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Key workers:	Dr Gemma Chope, Cranfield University, Dr Katherine Cools, Cranfield University, Dr John Hammond, Warwick HRI, Dr Andrew Thompson, Warwick HRI.
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Project co-ordinator:	David E. O'Connor, Allium and Brassica Centre Wash Road, Kirton Boston Lincs PE20 1QQ
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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

AUTHENTICATION

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

Dr Leon Terry
Cranfield University

Signature ..... Date .....

CONTENTS

GROWER SUMMARY

Headlines

- Onions can be cured at lower temperatures without adverse effects on dry weight, pungency, disease incidence or sprout growth and only marginal effects on the visual appearance enabling growers potentially cut costs.
- Short-term treatments with Ethylene and 1-MCP, a synthetic plant growth regulator, before curing and 1-MCP after curing reduced sprout growth of cv. Sherpa and extended storage life with no deleterious effects on bulb quality.

Background and expected deliverables

There is a large variation between the onion cultivars in their innate dormancy and storage, but little is known of what causes the variation. Little is known about the underlying cause of the variation.

Trials were carried out to test different low energy input curing regimes on ABA levels, postharvest quality, disease control and bulb flavour. Subsequently attempts were made to manipulate storage life, with a view to designing new energy efficient storage protocols and decision support systems, using information derived from potential biochemical and genetic markers influencing dormancy, sprout suppression and bulb quality. An onion microarray was also constructed to identify genes involved with curing and storage.

If the factors that control onion dormancy and sprout inhibition can be understood then this will provide a foundation for understanding postharvest bulb quality, eliminating MH as a sprout suppressant and providing an opportunity to evaluate the use of both targeted metabolomics and microarrays as a potential diagnostic/screening tool.

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. Already as a result of this project lower temperature curing has already been trialed successfully by growers.
- Improved consumer acceptability of product as it would be free from all pesticide residues (including MH) at harvest.

- Improved maintenance of flavour and quality through better storage and shelf-life techniques. Quality and shelf life has been shown to be at least as good with lower temperature curing.

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 direct costs. Potential cost reduction highlighted in demonstration trial section.

Other Benefits

- Reduction in emissions from direct firing of propane and electricity for fans and refrigeration. Saving c.6,500 tonnes CO_2 p.a.
- Environmental benefit through avoidance of field spraying onions prior to harvest with the sprout suppressant maleic hydrazide (MH).
- Reduction of waste. Currently 17.5% of bulb onions are below retail quality and are used for processing. The target is a 30% reduction.

Summary of the results and main conclusions

Introduction

Physiological, biochemical and transcriptional changes in onion bulbs during postharvest storage were investigated. Based on experiments using almost 2000 onions, there is evidence to support the modification of current curing practice in the UK, which has not been re-evaluated since it was proposed in the 1970s. Results suggest there is no detrimental effect on bulb storage life and quality when curing at 20°C (compared to the 28°C UK norm) and it may be beneficial. *It is therefore recommended that where pathogen load is low onions be cured at 20°C with considerable savings in energy and associated costs.*

Ethylene

Onion storage can already be extended by using continuous ethylene as a sprout inhibitor. To better understand the fundamental role of ethylene in onion storage and further reduce cost incurred when using continuous ethylene three experiments were carried out in successive years including over 2000 onions. Short 24 h treatments with ethylene and/or an ethylene binding inhibitor (1-methylcyclopropene; 1-MCP) before or after curing were applied

and physiological and biochemical parameters measured throughout storage. Sprout suppression was observed when ethylene and 1-MCP were applied for just 24 h; *however the most successful treatment was when ethylene and 1-MCP were applied in combination.*

Short treatment with ethylene for 24 h was found to immediately increase the respiration rate of onion bulbs. 1-MCP did not have this same effect on onion physiology. Higher concentrations of fructose, glucose, sucrose and fructans were measured mainly in the bottom portion of the bulbs treated with ethylene and/or 1-MCP which taken together with the physiological results signifies both an immediate and longer lasting response to short 24 h treatments. Short treatment with ethylene for 24 h had no deleterious effects on the concentration of antioxidant compounds (*viz*. individual flavonols). As a result of the changes in economic climate during this three year project, which impacted on the availability of important chemicals, *a new method for the quantification of sugars and fructooligosaccharides (fructans) in onion was developed and was published (Downes et al., 2010; Appendix 1)*.

Microarray

To further elucidate the mechanisms of onion dormancy and sprout suppression the underlying targeted metabolomic and transcriptomic changes, which occurred during curing and storage, were determined. *The first onion oligonucleotide microarray was constructed and used to determine differential gene expression in selected samples during onion curing and storage*. A new method for the quantification of hormones in onion was developed using LC-MS/MS. Targeted metabolomic and physiological analyses were supported by transcriptional changes; however, direct alignments between transcripts and metabolites were not possible due to the lack of comprehensive sequence data for onion. Results suggest that dormancy may be relatively short – ending just after curing with extended storage being a function of sprout suppression. Both molecular and biochemical biomarkers of dormancy were identified. For example, using chemometric analysis on 3 years data, the ratio of monosaccharides (fructose and glucose) to disaccharide (sucrose), along with the concentration of zeatin riboside (ZR) were identified as important factors in discriminating between sprouting and pre-sprouting samples. The mono to disaccharide ratio has the advantage that it is easier and more cost-effective than ZR to measure. *This relatively simple parameter could give important information on the dormancy / sprouting status of stored onion bulbs*.

The microarray was also used to further explain the mechanism(s) by which both ethylene and 1-MCP achieve sprout suppression. In total, 1228 probes (tentatively annotated as particular genes) were differentially regulated in onion immediately after treatment with ethylene and/or 1-MCP for 24 h before curing. Of these 1228, 180 probes were differentially regulated between treatment regimes. Analysis of onions after long term cold storage in continuous ethylene revealed 272 probes up regulated and 302 probes down regulated. Down regulation of an ethylene receptor and transcription factor involved in ethylene signalling was also found in onions treated with 1-MCP which may explain why ethylene and 1-MCP both result in sprout suppression. That said, differential expression was observed between onions treated with ethylene and/or 1-MCP. A cluster of probes was up regulated by 1-MCP but down regulated by ethylene in the presence and absence of 1-MCP suggesting some probes respond to ethylene possibly via a receptor not bound by 1-MCP. The fact that many temporal changes in expression were detected in the probes on the onion microarray suggests that *the possibility of generating a diagnostic microarray chip that could predict sprouting exists*. In order to create this, a more detailed time course between curing and the onset of sprouting would be necessary, together with more sequence data for onions.

Curing Temperature and Botrytis allii

In addition to the objectives of this project, the effect of reducing curing temperatures from 28 to 20ºC on the incidence of neck rot (*Botrytis allii*) was investigated. Onions were inoculated with a solution of *B. allii* conidial spores or water, cured at 20 or 28ºC for six weeks, and cold-stored (1ºC) for six months. Disease incidence and severity was recorded after curing and storage. Using a bespoke aluminium temperature block, the effect of temperature on growth and viability of *B. allii* in culture was also investigated. Minimal disease was present in non-inoculated onions cured at either temperature. In inoculated onions, curing at 28ºC delayed onset of disease until after cold storage. For inoculated onions cured at 20ºC, disease incidence remained stable with time but disease incidence increased during cold storage. In culture, *B. allii* grew over a broad temperature range (1 to 32ºC, optimum 20ºC) but sporulated within a more narrow range (12 to 31ºC, optimum 21ºC*). These results suggest that curing at 28ºC can delay disease onset, compared with curing at 20ºC, but does not kill the pathogen. Where there was no disease pressure, there was no difference between the two curing temperatures.*

Financial benefits

Commercial benefits

- Annual value in area of impact: 400,000 tonnes annual production with a farm gate value *ca*. £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).
- 30- 40% energy saving projected ~ £7 per tonne, with a total crop ~ £3m gross margin improvement per annum.

Market potential

- 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

- Curing at lower temperatures (20^{o}C) no problem, subject to low disease level and does not compromise bulb storage or quality.
- Time in storage can probably be extended with lower temperature curing.
- For reds, bulb colour is better at lower temperature curing.

  1-MCP may be useful in promoting better storage – approval & registration will be needed.

SCIENCE SECTION

PHYSIOLOGICAL, BIOCHEMICAL AND TRANSCRIPTIONAL ANALYSIS OF ONION BULBS DURING STORAGE.

Gemma A. Chope[1,2], Katherine Downes[1], John P. Hammond[2], Andrew J. Thompson[2] and Leon A. Terry[1].

[1] Plant Science Laboratory, Cranfield University, Bedfordshire, MK43 0AL, UK
[2] Warwick HRI, University of Warwick, Wellesbourne, Warwick, CV35 9EF

Introduction

Onions bulbs have evolved as a storage organ to allow the plant to over-winter. During the transition from dormancy to sprout suppression (endo-dormancy to eco-dormancy) and subsequent growth, the bulb undergoes the transition from sink organ to source, to sustain cell division in the meristematic tissue. The mechanisms controlling these processes are not fully understood.

Many characteristics change during onion bulb storage including water content, and the concentration of flavour compounds, carbohydrates, minerals and plant growth regulators. Changes in these characteristics are likely to be linked with respiration and remobilization of carbohydrates to provide energy for the growing sprout. All nutrients required for growth of the sprout must come from within the bulb; therefore changes in certain key characteristics could be used to predict the onset of sprouting. Different onion cultivars vary in their innate storage life, but relatively little is known of the fundamental basis of this variation other than largely empirical correlations to such traits as dry matter, pungency, skin quality and degree of polymerization of non-structural carbohydrates. Peaks and troughs in certain compounds are known to coincide with sprouting but there is currently no biochemical or molecular assay that anticipates sprouting.

Dry weight and concentrations of non-structural carbohydrates, pyruvate and phenolics can be used to assess onion bulb quality. These characteristics differ both between cultivars and during storage. Onion bulbs with high dry weight are more suitable for longer-term storage and tend also to contain higher concentrations of fructans (Darbyshire and Henry,

1979). Fructans represent a major carbohydrate reserve in onion bulbs, and in general, are enzymatically hydrolysed to fructose during the storage period, accounting for a concomitant increase in fructose concentration (Hurst *et al.*, 1985). Soluble sugars are required to provide energy for sprout growth, and so the concentration of soluble sugars decreases when sprouting occurs (Rutherford and Whittle, 1982). Quercetin 4'-glucoside and quercetin 3,4'-diglucoside are the dominant phenolic compounds in onion flesh, and have been shown to increase during curing (Downes *et al.*, 2010).

During over winter storage in temperate climates a gradual change in the relative composition of plant growth regulators occurs as the levels of growth inhibitors drop and the levels of growth promoters rise. Hormone activity in onions cvs. Rijnsberger (long-storing) and Lancastrian (short-storing) bulbs harvested in September was measured by Thomas (1969) and Thomas and Isenberg (1972). The following pattern existed; gibberellins (GAs) had a first peak in December, followed by peaks of cytokinins and auxins. High auxin activity persisted as sprouting continued. A second GA peak was accompanied by sprouting in March. This GA peak was more likely to be an effect of sprouting rather than a cause, as GA activity was low in both non-sprouted and internally sprouted bulbs (Thomas, 1969; Yamazaki *et al.*, 2002) and application of exogenous GAs and auxin failed to stimulate sprouting (Thomas, 1969). Abscisic acid has been identified as part of the inhibitory complex present in onion bulbs (Tsukomoto *et al.*, 1969) and has been demonstrated to play a functional role in maintenance of dormancy in *Allium wakegi* (a cross between Japanese bunching onion and shallot) through the application of exogenous ABA and fluridone, an inhibitor of ABA biosynthesis (Yamazaki *et al.*, 1999b). Other studies have related a reduction in ABA concentration with loss of dormancy in onion (Matsubara and Kimura, 1991; Chope *et al.*, 2006, 2007a) and *A. wakegi* (Yamazaki *et al.*, 1999a, b).

Sprout growth, and the suppression thereof, is therefore a major factor in determining the storage life of onions. To date, most strategies to delay sprouting and prolong storage have focused on crop husbandry, the storage environment and breeding programmes. In maritime climates such as the UK, onions for storage are brought inside to be artificially cured and dried after harvest because of the risk of skin staining caused by wet weather during this period (O'Connor, 1979; Gubb and MacTavish, 2002). Curing dries the thin outer layers of the bulb to form one or more complete outer skins, which act as a barrier against water loss and pathogen attack (O'Connor, 1979). Onions are then stored under refrigeration to further suppress sprout development. Current UK curing and drying is based on a method developed in the 1970s on cultivars that are very different from those used today (Shipway, 1977; O'Connor and Shipway, 1978). Improvements to current methods,

such as a reduction in the temperature and/or duration of the curing and drying periods, could deliver benefits in the form of energy savings and reduced green house gas emissions, whilst still producing a continuous supply of onion bulbs of a satisfactory quality.

Advances in biochemical and transcriptional analysis methods now enable detailed analyses of sprout development during storage. Onion has an estimated nuclear genome size of 15 290 to15 797 Mbp per 1C, and a 2C DNA amount of 31.69 to 33.2 pg (Arumuganathan and Earle, 1991; Ricroch and Brown, 1997), which is approximately 107 times larger than *Arabidopsis* (King *et al.*, 1998). Consequently, there is a paucity of publically available genetic information on onion and *Allium* species, yet some key genes in the sulphur assimilation pathway have been cloned (McCallum *et al.*, 2002; Eady *et al.*, 2008) and a collection of over 10,000 onion expressed sequence tags is available (Kuhl *et al.*, 2004).

Here we utilise these advances to assess the effect of curing at different temperatures in combination with different storage temperatures on onion bulb physiology, transcriptome and targeted metabolites. These data show novel changes in metabolites and PGRs, supported by changes in transcripts with homology to genes involved in the metabolism of these metabolites during curing and storage. These advances will contribute to improved storage methods for onions.

Materials And Methods

Plant Material

Experiments were carried out over three growing seasons at multiple sites using three UK-grown onion cultivars with varying storage potential *viz.* Red Baron (red, average-storing), Wellington (brown, long-storing) and Sherpa (brown, average-storing) grown according to normal commercial practice. In 2007, onions were grown on sandy soil (Elveden Farms, Nr. Thetford, Norfolk) and on sandy clay loam (A. Findlay's, Cardington, Bedfordshire.). The Norfolk site was drilled on 9th March 2007 at a rate of 47-48 plants m^{-2} and bulbs were hand-harvested at 100% fall-over into storage bins on 2nd September. The Bedfordshire site was drilled on 28th February 2007 at a rate of 37-38 plants m^{-2} and bulbs were machine-harvested at 80% fall-over on 16th September. In 2008, onions were grown on sandy soil (A. W. Mortier Farms Ltd., Woodbridge, Suffolk) and sandy clay loam (A. Findlay's). The Suffolk site 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 Bedfordshire site was drilled on 5th

March 2008 at a rate of 57 seeds m^{-2} and bulbs were machine-harvested at 100% fall-down on 17th September 2008. In 2009, onions were grown at A Findlay's from seeds drilled on 16th March 2009 at a rate of 54 seeds m^{-2}. Onions were harvested on 7th September 2009 at 100% fall-down. All onions were free from maleic hydrazide.

Experimental Design

The experimental design for each trial was a completely randomised design with three replicates. At each growing 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. In 2007, three different curing temperatures were used; *viz*. 20, 24 and 28˚C, while only 20 and 28˚C were used in 2008 and 2009 (Table 1). All curing treatments were carried out at RH 65-75% at Sutton Bridge Experimental Station (Lincolnshire, UK). Experimental bulbs were placed in nets among loose bulbs in 1 tonne wooden boxes for six weeks. Following the postharvest curing treatments the nets were removed from the boxes and transported to Cranfield University within 3h. Dry aerial parts and roots were removed and diseased and damaged bulbs were discarded prior to storage in open plastic trays. Onions were stored at 0 ± 1 ºC in 2007, at 1, 3 or 6 ºC in 2008, and at 1, 6 or transferred from 6 to 1ºC after 13 weeks storage in 2009. Samples were taken straight after harvest (day 0), after 6 weeks postharvest curing treatment and at regular intervals during cold storage (four in 2007, three in 2008, and two in 2009).

Table 1. Experimental design for the three year experiment.

Year	2007	2008	2008	2008	2009
Site	Norfolk / Beds	Suffolk	Suffolk	Beds	Beds
Cultivar	Red Baron Sherpa Wellington	Red Baron Wellington	 Sherpa 	Red Baron Sherpa Wellington	Red Baron Sherpa Wellington
Curing temp (˚C)	20, 24, 28	20, 28	20, 28	20, 28	20, 28
Storage temp (˚C)	1	1, 3, 6	1	1	1, 6, 6→1
Sample times	0, 1, 2, 3, 4, 5	0, 1, 2, 3, 4	0, 1, 2, 3, 4	0, 1, 2, 3, 4	0, 1, 2, 3

Four bulbs were pooled 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. From the remaining half, two 5 g vertical wedges were cut and immediately snap frozen in liquid nitrogen, one of which was stored at -40ºC prior to lyophilisation, and the other stored at -80ºC. Dry weight, sprout growth, sprout length, root growth and fructose, sucrose and glucose concentrations were measured in all samples. Samples selected in triplicate for microarray analysis were cvs. Sherpa and Wellington from 2007, cured at 20 and 28˚C, and sampled at time 0 (harvest), 1 (after curing), 3 (before sprouting) and 4 (sprouting). Fructans were analysed in these samples, and in all samples from 2008 and 2009. Flavonols were measured in the microarray samples for 2007. The plant growth regulators abscisic acid (ABA), zeatin riboside (ZR) and isopentenyladensoine (IPA) were also measured in the microarray samples and in all samples from Suffolk in 2008.

Sprout Length, Rooting, Disease Incidence and Dry Weight

Presence or absence of roots was recorded, and then bulbs were assessed for the presence of a sprout after being cut in half. If a sprout was present, the length of the sprout, and the length and height of the bulb was recorded (Chope *et al.*, 2006). Dry weight was recorded in lyophilised samples.

Pungency

Pungency was measured in all samples from 2007. Pyruvate concentration was measured according to Abayomi and Terry (2009), with slight modifications. Pooled samples (30 g) were homogenised in 45 mL of distilled water using a hand held blender (Braun, Type 4192, Spain). A second identical 30 g sample was left to stand at 4°C for 1 h in 5% trichloroacetic acid (TCA) and homogenised to estimate endogenous pyruvate concentration. The background pyruvate concentration as calculated from the TCA samples was subtracted from the water samples and expressed in µmoles pyruvate g^{-1} FW.

Non-Structural Carbohydrates

Non-structural carbohydrates (NSCs) were extracted according to Downes and Terry (2010). Briefly, fructans were initially extracted from freeze dried onion powder (150 mg) with HPLC grade water, then fructose, glucose and sucrose were extracted from the same sample using aqueous methanol. All NSCs were quantified using HPLC coupled with evaporative light scattering detection. In 2007 and 2008, fructans were analysed separately from fructose, sucrose and glucose using a gradient of ethanol in water (Downes and Terry, 2010), or

water (Davis *et al.*, 2007), as a mobile phase, respectively. In 2009, all NSCs were analysed together in a single run using a gradient of acetonitrile in water (Downes and Terry, 2010).

Flavonols

Flavonols were extracted and quantified according to Downes *et al.* (2010). Briefly, flavonols were extracted from freeze dried onion powder (150 mg) with acidified methanol and quantified using HPLC coupled with diode array detection.

Abscisic Acid, Zeatin Riboside and Isopentenyladenosine

Lyophilised bulb samples (200 mg) were extracted in HPLC-grade water (5 mL) for 12-18 h on a shaker at 4ºC. Before extraction, 50 ng of deuterated internal standards for ABA, ZR and IPA (d_4-ABA, d_3-DHZR and d_6-IPA, respectively, National Research Council of Canada, SK, Canada) were added. Samples were centrifuged and the pellet re-extracted with 1 mL water, and the supernatants pooled. Samples were passed through a pre-conditioned SepPak C18 cartridge (Waters, MA, USA), and eluted with 80% (v/v) methanol (Hou *et al.*, 2008), then evaporated to dryness and resuspended in 200 µL 100% methanol.

Samples were analysed using a Waters Alliance 2795 HPLC coupled to a Micromass Quattro quadrupole tandem mass spectrometer (Waters, MA, USA) with an electrospray ion source. Both the HPLC and the mass spectrometer were operated by MassLynx v4.0 SP3 software (Waters). Samples (10 µL) were separated on a Zorbax Eclipse XDB-C18 analytical column (3.5 µm, 2.1 x 100 mm, Agilent, CA, USA) with 1 mm C18 guard column (Optiguard, Optimize Technologies, OR, USA) maintained at 25˚C. The mobile phase consisted of HPLC-grade methanol (A), water (B) and 5% acetic acid (C). The gradient involved an increase/decrease in solvent A; 10-60%, 15 min; 60-99.2%, 15 min; 99.2-10%, 2 min; 10%, 8 min, at a constant proportion of solvent C (0.8%) at a flow rate of 0.2 mL min^{-1}. Mass spectrometry was carried out using multiple reaction monitoring (MRM), with a capillary potential of 2.75 kV, a source temperature of 120˚C, a desolvation temperature of 350˚C, cone gas and desolvation gas flow rates of 50 and 950 L h^{-1}, respectively, and a collision gas (Ar) pressure of 0.0 Pa. The MRM transition, cone voltage, collision energy, and retention window for each compound and internal standard are shown in Table 2. Calibration curves were prepared using a range of standard solutions containing an increasing amount of ABA, ZR and IPA with a constant amount of deuterated internal standards, and the amount of endogenous compounds were quantified in relation to the internal standard using the calibration curves generated.

Table 2. Multiple reaction monitoring transitions and conditions used for mass spectrometry. The interchannel delay time was 0.02 s, the interscan delay was 0.1 s and the dwell time was 0.05 s.

	MS conditions					
Molecule	Precursor m/z	Product m/z	Cone voltage (V)	Collision energy (eV)	Retention time (min)	Ionisation mode
ABA[a]	263	253	30	9	17.02	Negative
d_4-ABA	267	156	25	9	17.00	Negative
IPA[b]	336	204	26	10	16.99	Positive
d_6-IPA	342	210	25	11	16.87	Positive
ZR[c]	352	220	30	12	11.39	Positive
d_3-DHZR[d]	357	225	25	12	11.64	Positive

[a] abscisic acid; [b] isopentenyladenosine; [c] zeatin riboside; [d] dihydrozeatin riboside

Extraction of RNA

Total RNA was extracted from frozen, ground onion tissue (100 mg) homogenised in 1 mL extraction buffer (2% (w/v) CTAB (cetyl trimethylammonium bromide), 0.8 M NaCl, 20 mM Na_2EDTA, 0.2 M boric acid, adjusted to pH 7.6 with TRIZMA base, β-mercaptoethanol added to 1% (v/v) just prior to use) using a pestle and mortar. The mixture was transferred to a 2 mL microtube and incubated at 65ºC for 10 min, then allowed to return to room temperature. Chloroform (1 mL) was added and mixed before being centrifuged at 16.2 g for 5 min at room temperature. The aqueous phase was removed to a clean tube and an equal volume of precipitation buffer (0.5% (w/v) CTAB, 50 mM Na_2EDTA, 50 mM MES (2-(N-morpholino)ethanesulfonic acid), adjusted to pH 5.8 with NaOH, and filtered through a 0.2 µM sterile filter), mixed and incubated on ice for 30 min. Samples were centrifuged at 13 000 rpm for 20 min at 4ºC and the supernatant removed. The pellet was resuspended in SSTE (1.0 M NaCl, 0.5% SDS, 10 mM TrisHCl (pH 8.0), 1 mM Na_2EDTA (pH 8.0)) and briefly incubated at 37ºC, before being allowed to return to room temperature. Chloroform (1 mL) was added and mixed, before being centrifuged at 16.2 g for 5 min at room temperature. The aqueous phase was removed to a clean tube and an equal volume of isopropanol added and incubated on ice for 20 min. Samples were centrifuged at 16.2 g for 20 min at 4ºC and the supernatant removed. The pellet containing total nucleic acid was washed with 1 mL 70% (v/v) ethanol, then left to air dry and finally resuspended in 50 µL DEPC-treated

water. Then, 30 µL 8 M lithium chloride solution was added and the samples incubated on ice overnight to selectively precipitate RNA. Samples were centrifuged for at 16.2 g for 30 min at 4ºC, the supernatant was removed, the pellet washed with 0.5 mL 70% ethanol and resuspended in 15 µL RNase-free water. Sample purity and integrity were verified using the RNA 6000 Nano Assay on the Agilent 2100 BioAnalyzer and then treated with Baseline Zero DNase (Epicentre, Madison, WI, USA) according to the supplier's instructions.

Microarray Analysis

A total of 13,310 onion nucleotide sequences were available for the construction of a 60-mer oligonucleotide custom *Allium cepa* microarray. The majority were obtained from public databases; 13,154 from the Onion Gene Index (http://compbio.dfci.harvard.edu/cgi-bin/tgi/gimain.pl?gudb=onion) and 102 from GenBank, with the remaining 54 sequenced directly from onion bulb tissue. Microarrays were designed using Agilent Technologies e-array microarray design platform (https://earray.chem.agilent.com/earray/). Initially, a prototype chip was designed in a 4 x 44 K format where 60-mer oligonucleotide probes for ESTs and singletons were designed to both sense and anti-sense. Test hybridisations of RNA from a range of onion tissues (root, shoot, bulb and leaf) were used to orientate these probes, thus reducing the number of probes, so the final format was 8 x 15 K. The final array design consisted of 15,736 60-mer oligonucleotide probes in total, representing 536 internal control probes and 15,200 probes representing 13,310 unique onion sequences. In order to further our analyses of onion gene expression, the annotation for individual probes was populated with annotations from the closely related, fully sequenced, genome of rice (*Oryza sativa*). Translated blastx alignments were made between onion sequences downloaded from the Onion Gene Index (Release 2.0; http://compbio.dfci.harvard.edu/tgi/plant.html) and rice cDNA sequences from the Rice Genome Annotation project (Version 6.1; http://rice.plantbiology.msu.edu/index.shtml). The tblastx alignments were performed with an E-value cut-off of 0.01 (Altschul *et al.*, 1997). Annotations, including descriptions and Gene Onotology assignments were then cross-referenced from rice sequences with significant homology to onion sequences, allowing GO analysis and more informative descriptions on the putative role of onion genes.

Agilent Quick Amp Labeling Kit, One Color and Cyanine 3-CTP was used to amplify and label target RNA to generate complementary RNA with Agilent One Color RNA Spike-In Kit used as a positive control for monitoring microarray workflow from sample amplification and labelling to microarray processing. The cRNA was fragmented and hybridized to the chip using the Agilent Gene Expression Hybridisation Kit, and then washed with Gene Expression

Wash Buffers 1 and 2, according to the manufacturer's instructions. Microarrays were scanned on an Agilent G2565BA Microarray scanner equipped with Agilent Scan Control version A8.4.1 and Agilent Feature Extraction version 10.5.1.1 software. All microarray data have been submitted to the online database Gene Expression Omnibus for public access and long term storage (awaiting accession number).

Quantitative real time PCR validation of microarray results

The transcript levels of 16 cDNAs that were significantly and differentially expressed at different times in the microarray experiments were confirmed by real time quantitative PCR. The ThermoScript™ RT-PCR System for First-Strand cDNA Synthesis kit (Invitrogen, Cat. No. 11146-024) was used to produce cDNA from total RNA samples (1 µg) using a combination of random hexamers and oligo DT primers. Gene specific primers were designed using Primer 3 and PrimerSelect (Lasergene) software. The transcript abundance detected by an ABI Prism 7900HT sequence detection system (Applied Biosystems) controlled by SBS 2.1 software (Aplied Biosystems) using a SensiMix SYBR Green qPCR MasterMix (Bioline, London, UK). The qPCR was performed in 384 well plates using the "Standard Curve" method (Wong and Medrano, 2005) for mRNA quantification with normalisation to the endogenous control gene, tumour protein (TC4554; CUST_716_P1403527117; F TCCGACTACAGGAACAACCAG, R AAACTCCTCTGCCTTCTCAGC). The control gene was selected from six genes evaluated for stability within our samples using the geNorm software package (details). Quantitative PCR conditions, efficiency calculations and data normalisations were as described previously (Hammond *et al.*, 2006). Table 1 describes probes used for qPCR analysis to validate the microarray data.

Table 1 Primers used for qPCR analysis

Probe	Gene	Forward Sequence	Reverse Sequence
7_P1403527117	Gamma glutamyl transpeptidase	TTGGGGTTTAGTC AGGAGTTG	ATTACAGGGATAG TTCAGCAGTTAG
533_P1403527117	FAD-dependent oxidoreductase domain	ATGAACCACGACA CCCAGAG	CATTCCCACGCTT TCTCAAC
8043_P1404013528	Onion_GI_singleton_antisense	ATGCCCTGCTTTC TCTCAAG	GATGGAGGAGGA GGATACGG

Statistical Analysis

All statistical analyses were carried out using Genstat for Windows 12th Edition, Version 12.1.0.3338 (VSN International Ltd., Herts., UK) unless otherwise stated. Analysis of variance (ANOVA) was performed on the data specifying a nested treatment structure of a common baseline (observation before curing treatments was the baseline). Least significant difference values (LSD; p=0.05) were calculated from each analysis, for comparison of appropriate treatment means, using critical values of t for two-tailed tests. Results are significant to p<0.05 unless otherwise stated. Principal component analysis (PCA) was carried out on autoscaled data on the samples for which the maximum variables were recorded; these were the samples used for microarray analysis in 2007, all samples grown at Suffolk in 2008, and all samples in 2009. Microarray data analysis was performed using Genespring GX11 (Agilent Technologies). A fully balanced experimental design was used for the microarray experiment, with three biological replicates for each time (harvest, cured, before sprouting, sprouting), curing temperature (20, 28ºC) and cultivar (Wellington, Sherpa) combination (n = 42). Raw expression data were subject to quantile normalization, and then baseline normalization was applied to individual probes, by dividing probe signal values by the median probes signal across all samples. Significantly differentially expressed transcripts were selected using a one-way ANOVA (GeneSpring GX) with a Benjamini-Hochberg corrected p-value <0.05 and a fold-change cut-off > 2. Significantly differentially expressed transcripts were then grouped using the K-means clustering algorithm in GeneSpring GX.

Results

Storage Temperature, but not Curing Temperature Affects Sprout Development

The effect of curing temperature on sprout initiation and growth was tested over three years, curing samples at either 28°C (current commercial practice) or 20°C. There was no significant affect of curing temperature on either sprout growth or sprout initiation during storage (data not shown). The effect of storage temperature on sprout development was also recorded during storage. Onset of sprouting occurred between *ca*. 13 and 29 weeks of storage at 1ºC (current commercial practice). There was no significant difference in sprout incidence between the cultivars grown, but sprout growth preceded more rapidly in cv. Sherpa, and slowest in cv. Wellington. Onset of sprouting occurred significantly earlier in onions stored at 3ºC (2008) or 6ºC (2008 and 2009), *ca*. 13 weeks after curing and sprouts grew most quickly in onions stored at 6ºC. In 2009, a subsample of onion bulbs was

transferred from 6ºC to 1ºC after 13 weeks storage until the end of the experiment. When analysed at the end of storage (25 weeks), there was a significant effect of storage temperature with the greatest sprout growth at 6ºC, followed by transfer from 6ºC to 1ºC and the least sprout growth at 1ºC (sprouts reaching 59.6, 48.7 and 34% of bulb eight respectively).

No root growth occurred during the duration of the 2007 experiment. In the last two experimental years, the least root development occurred in the onion cv. Wellington, although this was only significant in 2009 (41.7, 31.9, 18.1% of Red Baron, Sherpa and Wellington bulbs, respectively, showed root growth after 25 weeks storage). Onions stored at 1 or 6ºC had the lowest incidence of root growth compared with 3ºC or transfer from 6 to 1ºC. Curing temperature did have an effect on root growth in 2009, with root development significantly greater in onions cured at 28°C (22.2%) than 20°C (38.9%).

Onion dry weight was significantly higher in bulbs from the Bedfordshire site (115.63 mg g^{-1} FW) than the Norfolk (110.27 mg g^{-1} FW) site in 2007, but in 2008, there was no difference between sites. In all years, dry weight was greater in the red onion cv. Red Baron (129.52 mg g^{-1} DW) compared with the brown onion cvs. Sherpa and Wellington (110.47 and 110.14 mg g^{-1} DW, respectively). Dry weight generally decreased during curing, although this was not significant, and subsequently remained stable during cold storage. There was no effect of curing temperature or storage temperature on dry weight. Disease incidence in all years was below 1%.

Onion Metabolome is Significantly Affected by Postharvest Storage

Targeted metabolomic profiling was used to investigate the effects of onion bulb curing and storage on carbohydrates, flavour compounds and PGRs, and enable comparisons with changes in sprout development during storage. Distinct sugar profiles were observed for the individual cultivars, which were consistent between the three years. Onions cv. Red Baron bulb composition was dominated by disaccharides (fructose 126.8, glucose 137.5, sucrose 193.5 mg g^{-1} DW), while onions cv. Sherpa contained more monosaccharides (fructose 138.0, glucose 160.90) than disaccharides (sucrose 160.1 mg g^{-1} DW), and cv. Wellington was between the two (fructose 140.3, glucose 164.3, sucrose 192.5 mg g^{-1} DW). Overall, fructose, sucrose and glucose concentrations were 1.4-fold higher in 2007 and 2008 than 2009. In all years, fructose increased during storage until the onset of sprouting, after which it decreased. In 2009, fructose was higher in onion cvs. Wellington and Sherpa (108.8 and 103.3 mg g^{-1} DW, respectively), than cv. Red Baron (95.3 mg g^{-1} DW). In 2007 and 2008,

glucose changed little during storage, but in 2009, both glucose and sucrose concentrations decreased dramatically (3 to 4-fold) to the onset of sprouting and then increased thereafter.

Onions contained fructans in degrees of polymerization ranging from 3 to 8, with the concentration decreasing in proportion with the increase in size, with the following ranges recorded across all three years; kestose 20 to 160, nystose 5 to 130, DP5 5 to 90, DP6 2 to 55, DP7 2 to 45, DP8 2 to 30 mg g^{-1} DW. In 2007, fructans did not vary with cultivar, but in 2008 and 2009, onions cv. Red Baron contained the highest concentrations of fructans. In all years, fructan decreased during curing and cold storage reaching a minimum level prior to the onset of sprouting (except kestose which increased during curing). In 2008, fructans were generally lower in onions stored at 1ºC, and the increase in the higher DPs after the onset on sprouting was greater in magnitude and occurred earlier in onions stored at 3 or 6 than 1ºC, but there was no main effect of storage temperature in 2009.

Field Site, but not Storage, Affects Onion Pungency

Pungency was measured in all samples in 2007; there was a significant effect of site, with onions from Bedfordshire (6.28 µmol pyruvate g^{-1} FW) more pungent than those from Norfolk (2.64 µmol pyruvate g^{-1} FW), however there was no effect of curing temperature, therefore pungency was not measured in subsequent years. Flavonols were measured in onion bulb tissue from the 2007 growing season. The major flavonols in onion flesh were quercetin 3,4'-diglucoside and quercetin 4'-glucoside. Isorhamnetin 3,4'-diglucoside, isorhamnetin 4'-glucoside, quercetin 3-glucoside and quercetin were present in very low concentrations. Quercetin 3,4'-diglucoside and quercetin 4'-glucoside were higher in cv. Sherpa (3.49 and 2.37 mg g^{-1} DW respectively) than cv. Wellington (2.66 and 1.66 mg g^{-1} DW). Quercetin 3,4'-diglucoside increased during curing at both temperatures, while quercetin 4'-glucoside increased during curing at 20ºC, but decreased at 28ºC.

