

**CRANFIELD UNIVERSITY**

**GEMMA AMY CHOPE**

**UNDERSTANDING THE MECHANISMS BEHIND ONION  
BULB DORMANCY IN RELATION TO THE POTENTIAL  
FOR IMPROVED ONION STORAGE**

**CRANFIELD HEALTH**

**Plant Science Laboratory**

**Ph.D THESIS**

**2006**

CRANFIELD UNIVERSITY

Cranfield Health

Plant Science Laboratory

Ph.D THESIS

Academic Years 2003-2006

GEMMA AMY CHOPE

UNDERSTANDING THE MECHANISMS BEHIND ONION BULB  
DORMANCY IN RELATION TO THE POTENTIAL FOR IMPROVED  
ONION STORAGE

Supervisors: Dr. Leon A Terry & Prof. Philip J White

December 2006

This thesis is submitted in partial fulfilment of the requirements  
for the degree of Doctor of Philosophy

© Cranfield University, 2006. All rights reserved. No part of this publication may be reproduced without  
the written permission of the copyright holder.

## ABSTRACT

Onion (*Allium cepa* L.) is the most economically important *Allium* crop. Onion cultivars adapted for growth in temperate regions require long days for bulb initiation, so the summer crop is stored over-winter. Deterioration in store is largely due to resumption of growth. Extended storage of onions is currently dependent on the synthetic sprout suppressant maleic hydrazide, whose future use is uncertain due to pressure to reduce residues in foods.

Abscisic acid (ABA) has previously been linked with dormancy in a relative of onion (*A. wakegi*). Bulb ABA concentration in three onion cultivars (*viz.* Renate, Ailsa Craig and SS1) with contrasting storage lives declined exponentially during controlled atmosphere (CA – 3% CO<sub>2</sub>, 5% O<sub>2</sub>; 2°C) storage at the same rate in each cultivar. Sprouting occurred at a minimal ABA concentration (*ca.* 50-120 ng g<sup>-1</sup> DW). It was proposed that extended periods of high concentrations of ABA may delay sprouting.

An ABA analogue (PBI-365) and exogenous ABA, were applied as preharvest foliar sprays (cvs. Renate, Carlos, Dinaro, Hysam, Red Baron and SS1), or as postharvest bulb soaks (cv. Hysam) with the aim of increasing endogenous ABA concentration and, thereby, extending the storage period. Endogenous bulb ABA concentration was not affected. Bulb ABA concentration again decreased during storage at a range of temperatures (4, 12 and 20°C) and sprouting occurred at minimal ABA concentration (*ca.* 75-150 ng g<sup>-1</sup> DW). After the onset of sprouting, ABA concentration increased again, probably due to synthesis by the sprout. The concentration of certain carbohydrates has been linked with storage potential. No straightforward relationship between ABA and non-structural carbohydrate (NSC; fructose, glucose, sucrose and fructans) metabolism could be determined. It was therefore postulated that ABA is more likely to play a role in mediating cell elongation rather than cell division, or that minimal ABA concentration could be a trigger for remobilisation of carbohydrates.

Controlled atmosphere (CA) is used to extend storage life of onions; however, shelf-life can be compromised. The effects of the transition between CA (3 % CO<sub>2</sub>, 5 % O<sub>2</sub>; 2°C) and air storage on ABA concentration, quality characteristics, respiration rate

(RR) and NSCs in three onion cultivars (*viz.* Renate, Carlos and SS1) were investigated. The RR of the short storing cultivar, SS1, was greatest by *ca.* 0.5-fold. The RR increased on removal from CA storage, with no accompanying decrease in carbohydrate concentration, indicating that the increased RR may have been a transient stress response. Storage of onions cv. SS1 for three weeks in air, followed by three weeks CA was as effective in suppressing sprout growth as six weeks continuous CA storage. Bulb ABA concentration decreased significantly between the time of harvest and after curing. Therefore the current practice of curing onions for extended periods at high temperatures could be reducing bulb ABA concentration, and therefore storage life.

The literature concerning the role of ethylene in onion storage is limited and conflicting. The effect of 1-methylcyclopropene (1-MCP; an ethylene perception inhibitor) on sprout growth in onions cv. SS1 stored at a range of temperatures (4, 12 and 20°C) was investigated, along with quality characteristics, NSCs and ABA. Sprout growth was reduced in onions treated with 1-MCP and stored at 4 or 12°C, but not at 20°C. Approximately 2-fold greater concentrations of sucrose, glucose and fructose were maintained in 1-MCP-treated bulbs stored at 12°C as compared with untreated bulbs. It appeared that 1-MCP reduced carbohydrate metabolism.

Both ethylene and ABA are highly likely to impact significantly on onion storage life. The results are discussed in relation to the potential to influence storage life by changes in horticultural practices, including the recommendation to re-evaluate the curing and drying protocol, and to investigate the possibility of delaying the start of CA storage.

## ACKNOWLEDGEMENTS

I would like to express my gratitude to Dr. Leon Terry and Prof. Philip White for their tireless support, guidance and patience throughout this project. I am also grateful to Allen Hilton, Balwinder Cook and Frank Davis at Cranfield University for technical support, and to the staff at Warwick HRI including Andrew Mead for statistical advice, and Sylvie Fairless and Peter Brooks for sourcing journals. This studentship was funded by the HDC, and I would like to thank them for the financial support.

This experience has been enhanced by all the friends I made at Warwick HRI, especially the occupants of office 131, who made me laugh, the Plant Mineral Nutrition group, including Helen, John, Rory, Will and Mark who made me very welcome and taught me valuable skills, not least of which was the art of white van driving.

Last, but not least, I would like to thank my parents and sister for their support, Adam for being there for me, and of course Kitty for keeping me company during long days in front of the computer.

## LIST OF CONTENTS

<b>ABSTRACT</b> .....	i
<b>ACKNOWLEDGEMENTS</b> .....	iii
<b>LIST OF CONTENTS</b> .....	iv
<b>LIST OF FIGURES</b> .....	x
<b>LIST OF TABLES</b> .....	xviii
<b>NOTATION</b> .....	xx
<b>1.0 CHAPTER ONE: INTRODUCTION</b> .....	1
1.1 Project background .....	1
1.2 Aim and objectives .....	1
1.2.1 Aim .....	1
1.2.2 Objectives .....	2
1.3 Thesis structure .....	2
<b>2.0 CHAPTER TWO: LITERATURE REVIEW</b> .....	6
2.1 <i>Allium cepa</i> L. ....	6
2.2 Economic importance of the onion crop in the UK .....	6
2.3 The onion lifecycle .....	8
2.3.1 Bulb initiation and formation .....	9
2.3.2 Onion bulb dormancy .....	10
2.4 Quality attributes of marketable onions .....	11
2.5 Strategies to delay sprouting .....	12
2.6 Pre-harvest factors that affect storage life .....	12
2.6.1 Pre-harvest nutrition .....	12
2.6.2 Growing season temperature .....	13
2.6.3 Maleic hydrazide .....	14
2.6.4 Crop maturity at harvest .....	15
2.6.5 Harvesting process .....	15

2.7	Postharvest factors that affect storage life .....	16
2.7.1	Curing and drying .....	16
2.7.2	Irradiation .....	17
2.7.3	Nitrous oxide .....	17
2.7.4	Storage environment .....	18
2.7.4.1	Storage temperature .....	18
2.7.4.2	Controlled atmosphere storage .....	19
2.7.4.3	Humidity .....	20
2.8	Alternative strategies to delay sprouting .....	20
2.9	Changes occurring in onion bulbs during storage and sprouting .....	21
2.9.1	Mitotic activity .....	21
2.9.2	Flavour precursors and pungency .....	22
2.9.3	Carbohydrates .....	24
2.9.4	Fresh weight and water loss .....	26
2.9.5	Respiration .....	26
2.9.6	Plant growth regulators .....	27
2.10	Conclusions .....	32
<b>3.0</b>	<b>CHAPTER THREE: TEMPORAL CHANGES IN ABSCISIC ACID CONCENTRATION AND QUALITY CHARACTERISTICS DURING CONTROLLED ATMOSPHERE STORAGE OF ONION BULBS OF THREE CULTIVARS WITH CONTRASTING STORAGE POTENTIAL .....</b>	<b>33</b>
3.1	Abstract .....	33
3.2	Introduction .....	34
3.3	Materials and methods .....	37
3.3.1	Plant material and storage regime .....	37
3.3.2	Experimental design .....	37
3.3.3	Sample preparation .....	38
3.3.4	Quantification of total fructans .....	38

3.3.5	Pyruvate assay for assessment of pungency .....	39
3.3.6	Bulb firmness and total soluble solids assessments .....	40
3.3.7	Mineral analysis .....	40
3.3.8	Abscisic acid (ABA) quantification .....	40
3.3.9	Statistical analysis .....	42
3.4	Results .....	42
3.4.1	Mineral concentrations .....	42
3.4.2	Sprout growth .....	44
3.4.3	Dry weight .....	46
3.4.4	Total soluble solids .....	46
3.4.5	Firmness .....	46
3.4.6	Pyruvate .....	47
3.4.7	Total fructans .....	49
3.4.8	Abscisic acid .....	49
3.5	Discussion .....	53
3.6	Conclusion .....	58
<b>4.0</b>	<b>CHAPTER FOUR: NEITHER PRE- NOR POSTHARVEST APPLICATION OF EXOGENOUS ABSCISIC ACID (ABA) OR AN ABA ANALOGUE AFFECTS ENDOGENOUS ABA CONCENTRATION OF ONION BULBS.....</b>	<b>60</b>
4.1	Abstract .....	60
4.2	Introduction .....	61
4.3	Materials and methods .....	63
4.3.1	Plant material .....	63
4.3.2	Experiment 1 – Pre-harvest foliar spray method .....	63
4.3.3	Harvest procedure .....	65
4.3.4	Experiment 2 - Postharvest bulb dip method .....	65
4.3.5	Storage regime and experimental design .....	65
4.3.6	Sample preparation .....	66



4.3.7	Physical assessments .....	66
4.3.8	Biochemical assessments .....	66
	4.3.8.1 NSC extraction .....	67
	4.3.8.2 NSC quantification .....	67
4.3.9	Statistical analysis .....	68
4.4	Results.....	68
4.4.1	Experiment 1 - Consequences of a pre-harvest foliar spray of ABA or PBI-365 .....	68
	4.4.1.1 Pre-storage cultivar characteristics .....	68
	4.4.1.2 Sprout growth .....	72
	4.4.1.3 Firmness .....	74
	4.4.1.4 Dry weight .....	74
	4.4.1.5 Total soluble solids .....	77
	4.4.1.6 Pyruvate concentration .....	77
	4.4.1.7 Non-structural carbohydrates .....	80
	4.4.1.8 Abscisic acid .....	89
4.4.2	Experiment 2- Consequences of postharvest bulb dip into solutions of ABA or PBI-365 .....	91
	4.4.2.1 Sprout growth .....	91
	4.4.2.2 Firmness and dry weight .....	92
	4.4.2.3 Total soluble solids and pyruvate concentration .....	93
	4.4.2.4 Abscisic acid .....	94
4.5	Discussion .....	95
4.6	Conclusion .....	100
<b>5.0</b>	<b>CHAPTER FIVE: THE EFFECT OF 1-METHYLCYCLOPROPENE (1-MCP) ON THE PHYSICAL AND BIOCHEMICAL CHARACTERISTICS OF ONION CV. SS1 BULBS DURING STORAGE .....</b>	<b>102</b>
5.1	Abstract .....	102

5.2	Introduction .....	102
5.3	Materials and methods .....	104
5.3.1	Plant material and storage regime .....	104
5.3.2	Application of 1-MCP.....	104
5.3.3	1-MCP quantification .....	105
5.3.4	Storage conditions and sampling regime .....	105
5.3.5	Sample preparation .....	106
5.3.6	Physical assessments .....	106
5.3.7	Biochemical assessments .....	106
5.3.8	Statistical analyses .....	107
5.4	Results.....	107
5.4.1	Effect of 1-MCP on physical properties of onion bulbs .....	107
5.4.1.1	Sprout growth .....	107
5.4.1.2	Firmness and dry weight .....	108
5.4.2	Effect of 1-MCP on biochemical properties of onion bulbs .....	110
5.4.2.1	Total soluble solids and pungency .....	110
5.4.2.2	Abscisic acid .....	111
5.4.2.3	Non-structural carbohydrates .....	112
5.5	Discussion .....	117
5.6	Conclusion .....	122
<b>6.0</b>	<b>CHAPTER SIX: THE EFFECT OF THE TRANSITION BETWEEN CONTROLLED ATMOSPHERE AND AIR STORAGE ON BULBS OF ONION CULTIVARS SS1, CARLOS AND RENATE .....</b>	<b>123</b>
6.1	Abstract .....	123
6.2	Introduction .....	123
6.3	Materials and methods .....	129
6.3.1	Plant material .....	129
6.3.2	Controlled atmosphere transition treatment .....	129
6.3.3	Experimental design .....	130

6.3.4	Sample preparation .....	130
6.3.5	Physical assessments .....	131
6.3.5.1	Measurement of respiration rate .....	131
6.3.6	Biochemical assessments .....	132
6.3.7	Statistical analysis .....	132
6.4	Results .....	133
6.4.1	Effect of the transition between CA and air storage on physical characteristics of onion bulbs .....	133
6.4.2	Effect of the transition between CA and air storage on biochemical characteristics of onion bulbs .....	137
6.5	Discussion .....	144
6.6	Conclusion .....	150
<b>7.0</b>	<b>CHAPTER SEVEN: GENERAL DISCUSSION AND CONCLUSIONS...</b>	<b>151</b>
7.1	Discussion .....	151
7.2	Recommendations for future experimental work .....	161
7.3	Implications for growers .....	169
7.4	Project conclusions .....	171
<b>8.0</b>	<b>CHAPTER EIGHT: LITERATURE CITED .....</b>	<b>173</b>
 <b>APPENDICES</b>		
<b>A.</b>	<b>APPENDIX A: VALIDATION OF THE ABSICISC ACID RADIO-IMMUNOASSAY .....</b>	<b>190</b>
<b>B.</b>	<b>APPENDIX B: STATISTICAL TABLES .....</b>	<b>196</b>
<b>C.</b>	<b>APPENDIX C: PUBLISHED LITERATURE .....</b>	<b>246</b>

## LIST OF FIGURES

### CHAPTER TWO

<b>Figure 2.1.</b> Annotated diagram of a cross-section of an onion bulb .....	9
<b>Figure 2.2.</b> Cross-sections of an onion cv. Renate bulb: A – with no sprout and B – with an external sprout .....	11
<b>Figure 2.3.</b> Chemical structure of: A – Uracil, B – Maleic hydrazide, C – Cytosine.....	14
<b>Figure 2.4.</b> Simplified pathway of flavour production in onion bulb tissue .....	23
<b>Figure 2.5.</b> Percentage sprouting and hormone activity of onions cv. Rijnsberger stored at 5-8°C (Thomas and Isenberg, 1972) .....	28
<b>Figure 2.6.</b> The synthesis of abscisic acid (ABA) from xanthoxin in higher plants. Solid arrows indicate the major pathway, dashed arrows indicate a minor pathway, AB=abscisic (Cutler and Krochko, 1999) .....	30
<b>Figure 2.7.</b> Metabolism of abscisic acid (ABA) .....	31

### CHAPTER THREE

<b>Figure 3.1.</b> Elemental content of onion cvs. Renate, Ailsa Craig and SS1 bulbs shortly after harvest (day 0, black bars, n=4), and at early (light grey bars, n=8) and late (dark grey bars, n=8) time points during controlled atmosphere storage (cv. Renate: days 40 and 230, cv. Ailsa Craig: days 81 and 129, and cv. SS1: days 40 and 81). Standard error bars are shown .....	43
<b>Figure 3.2.</b> Changes in the physical parameters; sprout length (expressed as a ratio of bulb height), total soluble solids and firmness ( $\text{N mm}^{-1}$ ), in bulbs of onion cvs. Renate ( $\blacktriangle$ ), Ailsa Craig ( $\bullet$ ) and SS1 ( $\blacksquare$ ) during controlled atmosphere storage. Drop down arrows indicate the final sample taken. LSD ( $P=0.05$ ) bars are shown, symbols correspond to cultivar; n=24 .....	45

**Figure 3.3.** Changes in the biochemical parameters; pyruvate, fructan, and ABA concentrations, in bulbs of onion cvs. Renate (▲), Ailsa Craig (●) and SS1 (■) during controlled atmosphere storage. Drop down arrows indicate the final sample taken. LSD bars ( $P=0.05$ ) are shown, symbols correspond to cultivar. LSD bar A is for comparison of time zero with all other times, bar B is for comparison of all times except time zero. Pyruvate:  $n=24$ , fructan and ABA: time zero  $n=4$ , all other times  $n=8$  .....48

## CHAPTER FOUR

**Figure 4.1.** A. 8'-methylene abscisic acid methyl ester. B. (+)-abscisic acid .....62

**Figure 4.2.** Sprout length of onion cvs. Carlos (●), Dinaro (○), Hysam (▼), Red Baron (□), Renate (■) and SS1 (□) treated with a pre-harvest spray of  $10^{-4}$  M ABA in 0.5% (v/v) Li-700,  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 109 days or 20°C for 98 days ( $n=5$ ). LSD bars ( $P=0.05$ ) are shown based on 321, 193 and 120 d.f for 4°C, 12°C and 20°C respectively .....73

**Figure 4.3.** Firmness ( $N\ mm^{-1}$ ) of onion cvs. Carlos (●), Dinaro (○), Hysam (▼), Red Baron (□), Renate (■) and SS1 (□) treated with a pre-harvest spray of  $10^{-4}$  M ABA in 0.5% (v/v) Li-700,  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 109 days or 20°C for 98 days ( $n=5$ ). LSD bars ( $P=0.05$ ) are shown based on 526, 556 and 551 d.f for 4°C, 12°C and 20°C respectively .....75

