks. Bulb samples were taken immediately before curing, after curing, and at four times during cold storage (seven months duration at 1 ± 0.5°C). Skin samples were taken after curing and after seven months cold storage. Bulb samples were analysed for disease incidence, dry weight, sprout growth, pyruvate, non-structural carbohydrates (fructose, sucrose and glucose) and phytohormones (ABA and cytokinins). Skin samples were analysed for colour, non-structural carbohydrates (fructose, sucrose and glucose), flavonols and anthocyanins.

- Curing at a lower temperature did not affect disease incidence.
- The proportion dry weight was not affected by treatment and so this indicates that curing at a lower temperature does not affect this parameter.
- Curing at a lower temperature did not affect pungency of onion bulbs [N.B. pyruvate levels were usually low].
- A method has been developed to extract and quantify ABA and cytokinins in onion samples using LC-MS/MS.
- Mean ABA concentration was significantly higher in cvs. Red Baron and Sherpa than in cv. Wellington.
- Mean ABA concentration was significantly (*P*>0.001) affected by treatment; 20ºC (106.7) > [24ºC (79.6) and 28ºC (62.0)].
- ABA declined during curing from 190.6 ng g-1 DW, with the greatest decrease occurring at 28ºC.
- Onset of sprouting occurred between outturn 3 and 4, shortly after the increase in ABA concentration.

- Wellington had the lowest ABA concentration – perhaps implying that it is more sensitive to ABA than the other two cultivars.
- Curing at lower temperatures had only marginal effects on the visual appearance of onion bulbs.
- Degradation of anthocyanins play a major role in determining changes in skin colour of onion cv. Red Baron during curing at high temperature
- Total flavonol concentration was positively correlated with total anthocyanin concentration and was negatively correlated with hue angle (H°, objective colour-redness) in the skin of onion cv. Red Baron.
- No significant correlations between H° and any biochemical parameters measured including total flavonols in the skin of brown onion cvs. Sherpa or Wellington.

1.1 Introduction

Long-term storability of onions is a result of first, dormancy induction and then sprout suppression (for a detailed literature review see HL0182 Annual Report 2008), and little attention has been given to understanding the underlying physiological and genetic mechanisms. The UK has led the world in many aspects of bulb onion drying and storage technology, which was based on 1970's research at Kirton EHS and MAFF Development Farm Project. In light of rising energy costs (the cost of commercial electricity increased by *ca*. 50%, and gas by *ca*. 65% between 2005 and 2006; Department of Trade and Industry, 2006) any reduction in the amount of gas and electricity used in the curing, drying and storage of onions would be desirable to industry. Current UK practice aims to remove surface moisture within three days of loading the store by heating at 30 ºC, followed by a further ten days at 24ºC (RH not to exceed 75%). The crop is then allowed to cool to approximately 15 ºC, with ventilation, for a few days until the necks are tight and dry. The temperature can then be slowly reduced until the desired long-term storage temperature is reached. The curing and drying procedure is based on a method developed in the 1970s, and therefore the

research that formed the basis of this procedure was carried out on cultivars that are very different from those used today. Thus, it is likely that alterations to current methods, such as a reduction in the temperature and duration of the curing and drying periods could deliver benefits in the form of energy savings and reduced carbon emissions, while still producing onion bulbs of a satisfactory quality standard.

Since that time, onion growers in the UK have invested heavily in technologically advanced refrigerated and controlled atmosphere (CA) storage systems designed to maintain availability of UK onions for prolonged periods. The current annual production of around 400,000 tonnes (value £52m) meets around two thirds of the UK annual requirement for onions. The use of refrigerated and CA storage systems is effective but is also energy intensive, depends upon the use of the sprout suppressant, MH. In the current climate of ever-increasing energy costs (up by 50% in 3 years), the viability of these energy intensive methods of UK onion production and storage are being threatened. There are also increasing pressures to from both consumers and retailers to eliminate residues in food, which makes the future use of MH as a sprout suppressant uncertain. The UK system of topping onions in the field, lifting into store and then drying at 28^{o}C may not be so well suited to earlier maturing modern cultivars and may be adversely affecting storage life.

Recent work (Chope *et al*., 2006; 2007a) has shown for the first time that a decline in endogenous abscisic acid (ABA) was correlated with storage life under both controlled atmosphere conditions and regular atmosphere conditions. It was also shown that the concentration of ABA in freshly harvested bulbs was approximately double that measured before versus after curing (Chope *et al*., 2007c). Extended curing times may also reduce ABA concentration. Yamazaki *et al*. (1999a; b) also demonstrated a functional role of ABA in maintaining bulb dormancy in *A. wakegi*. Thus, maintaining ABA concentration could extend dormancy and storage life. Studying the concentrations of ABA and other phytohormones, along with non-structural carbohydrates and other parameters such as dry weight and pungency in stored onions cured at different

temperatures will determine the effect of curing at different temperatures and build a clearer picture of the changes occurring in the bulb at the time of transition from dormancy/sprout suppression to sprout growth. The samples and data generated will be used in the microarray experiment (Part 2).

Onions are cured in order to form a complete, dry, outer skin which reduces water loss and suppresses incidence of disease, and can promote a darker skin finish. There is a dearth of empirical data on the effects of curing temperature on flavonol concentration in the skin of brown onions and on flavonol and anthocyanin concentration in the skin of red onions. Therefore, part of this study aimed to elucidate the compounds responsible for the change in onion skin colour when cured at different temperatures.

1.2 Materials and Methods

See HL0182 Annual Report 2008 for Materials and Methods. Additional methods are detailed below.

1.2.1 Sample preparation for onion skin analysis

Brown onions cvs. Sherpa and Wellington, and red onions cv. Red Baron were cured at 20, 24 or 28°C for six weeks. Replicated skin samples were analysed immediately after curing and again after seven months cold storage at 1 ± 0.5°C. Objective colour, fructose, sucrose and glucose concentrations and flavonols and anthocyanins were analysed.

1.2.2 Quantification of flavonols and anthocyanins in onion skin

Flavonols were extracted according to Giné Bordonaba and Terry (2008) with slight modifications. Anthocyanins were separated and quantified according to Manhita *et al*. (2006) and Giné Bordonaba and Terry (2008) with modifications.

1.2.3 Phytohormones and metabolites quantification by HPLC-MS/MS

The method for the extraction and quantification of phytohormones and their metabolites was adapted from that of Chiwocha *et al*. (2003). A number of extraction solvents were tested, and the following was selected. The following compounds can be detected in onion bulb extracts using this method: abscisic acid ABA), zeatin (Z), zeatin riboside (ZR), isopentenyladenine (2iP) and isopentenyladenosine (IPA) and their respective internal standards (Table 1.1).

Table 1.1 The ionisation mode, characteristic product to precursor ion transition, and retention time for phytohormones and metabolites, along with their respective deuterated internal standards.

Ionisation mode	Compound	Transition	RT (min)	Internal standard	Transition	RT (min)
Positive	Z	220 > 136	9.52	d_3-DHZ	225 > 136	9.17
Positive	ZR	352 > 220	11.30	d_3-DHZR	357 > 225	10.93
Negative	DPA	281 >171	11.89	d_3-DPA	284 > 174	11.84
Negative	IAAsp	289 >132	13.08	d_5-IAA	179 > 135	16.77
Negative	GA_3	345 > 221	13.78	d_2-GA_1	349 > 275	13.86
Negative	GA_1	347 > 273	14.05	d_2-GA_1	349 > 275	13.86
Positive	2iP	204 >136	13.57	d_6-2iP	210 > 137	13.46
Negative	ABA-GE	425 >263	14.29	d_5-ABA-GE	430 > 268	14.13
Negative	PA	279 >139	15.11	d_3-PA	282 > 142	14.96
Positive	IPA	336 >204	15.36	d_6-IPA	342 > 210	15.22
Negative	7`-OH-ABA	279 > 151	15.96	d_4-7`-OH-ABA	283 > 154	-
Negative	IAA	174 > 130	17.03	d_5-IAA	179 > 135	16.77
Negative	ABA	263 >153	18.13	d_4-ABA	267 > 156	17.98
Negative	GA7	329 >223	22.53	d_2-GA_4	333 > 215	22.73
Negative	GA4	331 >213	22.79	d_2-GA_4	333 > 215	22.73

Ground, lyophilized samples (200 mg) were mixed with HPLC-grade water (5 ml) to which 50 ng of each internal standard had been added and incubated at 3°C overnight on a shaker. Each sample was filtered using a 0.2 µM syringe filter. The pellet was re-suspended in 1 ml HPLC-grade water, vortexed to mix and then filtered. The extracts were pooled and loaded under vacuum onto a Waters C18 SPE cartridge equilibrated with 3 ml HPLC-grade water followed by 3 ml methanol. The column was then washed with 1 ml 20:0.1:79.9 (v/v/v) methanol : formic acid : water. The sample was eluted with 2 ml 80:20 (v/v) methanol : water. The samples were then lyophilised and re-suspended in 500 µl 100% methanol.

Samples (10 µl) were injected using a HPLC system consisting of an Alliance 2695 separation module (Waters) equipped with a 100 mm x 2.1 mm, 3.5 µm Eclipse XDB C18 column (Agilent), with a 12.5 mm x 2.1 mm, 5 µm Zorbax XDB-C8 guard column (Agilent). The mobile phase was a ternary system comprising acetonitrile (A), de-ionised water (B), and 5% glacial acetic acid in water (C). The gradient was of increasing acetonitrile content, constant glacial acetic acid concentration of 7 mM (pH 3.4), and a flow rate of 0.200 ml min^{-1}. The MS was a quadrupole tandem mass spectrometer (Micromass) outfitted with an electrospray ion source.

Following the recent decline in the availability of acetonitrile, the method has subsequently been adapted to use methanol in place of acetonitrile in the mobile phase.

1.3 Results

1.3.1 Physical Measurements

1.3.1.1 Dry weight

Onion bulbs cv. Red Baron had a significantly (P>0.001) higher overall mean dry weight than cvs. Wellington and Sherpa. In addition, the dry weight of bulbs grown at Findlay's was consistently higher (P>0.001) than the dry weight of those grown at Elveden. The proportion dry weight of all cultivars decreased during curing at all temperatures (Figure 1.1). There was no significant overall effect of curing temperature on dry weight, and generally little change in dry weight after curing.

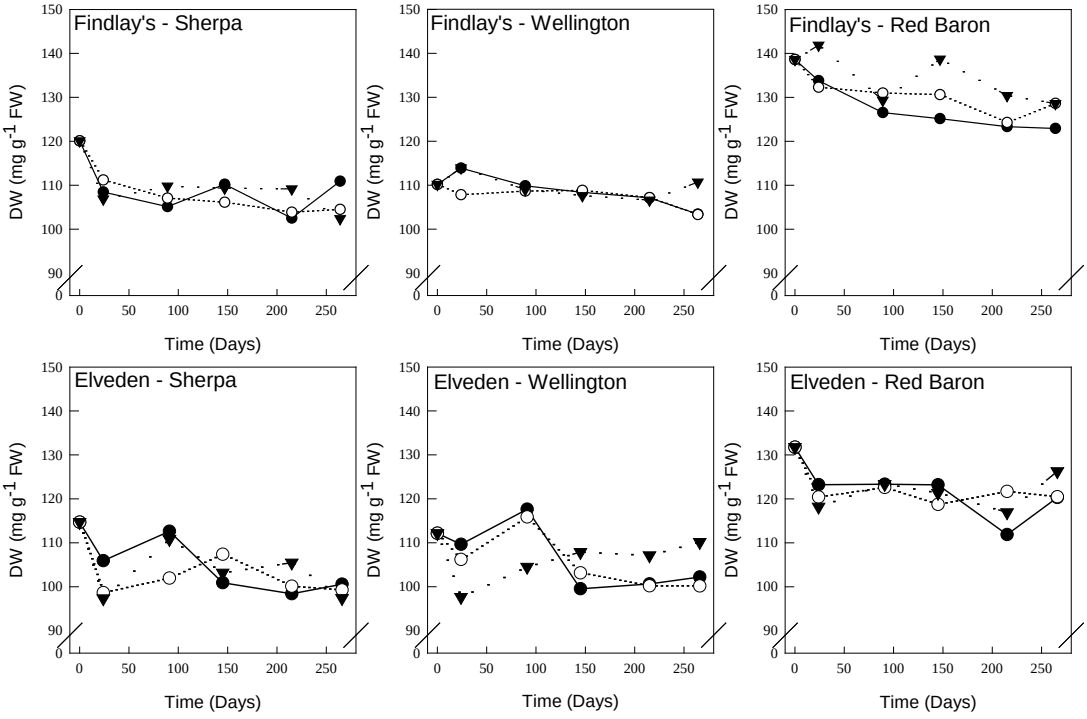


Figure 1.1 Proportion dry weight of onion cvs. Sherpa, Wellington and Red Baron, grown at Findlay's or Elveden and cured at 20ºC (closed triangles), 24ºC (open circles) or 28ºC (closed circles) for 6 weeks and subsequently stored at 0ºC. LSD bars (P<0.05) are shown.

1.3.1.2 Sprout length

Sprout growth began between outturns 4 and 5 (*ca*. 5 - 7 months after harvest) for all cultivars and there was no main effect of curing temperature or

cultivar (Figure 1.2), although sprouting occurred at the same time, sprouts in onions grown at Elveden were longer than in those grown at Findlay's.

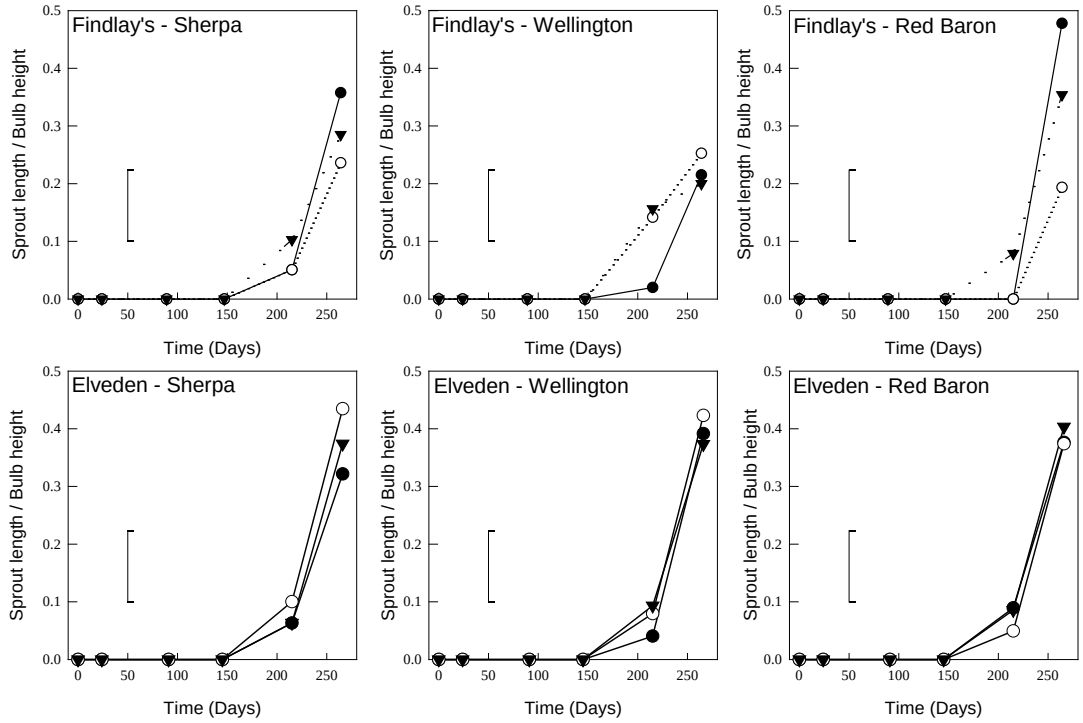


Figure 1.2 The sprout length of onion cvs. Sherpa, Wellington and Red Baron, grown at Elveden or Findlay's and cured at 20ºC (closed triangles), 24ºC (open circles) or 28ºC (closed circles) for 6 weeks and subsequently stored at 0ºC. LSD bars (P<0.05) are shown.

1.3.1.3 Disease incidence

No incidence of neck rot was been recorded.

1.3.2 Biochemical measurements

1.3.2.1 Pungency

For pyruvate concentration, there were significant main effects of cultivar and site (P<0.001), whereby onions cv. Red Baron were more pungent than onions cv. Sherpa and Wellington and onions grown at the Findlay's site contained more pyruvate than those grown at Elveden (Figure 1.3). There was no significant difference in the pyruvate concentration of onions cured at different temperatures; however, in general, a peak in the pyruvate concentration occurred around halfway through the cold storage period. This effect was more apparent in onions grown at the Findlay's site than at Elveden.

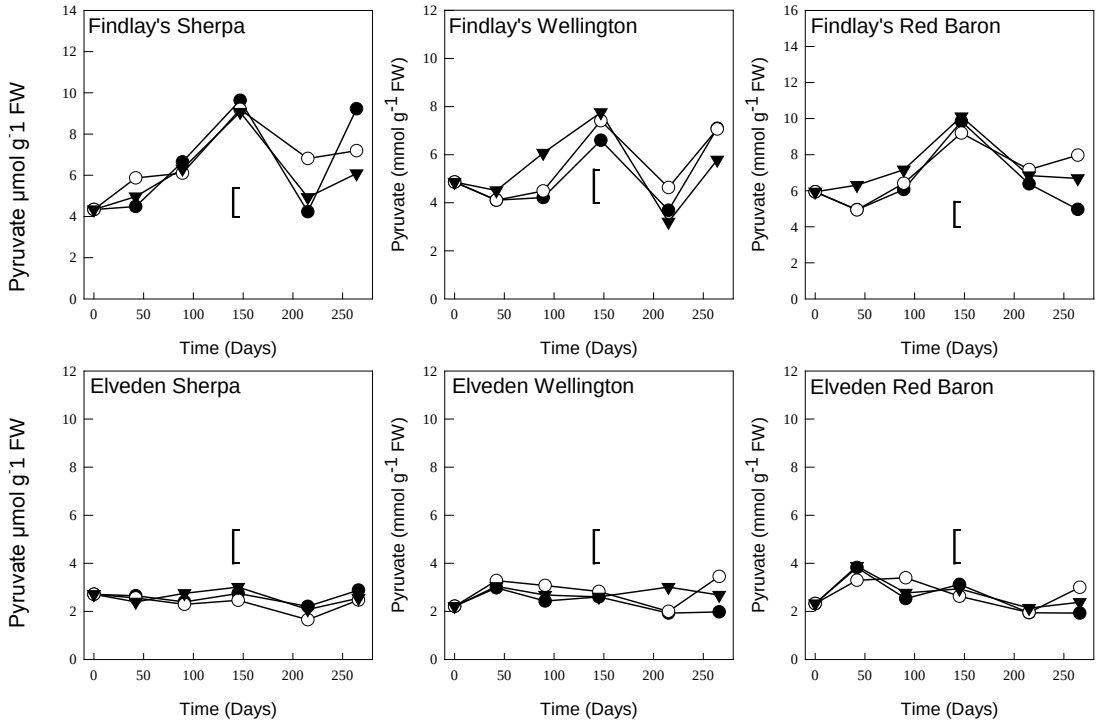


Figure 1.3 Pyruvate concentration of onion cvs. Sherpa, Wellington and Red Baron, grown at Findlay's or Elveden cured at 20ºC (closed triangles), 24ºC (open circles) or 28ºC (closed circles) for 6 weeks and subsequently stored at 0ºC. LSD bars (P<0.05) are shown.