PGRs Signal Sprout Initiation in Stored Onion Bulbs

In 2007, overall mean abscisic acid (ABA) concentration was greater in cvs. Red Baron and Sherpa (19.2 and 19.9 ng ABA g^{-1} DW, respectively) than Wellington (15.5 ng g^{-1} DW), however, there was no difference between cultivars in 2008. Overall, ABA decreased during curing and reached a minimum prior to the onset of sprouting, after which it increased. In 2007, ABA was significantly greater in onions cured at 20 than at 24 or 28ºC; however, there was no difference between curing temperatures in 2008. In 2007, onion cv. Sherpa contained the most zeatin riboside (ZR) (130.8) and cv. Wellington (97.8 ng g^{-1} DW) the least. The same trend was observed in 2008, but was not significant. There was no effect

of curing temperature on ZR concentration, but ZR decreased during curing, and then increased towards the end of storage. The concentration of isopentenyladensoine (IPA) did not vary according to cultivar or curing temperature. There was no change in IPA during curing, but the concentration increased during cold storage, reaching a peak around the time of onset of sprouting.

Chemometric Analysis Reveals Consistent Changes in Metabolites

Principal component analysis (PCA) was used to identify key variables within the large physiological and metabolomic data set for each experimental year. The PCA for 2007 showed that the samples could be grouped according to time, but did not separate according to cultivar or curing treatment (Figure 1). Samples before and after cold storage were separated on PC1 (47.04% of variation), while harvest and sprouting samples were separated from cured and pre-sprouting samples by PC2 (14.32% of variation). This trend was consistent for the 2008 (Figure 2) data, and similar for the 2009 (Figure 3) data, although the 2009 data set did not include any PGR data. These analyses show that samples taken at harvest are characterised by high fructan and ABA concentrations, and cold stored samples contain more fructose, sucrose and IPA. Sprouting samples were characterised by higher ZR and a higher ratio of monsaccharides to disaccharides.

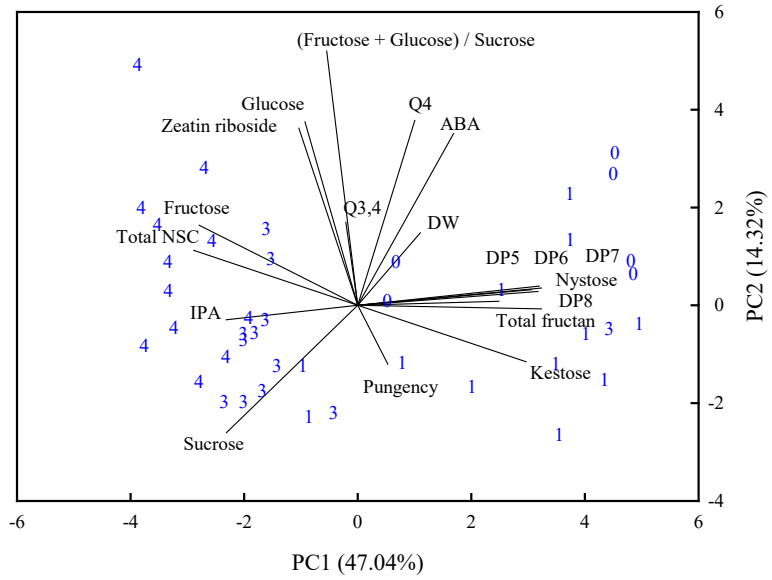


Figure 1. Principle component analysis biplot of onion cvs. Wellington and Sherpa bulbs cured at 20 or 28ºC and stored at 1ºC; after harvest (0), after curing (1), and before (3) and after (4) sprouting in cold storage in 2007.

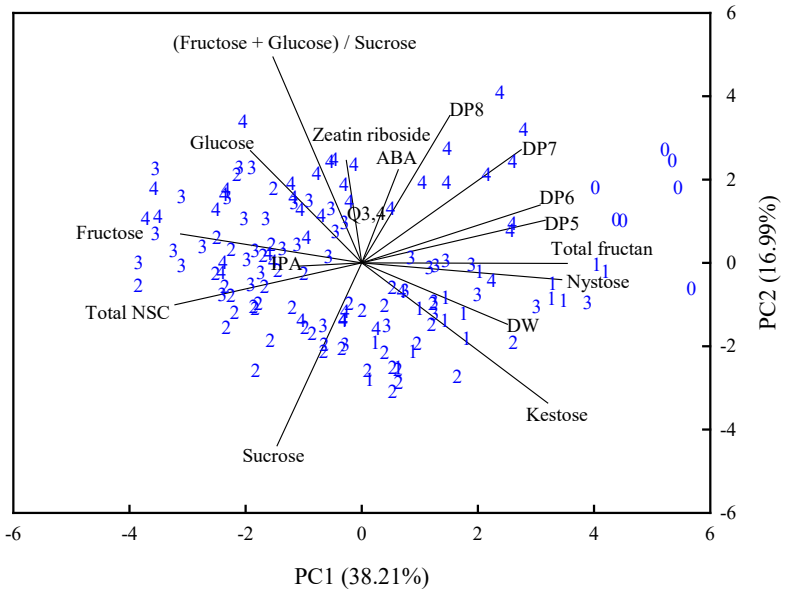


Figure 2. Principle component analysis biplot of onion cvs. Wellington and Sherpa bulbs cured at 20 or 28ºC and stored at 1, 3 or 6ºC; after harvest (0), after curing (1), and before (3) and after (4) sprouting in cold storage in 2008.

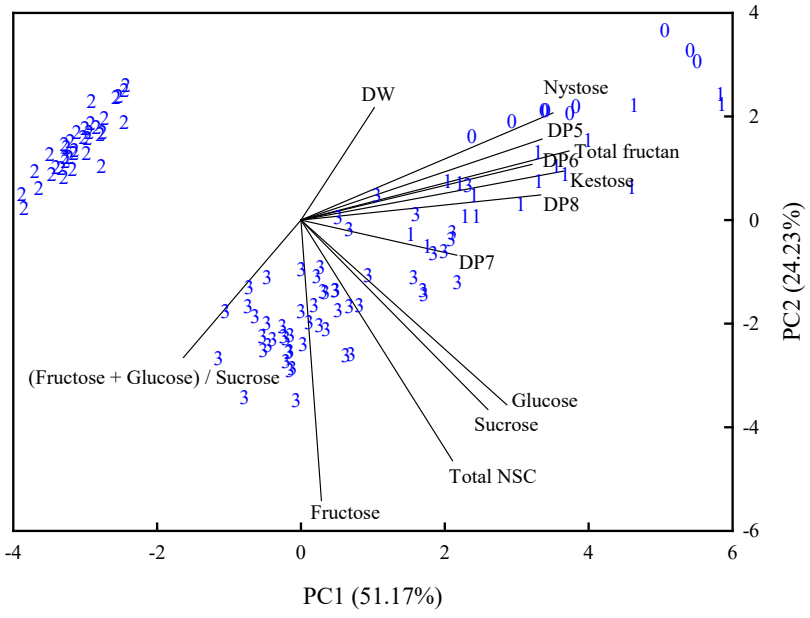


Figure 3. Principle component analysis biplot of onion cvs. Red Baron, Wellington and Sherpa bulbs cured at 20 or 28ºC and stored at 1, 3 or 6→1ºC; after harvest (0), after curing (1), and before (2) and after (3) sprouting in cold storage in 2009.

Effect of Postharvest Handling on Onion Gene Expression

Transcriptional profiles of onion bulbs during storage were determined to provide additional evidence for the metabolomic changes observed. Samples of RNA were prepared from onions of two cultivars at various physiological ages, *viz*, freshly harvested, cured, pre-sprouting and sprouting. Three biological replicates were hybridized for each cultivar and time combination. Curing temperature did not have an effect on the gene expression profile. In total, 3,111 and 1,406 probes were identified as being significantly (Benjamini and Hochberg (BH) corrected $p<0.05$) differentially expressed at one or more time points during curing and storage in onion cvs. Wellington and Sherpa, respectively. Of these, 1050 were common between the two cultivars, and 1036 remained after filtering for a fold change cut-off: >2.0. These entities were subjected to K-means clustering analysis, which revealed 8 clusters of temporal gene expression profiles, four representing up-regulated genes and four down-regulated genes (Figure 4). The microarray data was validated using qPCR revealing a strong correlation between the microarray fold changes and the qPCR fold changes of $r = 0.86$ (Figure 5).

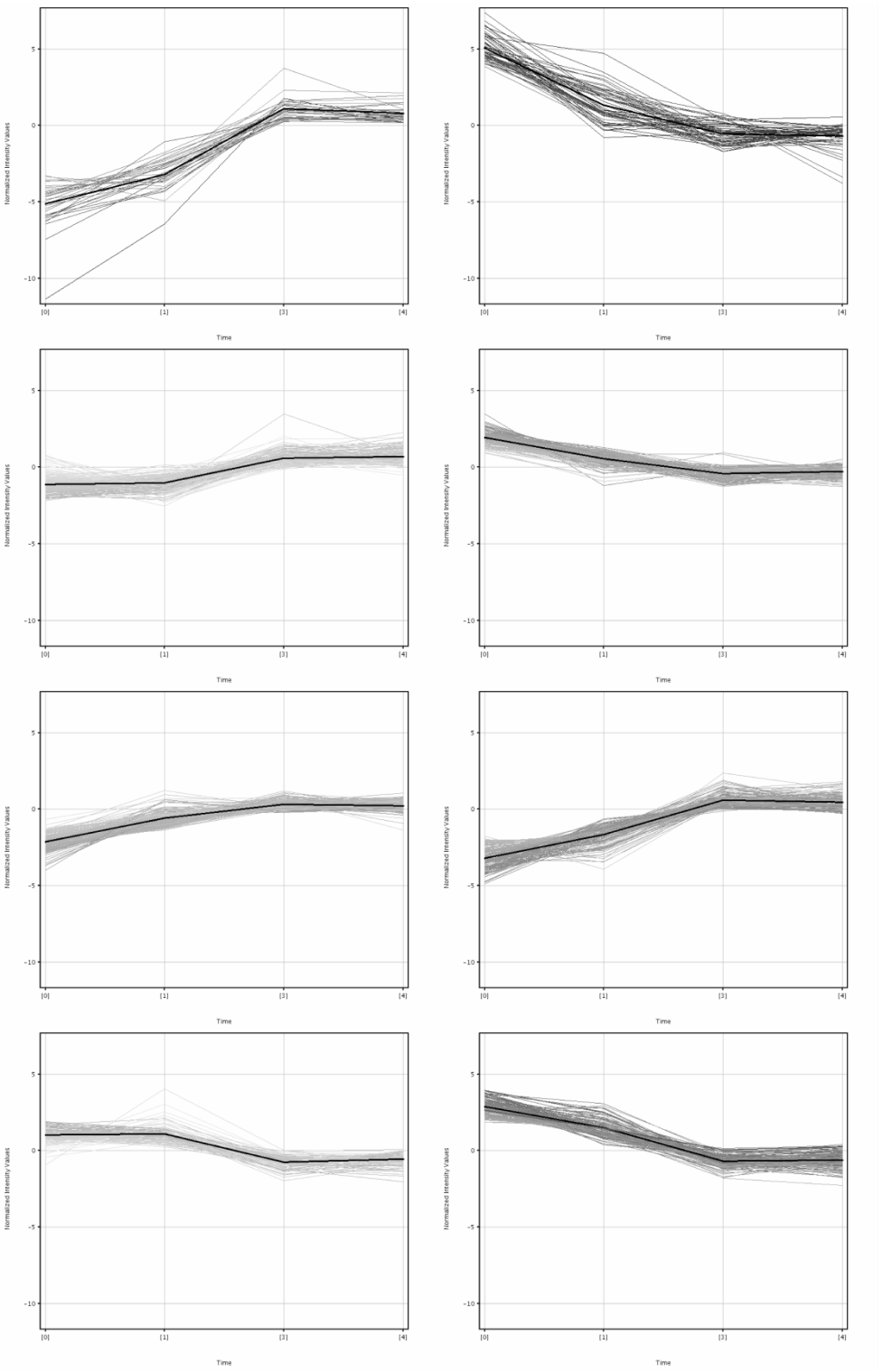


Figure 4. Cluster analysis for time course of gene expression. The altered gene expression was grouped by the K-means clustering algorithm.

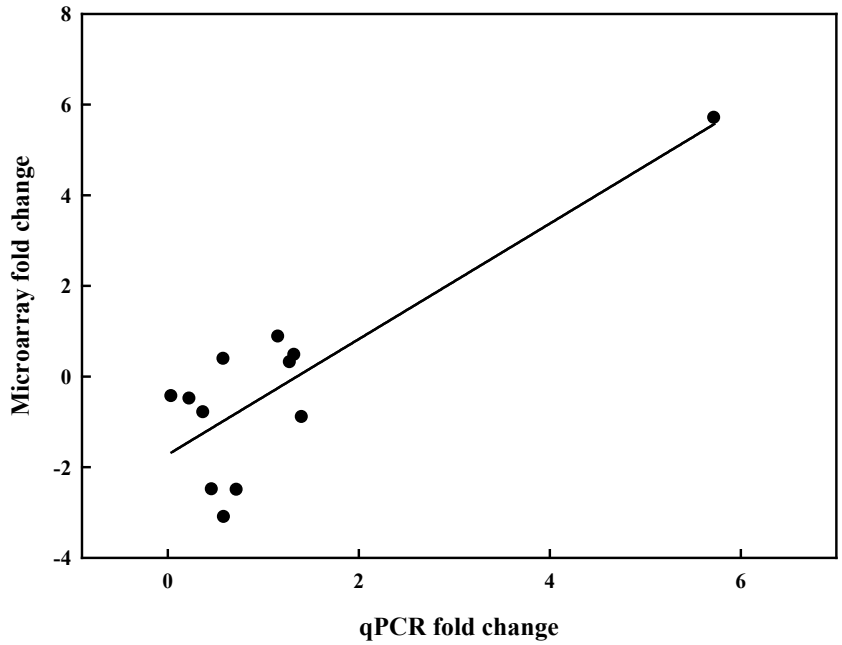


Figure 5 Correlation between the gene expressions of genes quantified using the onion microarray and qPCR. (r = 0.86, P < 0.001).

Every probe was compared with other known genes in GenBank non-redundant databases (Altshul *et al.*, 1997), and categorised into functional categories based on their homology to known genes. Three of the eight clusters contained probes with GO terms that were significantly (BH corrected p<0.05) over-represented. In cluster 1, GO terms for photosynthesis, thylakoid, plastid and cellular processes were significantly over-represented, and similarly, GO terms for thylakoid was significant in cluster 4, and RNA binding in cluster 7. These findings reflect the functional classification of the probes. Overall, *ca*. 34% of the probes were designated unclassified, and *ca*. 11% as housekeeping. The top 30 most down- and up-regulated genes are listed in Tables 2 and 3. The down-regulated genes are all from cluster 1, and most of the up-regulated genes are from cluster 0, with some from 2 and 5 (Tables 2 and 3). In cluster 1, many of the most highly down-regulated probes were annotated as having high homology to alliin lyase, which may suggest that this is the same gene, however, these probes were all designed to ESTs that map to different tentative consensus sequences. In addition, another probe annotated as having high homology with a gene involved in onion flavour metabolism, lachrymatory factor synthase, is also represented in this category. These probes account for the high representation of secondary metabolism in the functional categories (Table 4). In cluster 0, the largest category contained probes with homology to genes involved in stress and defence response. Probes annotated as being related to plant growth regulators appeared in cluster 2 (ethylene

responsive transcription factor, cyokinin-*O*-glucosyl transferase 1 and gibberellin receptor GID1L2), cluster 3 (ethylene receptor, S-ado met synthetase, auxin efflux carrier component), cluster 4 (auxin responsive gene family member), cluster 5 (gibberellin 20 oxidase 2), cluster 6 (auxin responsive gene family member, ethylene insensitive) and cluster 7 (auxin induced protein). Probes with homologies to genes involved in cell wall organisation were represented in clusters 2, 3 and 5 and probes with homologies to genes involved in the regulation of the cell cycle are most highly represented in the up-regulated clusters 2, 4 and 5.

Table 2. The 30 most down regulated genes postharvest in onion cvs. Sherpa and Wellington sampled at harvest, after curing at 20 or 28ºC for six weeks, and after 15 (non-sprouting) and 25 (sprouting) weeks storage at 1ºC.

Probe	Tentative annotation	Fold Change[a]	Onion sequence ID	Cluster
CUST_97_PI404013531	Chlorophyll A/B binding protein	322.9	X95687.1	1
CUST_10495_PI403527117	Alliin lyase precursor	266.5	BQ580283	1
CUST_2252_PI403527117	Starch synthase	256.8	TC6090	1
CUST_2105_PI403527117	Alliin lyase precursor	218.2	TC5943	1
CUST_9364_PI403527117	Alliin lyase precursor	197.6	BQ580052	1
CUST_312_PI403527117	Chlorophyll A/B binding protein	181.7	TC4150	1
CUST_3578_PI403527117	BURP domain containing protein	173.2	TC7416	1
CUST_10965_PI403527117	Starch synthase	169.5	BE205631	1
CUST_8035_PI404013528	Lipoxygenase	144.0	BI095683	1
CUST_11714_PI403527117	Alliin lyase precursor	138.6	BQ579865	1
CUST_6269_PI404013528	Alliin lyase precursor	137.7	BE205573	1
CUST_2477_PI403527117	Chlorophyll A/B binding protein	128.2	TC6315	1
CUST_1366_PI403527117	Chlorophyll A/B binding protein	118.8	TC5204	1
CUST_2054_PI403527117	3-hydroxy-3-methylglutaryl-coenzyme A reductase	116.2	TC5892	1

CUST_5317_PI403527117	Alliin lyase precursor	114.5	BQ579913	1
CUST_7489_PI403527117	Glutamate-cysteine ligase, chloroplast precursor	111.9	CF450735	1
CUST_6781_PI403527117	POEI3 - Pollen Ole e I allergen and extensin family protein precursor	102.3	CF435504	1
CUST_2601_PI403527117	POEI1 - Pollen Ole e I allergen and extensin family protein precursor	93.2	TC6439	1
CUST_5265_PI404013528	Adenine phosphoribosyltransferase	92.4	BQ580038	1
CUST_8043_PI404013528	Pherophorin-C2 protein precursor	90.3	BI095694	1
CUST_915_PI403527117	Annexin	85.8	TC4753	1
CUST_379_PI403527117	Chlorophyll A/B binding protein,	85.0	TC4217	1
CUST_14_PI404013531	Alliinase	70.2	Z12620.1	1
CUST_1269_PI403527117	MYB family transcription factor	69.3	TC5107	1
CUST_5044_PI403527117	Alliin lyase precursor	69.3	BQ580118	1
CUST_8097_PI403527117	3-hydroxy-3-methylglutaryl-coenzyme A reductase	65.4	CF449184	1
CUST_7_PI404013531	Alliinase	64.8	Z12621.1	1
CUST_42_PI404013531	Lachrymatory factor synthase	64.5	AB089203.1	1
CUST_342_PI403527117	Chlorophyll A/B binding protein	62.5	TC4180	1
CUST_102_PI404013531	Alliinase	61.9	M98267.1	1

[a] Fold change compared with expression at harvest, calculated as 2^x, where x=absolute value of (normalised condition 1 – normalised condition 2)

Table 3. The 30 most up regulated genes postharvest in onion cvs. Sherpa and Wellington sampled at harvest, after curing at 20 or 28ºC for six weeks, and after 15 (non-sprouting) and 25 (sprouting) weeks storage at 1ºC.

Probe	Tentative annotation	Fold Change[a]	Onion sequence ID	Cluster
CUST_7598_PI403527117	No significant match	8611.4	CF449784	0
CUST_1748_PI404013528	OsRCI2-6 - Hydrophobic protein LTI6B	434.9	AA508914	0
CUST_3047_PI403527117	LTPL129 - Protease inhibitor/seed storage/LTP family protein precursor	265.6	TC6885	0
CUST_2917_PI404013528	Alanine precursor	167.1	CF451215	0
CUST_1734_PI404013528	Glycine/proline-rich protein	139.1	AA451576	0
CUST_2079_PI403527117	Glycine-rich cell wall structural protein precursor	118.4	TC5917	0
CUST_1639_PI403527117	Caffeoyl-CoA O-methyltransferase	112.4	TC5477	0
CUST_5851_PI403527117	OsPOP4 - Putative Prolyl Oligopeptidase homologue	106.4	CF438948	0
CUST_11680_PI403527117	Integral membrane protein	105.7	CF443686	0
CUST_11157_PI403527117	Caffeoyl-CoA O-methyltransferase	104.6	CF443345	0
CUST_8031_PI404013528	Glutathione S-transferase	100.2	BI095676	0
CUST_7555_PI403527117	No significant match	96.5	CF450666	0
CUST_12036_PI403527117	Hypoxia-responsive family protein	88.0	CF441799	0
CUST_565_PI403527117	OsAPx2 - Cytosolic Ascorbate Peroxidase encoding gene 4,5,6,8	87.5	TC4403	0
CUST_3316_PI403527117	Glutathione S-transferase	82.1	TC7154	0
CUST_579_PI403527117	Stem-specific protein TSJT1	81.3	TC4417	0

CUST_10249_PI403527117	NADH-ubiquinone oxidoreductase chain 3	66.5	CF442073	0
CUST_11370_PI403527117	DUF538 domain containing protein	57.5	CF443772	0
CUST_9938_PI403527117	Endoribonuclease	57.2	CF445119	0
CUST_1014_PI403527117	Peroxidase precursor	50.4	TC4852	5
CUST_10976_PI403527117	Cysteine proteinase inhibitor 8 precursor	50.0	BE205657	0
CUST_10222_PI403527117	Pherophorin-C2 protein precursor	44.4	CF446675	2
CUST_995_PI403527117	Phosphate-induced protein 1 conserved region domain containing protein	44.3	TC4833	5
CUST_5445_PI403527117	HD domain containing protein 2	43.5	CF435292	5
CUST_4440_PI403527117	Multicopper oxidase domain containing protein	41.5	CF437212	0
CUST_2280_PI404013528	Gibberellin 20 oxidase 2	41.2	CF449051	5
CUST_7158_PI403527117	Kinase, pfkB family	39.6	CF437420	0
CUST_12100_PI403527117	Glycosyl hydrolase	36.6	CF446683	0
CUST_10751_PI403527117	Ubiquitin-conjugating enzyme	36.4	CF444379	0
CUST_917_PI403527117	Ribonucleoside-diphosphate reductase small chain	35.9	TC4755	5

[a] Fold change compared with expression at harvest, calculated as 2^x, where x=absolute value of (normalised condition 1 – normalised condition 2)

Table 4. Functional grouping of genes differentially expressed during onion curing and storage as a percentage of the probes in each K-means cluster.

Functional category	Cluster 0	1	2	3	4	5	6	7	Total up-regulated	Total down-regulated
Housekeeping	9.4	5.4	15.1	9.2	11.8	17.4	9.8	5.5	12.6	8.8
Stress / defence	21.9	1.8	1.0	11.2	2.6	7.6	4.5	10.3	4.8	8.5
Chaperones	-	7.1	0.5	0.7	1.0	0.7	0.9	0.7	0.7	1.7
Photosynthesis	-	19.6	1.5	0.7	0.5	2.1	0.9	2.1	1.5	3.7
Cell wall	-	-	2.5	5.9	0.5	2.1	-	1.4	1.6	2.6
Secondary metabolism	12.5	37.5	3.5	3.9	1.5	2.8	5.4	6.8	3.5	10.5
Cell death	-	1.8	0.5	0.7	-	-	-	-	0.1	0.6
Peptidase / kinase	9.4	-	7.5	5.9	7.2	2.8	7.1	6.8	6.3	5.7
Transport / secretion	6.3	-	8.0	8.6	9.7	9.0	7.1	8.9	8.9	6.5
Signalling	-	-	3.0	0.7	5.1	2.8	3.6	4.1	3.8	1.4
Metabolism	6.3	10.7	10.6	11.2	6.2	4.9	8.9	11.6	8.3	9.9
PGR-related	-	-	2.0	2.0	0.5	0.7	1.8	0.7	1.0	1.4
Cell cycle	-	1.8	5.0	0.7	3.6	4.2	1.8	0.7	3.5	1.1
Transcription factor	-	1.8	2.5	2.6	9.2	2.8	9.8	15.1	7.2	4.5
Phosphatase	-	-	1.0	-	1.0	-	0.9	1.4	0.9	0.3
Unclassified	34.4	12.5	35.7	36.2	39.5	40.3	37.5	24.0	35.2	32.7

Discussion

Biochemical and physiological analysis of onions of different cultivars (Wellington, Sherpa and Red Baron, not treated with maleic hydrazide) grown at different sites, cured at different temperatures (20, 24, 28ºC) and stored under different regimes (1, 3, 6, 6→1ºC) was carried out in order to study the mechanisms regulating postharvest onion sprout development. The underlying targeted metabolomic and transcriptomic changes which occured during curing and storage were determined. The first onion oligonucleotide microarray was constructed and used to determine differential gene expression in selected samples during onion curing and storage.

Biochemical and physiological analyses were supported by transcriptional changes; however, direct alignments between transcripts and metabolites were not possible due to the lack of comprehensive sequence data for onion. There were greater differences between samples at harvest and before sprouting, than between the samples taken before and after sprouting, with some significant changes occurring during the relatively short curing period. In addition, and in agreement with the biochemical and physiological analysis, no significant transcriptional differences could be found between the two curing temperatures. This suggests that there is no detrimental effect of reducing the curing temperature form 28 to 20ºC. The similar response of cvs. Sherpa and Wellington with storage time were also confirmed by the sprouting behaviour, although these varieties are reputed to have different commercial storage properties (Nigel Kingston, Syngenta, pers. comm). It is suggested that the reason for this may be that in this experiment, small changes in growth of the internal sprout leaves were monitored to give a true indication of the physiological state. If it is assumed that the onion bulb is in a dormant state at harvest, then the changes in gene expression that occur after this time are likely to represent the transition from endodormancy to sprout suppression, and to suggest that endodormancy may be relatively short – ending just after curing.

Bulb Mono to Disaccharide Ratio is a Potential Biochemical Marker for Sprouting

The changes in dry weight, pungency and concentrations of fructans and simple sugars and phenolics are consistent with those previously reported by (Chope *et al.*, 2006, 2007a; Downes *et al.*, 2010). Water-soluble carbohydrates in onion bulbs include glucose, fructose and sucrose, and a series of oligosaccharides called fructans. Changes in fructose, glucose and sucrose in stored onion bulbs are well reported and have been summarised by Chope *et*

al. (2007a). During storage in all years, the concentration of fructans decreased as they were enzymatically hydrolysed to form increasing amounts of fructose, as has previously been demonstrated by others (Suzuki and Cutliffe, 1989; Salama *et al.*, 1990; Pak *et al.*, 1995; Ernst *et al.*, 1998). It has been hypothesised that carbohydrate content is correlated with storage life. Suzuki and Cutliffe (1989) found a significant, but not large, positive correlation between fructan content and percent marketable bulbs in eight onion cultivars stored at 6 to 10ºC for four months. Higher fructose content at harvest was correlated with extended storage life in onion cv. Robusta bulbs stored at 4ºC for three months (Rutherford and Whittle, 1982). In this experiment, there was no significant effect of storage temperature on changes in sugars. Similarly, Benkeblia *et al.* (2002) found that the pattern of changes in total soluble sugar content of onion cv. Rouge Amposta bulbs was similar at 4, 10 and 20ºC, suggesting that the catabolism of carbohydrates is more dependent on physiological stage than temperature. In contrast, fructose concentration was higher in onion cv. Sentinel bulbs stored at 0 and 15ºC than in those stored at 30ºC, suggesting that hydrolysis of fructans increased at low temperatures (Salama *et al.*, 1990). Is it likely that the range of storage temperatures tested here (1 to 6 ºC) was too narrow to show any difference in sugar metabolism. It has previously been suggested that a peak in glucose (Benkeblia and Selselet-Attou, 1999) or sucrose (Benkeblia *et al.*, 2005) precedes sprouting, however, these phenomena seem to be cultivar specific and a universal biochemical marker of sprouting is yet to be identified. Using PCA, the ratio of monosaccharides (fructose and glucose) to disaccharide (sucrose), along with the concentration of ZR has been predicted as an important factor in discriminating between sprouting and pre-sprouting samples. The mono to disaccharide ratio has the advantage that it is easier and more cost-effective than ZR to measure. With the screening of a detailed time course during storage of a number of samples from a range of different cultivars it is possible that this relatively simple parameter could give important information on the dormancy / sprouting status of a stored onion bulb.

Association of Gene Expression Data with Dormancy and Sprout Suppression

Onions bulbs have evolved as a storage organ to allow the plant to overwinter. During the transition from dormancy to sprout suppression (endodormancy to ecodormancy) and subsequent growth, the bulb undergoes the transition from sink organ to source, to sustain cell division in the meristematic tissue. The mechanisms controlling these processes are yet to be elucidated.

Probes with tentative annotations relating to defence / stress related function formed the second largest functional category (following unclassified) in the highly up-regulated cluster

0. Similarly, a high proportion of defence-related transcript derived fragments were differentially expressed during the potato tuber lifecyle (Trindade *et al.*, 2004) and during endodormancy release in raspberry buds (Mazzitelli *et al.*, 2010). In the highly down-regulated cluster 1, *ca*. 20% of the probes were placed in the functional category related to photosynthesis. Bulb formation is well known to be influenced by photoperiod, and as light is perceived by the leaves and the signals transmitted to other plant parts, then the down-regulation of these light responsive elements could follow the die back and subsequent removal of the leaves at harvest.

It would be expected that expression of genes related to cell cycle regulation would increase during storage, as the meristematic cells begin to divide and elongate to form new sprout tissue. Indeed, up-regulated clusters 2, 4 and 5 contained 10, 7, and 6 probes, respectively, with tentative annotation for genes including histones and tubulins. It is also likely that the transition from dormancy to sprout suppression will also involve changes in the cell walls, as modifications are made to control the transport of reserve compounds and cell division resumes at the meristem. In general, cellulose synthase decreased postharvest (6 probes with tentative annotation for cellulose synthesis down-regulated), and indeed, cellulose concentration has been shown to decrease in onions cv. MBL87-WOPL stored at 6.6ºC for 12 weeks (Coolong *et al.*, 2008). Polygalacturonase and pectinesterase have been associated with a decrease in onion firmness caused by degradation of the middle lamella (Coolong *et al.*, 2008). In the results reported here, polygalacturonase and pectinesterase, along with beta galactosidase and pectate lyase, which are both involved in cell wall softening, were up-regulated after harvest (8 probes in clusters 2, 4 and 5). Taken together, this suggests that the cell walls are being degraded, and the bulb becoming softer, however, an increase in expression of the transcript does not necessarily translate to an increase in enzyme activity.

The fact that many temporal changes in expression were detected in the probes on the onion microarray suggests that the possibility of generating a diagnostic microarray chip that could predict sprouting exists. In order to create this, a more detailed time course between curing and the onset of sprouting would be necessary, together with more sequence data for onions. Although many probes with different expression patterns were identified, the relatively small coverage of the onion genome on the microarray used in our experiments means that genes with still greater differences in expression are yet to be discovered, and these in turn could provide breeders with targets to manipulate storage life. The samples in this study consisted of a longitudinal wedge of bulb tissue, which gives information about the real concentrations of each substance in the bulb at that time, but does not provide

information about transport within the bulb. Therefore, detailed spatial analysis will be necessary in the future, as certain parameters such as glucose and pyruvate have been shown to be differentially distributed in the bulb (Abayomi and Terry, 2009).

Plant Growth Regulators Mediated Control of Dormancy and Sprouting

During postharvest storage, a gradual change in the relative composition of plant growth regulators occurs as the levels of growth inhibitors drop and the levels of growth promoters rise (Thomas, 1969; Thomas and Isenberg, 1972). Abscisic acid has many physiological effects, many related to the response to water and cold-stress, including bulb and seed dormancy, inhibition of germination, stomatal closure and inhibition of cell elongation. The amount of ABA in the plant is a balance between synthesis and degradation. Plant development, environmental conditions such as drought stress, and other growth regulators affect these processes (Nambara and Marion-Poll, 2005). An effect of curing temperature on ABA concentration was only observed in bulb samples produced in 2007, suggesting that there may be an interaction with growing season. However, ABA concentration decreased during curing in both 2007 and 2008 and then increased after the onset of sprout growth, as was previously observed (Chope *et al.*, 2006, 2007a, b). Chope *et al.* (2006, 2007a, b) found that a decline in endogenous ABA was correlated with storage life under both controlled atmosphere and regular atmosphere conditions. Yamazaki *et al.* (1999a) also demonstrated a functional role of ABA in maintaining bulb dormancy in *A. wakegi*, a cross between Japanese bunching onion and shallot.

No probes with significant changes in expression pattern were annotated as being directly related to ABA. However, ABA is closely associated with the toleration of drought stress, which is undoubtedly experienced by a stored onion. Aquaporins are proteins that control water movement in and out of the cell, thus managing water relations (Chaumont *et al.*, 2005), and are thought to be partly controlled by ABA. Probes with putative annotations as aquaporins were identified in clusters 7 (highly down-regulated, n=5) and 4 (slightly up-regulated, n=2). Similarly, Mazzitelli *et al.* (2007) observed a down-regulation of ESTs with similarity to an aquaporin gene in raspberry buds during the transition from endo-dormancy to para-dormancy. Here, two probes were also up-regulated, however, aquaporins are part of a large and divergent gene family, which may be differentially regulated.

In this study, the cytokinins ZR, which decreased during curing, and IPA, which did not, were found to increase during storage and peaked with the onset of sprouting. Cytokinins stimulate cell division and this increase in growth promoting substances (Galuszka *et al.*, 2008), which begins immediately after curing suggests that there is a threshold above which

sprouting occurs, or, more likely that the onset of sprouting is controlled by a delicate balance between the concentrations of growth promoters (such as cytokinins) and growth inhibitors (such as ABA). A putative cyokinin-*O*-glucosyl transferase 1 was up-regulated in cluster 2. The glucosylated form of zeatin is thought to be important in transport, storage and protection against cytokinin oxidases, as *O*-glycosylation is reversible. This enzyme is likely to regulate the active and storage forms of zeatin. Therefore an increase in expression of this probe could represent an increase in transport of cytokinins, particularly as the increase occurs mostly between samples after curing and before sprouting. Similarly, the greatest changes in ZR and IPA occurred between these times, strongly suggesting that an increase in cytokinins is necessary to stimulate cell division at the meristem.

Ethylene is a plant growth regulator that is clearly fundamental to the postharvest physiology of many fresh produce types; however the literature on the role of ethylene in onion bulb dormancy and storage life is far from comprehensive. There are conflicting reports on how ethylene affects onion storage life. The observation that onion cv. Elba Globe bulbs produced ethylene at much greater amounts (actual amounts not specified) at the end of dormancy than at the beginning (Abdel-Rahman and Isenberg, 1974) suggests that ethylene may have a role in sprouting. In contrast, Benkeblia and Selselet-Attou (1999) found little variation in ethylene production (range of 4.4 – 4.6 nmol kg^{-1} hr^{-1}) of onion cv. Rouge Amposta bulbs during six months storage at 18°C and 70% RH. The dichotomy between these findings implies that production of ethylene by onion bulbs is likely to be cultivar dependent and that further investigation is required. An ethylene receptor, S-ado met synthetase (ACC synthase) (cluster 3) and ethylene insensitive, (a DNA binding protein which regulates transcription in response to ethylene and is essential for ethylene mediated responses) (cluster 6) were down-regulated. This suggests that onions become less sensitive to ethylene, and also produce less ethylene with time in storage, however, one probe with annotation relating to ethylene was up-regulated, *viz*. an ethylene responsive transcription factor (cluster 2). Onions are regarded as non-climacteric in their response to ethylene, and, as such, have consistently low endogenous ethylene levels and lack the dramatic change in ethylene production found in climacteric fruit which triggers ripening (Downes *et al.*, 2010). There is a paucity of research on endogenous ethylene production of stored onions, however, both the continuous application of ethylene gas has been shown to increase the storage life of onions (Bufler, 2009). More recently, a single 24 h treatment with ethylene before curing has been shown to suppress sprouting in onions cv. Sherpa (Downes *et al.*, 2010). Taken together, the efficacy of the single treatment and the potential reduction in sensitivity to ethylene throughout storage suggests that continuous ethylene treatment may not be necessary.