**Figure 4.4.** Dry weight of onion cvs. Carlos (●), Dinaro (○), Hysam (▼), Red Baron (□), Renate (■) and SS1 (□) treated with a pre-harvest spray of  $10^{-4}$  M ABA in 0.5% (v/v) Li-700,  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 109 days or 20°C for 98 days ( $n=5$ ). LSD bars ( $P=0.05$ ) are shown based on 525, 558 and 555 d.f for 4°C, 12°C and 20°C respectively .....76

**Figure 4.5.** Total soluble solids concentration onion cvs. Carlos (●), Dinaro (○), Hysam (▼), Red Baron (□), Renate (■) and SS1 (□) treated with a pre-harvest spray of  $10^{-4}$  M ABA in 0.5% (v/v) Li-700,  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 109 days or 20°C for 98 days (n=5). LSD bars ( $P=0.05$ ) are shown based on 526, 558 and 557 d.f. for 4°C, 12°C and 20°C respectively .....78

**Figure 4.6.** Pyruvate concentration of onion cvs. Carlos (●), Dinaro (○), Hysam (▼), Red Baron (□), Renate (■) and SS1 (□) treated with a pre-harvest spray of  $10^{-4}$  M ABA in 0.5% (v/v) Li-700,  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 109 days or 20°C for 98 days (n=5). LSD bars ( $P=0.05$ ) are shown based on 526, 559 and 557 d.f. for 4°C, 12°C and 20°C respectively .....79

**Figure 4.7.** Fructose concentration of onion cvs. Red Baron (●), Renate (○) and SS1 (▼) treated with a pre-harvest spray of  $10^{-4}$  M ABA in 0.5% (v/v) Li-700,  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 109 days or 20°C for 98 days (n=3). LSD bars ( $P=0.05$ ) are shown based on 120, 136 and 132 d.f. for 4°C, 12°C and 20°C respectively .....81

**Figure 4.8.** Sucrose concentration of onion cvs. Red Baron (●), Renate (○) and SS1 (▼) treated with a pre-harvest spray of  $10^{-4}$  M ABA in 0.5% (v/v) Li-700,  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 109 days or 20°C for 98 days (n=3). LSD bars ( $P=0.05$ ) are shown based on 120, 136 and 132 d.f. for 4°C, 12°C and 20°C respectively .....82

**Figure 4.9.** Glucose concentration of onion cvs. Red Baron (●), Renate (○) and SS1 (▼) treated with a pre-harvest spray of  $10^{-4}$  M ABA in 0.5% (v/v) Li-700,  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 109 days or 20°C for 98 days (n=3). LSD bars ( $P=0.05$ ) are shown based on 120, 136 and 132 d.f. for 4°C, 12°C and 20°C respectively .....83

**Figure 4.10.** Total sugars (glucose+sucrose+fructose) concentration of onion cvs. Red Baron (●), Renate (○) and SS1 (▼) treated with a pre-harvest spray of  $10^{-4}$  M ABA in 0.5% (v/v) Li-700,  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 109 days or 20°C for 98 days (n=3). LSD bars ( $P=0.05$ ) are shown based on 120, 136 and 132 d.f. for 4°C, 12°C and 20°C respectively .....85

**Figure 4.11.** Ratio of monosaccharide : disaccharide ((fructose+glucose)/sucrose) of onion cvs. Red Baron (●), Renate (○) and SS1 (▼) treated with a pre-harvest spray of  $10^{-4}$  M ABA in 0.5% (v/v) Li-700,  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 109 days or 20°C for 98 days (n=3). LSD bars ( $P=0.05$ ) are shown based on 120, 136 and 132 d.f. for 4°C, 12°C and 20°C respectively .....86

**Figure 4.12.** Total fructan concentration of onion cvs. Red Baron (●), Renate (○) and SS1 (▼) treated with a pre-harvest spray of  $10^{-4}$  M ABA in 0.5% (v/v) Li-700,  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 109 days or 20°C for 98 days (n=3). LSD bars ( $P=0.05$ ) are shown based on 120, 136 and 134 d.f. for 4°C, 12°C and 20°C respectively .....88

**Figure 4.13.** Abscisic acid concentration of onion cvs. Carlos (●), Dinaro (○), Hysam (▼), Red Baron (□), Renate (■) and SS1 (□) treated with a pre-harvest spray of  $10^{-4}$  M ABA in 0.5% (v/v) Li-700,  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 109 days or 20°C for 98 days (n=5). LSD bars ( $P=0.05$ ) are shown based on 524, 556 and 554 d.f. for 4°C, 12°C and 20°C respectively .....90

**Figure 4.14.** Sprout growth of onion cv. Hysam bulbs treated with a postharvest soak in  $10^{-4}$  M ABA in 0.5% (v/v) Li-700 (●),  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700 (▼) or water (○) and stored at 4°C for 215 days, 12°C for 109 days or 20°C for 67 days (n=3). LSD bars ( $P=0.05$ ) are shown based on 39 d.f. ....92

**Figure 4.15.** Firmness and dry weight of onion cv. Hysam bulbs treated with a postharvest soak in  $10^{-4}$  M ABA in 0.5% (v/v) Li-700 (●),  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700 (▼) or water (○) and stored at 4°C for 215 days, 12°C for 109 days or 20°C for 67 days (n=3). LSD bars ( $P=0.05$ ) are shown based on: firmness - 35, 36 and 35 d.f for 4°C, 12°C and 20°C respectively, dry weight - 39 d.f. ....93

**Figure 4.16.** Total soluble solids and pyruvate concentration of onion cv. Hysam bulbs treated with a postharvest soak in  $10^{-4}$  M ABA in 0.5% (v/v) Li-700 (●),  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700 (▼) or water (○) and stored at 4°C for 215 days, 12°C for 109 days or 20°C for 67 days (n=3). LSD bars ( $P=0.05$ ) are shown based on 39 d.f. ....94

**Figure 4.17.** ABA concentration of onion cv. Hysam bulbs treated with a postharvest soak in  $10^{-4}$  M ABA in 0.5% (v/v) Li-700 (●),  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700 (▼) or water (○) and stored at 4°C for 215 days, 12°C for 109 days or 20°C for 67 days (n=3). LSD bars ( $P=0.05$ ) are shown based on 39, 38 and 39 d.f. for 4°C, 12°C and 20°C respectively .....95

## CHAPTER FIVE

**Figure 5.1.** Sprout length in onions treated with  $1 \mu\text{l l}^{-1}$  1-MCP at 20°C for 24 hours (open symbols) and untreated onions (controls, closed symbols) stored at 4°C for 109 days, 12°C for 53 days or 20°C for 39 days. Controls n=5, 1-MCP n=10. LSD bars ( $P=0.05$ ) A=5,5; B=10,5; C=10,10 .....108

**Figure 5.2.** Firmness and dry of onions treated with  $1 \mu\text{l l}^{-1}$  1-MCP at 20°C for 24 hours (open symbols) and untreated onions (controls, closed symbols) stored at 4°C for 109 days, 12°C for 53 days or 20°C for 39 days. Controls n=5, 1-MCP n=10. LSD bars ( $P=0.05$ ) A=5,5; B=10,5; C=10,10 .....109

**Figure 5.3.** Total soluble solids (%) and pyruvate concentration of onions treated with  $1 \mu\text{l l}^{-1}$  1-MCP at 20°C for 24 hours (open symbols) and untreated onions (controls, closed symbols) stored at 4°C for 109 days, 12°C for 53 days or 20°C for 39 days. Controls n=5, 1-MCP n=10. LSD bars ( $P=0.05$ ) A=5,5; B=10,5; C=10,10 .....111



**Figure 5.4.** ABA concentration during storage in onions treated with  $1 \mu\text{l l}^{-1}$  1-MCP at  $20^{\circ}\text{C}$  for 24 hours (open symbols) and untreated onions (controls, closed symbols) stored at  $4^{\circ}\text{C}$  for 109 days,  $12^{\circ}\text{C}$  for 53 days or  $20^{\circ}\text{C}$  for 39 days. Controls  $n=5$ , 1-MCP  $n=10$ . LSD bars ( $P=0.05$ ) A=5,5; B=10,5; C=10,10.....112

**Figure 5.5.** Sucrose, glucose and fructose concentrations in onions treated with  $1 \mu\text{l l}^{-1}$  1-MCP at  $20^{\circ}\text{C}$  for 24 hours (open symbols) and untreated onions (controls, closed symbols) stored at  $4^{\circ}\text{C}$  for 109 days,  $12^{\circ}\text{C}$  for 53 days or  $20^{\circ}\text{C}$  for 39 days. Controls  $n=5$ , 1-MCP  $n=10$ . LSD bars ( $P=0.05$ ) A=5,5; B=10,5; C=10,10 .....114

**Figure 5.6.** Ratio of monosaccharide : disaccharide (glucose+fructose/sucrose), total sugar (sucrose+glucose+fructose) and total fructan concentration in onions treated with  $1 \mu\text{l l}^{-1}$  1-MCP at  $20^{\circ}\text{C}$  for 24 hours (open symbols) and untreated onions (controls, closed symbols) stored at  $4^{\circ}\text{C}$  for 109 days,  $12^{\circ}\text{C}$  for 53 days or  $20^{\circ}\text{C}$  for 39 days. Controls  $n=5$ , 1-MCP  $n=10$ . LSD bars ( $P=0.05$ ) A=5,5; B=10,5; C=10,10 .....116

## CHAPTER SIX

**Figure 6.1.** The respiration rate of onions cvs. Carlos (■), Renate (□) and SS1 (▣) recorded before storage (Day 0), after 21 days storage in CA (CA P1) or air (Air P1) and after CA for 42 days (CA/CA), CA for 21 days followed by air for 21 days (CA/Air), air for 21 days followed by CA for 21 days (Air/CA) and air for 42 days (Air/Air). Day 0  $n=8$ , CA P1 and Air P1  $n=4$ ; CA/CA, CA/Air, Air/CA and Air/Air  $n=2$ . LSD bars ( $P=0.05$ ) A=2,2; B=4,2; C=4,4 are shown .....134

**Figure 6.2.** The sprout length of onion cv. SS1 expressed as a percentage of bulb height after storage in; CA for 42 days (CA/CA), CA for 21 days followed by air for 21 days (CA/Air), air for 21 days followed by CA for 21 days (Air/CA) and air for 42 days (Air/Air);  $n=4$ . LSD bar ( $P=0.05$ ) is shown .....135

**Figure 6.3.** The sucrose, glucose and fructose concentration of onion cvs. Carlos (■), Renate (□) and SS1 (▣) measured at harvest, before storage (Day 0), after 21 days storage in CA (CA P1) or air (Air P1) and after CA for 42 days (CA/CA), CA for 21 days followed by air for 21 days (CA/Air), air for 21 days followed by CA for 21 days (Air/CA) and air for 42 days (Air/Air). Harvest n=12, Day 0 n=16, CA P1 and Air P1 n=8; CA/CA, CA/Air, Air/CA, Air/Air n=4. LSD bars ( $P=0.05$ ) A=4,4; B=8,4; C=8,8 are shown .....140

**Figure 6.4.** The total sugar (glucose, sucrose and fructose), monosaccharide to disaccharide ratio, and total fructan concentration of onion cv. Carlos (■), Renate (□) and SS1 (▣) measured at harvest, before storage (Day 0), after 21 days storage in CA (CA P1) or air (Air P1) and after CA for 42 days (CA/CA), CA for 21 days followed by air for 21 days (CA/Air), air for 21 days followed by CA for 21 days (Air/CA) and air for 42 days (Air/Air). Harvest n=12, Day 0 n=16, CA P1 and Air P1 n=8; CA/CA, CA/Air, Air/CA, Air/Air n=4. LSD bars ( $P=0.05$ ) A=4,4; B=8,4; C=8,8 are shown .....142

**Figure 6.5.** The ABA concentration of onion cvs. Carlos (■), Renate (□) and SS1 (▣) measured at harvest, before storage (Day 0), after 21 days storage in CA (CA P1) or air (Air P1) and after CA for 42 days (CA/CA), CA for 21 days followed by air for 21 days (CA/Air), air for 21 days followed by CA for 21 days (Air/CA) and air for 42 days (Air/Air). Harvest n=12, Day 0 n=16, CA P1 and Air P1 n=8; CA/CA, CA/Air, Air/CA, Air/Air n=4. LSD bars ( $P=0.05$ ) A=4,4; B=8,4; C=8,8 are shown .....144

## CHAPTER SEVEN

<b>Figure 7.1.</b> Cross-section of a potato tuber .....	155
<b>Figure 7.2.</b> Summary of the pathways involved in ABA biosynthesis and catabolism. Dashed arrow indicates export of xanthoxin from the plastid to the cytosol. ZEP=zeaxanthin epoxydase, NCED=nine <i>cis</i> -epoxycarotenoid dioxygenase; SDR=short chain alcohol dehydrogenase/reductase; AAO=abscisic aldehyde dehydrogenase; ABA=abscisic acid; PA=phaseic acid; DPA=dihydrophaseic acid (Nambara and Marion-Poll, 2005; Taylor <i>et al.</i> , 2005) .....	165
<b>Figure 7.3.</b> Simplified pathway of ethylene biosynthesis. SAM=S-adenosyl-L-methionine, MTA=5'-methylthioadenosine, ACC=1-amino-cyclopropane-1-carboxylic acid (Ecker, 1995; Wang <i>et al.</i> , 2002) .....	168

## LIST OF TABLES

### CHAPTER TWO

**Table 2.1.** The area of onions<sup>1</sup> harvested (1000 Ha), the yield (100 g Ha<sup>-1</sup>) and production (tonnes) from 2000 to 2005 in the UK and worldwide (FAOSTAT, 2006).....7

**Table 2.2.** The quantity (tonnes) and value (£1000<sup>1</sup>) of onions imported to, and exported from, the UK (FAOSTAT, 2006) .....8

### CHAPTER THREE

**Table 3.1.** The maximum and minimum abscisic acid (ABA) concentrations reported previously in bulbs of onions and related species .....36

**Table 3.2.** The mean elemental composition of onion cvs. Renate, Ailsa Craig and SS1 bulbs (n=20) compared with data for raw and sweet onions from the USDA database (U.S. Department of Agriculture, 2004) .....44

**Table 3.3.** The maximum and minimum abscisic acid concentrations on a dry weight ( $\pm$  S.E.) and fresh weight basis recorded in onion cvs. SS1, Ailsa Craig and Renate bulbs at the beginning and end of storage (Time zero: n=4, all other times: n=8) .....50

**Table 3.4.** The parameters ( $\pm$  S.E.) of the exponential functions ( $y = (A + B)R^x$ ) describing the change in ABA concentration during storage (days) in onion cvs. SS1, Ailsa Craig and Renate bulbs .....52

### CHAPTER FOUR

**Table 4.1.** The estimated storage life (months) of onion cvs. Carlos, Dinaro, Hysam, Red Baron, Renate and SS1 (Tim Crowther, Warwick HRI, pers. comm.) .....63

**Table 4.2.** Treatments applied to the onion plants in the field .....64

**Table 4.3.** Weather conditions in the week following pre-harvest treatment .....64

<b>Table 4.4.</b> The ranking of the cultivars Carlos, Dinaro, Hysam, Red Baron, Renate and SS1 according to mean pre-storage measurements (n=20) of dry weight, total soluble solids, pungency, firmness and abscisic acid .....	70
<b>Table 4.5.</b> The ranking of the cultivars Red Baron, Renate and SS1 according to mean pre-storage measurements (n=12) of glucose, sucrose, fructose and fructan concentration.....	71
<b>Table 4.6.</b> The time (days) taken for 50% of the bulbs to sprout at each storage temperature .....	72

## CHAPTER SIX

<b>Table 6.1.</b> Summary of the changes in carbohydrate composition of onion bulbs during storage.....	125
<b>Table 6.2.</b> The firmness ( $\text{N mm}^{-1}$ ) and dry weight ( $\text{mg g}^{-1}$ FW) of onion cvs. Carlos, Renate and SS1 measured at harvest, before storage (Day 0), after 21 days storage in CA (CA P1) or air (Air P1) and after CA for 42 days (CA/CA), CA for 21 days followed by air for 21 days (CA/Air), air for 21 days followed by CA for 21 days (Air/CA) and air for 42 days (Air/Air). Firmness LSD ( $P=0.05$ ) 12,16=5.903; 8,4=9.466. Dry weight LSD ( $P=0.05$ ) 12,16=7.941; 8,4=12.733 .....	136
<b>Table 6.3.</b> The total soluble solids (TSS) concentration (%) and pyruvate concentration ( $\mu\text{mol g}^{-1}$ FW) of onion cvs. Carlos, Renate and SS1 measured at harvest, before storage (Day 0), after 21 days storage in CA (CA P1) or air (Air P1) and after CA for 42 days (CA/CA), CA for 21 days followed by air for 21 days (CA/Air), air for 21 days followed by CA for 21 days (Air/CA) and air for 42 days (Air/Air). TSS LSD ( $P=0.05$ ) 12,16=0.8538; 8,4=1.3691. Pyruvate LSD ( $P=0.05$ ) 12,16=1.049; 8,4=1.682 .....	138

## NOTATION

<	less than
>	greater than
≤	less than or equal to
≥	greater than or equal to
%	per cent
=	equals
°C	degree Celsius
β	beta
γ	gamma
μl	microlitre
μM	micromolar
μmol	micromoles
1-MCP	1-methycyclopropene
AACC	American Association of Cereal Scientists
AAO	abscisic aldehyde dehydrogenase
ABA	abscisic acid
ABU	arbitrary unit
ACC	1-amino-cyclopropane-1-carboxylic acid
ACSO	<i>S</i> -alk(en)yl-L-cysteine sulphoxide
ADAS	Agricultural Development and Advisory Service
ANOVA	Analysis of Variance
AOAC	Association of Analytical Communities
BCPC	British Crop Production Council
BOC	British Oxygen Company
CA	controlled atmosphere
<i>ca.</i>	approximately
Ca	calcium
CaCl <sub>2</sub>	calcium chloride