1.3.2.2 Non-structural carbohydrates

There was no consistent significant effect of curing treatment on the concentrations of sucrose, glucose and fructose. The concentrations of fructose (Fig. 1.4) and sucrose (Fig. 1.5) increased during storage until the onset of sprouting after which they gradually declined.

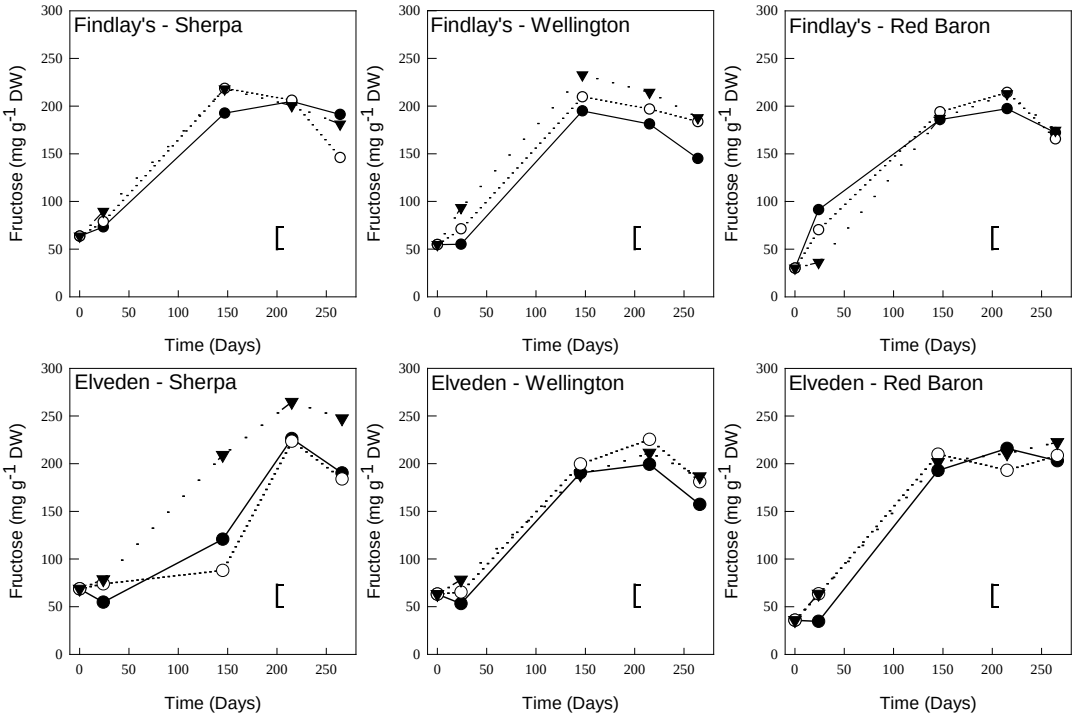


Figure 1.4 Fructose concentration of onion cvs. Sherpa, Wellington and Red Baron, grown at Findlay's or Elveden cured at 20ºC (closed triangles), 24ºC (open circles) or 28ºC (closed circles) for 6 weeks and subsequently stored at 0ºC. LSD bars (P<0.05) are shown.

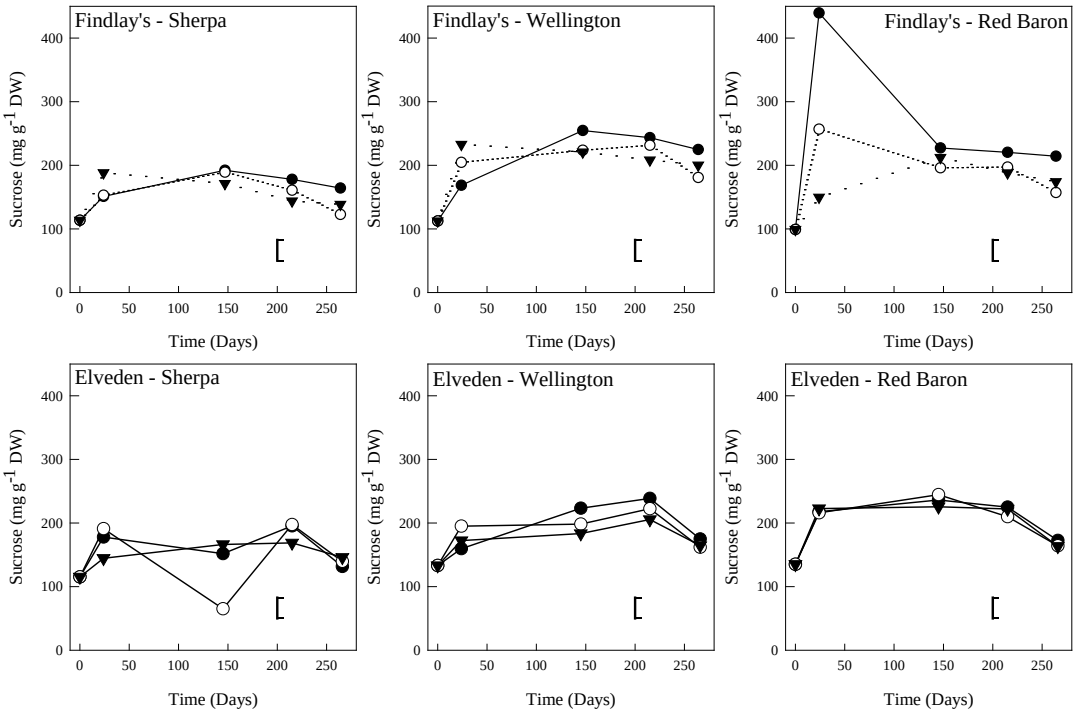


Figure 1.5 Sucrose concentration of onion cvs. Sherpa, Wellington and Red Baron, grown at Findlay's or Elveden cured at 20ºC (closed triangles), 24ºC (open circles) or 28ºC (closed circles) for 6 weeks and subsequently stored at 0ºC. LSD bars (P<0.05) are shown.

The concentration of glucose remained relatively stable during storage and was significantly greater (P>0.001) in onions cv. Red Baron than cvs. Sherpa and Wellington (Fig. 1.6). However, there was a general trend for a decrease in glucose concentrations towards the end of storage after onset of sprouting.

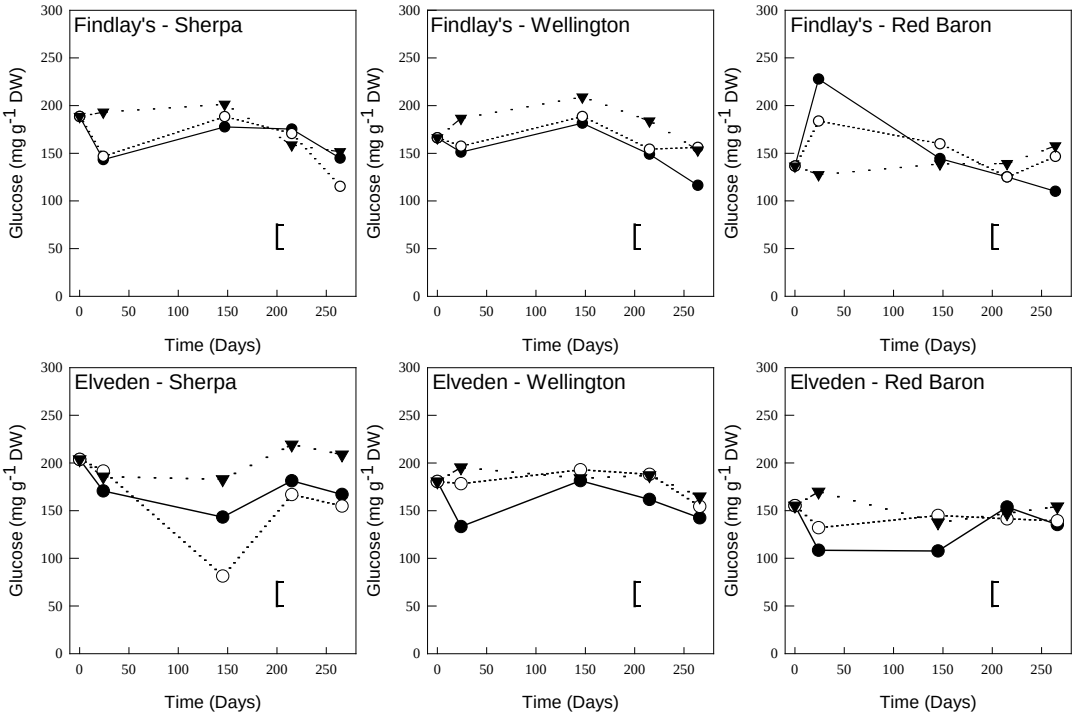


Figure 1.6 Glucose concentration of onion cvs. Sherpa, Wellington and Red Baron, grown at Findlay's or Elveden cured at 20ºC (closed triangles), 24ºC (open circles) or 28ºC (closed circles) for 6 weeks and subsequently stored at 0ºC. LSD bars (P<0.05) are shown.

1.3.2.3 Phytohormones and metabolites

As part of the method development, onion samples were spiked with known amounts of ABA and analysed by LCMS/MS (Figure 1.7). This showed that ABA could be detected against the background of the onion sample, and that the response of the detector increased in proportion to the increase in concentration of the target analyte.

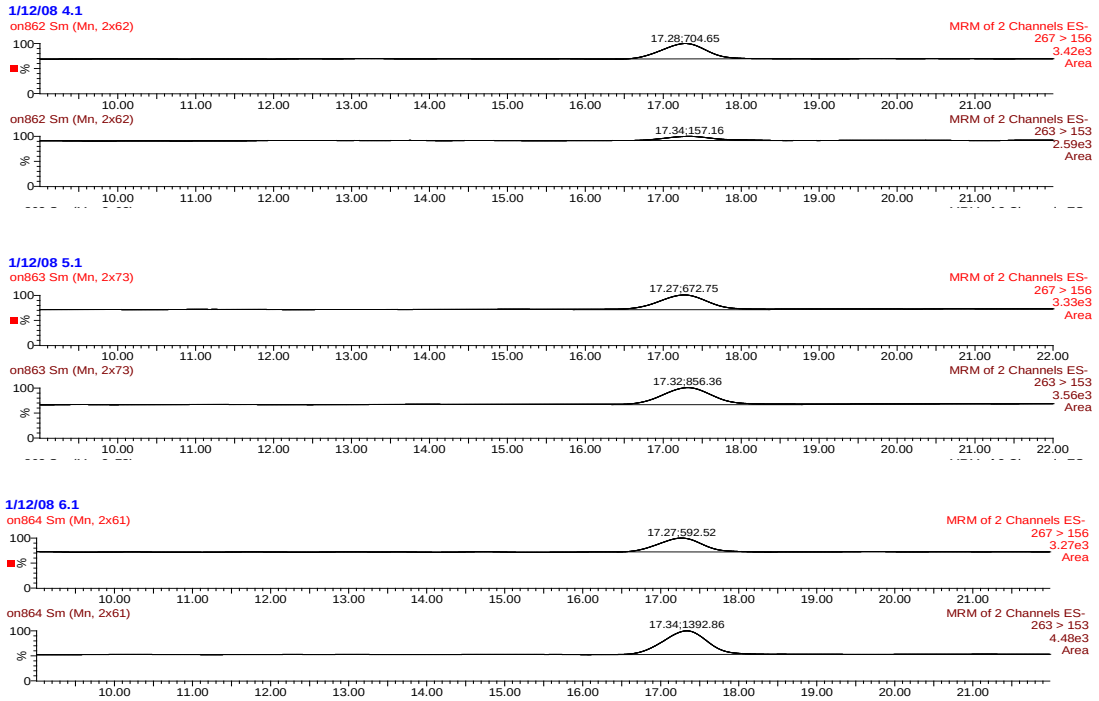


Figure 1.7 Chromatogram showing sample un-spiked (4.1), and spiked with 100 mg (5.1) or 200 mg ABA (6.1). 267>156: d4-ABA (internal standard), 263>153: ABA.

The mean ABA concentration was greater (P>0.001) in onions cv. Red Baron (98.1 ng g^{-1} DW) and Sherpa (106.7) than in cv. Wellington (63.7). There was a significant main effect of curing temperature, whereby onions cured at 20ºC had the greatest concentration of ABA, and those cured at 24 or 28ºC had lower concentrations (Fig. 1.8). There was a decline in ABA concentration during curing, with the greatest decrease occurring in those onions cured at 28ºC. There was a trend for a decline in ABA concentration during cold storage, followed by an increase, which was preceded by the onset of sprout growth.

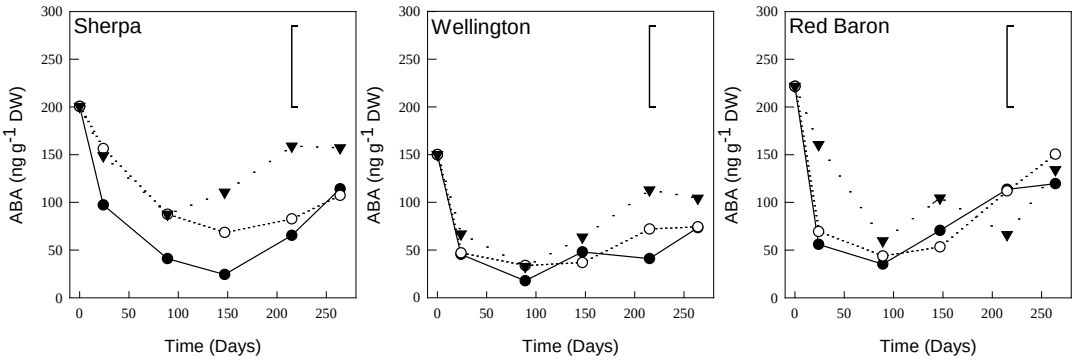


Figure 1.8 The ABA concentration of onion cvs. Sherpa, Wellington and Red Baron, grown at Elveden and cured at 20ºC (closed triangles), 24ºC (open circles) or 28ºC (closed circles) for 6 weeks and subsequently stored at 0ºC. LSD bars (P<0.05) are shown.

The concentration of the cytokinin zeatin (Fig. 1.9), and its conjugate zeatin riboside (Fig. 1.10) both tended to increase during cold storage. There was no significant difference between curing temperatures, but there was a decrease in zeatin and zeatin riboside concentration during curing, after which it increased. There was a main effect of cultivar on zeatin concentration, whereby onions cv. Red Baron contained the highest amount; however, this is likely to be due to the marked increase in zeatin concentration at the end of storage in this cultivar. There was a peak in zeatin riboside concentration at outturns 3, 4 and 5 for cvs. Wellington, Sherpa and Red Baron respectively.

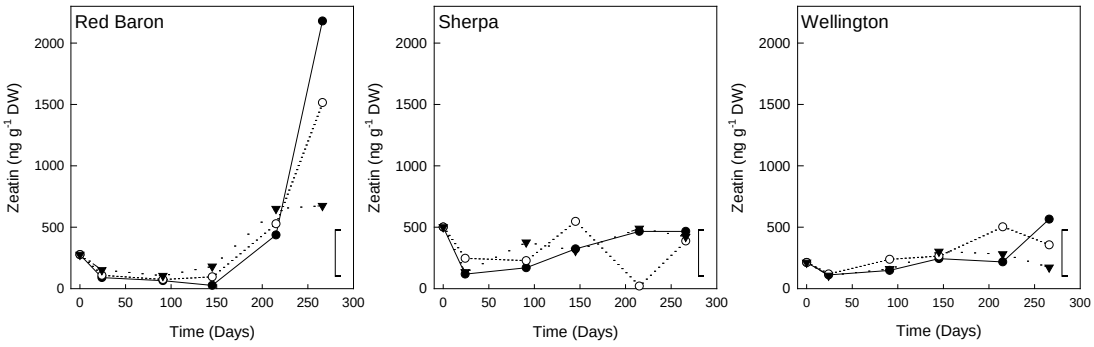


Figure 1.9 The zeatin concentration of onion cvs. Sherpa, Wellington and Red Baron, grown at Elveden and cured at 20ºC (closed triangles), 24ºC (open circles) or 28ºC (closed circles) for 6 weeks and subsequently stored at 0ºC. LSD bars (P<0.05) are shown.

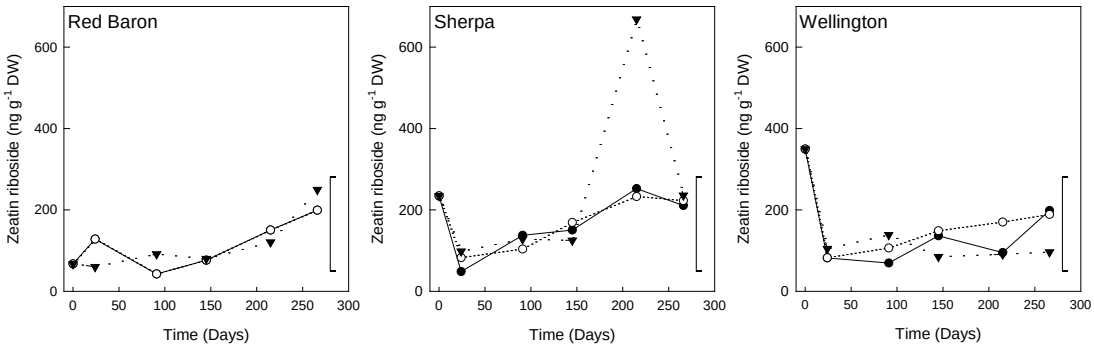


Figure 1.10 The zeatin riboside concentration of onion cvs. Sherpa, Wellington and Red Baron, grown at Elveden and cured at 20ºC (closed triangles), 24ºC (open circles) or 28ºC (closed circles) for 6 weeks and subsequently stored at 0ºC. LSD bars (P<0.05) are shown.

1.3.2.4 Preliminary chemometric analysis

Preliminary chemometric analysis was carried out on the data set for year 1 using two different techniques – Principal Component Analysis (PCA; Figure 1.11) and Canonical Variate Analysis (CVA; Figure 1.12). PCA is an unsupervised technique, whereas CVA is a technique which bases the analysis on grouping the data so that the differences within groups are minimised, and the differences between groups are maximised (Chope and Terry, 2009). The data tended to group into two sets – one containing fresh and cured onions, and the other containing stored onions, indicating a possible differentiation between dormancy and sprout suppression.