The concentrations of gibberellins and auxins were not measured, however, in cluster 2, a gibberellin receptor GID1L2 was up-regulated. Gibberellins (GAs) stimulate cell elongation, so an up-regulation in its receptors could be explained by meristematic tissue preparing for growth. One of the most significantly up-regulated probes in cluster 5 is annotated as a gibberellin 20 oxidase, which is involved in the production of active GAs. Taken together, this suggests a role for gibberellins in dormancy release. An auxin efflux carrier component (cluster 3) was down-regulated along with an auxin responsive gene family member (cluster 6) and an auxin induced protein (cluster 7). However, an auxin responsive gene family member was up-regulated (cluster 4). This suggests that as auxins may be more important in elongation of the sprout after initial growth has begun. This is contrary to results reported by Thomas (1969), who found that gibberellins were present in bulbs with advanced sprouts, and auxin in bulbs with small sprouts, PGRs reported by Thomas (1969) were quantified using bioassays, which may not have been sensitive enough to pick up changes occurring earlier, however, gene expression studies do not take posttranslational modifications into account.

Conclusion

Here we provide a detailed analysis of physiological, biochemical and transcriptional changes in onion bulbs during postharvest storage. These detailed analyses provide novel insights into key regulatory triggers for sprout dormancy release in onion bulbs and provide the potential for the development of biochemical or transcriptional markers for sprout initiation.

Based on data presented here, there is evidence to support the modification of current curing practice in the UK, which has not been re-evaluated since it was proposed in the 1970s. Artificial postharvest onion curing in the UK is typically performed at 28°C. However, evidence presented here suggests there is no detrimental effect on bulb storage life and quality caused by curing at 20°C, producing a considerable saving in energy and costs.

INVESTIGATIONS INTO THE EFFECT OF POSTHARVEST TREATMENTS ON ONION BULB QUALITY

Katherine Downes, Gemma A. Chope and Leon A. Terry.

Plant Science Laboratory, Cranfield University, Bedfordshire, MK43 0AL, UK

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; Chope and Terry, 2008). Currently, standard methods of suppressing sprouting in temperate climates include controlled atmosphere (CA), ambient or cold storage with/without controlled relative humidity in addition to the preharvest application of maleic hydrazide (1,2-dihydro-3,6-pyridazinedione; MH). The continued use of MH is uncertain as it remains the only chemical used on onions that leaves detectable residues (4–6 mg kg^{-1}) (Johnson, 2006). Additionally, MH is considered by some to be detrimental to the environment with concerns over the risk of it leaching into drinking water (Sorensen and Grevsen, 2001). Increasing pressure from retailers and consumers alike has driven the search for alternative methods to extend onion storage life.

Onions are regarded as non-climacteric with consistently low endogenous ethylene production during storage (<0.1 µL kg^{-1} h^{-1} at 0–5°C) (Suslow, 1998). However, the continuous application of ethylene at the supposedly saturated concentration of 10.2 µL L^{-1} and the preharvest application of Ethephon have both been shown to increase the storage and shelf-life of onions (Adamicki, 2005; Bufler, 2009). Ethephon is an ethylene yielding chemical which when applied to plants can elicit a response characteristic of ethylene treatment (Yang, 1969). Application of Ethephon (1.8 L ha^{-1}) sprayed directly onto onion foliage 2 weeks prior to harvest reduced sprout incidence 2-fold compared with controls after 22 weeks storage at 0–1°C (Adamicki, 2005). However, preharvest application of Ethephon has been found to significantly reduce yield by reducing bulb diameter and weight (Thomas and Rankin, 1982). Recently, systems which produce a continuous supply of ethylene (10 µL L^{-1}) have been introduced into onion and potato stores to suppress sprouting (Chope and Terry, 2008). The Restrain system uses a catalyst to convert ethanol into ethylene and water whereas the Biofresh system is essentially a sensor which uses pure ethylene gas to achieve correct ethylene levels in store (Biofresh, 2010; Restrain, 2010) Sprout suppression using ethylene treatment could help at least partly replace MH, thus eliminating the

perceived risk of chemical residues. In addition, the use of ethylene concentrations anywhere below 30,000 μL L^{-1} poses no hazard to workers (Briddon A., pers. comm.), and the potential integration of ethylene treatment with CA, and low or high temperature storage remains a possibility. In direct contrast to the positive effects of ethylene on onion storage, the blocking of ethylene perception by 1-methylcyclopropene (1-MCP) may be expected to have a deleterious effect on onion storage. This has been demonstrated by Bufler (2009) who found treatment with 1-MCP (0.25 μL L^{-1}, 5 h, 20°C) 4 weeks after harvest (2 weeks drying at 25°C plus 2 weeks storage at 18°C) reduced dormancy during storage at 18°C by 2 weeks compared with the control onions cv Copra. However, Chope *et al.* (2007a) found that a single 24 h treatment with 1-MCP (1 μL L^{-1}, 24 h, 20°C) after harvest (9 days drying at 30°C plus 2 weeks in ambient air) reduced sprout length and maintained higher glucose and fructose concentrations in onion cv. SS1 bulbs (low dry matter and thin skinned) stored at 12°C. When considered together, the results from these studies suggest that a contradiction may exist whereby both ethylene and 1-MCP have both been shown to reduce sprout incidence in onion. Although Chope *et al.* (2007a) found treatment with 1-MCP reduced sprout growth when stored at 12°C, the opposite effect was found when the onions cv. SS1 were subsequently stored at 20°C. It has been hypothesised that lower temperatures may reduce the affinity of the ethylene binding site for 1-MCP (Blankenship and Dole, 2003).

The aim of this study was to investigate whether a single 24 h treatment of ethylene or 1-MCP was sufficient to affect the physiological and biochemical properties of cold-stored onion bulbs of medium and long-storage potential. Treatments were applied before or after curing and subsequently stored in different cold storage regimes to investigate whether curing or cold storage conditions affected treatment efficacy. To elucidate the effects of ethylene and/or 1-MCP on onion bulb physiology and biochemistry, sprout length, respiration rate, antioxidant capacity, sugars, plant growth regulators and pungency were measured.

Three experiments were conducted in successive years investigating the effects of ethylene and 1-MCP on onion bulb physiology and biochemistry. The results from 2007/08 have been published in Postharvest Biology and Technology and can be found in Appendix A. Results from 2008/09 have been described in Section C and results from 2009/10 are described and summarised below.

Materials And Methods

Plant material and curing

Onion seeds cv. Sherpa were drilled on sandy clay loam (Alistair Findlay's, Cardington, Beds., UK; 1.2 x 0.3 ha) on 16th March 2009 at a rate of 54 seeds m^{-2} with pesticides applied as per commercial practice. Plants were machine-harvested at 100% fall-down on 8th September 2009. Onion bulbs were stored in 45 large nets (approx. 30 bulbs) and 15 half nets (approx. 15 bulbs) buried amongst loose bulbs in one tonne wooden crates for batch curing at the Sutton Bridge Experimental Unit (Lincs., UK). Bulbs were cured at 20°C for six weeks as per normal commercial practice in the UK with relative humidity controlled at 65 – 75%.

Experimental design

The experiment was a completely randomised design with three replicates taken from three sections of the field. There were five postharvest treatments per replicate viz. 1 µL L^{-1} 1-MCP before curing (MB), 10 µL L^{-1} ethylene before curing (EB), both ethylene and 1-MCP before curing (EMB), both ethylene and 1-MCP after curing (EMA) and control (no treatment). Treatments were applied in water-sealed air tight polypropylene chambers (88 cm x 59 cm x 59 cm) which housed two 8 x 8 cm electric fans (Nidec Beta SL, Nidec, Japan) to circulate the gases during treatments. Onions were treated in the chambers for 24 h at 20°C and the control bulbs held at 20°C in air. Levels of CO_2 were not controlled during treatment due to the short treatment time and low production of CO_2 by onions (0.13 – 0.17 mmol kg^{-1} h^{-1} at 0-5°C; 1.12 – 1.21 mmol kg^{-1} h^{-1} at 25-27°C) (Suslow, 1998). The 1-MCP was applied by adding 1.8 g SmartFresh (0.14%, Rohm and Haas, PA) to a 50 mL conical flask and sealed with Nescofilm (Bando Chemical Ind. Ltd., Kobe, Japan). To release 1 µL L^{-1} 1-MCP gas, 20 mL warm (50°C) water was injected into the conical flask through the Nescofilm using a needle and syringe prior to transfer to the chamber (Chope *et al.,* 2007a). Ethylene treatment (10 µL L^{-1}) was administered by injecting 3.25 mL ethylene (100% ethylene; SIP Analytical Ltd., Kent, UK) directly into the chamber via a tapped tube (polyvinyl chloride) followed by repeated full withdrawal-injection displacements to flush the ethylene into the chamber.

Pre-storage treated onions

After curing, onions were transported to CU within 2.5 h. Diseased or damaged bulbs were removed and the remaining bulbs randomly placed in individual plastic stackable crates and

stored in air for 31 weeks at 1 or 6°C in the dark. A third batch of treated onions were stored at 6°C then transferred to 1°C after assessment at 19 weeks. Four bulbs per treatment, storage temperature and replicate (*n* = 180) were selected randomly at each outturn, taken after harvest (day 0), immediately after curing (six weeks) then at intervals during cold storage (19 and 31 weeks after harvest) (*n* = 720).

Pre-storage and storage treated onions

After curing, a subset of the treated onion bulbs was transported to the Allium and Brassica Centre (ABC) (Lincs., UK) for continuous ethylene treatment. Onions pre-treated with EB, MB, EMB, EMA or no treatment were stored in 10 µL L^{-1} ethylene (supplied using pure ethylene gas) for 31 weeks at 6°C. Four bulbs per pre-storage treatment and replicate (*n* = 60) were selected at random at the end of storage (31 weeks). For logistical reasons, it was not possible for a subset of bulbs to be stored in continuous air at the ABC as a storage treatment control therefore the onions treated in continuous ethylene were compared with the control bulbs held at CU at 6°C.

Sample Preparation

For the CU samples, each onion bulb was halved and sprout growth and rooting recorded as a % of the bulb height in mm and as the presence or absence of roots, respectively. One half was then cut again into quarters and a 2.5g sample of the base section and 5 g sample of the remaining section weighed. The sections from each of four bulbs were pooled to give a 10 g base plate sample and 20 g top section, respectively. From the other half of the bulb, a whole bulb sample was taken and pooled to give a 20 g sample. The top, bottom and whole bulb samples were snap-frozen in liquid nitrogen and then stored at -40°C. Frozen tissue was lyophilised and powdered according to section A materials and methods. The whole samples were analysed for dry weight, sugar and fructan concentrations and the spatial sections analysed for dry weight, sugar and fructans and hormone concentrations. Only sprout and root growth were measured in the onions sent to the ABC for continuous ethylene treatment.

Shelf life

At the end of storage (31 weeks), ten bulbs from each treatment, storage temperature and replicate were removed from cold storage at CU and ABC and transferred and transferred to 20°C for 2 weeks to measure shelf life. Onions were halved and sprout length as a % of the bulb height and the presence/absence of rooting was recorded from all ten bulbs.

Physiological measurements

Respiration rate

Four onion bulbs were placed in 3 L jars with air tight lids and septum. The jars were sealed for 4 h at room temperature and gas samples removed with repeated full withdrawal-injection displacements using a 30 mL plastic syringe (Chope *et al.,* 2007a). Gas samples were analysed using gas chromatography (GC model 8340, DP800 integrator, Carlos Erba Instruments, Herts., UK) with hot wire detection for CO_2 analysis (Chope *et al.,* 2007a). The GC was calibrated using 10.06% CO_2 (10% CO_2, 2% O_2, 88% N_2; Certified Standard from BOC). The four onions were weighed and respiration rate calculated in mmoles kg^{-1} h^{-1}.

Biochemical measurements

Extraction and quantification of sugars and fructans

Extraction and quantification of non-structural carbohydrates was carried out according to section A materials and methods

Extraction and quantification of hormones

Extraction and quantification of hormones was carried out according to section A materials and methods

Statistical analysis

Analysis of variance was conducted according to section A materials and methods with modification. The first sampling time (day 0; before curing) consisted of four treatments and the outturns thereafter consisted of five treatments. In addition, due to the different storage temperatures the first two sampling times (day 0 and week 6; before and after curing) consisted of only one curing temperature whereas the outturns thereafter consisted of two storage temperatures; 1 and 6°C. This imbalance was resolved by including two baselines; the first time point was considered as baseline 1 and the first two time points considered as baseline 2 to which the remaining time points could be compared. After the third sampling outturn, a subset of onions stored at 6°C was transferred to 1°C creating a third storage temperature and therefore an additional imbalance. As a third baseline could not be included in the ANOVA, all data from onions stored at either 6 or 1°C was analysed together and the final outturn analysed again separately including all three storage temperatures; 1, 6 and 6 → 1°C. Hormone data was not normally distributed therefore data was log transformed before performing statistical analyses.

Results

Sprouting

Onions treated after curing with ethylene and 1-MCP and stored at 6°C had significantly shorter sprouts than the control bulbs 19 weeks after harvest (Table 1). However by week 31, sprout growth was no longer significantly different between treated and untreated onions. Although sprout incidence also appeared to be lower 19 weeks after harvest in onions treated with ethylene and 1-MCP after curing then stored at 6°C, the difference was not significant (P = 0.177) (Table 2). Sprout growth and sprout incidence was shorter and lower, respectively, in onions stored at 1°C 19 weeks after harvest compared to those stored at 6°C. This trend was still apparent 31 weeks after harvest with those onions transferred from 6 → 1°C after 19 weeks containing sprouts that were longer than those held at 1°C but shorter than those onions which remained at 6°C (Table 1). This same trend was reflected in the sprout incidence after 19 weeks but not maintained for 31 weeks (Table 2). Shelf life of the pre-storage treated onions was not affected by treatment or storage temperature (Table 1) and the sprout incidence for all onions was 100%.

Sprout growth of the onions stored at 6°C in continuous ethylene differed between the treatments with those treated with ethylene and 1-MCP before curing being on average the shortest (52.9% of bulb height) and those treated with ethylene and 1-MCP after curing having on average the longest sprouts (65.7% of bulb height). That said, the sprout length of the treated bulbs was not significantly different from the control bulbs (61.7% of bulb height). As the bulbs in continuous ethylene were stored at 6°C the sprout incidence was 100% for all onions 31 weeks after harvest plus shelf life. No significant differences between shelf life were found between treated and untreated onions stored in continuous ethylene (Table 3).

The overall means of sprout length between those stored in air at 6°C and those stored in continuous ethylene at 6°C showed that onions stored in air had significantly longer sprouts (69.3% of bulb height) 31 weeks after harvest compared with those held in ethylene (58.4% of bulb height). However, whether the onions were stored in air or continuous ethylene had no effect on shelf life sprout length (90.7 and 90.3% of bulb height, respectively).

Table 1 Sprout length (% of the bulb height) of cv. Sherpa measured 19 and 31 weeks after harvest plus two weeks shelf-life (20°C) treated before or after curing (six weeks at 20°C) with 10 µL L^{-1} ethylene and/or 1 µL L^{-1} 1-MCP for 24 h at 20°C then transferred to cold storage (1, 6 or 6-1°C) (*n* = 12).

Treatment	Sprout length (% of bulb height)							
	19 weeks		31 weeks			Shelf-life		
	1°C	6°C	1°C	6°C	6-1°C	1°C	6°C	6-1°C
Control	0.0^{a}	28.6^{b}	42.5^{abc}	72.3^{e}	55.3^{abcde}	78.4^{a}	86.8^{a}	93.9^{a}
EB	4.3^{a}	26.0^{b}	38.7^{a}	74.4^{e}	58.5^{bcde}	85.9^{a}	94.0^{a}	88.9^{a}
MB	0.0^{a}	37.6^{b}	39.4^{ab}	72.2^{e}	51.2^{abcd}	79.2^{a}	89.5^{a}	78.3^{a}
EMB	6.1^{a}	27.2^{b}	47.4^{abc}	66.9^{de}	58.3^{bcde}	84.5^{a}	90.7^{a}	83.7^{a}
EMA	5.0^{a}	9.1^{a}	43.0^{abc}	60.8^{cde}	48.7^{abcd}	79.4^{a}	92.4^{a}	81.3^{a}

Treatments: EB, ethylene before curing; MB, 1-MCP before curing; EMB, ethylene and 1-MCP before curing; EMA, ethylene and 1-MCP after curing. LSD (*P* = 0.05), 19 weeks = 14.45, 31 weeks = 19.49; shelf-life = 19.97.

Table 2 Sprout incidence (% of bulbs sprouted) of cv. Sherpa measured 19 and 31 weeks after harvest treated before or after curing (six weeks at 20°C) with 10 µL L^{-1} ethylene and/or 1 µL L^{-1} 1-MCP for 24 h at 20°C then transferred to cold storage (1°C) (*n* = 12).

Treatment	Sprout incidence (% of bulbs sprouted)				
	19 weeks		31 weeks		
	1°C	6°C	1°C	6°C	6-1°C
Control	0.0^{a}	50.0^{c}	91.7^{ab}	100.0^{b}	91.7^{ab}
EB	8.3^{ab}	58.3^{c}	91.7^{ab}	91.7^{ab}	100.0^{b}
MB	0.0^{a}	33.3^{bc}	83.3^{ab}	100.0^{b}	100.0^{b}
EMB	8.3^{ab}	33.3^{bc}	91.7^{ab}	83.3^{ab}	100.0^{b}
EMA	16.7^{ab}	16.7^{ab}	75.0^{a}	91.7^{ab}	100.0^{b}

Treatments: EB, ethylene before curing; MB, 1-MCP before curing; EMB, ethylene and 1-MCP before curing; EMA, ethylene and 1-MCP after curing. LSD (*P* = 0.05), 19 weeks = 32.70; 31 weeks = 22.66.

Table 3 Sprout length (% of the bulb height) of cv. Sherpa measured 31 weeks after harvest plus two weeks shelf-life (20°C) treated before or after curing (six weeks at 20°C) with 10 µL L^{-1} ethylene and/or 1 µL L^{-1} 1-MCP for 24 h at 20°C then transferred to continuous ethylene storage (6°C) (*n* = 12).

Treatment	Sprout length (% of bulb height)	
	31 weeks	Shelf-life
Control	61.7[ab]	91.4[ab]
EB	56.5[a]	78.0[a]
MB	55.6[a]	92.2[ab]
EMB	52.9[a]	95.4[b]
EMA	65.7[b]	94.2[b]

Treatments: EB, ethylene before curing; MB, 1-MCP before curing; EMB, ethylene and 1-MCP before curing; EMA, ethylene and 1-MCP after curing. LSD (*P* = 0.05), 31 weeks = 8.91; shelf-life = 15.20.

Rooting

The short 24 h pre-storage treatments had no significant effect on the rooting incidence of onion bulbs 31 weeks after harvest and after two weeks shelf-life. The only significant differences in rooting of onions stored in air were between storage temperatures, however this was only apparent after two weeks shelf life at 20°C. The onions held at 6°C or transferred from 6 → 1°C had lower root incidence than those stored at 1°C (Table 4).

There were no significant differences in rooting incidence between treated and untreated onions in those stored in continuous ethylene 31 weeks after harvest and after 2 weeks shelf life (Table 5). Comparing the onions held in air at 6°C with those held in continuous ethylene at the same temperature found root incidence was reduced in onions stored in air 31 weeks after harvest (31.7% of bulbs rooting) and after two weeks shelf life (15.9% of bulbs rooting) compared with those held in continuous ethylene (46.7% and 27.3% of bulbs rooting, respectively).

Table 4 Root incidence (% of bulbs rooting) of cv. Sherpa measured 31 weeks after harvest treated before or after curing (six weeks at 20°C) with 10 µL L^{-1} ethylene and/or 1 µL L^{-1} 1-MCP for 24 h at 20°C then transferred to cold storage (1, 6 or 6-1°C) (*n* = 12).

Treatment	Root incidence (% of bulbs rooting)					
	31 weeks			Shelf-life		
	1°C	6°C	6-1°C	1°C	6°C	6-1°C
Control	16.7^{a}	25.0^{ab}	50.0^{b}	23.3^{abc}	10.0^{abc}	10.7^{abc}
EB	16.7^{a}	33.3^{ab}	50.0^{b}	30.0^{bc}	3.3^{a}	33.3^{c}
MB	41.7^{ab}	25.0^{ab}	25.0^{ab}	31.0^{bc}	29.7^{bc}	6.7^{ab}
EMB	41.7^{ab}	41.7^{ab}	25.0^{ab}	27.7^{abc}	29.7^{bc}	6.7^{ab}
EMA	25.0^{ab}	33.3^{ab}	25.0^{ab}	33.3^{c}	6.7^{ab}	13.3^{abc}

Treatments: EB, ethylene before curing; MB, 1-MCP before curing; EMB, ethylene and 1-MCP before curing; EMA, ethylene and 1-MCP after curing. LSD (*P* = 0.05), 31 weeks = 28.85; shelf-life = 26.34.

Table 5 Root incidence (% of bulbs rooting) of cv. Sherpa measured 31 weeks after harvest plus two weeks shelf-life (20°C) treated before or after curing (six weeks at 20°C) with 10 µL L^{-1} ethylene and/or 1 µL L^{-1} 1-MCP for 24 h at 20°C then transferred to continuous ethylene storage (6°C) (*n* = 12).

Treatment	Root incidence (% of bulbs rooting)	
	31 weeks	Shelf-life
Control	41.7^{a}	26.7^{a}
EB	41.7^{a}	30.0^{a}
MB	41.7^{a}	30.0^{a}
EMB	41.7^{a}	20.0^{a}
EMA	66.7^{a}	30.0^{a}

Treatments: EB, ethylene before curing; MB, 1-MCP before curing; EMB, ethylene and 1-MCP before curing; EMA, ethylene and 1-MCP after curing. LSD (*P* = 0.05), 31 weeks = 27.85; shelf-life = 23.57.

Respiration rate

Overall, the respiration rate of onions treated with ethylene alone or in the presence of 1-MCP was 1.5-fold higher than untreated onions or those treated with 1-MCP (Figure 1). From the analysis of the final outturn including the onions swapped from 6 → 1°C at 19 weeks, the onions stored at 1°C had the highest respiration rate (0.1787 mmol CO_2 kg^{-1} h^{-1}) followed by onions stored at 6°C (0.1523 mmol CO_2 kg^{-1} h^{-1}) and 6 → 1°C (0.1162 mmol CO_2 kg^{-1} h^{-1}). The higher overall respiration rate of onion stored at 1°C was due to the high respiration rate of onions treated with ethylene and 1-MCP before curing (0.3109 mmol CO_2 kg^{-1} h^{-1}). Yet, onions treated with ethylene and 1-MCP after curing then stored at 1°C had the lowest respiration rate of all treatments and storage temperatures at 0.0960 mmol CO_2 kg^{-1} h^{-1} (Table 6).

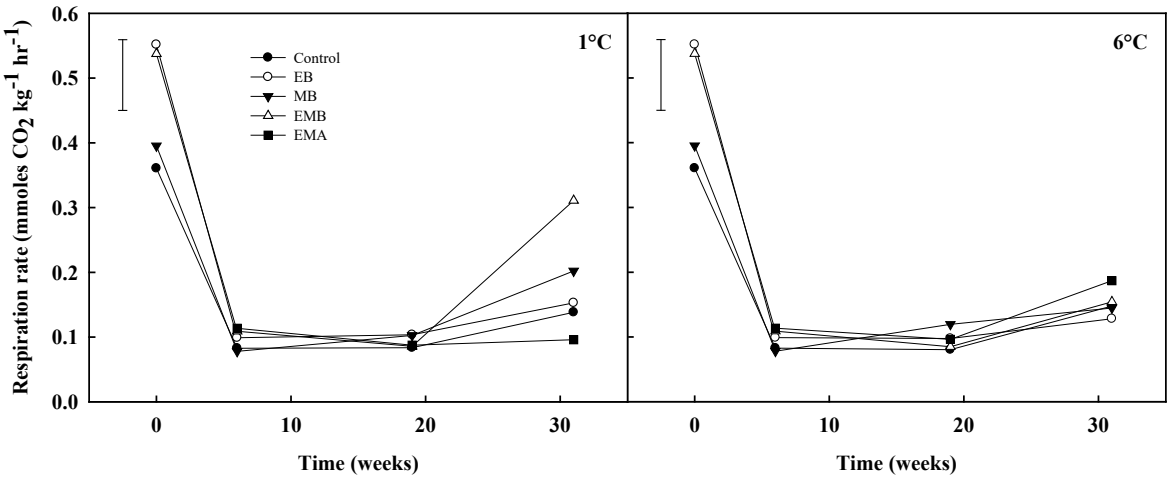


Figure 1 Respiration rate (mmol CO_2 kg^{-1} h^{-1}) of cv. Sherpa treated before or after curing (six weeks at 20°C) with ethylene and/or 1-MCP for 24 h at 20°C before being transferred to cold storage (1 or 6°C) (n = 12); LSD bars (P = 0.05) are shown.

Table 6 Respiration rate (mmol CO_2 kg^{-1} h^{-1}), fructose, glucose, sucrose and total fructans (mg g^{-1} DW) of cv. Sherpa measured 31 weeks after harvest treated before or after curing (six weeks at 20°C) with 10 µL L^{-1} ethylene and/or 1 µL L^{-1} 1-MCP for 24 h at 20°C then transferred to cold storage (1, 6 and 6-1°C) (*n* = 12).

	1°C					6°C					6-1°C				
	Control	EB	MB	EMB	EMA	Control	EB	MB	EMB	EMA	Control	EB	MB	EMB	EMA
Respiration rate	0.138^{ab}	0.153^{ab}	0.196^{b}	0.311^{c}	0.096^{a}	0.148^{ab}	0.128^{ab}	0.145^{ab}	0.154^{ab}	0.187^{b}	0.124^{ab}	0.102^{a}	0.100^{a}	0.128^{ab}	0.128^{ab}
Fructose	182.3^{de}	179.5^{cde}	136.1^{bc}	129.6^{b}	204.4^{e}	138.3^{bc}	140.8^{bcd}	135.4^{bc}	123.1^{ab}	138.6^{bcd}	158.4^{bcd}	134.2^{b}	80.9^{a}	115.5^{ab}	115.6^{ab}
Glucose	159.9^{ef}	149.5^{de}	124.8^{bcde}	101.6^{ab}	189.2^{f}	141.8^{cde}	140.1^{bcde}	140.4^{bcde}	109.8^{abc}	146.5^{cde}	157.5^{ef}	121.1^{bcde}	79.8^{a}	114.3^{abcd}	116.7^{abcd}
Sucrose	175.9^{cd}	142.6^{bc}	115.7^{ab}	109.9^{ab}	176.7^{cd}	168.7^{cd}	194.3^{d}	172.6^{cd}	155.2^{bcd}	180.7^{cd}	182.5^{cd}	139.2^{abc}	91.5^{a}	146.6^{bcd}	139.1^{abc}
Total fructans	101.7^{d}	$83.0b^{cd}$	72.7^{abcd}	31.6^{a}	86.9^{bcd}	109.9^{d}	116.2^{d}	84.1^{bcd}	37.9^{ab}	105.8^{d}	105.8^{d}	114.3^{d}	40.8^{abc}	99.8^{d}	90.1^{cd}

Treatments: EB, ethylene before curing; MB, 1-MCP before curing; EMB, ethylene and 1-MCP before curing; EMA, ethylene and 1-MCP after curing. LSD (*P* = 0.05), respiration rate = 0.0831; fructose = 44.45; glucose = 39.16; sucrose = 49.47; total fructans = 50.29.

Dry weight

Dry weight as a proportion of the fresh weight was not significantly different between treatments or storage temperatures. The only significant change in dry weight as a proportion of fresh weight was over time with a significant decrease observed during curing (122.87 – 114.62 mg g^{-1} DW). The bottom section of the onion bulbs had a higher proportion of dry weight (136.72 mg g^{-1} DW) than the top section (122.65 mg g^{-1} DW) and did not significantly change over time, whereas the proportion of dry weight in the top section decreased significantly (122.65 – 107.26 mg g^{-1} DW). No significant difference in dry weight was observed between onions stored at different temperatures; 1°C (Figure 2) and 6°C (Figure 3).

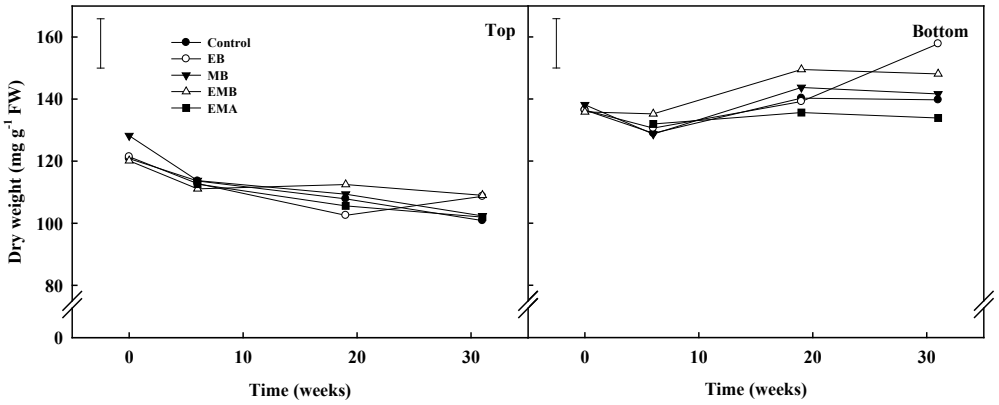


Figure 2 Dry weight (mg g^{-1} FW) of spatial sections of cv. Sherpa onion bulbs treated before or after curing (six weeks at 20°C) with ethylene and/or 1-MCP for 24 h at 20°C before being transferred to cold storage (1°C) (*n* = 12); LSD bars (*P* = 0.05) are shown.

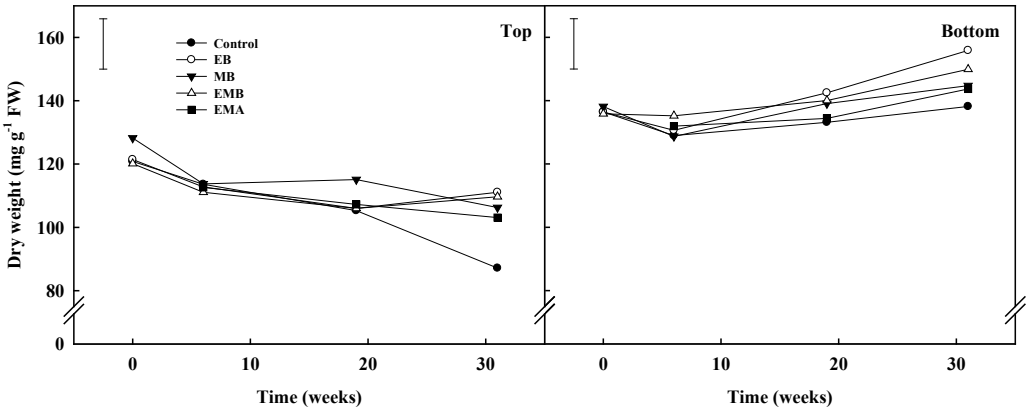


Figure 3 Dry weight (mg g^{-1} FW) of spatial sections of cv. Sherpa onion bulbs treated before or after curing (six weeks at 20°C) with ethylene and/or 1-MCP for 24 h at 20°C before being transferred to cold storage (6°C) (*n* = 12); LSD bars (*P* = 0.05) are shown.

Sugars

Onions treated with ethylene and 1-MCP before curing had overall significantly lower concentrations of fructose (90.4 mg g^{-1} DW), glucose (95.2 mg g^{-1} DW) and sucrose (113.9 mg g^{-1} DW) than onions treated with both gases after curing for fructose and glucose (112.0 and 143.9 mg g^{-1} DW, respectively) or the control bulbs for sucrose (137.9 mg g^{-1} DW). This was due to a lower concentration of fructose and sucrose in onions treated with ethylene and 1-MCP before curing at the end of storage (31 weeks) at 1°C (Figure 4) and was also observed in total fructan content. This low concentration of fructose, sucrose and total fructans at the end of storage at 1°C was also observed in onions treated with 1-MCP before curing however this was not significant over the whole storage period. There was a significant change in fructose, glucose, sucrose and total fructans over time; only fructose and sucrose increased significantly during curing (44.7 – 84.6 and 114 – 178.6 mg g^{-1} DW) while glucose remained steady (152.0 – 159.5 mg g^{-1} DW) and total fructans decreased (333.2 – 257.0 mg g^{-1} DW). During the first 13 weeks of cold storage, fructose, glucose, sucrose and total fructans all decreased (84.6 – 67.0, 159.5 – 70.3, 178.6 – 71.6 and 257.0 – 38.0 mg g^{-1} DW, respectively), then increased during the final 12 weeks (67.0 – 150.8, 70.3 – 140.4, 71.6 – 159.2 and 38 – 83.0 mg g^{-1} DW, respectively). Spatial sugar analysis revealed differences in sugar concentrations between the top and bottom sections except for sucrose content which did not differ significantly. Fructose and glucose were higher in the top section (156.5 and 168.7 mg g^{-1} DW) compared with the bottom section (82.6 and 107.2 mg g^{-1} DW); changes in fructose and glucose content over time followed the same trend in both sections. In contrast, total fructan content was higher in the bottom section (166.6 mg g^{-1} DW) compared with the top (144.8 mg g^{-1} DW). There was no significant effect of storage temperature on spatial sugar content (Figure 5 and 6). That said, in the whole onion bulbs, glucose concentration was not affected by storage temperature whereas fructose and sucrose concentrations were both lower in onions stored at 1°C compared with 6°C. However, after 31 weeks, fructose and glucose concentrations were lowest in onions stored at 6 - 1°C (120.9 and 117.9 mg g^{-1} DW) followed by 6°C (135.2 and 135.7 mg g^{-1} DW) and 1°C (166.4 and 145.0 mg g^{-1} DW). Total fructan content was not affected by storage temperature (Table 6).