CEPA	2- chloroethylphosphonic acid
CHO	carbohydrate
cm	centimetre
cM	centi Morgan
Co	Cobalt
CO <sub>2</sub>	carbon dioxide
cv.	cultivar
DEFRA	Department for Environment Food and Rural Affairs
<i>de novo</i>	anew
d.f.	degrees of freedom
DP	degree of polymerisation
DPA	dihydrophaseic acid
DW	dry weight
EEC	European Economic Community
ELSD	Evaporative Light Scattering Dectector
<i>et al.</i>	and others
FAO	Food and Agriculture Organisation of the United Nations
FID	Flame Ionisation Detection
FW	fresh weight
g	gram
GA	gibberellin
GC	Gas Chromatography
GC	guanine-cytosine
Gy	Gray unit
hr	hours
<sup>3</sup> H	tritium
ha	hectare
HCl	hydrochloric acid
HDC	Horticultural Development Council
HPLC	High Performance Liquid Chromatography

ICP-AES	Inductively Coupled Plasma – Atomic Emission Spectroscopy
<i>in vitro</i>	outside a living organism
<i>in vivo</i>	inside a living organism
kg	kilogram
kN	kiloNewton
kPa	kiloPascal
l	litre
LSD	least significant difference
M	molarity
m	metre
MAC	monoclonal antibody
MAFF	Ministry of Agriculture, Fisheries and Food
Mbp	mega base pairs
MCSO	methyl <i>S</i> -alk(en)yl-L-cysteine sulphoxide
mg	milligram
MH	maleic hydrazide
min	minute
ml	millilitre
mm	millimetre
mM	millimolar
MTA	5'-methylthioadenosine
MW	molecular weight
N	normality
N <sub>2</sub>	nitrogen
NA	not applicable
NaBH <sub>4</sub>	sodium borohydride
NaCl	sodium chloride
Na <sub>2</sub> HPO <sub>4</sub>	di-sodium hydrogen orthophosphate
NaH <sub>2</sub> PO <sub>4</sub>	sodium di-hydrogen orthophosphate
NaOH	sodium hydroxide



NCED	nine <i>cis</i> -epoxycarotenoid dioxygenase
ng	nanogram
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	ammonium sulphate
NSC	non-structural carbohydrate
nm	nanometre
nmol	nanomole
N <sub>2</sub> O	nitrous oxide
O <sub>2</sub>	oxygen
<i>P</i>	probability
PA	phaseic acid
PAHBAH	<i>p</i> -hydroxybenzoic acid hydrazide
PBI-365	8'-methylene ABA methyl ester
PBS	phosphate buffered saline
PCSO	propyl <i>S</i> -alk(en)yl-L-cysteine sulphoxide
pg	picogram
PRENCSO	1-propenyl <i>S</i> -alk(en)yl-L-cysteine sulphoxide
Q <sub>10</sub>	increase in the respiration rate produced by raising the temperature by 10°C
RIA	Radio-immunoassay
RH	relative humidity
RNA	ribonucleic acid
rpm	revolutions per minute
RR	respiration rate
RT-PCR	Reverse transcription – polymerase chain reaction
S	sulphur
SAM	<i>S</i> -adenosyl-L-methionine
SDR	short chain alcohol dehydrogenase/reductase
SDW	sterile distilled water
S.E.	standard error
SS1	Supasweet

Syn.	synonym
TLC	thin layer chromatography
TSS	total soluble solids
UK	United Kingdom
US	United States
USA	United States of America
USDA	United States Department of Agriculture
<i>viz.</i>	namely
v/v	volume by volume
w/w	weight by weight
WSC	water soluble carbohydrate
ZEP	zeaxanthin epoxydase

## 1.0 CHAPTER ONE

### Introduction

#### 1.1 Project background

Onion (*Allium cepa*) is an important crop that is grown worldwide. Cultivars adapted to temperate climates require long days for bulb initiation; therefore the summer crop must be stored over the winter. A major cause of loss of stored onions is re-growth in the form of a green sprout that eventually protrudes from the neck of the bulb. Maleic hydrazide (MH) is a synthetic sprout suppressant that inhibits sprout growth, but leaves a residue in the bulb. Concerns from retailers and consumers about residues in foodstuffs are increasing, so the future for the use of MH is uncertain, and other methods of sprout suppression will need to be sought. In the past, work to improve onion storage potential has concentrated on breeding, cultural practices and optimisation of the storage environment, with relatively little attention paid to the mechanisms behind onion bulb dormancy.

This project was funded by the Horticultural Development Council (HDC; CP 20) to investigate alternative targets for possible manipulation to control sprouting in store.

#### 1.2 Aim and objectives

##### 1.2.1 Aim

The aim of this PhD project was to determine how the biochemical and physical changes occurring in stored onion bulbs influence the storage potential of UK-grown bulbs, in relation to the possibilities for improved storage of onions.

### 1.2.2 Objectives

- To determine if differences exist in the initial concentrations and/or rate of degradation of abscisic acid (ABA) in bulbs of onion cultivars with different storage potential.
- To determine if the remobilisation of carbohydrates is affected by, and correlated with, the concentration of ABA in the bulb.
- To verify the effect, if any, on storage potential of bulb ABA concentration using a chemical analogue of ABA.
- To determine the effect of an ethylene inhibitor on onion storage life.
- To determine the effect of the transition between controlled atmosphere storage and air on bulbs of onion cultivars with different storage potential.
- To relate the observations made to the potential to influence onion storage life by changes in horticultural practices.

### 1.3 Thesis structure

The thesis is arranged into seven chapters. Chapter 2 is a review of existing literature. First, it describes the lifecycle of the onion plant and its importance as a crop species. The current strategies used to delay sprouting are then outlined. After this, the pre- and postharvest factors that affect onion storage life are described, followed by a consideration of the biochemical and physical changes that occur within stored onion bulbs as targets for manipulation to increase onion storage life.

The initial concentration and the rate of degradation of ABA during storage of onion cultivars with different storage lives had not been compared previously. Chapter 3 details the temporal changes in ABA concentration and other quality traits during controlled atmosphere (CA) storage of onion bulbs of three cultivars with contrasting

storage potential. Controlled atmosphere was used to extend to viable sampling period. Results from this work have been published as follows:

- Chope, G. A., Terry, L. A. and White, P. J. (2005). Temporal changes in abscisic acid concentration in onion bulbs during controlled atmosphere storage. In: *Frutic 05, Information and technology for sustainable fruit and vegetable production*, 12-16 September 2005, Montpellier, France. Oral presentation.
- Chope, G. A., Terry, L. A. and White, P. J. (2006). Effect of controlled atmosphere storage on abscisic acid concentration and other biochemical attributes of onion bulbs. *Postharvest Biology and Technology*, 39, 233-242. (See Appendix C).
- A poster was presented at the Advances in Applied Biology (AAB) Centennial Conference – *Advances in Applied Biology: Providing New Opportunities for Consumers and Producers in the 21<sup>st</sup> Century*. 15-17 December 2004, Oxford.

Synthetic analogues of ABA that resist degradation are available. The effect of synthetic analogues of abscisic acid on onion dormancy has not previously been investigated. Chapter 4 describes experiments aimed at increasing endogenous bulb ABA concentration via the application of exogenous ABA and an ABA analogue (PBI-365; 8'-methylene ABA methyl ester) in the form of a pre-harvest foliar spray (six cultivars) or a postharvest bulb soak (one cultivar only). An extended range of six cultivars were tested and onions from this experiment were stored at a range of temperatures (4, 12 or 20°C) to ensure that sprout growth would progress fully in all cultivars allowing measurements to be taken from bulbs at all physiological stages. Results from this work have been accepted for publication in *Plant Growth Regulation*.

- Chope, G. A., Terry, L. A. and White, P. J. Neither pre- nor postharvest application of exogenous abscisic acid (ABA) or an ABA analogue affects endogenous ABA concentration of onion bulbs. *Plant Growth Regulation*. Accepted for publication on 18 January 2007.

Literature concerning the effects of the plant growth regulator ethylene on stored onions is limited and conflicting, and more investigation is required to determine the role of ethylene in onion bulb dormancy. Chapter 5 reports an experiment to investigate the effect of the use of 1-methylcyclopropene (1-MCP), an inhibitor of ethylene perception, as a postharvest treatment of short storing onion cv. SS1 bulbs on sprout growth, quality characteristics, non-structural carbohydrates and ABA. Results from this work have been published in *Postharvest Biology and Technology*. Details on optimal methods for extraction and quantification of non-structural carbohydrates were submitted for publication in the *Journal of Agricultural and Food Chemistry*.

- Chope, G. A., Terry, L. A. and White, P. J. (2007). The effect of 1-methylcyclopropene (1-MCP) on the physical and biochemical characteristics of onion cv. SS1 bulbs during storage. *Postharvest Biology and Technology*. In press. (See Appendix C).
- Davis, F., Terry, L. A., Chope, G. A. and Faul, C. J. The effect of extraction procedure on measured sugar concentrations in onion (*Allium cepa* L.) bulbs. *Journal of Agricultural and Food Chemistry*. Submitted on 3 November 2006.

Controlled atmosphere is used to extend storage life of onions, however, upon removal from CA storage, the time to sprouting is accelerated. It is therefore essential that the onions from CA reach the consumer within a certain time constraint as shelf-life can be compromised. Chapter 6 describes the effect of the transition between CA and air storage on ABA concentration, quality characteristics, respiration rate and non-structural carbohydrate concentration in onion bulbs of three cultivars with contrasting storage potential. In addition the effect of the curing process on bulb ABA concentration is discussed. Results from this work have been published in *Postharvest Biology and Technology*.

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

Chapter 7 is a general discussion which integrates the results from previous chapters, proposes recommendations for future research, and considers the implications of the results in terms of the considerations for onion growers. The results from this thesis have formed the foundation of a recent successful application to DEFRA HortLINK entitled 'HL0181 - Sustaining UK fresh onion supply by improving consumer acceptability, quality and availability'.

## 2.0 CHAPTER TWO

### Literature Review

#### 2.1 *Allium cepa* L.

The common onion (*Allium cepa* L.) belongs to the genus *Allium* (family *Alliaceae*). The *Allium* genus comprises over 700 botanical species distributed throughout the temperate, warm temperate, boreal and tropical (mountainous areas only) zones of the world, predominantly in the Northern Hemisphere. Species within the genus are mostly perennial, bulbous plants. Their life cycles exhibit a variety of responses to seasonal and climatic changes according to the environment in which they live; consistent with the ecological diversification that has accompanied the evolution of *Alliums* (Fritsch and Friesen, 2002). For example, summer dormant species are adapted to dry summers and winter dormant species are adapted to cold regions (Brewster, 1994). Onions belong to the subgenus *Allium* and the section *Cepa*. The onion has been cultivated for around 5000 years and no longer exists as a wild species (Brewster, 1994). The direct wild ancestor of onion is not known. Wild species in the section *Cepa* are adapted to grow in sites with a shallow soil layer such as rock crevices, stony slopes and river banks (Fritsch and Friesen, 2002). Wild *Alliums* have a long annual growth cycle (spring to winter) and take between three and ten years to reach flowering maturity.

#### 2.2 Economic importance of the onion crop in the UK

Onions (*Allium cepa* L.) grown in the UK are produced for human consumption. Onions, leeks (*Allium porrum* L.) and shallots (*Allium cepa* L. *Aggregatum* Group) account for 4.3% of overall fruit and vegetable consumption in the UK (DEFRA, 2003). As well as being sold for the fresh market, onions are components in a wide variety of processed foods. Onions are the most economically important *Allium* crop, with their



value to the producer rising from £100 per tonne in 2000 to £156 per tonne in 2003 (FAOSTAT, 2006). An area of just over 11, 000 hectares of onions was harvested in the UK in 2005, accounting for 0.63% of worldwide production (Table 2.1).

**Table 2.1.** The area of onions<sup>1</sup> harvested (1000 Ha), the yield (100 g Ha<sup>-1</sup>) and production (tonnes) from 2000 to 2005 in the UK and worldwide (FAOSTAT, 2006).

Year	Area Harvested (Ha)		Yield (100g Ha <sup>-1</sup> )		Production (Tonne)	
	UK	World	UK	World	UK	World
2000	9, 100	2, 798, 742	431, 538	175, 041	392, 700	48, 989, 519
2001	8, 600	2, 899, 580	435, 930	175, 066	374, 900	50, 761, 749
2002	8, 390	2, 954, 209	337, 783	177, 496	283, 400	52, 436, 098
2003	8, 730	3, 020, 839	427, 950	177, 026	373, 600	53, 476, 640
2004	8, 590	3, 097, 149	396, 857	183, 790	340, 900	56, 922, 514
2005	11, 191	3, 172, 455	324, 508	180, 933	363, 157	57, 400, 277

<sup>1</sup>Onions harvested at a mature stage, not dehydrated.

The quantity of onions imported to the UK has increased *ca.* 1.8-fold, and the value 2.7-fold, between 2000 and 2004 (Table 2.2). Between March and July, bulbs that have been harvested in the UK and stored since the previous autumn are in direct competition with freshly harvested bulbs from the Southern hemisphere. Consumers reject soft bulbs with cracked skin in favour of imported bulbs. It is important for UK growers to compete at this time of year, so that this valuable market share is not lost to imports.

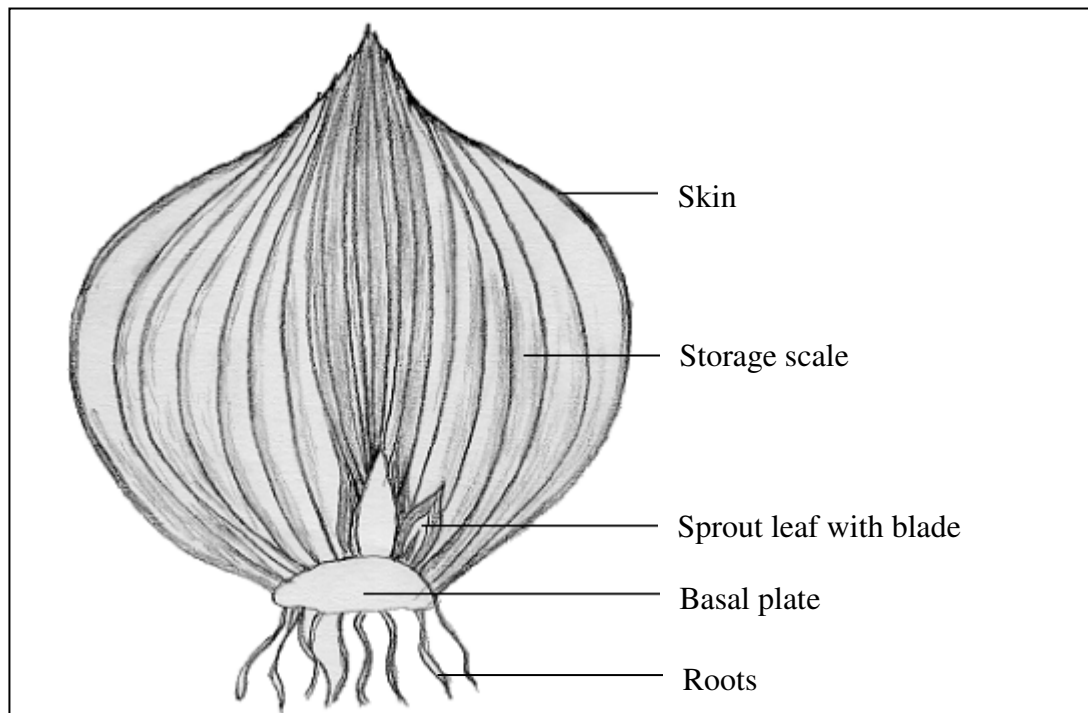
**Table 2.2.** The quantity (tonnes) and value (£1000<sup>1</sup>) of onions imported to, and exported from, the UK (FAOSTAT, 2006).

Year	Imports		Exports	
	Quantity (Tonnes)	Value (£1000)	Quantity (Tonnes)	Value (£1000)
2000	156, 718	25, 952	5, 406	1, 536
2001	231, 504	38, 001	4, 074	1, 642
2002	263, 379	48, 915	5, 284	2, 220
2003	272, 901	59, 422	12, 217	2, 666
2004	276, 508	67, 780	7, 791	3, 212

<sup>1</sup> Converted from US \$, where \$1 = £0.5133.

### 2.3 The onion lifecycle

Onions have a natural biennial lifecycle, but are grown as annual crops (Brewster, 1994). Selection for more rapid growth probably took place during domestication. Onions are propagated by seeds, bulbs, sets or transplants. An onion bulb is a storage organ, consisting of foliage leaf bases and swollen, bladeless inner sheaths (Figure 2.1). Wide variation in bulb characteristics, such as weight, shape, colour and flavour, exists between onion cultivars. White flowers with green stripes are produced between spring and early summer (Currah, 1981). A wide range of adaptations to photoperiod and temperature exist, indicating intense selection of cultivars within a range of environments.



**Figure 2.1.** Annotated diagram of a cross-section of an onion bulb.

### 2.3.1 *Bulb initiation and formation*

Bulb formation is the process whereby the leaves in the neck region of the sheath rapidly elongate (Brewster, 1977a). The leaf sheath cells then expand causing lateral swelling of the leaf sheath. Scale leaves are formed instead of leaves, which have a much reduced leaf blade in comparison to the sheath. The scale leaves then swell to form the storage tissue. As the bulb matures, two or three foliage leaf initials are laid down at the bulb base. These initials elongate to produce leaf blades in the following season when the bulb sprouts.