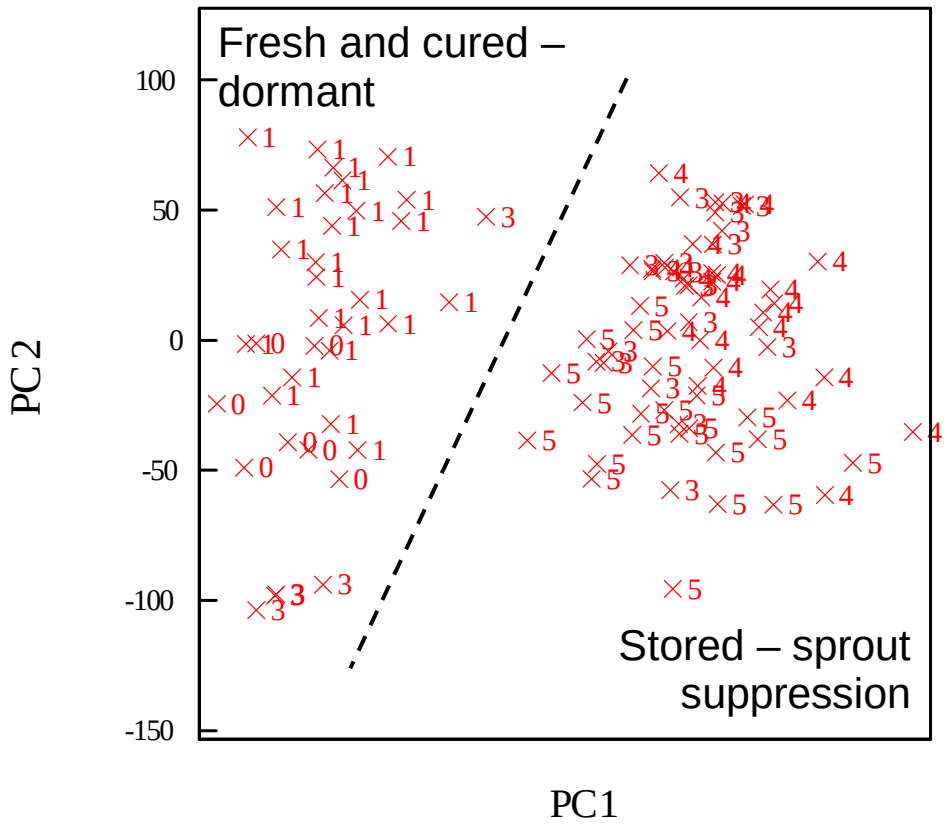


Figure 1.11 Principal component analysis of onion cvs. Red Baron, Sherpa and Wellington including the variates NSC, DW, pungency, ABA and labelled with outturn.

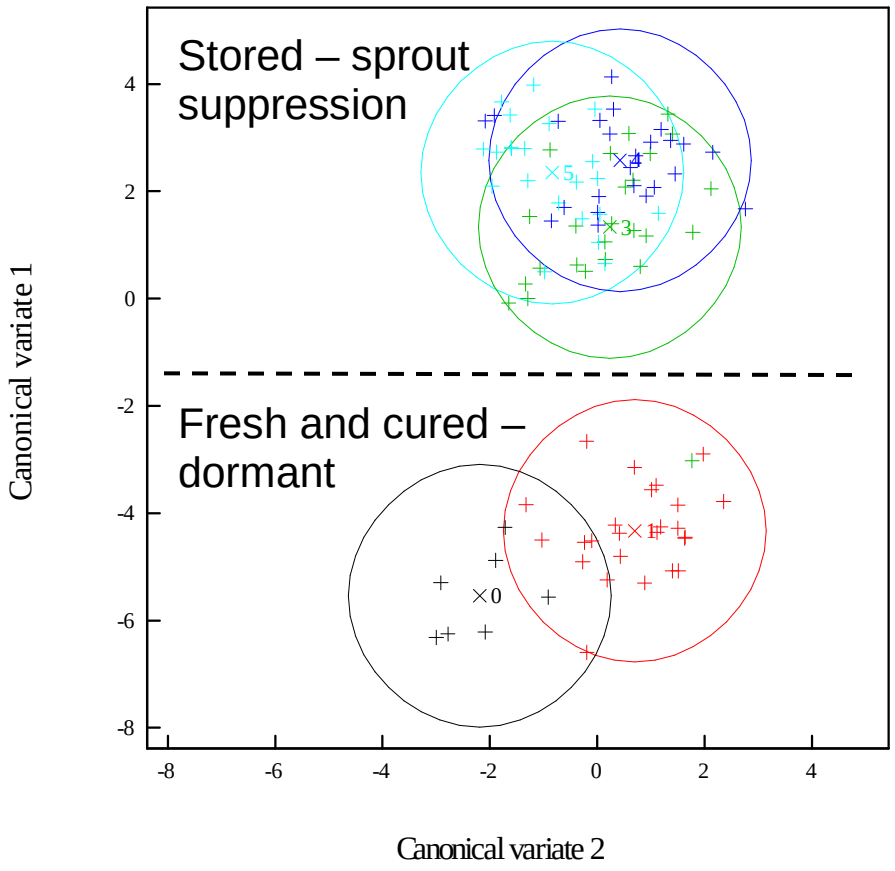


Figure 1.12 Canonical variate analysis of onion cvs. Red Baron, Sherpa and Wellington including the variates NSC, DW, pungency, ABA and grouped by outturn.

1.3.3 Onion skin analysis (Kate Downes)

1.3.3.1 Skin colour

During cold storage, the only mean change in the chroma was observed in the skin of onions cv. Red Baron which became significantly more vivid over time (21.09 – 25.27). Onion cv. Red Baron bulbs had significantly lower H° and darker skin compared to cvs. Wellington and Sherpa due to obvious colour differences between red and brown onions. The skin of onion cvs. Sherpa and Wellington cured at 28°C were darker and had a lower H° as compared to those

cured at 20°C although this was only significant immediately after curing (Table 1.2). In contrast, the skin of onions cv. Red Baron had a higher H° (i.e. less red) but no change in lightness when cured at 28°C, and again this was only significant when measured immediately after curing, and not after long-term cold storage. Mean skin thickness of onion cvs. Red Baron, Sherpa and Wellington were significantly different at 0.116, 0.078 and 0.133 mm, respectively. However, skin thickness was not correlated with colour. There were no significant differences in skin thickness between onions cured at different temperatures (Table 1.3).

Table 1.2 Chroma (C), Hue (H°) and Lightness (L*) of skins of onion cvs. Red Baron, Sherpa and Wellington cured for 6 weeks at 20, 24 and 28°C before being transferred to cold storage (1 ± 0.5°C) for seven months (*n* = 15).

		Chroma (C)			Hue angle (H°)			Lightness (L*)		
	Curing Temp (°C)	Red Baron	Sherpa	Wellington	Red Baron	Sherpa	Wellington	Red Baron	Sherpa	Wellington
After curing	20	20.93^{a}	30.74^{c}	31.22^{c}	13.15^{a}	58.86^{f}	58.46^{ef}	31.26^{a}	58.88^{ef}	57.70^{def}
	24	21.27^{a}	31.08^{c}	31.07^{c}	16.35^{ab}	54.05^{cde}	55.42^{cdef}	33.84^{a}	57.75^{def}	52.50^{b}
	28	21.07^{a}	30.70^{c}	31.34^{c}	18.35^{b}	53.41^{cd}	52.29^{c}	33.68^{a}	55.24^{bcd}	53.60^{bc}
After cold storage	20	25.70^{b}	31.07^{c}	32.14^{c}	17.79^{ab}	57.75^{def}	56.93^{cdef}	34.56^{a}	59.82^{f}	57.03^{cdef}
	24	23.93^{b}	32.24^{c}	32.35^{c}	15.04^{ab}	56.62^{cdef}	55.47^{cdef}	31.47^{a}	56.87^{cdef}	55.20^{bcd}
	28	26.19^{b}	32.90^{c}	32.72^{c}	17.58^{ab}	53.97^{cde}	53.66^{cd}	33.38^{a}	55.41^{bcde}	54.04^{bc}

LSD bars (*P* = 0.05); C = 2.50, H° = 4.72, L* = 3.52. Values followed by the same letter are not significantly different from each other calculated using the LSD.

Table 1.3 Skin thickness (mm) of onion cvs. Red Baron, Sherpa and Wellington immediately after being cured for 6 weeks at 20, 24 and 28°C (*n* = 15).

Curing temperature (°C)	Red Baron	Sherpa	Wellington
20	0.123^{cd}	0.070^{a}	0.132^{cd}
24	0.107^{bc}	0.075^{a}	0.126^{cd}
28	0.118^{cd}	0.088^{ab}	0.140^{d}

LSD (*P* = 0.05); 0.029. Values followed by the same letter are not significantly different from each other calculated using the LSD.

1.3.3.2 Non-structural carbohydrates

Fructose concentration was significantly higher in the skin of onions cv. Red Baron (ranging from 0.57 – 4.07 mg g^{-1} FW) compared to onion cvs. Wellington and Sherpa (ranging from 0.20 – 2.17 mg g^{-1} FW, respectively) whereas glucose concentrations were highest in the skin of cv. Sherpa (up to 31.06 mg g^{-1} FW). The concentration of fructose in onions cv. Red Baron cured at 28°C was significantly higher than in those cured at 20 and 24°C immediately after curing but then the levels of fructose in those cured at 28°C decreased during cold storage to similar concentrations found in onions cured 20 and 24°C. In contrast, skin fructose concentration of onions cv. Red Baron cured at 20°C was significantly lower than onions cured at 28°C immediately after curing but subsequently increased during cold storage to higher concentrations than those cured at 24°C. Glucose concentrations in the skin of onions cv. Wellington were significantly lower when cured at 28°C compared to 20°C immediately after curing although after cold storage these differences were no longer significant. Immediately after curing, no significant differences were found between fructose concentrations of onions cv. Sherpa, however after seven months cold storage, lower glucose concentrations were found in the skins of those cured at 24 rather than 20 or 28°C (Table 1.4). The sucrose concentration in onion bulb skins of all

three cvs. was less than 1 mg g^{-1} FW immediately after curing and was not significantly different between cultivars or treatments (data not shown). Sucrose was undetectable in skins after seven months in cold storage.

Glucose and fructose concentrations in the skin of onion cvs. Sherpa and Wellington were positively correlated (r = 0.80) with one another but the same relationship was not observed for cv. Red Baron. No significant correlations were found between colour data and sugars.

1.3.3.3 Flavonols

Mean quercetin concentration in onion cv. Sherpa skin was significantly higher than in cvs. Wellington or Red Baron. In contrast, quercetin 4-glucoside and quercetin 3,4-diglucoside concentrations were higher in onion cv. Red Baron skin compared to the brown cultivars. There was no main effect of treatment or time on quercetin or quercetin 4-glucoside concentrations. Concentrations of quercetin 3-glucoside were below detectable levels in onion skin. Quercetin glucoside concentrations in the skins of cv. Red Baron cured at 20°C decreased during cold storage. However, quercetin glucoside concentrations in onions cured at 24°C increased whereas those cured at 28°C remained steady (Table 1.5). The H° was negatively but weakly correlated with total flavonols (r = -0.64) in onions cv. Red Baron, but no significant correlations were found between colour data and flavonols in the skins of onion cvs. Sherpa and Wellington.

Table 1.4 Fructose and glucose concentrations (mg g^{-1} FW) in skins of onion cvs. Red Baron, Sherpa and Wellington cured for 6 weeks at 20, 24 and 28°C before being transferred to cold storage (1 ± 0.5°C) for seven months (*n* = 15).

		Fructose (mg g^{-1} FW)			Glucose (mg g^{-1} FW)		
	Curing Temp (°C)	Red Baron	Sherpa	Wellington	Red Baron	Sherpa	Wellington
After curing	20	0.89^{ab}	1.00^{ab}	1.22^{ab}	13.56^{cde}	23.53^{ef}	21.31^{def}
	24	1.83^{abc}	0.80^{ab}	0.30^{a}	15.48^{cde}	24.52^{ef}	10.75^{abcd}
	28	4.07^{d}	2.17^{bc}	0.30^{a}	24.26^{ef}	31.06^{f}	7.17^{ab}
After cold	20	3.21^{cd}	1.20^{ab}	0.84^{ab}	15.24^{cde}	17.91^{cde}	13.93^{cde}
storage	24	0.57^{ab}	0.20^{a}	0.53^{ab}	13.88^{cde}	6.29^{a}	9.44^{c}
	28	1.36^{abc}	0.54^{ab}	1.45^{abc}	16.68^{cde}	11.56^{cd}	18.28^{cde}

LSD (*P* = 0.05); fructose = 1.85, glucose = 11.0. Values followed by the same letter are not significantly different from each other calculated using the LSD.

Table 1.5 Quercetin, quercetin 4-glucoside and quercetin 3,4-diglucoside concentrations (mg g^{-1} FW) in onion skins of cvs. Red Baron, Sherpa and Wellington cured for 6 weeks at 20, 24 and 28°C before being transferred to cold storage (1 ± 0.5°C) for seven months (*n* = 15).

		Quercetin (mg g^{-1} FW)			Quercetin 4-diglucoside (mg g^{-1} FW)			Quercetin 3,4-diglucoside (mg g^{-1} FW)		
	Curing Temp (°C)	Red Baron	Sherpa	Wellington	Red Baron	Sherpa	Wellington	Red Baron	Sherpa	Wellington
After curing	20	6.76^{abc}	9.25^{abc}	6.38^{ab}	8.92^{b}	3.16^{a}	3.00^{a}	1.16^{cd}	0.30^{a}	0.19^{a}
	24	5.50^{ab}	10.15^{bc}	6.58^{ab}	6.59^{ab}	3.90^{ab}	2.02^{a}	0.47^{a}	0.54^{abc}	0.09^{a}
	28	6.26^{ab}	11.46^{c}	7.08^{abc}	3.01^{a}	2.42^{a}	1.58^{a}	0.53^{ab}	0.21^{a}	0.17^{a}
After cold storage	20	6.23^{ab}	9.55^{bc}	4.43^{a}	2.66^{a}	2.67^{a}	1.38^{a}	0.35^{a}	0.17^{a}	0.16^{a}
	24	7.30^{abc}	8.40^{abc}	8.70^{abc}	8.48^{b}	2.62^{a}	6.06^{ab}	1.20^{d}	0.18^{a}	0.40^{a}
	28	6.27^{ab}	7.85^{abc}	6.32^{ab}	5.63^{ab}	1.88^{a}	1.38^{a}	1.10^{bcd}	0.14^{a}	0.12^{a}

LSD (*P* = 0.05); quercetin = 4.85, quercetin 4-glucoside = 5.28, quercetin 3,4-diglucoside = 0.62. Values followed by the same letter are not significantly different from each other calculated using the LSD.

1.3.3.4 Anthocyanins

Onions cured at 28°C contained lower concentrations of all individual anthocyanins except cyanidin 3-laminariboside and cyanidin 3-(malonoyl)(acetoyl)glucoside compared to those cured at 20°C (Table 1.6). There was a significant interaction between storage time and temperature for peonidin 3-(malonoyl) glucoside as the concentration in skins cured at 28, 24 and 20°C decreased, remained steady and increased, respectively after seven months cold storage. Individual anthocyanins in the skins of onions cv. Red Baron were positively correlated with each other (data not shown), and a strong significant positive correlation was found between the two most abundant anthocyanins; cyanidin 3-(6''-malonylglucoside) and cyanidin 3-glucoside (r = 0.86). The H° was negatively correlated with total anthocyanins (r = -0.70) in onions cv. Red Baron.

Table 1.6 Anthocyanin concentrations (mg cyanidin 3-glucoside equivalents (C3GE) g^{-1} FW) assigned identity according to Wu and Prior (2005) in onion skins of cv. Red Baron cured for 6 weeks at 20, 24 and 28°C before being transferred to cold storage (1 ± 0.5°C) for seven months (*n* = 15).

Anthocyanin (mg C3GE g^{-1} FW)	Curing temperature (°C)					
	After curing			After cold storage		
	20	24	28	20	24	28
Cyanidin 3-(3''acetoyl)glucoside[1]	0.090^{c}	0.071^{bc}	0.029^{a}	0.052^{ab}	0.077^{bc}	0.033^{a}
Cyanidin 3-(3''malonoylglucoside)[2]	0.057^{c}	0.041^{b}	0.018^{a}	0.039^{b}	0.046^{bc}	0.024^{a}
Cyanidin 3-(6''malonoyl-laminariboside)[3]	0.242^{d}	0.197^{cd}	0.086^{a}	0.160^{bc}	0.198^{cd}	0.101^{ab}
Cyanidin 3-(6''malonylglucoside)[4]	1.650^{c}	1.070^{b}	0.450^{a}	0.910^{ab}	1.310^{bc}	0.590^{a}
Cyanidin 3-glucoside[5]	0.469^{b}	0.475^{b}	0.206^{a}	0.371^{ab}	0.627^{b}	0.293^{ab}
Cyanidin 3-laminariboside[6]	0.058^{abc}	0.080^{c}	0.042^{a}	0.072^{bc}	0.086^{c}	0.050^{ab}
Cyanidin 3-(malonoyl)(acetoyl)glucoside[7]	0.016^{ab}	0.023^{b}	0.009^{a}	0.023^{b}	0.017^{ab}	0.012^{a}
Cyanidin 3-(malonoyl)-glucoside-5-glucoside[8]	0.093^{bc}	0.089^{abc}	0.031^{a}	0.081^{abc}	0.131^{c}	0.045^{ab}
Peonidin 3-glucoside[9]	0.021^{b}	0.022^{bc}	0.009^{a}	0.016^{ab}	0.030^{c}	0.017^{ab}
Peonidin 3-(malonoyl)glucoside[10]	0.057^{d}	0.041^{c}	0.019^{a}	0.037^{bc}	0.050^{d}	0.031^{b}

LSD (*P* = 0.05); 1 = 0.028, 2 = 0.013, 3 = 0.066, 4 = 0.473, 5 = 0.216, 6 = 0.029, 7 = 0.008, 8 = 0.058, 9 = 0.008, 10 = 0.007. Values followed by the same letter are not significantly different from each other calculated using the LSD.

1.4. Discussion

It was hypothesised that the initial ABA concentration before curing and storage was positively correlated with storage life (Chope *et al*., 2006; 2007b; 2007c); however, this was not shown by the year 1 results, as onions cv. Wellington had the lowest initial concentration of ABA, but are reputed to be a very long-storing cultivar. However, in experiments presented herein there was no significant difference between cultivars, and onset of sprouting occurred at the same time for all cultivars. It was also proposed that curing at a high temperature reduced the concentration of ABA as suggested by Chope *et al*., (2007a), similarly, results presented here support this statement as ABA concentration was less in onions cured at higher temperatures (24 or 28°C) than lower temperature (20°C). Finally, it was hypothesised that the decrease in ABA concentration during high temperature curing would negatively affect onion storage life; however this was not shown by the year 1 results, as onset of sprouting was not significantly different for cultivar, site or curing treatment. This difference may become apparent in the results from the year 2 experiments where a range of storage temperatures are used which should highlight the differences in sprouting behaviour.