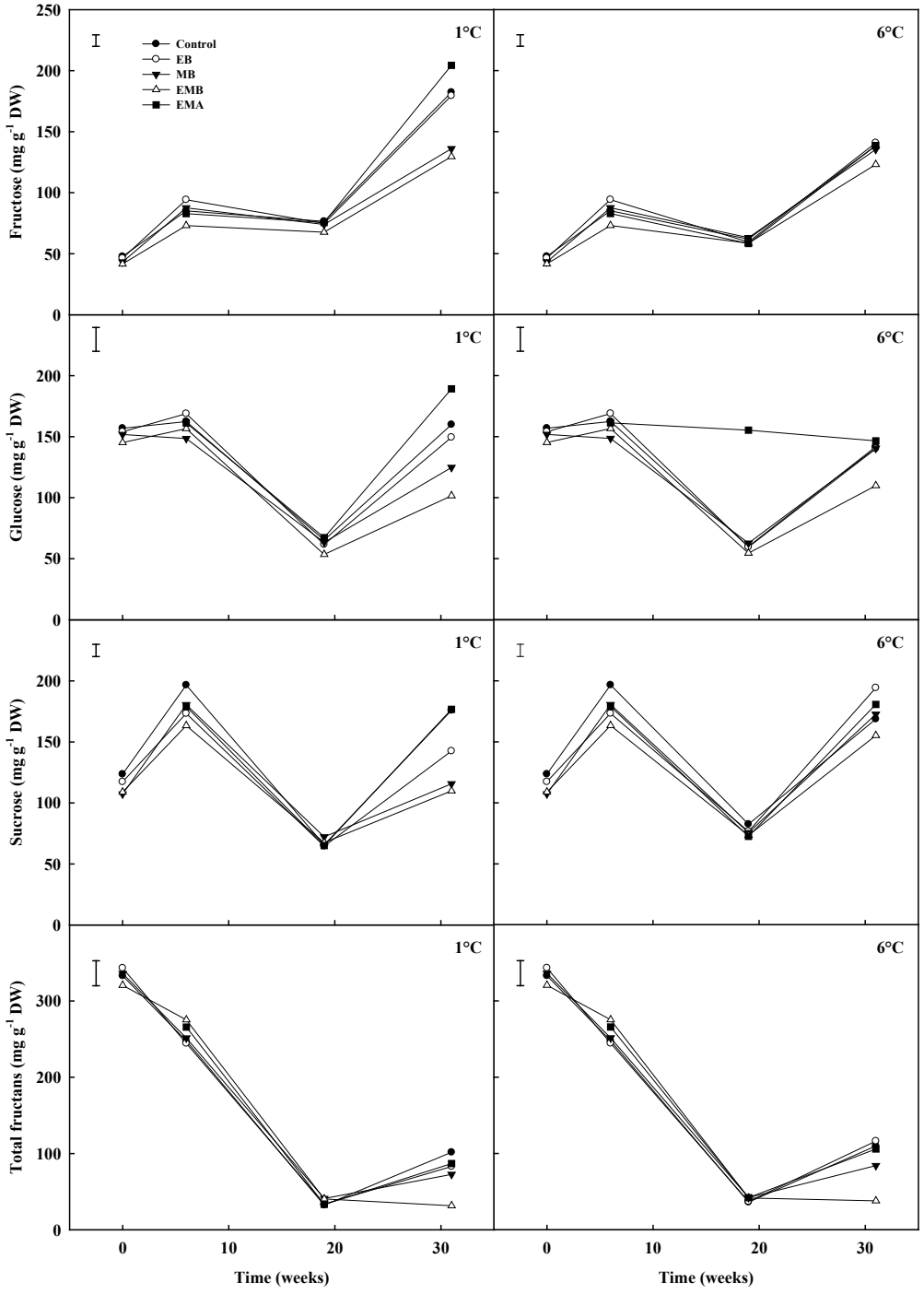


Figure 4 Fructose, glucose, sucrose and total fructan concentrations (mg g^{-1} DW) in cv. Sherpa onions treated before or after curing (six weeks at 20°C) with ethylene and/or 1-MCP for 24 h at 20°C before being transferred to cold storage (1 or 6°C) (n = 12); LSD bars (P = 0.05) are shown.

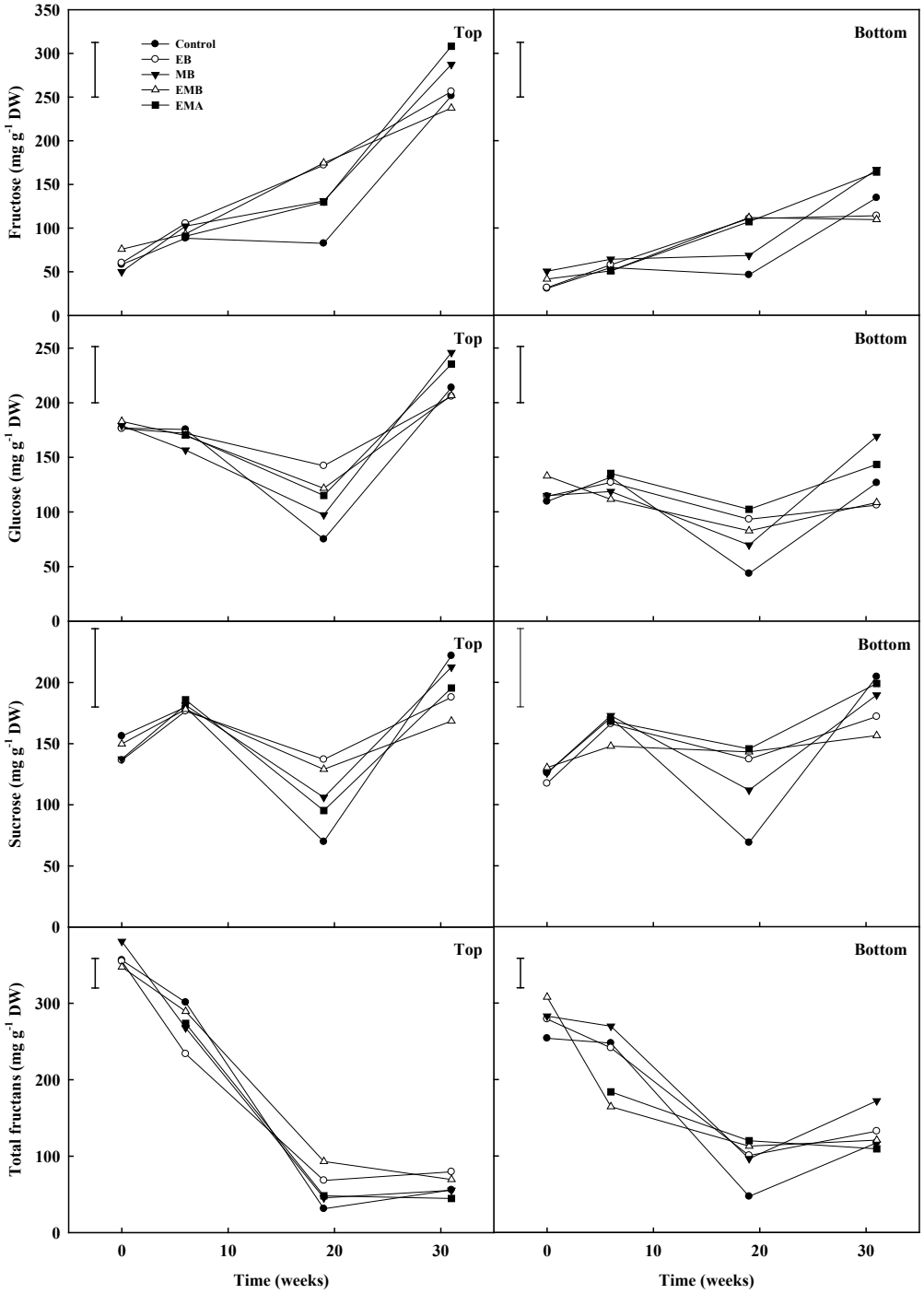


Figure 5 Fructose, glucose, sucrose and total fructan concentrations (mg g^{-1} DW) in spatial sections of cv. Sherpa onions treated before or after curing (six weeks at 20°C) with ethylene and/or 1-MCP for 24 h at 20°C before being transferred to cold storage (1°C) (n = 12); LSD bars (P = 0.05) are shown.

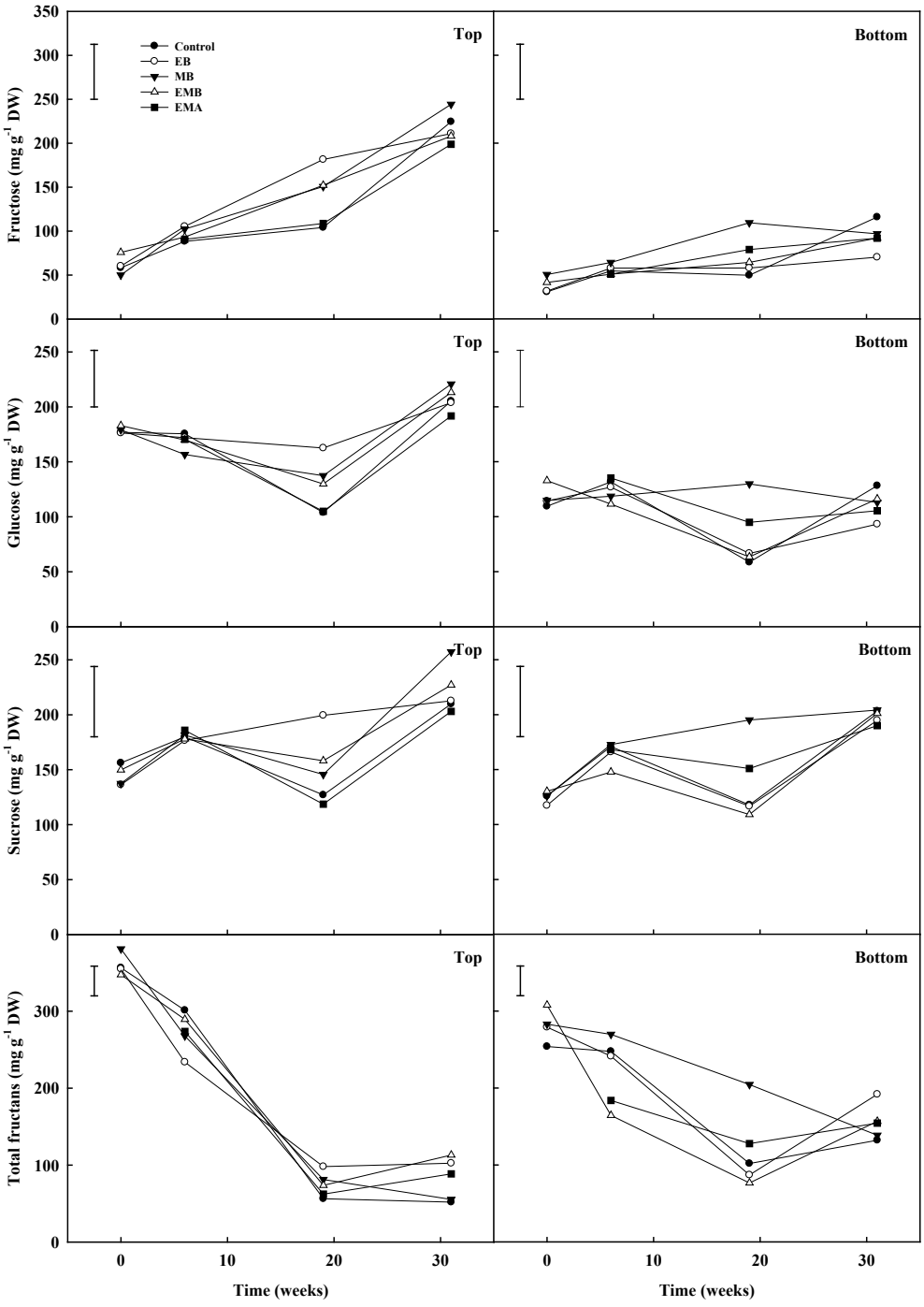


Figure 6 Fructose, glucose, sucrose and total fructan concentrations (mg g^{-1} DW) in spatial sections of cv. Sherpa onions treated before or after curing (six weeks at 20°C) with ethylene and/or 1-MCP for 24 h at 20°C before being transferred to cold storage (6°C) (n = 12); LSD bars (P = 0.05) are shown.

Hormones

Concentrations of ABA, IPA and ZR were significantly higher in the bottom section (45.8, 93.4 and 253 ng g^{-1} DW, respectively) compared with the top section of the onion bulbs (32.0, 47.3 and 86 ng g^{-1} DW, respectively). Overall ABA and ZR concentrations were higher at harvest (79.1 and 251 ng g^{-1} DW, respectively) compared with concentrations during storage (28.2 and 148 ng g^{-1} DW, respectively) however IPA content was only higher at harvest in the top section of the bulb. Onions treated with ethylene before curing contained overall lower concentrations of IPA. Onions treated with ethylene alone or with 1-MCP before curing contained higher concentrations of ZR throughout storage in the bottom section only (Figure 7).

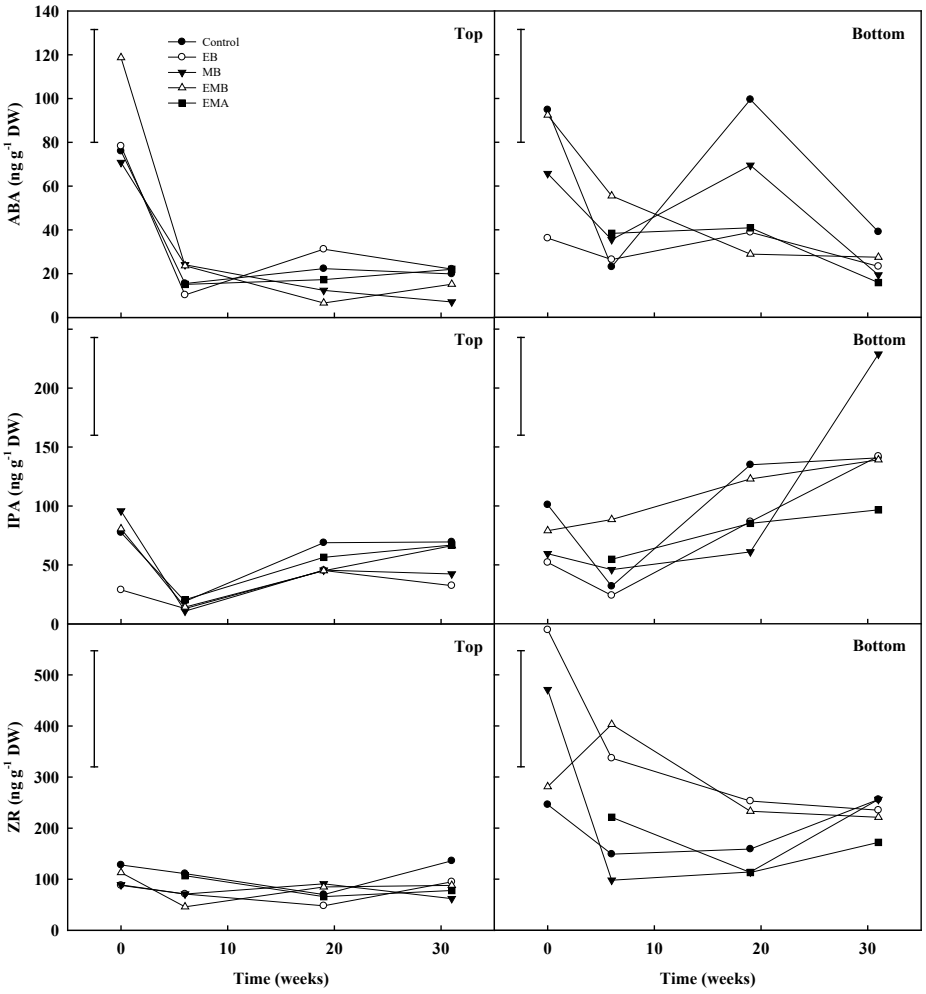


Figure 7 ABA, IPA and ZR concentrations (mg g^{-1} DW) in spatial sections of cv. Sherpa onions treated before or after curing (6 weeks at 20°C) with ethylene and/or 1-MCP for 24 h at 20°C before being transferred to cold storage (1°C) (*n* = 12); LSD bars (*P* = 0.05) are shown.

Discussion

This study and that described in Appendix A titled 'Postharvest application of ethylene and 1-methylcyclopropene either before or after curing affects onion (*Allium cepa* L.) bulb quality during long term cold storage' has demonstrated two important points; firstly, short treatments of ethylene and/or 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.

Cv. Sherpa treated with ethylene and 1-MCP after curing then stored at 6°C had shorter sprouts than the control bulbs 19 weeks after harvest however, there was no evidence that ethylene or 1-MCP alone had any significant effect on sprout growth. Treatment with ethylene and 1-MCP after curing only appeared to reduce sprout growth at 6°C, however this may have been due to the onions stored at 1°C being analysed too early as the average sprout length was only 3% of the bulb height. The use of ethylene and 1-MCP again reduced sprout growth in the onions held in continuous ethylene but only when applied before curing and the results were not significant. It has previously been shown that a short 24 h treatment of 1-MCP (1 μL L^{-1}) after curing was sufficient to inhibit sprout growth in cured onions cv. SS1 when stored at 4 and 12°C (Chope *et al.,* 2007a). However, Bufler (2009) found onions cv. Copra sprouted earlier when treated after curing (two weeks at 25°C) with 0.25 μL L^{-1} 1-MCP for 5 h (20°C) however the storage temperature was higher at 18°C, and the 1-MCP concentration and treatment duration were lower and shorter, respectively. When stored at 20°C, Chope *et al.* (2007a) 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 such that 1-MCP can have differential effects according to temperature (Watkins, 2006). The sprout suppression results may therefore differ due to the different temperatures used; 1 and 6°C, respectively. Differences in sprout growth between treatments in onions held at 1°C may have unfortunately occurred between sampling times; at 19 weeks the sprouts were too short and at 31 weeks the sprouts were already well established.

Onions stored at 1°C had shorter sprouts and reduced incidence compared with those stored at 6°C which agrees with Chope *et al.* (2007a). To the best of our knowledge,

and for the first time, a subset of onions was transferred from 6°C to 1°C at 19 weeks to investigate whether 1°C was required for the entire storage period. Onions transferred from 6-1°C had shorter sprouts than those held at 6°C for the full 31 weeks yet those held at 1°C for the full storage period still had the shortest sprouts. However those held at 6-1°C had lower rooting incidence than those at 1°C. Our aim was to swap the onions before the onset of sprouting however this was missed by approximately 1-2 weeks. Swapping the onions between storage temperatures a few weeks earlier may help to reduce sprout growth and incidence and at the same time reduce energy costs. The treatments of ethylene and 1-MCP were only effective at 6°C at 19 weeks but the effect of the swap from 6-1°C at 19 weeks on treatment efficiency is unknown as by 31 weeks there was no significant difference in sprout growth between treated and untreated onions at any temperature.

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; Johnson, 2006; Bufler, 2009). This was confirmed by the onions stored in continuous ethylene which had overall shorter sprouts than those held in air. Although, short treatments with ethylene after curing has been investigated by Bufler (2009) who found onions which had been held at 25°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 with 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 suppresses sprout growth after dormancy release, suggesting that 1-MCP and ethylene may elicit the same response via different pathways (Chope and Terry, 2008). Bufler (2009) found that 1-MCP broke dormancy at 18°C and suggested endogenous ethylene may play an important role in maintaining onion bulb dormancy. However, as the results presented herein show, sprout growth was reduced in cv. Sherpa when treated with 1-MCP. This suggests that endogenous ethylene may be involved in the break of dormancy or initiation of sprout growth and that 1-MCP may help to mitigate 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 all or certain types of receptors without being perceived resulting in little or no change in respiration rate. When treated with ethylene and 1-MCP, an increase in respiration rate was still observed even though it is thought that 1-MCP has a higher affinity for ethylene receptors (Blankenship and Dole, 2003). This suggests that ethylene is still binding to elicit a response therefore it is possible that 1-MCP may only bind to specific receptors as more than one ethylene receptor exists in other species such as *Arabidopsis thaliana* (Chang *et al.,* 1993; Hua *et al.,* 1995; Hua and Meyerowitz, 1998; Sakai *et al.,* 1998).

Sugar content was in agreement with results previously reported by Davis *et al.* (2007), Vågen and Slimestad (2008) and Chope *et al.* (2006), respectively. Onions treated with ethylene and 1-MCP after curing, then stored at 6°C had higher overall glucose concentrations at 19 weeks, coinciding with significantly shorter sprout length. Spatial analysis revealed the higher glucose content was due to higher concentrations in the bottom section of the bulb as well as higher fructose, sucrose and total fructans. It is unlikely, however, that sugar or fructan concentrations could be used as an early marker for storability since these differences were only observed at 19 weeks during sprouting itself and not at harvest. Spatial analysis showed the top section of the bulb contained higher concentrations of glucose, fructose and fructans in agreement with Abayomi and Terry (2009) yet higher dry weight was found in the bottom section. Sugars are stored in the whole bulb then translocated to the base plate during sprouting and rooting where they are subsequently used (Pak *et al.*, 1995).

The hormone ABA is thought to be involved in dormancy with concentrations in onion initially high at harvest which decreases to its lowest point during sprout initiation and increases again possibly due to synthesis by the growing sprout (Chope *et al.*, 2006). There were no differences in ABA concentrations of onions treated with ethylene and/or 1-MCP which agree with Chope *et al.* (2007a) who found no effect of 1-MCP on ABA concentrations even though differences in sprout growth were observed. Coleman (1998) found ABA decreased 4-fold in potato following a short 24 h treatment with 1.74 µmol l^{-1} ethylene which returned to normal levels after a few hours however

this was not observed herein. That said, differences in cytokinins were observed between treated and untreated onions. Ethylene treatment appeared to result in an increase in ZR in the bottom section and an overall decrease in IPA. Cytokinins are involved in cell division and are found to increase towards the end of storage in onion during sprout and root growth (Abdel-Rahman and Isenburg, 1974). It has been suggested that cytokinins play a direct role in the initiation of sprout growth as Miedema (1994) removed the roots of onion cvs. Rador and Stentor and found a reduction in sprout growth. Ethylene was the only treatment to effect cytokinin concentrations however these particular treatments did not result in reduced sprout or root growth. Although, it does suggest cross talk between ethylene and cytokinin which has been suggested previously (Chen *et al.*, 2005). Both cytokinins measured; ZR and IPA showed differential responses to ethylene. The two cytokinins appeared to behave quite differently with IPA being evenly distributed throughout the bulb and decreasing in response to ethylene whereas ZR was found in higher concentrations in the bottom section of the bulb and increased in response to ethylene. This suggests each cytokinin may play a different role in onion bulb physiology. It may be interesting to see whether it is possible to quantify any other cytokinins present in onion to help understand the exact role of each compound.

Conclusion

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 in cv. Sherpa. The mode of action of ethylene and 1-MCP is still unknown for onions although the results from this study have shown that both 1-MCP and ethylene reduce sprout growth but result in differential biochemical and physiological effects suggesting different mechanisms.

ETHYLENE AND 1-MCP DIFFERENTIALLY REGULATE GENE EXPRESSION DURING ONION SPROUT SUPPRESSION

Katherine Downes[1], Gemma A. Chope[1,2], John P. Hammond[2], Andrew J. Thompson[2] and Leon A. Terry[1].

[1] Plant Science Laboratory, Cranfield University, Bedfordshire, MK43 0AL, UK

[2] Warwick HRI, University of Warwick, Wellesbourne, Warwick, CV35 9EF

Introduction

Onion quality is dependent on the rate of internal sprout growth during storage. To eliminate the use of artificial chemicals such as maleic hydrazide, the use of the naturally occurring hormone, ethylene, has been found to reduce sprout growth in onion bulbs when applied continuously throughout storage (10 µL L^{-1}). In climacteric fruits, ethylene induces ripening, and, in direct contrast the ethylene binding inhibitor 1-MCP typically inhibits ripening (Watkins, 2006). On the other hand, onion is traditionally classified as non-climacteric, yet both ethylene and 1-MCP have been found to inhibit sprout growth (Chope *et al.*, 2007a; Bufler, 2009). Chope *et al.* (2007a) found that treatment with 1-MCP (1 µL L^{-1}) for 24 h after curing reduced sprout growth in onions cv. SS1 when stored at 4 or 12°C. Bufler (2009) found onions cv. Copra cured for 2 weeks at 25°C then held in continuous ethylene (10.6 µL L^{-1}) reduced sprout growth compared with those held in air. Although ethylene and 1-MCP have both been shown to reduce sprout growth, biochemical and physiological responses to each stimulus differ (Chope *et al.,* 2007b; Bufler, 2009). Ethephon is an ethylene yielding chemical which when applied directly to plants can elicit a response characteristic of ethylene treatment (Yang, 1969; Warner and Leopold, 1969). Application of Ethephon to onion plants two weeks prior to harvest was found to reduce sprout incidence by 5% of the initial weight after 32 weeks storage at 0°C, however no significant reduction in rooting was observed (Adamicki, 2005). Unlike Ethephon treatment, continuous ethylene exposure has been found to increase shelf-life after 14 days at 20°C (Adamicki, 2005; Johnson, 2006). The combination of ethylene and 1-MCP has, to the best of our knowledge, not been investigated in onion. Treating potatoes cv. Russet Burbank with 0.9 µL L^{-1} 1-MCP for 48 h prior to ethylene treatment was enough to prevent fry colour darkening for 4 weeks, a negative side effect of

ethylene treatment. It was suggested, but not tested, that ethylene is able to have an effect on sprout suppression in potato tubers after 1-MCP treatment due to the production of new ethylene receptors at the site of sprouting (eye) but to a lesser extent in the potato pith and cortex (Prange *et al.,* 2005). It is unknown whether a similar mechanism might occur in onions.

Onions are in the order Asparagales which possess some of the largest genomes of the eukaryotes especially in the genus *Allium* (Kuhl *et al.,* 2004). The onion is diploid and comprises a large nuclear genome of 16,415 Mbps (over 5 times that of the human genome) spread over eight chromosomes whereas chive has the smallest of the *Alliums* at 7,448 Mbps (Havey *et al.*, 2008; NCBI, 2008). It has been suggested that the large onion genome has resulted from intrachromosomal duplication and a very high level of dominant restriction fragment length polymorphisms (RFLPs) (McCallum *et al.,* 2001; Kuhl *et al.,* 2004). The large size has hindered plans to sequence the onion genome, however, 20,180 expressed sequence tags (ESTs) have been sequenced from cDNA mainly from a cross of inbred cultivars, Bringham Yellow Globe 15-23 x Alisa Craig 43 (NCBI, 2008). These ESTs have been used to develop the first onion microarray (Chope *et al.* unpublished). Although literature exists on the effect of ethylene and 1-MCP on climacteric fruits and vegetables at the molecular level, it is still unknown by which mechanisms exogenously applied ethylene and 1-MCP suppress sprout growth in onions.

The aim of this study was to investigate the most effective postharvest treatment for onion sprout suppression during storage and shelf-life by testing the effect of short 24 hour pre- and post-curing treatments; ethylene and/or 1-MCP with or without the addition of continuous ethylene during storage. In addition, using the first onion microarray, the mechanism by which ethylene and 1-MCP act at a molecular level was investigated.

Materials And Methods

Plant material and curing

Onion seeds cv. Sherpa (medium pungency, medium dry matter) were drilled on sandy clay loam (Alistair Findlay's, Cardington, Beds., UK; 1.2 x 0.3 ha) on 5 March 2008 at a

rate of 57 seeds m^{-2} with pesticides applied as per commercial practice. Plants were machine-harvested at 100% fall-down on 17 September 2008. Onion bulbs were stored in 72 large nets (approx. 60 bulbs) and 24 half nets (approx. 30 bulbs) buried amongst loose bulbs in one tonne wooden crates for batch curing at the Sutton Bridge Experimental Unit (Lincs., UK). Bulbs were artificially cured at either 20 or 28°C for six weeks as per normal commercial practice in the UK with relative humidity controlled at 65 – 75%.

Experimental design

The experiment was a completely randomised design with three replicates taken from three sections of the field. There were seven postharvest treatments per replicate *viz.* 1 µL L^{-1} 1-MCP before curing (MB), 10 µL L^{-1} ethylene before curing (EB), both ethylene and 1-MCP before curing (EMB), 1 µL L^{-1} 1-MCP after curing (MA), 10 µL L^{-1} ethylene after curing (EA), both 10 µL L^{-1} ethylene and 1 µL L^{-1} 1-MCP after curing (EMA) and control (no treatment). Treatments were applied according to section B materials and methods.

Pre-storage treated onions

After curing, onions were transported to Cranfield University (CU) within 2.5 h. Diseased or damaged bulbs were removed and the remaining bulbs randomly placed in individual plastic stackable crates and stored in air for 35 weeks at 0-1°C in the dark. At each sampling time, four bulbs per treatment, curing temperature and replicate (n = 168) were selected randomly, taken after harvest (day 0), immediately after curing (6 weeks) then at intervals during cold storage (17, 25 and 35 weeks after harvest) (n = 840).

Pre-storage and storage treated onions

After curing, a subset of the treated onion bulbs was transported (4 h) at ambient temperature to the Research Institute of Vegetable Crops (Skierniewice, Poland) for continuous air or ethylene treatment. Control bulbs and onions treated with EB, MB, EMB, EA and EMA cured at either 20 or 28°C for six weeks were placed in individual plastic trays and stored in air or continuous 10 µL L^{-1} ethylene for a further 29 weeks at 0-1°C in the dark. Six bulbs per pre-storage treatment, post-curing treatment and

curing temperature (*n* = 144) were selected at random at the end of storage (35 weeks after harvest).

Sample preparation

Sample preparation of the onions stored at CU was carried out according to section B materials and methods with slight modification. Two longitudinal wedges were cut and snap-frozen in liquid nitrogen and each then stored at -40°C for biochemical analysis or -80°C for RNA extraction. Sprout and root growth and disease incidence were measured in the onions sent to Poland for continuous ethylene treatment. These onions were also cut into two longitudinal wedges and snap-frozen in liquid nitrogen in Poland and returned to the UK on dry ice for microarray analysis.

Physiological measurements

Respiration rate

Respiration rate was measured according to section B materials and methods.

Biochemical measurements

Extraction and quantification of sugars

Extraction and quantification of non-structural carbohydrates was carried out according to section A materials and methods.

Microarray analysis

RNA extraction and microarray analysis was carried out according to section A materials and methods.

Quantitative real-time PCR validation

To validate the microarray results, transcript levels of cDNAs that were differentially expressed according to the microarray were confirmed using real time quantitative PCR. From the 18 total RNA samples, cDNA was synthesised using the ThermoScript™ RT-PCR System for First-Strand cDNA Synthesis kit (Invitrogen, Cat. No. 11146-024) and a combination of random hexamers and oligo DT primers. Primer 3 and PrimerSelect (Lasergene) software (DNAStar, Inc., Madison, WI) was used to

design gene specific primers and the transcript abundance detected using an ABI Prism 7900HT sequence detection system (Applied Biosystems, Carlsbad, CA) and SBS 2.1 software. The qPCR was performed in 384 well plates using MESA Green qPCR Mastermix Plus for SYBR assay and probe CUST_716_P1403527117 (tumour protein TC4554; F TCCGACTACAGGAACAACCAG, R AAACTCCTCTGCCTTCTCAGC) as the internal control gene for normalisation. Table 1 describes five probes differentially regulated in treated onion bulbs as compared to the control according to the microarray data. These five probes were used for qPCR analysis to validate the microarray data.

Table 1 Primers used for qPCR analysis

Probe	Gene	Forward Sequence	Reverse Sequence
3995_P14035271	ABTB1-Armadillo	TTGGCTCTTGCTC	ACCATCTTGCTGT
17	repeat	ATCTTTG	TGCTTTG
10973_P1403527	Monocopper oxidase	GATCGGAGAATTG	TTAGCTCGGCCAC
117		GGAAAGAC	ACAGAAG
2287_P14035271	LTPL121-Protease	CTGCACTCCTTGC	CTCCCAGCTTCAG
17	inhibitor / seed storage	CCTAAAC	TGTATCG
1126_P14035271	RNA polymerase	AAGTGGCGGTGGT	AGGCAGCAACAAA
17		CTGATAG	GATGGTAAG
2252_PI4035271	Starch synthase	ATGTTCGGGTTCT	GCCTCTTCTTCAC
17		TTGTTCAG	TTACTTTCCAG

Statistical analysis

Statistical analyses were conducted according to section A materials and methods. Microarray data analysis was performed using Genespring GX11 (Agilent). There were three replicates for each treatment (control, ethylene before curing, 1-MCP before curing, ethylene and 1-MCP before curing, continuous storage in air and continuous storage in ethylene) totalling 18 samples. The continuous treated samples (n = 6) were analysed separately to those treated before curing (n = 12). In all cases raw expression values were normalised against the median of the controls.

Results

Sprouting

Pre-storage treated onions

Average sprout growth at 25 and 35 weeks was 28.8 and 57.6% of bulb height, respectively, with curing temperature significantly affecting sprout length after 25 weeks only. Onions cured at 20°C had a mean sprout length of 37.8% of bulb height whereas those cured at 28°C were 19.8% of bulb height 25 weeks after harvest (Table 2). Significant differences between treatments were only observed after 25 weeks with the most significant reductions in sprout growth observed when ethylene and 1-MCP were used together before (19.3% of bulb height) or after (11.6% of bulb height) curing compared to the control bulbs (45.3% of bulb height). In addition, onions treated with 1-MCP before (22.9% of bulb height) or after (31.1% of bulb height) curing had significantly shorter sprouts than the control. That said, no significant interactions were found between treatments and curing temperatures, and onions treated with ethylene alone did not have significantly shorter sprouts compared to the control.

Table 2 Sprout length (% of the bulb height) of cv. Sherpa onions measured 25 and 35 weeks after harvest (six weeks curing then transferred to cold storage) treated before or after curing with 10 µL L^{-1} ethylene and/or 1 µL L^{-1} 1-MCP for 24 h at 20°C (*n* = 12).

Treatment	Sprout length (% of bulb height)			
	25 weeks		35 weeks	
	20°C	28°C	20°C	28°C
Control	51.3	39.2	55.4	58.9
EB	47.3	30.8	68.0	58.8
MB	39.8	6.1	58.0	42.9
EMB	24.8	13.9	60.7	53.5
EA	46.0	19.1	51.6	54.3
MA	40.1	22.0	62.5	56.1
EMA	15.3	7.9	61.6	64.0

Treatments: EB, ethylene before curing; MB, 1-MCP before curing; EMB, ethylene and 1-MCP before curing; EA, ethylene after curing EMA, ethylene and 1-MCP after curing. LSD (*P* = 0.05) = 22.86.

Pre-storage and storage treated onions

Significant interactions between pre-storage treatments and continuous storage treatments were observed in onions cured at 20°C only. Ethylene treatment throughout storage significantly reduced sprout growth (42.9% of bulb height) compared with controls held in air (59.4% of bulb height), irrespective of pre-storage treatments. Nevertheless, mean sprout length of onions pre-treated with ethylene and 1-MCP in combination before curing was even shorter at 28.8% of bulb height (Table 3). In contrast, onions treated with ethylene and 1-MCP after curing and then treated continuously with ethylene had longer sprouts (64.2% of bulb height), but those continuously stored in air had shorter sprouts at 37.6% of bulb height (Table 3).

Table 3 Sprout length (% of the bulb height) of cv. Sherpa measured 35 weeks after harvest treated before or after curing with 10 µL L^{-1} ethylene and/or 1 µL L^{-1} 1-MCP for 24 h at 20°C then transferred to air or continuous ethylene storage at 0-1°C (*n* = 6).

Pre-storage	Sprout length (% of bulb height)			
	Air		Ethylene	
	20°C	28°C	20°C	28°C
Control	63.1	56.2	49.3	38.9
EB	68.3	61.0	33.2	51.3
MB	64.1	61.0	41.9	49.7
EMB	63.4	60.1	28.8	37.4
EA	63.0	51.6	36.0	40.6
EMA	37.6	62.8	64.2	43.9

Treatments: EB, ethylene before curing; MB, 1-MCP before curing; EMB, ethylene and 1-MCP before curing; EA, ethylene after curing EMA, ethylene and 1-MCP after curing. LSD (*P* = 0.05) = 14.78.

Rooting

Pre-storage treated onions

The percentage of bulbs with roots was only significantly lower in onions treated with ethylene after curing at 28°C. However, more treatments resulted in a higher percentage of roots including onions treated with ethylene before curing at 20°C,

ethylene and 1-MCP after curing at 28°C, ethylene and 1-MCP before curing at 20°C and finally 1-MCP alone before curing at 20°C. There was no main effect of curing temperature or treatment on rooting; however, the interactions between treatment and curing temperature were significant (P = 0.028).

Pre-storage and storage treated onions

Apart from sprouting, continuous ethylene during storage also significantly reduced rooting incidence (18.1%) compared to the control (62.5%). Less rooting was also observed in those cured at 20°C (29.2%) compared with 28°C (51.4%). Onions treated with ethylene and 1-MCP after curing at 20°C and then stored in air had no rooting and were significantly lower than the control (83.3%) (Table 4); this treatment regime also had an inhibitory effect on sprout growth (Table 3). Onions cured at 20°C then stored in continuous ethylene had almost no rooting irrespective of the pre-storage treatment. Onions cured at 28°C then stored in continuous ethylene had high rooting incidence however rooting was absent in onions pre-treated with ethylene and 1-MCP before curing.

Table 4 Root incidence (% of bulbs with roots) of cv. Sherpa onions measured 35 weeks after harvest (six weeks curing then transferred to cold storage) treated before or after curing with 10 µL L^{-1} ethylene and/or 1 µL L^{-1} 1-MCP for 24 h at 20°C then transferred to air or continuous ethylene storage at 0-1°C (n = 6).