Bulb formation in onion plants occurs when both a threshold day length and accumulated thermal time have been reached (Lancaster *et al.*, 1996). In the case of two closely related cultivars commonly grown in New Zealand (Pukekohe Longkeeper and Early Longkeeper), these thresholds are *ca.* 13.5 hours and 590 degree days respectively.

Threshold values will vary according to cultivar, however the thermal time threshold is thought to be linked with the requirement for a minimum number of leaves to be initiated prior to bulbing. If the threshold thermal time is reached before critical day length then bulbing is delayed and subsequent bulbs have larger diameters and more leaves. Conversely, the threshold day length can be reached before threshold thermal time has accumulated, and in this case bulbing is delayed until the thermal time requirements have been met. Light spectral quality interacts with day length (Brewster, 1990). Short-day onions form bulbs under short day lengths at low latitudes; however their behaviour is typical of other onions in that bulbing accelerates with increased day length (Wickramasinghe *et al.*, 2000). Far-red light, and to a lesser extent blue light, promote bulbing, whereas red light inhibits it (Kahane *et al.*, 1992). Once bulbing has been initiated, temperature (including night temperature) is positively correlated with the rate of bulb development in an inductive day length (Brewster, 1990; Wheeler *et al.*, 1998).

### 2.3.2 *Onion bulb dormancy*

Mature onion bulbs enter a dormant period, when sprouting and rooting are not induced despite favourable conditions. For most cultivars, true dormancy is relatively short, and ends early on in the storage period. Apparent dormancy is maintained through a period of sprout suppression when internal changes occur. These prepare the plant for subsequent growth and eventually the bulb proceeds towards flowering and seed production. Sprouting occurs when the leaf primordia that are produced in stored onion bulbs develop green leaves rather than scale leaves (Abdalla and Mann, 1963). The blades of these leaves elongate, and eventually protrude from the neck of the bulb (Figure 2.2). The growth rate of the sprout inside the bulb varies according to cultivar and pre- and postharvest factors such as maturity at harvest and storage regime. Sprout growth, and the suppression thereof, is a major factor in determining the storage life of onions.



**Figure 2.2.** Cross-sections of an onion cv. Renate bulb: A – with no sprout and B – with an external sprout.

Bulbs with roots sprout earlier in dry storage than those whose roots have been removed (Miedema, 1994b). Therefore, the root system may provide substances that promote sprout growth or elongation. Cultivar differences in time to sprouting in store are more pronounced in de-rooted bulbs than in rooted bulbs (Miedema, 1994b). Cytokinins produced in the roots stimulate cell division in the sprout meristem or increase the sink activity of the sprout. Wounding of the growth plate also promotes sprouting and may do so by facilitating gas exchange and promoting respiration.

#### 2.4 Quality attributes of marketable onions

The aim of onion bulb storage is to meet consumer demands for extended availability of onions whilst maintaining product quality. The principal biological factors leading to onion bulb deterioration during storage are respiration, resumption of growth and pathogen attack.

Class I onions must not show any signs of external sprouting (Commission Regulation 1508/2001/EEC). Early signs of external shoot growth are permitted in Class II onions provided that the number or weight does not exceed 10% per unit of

presentation. Bulbs with watery scale and bacterial or fungal rots are deemed unfit for marketing.

## **2.5 Strategies to delay sprouting**

Storage life of onions depends on many factors such as cultivar and pre- and postharvest treatment. Long storing cultivars are available and are characterised by high dry matter content. New varieties of less pungent low dry matter onions grown for the fresh market for raw consumption are becoming more popular, but generally do not store very well (Hurst *et al.*, 1985). Research to develop strategies to delay sprouting has been focused on crop husbandry, the characteristics of the storage environment and breeding programmes.

## **2.6 Pre-harvest factors that affect storage life**

Pre-harvest treatment and conditions in the field have an important role to play in affecting storage life. These include pre-harvest nutrition, temperature during the growing season, application of maleic hydrazide, crop maturity at harvest and the harvesting process.

### *2.6.1 Pre-harvest nutrition*

Reduced nitrogen fertilisation caused onions cv. Hyton to mature later, with a later harvest date and reduced yield (Sorensen and Grevsen, 2001). Nitrogen deficiency also slightly reduced shelf-life. Drought-stressed onions matured earlier with increased dry matter concentration but reduced yield (Sorensen and Grevsen, 2001). A 75% water deficit in the top 25 cm soil profile was reported to reduce postharvest sprouting; however in these experiments storage of bulbs from all treatments was ended on the same

date despite different harvest dates. This meant that, although samples were taken simultaneously, the time in storage was not the same. Therefore, reduced sprouting could have been due to the later harvest date and, consequently, shorter storage time.

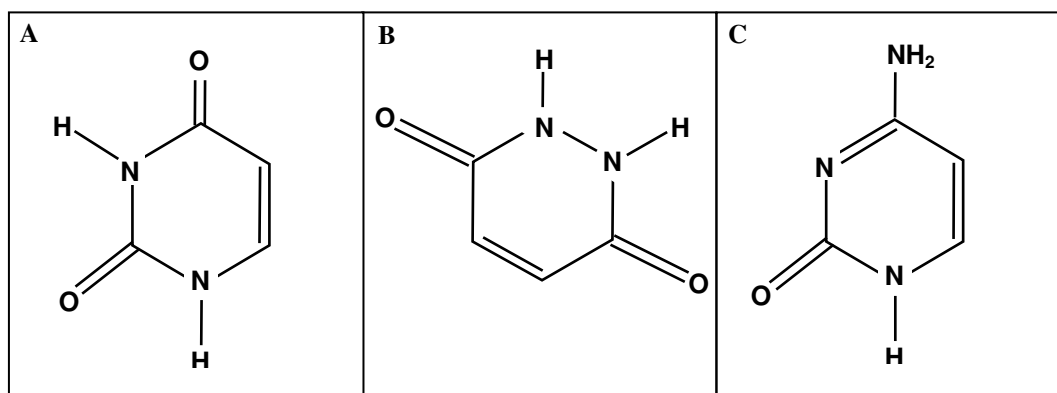
Sulphur nutrition impacts on dry weight and bulb quality. Onions grown in a hydroponic system with high sulphur nutrition were firmer than those grown with low sulphur nutrition, and had a higher fresh weight (Lancaster *et al.*, 2001). As greater bulb dry matter is correlated with improved storage attributes it is possible that sulphur fertilisation could improve storage life, although with the increasing demand for less pungent onions (see section 2.9.2) it is unlikely that this strategy would be adopted by growers.

#### 2.6.2 *Growing season temperature*

Storage potential varies between crops harvested in different years; even when identical cultural, drying and storage regimes are followed. This is presumably due to climatic variation between growing seasons (Rutherford and Whittle, 1982). Higher temperatures during the growing season reduce storage life (Rutherford and Whittle, 1982; Wheeler *et al.*, 1998; Sorensen and Grevsen, 2001). Wheeler *et al.* (1998) concluded that although the time until onset of sprouting (first visible sprout) was not affected by growing season temperature, the rate of increase in the number of sprouted bulbs increased with growing season temperature. However, a linear function was used to describe the relationship between the numbers of bulbs sprouted and the duration of storage, and onset of sprouting was calculated by extrapolation. A bulb was classed as sprouted after emergence of a visible sprout, therefore this method did not allow for differences that may exist between the sprout growth rate inside the bulb and the growth rate outside the bulb, and so the estimated time of onset of sprouting may not have been precise.

### 2.6.3 Maleic hydrazide

Maleic hydrazide (1,2-dihydro-3,6-pyridazinedione; MH) is a chemical isomer of uracil that is applied as a pre-harvest spray to inhibit subsequent sprouting of bulbs in store (Figure 2.3).



**Figure 2.3.** Chemical structure of: A – Uracil, B – Maleic hydrazide, C – Cytosine.

Responses to MH vary with cultivar (Sorensen and Grevson, 2001). Timing of application as well as dose is important when considering the residue level in the crop. A ban on the use of MH in Denmark is being considered because of the risk of it leaching into drinking water (Sorensen and Grevsen, 2001) and concerns over its mutagenic properties (Marcano *et al.*, 2004). The amount of MH used in onion production in Denmark was reduced by 75% in a three-year period from 1997 (Sorensen and Grevson, 2001).

Maleic hydrazide is incorporated into the RNA of cells where it is substituted for cytosine (Benkeblia, 2004). In normal root tip cells of *A. cepa* the ultrastructure of the nucleolus has mixed granular and fibrillar components. However in bulbs treated with MH, the granular components were centrally located and surrounded by the fibrillar components, an arrangement known as nucleolar segregation (Marcano *et al.*, 2004). This is a morphological manifestation of blocked transcription. In this way MH affects



the biosynthetic activity of the nucleolus. Maleic hydrazide caused a dose dependent reduction in the mitotic index (the mitotic index allows estimation of the frequency of cell division) compared to untreated bulbs whereby the mitotic index remains constant. The effect of MH concentration was 1.93-fold higher than the effect of the time of exposure. Maleic hydrazide was also capable of breaking chromosomes. However, MH had no effect on sugar and organic acid composition of onion bulbs cv. Sentinel (Salama *et al.*, 1990).

#### 2.6.4 *Crop maturity at harvest*

The developmental stage of the crop at harvest impacts on both yield and storage potential. The consensus in Europe and the USA is that the optimum harvest time for storage onions is at 80-90% tops down, sacrificing some yield for a greater number of intact skins (Gubb and MacTavish, 2002). If bulbs are harvested too soon the water content in foliage leaves and the neck is too high, which results in increased susceptibility to pathogen attack. Early harvested bulbs may not be dormant and would therefore be unsuitable for storage purposes. Maturity stage at harvest can influence initial bulb weight, respiration and incidence of sprouting, decay and cumulative weight loss. Rutherford and Whittle (1982) found that bulbs harvested early, dried and stored in the same manner as bulbs harvested later, had lower carbohydrate levels, which were reduced further during sprouting, which occurred earlier.

#### 2.6.5 *Harvesting process*

Physical damage to onion bulbs during harvesting must be minimised, especially for softer, less pungent onions, because wounding, particularly of the basal plate, causes accelerated sprout growth (Miedema, 1994b) and increases storage losses due to rotting (Herold *et al.*, 1998). Undercutting is usually performed prior to mechanised lifting. The

aerial parts and roots are removed before onions are stored in bulk, which aids airflow among the bulbs. In temperate countries, the crop is then moved directly into a heated, forced-air ventilation store for immediate curing (Gubb and MacTavish, 2002).

## **2.7 Postharvest factors that affect storage life**

Postharvest treatments and storage conditions have a significant impact on storage life. These include curing and drying, irradiation, nitrous oxide treatment, and aspects of the storage environment including temperature, gaseous composition of the atmosphere, and humidity.

### *2.7.1 Curing and drying*

Onions for storage are cured and dried after harvest (O'Connor, 1979; Gubb and MacTavish, 2002). It is important that the skin integrity, firmness, colour and flavour are maintained during curing. The purpose of curing is to dry the thin outer layers of the bulb to form one or more complete outer skins. These outer skins act as a barrier against water loss and infection from fungal pathogens such as *Botrytis allii* (neck rot) (Maude *et al.*, 1984), *Aspergillus niger* (black mould) and *Fusarium oxysporum* (basal rot), and bacterial pathogens such as *Erwinia carotovora* (soft rot) (Fenwick and Hanley, 1985). Curing is complete when the necks have dried out and are tightly closed, and the skins have an attractive colour (O'Connor, 1979). The time this takes depends on the temperature and relative humidity of the forced ventilating air and the maturation stage of the bulbs. Standard practice is to dry the bulbs in bulk stores using air at 30°C. After three to five days the temperature is lowered to 24°C and relative humidity (RH) to 70-75% to complete the curing process. The crop is then slowly cooled to the desired storage temperature.

### 2.7.2 Irradiation

Irradiation is not popular for food use in many countries, but is an effective method of long-term sprout control and reducing chemical residues (Gubb and MacTavish, 2002). Use of irradiation depends on consumer acceptance, the practicality of treating large volumes, and economics (Kleinkopf *et al.*, 2003).

Ionising radiation ( $^{60}\text{Co}$  source at a dose of 150 Gy) decreased the respiration rate of onions cv. Rouge Amposta, probably through degeneration of meristematic cells and the death of the sprout caused by the  $\gamma$ -radiation, which slows down the complete respiratory pathway including glycolysis (Benkeblia *et al.*, 2002). After 24 weeks storage at 20°C, 5% of irradiated bulbs had sprouted, compared with 75% of non-irradiated bulbs. However, refrigerated storage at 4°C for 24 weeks was as effective as ionising radiation in inhibiting sprout growth.

In general ascorbic acid and carbohydrate levels were higher in onion cv. Valencia sintética bulbs irradiated 30 days postharvest at 20°C with a  $^{60}\text{Co}$  source at a dose of 50 Gy, and stored in warehouse conditions (6 to 32°C and 40-50% RH) for *ca.* 43 weeks (Crocì *et al.*, 1995). This may have been due to increased ease in extracting these substances because of the irradiation or a delay in their metabolism.

### 2.7.3 Nitrous oxide

Nitrous oxide ( $\text{N}_2\text{O}$ ) is similar to carbon dioxide in terms of relative stability and high solubility in water, and is permitted for food use. Benkeblia and Varoquaux (2003) investigated the possibility of using nitrous oxide to extend the storage period of onions cv. Rouge Amposta. Nitrous oxide has been reported to cause reversible inhibition of oxygen consumption by mitochondria and respiration, to have anti-ethylene effects and to inhibit some bacteria and fungi. Respiration rates in bulbs treated for five days with 50 kPa, 80 kPa and 100 kPa  $\text{N}_2\text{O}$  and subsequently stored at  $18\pm 0.1^\circ\text{C}$  and  $65\pm 1\%$  RH, were half that of the untreated bulbs, whereas the respiration rate of bulbs treated for 10 and 15

days was approximately 0.8-fold less than that of the untreated bulbs (Benkeblia and Varoquaux, 2003). Five days treatment decreased rots, whereas ten and fifteen days of treatment increased rots. Total soluble sugars were less in untreated bulbs. There was no effect on visible sprouting, but internal sprouting was not assessed.

#### 2.7.4 *Storage environment*

Temperature, humidity and gaseous atmosphere can be manipulated to increase the storage life of onion bulbs. The most important of these is temperature. The storage regime chosen depends on the cultivar, target storage period and cost.

##### 2.7.4.1 *Storage temperature*

Temperature has a profound effect on the dormancy period and storage life of onion bulbs. In general, sprouting is inhibited both by low and by high temperatures, and encouraged at intermediate temperatures (Abdalla and Mann, 1963; Brewster, 1977b; Miedema, 1994a; Ernst *et al.*, 1999). Different cultivars respond differentially to temperature (Gubb and MacTavish, 2002). The optimum temperature range for sprouting in dry storage is 10-20°C for most cultivars, with some cultivars displaying a sharp optimum while others have a broader range. Moisture loss is greater at temperature ranges <10°C and >27°C.

In developed temperate countries, such as the UK, onions are kept in large, specialised stores. Ventilation is forced, and temperature is usually maintained around 5°C, but can be as low as -1°C. In warm climates, such as the tropics, high temperature storage is a practical option, but involves a compromise between sprouting losses and rotting losses (Ko *et al.*, 2002). High temperature storage conditions are generally 25-30°C and 60–75% RH. Ventilation of storage bins to reduce fluctuations in temperature and humidity reduced the rate of external sprouting, bacterial infection and dehydration over 31 weeks of high temperature storage in red onion cv. Baftain bulbs (Brice *et al.*,

1995). The high temperature inhibition of sprouting may be related to the dormancy observed in hot seasons in some wild *Alliums* (Gubb and MacTavish, 2002). Short-term (three weeks) high temperature postharvest treatments of 30 and 35°C significantly reduced the number of days to sprouting in dry storage at 15°C, when compared to those exposed to postharvest temperature treatments of 15 and 25°C, which in turn were not significantly different from one another (Miedema, 1994a). This indicates that exposure of onion bulbs to high temperatures during curing and drying may reduce the level of dormancy and therefore reduce storage time.

Short-term (two or three weeks) chilling treatments at 0 or 9°C decreased the time to sprouting in onion cv. Rouge Amposta bulbs subsequently stored at 18°C, with the 9°C treatment for three weeks having the greatest effect – 100% of bulbs in this treatment group had sprouted after 4-5 weeks, but after 8 weeks only 20% of non-chilled bulbs had sprouted (Benkeblia and Selselet-Attou, 1999a). The chilled bulbs also generally had a lower concentration of soluble sugars. Therefore, it is important that the chilling treatment is maintained long-term in order to extend storage life. Short-term chilling treatment may mimic the conditions that a dormant onion would experience over winter, with the return to a higher temperature being equivalent to the onset of spring and a trigger for release from dormancy.

#### 2.7.4.2 *Controlled atmosphere storage*

Controlled atmosphere (CA) storage involves manipulating the oxygen and carbon dioxide concentrations in the storage environment in addition to the temperature (Gubb and MacTavish, 2002). Low oxygen storage inhibits sprouting, decreases the incidence of neck rot and reduces weight loss. However, very low oxygen concentrations (0.7%) can cause high rates of sprouting after removal from storage, as well as off-odours and tissue breakdown. Also, high carbon dioxide concentrations (>10%) for short-term storage can cause accelerated softening, rooting and a putrid odour. A storage

atmosphere consisting of 5% CO<sub>2</sub> and 3% O<sub>2</sub> has been found to yield a good percentage of marketable bulbs and maintain quality (Adamicki and Kepka, 1974; Smittle, 1988).