During over winter storage in the UK a gradual change in the relative composition of plant growth regulators occurs as the levels of growth inhibitors (i.e. ABA) drop and the levels of growth promoters (such as cytokinins) rise. Hormone activity in onions cvs. Rijnsberger (long-storing) and Lancastrian (short-storing) bulbs was measured by Thomas (1969) and Thomas and Isenberg (1972), who reported the following pattern; gibberellins had a first peak in December, followed by peaks of cytokinins and auxins. High auxin activity persisted as sprouting continued. A second gibberellin peak was accompanied by sprouting in March and was more likely to be an effect of sprouting rather than a cause, as gibberellin activity was low in both non-sprouted and internally sprouted bulbs (Thomas, 1969; Yamazaki *et al*., 2002) and application of exogenous gibberellins and auxin failed to stimulate sprouting (Thomas, 1969). There is little current information on the content of endogenous cytokinins in onion bulbs. The cytokinin zeatin, and its conjugate, zeatin riboside, have been shown to decrease in concentration

during curing, and then subsequently increase during cold storage. Evidence for the growth promoting effect of cytokinins has been suggested by Miedema (1994) who found that bulbs with roots sprouted earlier in dry storage than those whose roots have been removed and cultivar differences in time to sprouting in store were more pronounced in de-rooted bulbs than in rooted bulbs. Therefore, the root system may provide substances that promote sprout growth or elongation. Cytokinins produced in the roots stimulate cell division in the sprout meristem or increase the sink activity of the sprout.

For the year 1 experiments, the proportion dry weight of freshly harvested onion bulbs was less than that of cured bulbs, this finding is supported by Hansen (1999) on onions cv. Hyduro and Hyton cured at 24 ºC for 5 weeks. This is perhaps counter-intuitive as moisture is lost during the curing process. However, the moisture loss is greater from the thin, outer skins than from the inner scales. Samples of bulb tissue were taken from the bulb with the outer scales removed; therefore the loss of moisture in this part of the bulb would not be reflected in the dry weight measurements taken. The reduction in proportion dry weight may therefore be due to increased respiration rate caused by the high temperatures during curing (Hurst *et al*., 1985). The proportion dry weight was not affected by treatment and so this indicates that curing at a lower temperature does not affect this parameter. The effect of curing on sugars was a trend for a decrease in glucose concentration and an increase in fructose and sucrose concentrations. The increase in fructose and sucrose concentrations is likely to be due to a breakdown in fructans.

The effect of curing temperature on pungency of onion bulbs was not known, but we have shown in the year 1 data that the pyruvate concentration of onions was not affected by curing temperature, and in fact, the differences between the sites overshadowed even the differences between cultivars. The lack of useful information gained from measuring pungency lead to the decision not to measure pyruvate concentration for all outturns in the year 2 experiments. Differences between the sites in terms of pungency, dry matter and NSC concentrations are likely to be due to the differences between the growing conditions at the two sites, including factors such as soil type (including sulphur content) and the prevailing weather.

Currently in the UK, standard postharvest curing practise of bulb onions involves heating at 28°C for six weeks. In the present study, onions were cured at different temperatures, which resulted in a range of brown and red onion skin colours to compare with skin biochemical composition. This study has demonstrated a link between the skin colour of onion cv. Red Baron and total anthocyanin and total flavonol concentrations using different curing temperatures. However, no significant correlation was observed between colour and biochemical data in the skin of brown onion cvs. Sherpa and Wellington.

Phenylpropanoids are derived from cinnamic acid, synthesised from phenylalanine via phenylalanine ammonia lyase (PAL). Flavonols and anthocyanidins are both synthesised further down the phenylpropanoid pathway via chalcone synthase (CHS) and can then be glycosylated into flavonol glucosides and anthocyanins. In onion flesh, PAL activity is affected by temperature as Benkeblia (2000) found reduced PAL activity when stored at 4°C compared to 20°C. In the skin of cv. Red Baron there were no overall significant differences in anthocyanin concentrations between the two time points; after curing (20, 24 or 28°C) and seven months after cold storage (1°C). It therefore appears unlikely that differences in anthocyanin concentrations in onion skin immediately after being cured at different temperatures (20, 24 and 28°C) are sufficiently different to influence PAL activity. It may be more plausible that differences in anthocyanin concentrations between curing temperatures are related to the rate of anthocyanin degradation rather than production. It has been shown that in the skin of red-wine grapes (*Vitis vinifera* L.) cv. Cabernet Sauvignon isotope labelled anthocyanins, specifically cyanidin and peonidin 3-glucoside (both found in red onion skin), decreased in concentration in response to seven days postharvest heat treatment (35°C) as compared to control (25 or 15°C) (Mori *et al*., 2007). This may suggest that curing onions at 28°C may result in both the degradation of anthocyanins in combination with the suppression of the activity of enzymes in the anthocyanin biosynthetic pathway. In onions cv. Red Baron a negative correlation existed between total flavonols and H° implying flavonol concentration in the skin may influence the colour of red onion skins. However, this relationship between flavonols and H° was not

present in the skin of brown onions. The higher concentration of quercetin glucosides found in the skin of cv. Red Baron provided a greater range of data which may explain the correlation found between total flavonols and H°, which was not found in cvs. Sherpa and Wellington. The difference in curing temperatures may not have been sufficient to provide a range of skin colours to detect a possible correlation between darkening of brown onions and quercetin glucoside concentration. Gennaro *et al*. (2002) suggested that flavonol glucosides in red onions were more resistant to storage than anthocyanins, thus indicating that more extreme conditions may be necessary to distinguish between flavonol concentrations at different curing temperatures in brown onions.

Two major flavonols were identified by Suh *et al*. (1999) in skin of Korean onions grown in the Mooan region (colour of cv. not stated); these were quercetin 4-glucoside and quercetin which agrees with the results presented herein, although small concentrations of quercetin 3,4-diglucoside were also measured. Quercetin was the major flavonol in the skin of all onion cvs. tested, however quercetin concentration was not influenced by curing temperature. In contrast, quercetin 4-glucoside and quercetin 3,4-diglucoside were both influenced by curing temperature in onions cv. Red Baron with higher concentrations found in onions cured at 20°C and lower at 24 and 28°C. This trend was reversed after seven months of cold storage. Flavonols are often produced in response to environmental stress therefore onions cured at 24 or 28°C may have endured a greater shock when transferred to cold storage (1°C) compared to onions cured at 20°C. Additionally, as with anthocyanin concentrations, onions cv. Red Baron cured at 20°C had higher concentrations of flavonol glucosides immediately after curing compared to 24 and 28°C, therefore flavonol glucoside production may already have peaked earlier than those cured at 24 and 28°C. Assuming flavonols degrade at the same rate when stored at the same temperature, this earlier peak in flavonol glucoside concentrations may have caused earlier degradation and therefore lower concentrations after seven months storage compared to onions cured at 24 and 28°C.

The most abundant sugar found in onion skin was glucose with much lower concentrations of fructose (16.38 and 1.25 mg g^{-1} FW, respectively) and

were in range of results published previously in the onion skin of cv. Hysam (Jaime *et al*., 2000). Sugar profiles differ between onion skin and flesh with lower concentrations of sugars and fructans in the skin (data not shown). The concentrations of sugars have previously been positively correlated with some anthocyanins in the flesh of red onions cv. Tropea and are thought to play a regulatory role in anthocyanin production (Gennaro *et al*., 2002). However no relationship between sugar concentrations and objective colour or flavonol/anthocyanin concentration was found.

1.5. Conclusions

At this stage in the project it is apparent that reducing the curing temperature had no adverse effect on sprouting or disease incidence, or on the quality parameters measured, in onion bulbs cold-stored for *ca*. 9 months. If confiormed in Year 2 and 3, this means that substantial reductions in the use of propane gas for heating during curing could be made without affecting quality.

P1.1 Complete temporal and spatial biochemical analysis for onion bulbs held under different storage regimes used for 3.1 and 4.1 (CU; 18 months). **- Completed**

P4.1 Select optimum curing regime to extend onion bulb storability and maintain quality and neck rot control (CU and ABC; 8 months). - **Completed**

Part 2: Gemma Chope – CU, Andrew Thompson, John Hammond – WHRI (Objective 2)

Executive Summary

A prototype onion-specific microarray was created using onion genetic information available in the public domain, along with some onion sequences isolated during this project. The prototype array was successfully tested with RNA from a range of onion tissues (shoot, root, bulb, leaf). Analysis of the results of this test hybridisation will allow the final design of the custom onion-specific microarray, maximising the number of samples that can be analysed on one chip.

2.1 Introduction

Advances in the field of molecular biology have meant that high throughput techniques, such as microarrays are available. A microarray consists of an orderly arrangement of thousands of spotted samples known as probes immobilized on a glass slide. The probes consist of cDNA derived from mRNA which represents expressed sequences rather than just genomic DNA. A typical microarray experiment involves the hybridisation of an mRNA molecule to the DNA molecule from which it originated. The amount of mRNA bound to each site indicates the expression level of that sequence.

It will be beneficial to assess molecular markers of sprout suppression and dormancy in conjunction with physical and biochemical traits, as this will further elucidate the genetic mechanisms underlying these physiological processes. Microarray technology would allow simultaneous screening of thousands of onion genes. The expression of these genes could then be compared with desirable bulb traits. In addition, this technique would reveal classes of genes whose expression alters during different environmental, temporal or spatial conditions.

There is a paucity of public genetic information on onion or any other *Allium* crops. Some key genes in the sulphur assimilation pathway have been cloned (McCallum *et al*., 2002) and recently, a set of over 10 000 onion

expressed sequence tags has become available (Kuhl *et al*., 2004). However, biochemical and molecular investigation are required before the exact function of these genes and the proteins they encode can be assigned (Jones *et al*., 2004). An onion-specific microarray was constructed using this information, and from sequences generated by this project. Microarray analysis will be accompanied by the detailed biochemical and physiological data generated by this project, as it is likely that post-transcriptional regulation of proteins occurs. A complex chemical profile including substances such as phytohormones and compounds concerned with carbohydrate would allow the application of chemometrics to the gene expression data. Chemometrics is a statistical technique whereby the chemical data collected can be related to the physiological state. In this way it is anticipated that the use of biochemical and physiological profiling in conjunction with microarray technology could identify the factors that are important in the transition from dormancy induction to sprout suppression.

2.2 Materials and methods

2.2.1 Extraction of RNA

RNA was extracted from various onion tissues, using a method appropriate to that tissue. See HL0182 Annual Report 2008 for details.

2.2.2 Design of prototype onion custom microarray

A prototype custom microarray for onion, 'onion test array', was designed using Agilent e-array (https://earray.chem.agilent.com/earray/) according to the sequences produced during this project (n=41) and publicly available sequence data from Genbank (n=103) (http://www.ncbi.nlm.nih.gov/) and ESTs from the Gene Index Project (http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/gimain.pl?gudb=onion) (n=12231). The prototype array was designed to contain both sense and anti-sense probes for singletons and sequences generated during HL0182 giving a total of 20547 60-mer probes. The slide was designed as a 4 x 44K format.

Hybridisation of RNA from a mixture of onion tissues will allow the direction of these sequences to be confirmed, and the microarray can then be designed as an 8 x 15K format, thus maximised the number of samples per slide.

2.2.3 Amplification, labelling and hybridisation of microarray

A pool of RNA samples was prepared as detailed in Table 2.1 below. Prior to pooling, samples with a low RNA concentration were lyophilized and re-suspended in DEPC-treated water to increase the concentration. All concentrated samples were run in 1 µl aliquots on a chip in a 2100 Bioanalyzer machine (Agilent) according to the manufacturer's instruction to check for degradation before being used in the mixtures. The Bioanalyzer uses a combination of microfluidics, capillary electrophoresis, and fluorescence to evaluate both RNA concentration and integrity. The pooled samples were labelled with Cy-3 dye using the Agilent One-Color Microarray-Based Gene Expression Analysis (Quick Amp Labelling) kit and the Agilent One Color RNA Spike-In kit. Labelled samples were also run on the Bioanalyzer to check the quality of the mRNA.

Table 2.1 RNA samples used to test prototype microarray.

Mixture	Tissue	A 260/280	A 260/230	C (ng μl^{-1})
Mix 1 – RNA from all tissues	Bulb – Brown	2.14	2.22	2971.7
	Bulb – Brown	2.05	2.50	2213.9
	Root – Brown	2.14	2.21	697.2
	Root – Red	2.11	1.60	397.0
	Meristem - Red	2.17	1.72	2051.8
	Meristem - Brown	2.19	1.97	1178.8
	Dehydrated Bulb - Red	2.10	1.16	382.5
	Dehydrated Bulb - Brown	2.09	1.89	244.5
	Leaf - Red	2.15	1.20	782.6

	Leaf - Brown	2.18	2.23	1629.6
Mix 2 – RNA from bulb tissue	Shoot - Brown	2.22	2.20	943.1
	Shoot - Red	2.17	2.13	798.4
	Bulb - Brown	2.14	2.22	2971.7
	Bulb - Brown	2.05	2.50	2213.9
	Dehydrated Bulb - Red	2.10	1.16	382.5
	Dehydrated Bulb - Brown	2.09	1.89	244.5
	Bulb - Brown	2.26	1.23	942.7
Mix 3 – RNA from all tissues except bulb	Root - Brown	2.14	2.21	697.2
	Root - Red	2.11	1.60	397.0
	Meristem - Red	2.17	1.72	2051.8
	Meristem - Brown	2.19	1.97	1178.8
	Leaf – Red	2.15	1.20	782.6
	Leaf – Brown	2.18	2.23	1629.6
	Shoot - Brown	2.22	2.20	943.1
	Shoot – Red	2.17	2.13	798.4

Samples of mix 1 and mix 2 were hybridised to the 4 x 44K microarray slide. Mix 1 was hybridised in triplicate, and mix 2 as a single, this provided three technical replicates of mix 1 (all tissues) and a single experiment to look at the pattern seen when using just bulb tissue. This will allow assessment of the feasibility of extracting RNA from bulb tissue for use on the microarray, or whether it will be necessary to collect meristematic tissue.

2.3 Results

2.3.1 Design of prototype onion microarray

Efforts to clone genes of interest from onion, such as those in the ethylene and ABA biosynthesis and degradation pathways, were unsuccessful, despite the conservation of these genes. A number of different primer pairs designed to conserved regions (Table 2.2) and PCR conditions

were tested. In addition, these reactions were tested on cDNA extracted from stressed bulb tissue (dehydrated and dehydrated) and on genomic DNA.

Table 2.2 Primer sequences to amplify NCED genes from onion cDNA.

Primer Pair Sequences (5' to 3') (F = forward; R = reverse) (S = C/G; Y = C/T; N = A/G/C/T; R = A/G; B=T/C/G; V=A/C/G; M=A/C; K=T/G)	**Name**	**Target species / gene**	**Tm**	**% GC**
F - GAC GTC ATC AAG AAG CCT TAC C	Millar *et al*.	Barley NCED	50	50
F - ATG TCA TCA AGA AGC CGT ACC TC	(2006)		50	47
F - CTC GAA TCG ATC GGA CCA GCT CT	Chono *et al*.	Barley NCED	54	56
R - GTG ATG AGT AAC CGC CGC TAA CTG	(2006)		54	54
F - CCT CCG TTG CAG GTT GCA GGT AAC A	Chono *et al*.	Barley NCED	56	56
R - GCC GTT GCC TCT ATC GTG CTG TTG A	(2006)		56	56
F - CTC TCT CGC AAC AAC AAA ACC CAC G	Chono *et al.*	Barley NCED	54	52
R - CTG GCA ACT TCC TCT TTC CAT GTC C	(2006)		54	52
F - AGT TGT TGT GTC ACC CAG TCC AG	Voisin *et al*.	Maize	52	52
R - CAC GCA CCG ATA GCC ACA	(2006)	NCED/VP14	47	61
F - TTY GAY GGN GAY GGN ATG GT	Burbridge *et al*.	Tomato NCED	48	75
R - CAN SCR AAR TCR TGC ATC AT	(1999)		45	62
F - GCS GGS CAC CAC CCT STT CG	Chope	Various monocot	57	52
R - GTC GTG GAT CAT SGT SGG CTG STC		species, NCED	57	45
F - CGT CAT CAA GAA GCC GTA CC	Chope	Various monocot	49	55
R - GTT CCA GAG GTG GAA GCA GA		species, NCED	49	55
F - GTT CAA GCT CCA GGA GAT GC	Chope	Various monocot	49	55
R - GCC GTA CTC GAA CTT GGT GA		species, NCED	49	55
R - AAT GGC CTT GGG GAA GAC GG	Chope	Various monocot	51	60
F - CTC AAC TTC TTC CAG CGC GC		species, NCED	51	60
R - TTG GCG AAG CCG GAC ACC TT	Chope	Various monocot	51	60
F - TGG TGG TGA TCG GCT CCT GCA T		species, NCED	53	59
R - ATC TCG ACG TCG GCG GAC TT	Chope	Various monocot	51	60
F - CCG GCC TCG TCT ACT TCA AC		species, NCED	51	60
R - AAG GTG TTG ACG CAN TGG CC	Chope	Various monocot	50	57
F - GTA CGG NCC CAT CTT CAA GA		species, CYP707	48	52
R - ATG ACG CCG ATG ACG TTG TC	Chope	Various monocot	49	55
F - TAC NCT GAC GCT GGA GAA GG		species, CYP707	50	57
R - CGG GGT TGT GGT GGA TGT TC	Chope	Various monocot	51	60

F - TCC TCA AGG CCG TCA CCG AA		species, CYP707	51	60
F - GGY CTG GCV GAA AAY CAG CT	Chope	Various monocot	50	58
R - AGS GTT TCR TTB GCR CCS GT		species, ACS	50	58
R - RAT CAG RAA BGC RTC RCC CG			50	58
F - ATG GGN CTN GCN GAR AAY CA	Subramanian *et*	Wheat ACS	48	52
R - CAN ARN CCR AAR CTN GAC AT	*al*. (1996)		45	45
R - AAC CAN CCN GGC TC			38	64
F - TAY TTY GAY GGN TGG AAR GC	Botella *et al*.	Mung bean ACS	46	47
R - RTC CAT RTT NGC RAA RCA	(1992)		39	41
F - ATG GGN YTG CNG ARA A			38	50
R - ARC ANA CNC KRA ACC A			37	46
F - CCA GAT GGG CCT CGC CGA GAA C	Gallie and Young	Maize ACC	57	68
R - GTT GGC GTA GCA GAC GCG GAA CCA	(2004)		57	62
F - CTC ATG CTG CTG CTC CAG GAC GAC	Gallie and Young	Maize ACO	57	62
R - CCT CGA ACC GTG GCT CCT TGG CCT	(2004)			
CGA ACT T			63	61
F - CGA CGC GTT CGA GGA GGG GT	Chope	Maize VP14	55	70
R - GGC GCG CTC CTG GGA GAG GC			59	80
F - GCC TCT CCC AGG AGC GCG CC			59	80
R - CAT GGC GCA GTC GAG CTG GC			55	70
R - AAA CAG AAG CAG TCC GGC AC			49	55
F - CTC TGG AAC TCG TGG GAG GAG C			55	63
R - GAA CCG GCC CTC GCC GTA CT			55	70
F - GAC AAC GTC ATC GGC GTC AT	Chope	Various monocot	49	55
R - AGC ATC TCS AGC TTG GCS AG		species, CYP707	51	60
F - TCT TCT TCC AGC AGG GCG ACT A			52	54
R - ATG ACG CCG ATG ACG TTG TC			49	55
F - GAC CAG CCC ACC ATG ATC CAC	Chope	Various monocot	53	61
R - TCG TTG AAG ATG GAG TCG GC		species, NCED	49	55
R - GTC GTG GAT CAT GGT GGG CT			51	60
F - GCC AAC GCC GGC CTC GTC TA			55	70
F - AAC TGG GGT TTC TTT GAC	Pan and Lou	Mulberry ACO	39	41
R - GCT TTC ATG GCT TAA ATC	(2008)		42	42
F - ACC AAY CCN TCN AAY CCN YTR GG	Gomez-Jimenez	Chick-pea ACO	53	54
	and Matilla			
R - CAN ARN CCR AAR CTY GAC AT	(2006)		45	45
F - GCN MTN TTY TTY CAN CAS GG	Chope	Various monocot	47	50
R - GCS GCG AAN AKN CAN CCG AT		species, CYP707	51	60

The onion EST database contains sequences with similarity to genes coding for proteins involved in ethylene and ABA metabolism (Table 2.3), and full length sequences from Genbank coding for genes involved in carbohydrate (e.g. fructan:fructan 6G fructosyltransferase), flavour precursor metabolism (e.g. alliinase, lachrymatory factor synthase), among others. In addition, one of the sequences generated by this project had homology to an aquaporin (Tonoplast Intrinsic Protein) gene which is implicated in ABA response (Li *et al*., 2008).