Pre-storage	Root incidence (% of bulbs with roots)			
	Air		Ethylene	
	20°C	28°C	20°C	28°C
Control	83.3	50.0	0.0	16.7
EB	33.3	83.3	0.0	16.7
MB	83.3	66.7	0.0	83.3
EMB	66.7	83.3	0.0	0.0
EA	50.0	83.3	0.0	33.3
EMA	0.0	66.7	33.3	33.3

Treatments: EB, ethylene before curing; MB, 1-MCP before curing; EMB, ethylene and 1-MCP before curing; EA, ethylene after curing EMA, ethylene and 1-MCP after curing. LSD (P = 0.05) = 40.30.

Respiration rate

Respiration rate was measured in pre-storage treated onions. Onion bulb respiration rate was affected by curing with a significant 6-fold decrease in the six weeks. Respiration rate of onions cured at 20°C was significantly lower than those cured at 28°C (0.0978 and 0.1220 mmol CO_2 kg^{-1} h^{-1}, respectively). Before curing, control bulbs had the lowest respiration rate followed by onions treated with 1-MCP, ethylene and 1-MCP in combination and ethylene alone however, this was not significant (Figure 1).

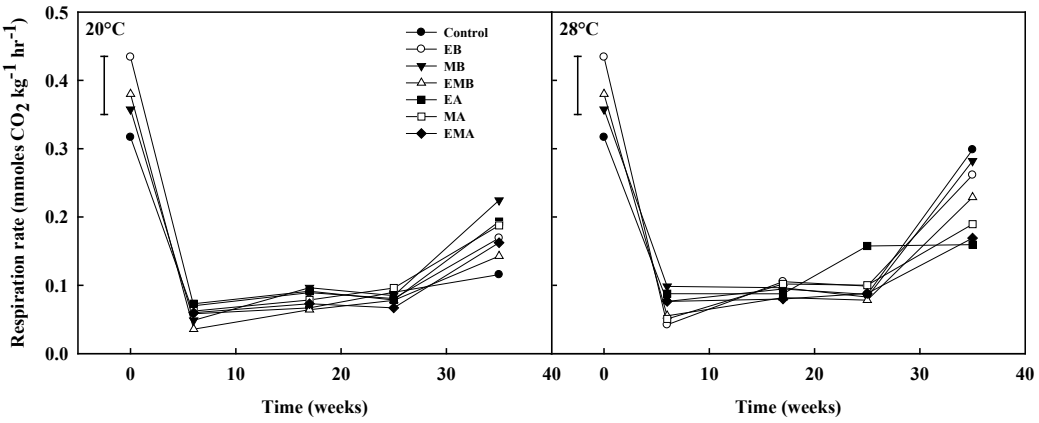


Figure 1 Respiration rate (mmol CO_2 kg^{-1} h^{-1}) of onions cv. Sherpa treated with ethylene (10 µL L^{-1}) before (EB) or after (EA) curing, 1-MCP (1 µL L^{-1}) before (MB) or after (MA) curing or ethylene and 1-MCP in combination before (EMB) or after (EMA) curing for 24 h at 20°C (*n* = 12); LSD bars (*P* = 0.05) are shown.

Dry weight

Pre-storage treated onions

Onion dry weight was not affected by pre-storage treatments. However, onion dry weight was affected by curing temperature and time. There was no change in dry weight of onions cured at 20°C, but those cured at 28°C had significantly higher dry weight before curing than the mean value of all post-curing onions (116.13 – 110.25 mg g^{-1} FW).

Pre-storage and storage treated onions

No significant differences in dry weight were found between pre-storage treatments, storage treatments or curing temperatures. Mean dry weight was 134.6 mg g^{-1} FW.

Non-structural carbohydrates

Sugars were measured in all pre-storage treated onions. Glucose content of onions treated before curing with both ethylene and 1-MCP was lower throughout storage, however by 25 weeks glucose had increased in line with the control. Onions treated after curing had higher sucrose at 25 weeks (254.1 mg g^{-1} DW) than those treated before curing (236.6 mg g^{-1} DW) and the control (212 mg g^{-1} DW). Fructose concentrations tended not to vary much between treatments, but at the end of storage onions treated with ethylene after curing had lower fructose concentrations (145.0 mg g^{-1} DW) than the control (190.1 mg g^{-1} DW) (Figure 2).

Sucrose and total fructans were the only non-structural carbohydrates affected by curing temperature. Onions cured at 28°C had higher sucrose content (206.0 mg g^{-1} DW) but lower total fructans (187.9 mg g^{-1} DW) than those cured at 20°C (188.7 and 203.7 mg g^{-1} DW, respectively). The lower concentration of total fructans in onions cured at 28°C was due to lower nystose, DP5 and DP6 content (Figure 2). Sucrose and total fructan content was 1.2-fold and 1.5-fold higher, respectively after 25 weeks in onions treated with ethylene and 1-MCP after curing. This peak was also observed in onions treated with 1-MCP after curing but only contained higher total fructans (1.5-fold increase). The difference in total fructan content between treatments was due to the largest fructans DP6 – DP8 (Figure 2).

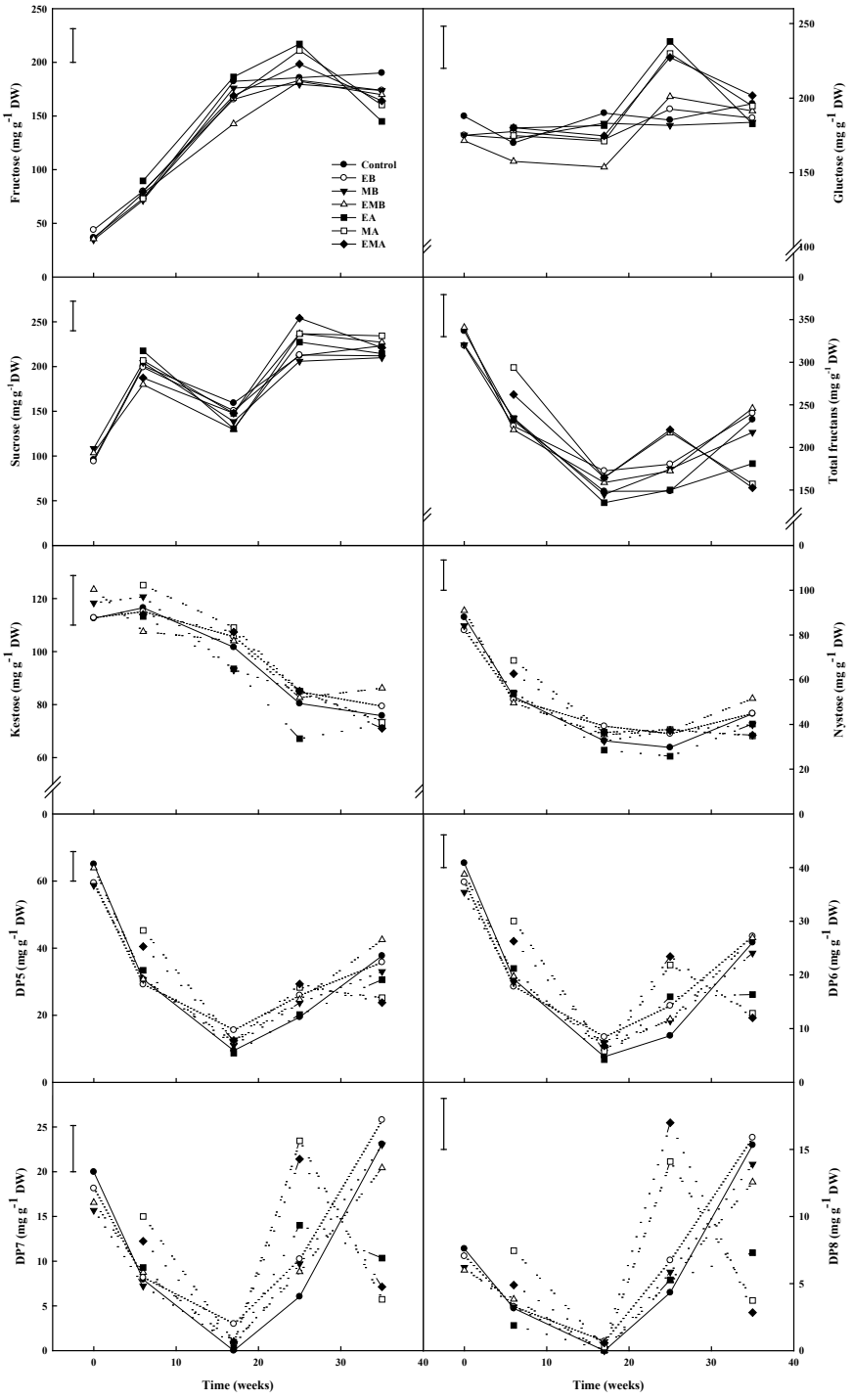


Figure 2 Fructose, glucose, sucrose and total fructans (mg g^{-1} DW) of onions cv. Sherpa treated with ethylene (10 µL L^{-1}) before (EB) or after (EA) curing, 1-MCP (1 µL L^{-1}) before (MB) or after (MA) curing or ethylene and 1-MCP in combination before (EMB) or after (EMA) curing for 24 h at 20°C (*n* = 12); LSD bars (*P* = 0.05) are shown.

Microarray

In total 1228 probes with differential changes in expression were observed in response to ethylene and/or 1-MCP treatment as compared with the control. These probes were clustered into eight different categories depending on their degree of response to each stimulus. Six of the clusters revealed 1048 probes that were similarly up or down-regulated whether the onions were treated with ethylene and/or 1-MCP. The remaining 180 probes were divided into three clusters which showed differential expression when treated with ethylene and/or 1-MCP (Figure 3). Cluster 0 revealed 71 probes down-regulated by ethylene or 1-MCP alone, but no change was observed in the expression of these probes in onion treated with ethylene and 1-MCP in combination. Cluster 6 included 87 probes down-regulated by 1-MCP whether bulbs were in the presence of ethylene or not. Finally, cluster 8 included 22 probes all down-regulated by ethylene irrespective of whether 1-MCP was present or not. All probes were classified into functional categories (Table 5) based on their tentative annotation using other known genes in GenBank non–redundant database. Although probes characterised as being related to plant growth regulators (PGR) included those associated with auxins, cytokinins and ethylene, the only PGR probes which were differentially expressed between ethylene and 1-MCP treatments were gibberellin receptors and gibberellin oxidase. Table 6 details the 30 most up- or down-regulated probes after short treatment pre- or post-curing with ethylene and/or 1-MCP treatments. The fold changes in Table 6 should not strictly be considered as quantitative however the correlation between the gene expression of five genes quantified using the onion microarray and qPCR was reasonable ($r = 0.68$, $P < 0.001$) (Figure 4). The majority of the genes identified as down regulated in the array data were down regulated in the qPCR data and the same for the up-regulated genes.

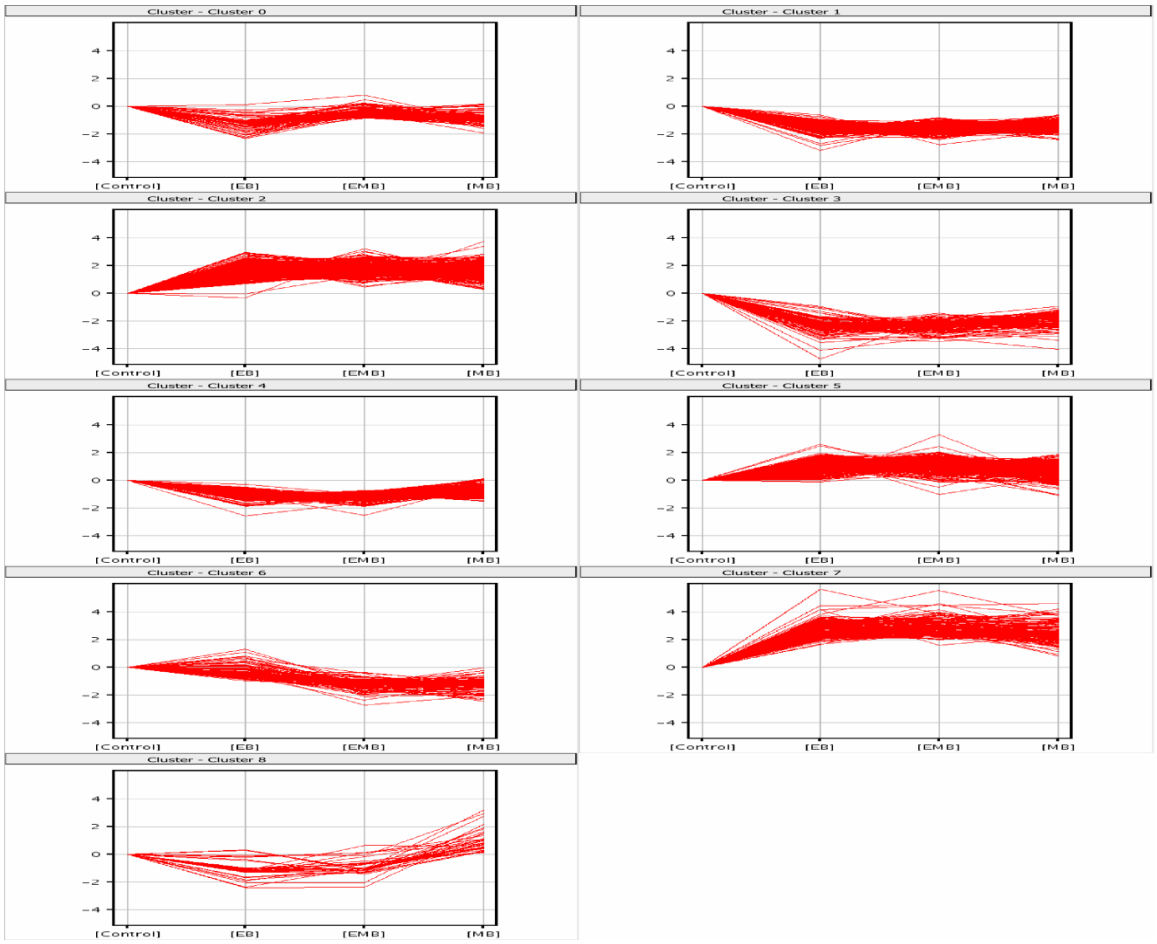


Figure 3 K-means cluster analysis of altered onion gene expression following treatment before curing for 24 h at 20°C with ethylene (EB), 1-MCP (MB), ethylene and 1-MCP (EMB) or untreated (control).

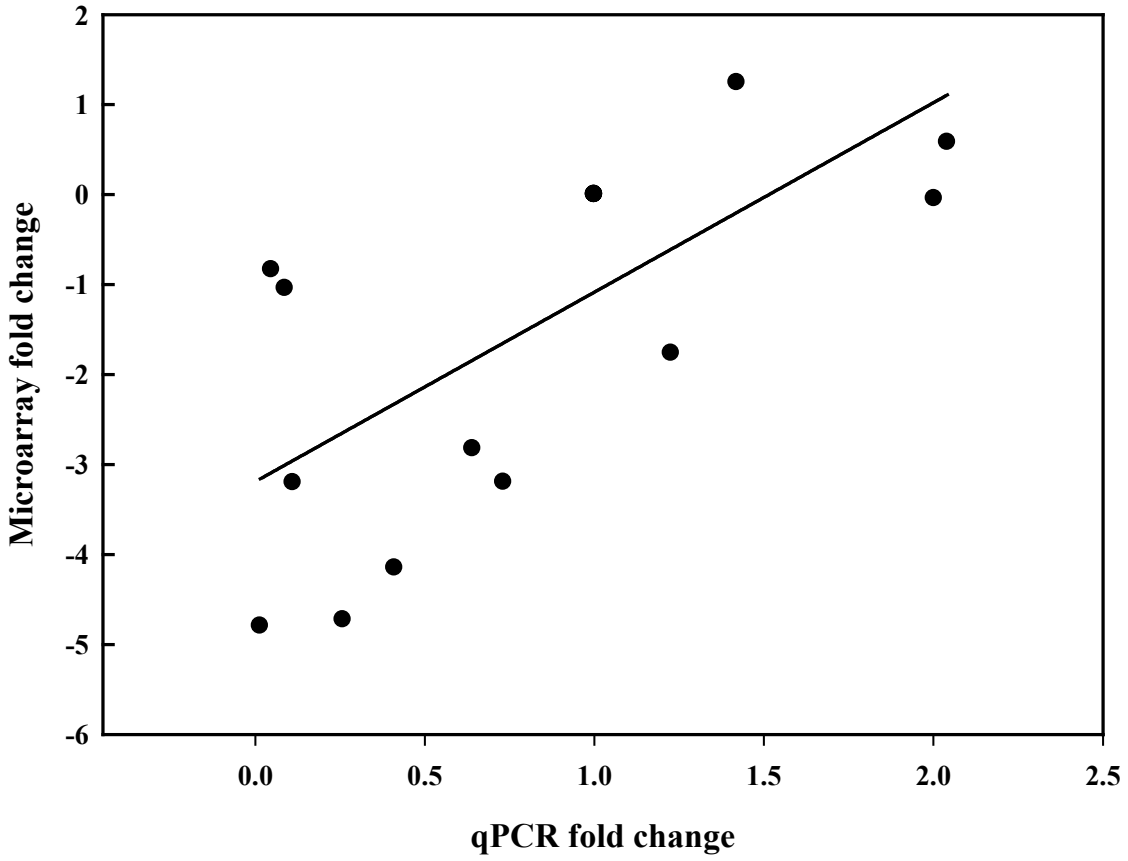


Figure 4 Correlation between the gene expressions of five genes quantified using the onion microarray and qPCR. The expression of the five genes was quantified for each onion treatment ($r = 0.68$, $P < 0.001$).

Table 5 Functional classification of onion probes differentially expressed when treated before curing for 24 h at 20°C with ethylene, 1-MCP, ethylene and 1-MCP or untreated (control). Each cluster corresponds to a group of genes similarly expressed in response to certain treatments as described in Figure 3.

Functional category	Cluster									
	0	1	2	3	4	5	6	7	8	Row Totals
Housekeeping	11	20	40	17	49	37	21	14	6	215
Stress and defence	3	10	6	4	5	8	6	9	3	54
Chaperones	0	3	3	2	5	0	2	0	0	15
Photosynthesis	0	1	4	1	5	3	2	1	0	17
Cell wall metabolism	0	3	2	1	4	3	2	3	0	18
Secondary metabolism	3	8	7	1	4	3	3	4	2	35
Cell death	1	0	2	0	2	1	0	0	0	6
Peptidase / Kinase	8	11	29	10	13	27	6	19	3	126
Transport	4	10	24	3	9	20	3	15	2	90
Signalling	3	0	4	1	8	6	2	4	0	28
Metabolism	8	22	29	4	20	23	8	13	2	129
Plant Growth Regulators	1	6	7	0	5	2	2	5	0	29
Cell cycle	1	1	2	0	5	3	0	1	1	13
Transcription factor	9	11	34	2	15	15	6	14	1	107
Unclassified	19	44	75	19	49	64	21	34	2	327
Phosphatase	0	2	3	1	2	4	3	4	0	19
Row Totals	71	152	271	66	200	219	87	140	22	1228

Table 6a The 30 most highly up and down-regulated onion probes compared with controls after treatment with ethylene before curing (EB), 1-MCP before curing (MB) or ethylene and 1-MCP before curing (EMB) (continued in Table 6b).

Probe	Tentative annotation	Fold change[a]	Regulation	Onion Sequence ID	Treatment
CUST_792_PI403527117	integral membrane protein	51.1	up	TC4630	EB
CUST_2054_PI403527117	3-hydroxy-3-methylglutaryl-coenzyme A reductase	48.1	up	TC5892	EMB
CUST_390_PI403527117	transferase family protein	42.0	up	TC4228	MB
CUST_5592_PI403527117	WD domain, G-beta repeat domain containing protein	33.9	up	CF447771	MB
CUST_3008_PI403527117	retrotransposon protein	28.9	up	TC6846	MB
CUST_2287_PI403527117	LTPL121 - Protease inhibitor/seed storage/LTP family protein precursor	26.4	down	TC6125	EB
CUST_10068_PI403527117	1-aminocyclopropane-1-carboxylate oxidase homolog 4	24.7	up	CF438875	EMB
CUST_7201_PI403527117	starch synthase	23.8	down	CF437167	EMB
CUST_160_PI403527117	per1-like family protein	22.6	up	TC3998	EMB
CUST_3247_PI403527117	CHIT5 - Chitinase family protein precursor	21.8	up	TC7085	EMB
CUST_4826_PI403527117	protein kinase family protein	21.0	up	CF438357	MB
CUST_7052_PI403527117	S-formylglutathione hydrolase	18.4	up	CF448815	MB
CUST_160_PI403527117	per1-like family protein	18.2	up	TC3998	EB
CUST_11478_PI403527117	stress responsive protein	18.1	up	BI095628	EMB
CUST_10708_PI403527117	amino acid transporter	17.9	up	CF440190	EB
CUST_10973_PI403527117	monocopper oxidase	17.7	down	BE205651	EB

Table 6b The 30 most highly up and down-regulated onion probes compared with controls after treatment with ethylene before curing (EB), 1-MCP before curing (MB) or ethylene and 1-MCP before curing (EMB) (continued from Table 6a).

Probe	Tentative annotation	Fold change[a]	Regulation	Onion Sequence ID	Treatment
CUST_1451_PI403527117	dihydrodipicolinate synthase, chloroplast precursor	17.1	up	TC5289	MB
CUST_36_PI403527117	peroxidase precursor	16.6	up	TC3874	MB
CUST_10095_PI403527117	Ser/Thr protein phosphatase family protein	16.3	up	CF440115	MB
CUST_600_PI403527117	EF hand family protein	15.9	up	TC4438	EMB
CUST_6021_PI403527117	OsWRKY48 - Superfamily of TFs with WRKY and zinc finger domains	15.3	down	CF439568	EMB
CUST_6801_PI403527117	zinc finger family protein	15.3	down	CF435756	EB
CUST_9681_PI403527117	alpha-soluble NSF attachment protein	15.1	up	BQ580069	EMB
CUST_792_PI403527117	integral membrane protein DUF6 containing protein	15.0	up	TC4630	EMB
CUST_11354_PI403527117	mitochondrial carrier protein	14.8	up	CF441173	MB
CUST_2054_PI403527117	3-hydroxy-3-methylglutaryl-coenzyme A reductase	14.5	up	TC5892	EB
CUST_160_PI403527117	per1-like family protein	14.4	up	TC3998	MB
CUST_10095_PI403527117	Ser/Thr protein phosphatase family protein	12.7	up	CF440115	EB
CUST_4897_PI403527117	aldehyde dehydrogenase	12.6	up	CF442148	EB
CUST_10269_PI403527117	myristoyl-acyl carrier protein thioesterase, chloroplast precursor	12.6	up	CF445478	EB

[a] Fold change compared with expression of control, calculated as 2^x, where x = absolute value of (normalised treatment – normalised control)

There were 574 probes up or down-regulated in response to continuous ethylene storage, of which 272 which were up-regulated and 302 were down-regulated (Figure 5). Functional characterisation of these probes revealed a large portion involved in photosynthesis were down-regulated (6.6%) whereas very few were up-regulated (0.37%) in response to continuous ethylene. Probes characterised as being related to PGRs revealed down regulation of an auxin efflux carrier component and a cytokinin dehydrogenase by continuous ethylene. More probes related to PGRs were up-regulated in response to continuous ethylene including gibberellin 2-beta-dioxygenase, ethylene-insensitive 3, auxin-responsive gene family member and importantly 1-aminocyclopropene-1-carboxylate oxidase (ACO).

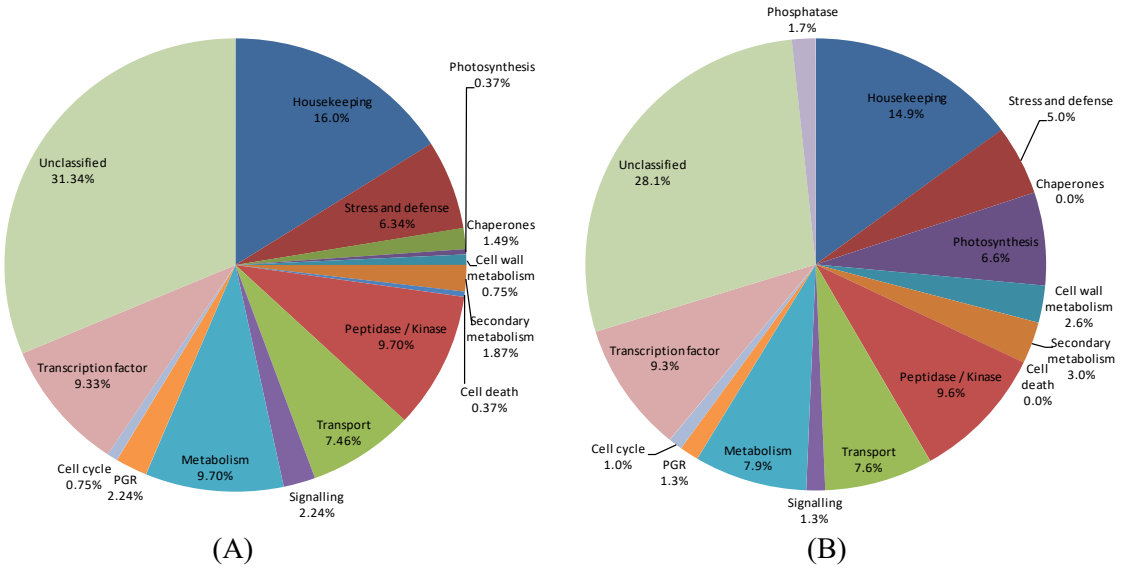


Figure 5 Functional classification of (A) 272 probes up-regulated by continuous ethylene treatment, (B) 302 probes down-regulated by continuous ethylene treatment.

Discussion

Onions were treated before or after curing (6 weeks at 20 or 28°C) with short 24 h treatments of ethylene and/or 1-MCP before being transferred to cold storage (1°C) at CU for a further 29 weeks. A subset of these onions was transported to Poland following curing and treatment to be cold stored (1°C) in air or continuous ethylene for 29 weeks. Biochemical, physiological and molecular techniques were used to identify the most

successful methods of onion sprout suppression and the transcriptomic changes which occurred following each treatment to help identify possible mechanisms for sprout suppression. To the best of our knowledge, this is the first published microarray study conducted on non-climacteric produce.

Onions treated with ethylene and 1-MCP after curing had the shortest sprout length after 25 weeks in storage at CU and this was also found after 35 weeks in continuous air in Poland. The shorter sprout growth in onions treated with ethylene and 1-MCP after curing is also supported by the reduced utilisation of sucrose and the larger fructooligosaccharides; DP6, DP7 and DP8 which were significantly higher at 29 weeks. Treatment with ethylene and 1-MCP after curing resulted in no root growth in the onions held in continuous air in Poland. Although treatment with ethylene and 1-MCP before curing and 1-MCP before curing at 28°C also resulted in shorter sprouts in the CU onions, this was not supported by the onions sent to Poland. Comparison between onions stored in continuous air or ethylene revealed those stored in ethylene had reduced sprout growth in agreement with Bufler (2009). This was supported by the microarray data which showed those onions treated with continuous ethylene had a higher proportion of down-regulated probes characterised as being involved in photosynthesis suggesting sprout growth was at an earlier stage physiologically and at the transcriptomic level. Since the short 24 h treatments did not affect sprout growth after 35 weeks, long term storage of onions may require longer ethylene treatments, however onions which are intended for storage up to approximately 4-5 months may not require extended ethylene treatment but may benefit from a short 24 h treatment with ethylene and 1-MCP after curing which would result in cost savings. Short treatments with ethylene and 1-MCP individually have both been shown to reduce sprout growth in onion (Chope *et al.*, 2007a) although no works have investigated the effect of both ethylene and 1-MCP applied together. In potato, 1-MCP has been used to reduce the effect of ethylene on fry colour darkening; 1-MCP did not interfere with ethylene-induced sprout suppression and ethylene did not cause such a dark fry colour when pre-treated with 1-MCP (Prange *et al.*, 2005). Prange *et al.* (2005) suggested that the 1-MCP may bind to the ethylene receptors and the continuous ethylene then regulates sprout growth by binding to newly formed ethylene receptors in the sprout eyes where mitotic activity is highest. It is possible that at these sites of high mitotic activity (e.g. potato eyes), in addition to the production of new ethylene receptors, greater 1-MCP metabolism may occur since Huber *et al.*, (2010) found 1-MCP may be metabolised *in planta*. In this study, treatment of onions with ethylene and 1-MCP together resulted in higher sucrose and fructan concentrations not found in those treated with ethylene alone. Also, sprout growth was reduced in onions treated with ethylene and 1-MCP together but not in those treated with each compound separately. The study herein therefore suggests that

ethylene and 1-MCP applied together for a short 24 h treatment affects onion bulb physiology and biochemistry differently than when applied individually suggesting ethylene and 1-MCP are both binding and eliciting a response. It is possible that there may be different receptors in onion that may not necessarily all bind 1-MCP and hence both ethylene and 1-MCP may elicit a different response. This is a plausible since there are five known receptors identified in Arabidopsis: ETR1, ETR2, ERS1, ERS2 and EIN4 (Chang *et al.,* 1993; Hua *et al.,* 1995; Hua and Meyerowitz, 1998; Sakai *et al.,* 1998) and it is unknown to date whether 1-MCP binds similarly to each.

Ethylene has previously been shown to increase respiration rate in onion (Ecker, 1987); in this study respiration rate of the treated onions was highest after treatment with ethylene and lowest after treatment with 1-MCP. The respiration rate of onions treated with both ethylene and 1-MCP lay between those treated with either ethylene or 1-MCP alone suggesting the physiological response of onions to ethylene in the presence of 1-MCP was not as great as when 1-MCP was absent. At the transcriptional level, there were three clusters containing 180 probes in total which showed a differential response to ethylene and/or 1-MCP suggesting ethylene and 1-MCP probably do not elicit the same response by being perceived as the same molecule. In climacteric fruits, 1-MCP blocks ethylene action and is therefore not perceived as an ethylene molecule and it is unlikely that this is any different for onion. Cluster 0 contained probes down-regulated by exogenous ethylene but not in the presence of 1-MCP, suggesting these probes may only respond to a specific ethylene receptor or group of receptors which bind 1-MCP. On the other hand, cluster 8 contained a set of probes only up-regulated by 1-MCP alone but down-regulated by ethylene alone or in combination with 1-MCP. This suggests these probes respond to ethylene perception by a receptor or group of receptors not bound by 1-MCP. It is worth noting that differences in ethylene and 1-MCP concentration, treatment duration and temperature may result in differential gene expression since physiological and biochemical responses differ depending on these parameters (Blankenship and Dole, 2003; Watkins, 2006; Chope *et al.,* 2007; Bufler, 2009).

All microarray probes with differential expression characterised as being involved with ethylene all showed a similar pattern in expression when treated with both ethylene and/or 1-MCP. Ethylene and 1-MCP both appeared to have an effect on ethylene perception by down regulating a gene coding for an ethylene receptor. Citrus fruit are non-climacteric with low and continuous production of ethylene which is autoinhibited following propylene treatment (Katz *et al.*, 2004). Although citrus exhibit climacteric-like characteristics in the early stages of development, during the non-climacteric later phase, CsERS1 (Citrus

Ethylene Response Sensor 1) expression remained constant following ethylene treatment (20 µL L^{-1}). Treatment with 1-MCP was only conducted after harvest when the citrus fruits were in climacteric-like phase, however 1-MCP was found to down regulate CsERS1 interfering with the autocatalytic production of ethylene. The results herein suggest that ethylene may actually reduce the expression of an ethylene receptor in onion. The other citrus ethylene receptor CsETR1 (Citrus Ethylene Receptor 1) was not affected by ethylene or 1-MCP and the authors concluded that this specific receptor may not be regulated by ethylene (Katz *et al.*, 2004). Similarly, Rasori *et al.* (2002) found no change in the regulation of ETR1, but down regulation of ERS1 after treatment with 1 µL L^{-1} 1-MCP (25°C for 24 h) in climacteric peaches cv. Maria Marta.

The results in the present study show that 1-MCP down regulated an ethylene receptor in onion although this was also found after ethylene treatment. Taken together with previous findings this suggests that exogenously applied ethylene and/or 1-MCP may regulate ethylene perception by down regulating the production of some but not all ethylene receptors. That said, Ma *et al.* (2009) found that treatment of broccoli florets with 2.5 µL L^{-1} 1-MCP for 12 h decreased gene expression of the broccoli ethylene receptors ETR1 and ETR2. Although non-climacteric, broccoli also exhibits climacteric-like reactions to ethylene including chlorophyll degradation, highlighting the lack of information on the effect of ethylene or 1-MCP on true non-climacteric produce at the molecular level. In Arabidopsis, the absence of ethylene usually results in the rapid degradation of EIN3 (ethylene insensitive 3) (Guo and Ecker, 2003), a transcription factor acting downstream of the ethylene receptors in the ethylene signalling pathway (Alonso *et al.* 1999). However the results herein have found that the presence of ethylene and 1-MCP appears to down-regulate *EIN3*. This down regulation of both an ethylene receptor and ethylene transcriptional regulators by both ethylene and 1-MCP may help to explain why both compounds result in sprout suppression (Chope *et al.*, 2007a) by down regulating the perception and signalling events of the 'ripening' hormone ethylene. In direct contrast, gene expression analysis of onion treated with continuous ethylene for 29 weeks revealed up regulation of *EIN3* as well as ACO which is involved in ethylene biosynthesis. As well as an increase in ethylene biosynthesis, an increase in gibberellin 2-beta-dioxygenase expression was observed which is involved in gibberellin biosynthesis. The probe annotated as cytokinin dehydrogenase was down-regulated; cytokinin dehydrogenase is an enzyme which deactivates cytokinins through the cleavage of their side chains (Galuszka *et al.*, 2001). Although after 29 weeks in cold storage, sprout growth of onions stored in continuous ethylene were significantly shorter and rooting was reduced than those held in continuous air, it is possible that at this advanced stage of storage, ethylene was no longer having an inhibitory effect on sprout growth and

root growth. Chope *et al.* (unpublished) found that onions may become less sensitive to ethylene and produce less endogenous ethylene the longer they are in storage. This was evidenced by a steady down regulation of ACS (1-aminocyclopropene-1-carboxylate synthase), involved in ethylene biosynthesis and EIN3, a transcriptional regulator. It would be interesting to investigate at what stage of storage the inhibitory effects that ethylene has on probes involved in plant growth regulation cease.

Conclusion

In conclusion, experiments carried out at both Cranfield University and Poland showed that treating onions with ethylene and 1-MCP after curing for just 24 h consistently reduced sprout and root growth for 25 weeks. Long term storage over 25 weeks may require extended periods of ethylene treatment although beyond this transcriptional changes suggested that continuous ethylene no longer controlled onion plant growth regulators. Previous hypotheses have suggested ethylene and 1-MCP may each be able to elicit a response in non-climacteric produce due to the production of new ethylene binding sites. This is certainly a possibility yet in addition, physiological, biochemical and transcriptional data herein suggests that ethylene and 1-MCP may bind to different ethylene receptors. It appeared that ethylene and/or 1-MCP down-regulated probes tentatively annotated as ethylene receptors as well as ethylene transcriptional regulators (EIN3). Further research is required into the structure of different ethylene receptors to investigate whether 1-MCP can bind all receptors. Since microarray data was only gathered from onions immediately after treatment at the beginning of storage or at the end of storage in continuous ethylene, it would be interesting to further investigate the dynamic effect ethylene/1-MCP has at the transcriptional and indeed the metabolic level.