#### 2.7.4.3 Humidity

The relative humidity of the storage environment is a compromise between maintaining a level below that at which pathogens are encouraged and above that at which water is rapidly lost from the bulbs (Hole *et al.*, 2000). The outer skins that protect against water loss tend to crack and fall off at <55% RH, and pathogen attack is encouraged at >80% RH, therefore 55-80% RH is desirable in the storage environment. When the water content of the skin is in equilibrium with the water vapour pressure of the surrounding atmosphere, water will be adsorbed or desorbed depending on the relative pressure. Changes in humidity, therefore, have an impact on the properties of onion skins. This is significant as the ability of onion bulbs to withstand physical abuse during postharvest and post storage handling depends on the mechanical properties of the skins. Hole *et al.* (2000) found that humidification increased the resistance of skins to breaking. Compliant skins are better able to withstand the rigours of commercial handling. Manipulation of humidity to condition skins reversibly prior to post storage handling may have a positive impact on bulb quality.

## 2.8 Alternative strategies to delay sprouting

Increasing pressure from both consumers and retailers to provide food with little or no chemical residues means that the continuing use of MH to extend onion storage life is far from certain, and alternative strategies must be explored. In order to identify potential targets for manipulation to suppress sprouting in store, it is useful to examine what changes occur in stored onion bulbs.

## 2.9 Changes occurring in onion bulbs during storage and sprouting

Many characteristics change during 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 remobilisation 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. Peaks and troughs in certain substances are known to coincide with sprouting but there is currently no biochemical assay that anticipates sprouting.

### 2.9.1 Mitotic activity

Control of cell division in the shoot meristem does not appear to be a key regulatory process in bulb dormancy and sprouting (Carter *et al.*, 1999). Expression of *histone 2A*, a cell cycle regulated gene whose expression pattern is linked with cell division activity, is barely detectable in the leaf tissue at the end of the growing period, indicating minimal mitotic activity, and the concentration in the bulb was about 2.5-fold greater than that in the root (Carter *et al.*, 1999). Expression levels in all parts of the bulb (outer, middle and inner) decreased from 100 days before harvest until harvest time. During storage, expression levels remained constantly low in the outer and mid bulb but levels in the inner bulb peaked at 140–160 days postharvest, coinciding with the appearance of external sprouts, and decreased thereafter. A peak in expression of *histone 2A* appeared at the same time point (March to April) in cultivars with varying storage potential, and did not correspond with the developmental stage of the bulbs. Similarly, after studying mitotic divisions in the shoot apex of onion cv. Excel bulbs, Abdalla and Mann (1963) concluded that as sprouting depends on the elongation of leaves initiated before harvest; cell division in the shoot apex is unlikely to be related to sprout growth. Indeed, onion bulbs are morphologically active throughout storage (Dahlhelm and

Matjeko, 1990). The genetic basis of control of sprout leaf elongation is not known, but will play a large part in the determination of storage capacity.

### 2.9.2 *Flavour precursors and pungency*

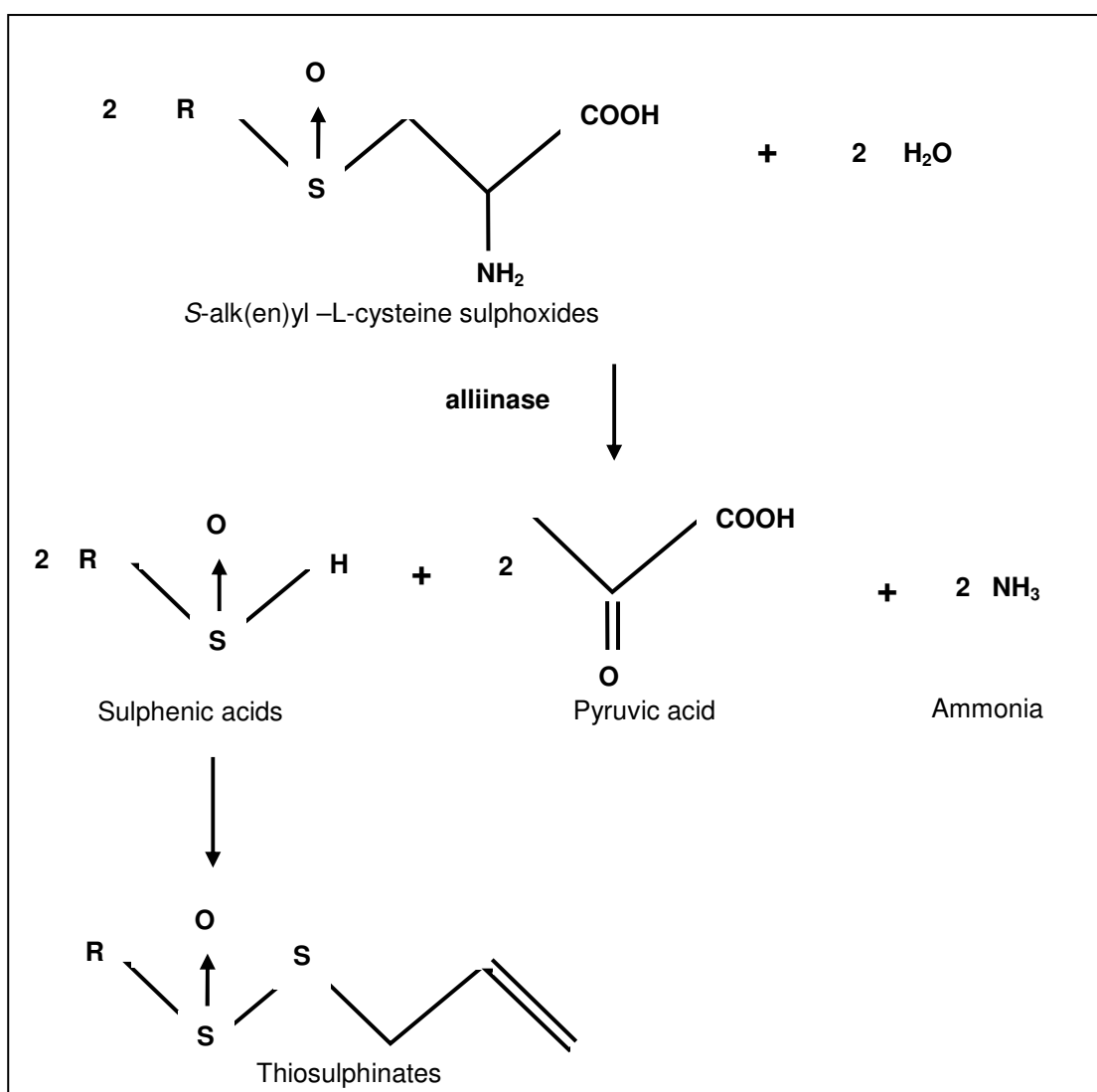
Onions, and other *Alliums*, are eaten for their unique taste and the medicinal properties of their flavour compounds (Randle, 1997; Griffiths *et al.*, 2002). The flavour compounds are secondary metabolites whose biosynthesis involves the metabolism of cysteine and glutathione, which are essential pathways for uptake of sulphur and detoxification (Jones *et al.*, 2004). Sulphate is taken up by the roots, reduced to sulphite and assimilated into cysteine. The tripeptide glutathione is formed and converted into *S*-2-carboxypropyl glutathione which is metabolised through many  $\gamma$ -glutamyl peptides, terminating in *S*-alk(en)yl-L-cysteine sulphoxide (ACSO) synthesis (Block, 1992; Kopsell and Randle, 1997). The ACSOs make up 1-5% of the dry mass of an onion bulb (Jones *et al.*, 2004), representing major biosynthetic activity. The role of flavour compounds is something of a mystery. Putative roles include storage of sulphur (Lancaster *et al.*, 2001) and carbon, and defence (Jones *et al.*, 2004). When onions are grown at low sulphur nutrition, sulphur is preferentially partitioned into ACSOs in the cell contents (Randle *et al.*, 1995; Lancaster *et al.*, 2001).

It is the ACSOs that are responsible for the characteristic flavour and odour of onions. Intact onion cells have no flavour, but when cells are disrupted the vacuolar enzyme alliinase (*S*-alk(en)yl-L-cysteine sulphoxide lyase) hydrolyses the flavour precursors (ACSOs) present in the cytoplasm. The products of this reaction are pyruvate, ammonia and unstable alk(en)yl sulphenic acids (Uddin and MacTavish, 2003), which spontaneously condense in pairs to form thiosulphinates that contribute to perceived flavour (Briggs and Goldman, 2002) (Figure 2.4). The three major ACSOs present in onion are methyl (MCSO), propyl (PCSO) and 1-propenyl (PRENCOSO) cysteine sulphoxide. PRENCOSO gives rise to the lachrymatory factor, thiopropanal *S*-oxide (Lancaster *et al.*, 1998; Kopsell *et al.*, 1999). The production of the lachrymatory factor



was thought to be spontaneous, but further investigation has revealed that it is specifically synthesised by an enzyme known as lachrymatory factor synthase (Imai *et al.*, 2002).

The composition and concentration of ACSOs determines the nature and intensity of flavour and odour. Total ACSO content is also positively correlated with enzymatically produced pyruvate (Kopsell *et al.*, 1999). Variations in flavour between cultivars, and changes that may occur in flavour during storage, are due to the differences and differential changes in the ACSOs present in bulbs and their alliinase activity.



**Figure 2.4.** Simplified pathway of flavour production in onion bulb tissue.

A highly significant correlation exists between threshold olfactory perception (the minimum concentration of onion juice in water that could be detected by 70% of the judges) and enzymatically produced pyruvic acid (Schwimmer and Weston, 1961; Schwimmer and Guadagni, 1962). This suggested that the same enzyme system that produces volatile odour compounds when the cells of onion are disrupted, also gives rise to pyruvic acid, and that pyruvic acid concentration was a good indicator of pungency. Selection for less enzymatically produced pyruvate resulted in a milder onion (Havey and Randle, 1996; Havey, 1999). Relative pungency is dependent on both genetic and environmental factors (Havey and Randle, 1996; Havey, 1999). It is possible for pungency to increase, decrease or stay the same during storage (Uddin and MacTavish, 2003).

### 2.9.3 Carbohydrates

Water-soluble carbohydrates in onion bulbs include glucose, fructose and sucrose, and a series of oligosaccharides called fructans (Darbyshire and Henry, 1978), and constitute 60-80% of the dry weight (Rutherford and Whittle, 1982). During storage at 15-16°C, fructose levels increased with time (Salama *et al.*, 1990) and fructan levels decreased (Suzuki and Cutliffe, 1989; Ernst *et al.*, 1998). The decrease in fructan concentration was shown to begin two weeks prior to harvest in onion cv. Rijnsberger Hysam, possibly due to the use of storage carbohydrates in respiration to compensate for reduced photosynthetic ability due to the loss of green leaves (Pak *et al.*, 1995). Fructan content in onion bulbs tended to decrease during refrigerated, ambient atmosphere (Suzuki and Cutliffe, 1989; Pak *et al.*, 1995; Ernst *et al.*, 1998; Benkeblia *et al.*, 2000), and low oxygen storage (Ernst *et al.*, 2003). Fructose concentration was higher in the outer scales of the bulb than the inner scales (Darbyshire and Henry, 1978; Salama *et al.*, 1990), similarly, Salama *et al.* (1990) found that glucose concentration was higher in the outer scales than in the inner scales; however Darbyshire and Henry (1978) and Pak *et al.* (1995) found no difference. A maximum soluble sugar concentration occurred between

five and eight weeks after harvest (Salama *et al.*, 1990; Benkeblia *et al.*, 2002). Postharvest, sucrose synthase activity decreased very slightly in the shoot, and increased in the bulb base, while activity in the inner and outer scales was consistently low (Pak *et al.*, 1995).

It has been postulated 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 6-10°C for four months. Higher fructose content at harvest was correlated with extended storage life in onion cv. Robusta bulbs stored at 4°C for three months (Rutherford and Whittle, 1982). This is in agreement with a lower proportion of bulbs sprouting per day in bulbs with higher carbohydrate concentrations at harvest, as observed by Wheeler *et al.*, (1998).

The pattern of changes in total soluble sugar content of onion cv. Rouge Amposta bulbs is similar at 4, 10 and 20°C, with a maximum concentration occurring at approximately seven weeks storage (Benkeblia *et al.*, 2002). The maximum soluble sugar concentration was less in the bulbs stored at 4°C, as were the concentrations of tetra, penta and hepta saccharides, probably due to decreased enzymatic hydrolysis of fructan polymers by depolymerases. The similarity in the pattern of change in total soluble sugars at different temperatures suggests 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 15°C than in those stored at 30°C, suggesting that hydrolysis of fructans increased at low temperatures (Salama *et al.*, 1990). A net increase in total sugars was observed at 0°C, and a net decrease observed at 15 and 30°C.

#### 2.9.4 Fresh weight and water loss

Water accounts for 80-93% of the fresh weight of freshly harvested onions. The actual amount depends on cultivar and growing conditions. Water loss during curing and drying is rapid and is around 5% of fresh weight (Gubb and MacTavish, 2002). Water loss continues throughout storage because of evaporation and low-level respiration.

#### 2.9.5 Respiration

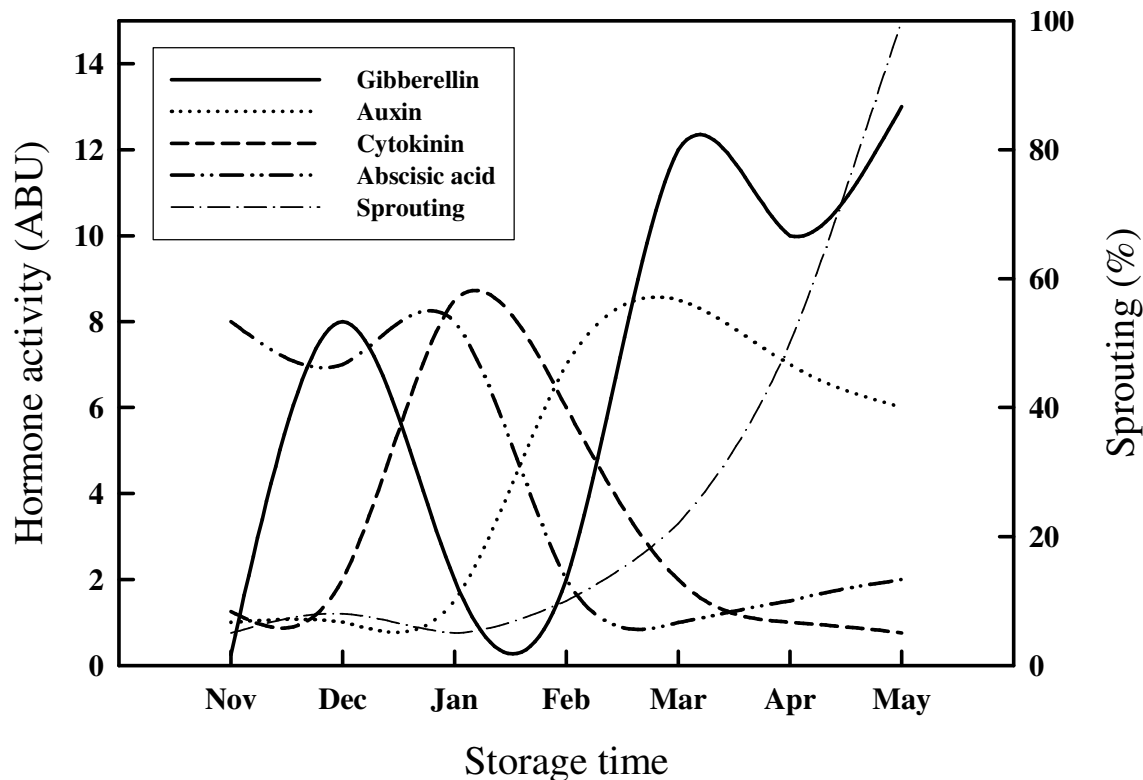
Staining with the redox reagent 2,3,5-triphenyl tetrazolium chloride, which is reduced to an insoluble red formazan derivative in tissues of high metabolic activity, revealed a changing pattern of respiration activity during storage (Carter *et al.*, 1999). At harvest (mid-October) staining was pale and confined to the meristematic region, indicative of a low metabolic rate and a high level of dormancy. In January, an increase in metabolic rate was indicated by increased staining of the root tips. In March and April rapid metabolic activity occurred in the meristem regions at the base of each growing shoot and in growing sprout leaves. After sprout emergence, staining intensity decreased, representing a return to a lower metabolic rate.

The  $Q_{10}$  of a respiration rate represents the increase in the respiration rate produced by raising the temperature by 10°C. Benkeblia *et al.* (2000) observed that the  $Q_{10}$  of onion cv. Rouge Amposta bulbs  $O_2$  and  $CO_2$  respiration rates were 1.67 and 1.84 respectively. The respiration rate of oxygen increased throughout storage, doubling within 15 weeks at 20°C and 20 weeks at 10°C. At 4°C the increase was only slight. Respiration rate is dependent on the physiological state of the bulb (Ogata, 1961; Benkeblia *et al.*, 2000). The respiration rate of a sprouted bulb is greater than that of a non-sprouted bulb sampled simultaneously.

### 2.9.6 *Plant growth regulators*

During over winter storage in the UK a gradual change in the relative composition of plant growth regulators occurs as the levels of growth inhibitors drop and the levels of growth promoters rise. Hormone activity in onions cvs. Rijnsberger (long-storing) and Lancastrian (short-storing) bulbs was measured by Thomas (1969) and Thomas and Isenberg (1972). The following pattern existed (Figure 2.5); 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 (ABA) has been identified as an inhibitory substance in onion bulbs (Tsukamoto *et al.*, 1969). The concentrations of inhibitors in bulbs with internal signs of sprouting were low when compared with the levels in non-sprouting or fully sprouted bulbs. More inhibitor was present in the long-storing cultivar at the beginning of the storage period than in the short-storing cultivar (Thomas, 1969).