Table 2.3 Onion EST sequences similar to genes involved in ABA and ethylene metabolism.

Sequence	Description
CF444933	Similar to ABA 8'hydroylase 3, *Arabidopsis thaliana*, partial (38%)
TC4335	Similar to auxin and ethylene responsive GH3-like protein, *Capsicum chinense*, partial (37%)
TC4472	Similar to ethylene signal transcription factor, *Musa acuminata*, partial (15%)
TC5518	Similar to ethylene responsive protein, *Glycine max*, partial (24%)
TC5879	Similar to 24-methylenesterol C-methyltransferase 2, *Arabidopsis thaliana*, partial (78%)
TC7737	Similar to 5,10-methylenetetrahydrofolate dehydrogenase, *Pisum sativum*, partial (98%)
TC7887	Similar to ethylene receptor homolog, *Nicotiana tabacum*, partial (41%)
CF447201	Similar to ethylene-responsive transciptional coactivator-like protein, *Retama raetam*, partial (98%)
CF434594	Similar to ethylene receptor, Gladiolus hybrid cultivar 'Traveler', partial (23%)
CF439540	Similar to ethylene-overproducer1-like protein, *Solanum lycopersicum*, partial (17%)

CF435331	Similar to ethylene receptor 5, *Malus domestica*, partial (39%)
CF450829	Homologue to ethylene receptor, Gladiolus hybrid cultivar 'Traveler', partial (32%)
CF450202	Similar to ethylene-responsive elongation factor EF-Ts precursor, *Solanum lycopersicum*, partial (33%)
CF449770	Weakly similar to ethylene-induced esterase, Citrus sinensis, partial (50%)
CF435450	Similar to 24-methylenesterol C-methyltransferase 3, *Arabidopsis thaliana*, partial (18%)
CF443294	Similar to 24-methylenesterol C-methyltransferase 2, *Arabidopsis thaliana*, partial (57%)
CF441717	Similar to ethylene receptor, *Ziziphus jujuba*, partial (8%)
CF443937	Similar to ethylene signal transcription factor, *Musa acuminata*, partial (31%)

2.3.2 Amplification, labelling and hybridisation of microarray

The purity and quality of the concentrated RNA to be used on the onion test array was determined using a 2100 Bioanalyzer machine (Agilent, CA, USA) (Figure 2.1).

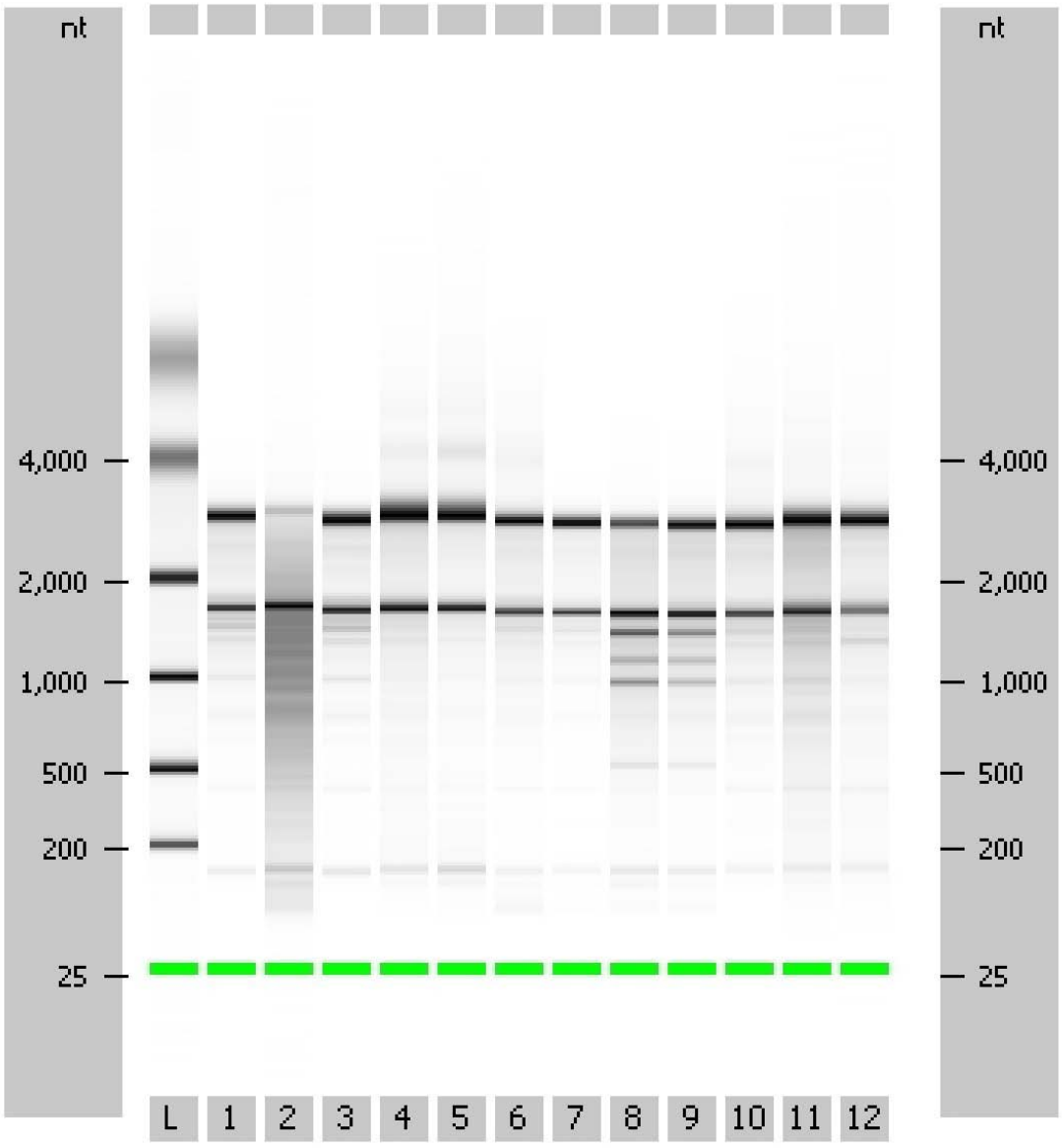


Figure 2.1 Gel electrophoresis image of the concentrated RNA samples run on the Bioanalyzer chip.

On the electropherogram, a degraded RNA sample shows a large number of small bands, which appear as a grey smear, whereas intact RNA shows two distinct bands that represent the 18S and 25S eukaryotic ribosomal RNA genes. Only RNA samples that showed distinct 25S and 18S bands where the relative intensity of the 25S band compared to the 18S band was approximately 2:1. The sample in lane 2 was not used in the pooled sample. The samples in lanes 8 and 9 are taken from the shoots from

geminating seed, and the extra bands present represent RNA from the chloroplasts.

The efficiency of the reaction to produce Cy-3-labelled cRNA was deteremined by calculating the cRNA yield and specific activity of each sample (Table 2.4). The yield should be >1.65 µg and the specific activity should be >9.0 (pmol Cy-3 μg^{-1} RNA).

Table 2.4 The properties of the labelled cRNA.

No	RNA sample	[Cy-3] (pmol μl^{-1})	A 260/280	[RNA] (ng μl^{-1})	cRNA yield (µg)	Specific activity (pmol Cy-3 μg^{-1} RNA)
1	Mix 1 All	4.9	2.22	438.1	13.14	11.18
2	Mix 1 All	4.7	2.20	441.3	13.24	10.65
3	Mix 2 Bulb	2.7	2.26	305.9	9.18	8.83
4	Mix 3 Not bulb	3.8	2.24	386.7	11.60	9.83

The purity and quality of the amplified and labelled mRNA was also determined 2100 Bioanalyzer machine using the mRNA nano assay (Figure 2.2). The majority of the fragments present in good quality mRNA should fall within the size range 100 – 2000 base pairs, indicated by a smear in this area. The samples prepared here are all of sufficiently high quality for use on the microarray. The second small peak in the electropherogram indicate ribosomal RNA, the quantity of this should not exceed 5% of the total sample.

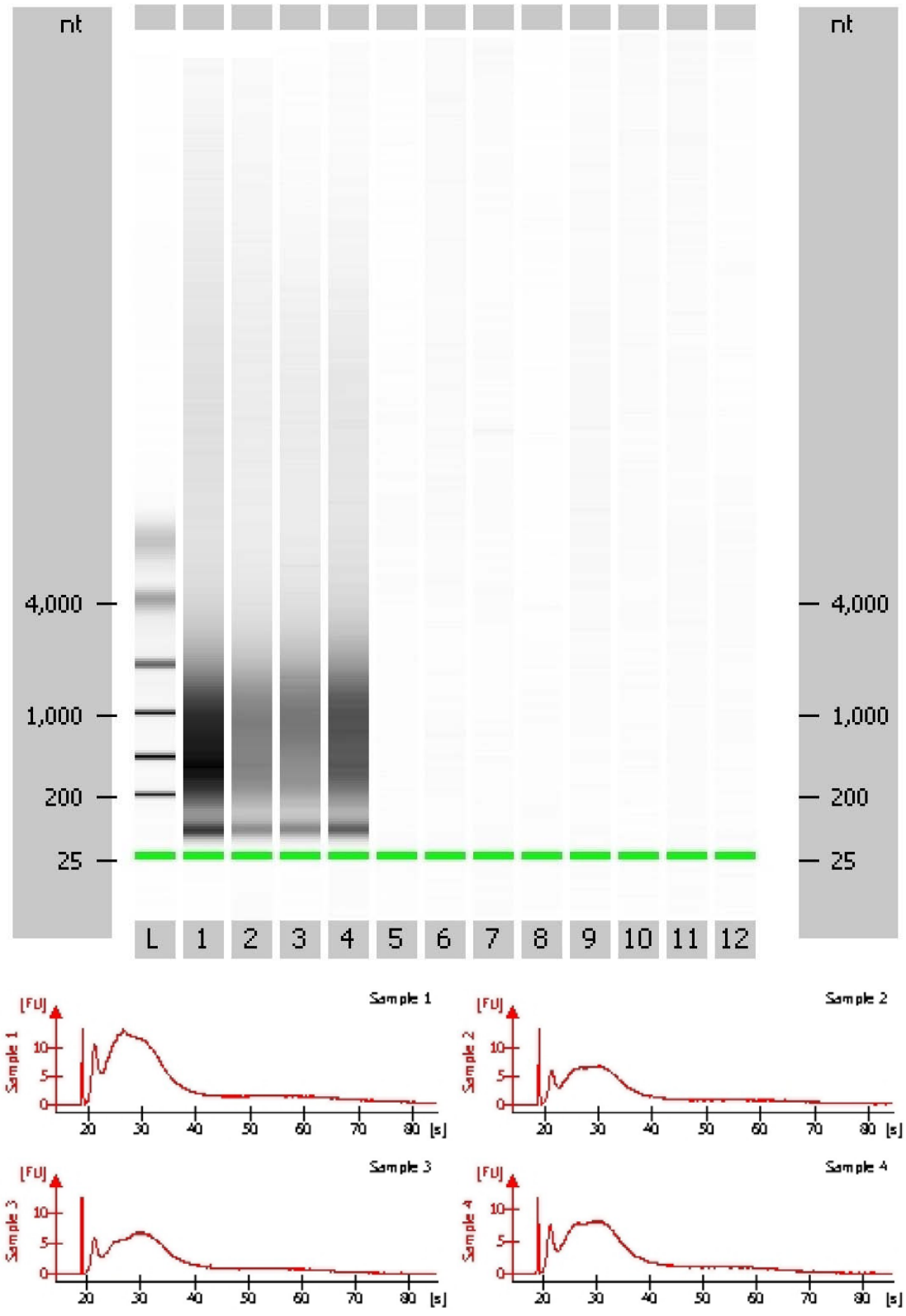


Figure 2.2 Gel electrophoresis image and electropherogram of the amplified and labelled RNA samples run on the Bioanalyzer chip. Lane 1 – Mix 1, Lane 2 – Mix 1, Lane 3 – Mix 2, Lane 4 – Mix 3.

2.3.3. Microarray image analysis

The onion test array was read using the Agilent Microarray Scanner. The bright spots indicate where cRNA from the onion sample has hybridized to the 60-mer probes on the surface of the microarray (Figure 2.3).

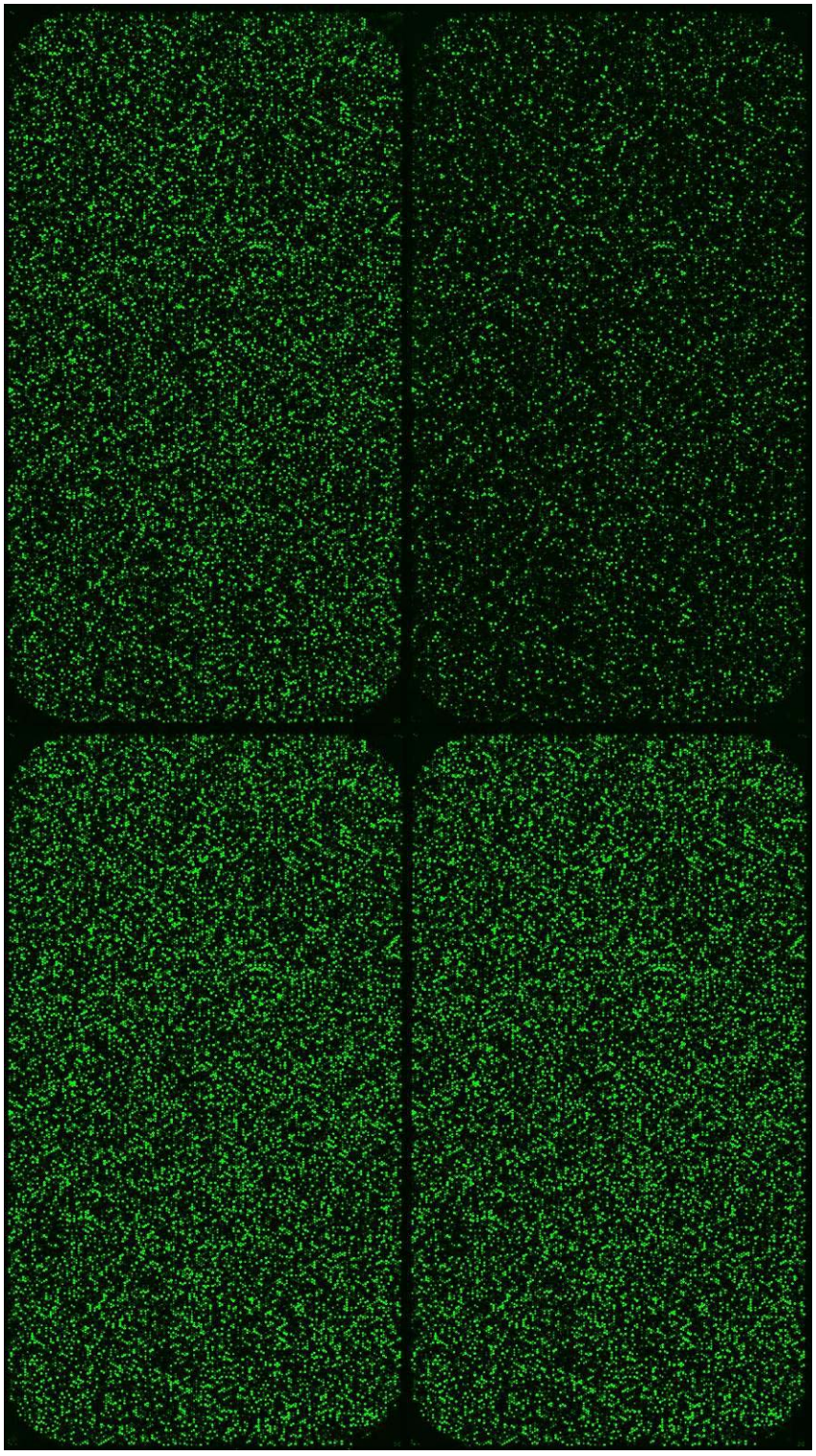


Figure 2.3 Microarray image of onion test microarray (4 x 44K format).

2.4 Discussion

Onion is a diploid (2n = 2x = 16) with one of the largest nuclear genomes of all cultivated species (>16 giga base pairs per 1C), with a low gene density of approximately 1 per 168 kb of genomic DNA (Jakše *et al*. (2008). It is likely that this is the reason that we were unable to clone target sequences from onion. However, the available sequence data from the public domain, coupled with sequences generated in this project should still provide sufficient markers to infer changes in gene expression during the transition form dormancy to sprout suppression and sprout growth in stored onions.

2.5 Conclusions

The onion test array was designed, and onion cRNA from a range of tissues, and from bulb tissue alone, were successfully hybridised to the 60-mer probes on the array. The data from the onion test array will be analysed to optimize the chip design.

P2.1 Complete selection and addition of targeted genes for inclusion into onion oligonucleotide microarray and design (WHRI, 12 months).- **Completed**

Part 3: Kate Downes, Leon Terry – CU (Objective 3)

Executive Summary

Onions cv. Sherpa were grown at Findlay's and treated for 24 h at 20°C with ethylene (10 µl l^{-1} ethylene) or 1-MCP 1 µl l^{-1} for 24 h at 20°C before or after curing at 28C for 6 weeks. Untreated bulbs were controls. Bulbs were cold-stored for 7 months at 1 ± 0.5°C. Samples were taken at before and after curing and at four times during cold storage. Samples were analysed for dry weight, sprout growth, pyruvate, ethylene and 1-MCP evolution, respiration rate, non-structural carbohydrates (fructose, sucrose and glucose), phenolics, flavonols and antioxidants.