THE EFFECT OF POSTHARVEST HANDLING OF ONION (*ALLIUM CEPA* L.) ON *BOTRYTIS ALLII* INFECTION

G. A. Chope[1], N. K. B. Adikaram[2], K. Downes[1], and L. A. Terry[1]

[a]Plant Science Laboratory, Cranfield University, Bedfordshire, MK42 0AL, UK
[b]Department of Botany, University of Peradeniya, Peradeniya 20400, Sri Lanka

Introduction

In the UK, neck rot caused by *Botrytis allii* is one of the principal causes of losses of marketable onions in store (Gunkel *et al.*, 1971; Maude & Presly, 1977a;). Infection occurs prior to harvest via dead leaf tissue near the bulb neck, or through wounds in living neck tissue caused by mechanical harvesting (Walker, 1926). Succulent neck tissue is especially susceptible to infection after wounding (Harrow & Harris, 1969). The disease is symptomless in plants, spreading both within individual plants and through the crop during the course of growing season (Maude & Presley, 1977b). Postharvest neck rot is thought to be due to progressive but latent foliage infections followed by incomplete or delayed bulb drying. Infected onions then develop neck rot symptoms during storage.

Direct harvesting, with mechanical removal of foliage, followed by postharvest drying at uncontrolled ambient temperatures (18ºC) resulted in an increase in the incidence of onion neck rot (Maude *et al.*, 1984), possibly due to the redistribution of residual inoculum by the violent removal of the crop foliage. Infected seeds have been shown to be the main source of *B. allii* in the onion crop (Maude & Presly, 1977a) although the disease only becomes evident in store. Seedlings raised from diseased seeds become infected by mycelial invasion of the cotyledon leaf tips from seed coats, many of which remain attached to the cotyledons when seedlings emerge from the soil (Maude & Presly, 1977a).

The optimal temperature for growth of *B. allii* was determined to be 20 or 25ºC on agar (Alderman & Lacy, 1983) and 20ºC on onion bulb tissue (Munn, 1917). Temperature also has an influence on pathogenicity, where inoculation with conidia produced at low temperature (-2ºC) caused a higher incidence and larger areas of disease than those produced at higher temperatures (Bertolini & Tian, 1997). Precise information on *B. allii* growth, inoculum production and survival in relation to the temperature range at which onions are cured and stored is not available.

In the UK, onions are cured to form a complete dry outer skin which conveys many benefits including reduction of disease incidence and prevention of excessive water loss from the outer scales of the bulb, resulting in reduced shriveling (Downes *et al.*, 2009). Traditionally, curing was completed in the field, but wet conditions during field curing can cause skin staining (Wright *et al.*, 2001). To improve onion quality, commercial value and appearance, windrowing and field drying in boxes or wire cages were replaced by enclosed artificial drying. Current practice in the UK is to cure onions for 3-6 weeks at 28ºC and 65-75% RH. Neck rot disease has been reduced in the UK since the introduction of forced air curing which effectively seals the bulb neck (Langston 2001). The disease was substantially reduced if topped onions were dried at 30ºC and this treatment was most effective if the crop was removed from the field for drying within 48 h of topping, thus avoiding severe infection of the damaged green tissues of the necks of onions (Maude *et al.* 1984). Although more expensive, artificial drying reduces skin-staining and fungal attack which results in improved skin appearance (O'Connor 1979). Brown onions gain their colour from flavonols, mainly quercetin and its derivatives (Griffiths *et al.*, 2002), whereas red onion skin contains anthocyanins (Wu & Prior, 2005). During skin drying and storage, quercetin glucosides are converted into quercetin (Hirota *et al.*, 1998). The antifungal agent, 3,4 dihydroxy benzoic acid (3,4 DHBA; protocatechuic acid) is synthesized through oxidation of quercetin during skin browning, similarly, 3,4 DHBA has been observed in the dry scales of red onions (Link *et al.*, 1929; Ng *et al.*, 2000).

It has been proposed that the current UK curing temperature of 28ºC could be reduced to 20ºC without compromising product quality, however, the effect of this on neck rot disease should be investigated. Therefore the present study was undertaken to monitor neck rot disease in onions inoculated with / without *B. allii* and cured at 20 or 28ºC both immediately after curing, and after six months cold storage (1ºC). In vitro experiments using a custom temperature block were performed to investigate growth, sporulation and viability of *B. allii* over a range of temperatures to include those involved in curing and storage of onions. Studies were also made to examine the behaviour of *B. allii* in response to some fungitoxic flavonoids found in onion scales.

Materials And Methods

Neck rot development in onion cured at 20 or 28˚C following artificial inoculation with B. allii

Two brown onion cultivars and one red cultivar with differing susceptibility to neck rot, Kamal, Vision and Arthur (susceptible), were grown on sandy soil (A.W. Mortier Farms, Woodbridge, Suffolk) from seeds drilled on 21st March 2009 at a rate of 58 seeds m^{-2} with pesticides applied as per commercial practice. Plants were harvested at 100% fall-down on 1st September 2009.

Botrytis allii (IMI number: 292066) was purchased as a freeze dried sample (CABI), and re-suspended in 300 µL sterile distilled water. The suspension was used to inoculate Petri dishes (9 cm) containing potato dextrose agar (PDA; Oxoid) with 1 mg mL^{-1} streptomycin. Plates were sealed with Parafilm and incubated at 22ºC (12 hour UV cycle; Sanyo Versatile Environmental Test Chamber) for 8 days. The resulting conidia were used to inoculate fresh PDA Petri dishes, which were incubated under the same conditions. Conidia suspensions were prepared from these second generation plates. Plates were flooded with 2 mL sterile distilled water containing 0.05% Tween 80 (v/v) (Sigma) and the resulting conidia suspension was filtered through glass wool and the filtrate divided into three replicates, each of which were subsequently diluted to a concentration of 5 x 10^4 conidia mL^{-1} with sterile distilled water.

The neck of the onion was cut with a clean scalpel blade to reveal living tissue and 100 µL of either conidia suspension or sterile distilled water was pipetted onto the open wound. Onions were left to air dry for 30 mins, and then divided between two curing treatments, 20 or 28ºC with relative humidity controlled at 45-55% for six weeks. After curing, a subset of onions was transferred to 20ºC for six weeks (n=360). The remaining bulbs were stored at 1 ± 0.5ºC for six months. The experiment was a completely randomised design with three replicates each consisting of 10 bulbs for each inoculation treatment, curing temperature and sampling time combination.

Disease incidence and disease coverage were analysed after harvest (before *B. allii* inoculation; T0; n=90), after curing (T1; n=360) and after cold storage (T2; n=360). Bulbs were cut in half vertically, and disease incidence was recorded as the percentage of bulbs showing symptoms. Disease coverage was assessed as the area of the cut surface of infected bulbs covered by the disease lesion. For all inoculated bulbs, after disease assessments, a square (*ca*. 0.5 mm^2) of tissue was cut from the bulb neck and placed on a Petri dish (9 cm) containing PDA and 1 mg mL^{-1} streptomycin. In addition, a sterile loop was used to transfer conidia (where present) onto another Petri dish. The cultures were incubated at 22ºC for eight days to determine whether *B. allii* could be cultured from these samples.

Isolation of fungi from onions cured at 20 and 28ºC and inoculated with B. allii

Neck rot symptom free onions cv. Kamal inoculated with *B. allii*, cured at 20 (n=12) or 28ºC (n=12), and stored for 11 weeks at 1ºC were selected. The protruding portion (1.5 cm) of the neck region was cut and removed from each bulb. After peeling away two outer scales, slices (0.5 cm^2) were cut from the neck region (n=5 per bulb) and surface sterilized with 10% sodium hypochlorite for 3 minutes then rinsed with sterile distilled water. The tissue was placed on PDA (1 mg mL^{-1} streptomycin sulphate) plates (n=5 per plate) and the plates incubated at 22ºC for 5 days. A record was made of the species and number of colonies present. The experiment was repeated on diseased onions cv. Arthur and Vision inoculated with *B. allii*, cured at 20 or 28ºC, and stored for 11 weeks. Fungi, other than *B. allii*, were isolated and sub-cultured on PDA and identified. Dual cultures containing *B. allii* and each of the other isolates were prepared on PDA, incubated at 22ºC, and observed daily to determine whether any of these fungi suppressed growth of *B. allii*.

Effect of temperature on growth and sporulation of B. allii

One hundred glass tubes (2.5 cm diameter, 7.5 cm tall) were sterilized by dipping in 2-propanol for 1 h. The PDA medium (12 mL) was poured into each tube under aseptic conditions and the tubes plugged with sterile cotton wool, then 5 µL aliquots of a conidia suspension (5 x 10^4 conidia mL^{-1}) of *B. allii* were applied to the centre of the agar surface. A custom aluminium temperature block (length 86 cm, width 20.5 cm, height 12 cm) with five rows of 20 cylindrical holes (2.5 cm diameter, 7.5 cm deep) and connected to hot water circulating equipment at one end and coolant at the other, was calibrated to a temperature range from 16 to 33ºC, with a temperature difference of 1ºC between each hole. The culture tubes were placed in the holes (n=5 per temperature). The temperature block was seated on, and covered with, a heat insulating rigifoam frame. After 5 days incubation, the culture tubes were removed and the radial growth of the colonies was measured at two right angle points. In tubes where the colonies had reached the periphery of the medium and continued to grow upwards along the inner wall of the tube, the distance of extra growth was added to the diameter of the tube and taken as the colony diameter. The average diameter of each colony was calculated. There was no growth at temperatures ≥ 29ºC during the first 5 days. To determine whether cultures incubated at these temperatures were still alive; the culture tubes were transferred to temperatures 19 to 22ºC, the optimal temperature range for growth, and observed for a further 14 days. The experiment was repeated at a temperature range of 1 to 15ºC. The culture tubes at 1 to 9ºC which had not shown growth after 5 days,

were incubated at the same temperatures for a 9 days after which the colony diameter was determined.

To determine sporulation, the mycelium was scraped from each tube and suspended in 2.5 mL portions of sterile distilled water separately. The remaining mycelium in the tubes was washed off with another 2.5 mL portion of fresh sterile distilled water and the two were mixed. The suspension was vortexed for 2 mins and filtered through glass wool. The number of conidia in the suspension was counted using a heamocytometer. Four drops from a suspension made from each replicate tube were used for counting conidia produced at each temperature.

Effect of temperature on viability of B. allii conidia

Thirty two *B. allii* cultures were grown on PDA in glass tubes (2.5 cm diameter and 7.5 cm tall) at 20ºC for 6 days until sporulation, as described previously, after which, conidia were harvested from four cultures and percentage germination was determined as described below. The remaining culture tubes were incubated at 20, 28, 29, 30, 31, 32 or 33ºC in the temperature block for 5 days. The cultures were removed and suspensions of conidia were prepared by scraping mycelia, and suspending in sterile distilled water. The number of conidia in each suspension was adjusted to 5 x 10^4 conidia mL^{-1}. Drops (20 µL) were placed on clean glass slides (2 drops per slide and two slides per treatment) and incubated in moist chambers for 18 h at 20ºC. At the end of the incubation period, a drop of lactophenol/cotton blue was added to each drop to stop germination. Counts were made on at least 100 conidia from each drop for germination and the percentage germination was determined. Another set of cultures were exposed to the same temperatures (20, 28 to 33ºC) for a period of 10 days and percentage germination of conidia was determined.

Antifungal activity of 3,4 dihydroxy benzoic acid, 2,4,6 trihydroxy benzoic acid and quercetin

A stock solution of 0.05 mM 3,4 dihydroxy benzoic acid (3,4 DHBA; Sigma-Aldrich) was prepared in sterile distilled water by shaking and vortexing vigorously for 10 min. A series of 3,4 DHBA solutions were prepared by diluting the stock solution with volumes of sterile distilled water and a conidia suspension of *B. allii* to each in such a way that the final concentrations were 0.005 mM, 0.01 mM, 0.02 mM, 0.03 mM, 0.04 mM of 3,4 DHBA and 5 x 10^4 conidia mL^{-1}. A suspension of conidia (5 x 10^4 conidia mL^{-1}) in sterile distilled water served as the control. Drops (20 µL) were applied to clean glass slides (2 drops per slide and 2 slides per solution) and incubated at 20ºC under humid conditions for 18 h and

percentage germination was determined for each solution as described previously. The experiment was repeated.

The germination experiment was also carried out with a series of preparations containing 0.005 mM, 0.01 mM, 0.02 mM, 0.03 mM, 0.04 mM and 0.05 mM 2,4,6 trihydroxy benzoic acid monohydrate (2,4,6 THBA; Sigma-Aldrich), which was not completely soluble in water but formed a slightly cloudy suspension. Similarly, a conidia germination assay for one *Penicillium* isolate and the *Giocladium* isolate was also carried out for 3,4 DHBA, and for one *Penicillium* isolate for 2,4,6 THBA.

Statistical analysis

All statistical analyses were carried out using Genstat for Windows 12th Edition, Version 12.1.0.3338 (VSN International Ltd.). Analysis of variance was performed on the data specifying a nested treatment structure of a common baseline (observation before curing was the baseline). Least significant difference values (LSD; P=0.05) were calculated from each analysis, for comparison of appropriate treatment means, using critical values of t for two-tailed tests. Results are significant to P<0.05 unless otherwise stated

Results

Neck rot development in onion bulbs artificially inoculated with B. allii after curing at 20 or 28ºC

No disease was recorded in freshly harvested onions prior to inoculation. For both disease incidence and severity there was a significant effect of treatment, curing temperature and time (P<0.001), where inoculated bulbs contained more disease than non-inoculated, and curing at 28ºC delayed onset of disease until after cold storage. After curing, no disease was recorded in inoculated or non-inoculated onions cured at 28ºC. The only disease recorded in non-inoculated bulbs cured at 20ºC was in cv. Kamal (Table 1). However, after curing, disease was recorded in all cultivars where bulbs were inoculated and cured at 20ºC. There was no significant difference in disease incidence or severity between cultivars. For inoculated onions cured at 20ºC, disease incidence remained stable during time, but disease severity increased during cold storage. Disease severity of onions cv. Arthur and Vision after cold storage was almost twice as much in onions cured at 20 than 28ºC.

Table 1. Disease incidence (percentage of bulbs infected) and severity (% of cut surface covered by lesion) of onions inoculated with / without *B. allii* and cured at 20 or 28˚C for 6 weeks immediately after curing and after six months cold storage ($LSD_{0.05}$ incidence = 1.895; severity = 9.617).

			After curing		After cold storage	
Cultivar	**Treatment (+/- *B. allii* inoculation)**	**Curing temperature (ºC)**	**Disease incidence (%)**	**Disease severity (%)**	**Disease incidence (%)**	**Disease severity (%)**
Arthur	+	20	9.00	7.03	7.67	96.17
	+	28	0.00	0.00	7.33	49.63
	-	20	0.00	0.00	0.00	0.00
	-	28	0.00	0.00	0.00	0.00
Kamal	+	20	7.33	8.51	8.33	74.35
	+	28	0.00	0.00	6.67	69.24
	-	20	0.33	10.00	0.00	0.00
	-	28	0.00	0.00	0.00	0.00
Vision	+	20	9.67	6.62	8.67	82.06
	+	28	0.00	0.00	6.33	45.00
	-	20	0.00	0.00	0.33	3.33
	-	28	0.00	0.00	0.00	0.00

Of the sub-set of samples stored for a further six weeks at 20ºC after the initial curing treatment at 20 or 28ºC, disease was only recorded in those bulbs originally cured at 20ºC. Similarly, *B. allii* was only cultured on PDA in Petri dishes inoculated with tissue or spore scrapings from inoculated onions cured at 20ºC.

Isolation of B. allii from onions cured at 20 and 28ºC and inoculated with B. alli followed by storage at 20ºC

Growth of several fungi was observed on PDA from the neck scale tissue pieces sampled from onions cured at both temperatures. Comparatively few *B. allii* colonies were present among numerous colonies of other fungi (Table 2). Among the seven other fungal isolates, six were culturally different Penicillia and one was a *Gliocladium* sp. Overall, a mean of 0.47 colonies of *B. allii* were cultured per plate, compared with 4.19 colonies of other fungi. There

was no significant effect of curing temperature on the number of colonies of *B. allii* or other fungi. The isolations carried out with onions with and without neck rot symptoms were not significantly different. All six *Penicillium* isolates and *Giocladium* sp. grew faster compared than *B. allii* when cultured together on PDA, thus restricting the expansion of the *B. allii* colony at the centre. A *Giocladium* sp. isolated was among the fastest to grow on PDA and initially restricted *B. allii* growth and finally completely overran the *B. allii* colony.

Table 2. Fungi isolated from the neck region of onions cv. Kamal (no visible neck rot symptoms) and Arthur, Vision (visible symptoms) inoculated with *B. allii* prior to curing at 20 or 28ºC for six weeks, ands stored at 1ºC for 11 weeks. Values are the means (n=12). Numbers in parentheses represent the percentage of the total number of colonies. $LSD_{0.05}$ number of colonies = 1.269.

Cultivar	Curing temperature (ºC)	No. of colonies			
		B. allii		Other fungi	
Arthur	20	0.25	(6.7%)	3.50	(93.3%)
	28	0.25	(5.1%)	4.67	(94.9%)
Kamal	20	0.70	(10.6%)	5.90	(89.4%)
	28	0.10	(2.3%)	4.30	(97.7%)
Vision	20	0.08	(1.9%)	4.08	(98.1%)
	28	1.42	(32.1%)	3.00	(67.9%)

Effect of temperature on growth, sporulation and viability of B. allii

The growth and sporulation of *B. allii* were determined at a temperature range from 1 to 33ºC after five days incubation. The fungus grew at all temperatures between 9 and 28ºC with strong growth between 15 and 25ºC, and maximum colony growth at 20 to 21ºC (Fig. 1). No growth occurred ≤9ºC or ≥29ºC within 5 days. However, prolonged incubation for an extra nine days at temperatures from 1 to 9ºC resulted in slow expansion of colony growth. *B. allii* growth sharply dropped when the temperature was reduced from 15 to 14ºC. Sporulation occurred only over a narrow range of temperatures from 17 to 24ºC within 5 days. Even after prolonged incubation the fungus did not produce conidia at temperatures below 11ºC. The optimum temperature range for sporulation was 19 to 21ºC. Growth was resumed in cultures previously grown at temperatures 29 to 32ºC (but not in those grown at 33ºC) after eight days incubation at optimal temperature (*ca*. 20ºC), although growth was

slow, producing only sparse mycelium. Sporulation occurred only in those cultures previously incubated at 29 to 31ºC.

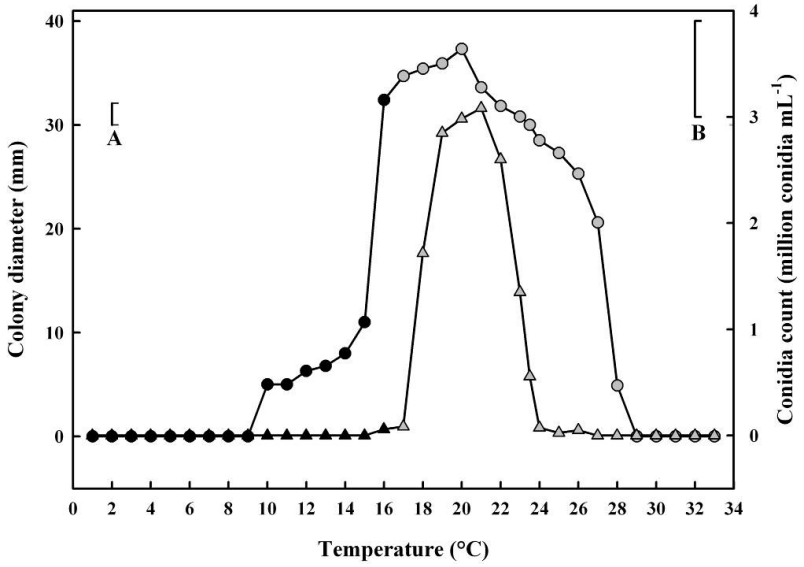


Figure 1. Colony growth (circles) and sporulation (triangles) of *B. allii* incubated at 1 to 33ºC for 5 days (Grey / black fill represents data from separate experiments). $LSD_{0.05}$ bars are shown; A = colony diameter, B = spore count.

Viability of conidia was tested following periods of 5 and 10 days of exposure of cultures to temperatures between 28 and 32ºC. Exposure of *B. allii* to temperatures above 28ºC for 5 days resulted in stimulation of significantly more conidia to germinate, with this effect being the greatest at 29ºC (Table 3). Similarly, stimulation of germination was also observed in cultures exposed to 28 or 29ºC for 10 days. However, prolonged exposure to temperatures higher than 30ºC progressively reduced the viability of conidia.

Table 3. Percentage germination of *B. allii* conidia harvested from cultures exposed to temperatures from 28 to 32ºC for 5 or 10 days. $LSD_{0.05}$ 5 days = 9.03; 10 days = 6.78.

Temperature (ºC)	Percentage germination		
	0 days	5 days	10 days
20	58.4	44.2	13.2
28	58.4	72.5	70.3
29	58.4	87.7	86.0
30	58.4	76.5	47.2
31	58.4	79.1	28.7
32	58.4	36.9	12.4

Germination of B. allii and selected Penicillium isolates in quercetin and its oxidative products

A solution of 0.05 mM 3,4 DHBA reduced germination of *B. allii* by over 80% and the germ-tube length of germinated conidia by 95% (Fig. 2). There was a progressive increase in both germination and germ-tube growth when the concentration was reduced from 0.050 to 0.005 mM. The highest concentration of 3,4 DHBA that would be tested was 0.050 mM, as it was insoluble above this level. Germination was significantly inhibited by 0.040 mM and 0.050 mM compared with 0.000, 0.010 mM and 0.005 mM 3,4 DHBA solutions, and germ-tube length was significantly shorter for all 3,4 DHBA concentrations compared with the control. The germination of an isolate of *Penicillium* spp. and *Giocladium* was stimulated by 3,4 DHBA at lower concentrations, below 0.04 mM (Table 4). However, there was a slight inhibition when the concentrations are higher around 0.05 mM.

Table 4. The effect of 3,4-dihydroxybenzoic acid (3,4 DHBA) on percentage germination of *Penicillium* and *Giocladium* isolates. $LSD_{0.05}$ = *Penicillium* = 14.64; *Giocladium* = 10.80.

Concentration of 3,4 DHBA (mM)	**Percentage germination**	
	Penicillium	***Giocladium***
0.000	0.0	0.0
0.005	30.3	12.5
0.010	44.0	32.9
0.020	28.4	29.8
0.030	23.3	39.8
0.040	2.8	12.8
0.050	0.0	5.4

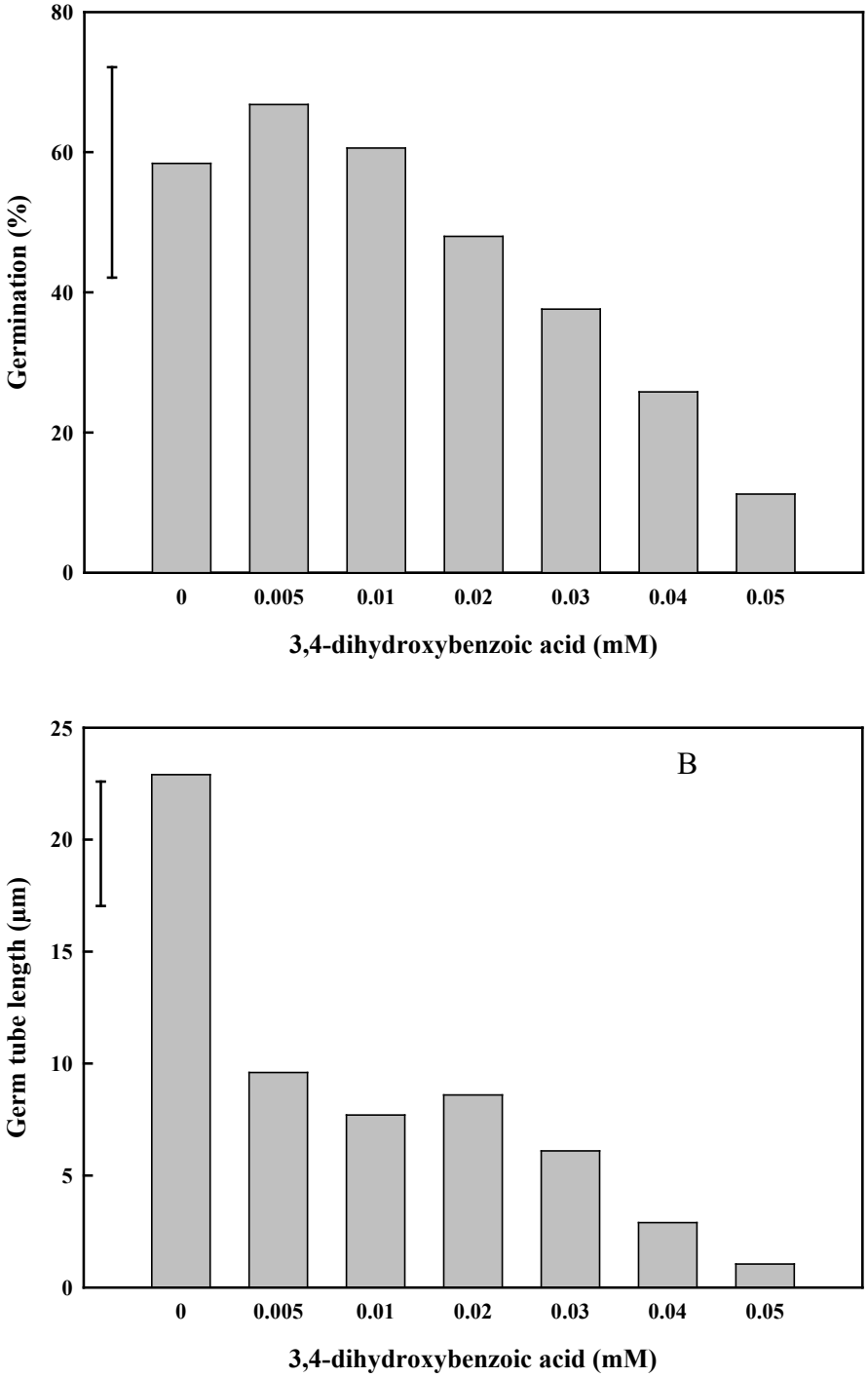


Figure 2. Effect of 3,4 DHBA on percentage germination (A) and germ tube length (B) of *B. allii*. $LSD_{0.05}$ bars are shown.

All concentrations of 2,4,6 THBA tested (0.005 mM to 0.050 mM) inhibited germination of *B. allii* completely (Table 5). Germination of the *Penicillium* isolate was also inhibited by 2,4,6 THBA at concentrations of 0.020 mM and above.

Table 5. The effect of 2,4,6 trihydroxybenzoic acid (2,4,6 THBA) on percentage germination of *B. allii* and a *Penicillium* isolate. $LSD_{0.05}$ *B. allii* = 3.78; *Penicillium* = 8.05.

Concentration of 2,4,6 THBA (mM)	Percentage germination	
	B.allii	***Penicillium***
0.000	16.9	1.5
0.005	0.0	7.8
0.010	0.0	15.0
0.020	0.0	0.0
0.030	0.0	0.0
0.040	0.0	0.0
0.050	0.0	0.0

Discussion

The effect of reducing the temperature at which UK onions are cured from 28 to 20ºC on the incidence of neck rot in store was investigated both *in vitro* and *in vivo*. Immediately after curing, disease symptoms were not evident in onions inoculated with *B. allii* prior to curing at 28ºC compared with a high incidence of disease in those cured at 20ºC. Similarly, *B. allii* could not be cultured from onion cured at 28ºC. However, this effect did not persist during cold storage at 1ºC for six months. Previously, Maude *et al.* (1984) found that after around three months storage (unspecified temperature) neck rot was increased in onions cv. Rijnsberger grown from treated infected seed cured at ambient temperature (ca. 18ºC) for six days compared with 30ºC, although the differences were relatively small (3.3% versus 1.3%). The authors also found similar results for inoculated (1 x 10^6 spores mL^{-1} at 22.7 mL spores m^{-1}). The reason for the discrepancies between the results presented here may be due to the much shorter curing time, and the use of ambient air rather than air at a controlled temperature.

There was no difference observed between the cultivars, even though one was a susceptible variety. This is likely to be due to the very high disease pressure that the bulbs were subjected to, which would mask any subtle differences in disease resistance.

The optimum temperature for growth and sporulation of *B. allii in vitro* was shown to be around 20ºC, the proposed new curing temperature, which explains the presence of disease symptoms in the inoculated onions cured at 20ºC. However, transferring inoculated onions from 28 to 20ºC for six weeks did not result in the onset of diseases symptoms. Results from the temperature block experiments show that culturing *B. allii* at 28ºC does not kill the pathogen, but slows its growth while maintained at that temperature. The fungus may have required longer incubation at 20ºC to compensate for the long exposure to high temperature during curing. Incubation temperature has been shown to have an effect on sporulation where larger spores were produced more slowly but in greater volumes at -2ºC compared with 20ºC (Tian & Bertolini, 1996). In this study, it was demonstrated that short exposure to high temperatures, including 28ºC, for up to ten days stimulated germination and therefore increased viability of conidia. This suggests that curing at 28C must be carried out for the relatively long period of six weeks, otherwise there is a risk that disease onset and severity could progress rapidly in storage. Another possible mechanism for the suppression of disease symptoms by curing at 28C is competition by other species. It was observed that there appeared to be greater presence of *Penicillium* sp. On the skins of onions cured at 28ºC, however, when samples were taken and cultured, there was no difference in the number of colonies of other species (which comprised *Penicillium* and *Gliocladium*) cultured from onions cured at 28 or 20ºC. These species were shown to out-compete *B. allii* in dual culture, so if these species are adapted to grow faster at a higher temperature, or are able to grow more easily when *B. allii* growth is inhibited at higher temperatures, then this could further reduce growth of *B. allii* at 28ºC. This hypothesis is also supported by the findings of Kohl *et al.* (1991), who found that in field trials of onions artificially inoculated with *Botrytis aclada*, application of *Gliocladium roseum* during harvest reduced the incidence of neck rot after three months storage at 9ºC.

Conclusion

In conclusion, these results suggest that curing at 28ºC can delay disease onset, compared with curing at 20ºC, but does not kill the pathogen. Where high disease pressure is not present, there is no difference between the two curing temperatures.

FATE OF FLAVONOIDS IN THE OUTER SKINS OF ONION (*ALLIUM CEPA* L.) THROUGHOUT CURING

Downes, K., Chope, G.A. and Terry, L.A.*

Plant Science Laboratory, Cranfield University, Bedfordshire, MK43 0AL, UK.

Introduction

Due to the maritime climate of the UK, onions are not usually cured outside in the field like in many other parts of the world, but are cured artificially, in closed environments. The purpose of curing is to remove excess water from the outer skins and seal the neck of the bulb, which acts as a barrier against pathogens (e.g. *Botrytis allii*) and minimises weight loss from the flesh (Thompson, 1982). Various curing regimes have been proposed (Whitwell, 1970; Thompson, 1972; O'Connor, 1979); nonetheless the consensus is that relative humidity (RH) should be controlled at *ca*. 75% as excessive water loss tends to occur below 60% RH which can result in skin splitting (Bleasdale *et al.*, 1969). Current practise involves curing onions at 28 °C for three to six weeks, however reducing this temperature to 20 °C would reduce energy use and therefore costs by 35% (D. O'Connor, pers. comm.). Apart from the aforementioned reasons, curing also creates a darker brown skin appearance which is appreciated by the consumer. Therefore, changing the curing temperature and hence the skin appearance may also influence the appeal of the product to consumers. Recent work by Downes *et al.* (2009) showed that the skins of red onions cured at 28 °C were less red than those cured at 20 °C, and also contained lower concentrations of certain anthocyanins, possibly caused by an increased rate of degradation of these compounds at the higher temperature. Additionally brown onions were found to be darker when cured at 28 °C compared to 20 °C, however, the reason for this was not elucidated as no interaction with flavonols, sugars or dry weight was identified by the authors.

The pigment of red onions results from anthocyanins which are mainly comprised of cyanidin and peonidin derivatives, however the pigment of brown onions has yet to be conclusively identified. Onion skins also contain phenolic acids; mainly ferulic, gallic and protocatechuic, and these may contribute to brown and red skin colour; however their concentrations in the skin of red onions (unknown cultivar) were 240-, 20-, and 40-fold respectively, lower than that of quercetin (Prakash *et al.*, 2007). It has been postulated that the pigmentation of brown onions is derived from flavonols, specifically quercetin and its derivatives, although the maximum absorbance of these compounds is at 370 nm, whereas that of dried outer skin

of brown onions is around 450 nm (Ito *et al.*, 2009). Ito *et al. (8)* identified a novel yellow pigment as a xanthylium, specifically 9-carboxy-1,3,6,8-tetrahydroxyxanthylium, which they named cepaic acid. A mechanism was hypothesised whereby cepaic acid is synthesised from quercetin via a multi-level pathway which involves oxidation. Quercetin can be oxidised via peroxidase (POX), resulting in the synthesis of many antifungal phenolic compounds such as 3,4-dihydrobenzoic acid, which increases during the skin browning process (Takahama and Hirota, 2000). Onions that do not synthesise flavonoids are prone to pathogenic infection suggesting the browning process is linked to the production of some preformed antifungal compounds (Walker and Link, 1929; Walker and Stahmann, 1955).

The major sugars found in onion skin are fructose and glucose (Downes *et al.*, 2009). It has been suggested that sugars may play a regulatory role in the production of flavonoids since both anthocyanindins and flavonols can exist as sugar conjugates (Gennaro *et al.*, 2002). In addition, sugars can react with amines to produce brown products via the Maillard reaction which is common in foodstuffs occurring at elevated temperatures or after prolonged periods of storage (Nursten, 2005).

The aim of this study was to elucidate the compounds responsible for the difference in colour between onions cured at 20 and 28 °C by monitoring dynamic biochemical changes in the skin at set intervals during curing. Three years of data were investigated however the results from 2007 were published in Postharvest Biology and Technology and can be found in Appendix A. Results from 2008 and 2009 are described below and use parametric and non-parametric multivariate analysis to verify the relationship between biochemical and physiological data.

Materials And Methods

Plant material

In 2008, one red and two brown onion cultivars were selected *viz.* 'Red Baron', 'Sherpa' and 'Wellington', respectively, and grown on sandy clay loam (Alastair Findlay's, Cardington, Beds., UK; 1.2 x 0.3 Ha). Onions were grown from seeds drilled on 5th March 2008 at a rate of 57 seeds m^{-2} with pesticides applied as per commercial practice. Plants were hand-harvested at 100% fall-down on 17th September 2008. In 2009, onion cultivars 'Red Baron', 'Sherpa' and 'Wellington', and an additional red cultivar, 'Kamal', were grown on the same site as in 2008. Onions were grown from seeds drilled on 16th March 2009 at a rate of 54

seeds m^{-2} with pesticides applied as per commercial practice. Onions were hand-harvested on 7th September 2009 at 100% fall-down.

Experimental design

Two growing seasons were used, each focusing on different aspects of the curing process. In 2008, onion skins were studied at three time points; before curing, after curing and after long term cold storage at 1 ± 0.5 °C for 35 weeks to investigate the effects of curing and storage on skin appearance and to identify the principal compounds responsible for these changes. In 2009, skin was sampled throughout curing at weekly intervals to identify the temporal profile of the changes recorded in 2008.

Both experiments were completely randomised with three replicates harvested from three equal plots of the field. Bulbs were harvested into nets and buried amongst other loose bulbs in one tonne capacity wooden crates for curing. In 2008, three crates of onions, one per replicate, were each cured at two curing temperatures; 20 or 28 °C for six weeks (65-75% RH), at Sutton Bridge Experimental Unit (Lincs., UK). After curing, all netted onions were transported to Cranfield University within 2.5 h. Diseased or damaged bulbs were removed and the remaining bulbs transferred to individual stackable plastic trays and stored for 35 weeks at 1 ± 0.5 °C. Five bulbs per cultivar, curing temperature and replicate were taken for analysis immediately before (n = 45) and after (n = 90) curing and after 35 weeks of cold storage (n = 90). In 2009, nets were divided between two crates, one at 20 and the other at 28 °C, to facilitate frequent sample collection during curing. Skin from three bulbs per cultivar, curing temperature and each of the three replicates was analysed before curing (n = 36) and after six, 13, 20 and 41 days of curing (n = 72).