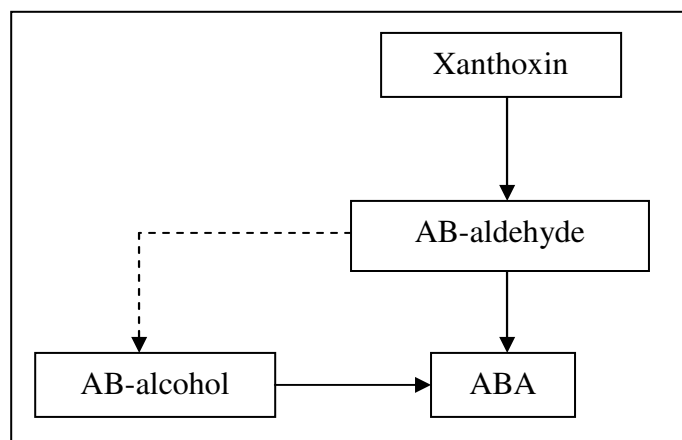
**Figure 2.5.** Percentage sprouting and hormone activity of onions cv. Rijnsberger stored at 5-8°C (Thomas and Isenberg, 1972).

Therefore before an external sprout is visible, important internal changes occur. The roles and mode of action of plant growth regulators are unknown, but it is probably a complex phenomenon involving the combined action of several endogenous hormones (Gubb and MacTavish, 2002).

Continuous exposure of onion bulbs to ethylene in store has recently been put forward as a method of sprout suppression (Johnson, 2006). However, there are conflicting reports on the effect of ethylene during storage. Abdel-Rahman and Isenberg (1974) observed that onion cv. Elba Globe bulbs produced ethylene at much greater concentrations at the end of dormancy than at the beginning. In contrast, Benkeblia and Selselet-Attou (1999b) found little variation in the ethylene production by onions cv. Rouge Amposta. Ethephon (2-chloroethylphosphonic acid, CEPA) degrades to form ethylene in an alkaline solution (Yang, 1969). Application of ethephon to plants has been effective in causing responses characteristic of ethylene treatment (Benkeblia and

Selselet-Attou, 1999b). A combination of cold storage (9°C for three weeks) and the injection of 1 ml of a 100 mg l<sup>-1</sup> solution of ethephon into the centre of the bulb caused onion cv. Rouge Amposta bulbs stored at 18°C to sprout earlier (50% sprouting after 2 months, 100% after 4 months) than those treated with ethephon alone and untreated controls (50% sprouting after 3 months, 100% after 6 months). It is likely that it was the effect of the chilling treatment that reduced the storage life of the bulbs; however, as no bulbs were subjected to chilling alone, this cannot be proven. Injection of bulbs with ethephon alone had no effect on sprouting, but when applied in combination with exogenous ABA it reduced the effect of ABA on the dormant period (Abdel-Rahman and Isenberg, 1974).

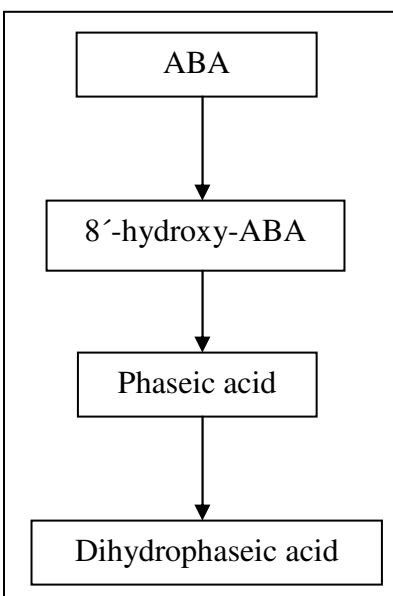
Absciscic acid is a naturally occurring phytohormone. The ABA biosynthesis pathway (Figure 2.6) begins in chloroplasts and other plastids with the cleavage of a C<sub>40</sub> carotenoid precursor to form xanthoxin. In the cytoplasm, xanthoxin is converted to ABA via abscisic alcohol (Cutler and Krochko, 1999; Taylor *et al.*, 2005). ABA 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.



**Figure 2.6.** The synthesis of abscisic acid (ABA) from xanthoxin in higher plants. Solid arrows indicate the major pathway, dashed arrows indicate a minor pathway, AB=abscisic (Cutler and Krochko, 1999).

The predominant pathway for ABA metabolism is hydroxylation at the 8' position, catalysed by the enzyme abscisic acid 8'-hydroxylase, to give 8'-hydroxy-ABA which is unstable and readily converts to phaseic acid (PA). In turn, PA can be reduced to dihydrophaseic acid (Cutler and Krochko, 1999; Nambara and Marion-Poll, 2005) (Figure 2.7). Phaseic acid has little hormonal activity in most assays and can be isolated, whereas 8'-hydroxy-ABA still has some hormonal action but can not be easily isolated due to its instability.





**Figure 2.7.** Metabolism of abscisic acid (ABA).

Endogenous ABA is found in all onion tissues *viz.* leaf, growing tip, bulb and leaf sheath (Matsubara and Kimura, 1991), and accumulates in all tissues during the growing period. The actual concentration of ABA varies according to plant age and tissue type.

The endogenous ABA concentration is affected by tissue water status and is high in water-stressed plants. During bulb formation storage molecules accumulate, which facilitates changes in tissue water status. *Allium wakegi* (a cross between Japanese bunching onion and shallot) plants do not form bulbs under short day conditions, and the endogenous ABA concentration remained low when plants were subjected to short days - a maximum concentration of  $5 \text{ ng g}^{-1} \text{ FW}$  compared to a maximum concentration of  $20 \text{ ng g}^{-1} \text{ FW}$  in plants subjected to long day conditions was observed (Yamazaki *et al.*, 1999a). The ABA concentration increased during bulb development, reaching a maximum two weeks after harvest: a rise from  $1 \text{ ng g}^{-1} \text{ FW}$  to  $13 \text{ ng g}^{-1} \text{ FW}$  over two months. This change was associated with changes in water status during bulb formation (Yamazaki *et al.*, 2002). However, *in vitro* studies do not support the theory that ABA is the primary cause of bulbing; addition of exogenous ABA ( $0.1\text{-}5 \text{ mg l}^{-1}$ ) to *in vitro* cultured onion plants did not induce bulbing, although the number of leaves decreased (Matsubara and Kimura, 1991). In addition, fluridone (an inhibitor of ABA biosynthesis) treatment of A.

*wakegi* plants reduced endogenous ABA concentration, but did not inhibit bulb scale formation or affect leaf sheaf ratio (ratio of leaf sheath length to the length the oldest unexpanded leaf) relative to untreated control plants (Yamazaki *et al.*, 1999a).

Abscisic acid has been associated with dormancy in onions and other plants with storage organs such as potato (Suttle and Hultstrand, 1994; Destefano-Beltrán *et al.*, 2006a; Destefano-Beltrán *et al.*, 2006b). Current understanding is that ABA is synthesised in the leaves and translocated to the bulb throughout growth. Treatments to prematurely kill off the aerial parts of onion plants resulted in increased sprouting of bulbs in store (Thomas and Isenberg, 1972), perhaps due to a reduction in accumulation of ABA in the bulb because ABA cannot be translocated from dead leaves. Bulb ABA concentration reached a maximum shortly after harvest (Yamazaki *et al.*, 2002). Onions are harvested when the aerial parts of the plant have fallen and would be expected to be less metabolically active. This may suggest that ABA can be synthesised in other parts of the onion plant when the aerial parts have been removed or ceased to be metabolically active.

## 2.10 Conclusions

The pre- and postharvest factors affecting onion bulb storage life and the biochemical and physical changes that occur in stored onion bulbs have been considered. Most research concerned with extending onion storage life has concentrated on breeding programmes, crop husbandry and optimisation of the storage environment in terms of temperature, humidity and the concentrations of oxygen and carbon dioxide. If an alternative method to the use of the synthetic sprout suppressant maleic hydrazide is to be identified, then further study of the physiology behind onion bulb dormancy and storage life is required. The information gained from these investigations has the potential to identify novel targets for manipulation to improve onion storage potential.

### 3.0 CHAPTER THREE

#### **Temporal changes in abscisic acid concentration and quality characteristics during controlled atmosphere storage of onion bulbs of three cultivars with contrasting storage potential**

##### **3.1 Abstract**

Onion bulbs (*Allium cepa* L.) of cultivars with long, medium and short storage lives, viz. Renate, Ailsa Craig and SS1, respectively, were stored in controlled atmosphere (CA) conditions (3% CO<sub>2</sub>; 5% O<sub>2</sub>; 2°C). Bulb abscisic acid (ABA) concentration, pyruvate, fructans, total soluble solids (TSS) and firmness were measured throughout storage.

In all cultivars, bulb ABA concentration declined exponentially during storage. The greatest decrease in ABA concentration occurred during the first 80 days of storage. The pattern of decline was similar for the long, medium and short-storing onion bulbs, although onion cv. SS1 bulbs had the lowest initial ABA concentration. Onion bulb ABA concentration at harvest (measured on a fresh weight basis) may prove to be an indicator of storage life. The ABA concentration at harvest (DW) may be indicative of a greater difference in sprouting during storage between cv. SS1 and the other cultivars than between cvs. Renate and Ailsa Craig.

It is hypothesised that the storage potential of bulbs of different onion cultivars is inversely related to the time at which they reach a minimal ABA content. Thus, the storage life of short-storing cultivars (e.g. cv. SS1) might be prolonged by reducing the rate of ABA degradation, and this could help extend the period for supplying these onions from temperate regions. Onion bulbs of cvs. Renate, Ailsa Craig and SS1 were characterised by high, intermediate and low concentrations of pyruvate, fructan and total soluble solids, respectively.

### 3.2 Introduction

The aim of onion bulb storage is to meet consumer demand for extended availability of onions whilst maintaining product quality. The principal biological factors leading to onion bulb deterioration are respiration, resumption of growth and pathogen attack. In onion bulbs a dormant period, when sprouting and rooting can not be induced, is followed by a period of internal changes that prepare the bulb for breaking of dormancy and subsequent growth. Out of storage, the bulb then proceeds towards flowering and seed production (Komochi, 1990).

Many biochemical characteristics change during storage. These include changes in water content, the concentrations of flavour-related compounds (Uddin and MacTavish, 2003), organic acids (Salama *et al.*, 1990), carbohydrates (Rutherford and Whittle, 1982; Jaime *et al.*, 2001; Benkeblia *et al.*, 2005a), plant growth regulators (Thomas and Isenberg, 1972) and phenolics (Benkeblia, 2000). Biochemical changes during storage are likely to be linked with respiration. All nutrients required for growth of the sprout must come from within the bulb; therefore, changes in certain key characteristics might be used to predict the onset of sprouting.

Onions are eaten for their unique taste and the supposed health giving properties of their sulphur containing flavour compounds, S-alk(en)yl-L-cysteine sulphoxides (ACSOs). Total ACSO content is positively correlated with enzymatically produced pyruvate (Kopsell *et al.*, 1999), which in turn is positively correlated with pungency (Schwimmer and Weston, 1961; Crowther *et al.*, 2005).

Abscisic acid has been associated with dormancy in onions (Matsubara and Kimura, 1991). The absolute concentration of ABA varies according to plant age and tissue type, and is also influenced by environmental challenges such as drought stress prior to the falling of green leaves at bulb maturity. The postharvest concentration of ABA in the bulb basal leaf sheath has been positively correlated with the number of days to sprouting in *A. wakegi* Araki L., a cross between Japanese bunching onion and shallot (Yamazaki *et al.*, 2002). Ranges of ABA concentrations in onion and related species have been reported by other authors (Table 3.1), however the changes in ABA concentration in onion bulbs of cultivars with different storage potentials have not been investigated.

The aim of this experiment was to detail the changes in onion bulb ABA concentration, as well as characteristics associated with quality (fructan, pyruvate, total soluble solids (TSS) and firmness), in three onion cultivars with different storage potentials. The relationship between these changes and the onset of sprouting was identified with the aim of identifying a biochemical predictor of sprouting incidence or a potential target for biochemical or genetic manipulation to delay sprouting.

**Table 3.1.** The maximum and minimum abscisic acid (ABA) concentrations reported previously in bulbs of onions and related species.

Tissue	Range of ABA concentrations (ng g <sup>-1</sup> FW)	Days in store	Storage conditions	Extraction method	Quantification method	Reference
<i>A. cepa</i> cv. Awaji Chūkodaka lyophilised central bulb tissue	9 - 1	0 - 30	25°C	Zones on thin-layer paper chromatogram eluted with acetone : methanol (9:1, v/v)	Gas chromatography with electron capture detector	Matsubara and Kimura (1991)
<i>A. wakegi</i> cv. Araki whole bulb tissue	13 - 6	14 - 42	In a room at ambient temperature	Extraction with 80% (v/v) acetone, followed by clean up and semi-preparative HPLC	As above	Yamazaki <i>et al.</i> (1995)
<i>A. wakegi</i> cv. Araki whole bulb tissue	33 - 10	14 - 42	Hung in plastic nets under an outdoor shelter in Japan	As above	As above	Yamazaki <i>et al.</i> (1999b) Yamazaki <i>et al.</i> (2002)

### 3.3 Materials and methods

#### 3.3.1 *Plant material and storage regime*

Short, intermediate and long-storing onion (*Allium cepa* L.) cultivars; SS1, Ailsa Craig and Renate, respectively, were spring-drilled on 18 March 2003 in four rows per bed using a tape seeder at a rate of 35 seeds m<sup>-1</sup> and grown in a sandy loam soil field at FB Parrish & Son (Beds., UK) using standard agronomic practices. Two treatments of additional sulphur and/or calcium at rates of 100 kg ha<sup>-1</sup> of sulphur and 300 kg ha<sup>-1</sup> of calcium were applied in four combinations including a negative control. Sulphur, in the form of agricultural gypsum, was applied uniformly over the plot area at the time of drilling. Calcium was applied evenly by hand in the form of 77% calcium chloride (CaCl<sub>2</sub>) flakes (Kemira, Cheshire). Onion bulbs were harvested (cv. SS1 on 19 August 2003; cvs. Renate and Ailsa Craig on 2 September 2003) into standard 25 kg plastic nets and dried in bin driers with ambient air for five weeks (cv. SS1) and three weeks (cvs. Ailsa Craig and Renate) as per standard practice in the UK. The dry aerial parts and roots were removed, and any diseased or damaged bulbs discarded prior to storage. Bulbs were held under industry standard controlled atmosphere (CA) conditions (3 % CO<sub>2</sub> and 5 % O<sub>2</sub>; Smittle, 1988) using an Oxystat 2 CA system, attached to an Oxystat 2002 Controller, and Type 770 fruit store analyser (David Bishop Instruments, Sussex, UK). This system was self-calibrating every 24 hr against 5% CO<sub>2</sub> in N<sub>2</sub> (British Oxygen Co., Surrey, UK). Bulbs were stored at 2 ± 1°C inside two rigid polypropylene fumigation chambers (88 x 59 x 59 cm). Relative humidity was not measured.

#### 3.3.2 *Experimental design*

The experiment was conducted as a completely randomised design with the assumption that the storage containers were identical and the samples were taken randomly. Bulbs were divided equally between the two storage containers. Bulbs

were removed from storage at regular intervals. Samples of cv. Renate were taken after 0, 40, 111, 166, 188, 209 and 230 days, cv. Ailsa Craig after 0, 26, 53, 81, 111 and 129 days, and cv. SS1 after 0, 14, 26, 40, 53, 66 and 81 days. At each sampling date (except time 0) for each cultivar, three bulbs from each treatment (+Ca-S, +Ca+S, -Ca+S and -Ca-S) were sampled from each storage chamber. At sampling time 0, four sets of six bulbs were sampled. Samples from each set of three bulbs from each sampling time and treatment were combined and lyophilised prior to ABA, fructan and mineral analysis (except at time 0 where sets of 6 bulbs were combined); therefore, values represent the mean of the replicates for each sampling date and cultivar. Mineral analysis was carried out on samples from cv. Renate: days 0, 40 and 230, cv. Ailsa Craig: days 0, 81 and 129, and cv. SS1: days 0, 40 and 81. Dry weight measurements were made on lyophilised samples. The growth of a green sprout within the bulb indicated the end of the storage period for marketable onion bulbs. Incidence of sprouting was recorded and expressed as the ratio of the height of the first appearing green leaves inside the bulb as a percentage of the bulb height.

### 3.3.3 *Sample preparation*

Onion bulbs were sliced vertically in half from the neck to the base. A *ca.* 5 mm thick equatorial slice was cut from the middle of each half. Juice was expressed from the equatorial slice using a hand-operated press (Randle and Bussard, 1993) and allowed to stand for 15 min. A sample of juice was collected in a 1.5 ml Eppendorf tube and frozen at -20°C for pyruvate and TSS measurements. In addition, a section was cut from a basal quarter of the bulb (Thomas and Isenberg, 1972) and was snap-frozen in liquid nitrogen and kept at -40°C until the sample was lyophilised (Edwards Super Modulo, Sussex, UK) for mineral analysis, fructan and ABA assays.

### 3.3.4 *Quantification of total fructans*

Total fructan concentration in ground lyophilised bulb tissue was measured using a fructan assay kit (Megazyme, Co. Wicklow, Republic of Ireland) according to



the manufacturer's instructions (AOAC method 999.03, AACC method 32.32, O'Donoghue *et al.*, 2004). The assay uses highly purified specific enzymes to hydrolyse sucrose, starch and fructans. The sample is first incubated with sucrase to hydrolyse sucrose to fructose and glucose. These reducing sugars are then reduced to sugar alcohols with alkaline NaBH<sub>4</sub> solution, which is then neutralised with acetic acid. The sample is incubated with fructanase (*exo*-inulinase) to hydrolyse fructans to fructose and glucose, which are subsequently measured by the PAHBAH (*p*-hydroxybenzoic acid hydrazide) method.