- Ethylene and 1-MCP before curing and 1-MCP after curing reduced sprout growth of cv. Sherpa
- There is no indication that the ethylene and 1-MCP treatments had any deleterious effects on bulb quality.
- Treatment, prior to curing, with ethylene or 1-MCP improved the efficiency of absorption.
- The mode of action of ethylene and 1-MCP is unknown, but results from this study suggest that 1-MCP and ethylene may protect against or down regulate the production of endogenous ethylene which may play a role in the break of dormancy or initiation of sprout growth.

3.1 Introduction

The storability of onion bulbs is dependent on the rate of internal sprout growth which is controlled, in part, by endogenous hormones (Chope *et al*., 2006). The aim of this study was to investigate whether a single 24 h treatment of ethylene or 1-MCP was sufficient to effect the physiological and biochemical properties of cold-stored onion bulbs of medium and long-storage potential. Treatments were applied before or after curing to investigate whether curing affected treatment penetration and efficacy. In order to

elucidate the effects of ethylene and 1-MCP on onion bulb physiology and biochemistry, sprout length, respiration rate, antioxidant capacity, sugars and pungency were measured.

3.2 Materials and methods

See HL0182 Annual Report 2008 for Materials and Methods.

3.3 Results

3.3.1 Physical measurements

3.3.1.1 Dry weight

No significant difference was found between the mean dry weights of cvs. Sherpa and Wellington. Overall mean dry weight decreased significantly during cold storage (113.74 – 109.58 mg g^{-1} DW) although the greatest reduction occurred during curing (122.44 – 113.74 mg g^{-1} DW). Dry weight was significantly higher in both onion cvs. treated with ethylene before curing (127.16 mg g^{-1} DW) compared to control bulbs (101.49 mg g^{-1} DW) 38 weeks after harvest.

3.3.1.2 Sprout length

Onions cv. Sherpa had significantly longer sprouts than cv. Wellington 38 weeks after harvest (48.4 and 39.5 % of bulb length, respectively). Sprout growth of onions cv. Sherpa treated before curing with ethylene or after curing with 1-MCP was significantly reduced as compared to the control 31 weeks after harvest (Table 3.1). Sprout length of onions treated with ethylene before curing was still significantly shorter than the control 38 weeks after harvest. Sprout length of onions cv. Wellington was not affected by treatment.

Table 3.1 Sprout length (% of the bulb height) of onions cvs. Sherpa and Wellington 31 and 38 weeks after harvest treated before or after curing with 10 µl l^{-1} ethylene or 1 µl l^{-1} 1-MCP (*n* = 12) for 24 h at 20°C.

Treatment	Sprout length (% of bulb height)			
	Sherpa		Wellington	
	31 weeks	38 weeks	31 weeks	38 weeks
Control	29.4^{cd}	50.0^{gh}	18.2^{abc}	38.3^{defg}
EB	11.8^{a}	38.6^{defg}	19.5^{abc}	42.4^{efg}
EA	25.4^{bc}	49.6^{fgh}	8.6^{a}	41.2^{defg}
MB	15.4^{ab}	44.2^{efg}	15.5^{ab}	38.1^{def}
MA	12.2^{a}	59.1^{h}	15.9^{ab}	37.5^{de}

Treatments: EB, ethylene before curing; EA, ethylene after curing; MB, 1-MCP before curing; MA, 1-MCP after curing.

LSD (*P* = 0.05) = 11.84. Values followed by the same letter are not significantly different from each other calculated using the LSD.

3.3.1.3. Ethylene and 1-MCP evolution

Ethylene efflux immediately after treatment before or after curing was greater from onions cv. Sherpa and Wellington treated before curing (10.13 and 9.06 nmol kg^{-1} h^{-1}, respectively) compared to those treated after curing (8.18 and 8.06 nmol kg^{-1} h^{-1}, respectively) (Table 3.2). Irrespective of treatment time, mean ethylene efflux from onions cv. Sherpa was higher than from onions cv. Wellington.

Efflux of 1-MCP following treatment before or after curing was greater from onions cv. Sherpa and Wellington treated before curing (1.90 and 0 nmol kg^{-1} h^{-1}, respectively) than after curing (1.630 and 0.947 nmol kg^{-1} h^{-1}, respectively). No ethylene or 1-MCP was detected from control bulbs. In addition, when analysed after curing, no ethylene or 1-MCP was detected from onions treated with ethylene or 1-MCP before curing (data not shown).

Ethylene or 1-MCP was only detected from onion bulbs which were treated immediately before analysis.

Table 3.2 Ethylene and 1-MCP gas efflux (nmol kg^{-1} h^{-1}) immediately after treatments before and after curing with 10 µl l^{-1} ethylene and 1 µl l^{-1} 1-MCP.

Treatment	Sprout length (% of bulb height)			
	Sherpa		Wellington	
	31 weeks	38 weeks	31 weeks	38 weeks
Control	29.4^{cd}	50.0^{gh}	18.2^{abc}	38.3^{defg}
EB	11.8^{a}	38.6^{defg}	19.5^{abc}	42.4^{efg}
EA	25.4^{bc}	49.6^{fgh}	8.6^{a}	41.2^{defg}
MB	15.4^{ab}	44.2^{efg}	15.5^{ab}	38.1^{def}
MA	12.2^{a}	59.1^{h}	15.9^{ab}	37.5^{de}

Treatments: EB, ethylene before curing; EA, ethylene after curing; MB, 1-MCP before curing; MA, 1-MCP after curing.
LSD (*P* = 0.05) = 11.84. Values followed by the same letter are not significantly different from each other calculated using the LSD.

3.3.1.4. Respiration rate

The respiration rate of cured onions was significantly lower than that of freshly harvested bulbs. The mean respiration rate of onions cv. Sherpa was significantly higher than cv. Wellington during storage (0.1368 and 0.1146 mmoles CO_2 kg^{-1} h^{-1}, respectively). There was a significant increase in the mean respiration rate of cv. Sherpa between 21 and 31 weeks after harvest whereas the mean respiration rate of cv. Wellington remained stable from 13 weeks until the end of storage (38 weeks after harvest).

Respiration rate was 1.8-fold higher in onion cvs. Sherpa and Wellington within 4 h of treatment with ethylene before and after curing compared to the control. Before curing, respiration rate of 1-MCP treated

onions cv. Sherpa was 1.5-fold higher than the control but significantly lower than that of ethylene-treated onions. The same pattern was observed for cv. Wellington, although this was not significantly different from the control bulbs (Figure 3.1).

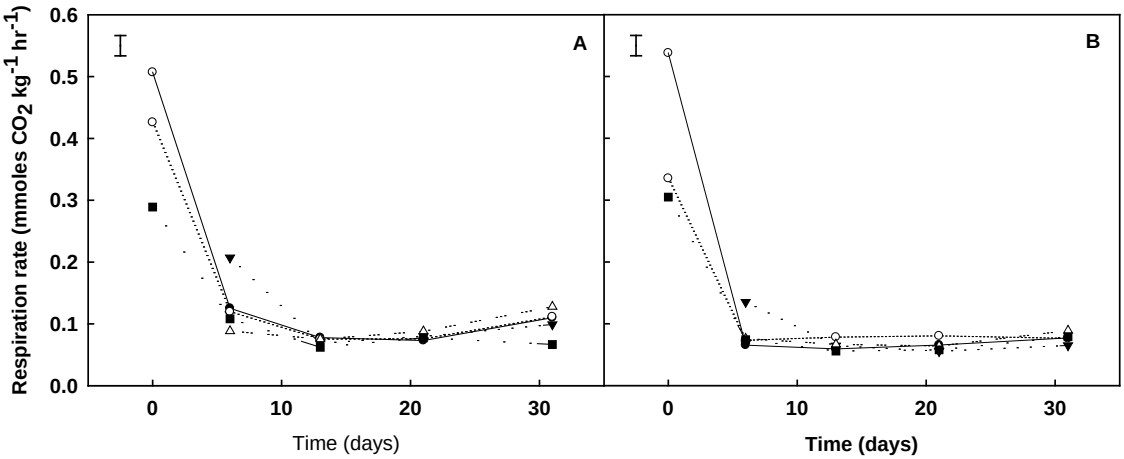


Figure 3.1 Respiration rate (mmol CO_2 kg^{-1} h^{-1}) of onion cvs. Sherpa (A) and Wellington (B) treated with 10 µl l^{-1} ethylene (closed symbols) 1 µl l^{-1} 1-MCP (open symbols) for 24 h at 20ºC before (circle) or after (triangle) curing or untreated (square); *n* = 12; LSD bars (*P* = 0.05) are shown.

3.3.2 Biochemical measurements

3.3.2.1 Fructose, glucose and sucrose

To compare the effect of ethylene and 1-MCP on carbohydrate profile, fructose, glucose and sucrose were measured (Figure 3.2). No significant difference in mean fructose, glucose and sucrose concentration was found between cultivars. Fructose concentrations of onions treated with either ethylene or 1-MCP before curing were not significantly different to control bulbs. However, after 6 weeks of curing, the mean fructose concentration of both cultivars was *ca.* 2-fold higher in onion bulbs treated before curing as compared to the control. There was no increase in fructose concentration observed in onions treated after curing with ethylene or 1-MCP.

Mean glucose and sucrose concentrations for both cultivars were significantly higher immediately after being treated before curing with ethylene

(169.7 and 108.9 mg g^{-1} DW, respectively) or 1-MCP (175.3 and 115.3 mg g^{-1} DW, respectively) than in control bulbs (155.9 and 89.2 mg g^{-1} DW, respectively). Mean glucose and sucrose concentrations were also higher after curing when treated with ethylene before curing (170.1 and 213.0 mg g^{-1} DW, respectively), 1-MCP before curing (166.4 and 197.8 mg g^{-1} DW, respectively) or 1-MCP after curing (162.6 and 203.5 mg g^{-1} DW, respectively) as compared to the control bulbs (134.0 and 177.2 mg g^{-1} DW, respectively). By the end of storage there were no significant differences in fructose, glucose or sucrose concentrations between treated and control bulbs.

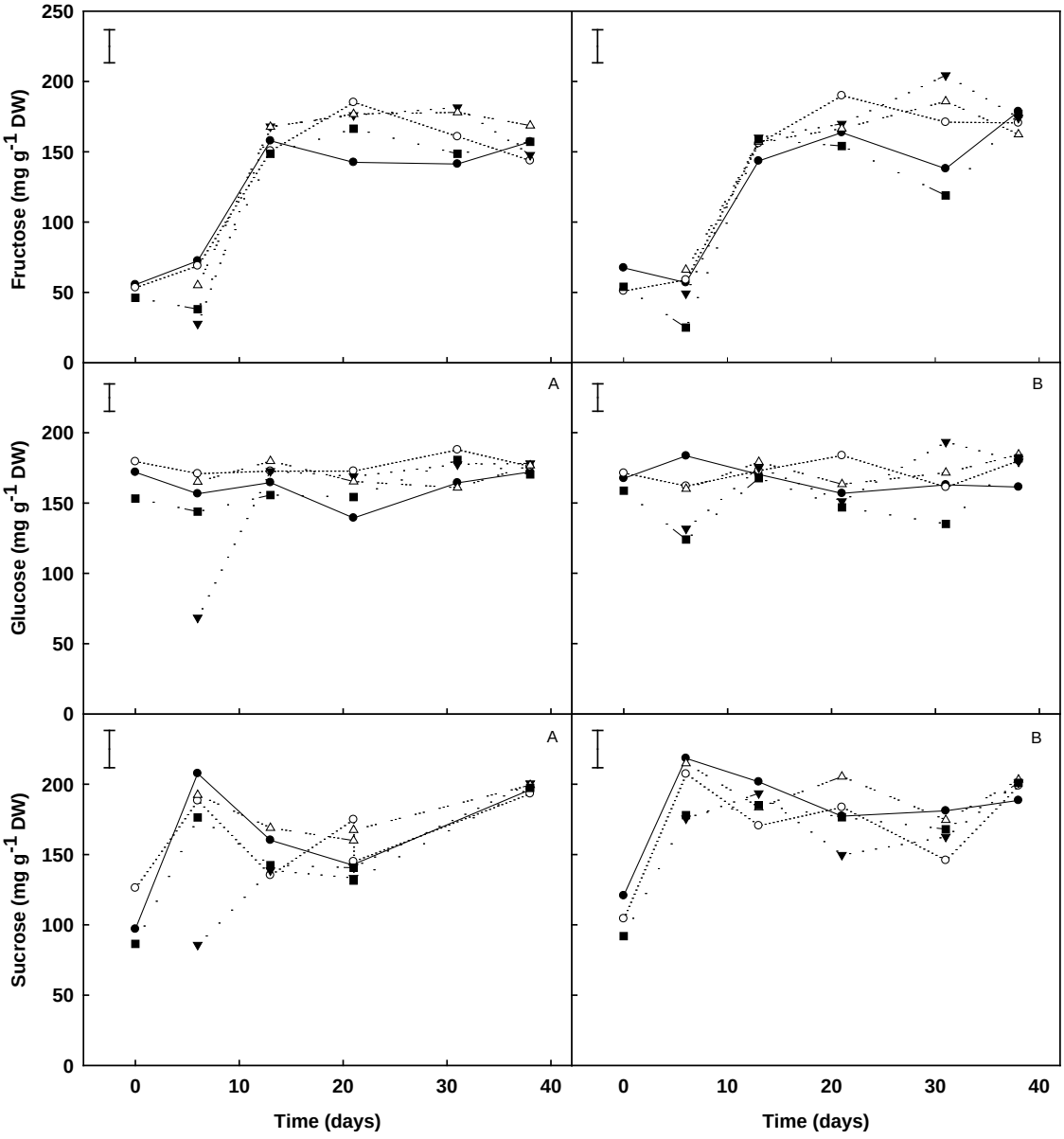


Figure 3.2 Fructose, glucose and sucrose concentrations (mg g^{-1} DW) in onions cvs. Sherpa (A) and Wellington (B) treated with 10 µl l^{-1} ethylene (closed symbols) 1 µl l^{-1} 1-MCP (open symbols) for 24h at 20ºC before (circle) or after (triangle) curing or untreated (square); *n* = 12; LSD bars (*P* = 0.05) are shown.

3.3.2.2 Total phenolics, flavonoids and antioxidant capacity

Total phenolic and total flavonoid concentrations were on average significantly higher in onions cv. Sherpa than cv. Wellington, although their trend over time was similar. Mean data for both cvs. indicated that onions treated with 1-MCP before curing had significantly higher TP at 0.75 mg GAE g^{-1} FW compared to the control (0.54 mg GAE g^{-1} FW). However onions treated before curing with ethylene had higher TP 38 weeks after harvest. There was no significant effect of treatment on TF concentrations. TAC was not significantly different between onion cvs. Sherpa and Wellington. Onions treated with 1-MCP before curing had significantly higher TAC after treatment compared to the control however returned to control concentrations after curing and for the remaining storage duration causing a significant interaction between baseline and treatment (Figure 3.3).

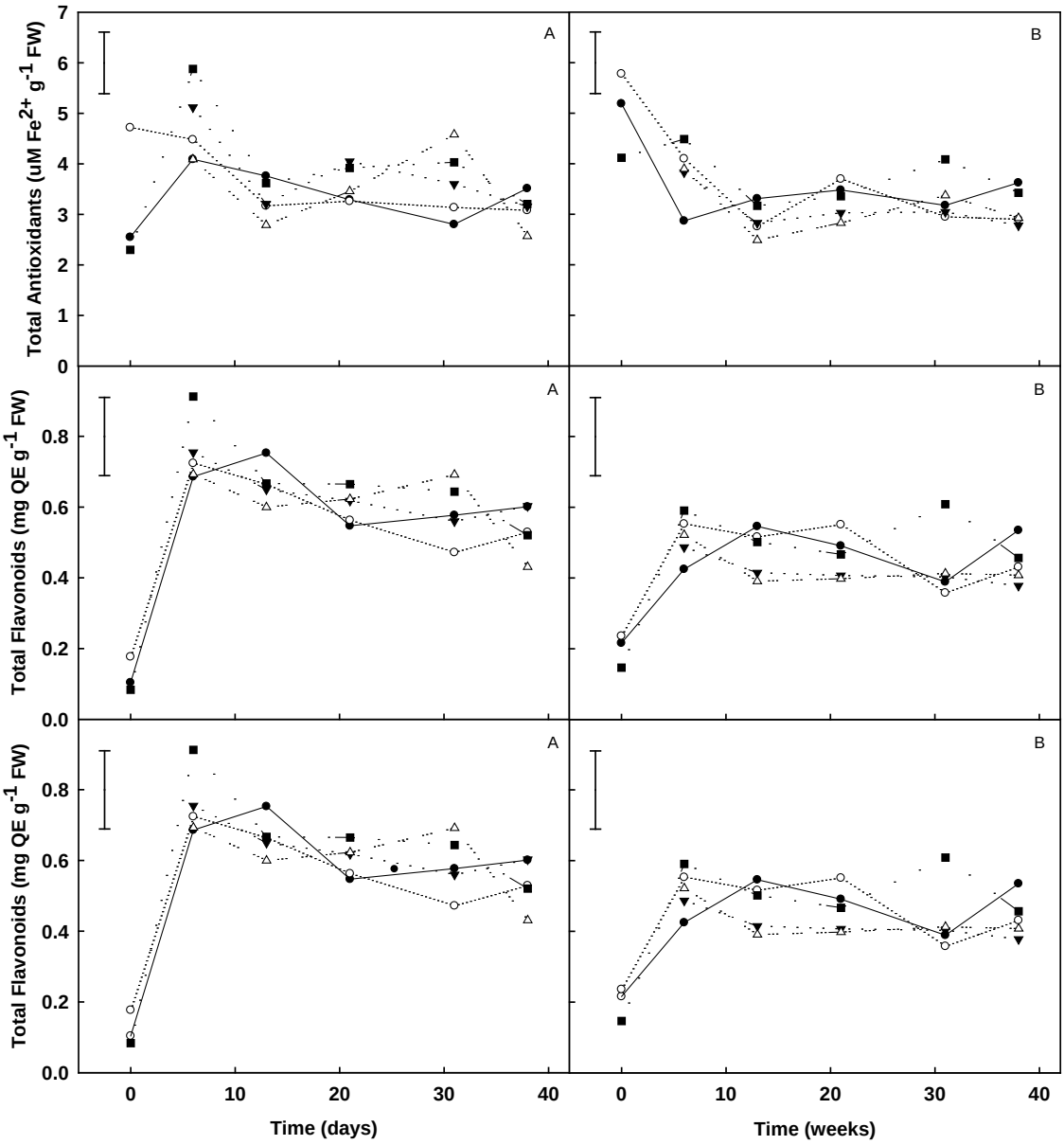


Figure 3.3 TP, TF and TAC of onions cvs. Sherpa (A) and Wellington (B) treated with 10 µl l^{-1} ethylene (closed symbols) 1 µl l^{-1} 1-MCP (open symbols) for 24h at 20ºC before (circle) or after (triangle) curing or untreated (square); *n* = 12; LSD bars (*P* = 0.05) are shown.