Colour measurement and sample preparation

Loose outer skins and dry roots were removed as per commercial practice. Objective colour (chroma (C*), hue angle (Hº) and lightness (L*)) was assessed using a Minolta CR-400 colorimeter and DP-400 data processor (Minolta Co. Ltd., Japan) as the mean of three measurements taken from around the equator of the onion bulb (Downes *et al.*, 2009). The first layer of intact dry outer skin from each bulb was removed and a sample (250 mg) was retained and stored at -40 °C. All biochemical analysis was conducted on fresh skins, however a portion was lyophilised for analysis of dry weight using an Alpha 1-4 Christ LDC-1 freeze-dryer and pump (Edwards Super Modulo, Sussex, UK).

Biochemical analysis

Quantification of sugars

Sugars were extracted and quantified according to Section A materials and methods

Quantification of flavonols

Flavonols were extracted and quantified according to Section A materials and methods

Quantification of anthocyanins

Anthocyanins were separated and quantified according to Giné Bordonaba and Terry (2008) with modifications (Downes *et al.*, 2009). The same extract and HPLC system was used to measure anthocyanins and flavonols. The undiluted extracts (10 µL) were injected into an Agilent ZORBAX Eclipse XDB-C18 column, 4.6 mm × 250 mm, 5µm particle size (Part no. 990967-902). The mobile phase consisted of HPLC grade water with 2.5% acetonitrile and 5% formic acid (A) and acetonitrile (B). The program involved a linear increase/decrease of solvent B: 5–10%, 9.5 min; 10–18%, 9.2 min; 18–65%, 3.3 min; 65%, 3min at a flow rate of 1mL min^{-1} and a column temperature of 35 °C. Anthocyanins were detected at a wavelength of 520 nm. Each peak was assigned an identity according to Downes *et al.* (2009) however only the three main anthocyanins; cyanidin 3'-glucoside, cyanidin 3-(6''-malonoyl-laminariboside) and cyanidin 3-(6''-malonoylglucoside) according to Fossen *et al.* (1996) and Wu and Prior (2005) were included. Anthocyanin concentrations were expressed as mg of cyanidin 3'-glucoside (Extrasynthese, Lyon, France) equivalent per g FW (mg C3GE g^{-1} FW).

Statistical analysis

All statistical analyses were conducted using Genstat for Windows version 10.1.0.147 (VSN International Ltd., Herts., UK). Analysis of variance (ANOVA) was performed on the data specifying a nested treatment structure of a common baseline (observation before curing at day 0 was the starting point for both curing temperature treatments), plus a factorial combination of time and curing temperature for each year separately. Least significant differences (LSD; $P = 0.05$) were calculated for each analysis. Significant differences are quoted at $P < 0.05$ for all physiological and biochemical parameters for both 2008 and 2009. Correlation coefficients (Pearson's) were calculated between all biochemical and physiological data sets. To further identify relationships between the biochemical and physiological data sets, principle component analysis (PCA; an unsupervised multivariate technique) and partial least squares discriminant analysis (PLSDA; a supervised multivariate technique) were adopted. As anthocyanins were only detected in red onions ('Red Baron' in

2008 and 'Kamal' and 'Red Baron' in 2009), data sets for red onions ('Kamal' and 'Red Baron') and brown onions ('Sherpa' and 'Wellington') were analysed separately for correlations and multivariate analysis. For the brown onion cultivars, nine variables (C*, dry weight, fructose, glucose, H°, L*, quercetin, quercetin and quercetin 3,4'-diglucoside) were considered as analytical data for PCA whereas the addition of nine individual anthocyanins took the number of variables for the red onion cultivars to 18.

Results And Discussion

In the UK, onions are currently cured at 28 °C although reducing the curing temperature to 20 °C could reduce energy inputs and therefore costs. Previous works by Chope *et al.* (unpublished) found curing onion cvs. 'Red Baron', 'Sherpa' and 'Wellington' at the lower temperature of 20 °C compared with 28 °C had no significant effect on sprout growth, sprout incidence, bulb dry weight, pungency or disease incidence. In fact, onions cured at 20 °C had lower root incidence than those cured at 28 °C. The aim of this study was to investigate the effect of different curing temperatures on skin composition and to the best of our knowledge, for the first time, measure skin biochemistry and physiology throughout the curing process.

There was no main effect of curing on quercetin, quercetin 3,4'-diglucoside or quercetin 4'-glucoside concentrations in 2008 and 2009 (Table 1 and Figure 1). Nevertheless, in 2009, mean quercetin 3,4'-diglucoside concentration for all cultivars throughout curing at 28 °C was significantly lower (0.72 mg g^{-1} FW) than throughout curing at 20 °C (1.21 mg g^{-1} FW). Freshly harvested brown onions 'Sherpa' and 'Wellington' contained higher concentrations of quercetin and a wider range (from *ca.* 10 – 55 mg g^{-1} FW) of values compared with cured onions. Thus, skin samples from brown onions cured at 20 °C contained quercetin concentrations below *ca.* 30 mg g^{-1} FW and those cured at 28 °C were even lower at *ca.* 4 – 10 mg g^{-1} FW. This trend (quercetin before curing > 20 °C > 28 °C) was also observed for H° with the skins of freshly cured brown onions having the highest H° (*ca.* 70 – 80 indicating a yellow colour) followed by those cured at 20 °C (*ca.* 60 – 70) and 28 °C (*ca.* 55 – 65) becoming successively browner and resulting in a positive correlation between quercetin and H° ($r = 0.69$; $n = 30$). This data is presented in Figure 2 and includes samples taken before curing and from onions cured at 20 or 28 °C both immediately after curing and after seven months cold storage. It may therefore have been assumed that the range of quercetin concentrations or H° values would be greater in cured and stored onion skin than those before curing, yet this was not the case. This suggests that curing results in a more uniform

skin colour and quercetin content which persists throughout cold storage as highlighted in Tables 1 and 2. Commercially, uniformity of colour is a desirable characteristic and reducing curing temperatures to 20 °C seems to create as uniform a colour as 28 °C. Although there was no significant correlation between H° and quercetin in the 2009 data; the PCA of brown onion cultivars (Figure 3) highlights the relationship between H° and flavonol content.

Table 1 Quercetin, Quercetin 4'-glucoside and Quercetin 3,4'-diglucoside Concentrations (mg g^{-1} FW (DW)) in Onion Skins of 'Red Baron', 'Sherpa' and 'Wellington' Cured for Six Weeks at 20 or 28 °C Before Being Transferred to Cold Storage (1 ± 0.5 °C) for Seven Months in 2008 (*n* = 15).

		Quercetin (mg g^{-1} FW (DW))			Quercetin 4'-glucoside (mg g^{-1} FW (DW))			Quercetin 3,4'-diglucoside (mg g^{-1} FW (DW))		
	Curing Temp (°C)	Red Baron	Sherpa	Wellington	Red Baron	Sherpa	Wellington	Red Baron	Sherpa	Wellington
Before curing	n/a	11.5[a] (14.1)	35.2[b] (43.8)	16.5[a] (19.6)	12.0[a] (14.7)	9.7[a] (12.1)	7.7[a] (9.1)	2.72[a] (3.30)	0.60[a] (0.75)	0.30[a] (0.35)
After curing	20	7.6[a] (8.9)	16.5[a] (18.5)	14.8[a] (17.4)	7.9[a] (9.2)	9.6[a] (10.7)	4.8[a] (5.7)	0.37[a] (0.43)	0.61[a] (0.68)	0.35[a] (0.41)
	28	8.9[a] (10.1)	9.6[a] (10.8)	8.0[a] (9.1)	20.3[a] (23.0)	5.5[a] (6.2)	5.0[a] (5.8)	2.48[a] (2.83)	0.23[a] (0.26)	0.43[a] (0.50)
After cold storage	20	9.3[a] (11.6)	7.6[a] (10.2)	8.6[a] (10.7)	20.4[a] (25.4)	3.7[a] (4.6)	13.7[a] (17.1)	5.04[b] (6.27)	0.13[a] (0.16)	1.48[a] (1.84)
	28	11.0[a] (14.0)	5.6[a] (6.3)	7.3[a] (9.1)	12.9[a] (16.2)	3.3[a] (3.7)	3.5[a] (7.0)	0.93[a] (1.17)	0.14[a] (0.16)	0.64[a] (0.80)

LSD (*P* = 0.05); quercetin =11.51 (13.88), quercetin 4'-glucoside =17.25 (20.79), quercetin 3,4'-diglucoside = 3.669 (4.541).

Values followed by the same letter are not significantly different from each other calculated using the LSD.

Table 2 Chroma (C*), Hue Angle (H°) and Lightness (L*) of Skins of Onion 'Red Baron', 'Sherpa' and 'Wellington' Before Curing, After Six Weeks Curing (20 or 28 °C) and After Cold Storage (1 ± 0.5 °C) for Seven Months in 2008 (*n* = 15).

		Chroma (C*)			Hue angle (H°)			Lightness (L*)		
	Curing Temp (°C)	Red Baron	Sherpa	Wellington	Red Baron	Sherpa	Wellington	Red Baron	Sherpa	Wellington
Before curing	n/a	16.34^{a}	27.11^{ef}	27.66^{efg}	14.15^{a}	74.10^{f}	75.23^{f}	32.54^{a}	61.14^{d}	59.62^{cd}
After curing	20	21.25^{b}	27.71^{efg}	28.10^{fghi}	12.23^{a}	65.20^{de}	67.72^{e}	32.31^{a}	60.06^{cd}	59.14^{cd}
	28	22.62^{bc}	28.01^{efgh}	29.74^{ghij}	14.48^{a}	58.53^{bc}	62.69^{cde}	33.24^{a}	56.41^{bc}	56.62^{bc}
After cold storage	20	25.47^{de}	30.42^{hij}	30.65^{ij}	12.26^{a}	61.37^{bcd}	60.77^{bcd}	34.67^{a}	61.64^{d}	58.75^{bcd}
	28	24.55^{cd}	31.25^{j}	31.94^{j}	15.55^{a}	58.43^{bc}	57.30^{b}	34.45^{a}	58.78^{bcd}	55.19^{b}

LSD (*P* = 0.05); C* = 2.542, H° = 5.046, L* = 3.920.

Values followed by the same letter are not significantly different from each other calculated using the LSD.

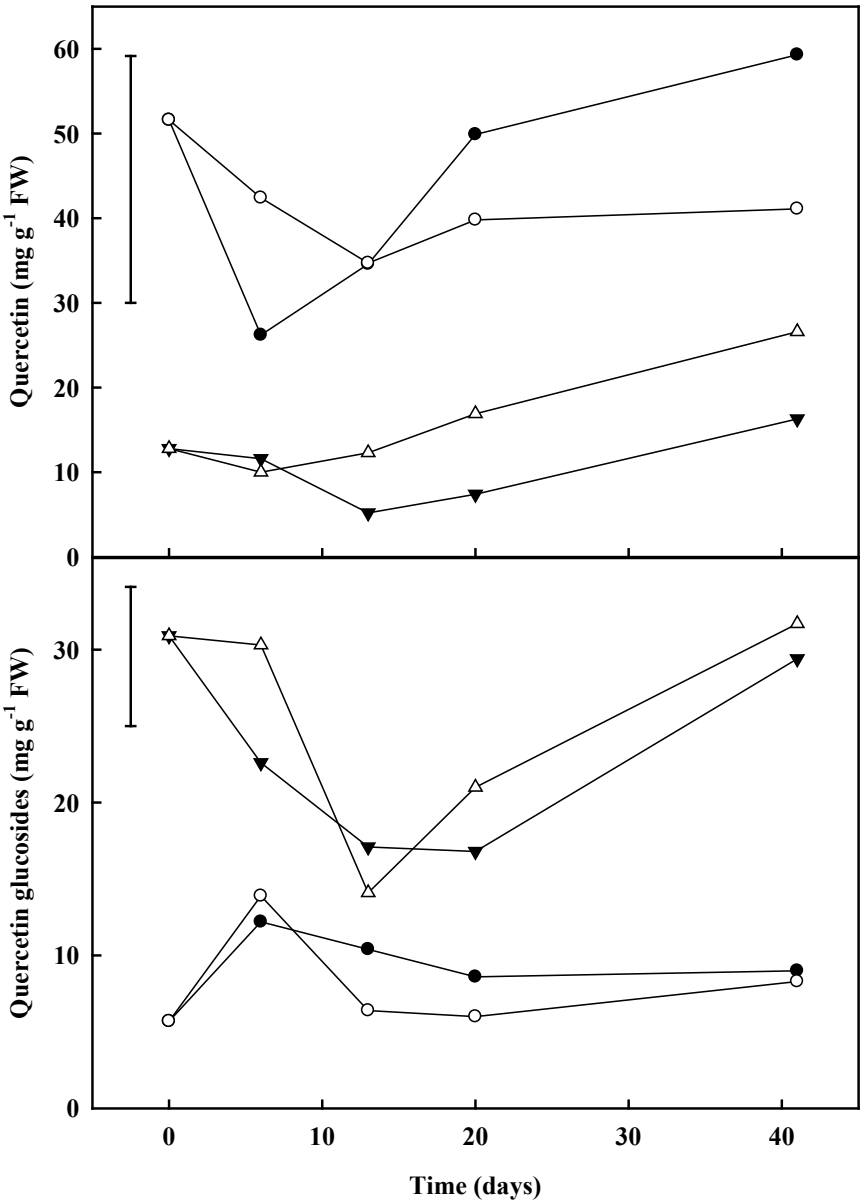


Figure 1 Flavonol Concentrations in the Skins of Brown (Mean of 'Sherpa' and 'Wellington'; Circles) and Red (Mean of 'Kamal' and 'Red Baron'; Triangles) Onion in 2009 During 42 Days of Curing at 20 (Closed Symbols) or 28 °C (Open Symbols) (*n* = 18).

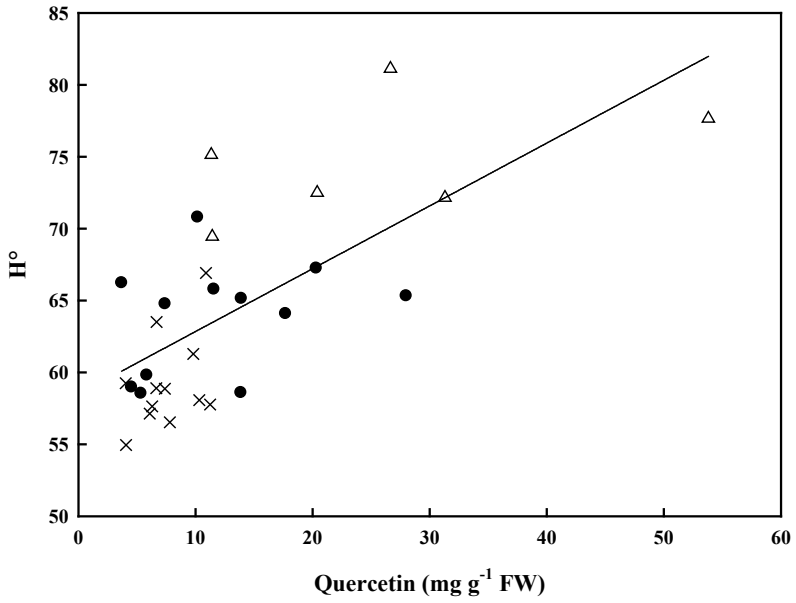


Figure 2 Correlation Between H° and Quercetin Content of Onion Skin 'Sherpa' and 'Wellington' Before Curing (Open Triangle), After Curing at 20 °C (Closed Circle) or 28 °C (Cross) in 2008 (r = 0.68; $p < 0.001$).

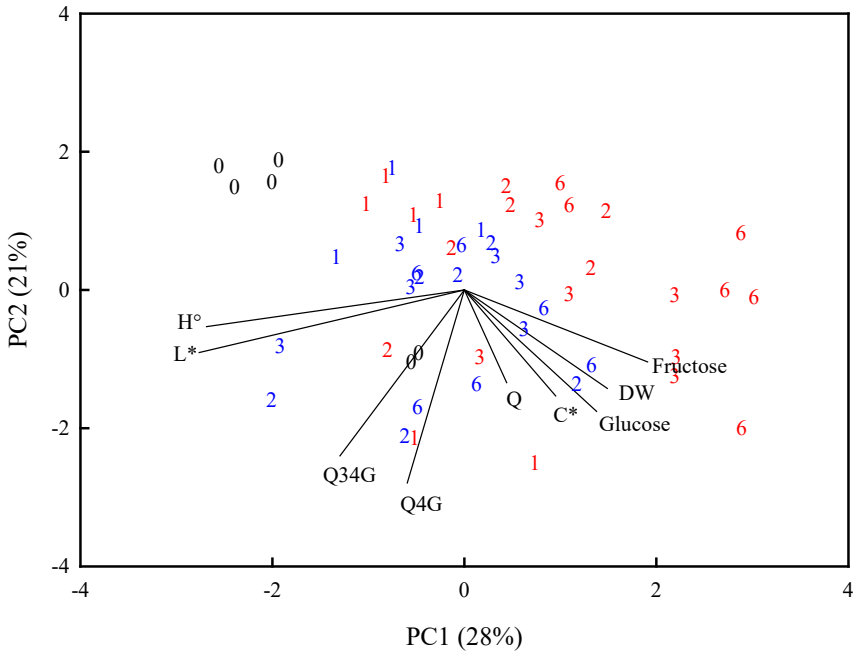


Figure 3 Principle Component Analysis Biplot of All Brown Onion Cultivars in 2009, Labelled by Number of Weeks Curing (0, 1, 2, 3 and 6 Weeks) and by Curing Temperature; Before Curing (Black), 20 °C (Blue) or 28 °C (Red).

Although both 2008 and 2009 data was analysed using PCA, only 2009 data has been presented since the same trends were found for both years. The skins of brown onion cultivars showed a clear separation of samples on PC1 (captured 28% of the variance) and PC2 (captured 21% of the variance) accounting for, in total, almost 50% of the variance. The inclusion of a third principal component did not improve sample clustering. Samples were mainly separated according to time and curing temperature (Figure 3). According to the biplots, the most important variables for differentiating between samples from different time points throughout curing were fructose, glucose, dry weight and C* which all increased with time. In contrast, the most important variables for distinguishing between curing temperatures were H°, L* and quercetin glucoside which were all higher in skins cured at 20 °C. Takahama and Hirota (2000) suggested that quercetin is formed from the deglucosilation of quercetin glucosides on the border between the dry brown skin and the drying section, since deglucosilation requires water. Since the 2009 data was recorded at weekly intervals throughout the early stages of curing (weeks 0, 1, 2, 3, 6), the quercetin glucosides may not yet have been deglucosilated into quercetin. In 2008, correlations between H° and individual quercetin glucosides (quercetin 4'-glucoside and quercetin 3,4'-diglucoside) were very low (r = 0.43 and 0.14, respectively) however the correlation between total flavonols and H° was reasonable (r = 0.66) thus suggesting that both quercetin aglycon and quercetin glucoside concentrations may contribute to brown onion skin colour. The 2008 data was recorded before curing, after curing and after cold storage; 80% of the samples were from the two latter time points by which time the quercetin glucosides may have already been converted to quercetin. Gökçe *et al.* (2010) correlated onion scale colour with total phenolics and total antioxidant capacity; this said, although significant, the correlations did not rise above $r = 0.42$. The lack of strong correlations may have been due to the use of total phenolics which, apart from quercetin, takes account of isorhamnetin, kaempferol and other phenolic acids found in red onion skin such as gallic acid, ferulic acid and protocatechuic acid as well as vitamin C and reducing sugars (including fructose and glucose) which may not all contribute to skin colour changes (George *et al.*, 2005; Singh *et al.*, 2009).

Onion skin L* was affected by curing temperature, where onions cured at 28 °C became significantly darker than those cured at 20 °C in both years (Table 2 and Figure 4). The H° of brown skin significantly decreased throughout curing at 20 °C (73.79 – 68.34) in 2009 and after curing at 20 °C and cold storage (74.6 – 63.77) in 2008, turning from yellow to orange. Those cured at 28 °C showed a greater change becoming more orange/brown throughout curing in 2009 (73.79 – 62.93) and after curing at 28 °C and after cold storage (74.67 – 59.24) in 2008. During curing, C* and L* decreased steadily week by week whereas the

largest change in red and brown skin H° was in the first six days of curing. In 2009, the PCA of the brown onion cultivars confirmed that at 20 °C onion skins were paler (higher L*), more yellow (higher H°) and contained higher concentrations of flavonol glucosides than the onions cured at 28 °C. It has previously been hypothesised (Hirota *et al.*, 1998) that the formation of brown pigmentation during onion skin drying may be due to the conversion of quercetin glucosides into the aglycon form since this conversion occurs during dry skin formation. On the other hand, Ito *et al.* (2009) found quercetin and its glucosides are unlikely to contribute themselves to skin colour since they are too pale and have a maximum wavelength of 370 nm whereas the pigmented compound in the dry scales of yellow onions had a maximum wavelength of 450 nm. Under oxidative conditions, Makris and Rossiter (2000) found the major degradative product of quercetin was 3,4-dihydroxybenzoic acid (protocatechuic acid).

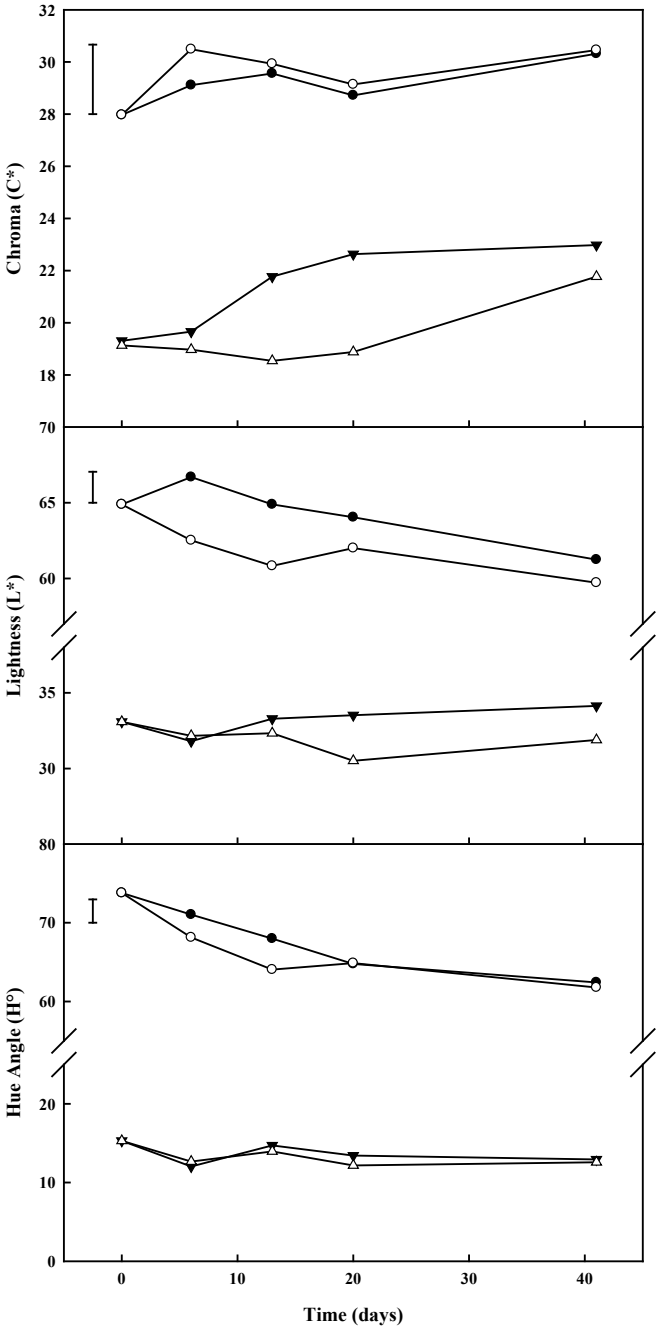


Figure 4. Chroma (C*), Hue Angle (H°) and Lightness (L*) of Skins from Brown (Mean of 'Sherpa' and 'Wellington'; Circles) and Red (Mean of 'Kamal' and 'Red Baron'; Triangles) Onion in 2009 During 42 Days of Curing at 20 (Closed Symbols) or 28 °C (Open Symbols) (*n* = 18).

A relationship between colour and quercetin glucoside content was also found in the skins of the red onions using multivariate analysis. The PCA on the red onion physiological and biochemical data showed a separation of samples according to time in PC1 (capturing 28% of the variance; data not shown) on the other hand PC2 only captured a further 16% of the variance. Again, the inclusion of a third principal component did not improve sample clustering. When samples were labelled according to curing temperature no clear separation was observed. Therefore, PLSDA (a supervised multivariate technique) was employed with clustering biased towards curing temperature (Figure 5). The most important variables contributing to clustering according to curing temperature were C*, L*, quercetin and cyanidin 3'-glucoside. The profile of anthocyanin content in the flesh and skins of red onion are comparable unlike flavonols as discussed earlier (Wu and Prior, 2005; Downes *et al.*, 2009). The PLSDA revealed that red onions cured at 20 °C contained a higher concentration of quercetin in the skins and had a lower C* value corresponding to a less saturated appearance compared with those cured at 28 °C. Like the relationship between quercetin / quercetin glucoside concentrations and colour in brown onions, the same explanation can be applied to the red onion skins where oxidation of quercetin into highly pigmented oxidative products may increase the strength of colour in the red onion skins and therefore result in a higher C* value. The difference in skin colour of the 2008 brown onion cultivars cured at different temperatures was qualified by differences in H° although the high red pigmentation of the red onions due to the anthocyanin content may have masked the effect of changes in flavonol content on H° and therefore only changes in colour saturation (C*) between curing temperatures were recorded. However, differences in L* were also found in the PLSDA of the 2009 data where onion skins cured at 28 °C were lighter than those cured at 20 °C although this is more likely to be due to the degradation of cyanidin 3-(6'' malonoyl-laminariboside)) and cyanidin 3-(6''-malonoylglucoside) at the higher temperature also found in 2008 (Figure 3), both of which have previously been shown to be sensitive to curing temperature (Downes *et al.*, 2009). This concludes that curing at 20 °C reduces the degradation of skin anthocyanin content preserving a more red appearance. Not only would reducing the curing temperature to 20 °C save energy costs but also potentially create an improved red onion appearance.

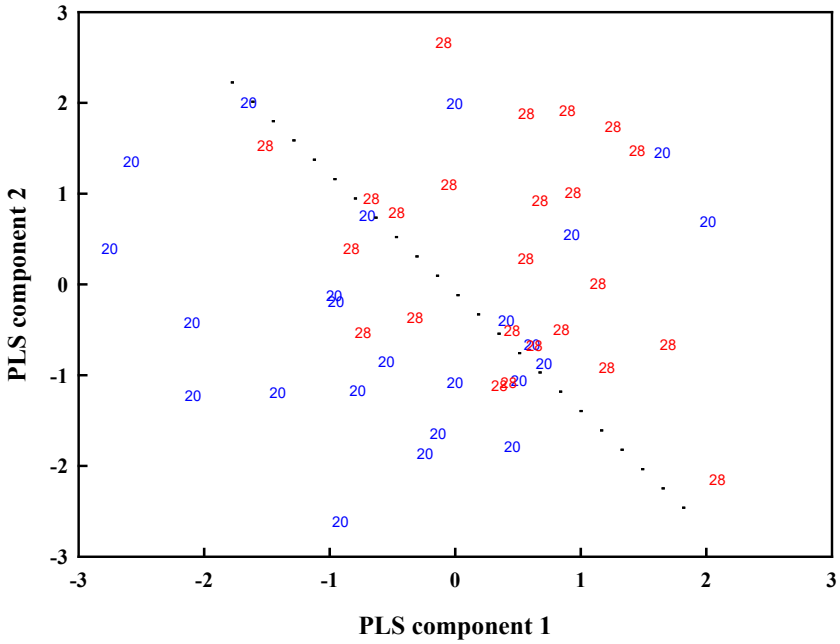


Figure 5 Partial Least Square Discriminant Analysis of All Red Onion Cultivars in 2009 Discriminated According to Curing Temperature; Cured at 20 °C (Blue) or Cured at 28 °C (Red).

In 2009, cyanidin 3-(6''-malonoylglucoside) concentrations in 'Red Baron' onions peaked after six days in those cured at 28 °C and after 20 days in those cured at 20 °C (Figure 6). This variation in anthocyanin production between curing temperatures could not be due to differences in the rate of skin water loss since there were no significant differences in skin dry weight between onions cured at different temperatures. In 2009, monitoring dry weight throughout curing showed that the largest decrease in skin water content occurred within the first six days (712.3 – 793.8 mg g^{-1} FW). Therefore, standard UK practise of curing for up to six weeks may not indeed be necessary. To the best of our knowledge this is the first time dynamic changes in skin water content has been measured during curing.

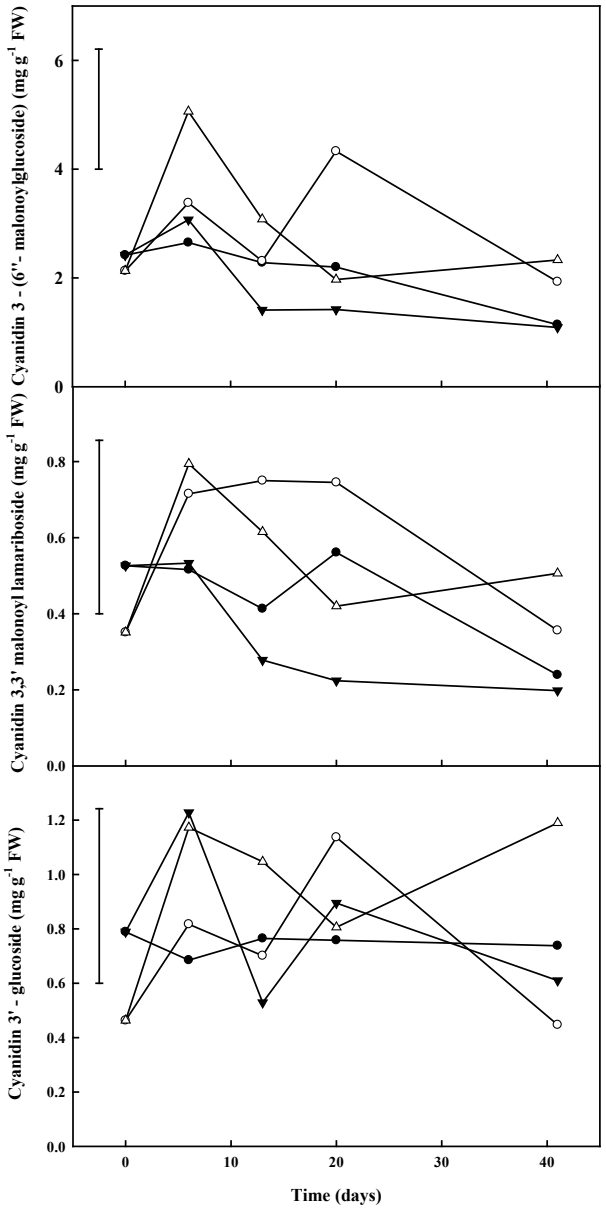


Figure 6 Anthocyanin Concentrations in Skins from Red Onion 'Kamal' (Closed Symbols) and 'Red Baron' (Open Symbols) in 2009 During 42 Days of Curing at 20 (Circles) or 28 °C (Triangles) (*n* = 9).

Sugars were measured in the skins of red and brown onions as some biproducts of the Maillard reaction are pigmented as well as the regulatory role sugars possibly play in the production of anthocyanins and flavonol glucosides (Gennaro *et al.*, 2002; Nursten, 2005). Fructose and glucose concentrations in onion skins were not affected by cultivar or curing temperature in either 2008 or 2009. Gennaro *et al.* (2002) identified a positive relationship between anthocyanin concentration and free sugar concentrations in the edible portion of

red onion cv. Tropea. Sugar concentrations were not correlated with anthocyanin or flavonol concentrations nor was a relationship with colour or dry weight identified in both 2008 and 2009 in the skins of any onion cultivars agreeing with Downes *et al.* (2009). Gökçe *et al.* (2010) found that soluble solids in onion scales were positively and significantly correlated with total phenolics and total antioxidant capacity but the correlations were weak (0.41 and 0.43, respectively).

Conclusion

In conclusion, fructose and glucose concentrations appeared to play no role in explaining the difference in onion skin appearance when bulbs were cured at different temperatures. Quercetin and its glucosides appear to be linked to the intensities of brown pigmentation in onions cured at different temperatures. Skins cured at 28 °C became darker and browner possibly due to the conversion of quercetin and its glucosides into brown oxidative products. This same relationship between skin flavonol content and colour intensity was also observed in the skins of red onion cultivars. Additionally, curing at 28 °C resulted in greater reduction in anthocyanin content therefore reducing the curing temperature to 20 °C may not only save costs but also improve red onion appearance. Finally, dynamic changes in skin biochemistry and physiology throughout curing appeared to mainly occur in the first few weeks. Since the only significant change in skin dry weight also occurred in the first week of curing, standard UK practise of up to six weeks could be reduced resulting in additional cost reductions.

GENERAL CONCLUSION

To reduce energy consumption and therefore costs, the results herein suggest that curing at 28°C may be unnecessary and reducing this to 20°C could be adopted as the standard UK curing temperature as long as pathogen load is low.

The results from this project have found that treating onions for only 24 h with ethylene or 1-MCP is sufficient to reduce sprout growth as compared with the control for up to approximately 4 months after harvest although this is cultivar dependent. However, onions intended for long term storage beyond 4 months would still require continuous ethylene treatment to suppress sprout growth. Reducing the use of ethylene would save costs for growers. In the UK, onions are not yet commercially treated with 1-MCP since 1-MCP is only approved for use in the UK on apples, tomatoes and some stone fruit. This study found that ethylene and 1-MCP applied for 24 h in unison was the most effective treatment for the reduction of sprout growth and therefore commercialisation of the postharvest use of 1-MCP on onions could be beneficial.

Identification of both molecular and biochemical biomarkers at the transition from dormancy to sprout suppression may in the future prove useful for predicting storage life and therefore managing required storage regimes so that they are better aligned with market requirements.

APPENDIX 1
GROWER DEMONSTRATION TRIALS

These comprised two sets of trials

- Bulk storage & energy saving potential trial

- 'Syngenta' variety trial

Bulk Storage Trials

Alongside the main scientific work bulk demonstration trials were carried out by ABC to illustrate to the grower partners the potential effects of the different curing regimes on bulk samples. In total some 25000kg bulbs were stored, graded and sampled over the 3 years and the size of these bulk samples gives some confidence in the overall conclusions. However it should be stressed these trials, although they were repeated over the three years of the project were not replicated, therefore caution is necessary in interpreting any results presented.

In summary, bulbs of the same varieties (Red Baron, Sherpa, Wellington in Yrs 1-3 Kamal, Vision & Arthur added in Yr3) from the same sites were harvested at the same time as bulbs for the main scientific work (see science section). At each site at least 0.5 tonne of each variety was harvested into bulk bins and dried/cured at Sutton Bridge; at three temperatures 20, 24 & 28°C in Yr1; the 24°C treatment was dropped in Yrs 2&3 in line with the main experimental work. During this curing/drying period time temperature, humidity and energy input was continuously monitored.

Until this point their treatment mirrored that of the bulbs used for the scientific experiments.

After curing, the bins were transported to Allium and Brassica Centre for long term storage in their experimental box store. In Yrs1& 2 the storage temperature was set at 3°C with continuous commercial *Restrain* ethylene

treatment; in Yr 3 the storage temperature was set at 6ºC with the intention to promote earlier sprouting. Before and after curing, and approx 4 months into storage, 3x50 (+60mm) 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 between early and late May depending on year and then transported to Moulton Bulb Co. for grading over a commercial line into <60, 60-80, >80mm sizes. The proportion of rots and rejects were also recorded as weights.