### 3.3.5 Pyruvate assay for assessment of pungency

Total pyruvate concentration was measured as an indicator of pungency based on the method of Schwimmer and Weston (1961) adapted for microplates (T. Crowther, Warwick HRI, pers. comm.). Frozen onion juice was allowed to thaw. The liquid was separated from the semi-solid matter by centrifugation at 8000 rpm for 4 min (Desaspeed MH-2, Sarstedt, Leics., UK). A 100 µl sample of onion juice (200 µl when analysing cv. SS1) was placed in a 10 ml tube to which 4 ml (3.9 ml when analysing cv. SS1) 0.375% (v/v) trichloroacetic acid solution was added. A lower dilution factor is required for accurate analysis of low pungency cultivars, such as cv. SS1, due to the low pyruvate concentrations present. A 30 µl aliquot of the sample solution was added to a microplate well (Cellstar 96, Greiner Bio-One, Gloucs., UK) in quadruplicate. Then, 50 µl of a 0.0025% (v/v) solution of 2, 4-dinitrophenylhydrazine in 2N HCl (Spa Contract Synthesis, Warks. UK) was added to each well. The microplate was incubated at 37°C for 10 min, and 150 µl 6N NaOH was added to each well. The absorbance of the samples was immediately read at 450 nm using a microplate reader (BP808, Biohit, Devon, UK) along with blanks (empty wells). The concentration of pyruvate in the sample, expressed as µmol g<sup>-1</sup> FW, was calculated using a calibration curve constructed using the absorbance of a range of pyruvic acid standards (1, 2, 4, 8, 12 and 16 µmol ml<sup>-1</sup>) assayed in eight replicates with each batch.

### 3.3.6 *Bulb firmness and total soluble solids assessments*

The firmness of each onion bulb was assessed according to Lancaster *et al.* (2001) with slight modifications. A 10 mm flat head probe was mounted onto the crosshead of an Instron Series IX 4301 Universal Testing Machine (Instron, Bucks., UK) fitted with a 0.1 kN load cell. The onion bulb was placed horizontally in a V-shaped rest. A compression program was set to perform a 3 mm extension at a crosshead speed of 50 mm min<sup>-1</sup>. The gradient of the linear zone of the force-deformation curve indicated the resistance to pressure, and, therefore, bulb firmness. The mean of two measurements were used for analysis, with the second measurement taken at right angles to the first.

The TSS content of thawed onion juice was measured using a digital hand held refractometer (Palette 100, Atago Co. Ltd., Tokyo, Japan).

### 3.3.7 *Mineral analysis*

Mineral concentrations were measured in ground, lyophilised bulb tissue. The samples were ashed and then digested with 1 ml concentrated nitric acid (ADAS, 1985). The intensity of ion response was measured by ICP-AES (Inductively coupled plasma – atomic emission spectroscopy; Ultima 2, Jobin Yvon, London, UK) and concentrations were determined by comparison with external standards. Results were expressed as total ion concentrations of boron, iron, copper, calcium, magnesium, potassium, manganese, sodium, zinc, phosphorus and sulphur.

### 3.3.8 *Abscisic acid (ABA) quantification*

Abscisic acid was quantified using a radio-immuno assay (RIA) according to Quarrie *et al.* (1988) with some modifications (the assay was validated according to Dorffling and Tietz (1983) and Rosher *et al.* (1985), see Appendix A). All reagents were purchased from Sigma (Dorset, UK) unless otherwise stated. Ground lyophilised bulb tissue (50 mg) was extracted overnight in 1:20 parts tissue to sterile

distilled water (SDW) at 4°C in the dark on a suspension mixer (802/TW, Luckham Ltd., Sussex, UK). Extracted samples were vortexed and then centrifuged at 3000 rpm (MSE Mistral 2000, Sanyo Gallenkamp, Leics., UK) for 10 min at 4°C.

A solution of DL-cis, trans-[G-<sup>3</sup>H] ABA (Amersham International, Bucks., UK) in 100% ethanol was diluted 10-fold in SDW and frozen at -20°C in 500 µl aliquots. This stock solution was diluted further to 4.8 µl ml<sup>-1</sup> in phosphate buffered saline (PBS; 50 mM NaH<sub>2</sub>PO<sub>4</sub> adjusted to pH 6.0 with 50 mM Na<sub>2</sub>HPO<sub>4</sub>, and 100 mM NaCl) containing 5 mg ml<sup>-1</sup> bovine γ-globulin to act as a co-precipitant with the cell line supernatant monoclonal antibody for (+)-ABA, MAC252 (Barrieu and Simonneau, 2000). MAC252 was diluted 1:1000 in PBS containing 5 mg ml<sup>-1</sup> bovine serum albumin and 4 mg ml<sup>-1</sup> soluble polyvinylpyrrolidone (MW 40000) to enhance binding of the antibody. Incubations were carried out in duplicate. A 50 µl sample or ABA standard, 100 µl <sup>3</sup>H ABA, 100 µl MAC252, and 200 µl 100% PBS were added to 2 ml microtubes with push-in caps (Sarstedt, Leics., UK), and incubated in the dark at 4°C for 45 min. Then, to precipitate the antibody 500 µl saturated (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> was added. The tubes were closed, inverted and incubated at room temperature for 30 min. The precipitated antibodies were pelleted by centrifugation for 4 min at 8800 g (Eppendorf Centrifuge 5413, Eppendorf UK Ltd., Cambs., UK). The pellet was washed with 1 ml 50% saturated (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (v/v), and re-centrifuged. One hundred µl SDW was added and the mixture was left for 10 min before vortexing to resuspend the pellet. EcoScint H (1.2 ml; National Diagnostics, E. Yorks., UK) was added to convert β-radiation emitted by the bound <sup>3</sup>H ABA into light. The tubes were placed inside 20 ml plastic screw top scintillation vials (National Diagnostics), wiped with a damp cloth to remove static, and counted on a <sup>3</sup>H program in a liquid scintillation counter (LS 6000TA, Beckman Coulter Ltd., Bucks., UK). Concentrations of ABA were calculated from the radioactivity (counts per minute) present in the pellets. The calibration curve was produced from two replicates of five unlabelled ABA standards ranging from 62.5 to 2000 pg per tube assayed with each batch.

### 3.3.9 *Statistical analysis*

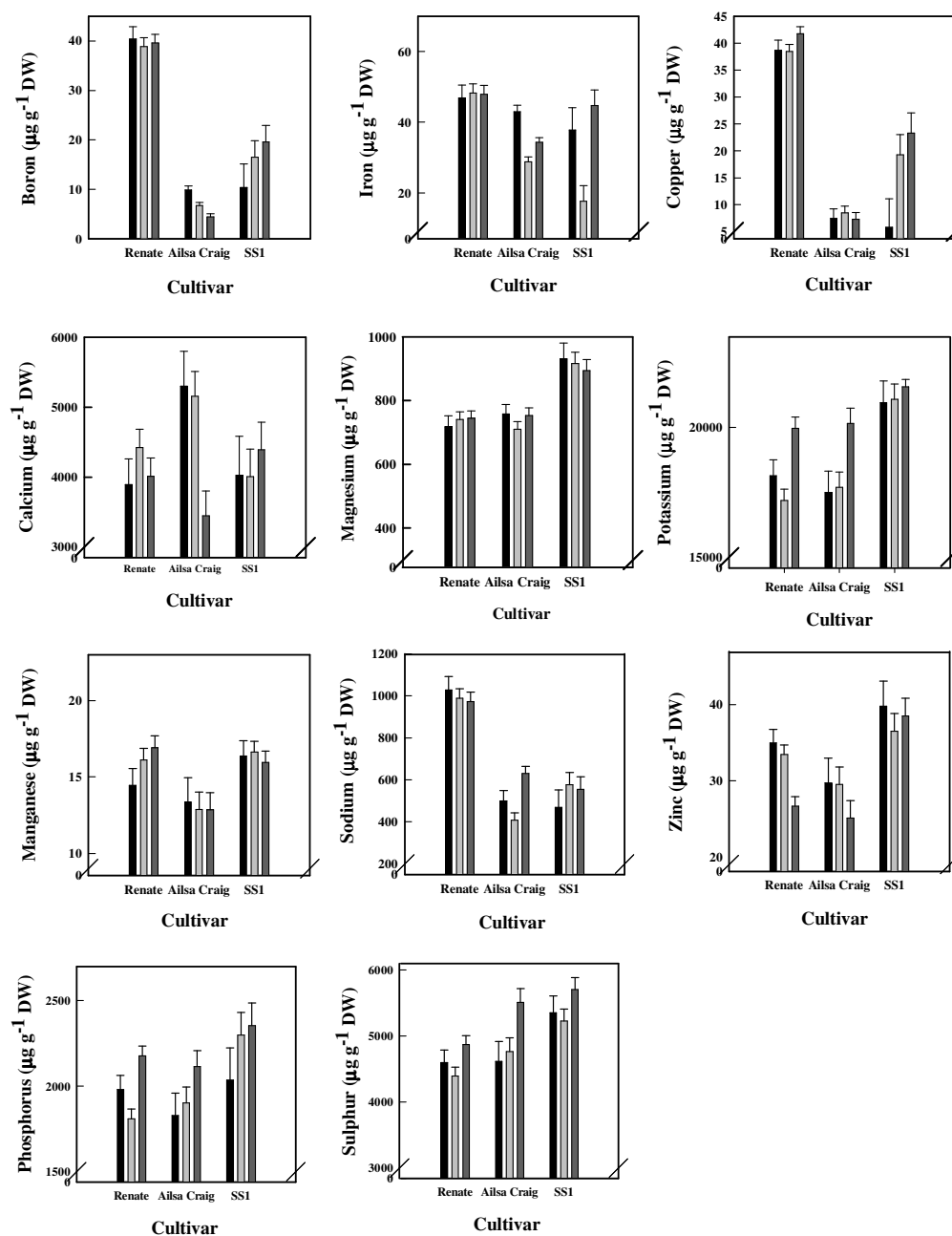
All statistical analyses were carried out using Genstat for Windows Version 7.1.0.198 (VSN International Ltd., Herts., UK). Least significant difference values (LSD;  $P=0.05$ ) were calculated for mean separation using critical values of  $t$  for two-tailed tests. Non-linear regression against an exponential standard curve was used to model the change in ABA concentration over time.

## 3.4 **Results**

The application of additional calcium and sulphur to onion plants in the field did not affect total bulb sulphur or calcium concentrations. The mineral treatments had non-significant and/or inconsistent effects on the other parameters measured and the main effects identified by ANOVA were due to cultivar and storage time. Therefore, the relationship between temporal changes in quality characteristics, ABA and fructan and the onset of sprout growth in each cultivar are presented.

### 3.4.1 *Mineral concentrations*

The variation in mineral content between cultivars was greater than the variation due to time (Figure 3.1). All elements measured except for calcium were present in significantly different ( $P<0.005$ ) concentrations in onion cvs. Renate, Ailsa Craig and SS1 bulbs. There was no consistent pattern according to which cultivar had the highest and lowest concentration of each element, although cv. Ailsa Craig did not have the highest concentration of any of the elements measured. Total sulphur concentration was greatest in cv. SS1, followed by cv. Ailsa Craig and the least in cv. Renate. To enable comparison with other published data on the elemental composition of onions, the data was calculated on a fresh weight basis using the mean dry weights of each cultivar (Table 3.2).



**Figure 3.1.** Elemental content of onion cvs. Renate, Ailsa Craig and SS1 bulbs shortly after harvest (day 0, black bars,  $n=4$ ), and at early (light grey bars,  $n=8$ ) and late (dark grey bars,  $n=8$ ) time points during controlled atmosphere storage (cv. Renate: days 40 and 230, cv. Ailsa Craig: days 81 and 129, and cv. SS1: days 40 and 81). Standard error bars are shown.

**Table 3.2.** The mean elemental composition of onion cvs. Renate, Ailsa Craig and SS1 bulbs (n=20) compared with data for raw and sweet onions from the USDA database (U.S. Department of Agriculture, 2004).

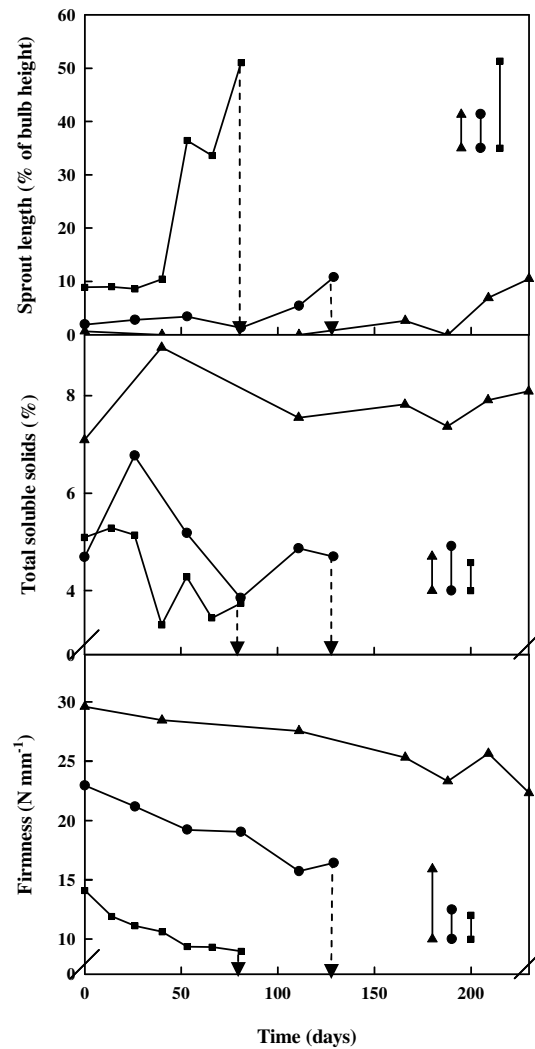
Element	Cultivar				
	Renate	Ailsa Craig	SS1	Raw onion <sup>1</sup>	Sweet onion <sup>1</sup>
Concentration ( $\mu\text{g g}^{-1}$ FW)					
Boron	5.80	0.65	1.16		
Calcium	603.44	453.35	292.24	220	200
Copper	5.81	0.79	1.28	0.38	0.56
Iron	7.03	3.40	3.12	1.9	2.6
Potassium	2708.48	1876.44	1492.38	1440	1190
Magnesium	108.09	74.17	63.90	100	90
Manganese	2.34	1.31	1.14	1.32	0.76
Sodium	145.68	51.86	38.40	30	80
Phosphorus	292.53	198.58	159.21	270	270
Sulphur	679.73	506.62	382.10		
Zinc	4.54	2.80	2.66	1.6	1.3

Blank cells – no data available

<sup>1</sup>Data from USDA database (U.S. Department of Agriculture, 2004).

### 3.4.2 Sprout growth

Sprout length increased significantly during storage in onion cvs. Renate ( $P=0.009$ ), Ailsa Craig ( $P=0.03$ ) and SS1 ( $P<0.001$ ) bulbs (Figure 3.2). The use of CA extends storage life of onion bulbs; however, as relative humidity was high within the storage chambers, disease often ended storage life before sprouting occurred. This was most pronounced in onion cvs. Ailsa Craig and SS1 bulbs.



**Figure 3.2.** Changes in the physical parameters; sprout length (expressed as a percentage of bulb height), total soluble solids and firmness ( $\text{N mm}^{-1}$ ), in bulbs of onion cvs. Renate ( $\blacktriangle$ ), Ailsa Craig ( $\bullet$ ) and SS1 ( $\blacksquare$ ) during controlled atmosphere storage. Drop down arrows indicate the final sample taken. LSD ( $P=0.05$ ) bars are shown, symbols correspond to cultivar;  $n=24$ .

### 3.4.3 *Dry weight*

Dry weight changed significantly ( $P < 0.001$ ) over time in onion cv. Renate bulbs. However this was due to a very high dry weight measurement taken on day 209. No other differences between means were significant, strongly suggesting that this high value might be anomalous and not biologically significant. Dry weight did not change significantly in cvs. Ailsa Craig or SS1, therefore, the overall means are representative of the differences in dry weight between the cultivars. The mean dry weights were cv. Renate:  $147 \pm 5.83 \text{ mg g}^{-1} \text{ FW}$ , cv. Ailsa Craig:  $100 \pm 1.84 \text{ mg g}^{-1} \text{ FW}$  and cv. SS1:  $71 \pm 1.72 \text{ mg g}^{-1} \text{ FW}$ , and were significantly different ( $P < 0.001$ ).

### 3.4.4 *Total soluble solids*

Total soluble solid (TSS) concentration changed significantly ( $P < 0.001$ ) over time in bulbs of cvs. SS1 ( $P < 0.001$ ), Ailsa Craig ( $P < 0.001$ ) and Renate ( $P = 0.002$ ) (Figure 3.2). In all cultivars, a maximum TSS concentration occurred in the first 40 days of CA storage. There was a difference in the pattern of change in the TSS concentration of onions cv. Renate and the other two cultivars. A net 1.1-fold increase occurred in bulbs of cv. Renate ( $P = 0.002$ ) between the beginning and end of storage, but no net difference was observed in cv. Ailsa Craig. A net decrease to 0.7-fold of the original TSS concentration occurred in bulbs of cv. SS1. The TSS concentration did not change significantly ( $P < 0.05$ ) after 111 days of storage in bulbs of onion cvs. Renate and Ailsa Craig, and after 53 days in cv. SS1. The TSS concentration was much greater in bulbs of cv. Renate than cvs. Ailsa Craig or SS1.