3.3.2.3 Flavonols

The sum of all flavonols; quercetin, quercetin glucosides and isorhamnetin glucosides, indicated that cv. Sherpa had significantly higher flavonol concentrations than cv. Wellington and that the most significant

interactions between factors were observed during curing (data not shown). There was no effect of treatment on flavonol concentration. In general, quercetin concentrations appeared to decrease during storage whereas isorhamnetin concentrations where seen to increase following curing especially in the final weeks of cold storage. Total flavonol diglucoside concentrations were approximately 1.6-fold higher than monoglucoside concentrations in onion flesh during storage.

3.3.2.4 Pyruvate concentration

Pyruvate concentration was significantly higher in onions cv. Sherpa than cv. Wellington at 5.71 and 5.02 µmol g^{-1} FW, respectively. Pyruvate concentrations 38 weeks after harvest were significantly higher in onions cv. Sherpa treated with ethylene before curing (5.71 µmol g^{-1} FW) and onions cv. Wellington treated with ethylene after curing (5.41 µmol g^{-1} FW) or 1-MCP before curing (4.71 µmol g^{-1} FW) as compared to control bulbs.

3.4 Discussion

The completed year 1 study has demonstrated two important points; firstly short treatments of ethylene and 1-MCP for just 24 h had a beneficial effect on onion bulb physiology and biochemistry as evidenced by changes in sprouting, dry weight, respiration rate and sugars. Secondly, treatment timing and cultivar were important factors affecting the degree of change measured in the onion bulbs.

Onions cv. Sherpa treated before curing with ethylene or before or after curing with 1-MCP had shorter sprouts 31 weeks after harvest compared to the control bulbs. It has previously been shown that short 24 h treatments of 1-MCP (1 µl l^{-1}) are sufficient to inhibit sprout growth in cured onions cv. SS1 when stored at 4 and 12°C (Chope *et al*., 2007b). Bufler (2008) found onions cv. Copra sprouted earlier when treated with 0.25 µl l^{-1} 1-MCP for 5 h (20°C) however the storage temperature was 18°C as well as differences in 1-MCP concentration and treatment duration. When stored at 20°C, Chope *et al*. (2007b) found onions cv. SS1 treated with 1-MCP had longer sprouts than the

control. Storing onions at low temperatures may inhibit ethylene binding site production as it has been previously reported that synthesis of new binding sites may be temperature dependent (Watkins, 2006). 1-MCP can have differential effects according to temperature; ripening inhibition of apple fruit was excellent at temperatures between 20 – 24°C whereas below 15°C the beneficial effects were lost (Watkins, 2006).

Very short ethylene treatments before curing have not previously been investigated although it is well documented that inhibition of sprout growth can be achieved in onion and potato using continuous ethylene treatment throughout storage (10 and 4 µl l^{-1}, respectively) (Prange *et al*., 2005; Bufler, 2008). However shorter treatments with ethylene after curing has been investigated by Bufler (2008) who found onions which had been held at 18°C for two weeks after harvest and then treated with ethylene for two weeks sprouted at the same time as those stored in air. The precise role that ethylene plays during onion storage is not yet understood. Appreciating the differences between onion and potato, continuous ethylene exposure during storage of potatoes (4 µl l^{-1}, 23-33 weeks, 4-13°C) has been shown to reduce their true dormancy (defined as the number of days from planting to shooting) compared to those treated with 1-MCP (1 µl l^{-1}, 48 h) and control tubers (air) (Pruski *et al*., 2006). This implies that ethylene is involved in controlling the release from dormancy but that it also suppresses sprout growth, suggesting that 1-MCP and ethylene may elicit the same response via different pathways (Chope and Terry, 2008). Bufler (2008) found that 1-MCP broke dormancy at 18°C and suggested endogenous ethylene may play an important role in maintaining bulb dormancy. However as the results presented herein show sprout growth is reduced when treated with 1-MCP and stored at 0-1°C. This suggests endogenous ethylene may be involved in the break of dormancy or initiation of sprout growth and that 1-MCP may protect against this action. High concentrations of exogenous ethylene may also protect against endogenous ethylene by negatively regulating its production. Stimulated ethylene production is thought to be due to a lack of negative feedback of ethylene biosynthesis (Watkins, 2006). This theory may help to explain the increase in onion respiration rate when treated with ethylene but little or no change when treated with 1-MCP. Exogenous ethylene may be perceived by

receptors causing an increase in metabolic activity resulting in increased respiration rate whereas 1-MCP may block the receptor without being perceived resulting in little or no change in respiration rate. It has previously been shown that short periods of ethylene induce sprouting (Bufler, 2008). From the biochemical and physiological data in this study in particular respiration rate, it appears that dormancy may not have fully begun before curing. By treating onions with ethylene before the initiation of dormancy may explain why a short 24 h treatment may have inhibited rather than initiated sprout growth as it may not be possible to interfere with dormancy before it has commenced.

Higher concentrations of fructose, glucose and sucrose concentrations were observed in onions treated before curing and were maintained until the end of curing signifying both an immediate and long lasting response to such a short pre-curing treatment of ethylene. Onions cv. SS1 treated after curing with 1 µl l^{-1} 1-MCP for 24 h then stored at 12°C for 51 days maintained higher concentrations of glucose, fructose and sucrose compared to the control bulbs (Chope *et al*., 2007b). In taste panels fructose and glucose have been positively correlated with likeability and sweetness, therefore ethylene and 1-MCP treatments may influence taste preference (Terry *et al*., 2005). However once transferred to cold storage differences in sugars between treated and untreated bulbs was reduced or no longer significant. Dry weight was significantly higher in onion cvs. Sherpa and Wellington treated before curing with ethylene 38 weeks after harvest. The delay in sprout growth found in cv. Sherpa treated before curing with ethylene may have delayed the breakdown of fructans causing an increase in sugars. However, this does not explain the higher dry weight in cv. Wellington treated before curing with ethylene as no delay in sprout growth was observed here. Sprout growth inhibition was found in onions cv. SS1 treated after curing with 1 µl l^{-1} 1-MCP for 24 h then stored at 4 or 12°C and also contained overall higher dry weight as compared to the control bulbs (Chope *et a*l., 2007b). Onion cvs. with high dry matter such as cv. Shakespeare contain higher concentrations of fructans than low dry matter cv. Sherpa measured using matrix-assisted laser desorption ionization – mass spectrometry (MALDI-TOF) (Davis *et al*., 2007). High dry matter has been linked to increased storability (Darbyshire and Henry, 1979) suggesting high

dry matter content of onions treated with 1-MCP may be directly linked to their extended storability.

It is important to the retailer and consumer that onion quality is not reduced by any additional treatments. Accordingly, individual flavonols, TF, TP, TAC and pyruvate were generally not reduced by any of the treatments 38 weeks after harvest when compared to the control. Quercetin and isorhamnetin glucoside concentrations were in the same range as previously reported by Vågen and Slimestad (2008).

This study has demonstrated that the efficiency of the treatments was influenced by timing and cultivar. The curing process appeared to reduce ethylene and 1-MCP absorption. A lower concentration of ethylene gas was released from onions cv. Wellington after treatment compared to the average-storing onions cv. Sherpa. Therefore, application before curing may improve the efficiency of gaseous treatments, which could lower costs as current practice involves continuous ethylene application during post-curing storage. Chope *et al*. (2007b) proposed that skin thickness may play an important role in the ability of treatment gases to reach the metabolically active meristem plate where sprouts are initiated. Onions cv. Wellington are long-storing, with thicker outer skin than onions cv. Sherpa (Downes *et al*., submitted) which may therefore be a more effective barrier against ethylene or 1-MCP influx. There was no effect of 1-MCP (1 µl l^{-1}) on sprout growth of the high dry matter, high pungency onion cv. Renate (L. Terry, pers. comm.). It may therefore be more important that thick skinned cultivars such as Wellington be treated before curing to aid absorption but less important for the thinner skinned, low pungency onions such as cv. SS1.

3.5 Conclusions

Ethylene treatment is currently approved (Control of Pesticides Regulation (SI 1986 No. 1510)) for use in the UK as a method of onion sprout suppression using continuous treatment throughout storage yet it has been shown in this study that a short 24 h treatment is sufficient to delay sprouting. The mode of action of ethylene and 1-MCP is unknown although the results from this study suggest that 1-MCP and ethylene may protect against or down

regulate the production of endogenous ethylene which may play a role in the break of dormancy or initiation of sprout growth. To further understand the mechanism of sprout suppression using ethylene, molecular techniques are required to determine differences in gene regulation in response to ethylene and 1-MCP.

P3.1 Complete evaluation of sprout suppressants/promoters (CU; 8 months). **- Completed**

4.1 Year 2 - Milestones.

P1.2 Construct chemometric model for onion dormancy and sprout suppression (CU; 24 months).

P2.2 Complete design and fabrication of onion oligonucleotide microarray (WHRI; 24 months).

P1.3 Identify markers (chemometric and molecular) for dormancy induction, sprout suppression and attributes related to bulb quality (CU and WHRI; 24 months)

P3.2 Complete first ethylene and 1-MCP experiment (CU; 20 months).

P4.2 Establish the optimum curing regime with lowest energy input, which is still capable of providing sufficient control of postharvest neck rot (CU and ABC, 20 months).

4.1.1 Plant material

Three onion cultivars with varying storage potential were selected *viz.* Red Baron (average-storing), Wellington (long-storing) and Sherpa (average-storing) and grown to normal commercial practice on three sites: A. W. Mortier Farms Ltd., Woodbridge, Suffolk (sand); A. Findlay's, Cardington, Beds. (sandy clay loam); Allpress Farms Ltd., Chatteris, Cambs. (peat). The site at Suffolk was drilled on 13th March 2008 at a rate of 57 seeds m^{-2} and bulbs were hand-harvested at 90% fall-over into bins on 1st September 2008. The site at Findlay's was drilled on 5th March 2008 at a rate of 57 seeds m^{-2} and were machine-harvested at 100% fall-over on 17th September 2008. The site at Cambs. was drilled on 23rd March 2008 at a rate of 57 seeds m^{-2} and were machine-harvested at 100% fall-over on 13th October 2008.

4.1.2 Experimental design – Gemma Chope, Leon Terry - CU

For year 2, the experiments conducted were divided into two parts – Experiment 4A and 4C. Experiment 4A consisted of two sites (Suffolk and Findlay's), three cultivars (Red Baron, Wellington and Sherpa) and two curing treatments (20 or 28ºC). Experiment 4C consisted of one site (Suffolk), two cultivars (Red Baron and Wellington), two curing temperatures (20 or 28ºC) and three storage treatments (1, 3 or 6ºC).

Both experiments were completely randomised designs with three replicates. At each site the plot was divided into three sections with onions harvested from each of the three sections being kept separate and treated as replicate blocks to be taken from the field to the store. Samples were taken straight after harvest (day 0), after 6 weeks postharvest curing treatment and at three intervals during cold storage. Five bulbs were sampled for each replicate and treatment combination. Each bulb was cut in half from top to bottom. One half of each bulb was used to provide tissue for pungency analysis where applicable. From the remaining half, two slices weighing 5 g were cut, and immediately snap frozen in liquid nitrogen. For each replicate, samples from each of the five bulbs were pooled. One set of samples were stored at -40ºC prior to lyophilisation, and the other stored at -80ºC for use as a source of RNA.

The following postharvest curing treatments were applied, beginning 4th September 2008 for onions from the Suffolk site, on 17th September 2008 for onions from the Findlay's site, and on 14th September 2008 for onions from the Cambs. site. Experimental bulbs were placed in nets among loose bulbs in 1 tonne wooden boxes.

- 28ºC for six weeks. RH 65-75%.
- 20ºC for six weeks. RH 65-75%.

Data on the temperature, heating hours, humidity and humidifier hours for the curing rooms are presented in the Appendix. Following the postharvest curing treatments the nets were removed from the boxes, and the experimental onions were placed into plastic crates and stored at 1, 3 or 6 ± 1 ºC at Cranfield University. In addition, onions from the bulk boxes were

transported to the Allium & Brassica Centre for commercial storage and evaluation of sprouting and deterioration to take place around the end of May.

4.1.3 **Experimental design** – Kate Downes, Leon Terry - CU

For year 2, the experiments were divided into two parts – Experiment 4B and 5. Experiment 4B consisted of one site (Findlay's), one cultivar (Sherpa), two curing treatments (20 or 28ºC) and seven treatments (1 µl l^{-1} 1-MCP prior to curing, 10 µl l^{-1} ethylene prior to curing, 1 µl l^{-1} 1-MCP and 10 µl l^{-1} ethylene simultaneously prior to curing, 1 µl l^{-1} 1-MCP post-curing, 10 µl l^{-1} ethylene post-curing, 1 µl l^{-1} 1-MCP and 10 µl l^{-1} ethylene simultaneously prior to curing and control). Experiment 5 consisted of one site (Allpress), two cultivars (Red Baron and Sherpa) and three treatments (1 µl l^{-1} methyl jasmonate before curing, 1 µl l^{-1} methyl jasmonate after curing and control).

The experiment was a completely randomised design with three replicates taken from the top, middle and bottom of the field. Each replicate batch was split into bags to be divided between treatments. All bagged onions were buried amongst bulk crates of onions to simulate commercial curing conditions and air flow. Gas treatments were conducted in air tight polypropylene chambers (88 cm x 59 cm x 59 cm) with tapped tubes for gas injection by syringe. All gas treatments were administered for 24 h at 20°C and untreated control bulbs held at 20°C. After curing all onions were transported to Cranfield University (Beds, UK) for cold storage at 1 ± 1°C. Diseased or damaged bulbs were removed and the remaining bulbs placed in individual plastic stackable crates and randomly distributed throughout the cold room. Outturns of four bulbs per treatment and replicate were taken before and after curing at intervals throughout cold storage. In addition, a total of 24 nets of onions each containing *ca*. 30 onions cv. Sherpa from experiment 4B were transported to Skierniwice, Poland for storage in +/- continuous ethylene treatment. The following treatments from both curing temperatures were included in the trial, with each cabinet containing six nets of each: control, ethylene before curing, 1-MCP before curing, ethylene after curing, 1-MCP after curing, ethylene and 1-MCP after curing.

5.1 Technology transfer

1) Paper entitled ‘Effect of curing temperature on the biochemical composition of skin from three UK-grown onion (*Allium cepa* L.) cultivars’ has been submitted to Postharvest Biology and Technology in April 2009 (Downes, Chope and Terry)

2) Paper entitled ‘Effects of postharvest application of ethylene and 1-methylcyclopropene either before or after curing on onion (*Allium cepa* L.) bulb quality during long-term cold storage’ to be submitted to Postharvest Biology and Technology (submitted to Consortium for approval) (Downes, Chope and Terry)

3) Oral presentations were given at the 6th International Postharvest Symposium, Antalya, Turkey, 8th-12th April 2009. Abstracts are detailed below.

- Effect of curing at different temperatures on phytohormone and biochemical composition of onion cv. Red Baron during long-term postharvest storage. Gemma A. Chope and Leon A. Terry

- Relationship between colour and biochemical composition of skin from onion cv. Red Baron bulbs cured at different temperatures Katherine Downes, Gemma A. Chope and Leon A. Terry

6.1 References

Benkeblia, N., 2000. Phenylalanine ammonia-lyase, peroxidase, pyruvic acid and total phenolics variations in onion bulbs during long-term storage. *Lebensm.-Wiss. U.-Technol*. 33, 112-116.

Botella, J.R., Arteca, J.M., Schlagnhaufer, C.D., Arteca, R.N. and Phillips, A.T. (1992). Identification and characterization of a full-length cDNA encoding for an auxin-induced 1-aminocyclopropane-1-carboxylate synthase from etiolated mung bean hypocotyl segments and expression of its mRNA in response to indole-3-acetic acid. *Plant Molecular Biology*, 20, 425-436.

Bufler, G. (2008). Exogenous ethylene inhibits sprout growth in onion bulbs. *Annals of Botany*. 103, 23-28.

Burbridge, A., Grieve, T. M., Jackson, A., Thompson, A., McCarty, D. R. and Taylor, I. B. (1999). Characterization of the ABA-deficient tomato mutant *notabilis* and its relationship with maize *Vp14*. *The Plant Journal*, 17, 427-431.

Chiwocha, S. D. S., Abrams, S. R., Ambrose, S.J., Cutler, A. J., Loewen, M., Ross, A. R. S. and Kermode, A. R. (2003). A method for profiling classes of plant hormones and their metabolites using liquid chromatography-electrospray ionisation tandem mass spectrometry: an analysis of hormone regulation of thermodormancy of lettuce (*Lactuca sativa* L.) seeds. *Plant Journal*, 35, 405-417.

Chono, M., Honda, I., Shinoda, S., Kushirt, T., Kaniya., Y., Nambara., E., Kawakami, N., Kaneko, S. and Watenabe, Y. (2006). Field studies on the regulation of abscisic acid content and germinablity during grain development of barley: molecular and chemical analysis of pre-harvest sprouting. *Journal of Experimental Botany*, 57, 2421-2434.

Chope, G. A., Terry, L. A. and White, P. J. (2006). Effect of controlled atmosphere storage on abscisic acid concentration and other biochemical attributes of onion bulbs. *Postharvest Biology and Technology*, 39, 233-242.

Chope, G.A., Terry, L. A. and White, P. J. (2007a). Neither pre- nor postharvest application of exogenous abscisic acid (ABA) or an ABA analogue affects endogenous ABA concentration of onion bulbs. *Plant Growth Regulation*, 52, 117-129.

Chope, G. A., Terry, L. A. and White, P. J. (2007b). The effect of 1-methylcyclopropene (1-MCP) on the physical and biochemical characteristics of onion cv. SS1 bulbs during storage. *Postharvest Biology and Technology*, 44, 131-140.

Chope, G. A., Terry, L. A. and White, P. J. (2007c). The effect of the transition between controlled atmosphere and regular atmosphere storage on bulbs of onion cultivars SS1, Carlos and Renate. *Postharvest Biology and Technology*, 44, 228-239.

Chope, G.A and Terry, L.A. (2008). The role of abscisic acid and ethylene in onion bulb dormancy and sprout suppression. *Stewart Postharvest Review,* 4, 1-7.

Darbyshire, B. and Henry, R. J. (1979). The association of fructans with high percentage dry weight in onion cultivars suitable for dehydrating. *Journal of the Science of Food and Agriculture*, 30, 1035-1038.

Davis. F., Terry, L. A., Chope, G. A. and Faul, C. F. J. (2007). Effect on extraction procedure on measured sugar concentrations in onion (*Allium cepa* L.) bulbs. *Journal of Agricultural and Food Chemistry*, 55, 4299-4306.