Very little external visible sprouting was observed at this stage. Therefore 50 bulbs from each bulk bin were sampled and measured to estimate internal sprouting and then further samples (60-80mm size) were kept to estimate sprouting/shelf life

For the Yr1& 2 trials all partners were invited to view the graded bulk samples (60-80mm). 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).

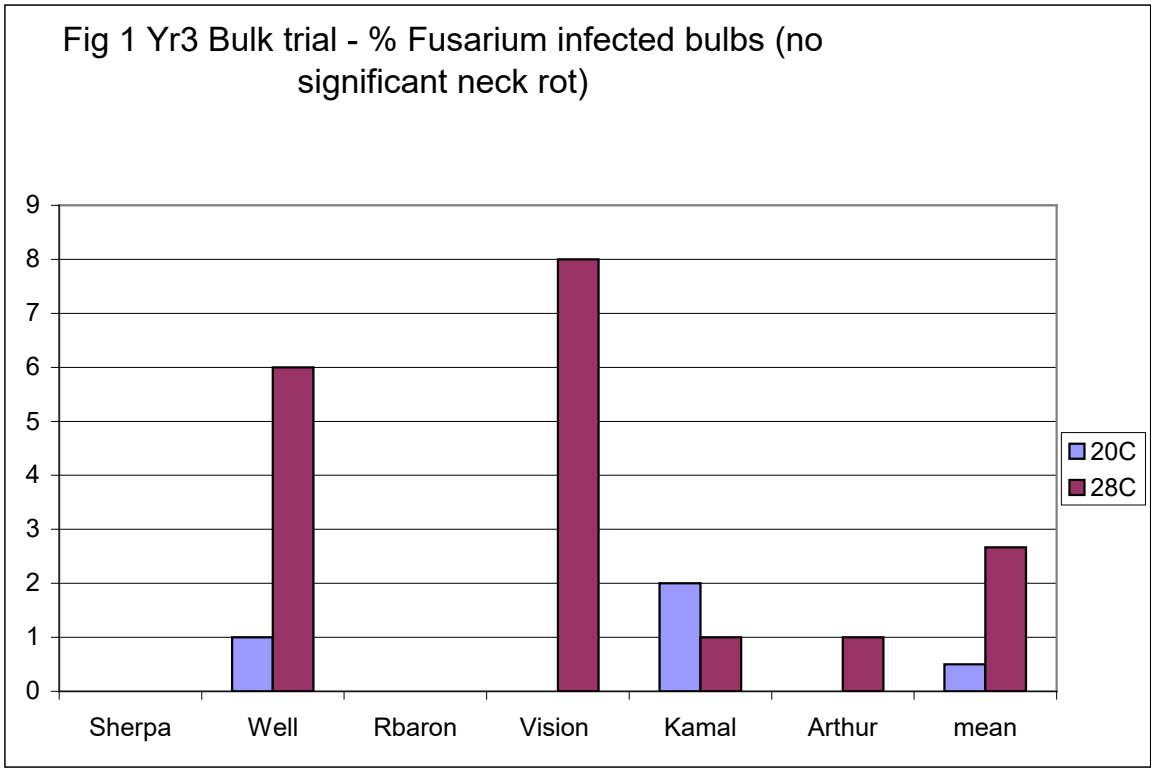
Results & Discussion

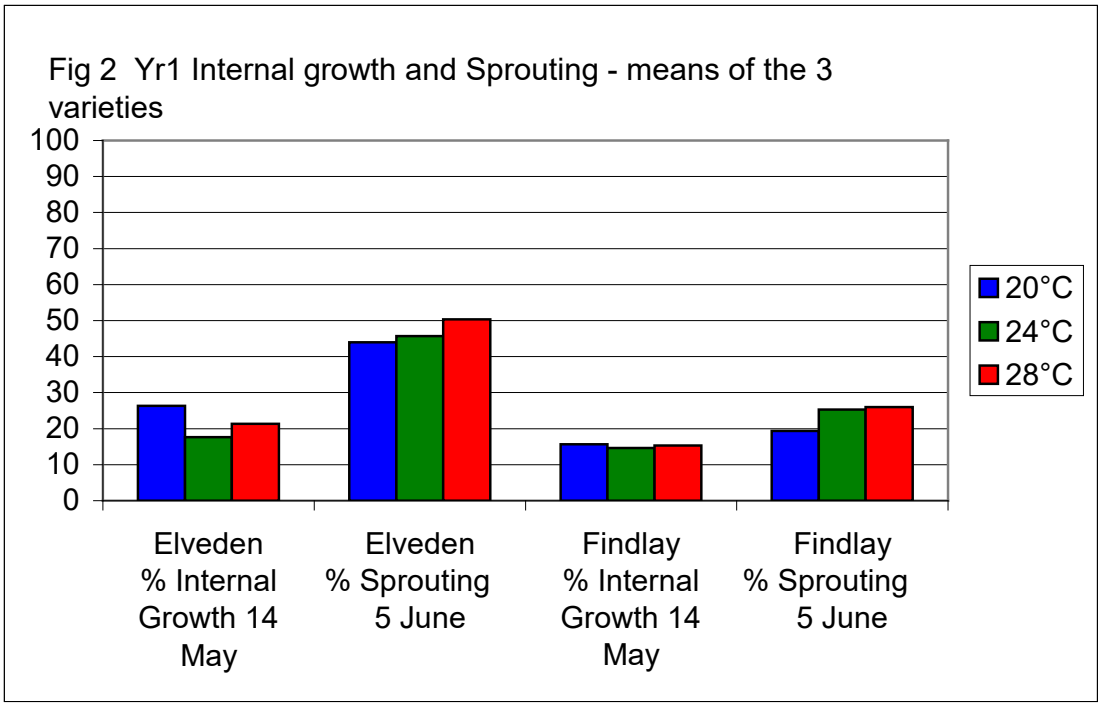
Overall from all years there were only small differences between curing temperatures for any trait with no obvious difference in weight loss. Essentially this mirrored the findings in the main science section. Similarly at grade out it might be expected that more neck rot disease would be visible at the lower curing temperatures but there was no evidence

of this from these trials and overall neck rot levels were very low irrespective of curing temperature. In Yr3 there was some evidence that fusarium rot was worse at 28°C (Fig1) but this was not noticed in previous trials. The grade out summary for Yr1 is shown in Table 1.

Table 1 Yr1 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 shelf life/visible sprouting in Yr1 is shown in Figure 2 averaged over the 3 varieties and again no consistent differences were detected between different curing temperatures. In Yr 3, shelf life/visible sprouting was better at 20°C than 28°C.

During the grower viewing/demonstration of the graded bulks the results from the coded display were collected and collated. In Yr1 (three curing temps) on average growers were only about 50% accurate at estimating the correct curing temperature, this improved somewhat in Yr2 (two curing temps). The summary of the Yr1 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. 3 out of the top 4 ranked samples were cured at 20°C and 4 out of 5 worst ranked samples were cured at 28°C. The results in Yr2 where only 20 & 28°C curing temperatures were used were similar, with 20°C being generally preferred.

Table 2 Summary of grower scoring of blind display of bulk bulbs 15 May (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

Bulk storage energy saving

As indicated above energy inputs were measured during the curing/drying phase of the bulk samples. During the storage phase all samples were stored together so any differences in energy inputs /costs can only be taken from the first curing phase. Averaged over the 3 years, energy input (heating) for the 20°C treatment as measured for the Sutton Bridge experimental stores was almost exactly one third of that used at 28°C. This figure was then used to calculate a notional cost (& CO2) saving if commercial drying followed this pattern.

The energy saving/cost comparisons below (Table 3) are based on a number of assumptions

- Costs of drying at 28°C based on commercial practice
- Drying at 20°C based on projections from relative energy usage at Sutton Bridge exp stores - approx 0.33 of energy used at 28°C(mean over 3 years)
- Fan usage same at both temperature regimes
- Overall storage life, bulb quality grading losses similar for both drying temperatures (broadly as found during project)
- For CO2 emissions some additional figures have been included to indicate the relative saving in relation to the CO2 cost of imports from NZ

Table 3 Calculation of Cost & CO2 savings

Table 3	Drying at 28°C	Drying At 20°C
Energy cost saving (curing only)		
Estimated energy use	**403. 96MJ/tonne =113.12kWhr/tonne** **based on 22 litres propane/tonne**	**128MJ/tonne =35.98kWhr/tonne**
Cost/tonne	**£4.18/tonne** **based on** propane@19p/litre	**£1.39/tonne**
Notional saving	**£2.80/tonne**	

CO2 savings		
	Drying at 28°C	**Drying At 20°C**
CO2 emissions	**23.30 kgCO2/tonne**	**7.41kgCO2/tonne**
Based on Defra 2008 conversion for gas 0.206kg CO2/kWhr	**2,330,000kg/100000tonnes** =2330 tonnes CO2/100000tonnes onions	**= 741 tonnes CO2/100000tonnes onions**
Notional saving	**1589 tonnes CO2/100000 tonnes onions**	
UK Annual production 400000tonnes	**6356 tonnes CO2**	
Comparable emissions* Estimated CO2 emissions for bulb production incl harvest but not storage UK 170kgCO2/tonne NZ 185kgCO2/tonne		
Estimated emissions for transport from NZ to UK **124.9 kgCO2/tonne**		**Based on shipping rate 0.114MJ/tonne-km (17840km NZ –UK)**

CONCLUSIONS

Overall the bulk trials seemed to suggest small overall differences between curing temperatures for weight loss, sprouting and disease incidence, with larger differences occurring between varieties and sites. However where differences occurred, drying/curing at

20°C generally produced better quality bulbs with less internal sprouting and improved shelf life compared to 28°C. At the start of the project grower partner concerns that poorer bulb colour would be a problem at lower curing temperatures were not borne out by the results. In fact grower preferences on bulb quality seemed to favour lower curing temperatures especially for the red varieties.

Energy savings when curing at 20°C were significant, especially when taking into account that bulb quality has tended to be better at the lower curing temperatures. CO2 emissions and saving have been calculated and whilst very small, from a national perspective, give an indication of what can be achieved by relatively small changes in current practice.

APPENDIX 2

'SYNGENTA' VARIETY TRIALS

The aim was to understand any potential differences, (using a much wider range of varieties than the three traditional varieties used in the main work) from the two different extreme curing temperatures 20& 28°C and 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: hence Dutch practice tends to need less forced drying in store). The intention was to grow each year around 15 different varieties at sites in the UK and Netherlands. Seed was supplied and the trials overseen by Syngenta. The trials were grown in UK (by NIAB in Suffolk) and at Lelystad in Holland.

In Yr1 fourteen varieties were grown in UK but only seven in Holland. The range represented traditional, new and experimental varieties. 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. However because this trial resulted in the one occasion when neck rot disease proved a problem the results are presented. In Yrs 2&3 the same 15 varieties were grown at each site so more comparisons could be made.

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 separate bins but in the same store as the bulk storage trial described above. At approximately the same time as bulk storage records were taken, 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.

Results & Discussion

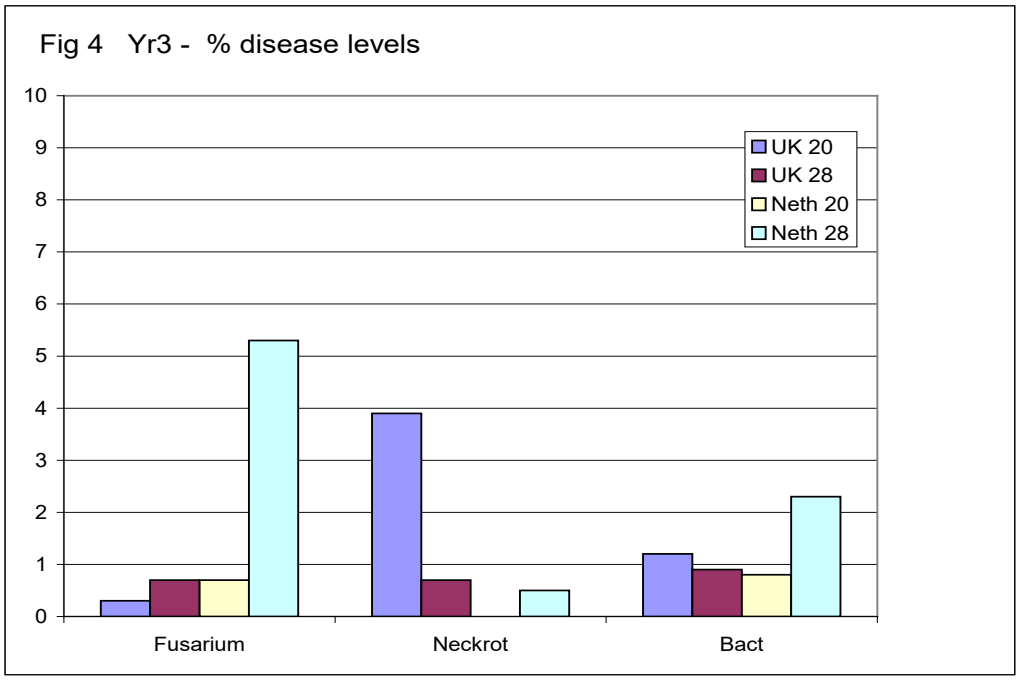
The results from Yr1 are presented in Table 4. What is immediately apparent is the high level of rotting after curing at 20°C although this only seems to have happened with the UK grown onions. This highlights the potential risk from curing at lower temperatures if the disease is present in high levels in the crop. Because the nature of this disease is that symptoms are rarely obvious in the crop this does represent a potentially serious risk but also an opportunity in future research to develop new detection methods.

Table 4 Summary of Yr1 Syngenta Variety trial

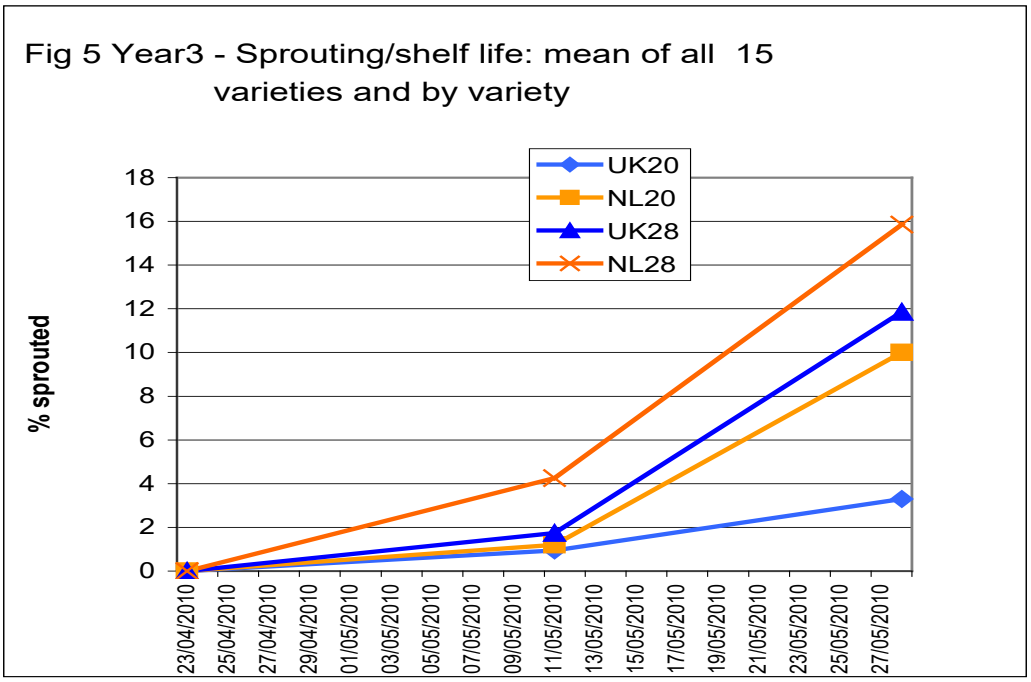
Variety ID	Variety name	Production site	% rotting after curing at 20°C	% rotting after curing at 28°C	Relative shoot length after curing at 20°C	Relative shoot length after curing at 28°C
KA	Hyfort	NIAB	60.0	2.0	0.29	0.34
KJ	Wellington	NIAB	12.0	0.0	0.34	0.33
DK	Sherpa	NIAB	6.0	6.0	0.46	0.49
KP	Centro	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

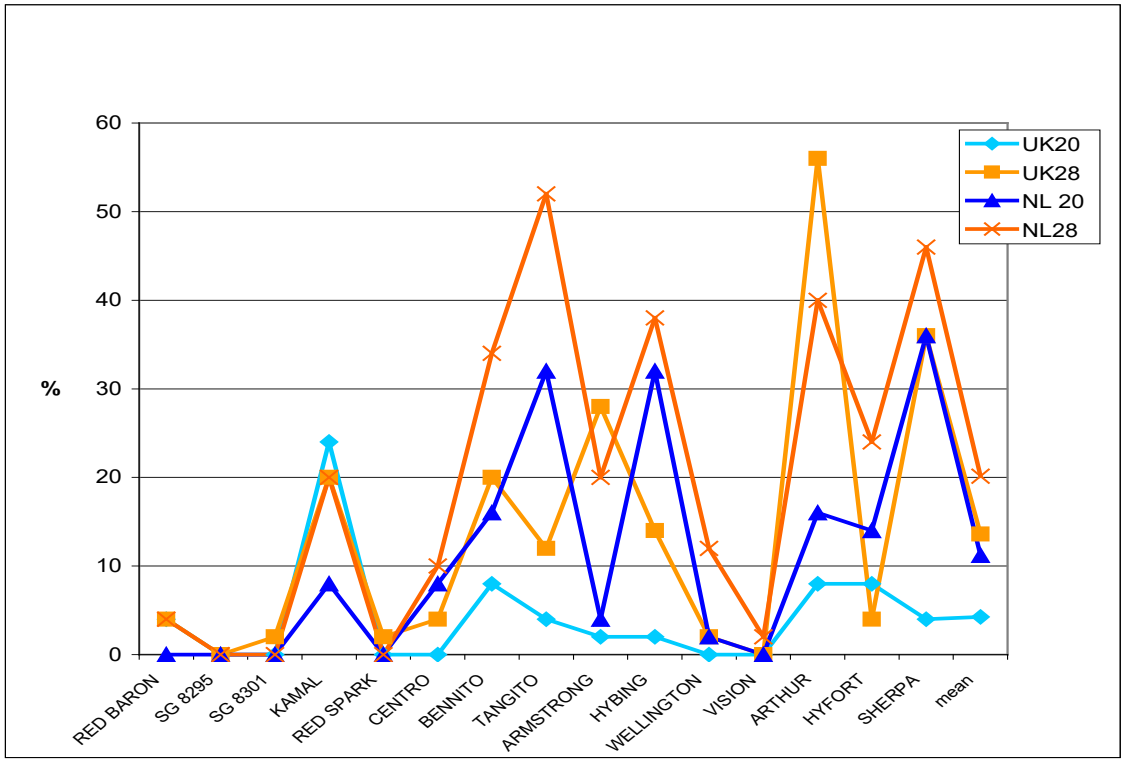
In Yrs 2 & 3 the varieties used were identical and the results broadly similar. A summary of results is shown in Figs 4 & 5. Disease levels were relatively low with the main problem

being fusarium being apparent at the 28°C curing treatment. A low level of neck rot was apparent at UK onions cured at 20°C.



Variety was the main influence on storage and shelf life but there were differences between UK and Dutch grown onions at the different curing temperatures. Onions cured at 20°C stored better with better shelf life than those cured at 28°C. and UK grown onions stored better than Dutch grown onions.





CONCLUSION

The trials showed some differences for actual storage potential for the two different temperatures. Generally curing at 20°C was at least as good, and sometime distinctly better, than 28°C for storage and shelf life. This was broadly in line with the results from bulk storage.

One trial pointed to the potential risk from neck rot at the lower curing temperature.

There was no obvious evidence that any of the more modern mainly earlier maturing varieties behaved radically differently under the different curing regimes than the main traditional varieties.

Generally Dutch grown bulbs stored less well than UK bulbs irrespective of curing temperature.

APPENDIX 3

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Effect of curing at different temperatures on biochemical composition of onion (*Allium cepa* L.) skin from three freshly cured and cold stored UK-grown onion cultivars

Katherine Downes, Gemma A. Chope, Leon A. Terry*

Plant Science Laboratory, Cranfield University, Bedfordshire MK43 0AL, UK

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ABSTRACT

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. Currently in the UK, standard curing practises for onions involves heating at 28 °C for six weeks (65–75% RH), however, reducing curing temperatures may help to reduce energy usage. There is little 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, the aim of this study was to elucidate the compounds responsible for the change in onion skin colour when cured at different temperatures.

Brown 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 after seven months cold storage at 1 ± 0.5 °C. Measurement of objective colour showed that skin of cvs. Sherpa and Wellington was darker and had a lower hue angle (H^*) immediately after being cured at 28 °C compared to 20 °C. In contrast, skin of cv. Red Baron had a higher H^* but no change in lightness (L^*) when cured at 28 °C compared to 20 °C. Fructose, sucrose and glucose concentrations were analysed as they are thought to play a role in regulating the synthesis of flavonols and anthocyanins, both coloured compounds found in onion skin; however no significant correlations were found between colour data and sugar concentrations. Flavonols were measured in the skin of all cvs. and anthocyanins in the skin of cv. Red Baron. Quercetin glucoside and anthocyanin concentrations in the skin of onions cv. Red Baron immediately after curing were higher in those cured at 20 °C. Total flavonols and total anthocyanins were negatively correlated with H^* in the skin of onions cv. Red Baron, but there was no similar correlation between total flavonols and H^* for onion cvs. Sherpa and Wellington. This suggests that anthocyanins and flavonols may play a major role in varying skin colour of red onions cv. Red Baron cured at different temperatures; however, the difference between curing temperatures may not have been sufficient to represent a correlation between darkening of cvs. Sherpa and Wellington and flavonol concentration. Further investigation is therefore required to fully elucidate the compounds responsible for colour changes observed in brown onions.

1. Introduction

In the UK and similar maritime climates, onions are often cured to form a complete dry outer skin which conveys many benefits including reduction of disease incidence and prevention of excessive water loss from the outer scales of the bulb, resulting in reduced shrivelling. Neck rot disease caused by *Botrytis allii* has been reduced in the UK since the introduction of forced air curing which effectively seals the bulb neck (Langston, 2001). Curing also dries the muddy outer skins which can then be removed easily resulting in a cleaner finish for all onion skin colours. In addition, curing improves the skin appearance of brown onions by turning them an attractive golden-brown colour which is appreciated by consumers. Traditionally, curing was completed in the field, but extended wet conditions during field curing can cause skin staining (Wright et al., 2001). To improve onion quality, commercial value and appearance, windrowing and field drying in boxes or wire cages were replaced by enclosed artificial drying. Although more expensive, artificial drying reduces skin staining and fungal attack resulting in improved skin appearance (O'Connor, 1979). The mild, wet maritime climate in the UK necessitates that onions be cured for 3–6 weeks at 28 °C (65–75% relative humidity (RH)).

Flavonoids can be categorised into flavonols and anthocyanins, both found in onion skins. Brown onions gain their colour from flavonols, mainly quercetin and its derivatives (Griffiths et al.,

* Corresponding author. Tel.: +44 7500 766 490.
E-mail address: l.a.terry@cranfield.ac.uk (L.A. Terry).

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Postharvest application of ethylene and 1-methylcyclopropene either before or after curing affects onion (*Allium cepa* L.) bulb quality during long term cold storage

Katherine Downes, Gemma A. Chope, Leon A. Terry*

Plant Science Laboratory, Cranfield University, Bedfordshire MK43 0AL, UK

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ABSTRACT

The storability of onion bulbs is dependent on the incidence and rate of sprout growth. Exogenous ethylene applied continuously has been demonstrated to act as a sprout suppressant in onion. However, the ethylene binding inhibitor, 1-methylcyclopropene (1-MCP), can also suppress sprouting in onion. Given this seemingly contradictory result, the precise role that ethylene plays during onion storage and the effect of curing on its efficacy is not understood.

'Sherpa' and 'Wellington' onion bulbs were treated before or after curing (28 °C for 6 weeks) with a single dose of 10 μL L^{-1} ethylene or 1 μL L^{-1} 1-MCP for 24 h at 20 °C, or no treatment (control). Replicated out-turns were sampled during 38 weeks storage at 0–1 °C. Sprout growth (31 weeks after harvest) was reduced in 'Sherpa' treated before curing with ethylene or before or after curing with 1-MCP. However, sprout growth of 'Wellington' was not affected by any treatment. Following treatment, the cured, thick-skinned 'Wellington' released a lower concentration of treatment gas compared with the newly harvested, thin-skinned 'Sherpa'. Onion bulb respiration rate increased immediately after being treated with ethylene but to a lesser extent or not at all when treated with 1-MCP. Fructose concentrations of onions treated with ethylene or 1-MCP before curing were not significantly different, however, after curing concentrations were about 2-fold higher compared with the control. Mean glucose and sucrose concentrations for both cultivars were higher immediately after being treated before curing with ethylene or 1-MCP than control bulbs. It appears that inhibition of sprout growth can be achieved using just a short 24 h treatment with ethylene or 1-MCP. However, skin thickness or permeability, which is dependent on cultivar and curing, may affect ethylene or 1-MCP influx and therefore efficacy of sprout suppressant action.

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). Currently, standard methods of suppressing sprouting in temperate climates include controlled atmosphere (CA), ambient or cold storage with/without controlled relative humidity in addition to the preharvest application of maleic hydraziде (1,2-dihydro-3,6-pyridazinedione; MH). The continued use of MH is uncertain as it remains the only chemical used on onions that leaves detectable residues (4–6 mg kg^{-1}) (Johnson, 2006). Additionally, MH is considered by some to be detrimental to the environment with concerns over the risk of it leaching into drinking water (Sorensen and Grevsen, 2001). Increasing pressure from retailers and consumers alike has driven the search for alternative methods to extend onion storage life.

Onions are regarded as non-climacteric with consistently low endogenous ethylene production during storage (<0.1 μL kg^{-1} h^{-1} at 0–5 °C) (Suslow, 1998). However, the continuous application of ethylene at the supposedly saturated concentration of 10.2 μL L^{-1} and the preharvest application of Ethephon have both been shown to increase the storage and shelf-life of onions (Adamicki, 2005; Bufler, 2009). Ethephon is an ethylene yielding chemical which when applied to plants can elicit a response characteristic of ethylene treatment (Yang, 1969). Application of Ethephon (1.8 L ha^{-1}) sprayed directly onto onion foliage 2 weeks prior to harvest reduced sprout incidence 2-fold compared with controls after 22 weeks storage at 0–1 °C (Adamicki, 2005). However, preharvest application of Ethephon has been found to significantly reduce yield by reducing bulb diameter and weight (Thomas and Rankin, 1982). Recently, systems which produce a continuous supply of ethylene (10 μL L^{-1}) have been introduced into onion and potato stores to suppress sprouting (Chope and Terry, 2008). Sprout suppression

* Corresponding author. Tel.: +44 7500 766 490.
E-mail address: l.a.terry@cranfield.ac.uk (L.A. Terry).

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A new acetonitrile-free mobile phase method for LC–ELSD quantification of fructooligosaccharides in onion (*Allium cepa* L.)

Katherine Downes, Leon A. Terry*

Plant Science Laboratory, Cranfield University, Bedfordshire MK43 0AL, UK

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ABSTRACT

Onion soluble non-structural carbohydrates consist of fructose, glucose and sucrose plus fructooligosaccharides (FOS) with degrees of polymerisation (DP) in the range of 3–19. In onion, sugars and FOS are typically separated using liquid chromatography (LC) with acetonitrile (ACN) as a mobile phase. In recent times, however, the production of ACN has diminished due, in part, to the current worldwide economic recession. A study was therefore undertaken, to find an alternative LC method to quantify sugars and FOS from onion without the need for ACN. Two mobile phases were compared; the first taken from a paper by Vågen and Slimestad (2008) [3] using ACN mobile phase, the second, a newly reported method using ethanol (EtOH). The EtOH mobile phase eluted similar concentrations of all FOS compared to the ACN mobile phase. In addition, limit of detection, limit of quantification and relative standard deviation values were sufficiently and consistently lower for all FOS using the EtOH mobile phase. The drawback of the EtOH mobile phase was mainly the inability to separate all individual sugar peaks, yet FOS could be successfully separated. However, using the same onion extract, a previously established LC method based on an isocratic water mobile phase could be used in a second run to separate sugars. Although the ACN mobile phase method is more convenient, in the current economic climate a method based on inexpensive and plentiful ethanol is a valid alternative and could potentially be applied to other fresh produce types.

In addition to the mobile phase solvent, the effect of extraction solvents on sugar and FOS concentration was also investigated. EtOH is still widely used to extract sugars from onion although previous literature has concluded that MeOH is a superior solvent. For this reason, an EtOH-based extraction method was compared with a MeOH-based method to extract both sugars and FOS. The MeOH-based extraction method was more efficacious at extracting sugars and FOS from onion flesh, eluting significantly higher concentrations of glucose, kestose, nystose and DP5–DP8.

1. Introduction

Onion bulbs contain the water soluble carbohydrates fructose, glucose, sucrose and fructans constituting 60–80% of the dry weight [1]. Fructans are oligo- and polysaccharides in which fructosyl units are bound to sucrose by a β-linkage, whereas fructooligosaccharides (FOS) generally only refer to the short chain fructans composed of kestose, nystose and fructofuranosylnystose [2]. FOS are used not only as energy reserves but additionally as osmoregulators due to their solubility in water. The main FOS found in onions are neokestoses which have fructose elongations up to DP19 (degree of polymerisation) from either side of the sucrose unit [3].

The structural and non-structural carbohydrate profile of onion bulbs varies greatly between cultivars [4–6] and throughout storage [5,7]. High fructan concentrations have been associated with increased postharvest storage life potential [8]. In addition, changes in the carbohydrate profile of onion bulbs are important for taste preference as concentrations of fructose and glucose have been positively correlated with likeability and sweetness [9].

Davis et al. [5] investigated the efficacy of different extraction methods for the quantification of sugars and fructans. Three extraction methods were compared and the most efficacious method at extracting sugars and fructans was that described by O'Donoghue et al. [4] with modification. The major differences between these extraction procedures were the solvent used; the O'Donoghue method utilising 62.5% (v/v) methanol (MeOH) whereas the other two methods used aqueous ethanol (EtOH) [10,11]. Due to the higher polarity of the MeOH mixture, fructose, glucose and sucrose tend to be more soluble in MeOH-based solutions than EtOH extraction solvents [5].

* Corresponding author at: Plant Science Laboratory, Cranfield University, Bedfordshire MK43 0AL, UK. Tel.: +44 7500 766 490.
E-mail address: l.a.terry@cranfield.ac.uk (L.A. Terry).

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Technology and Knowledge Transfer

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Downes, K., Chope, G.A. and Terry, L.A. (2009). Effect of curing at different temperatures on biochemical composition of onion (*Allium cepa* L.) skin from three freshly cured and cold stored UK-grown onion cultivars. *Postharvest Biology and Technology,* 54, 80-86.

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Chope, G.A., Adikaram, N., Downes, K. and Terry, L.A. The effect of postharvest handling of onion (Allium cepa L.) on *Botrytis allii* infection. *Plant Pathology* (to be submitted)

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Conference proceedings

Downes, K., Chope, G.A., Terry, L.A., (in press). Relationship between colour and biochemical composition of skin from onion cv. Red Baron bulbs cured at different temperatures. Sixth International Postharvest Symposium, 8-12 April 2009, Antalya, Turkey. Acta Hortic.

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Book chapters

Chope, G.A., Downes, K. and Terry, L.A. (*in press*). *Allium* vegetables (onion, garlic, shallot and leek). In: *Health promoting properties of fruits and vegetables,* edited by Terry, L.A., CABI publishing, Wallingford, UK.

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Oral presentations

Chope, G.., Adikaram, N., Downes, K. Terry, L. A. The effect of curing temperature on *Botrytis allii* infection of onion bulbs. XV International Botrytis Symposium, 31 May – 4 June 2010, Cadiz, Spain.

Chope, G., Downes, K., Terry, L. A. Sustaining UK fresh onion supply by improving curing and storage practise. The 6th UK Onion and Carrot Conference, 18-19 November 2009, Peterborough, UK.

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Downes, K., Chope, G.A., Terry, L.A. The role of ethylene in onion storage. Postgraduate Cranfield Health Conference, 16 September 2009, Cranfield, UK. (**Best oral presentation winner**).

Chope, G., Terry, L. A. Effect of curing at different temperatures on phytohormone and biochemical composition of onion cv. Red Baron during long-term postharvest storage. Sixth International Postharvest Symposium, 8-12 April 2009, Antalya, Turkey.

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Chope, G.A. and Terry, L.A. The role of ethylene and abscisic acid in onion bulb dormancy. Fifth International Symposium on Edible Alliaceae, 29th October - 1st November 2007, Lelystad, The Netherlands.

Poster presentations

Chope, G.A., Downes, K., Terry, L.A. Role of plant growth regulators in onion storage. 28th International Horticultural Congress, 22-27 August 2010, Lisbon, Portugal.

Downes, K., Chope, G.A., Terry, L.A. Effect of ethylene and 1-MCP treatment on the biochemistry and physiology of onion bulbs (Allium cepa L.) during storage. Postharvest Unlimited, 4-7 November 2008, Potsdam, Germany.

Terry, L.A., Chope, G.A., O'Connor, D., Thompson, A.J., Hammond, J.P., Downes, K. Sustaining UK Fresh Onion Supply by Improving Consumer Acceptability, Quality and Availability. Horticultural LINK 2007, 28 November 2007, Millbank, UK.

Exploitation

Since this project was also supported by HDC, there is already a grower summary which was published in HDC News as well as by British Onions in July 2010.

Progress with the project was presented at the Biennial Onion and Carrot Conference in 2009 and is in planning for 2011 also and possibly in the form of grower workshops.

It is also planned to produce a further article for HDC News in November, once this final report has been concluded.

As noted in “Uptake and Impact” this project is very much “self exploiting” as a result of the large consortium represented which in turn represents all the major onion growers in the U.K.

Highlighted during this work was the wastage which occurs when more than 3 – 4% of internal problems occur, and which cannot be seen on a grading line. Additionally a major cause of wastage (Botrytis allii or neck rot) was seen as a significant factor in these problems and likely to be increased by reduced temperature curing.

Further discussion with the consortium has led to a TSB proposal ” Seed to Store: A Holistic Approach to Controlling Internal Rots in Onions” which includes an in depth approach to detection and control of neck rot plus non invasive detection of internal defects.

No control measure will ever be totally 100% effective but if it were possible to reject automatically the few percent of defects which “escape” then entire stores of 500 – 2000 tonnes would avoid total rejection when >4% internal defects are found, which is not infrequent.

Conclusion of this “follow on” project will solve what is currently the most serious concern of U.K. growers. It should be noted that these concerns are common with production in all Northern European Countries and elsewhere.

Uptake and Impact

The U.K. onion industry is well co-ordinated through both consultants and packers.

Co-ordination is the result of rationalisation that has taken place in terms of supplying the major multiples, which is now through 5 main packhouses.
P.G. Rix (Stourgarden) supplying Tesco
G's Growers supplying Tesco and M & S
S.E. Produce (Beds Growers) supplying ASDA
Moulton Bulb CO. as main ASDA supply, Waitrose and Co-op
Rustler Produce supplying J. Sainsbury

These packhouses process in excess of 200,000 tonnes British onions (and also additional imports for supply continuity) which is a large proportion, over 50% of the U.K. crop.

This quantity in turn is supplied by the leading U.K. growers since requirement of these packhouses is for the best quality.

Before completion of the project around 2000 tonnes had been dried commercially at the lower temperature and results very encouraging.

In addition, to evaluate and control Botrytis allii a following T.S.B. Project has been proposed and supported.

Subject to concluding the specific disease issue highlighted in this research, the impact will be that most of the U.K. crop will be cured at a lower temperature.

Use of Ethylene/1-MCP will require further on farm evaluation (excl 1-MCP where there is no current approval).

As an entirely separate note, this project has brought together the senior personnel of the various packing operations and much is gained in terms of mutual assistance, co-operation and understanding.