Downes, K., Chope, G.A. and Terry, L.A. (in press). Effect of curing temperature on the biochemical composition of skin from three UK-grown onion (*Allium cepa* L.) cultivars. *Postharvest Biology and Technology*

Gallie, D.R. and Young, T.E. (2004). The ethylene biosynthetic and perception machinery is differentially expressed during endosperm and embryo development in maize. *Molecular Genetics and Genomics*, 271, 267-281.

Gennaro, L., Leonardi, C., Esposito, F., Salucci, M., Maiani, G., Quaglia, G., Fogliano, V., 2002. Flavonoid and carbohydrate contents in Tropea red onions: Effects of homelike peeling and storage. *Journal of Agricultural and Food Chemistry*, 50, 1904-1910.

Giné Bordonaba, J.G. and Terry, L.A. (2008). Biochemical profiling and chemometric analysis of seventeen UK-grown black currant cultivars. *Journal of Agricultural and Food Chemistry*, 56, 7422-7430.

Gomez-Jimenez, M.C. and Matilla, A.J. (2006). Cloning of a cDNA-encoding ACC synthase and its mRNA expression during zygotic embryogenesis of chick-pea (*Cicer arietinum* L.) seeds. *Plant Growth Regulation*, 50, 101-110.

Hansen, S. L. (1999). Content and composition of dry matter in onion (*Allium cepa* L.) as influenced by developmental stage at time of harvest and long-term storage. *Acta Agirculturae Scandinavica, Section B, Soil and Plant Science,* 49, 103-109.

Hurst, W. C., Shewfelt, R. L. and Schuler, G. A. (1985). Shelf-life and quality changes in summer storage onions (*Allium cepa*). *Journal of Food Science*, 50, 761-772.

Jakše, J., Meyer, J.D.F., Suzuki, G., McCallum, J., Cheung, F. Town,C.D. and Havey, M.J. (2008). Pilot sequencing of onion genomic DNA reveals fragments of transposable elements, low gene densities, and significant gene enrichment after methyl filtration. *Molecular Genetics and Genomics*, 280, 287-292.

Jaime, L., Martínez, F., Martín-Cabrejas, M.A., Mollá, E., López-Andréu, F.J., Waldron, K.W., Esteban, R.M., 2000. Study of total fructan and fructooligosaccharide content in different onion tissues. *Journal of the Science of Food and Agriculture,* 81, 177-182.

Jones, M. G., Hughes, J., Tregova, A., Milne, J., Tomsett, A. B. and Colin, H. A. (2004). Biosynthesis of the flavour precursors of onion and garlic. *Journal of Experimental Botany*, 55, 1903-1918.

Kuhl, J. C., Cheung, F., Yuan, Q., Martin, W., Zewdie, Y., McCallum, J., Catanach, A., Rutherford, P., Sink, K. C., Jenderek, M., Prince, J. P., Town, C. D. and Havey, M. J. (2004). A unique set of 11,108 onion expressed sequence tags reveals expressed sequence and genomic differences between the monocot orders Asparagales and Poales. *The Plant Cell*, 16, 114-125.

Li, G-W., Peng, Y-H., Yu, X., Zhang, M-H., Cai, W-M., Sun, W-N. and Su, W-A. (2008). Transport functions and expression analysis of vacuolar membrane aquaporins in response to various stresses in rice. *Journal of Plant Physiology*, 165, 1879-1888.

Manhita, A.C., Teixeira, D.M. and de Costa, C.T. (2006). Applications of sample disruption methods in the extraction of anthocyanins from solid or semi-solid vegetable samples. *Journal of Chromatography A*, 1129, 14-20.

McCallum, J., Pither-Joyce, M. and Shaw, M. L. (2002). Sulfur deprivation and genotype affect gene expression and metabolism of onion roots. *Journal of the American Society for Horticultural Science*, 127, 583-589.

Miedema, P. (1994). Bulb dormancy in onion. III. The influence of the root system, cytokinin and wounding on sprout emergence. *Journal of Horticultural Science*, 69, 47-52.

Millar, A. A., Jacobsen, J. V., Ross, J. J., Helliwell, C. A., Poole, A. T., Scofield, G., Reid, J. B. and Gubler, F. (2006). Seed dormancy and ABA metabolism in Arabidopsis and barley: the role of ABA 8'-hydroxylase. *The Plant Journal*, 45, 942-954.

Mori, K., Goto-Tamamoto, N., Kitayama, M., Hashizume, K., 2007. Loss of anthocyanins in red-wine grape under high temperature. *Journal of Experimental Botany,* 58, 1935–1945.

Pan, G. and Lou, C. (2008). Isolation of an 1-aminocyclopropane-1-carboxylate oxidase gene from mulberry (*Morus alba* L.) and analysis of the function of this gene in plant development and stresses response. *Journal of Plant Physiology*, 365, 1204-1213.

Prange, R.K., Daniels-Lake, B.J., Jeong, J-C. and Binns, M. (2005). Effects of ethylene and 1-methylcyclopropene on potato tuber sprout control and fry color. *American Journal of Potato Research*, 82, 389-397.

Pruski, K., Prange, R.K., Daniels-Lake, B.J., Nowak, J., Astatkie, T. and Ronis, D.H. (2006). Growth-room and field studies with seed tubers treated with ethylene and 1-methylcyclopropene (1-MCP) during storage. *American Journal of Potato Research*, 83, 149-160.

Subramaniam, K., Abbo, S. and Ueng, P.P. (1996). Isolation of two differentially expressed wheat ACC synthase cDNAs and the characterization of one of their genes with root-predominant expression. *Plant Molecular Biology*, 31, 1009-1020.

Suh, H.J., Lee, J.M., Cho, J.S., Kim, Y.S., Chung, S.H., 1999. Radical scavenging compounds in onion skin. *Food Research International*. 32, 659-664.

Terry, L.A., Law, K.A., Hipwood, K.J. and Bellamy, P.H. (2005). Non-structural carbohydrate profiles in onion bulbs influence taste preference. In: Frutic 05, Information and Technology for Sustainable Fruit and Vegetable Production, Montpellier, France, September 12-16.

Thomas, T. H. (1969). The role of growth substances in the regulation of onion bulb dormancy. *Experimental Botany*, 20, 124-137.

Thomas, T. H. and Isenberg, F. M. R. (1972). Hormone physiology of onion bulbs during dormancy. *Experimental Horticulture*, 23, 48-51.

Vågen, I.M. and Slimestad, R. (2008). Amount of characteristic compounds in 15 cultivars of onion (*Allium cepa* L.) in controlled field trials. *Journal of the Science of Food Agriculture*, 88, 404-411.

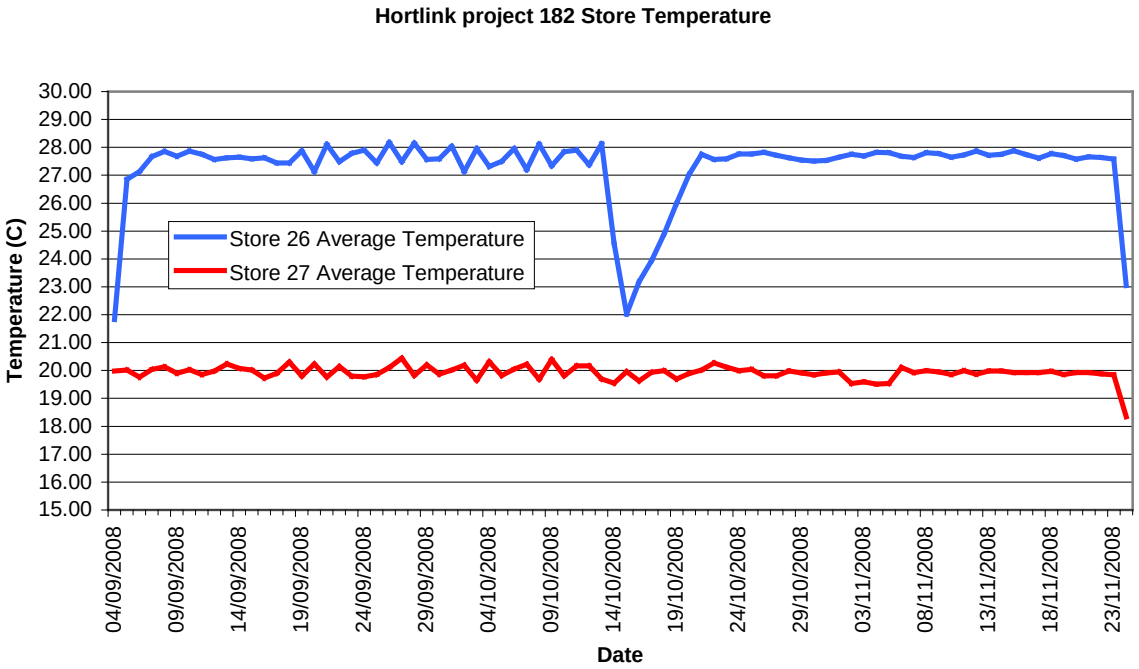
Voisin, A-S., Reidy, B., Parent, B., Rolland, G., Redondo, E., Gerentes, D., Tardieu, F. and Muller, B. (2006). Are ABA, ethylene or their interaction involved in the response of leaf growth to soil water deficit? An analysis using naturally occurring variation or genetic transformation of ABA production in maize. *Plant, Cell and Environment*, 29, 1829-1840.

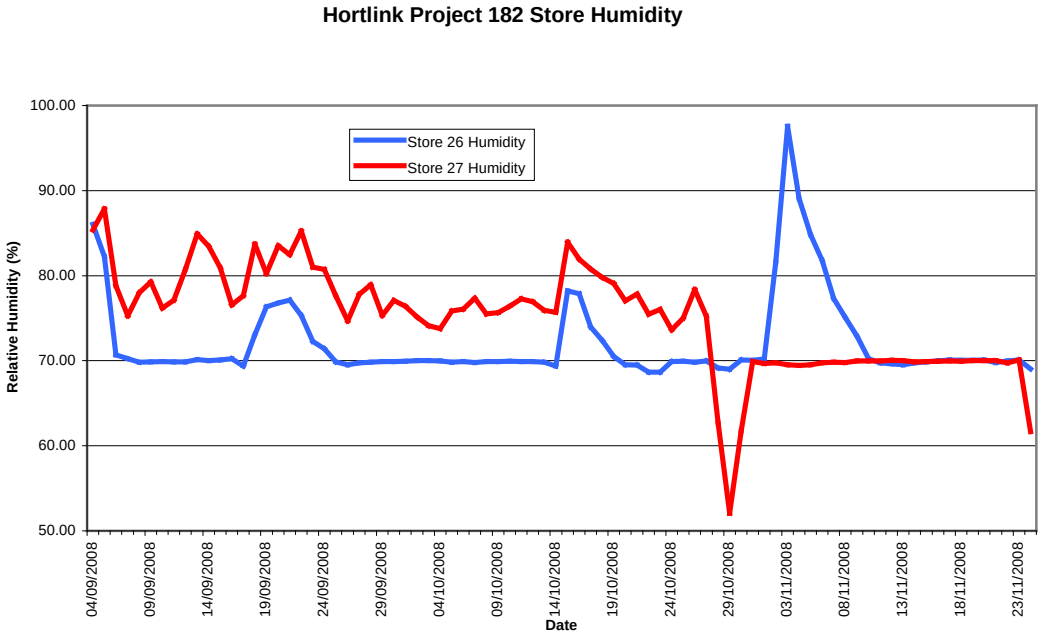
Watkins, C.B. (2006). The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. *Biotechnology Advances*, 24, 389-409.

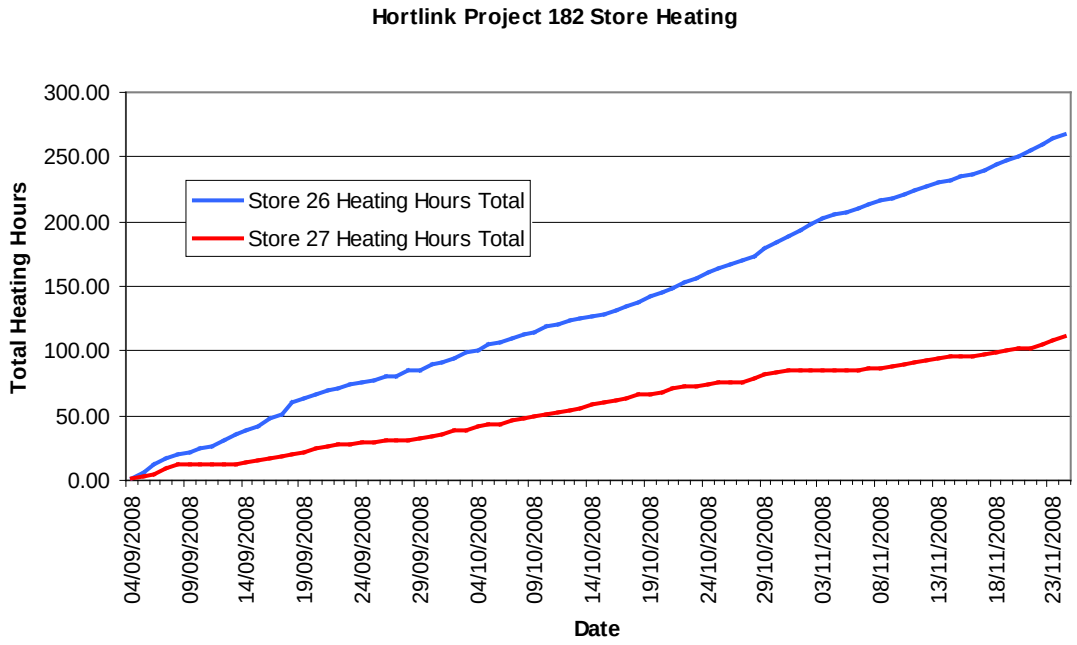
Wu, X. and Prior, R. L. (2005). Identification and characterization of anthocyanins by high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry in common foods in the United States: Vegetables, nuts, and grains. *Journal of Agricultural and Food Chemistry,* 53, 3101-3113.

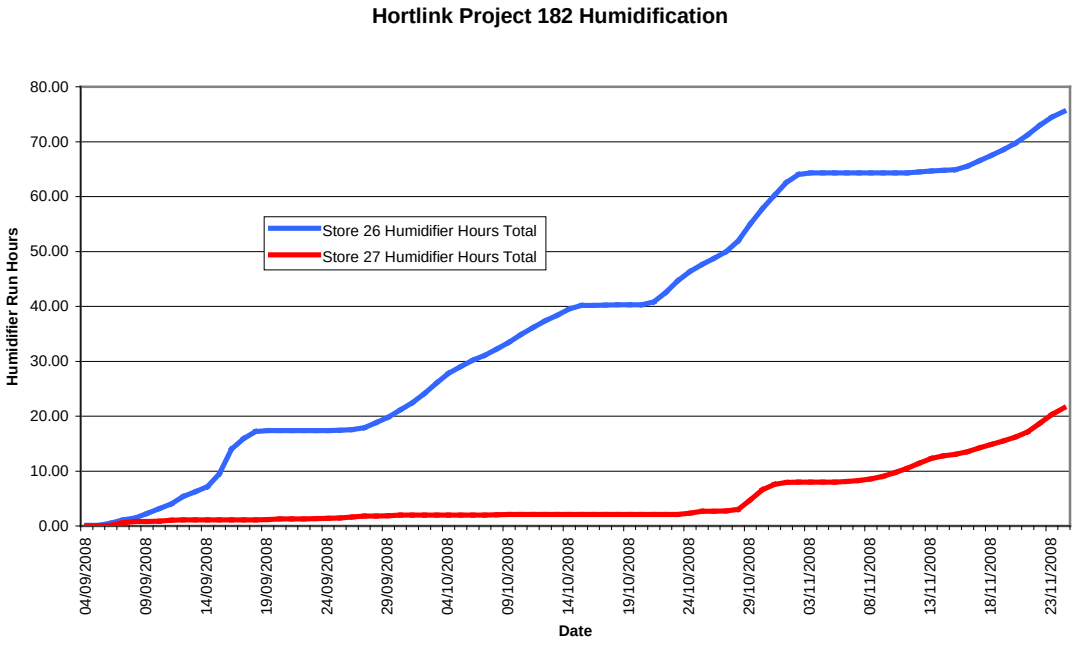
Yamazaki, H., Nishijima, T., Koshioka, M. and Miura, H. (2002). Gibberellins do not act against abscisic acid in the regulation of bulb dormancy of *Allium wakegi* Araki. *Plant Growth Regulation*, 36, 223-229.

APPENDIX


Hortlink project 182 Store Temperature
Temperature (C)
30.00
29.00
28.00
27.00
26.00
25.00
24.00
23.00
22.00
21.00
20.00
19.00
18.00
17.00
16.00
15.00
Store 26 Average Temperature
Store 27 Average Temperature
04/09/2008
09/09/2008
14/09/2008
19/09/2008
24/09/2008
29/09/2008
04/10/2008
09/10/2008
14/10/2008
19/10/2008
24/10/2008
29/10/2008
03/11/2008
08/11/2008
13/11/2008
18/11/2008
23/11/2008
Date


Hortlink Project 182 Store Humidity
Relative Humidity (%)
100.00
90.00
80.00
70.00
60.00
50.00
Store 26 Humidity
Store 27 Humidity
04/09/2008
09/09/2008
14/09/2008
19/09/2008
24/09/2008
29/09/2008
04/10/2008
09/10/2008
14/10/2008
19/10/2008
24/10/2008
29/10/2008
03/11/2008
08/11/2008
13/11/2008
18/11/2008
23/11/2008
Date


Hortlink Project 182 Store Heating
Total Heating Hours
300.00
250.00
200.00
150.00
100.00
50.00
0.00
Store 26 Heating Hours Total
Store 27 Heating Hours Total
04/09/2008
09/09/2008
14/09/2008
19/09/2008
24/09/2008
29/09/2008
04/10/2008
09/10/2008
14/10/2008
19/10/2008
24/10/2008
29/10/2008
03/11/2008
08/11/2008
13/11/2008
18/11/2008
23/11/2008
Date


Hortlink Project 182 Humidification
Humidifier Run Hours
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Store 26 Humidifier Hours Total
Store 27 Humidifier Hours Total
04/09/2008
09/09/2008
14/09/2008
19/09/2008
24/09/2008
29/09/2008
04/10/2008
09/10/2008
14/10/2008
19/10/2008
24/10/2008
29/10/2008
03/11/2008
08/11/2008
13/11/2008
18/11/2008
23/11/2008
Date

Power Consumption

CE26 - 28 C

	Fridge	Heater	Humidifier	Louvre
Totals	0.00	265.80	75.38	6.81
Power Rating (kW)		6.00	0.01	0.00
Fan run hours will be the same for each store				
Power Consumption (kWh)	0.00	1594.80	0.45	0.02

CE27 - 20 C

Fan	Fridge	Heater	Humidifier	Louvre
1940.84	26.34	108.65	21.50	50.21
0.55		6.00	0.01	0.00
1067.46	0.00	651.87	0.13	0.13