### 3.4.5 *Firmness*

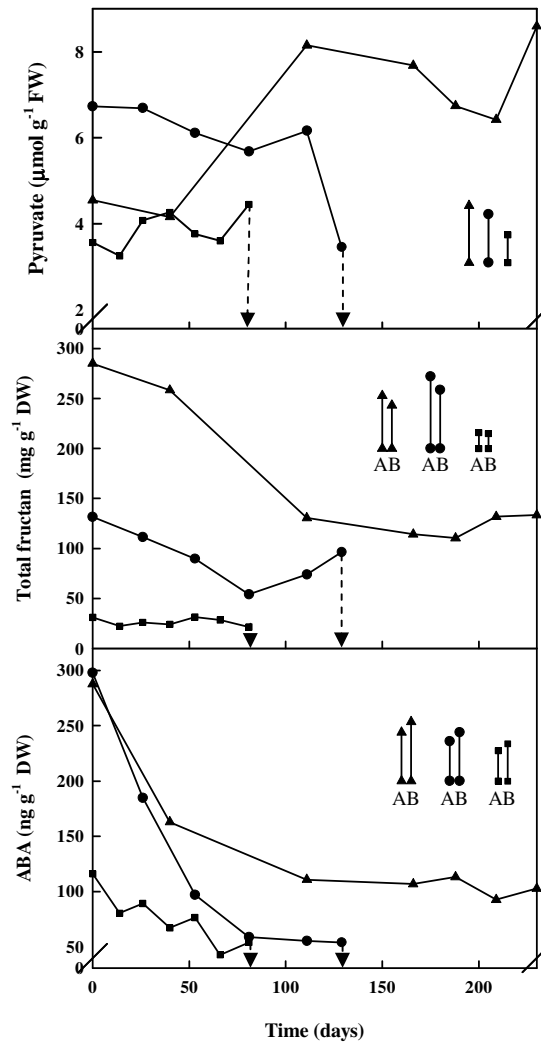
There was a significant difference ( $P < 0.001$ ) in the firmness ( $\text{N mm}^{-1}$ ) of bulbs of cvs. Renate, Ailsa Craig and SS1 when analysed together before storage. The bulbs of cv. Renate were 1.3-fold and 2-fold firmer than those of cvs. Ailsa Craig



and SS1, respectively. This pattern persisted throughout storage (Figure 3.2). The firmness of bulbs of cvs. Ailsa Craig and SS1 changed significantly over time ( $P<0.001$ ). In all cultivars the greatest decrease in firmness occurred between the beginning of CA storage and the first sampling date.

#### 3.4.6 Pyruvate

Pyruvate concentration changed significantly ( $P<0.001$ ) over time in bulbs of cvs. Ailsa Craig and Renate, and cv. SS1 ( $P=0.001$ ) (Figure 3.3). The pyruvate concentration in bulbs of cvs. Renate and SS1 showed a net increase of 1.9-fold and 1.2-fold, respectively, over the storage period. In contrast, a net 1.9-fold decrease in pyruvate was observed in bulbs of cv. Ailsa Craig. A sigmoidal pattern of change in pyruvate concentration occurred in bulbs of cvs. Renate and SS1. Two peaks in pyruvate concentration occurred during the storage period, with the first occurring in the middle of storage time and the second at the end of storage. Throughout storage, the pyruvate concentration of bulbs cv. Ailsa Craig was consistently higher than that of bulbs cv. SS1. When all cultivars were analysed together before bulbs were placed into storage (day 0), onion cv. Ailsa Craig bulbs had a significantly ( $P=0.003$ ) greater pyruvate concentration ( $6.73 \mu\text{mol g}^{-1}$  FW) than bulbs of cv. SS1 ( $3.57 \mu\text{mol g}^{-1}$  FW).



**Figure 3.3.** Changes in the biochemical parameters; pyruvate, total fructan, and ABA concentrations, in bulbs of onion cvs. Renate (▲), Ailsa Craig (●) and SS1 (■) during controlled atmosphere storage. Drop down arrows indicate the final sample taken. LSD bars ( $P=0.05$ ) are shown, symbols correspond to cultivar. LSD bar A is for comparison of time zero with all other times, bar B is for comparison of all times except time zero. Pyruvate:  $n=24$ , fructan and ABA: time zero  $n=4$ , all other times  $n=8$ .

### 3.4.7 Total fructans

The fructan content in bulb samples at day 0 was significantly different ( $P < 0.001$ ) in each of the three cultivars tested (Figure 3.3). Bulbs cv. Renate had the highest proportion of fructan per gram dry weight ( $285.0 \pm 20.84 \text{ mg g}^{-1} \text{ DW}$ ), followed by cvs. Ailsa Craig ( $131.3 \pm 28.11 \text{ mg g}^{-1} \text{ DW}$ ) and SS1 ( $31.0 \pm 7.24 \text{ mg g}^{-1} \text{ DW}$ ). Fructan concentration significantly ( $P < 0.001$ ) changed over time in onion cv. Renate bulbs. The decrease was most rapid between day 40 and day 111, and no other differences between fructan concentration during storage were significant. Fructan content of cvs. Ailsa Craig and SS1 bulbs did not change significantly during storage.

### 3.4.8 Abscisic acid

Bulb ABA concentration declined significantly during storage for all cultivars (Renate and Ailsa Craig  $P < 0.001$ ; SS1  $P = 0.003$ ; Figure 3.3; Table 3.3). The initial ABA concentrations in onion cv. Ailsa Craig ( $298 \text{ ng g}^{-1} \text{ DW}$ ) and cv. Renate ( $288 \text{ ng g}^{-1} \text{ DW}$ ) bulbs, were significantly greater (LSD ( $P = 0.05$ ) = 162.1) than that present in onion cv. SS1 bulbs ( $116 \text{ ng g}^{-1} \text{ DW}$ ). When analysed together for days 0, 26, 53 and 81, cultivar accounted for the majority of the variation in bulb ABA concentration between cvs. SS1 and Ailsa Craig ( $P < 0.001$ ). When analysed together with cvs. Renate (days 0 and 40) or Ailsa Craig (days 0, 26, 53 and 81), ABA concentration in cv. SS1 was always significantly lower ( $P < 0.001$ ).

**Table 3.3.** The maximum and minimum abscisic acid concentrations on a dry weight ( $\pm$  S.E.) and fresh weight basis recorded in onion cvs. SS1, Ailsa Craig and Renate bulbs at the beginning and end of storage (Time zero: n=4, all other times: n=8).

Cultivar	ABA concentration (ng g <sup>-1</sup> DW)		ABA concentration (ng g <sup>-1</sup> FW) <sup>1</sup>	
	Maximum	Minimum	Maximum	Minimum
	SS1	116.0 <sup>2</sup> (13.25)	42.8 <sup>3</sup> (9.37)	8.2 <sup>2</sup> (4.8)
Ailsa Craig	297.9 <sup>2</sup> (17.23)	54.1 <sup>4</sup> (12.18)	29.8 <sup>2</sup> (12.8)	5.4 <sup>4</sup> (2.3)
Renate	287.7 <sup>2</sup> (21.24)	92.7 <sup>5</sup> (15.02)	42.3 <sup>2</sup> (22.0)	13.6 <sup>5</sup> (7.1)

<sup>1</sup> Values calculated from mean DW for each cultivar; cv. Renate:  $147 \pm 5.83$  mg g<sup>-1</sup> FW, cv. Ailsa Craig:  $100 \pm 1.84$  mg g<sup>-1</sup> FW and cv. SS1:  $71 \pm 1.72$  mg g<sup>-1</sup> FW.

<sup>2</sup> Measured at day 0. <sup>3</sup> Measured at day 66. <sup>4</sup> Measured at day 129. <sup>5</sup> Measured at day 209.

In order to describe the relationship between storage time (x) and ABA concentration (y), non-linear regression was applied to the data. For all cultivars the relationship could be described by a negative exponential function (equation 1), where R is a measure of the rapidity of the decline in ABA concentration (if R is close to 1 the decline will be slow, and as R approaches zero the rate of decline will increase). The minimum ABA concentration (when  $x=\infty$ ) is given by A, and the ABA concentration when  $x=0$  is given by A+B. The ABA concentration at time x is given by y.

$$y = (A + B)R^x \quad (1)$$

An alternative form of equation 1, is equation 2 where  $K = -\log(R)$ .

$$y = (A + B)e^{-Kx} \quad (2)$$

The data for cvs. Ailsa Craig and Renate closely fitted an exponential function ( $P < 0.001$ ). The data for cv. SS1 also fitted an exponential function ( $P = 0.036$ ), but less closely. Grouped regression analysis of the curves describing the decline in bulb ABA concentration in cvs. SS1, Ailsa Craig and Renate over time showed that 98.0% of the variation between the curves was accounted for by allowing separate linear parameters (i.e. A and B), and that the same value of R could be used to describe each of the curves ( $P < 0.001$ ) (Table 3.4).

**Table 3.4.** The parameters ( $\pm$  S.E.) of the exponential functions ( $y = (A + B)R^x$ ) describing the change in ABA concentration during storage (days) in onion cvs. SS1, Ailsa Craig and Renate bulbs.

Parameter	Grouped regression			Individual regression		
	Renate (n=7)	Ailsa Craig (n=6)	SS1 (n=7)	Renate (n=7)	Ailsa Craig (n=6)	SS1 (n=7)
R		$0.97 \pm 0.002$		$0.97 \pm 0.003$	$0.98 \pm 0.004$	$0.98 \pm 0.022$
B	$184.6^2$	$262.9^2$	$68.2^2$	$184.7 \pm 7.94$	$263.9 \pm 14.8$	$75.6 \pm 39.5^4$
A	$102.0^2$	$39.1^2$	$45.2^2$	$102.9 \pm 3.69$	$37.3 \pm 12.1$	$35.9 \pm 42.9^4$
Percentage variance <sup>1</sup>		98.0		98.9	98.7	71.1
Half life <sup>3</sup> (days)		$26.2 \pm 1.90$		$24.8 \pm 3.01$	$27.1 \pm 4.13$	$35.0 \pm 39.5^4$

<sup>1</sup>Percentage variance =  $100 * (1 - ((\text{residual mean square}) / (\text{total mean square})))$  = adjusted  $R^2$  value expressed as a percentage.

<sup>2</sup>Standard error = 9.97.

<sup>3</sup>Half life =  $\log(0.5) / \log(R)$ .

<sup>4</sup>The large standard errors are representative of the poorer fit of the data for cv. SS1 to the regression, compared with the data for cvs. Ailsa Craig and Renate.

The time taken for the ABA concentration to decrease by half of that present before storage ('half life'),  $A + (B/2)$ , can be calculated using equation 3, which is derived from equation 2.

$$\text{half life} = \log(0.5) / \log(R) \quad (3)$$

The half lives were calculated from each individual regression, and an overall half life was also calculated from the common R parameter derived from the combined regression analysis. The common R value was  $26.2 \pm 1.90$  days (Table 3.4).

### 3.5 Discussion

The sulphur and calcium treatments, applied at the time of drilling, did not affect bulb sulphur or calcium content. Other authors have found that sulphur treatments did not affect dry matter (O'Donoghue *et al.*, 2004), carbohydrates or TSS (Lancaster *et al.*, 2001). Field, hydroponic and tissue culture trials worldwide have shown that attempting the manipulation of the sulphur content of onions by varying the sulphur supply during growth has mixed results and is highly dependent on factors such as cultivar, the extent of variation of sulphur supply, and other seasonal and environmental influences (Randle, 1992; Randle *et al.*, 1993; Hamilton *et al.*, 1998; Kopsell *et al.*, 1999; Lancaster *et al.*, 2001; Coolong *et al.*, 2004; O'Donoghue *et al.*, 2004). In general, manipulation of the sulphur supply has an effect on parameters such as firmness, pungency, ACSOs and dry matter when the treatment is applied constantly e.g. in a hydroponic system, or when applied in the field towards the end of the growing season when bulbing has been initiated.

Onion cv. SS1 bulbs had a greater concentration of total sulphur than cv. Ailsa Craig and Renate. This is perhaps surprising as cv. SS1 was the least pungent of the three cultivars, and pungency is determined by the availability of sulphur-containing flavour precursors (ACSOs). However, all mineral analyses were performed on lyophilised

tissue. When the concentrations are calculated on a fresh weight basis (using the mean dry weight percentage), free sulphur concentration was least in cv. SS1 bulbs (Table 3.2). Free mineral content should not change over the storage period and therefore the different concentrations recorded at different storage durations is more likely to represent the natural variation within the population than real changes in mineral concentration. Onion cv. Ailsa Craig bulbs showed the greatest variability over time, and this could be attributed to the fact that it is a 'garden' variety rather than a commercially grown cultivar. The data from the USDA database (U.S. Department of Agriculture, 2004) is comparable with that recorded in this investigation; however, onion cv. Renate bulbs contained approximately 2-fold the concentration of calcium, copper, iron, potassium and sodium. The onion cultivar used to obtain data for the USDA database is unknown; it is possible that it is a lower dry matter cultivar than cv. Renate which would explain these results.

Dry matter content is the primary characteristic of onion bulb quality, determining, in part, the end use (e.g. as salad, cooking or dehydrated onions), storage life, pungency and firmness (Sinclair *et al.*, 1995a). Onions with high dry matter content tend to be much firmer and store for longer periods before shoot growth and disease incidence deplete the number of marketable bulbs (Darbyshire and Henry, 1979; Rutherford and Whittle, 1982; Suzuki and Cutliffe, 1989). Ranking the cultivars by dry matter from the highest to the lowest (cv. Renate > cv. Ailsa Craig > cv. SS1), matches the ranking in terms of storage life from longest to shortest. Similarly, the greater the dry matter content, the firmer the bulb (Figure 3.2).

Total soluble solids is defined as the total of all the solids that dissolve in water, including sugars, salts, protein and organic acids, and the refractometer reading is the sum total of these. Thus, TSS is not a direct measure of sugar content and is unrelated to the perceived sweetness of some onion cultivars (Crowther *et al.*, 2005). Onion cv. SS1 bulbs are marketed as having a sweet flavour and are intended to be consumed raw, but have a lower TSS concentration than cv. Renate bulbs. High dry matter bulbs are able to accumulate higher TSS concentrations without taking up more water because of their ability to synthesise and store highly polymerised fructans (Sinclair *et al.*, 1995b). Net



TSS concentration did not change between the beginning and end of storage in bulbs of cvs. Renate and Ailsa Craig, which is consistent with the observations by Rutherford and Whittle (1982) on Rijnsberger-type onions stored at 4°C. A peak in TSS concentration occurred between *ca.* 4 and 6 weeks in all cultivars, followed by a general decline towards the end of the storage period. This is comparable to the peaks observed by other authors after 6 - 8 weeks storage at 4, 10 and 20°C in cv. Rouge Amposta bulbs (Benkeblia *et al.*, 2002) and at 5 weeks at 0, 15 and 30°C in cv. Sentinel bulbs (Salama *et al.*, 1990); both of these peaks being followed by a decline until the end of the storage period (24 and 20 weeks, respectively).

Pyruvate is a reliable indicator of pungency, and is a stable product from the hydrolysis of alk(en)yl cysteine sulphoxides (ACSOs) by the enzyme alliinase that occurs when onion cells are disrupted. The activity of alliinase has been shown to decrease at low O<sub>2</sub> (2%) and high CO<sub>2</sub> (8%) concentrations (Uddin and MacTavish, 2003). Thus, changes in pyruvate concentration during storage could be due to differences in availability of ACSOs or in the activity of alliinase. The cultivars used in this experiment can be ranked in order of pungency using the mean pyruvic acid concentration (Schwimmer and Weston, 1961; Crowther *et al.*, 2005) taken across the entire storage period; cv. Renate > cv. Ailsa Craig > cv. SS1. The pyruvate concentration of bulbs cv. Ailsa Craig decreased over the storage period. This is consistent with the findings of others, who reported a decrease in the pyruvate concentration in bulbs of cv. Hysam after 9 weeks in CA storage (either 2 % O<sub>2</sub>, 2% CO<sub>2</sub> or 2% O<sub>2</sub>, 8% CO<sub>2</sub>) (Uddin and MacTavish, 2003) or various long-day onion cultivars stored at 5°C for 24 weeks (Kopsell and Randle, 1997). In contrast, pyruvate concentration increased overall in cvs. Renate and SS1 during storage, which is in agreement with that observed for bulbs cv. Hysam stored under ambient atmosphere conditions for 9 weeks (Uddin and MacTavish, 2003) and in bulbs cv. Granex-Grano bulbs stored at 4°C for 24 weeks (Hurst *et al.*, 1985).

Onion cultivars characterised by low dry matter content tend to accumulate simple sugars such as glucose and fructose during bulb formation, whereas those characterised by high dry matter tend to accumulate more fructans. A positive relationship between

fructan content at harvest and storage life has been reported (Suzuki and Cutliffe, 1989; O'Donoghue *et al.*, 2004). Supporting this relationship, bulb fructan concentration before storage was greatest in the cultivar with the longest storage potential, cv. Renate, and least in the cultivar with the shortest storage potential, cv. SS1.

The rate of fructan metabolism was reduced in bulbs of onion cv. Sherpa stored under low oxygen concentrations at 2°C compared to ambient atmosphere, with the effect being greater at 0.5% O<sub>2</sub> than 1.0% O<sub>2</sub> (Ernst *et al.*, 2003). The oxygen concentration of 5% used in the present study may explain the earlier decrease in fructan concentration at *ca.* 16 weeks rather than 36 weeks in bulbs cv. Renate by allowing metabolism of fructans earlier in the storage period. However, the CO<sub>2</sub> concentration was 10-fold greater in the present study suggesting that the inhibitory effect of high CO<sub>2</sub> concentration on respiration was overcome by the high O<sub>2</sub> concentration. Fructan concentration would be expected to decrease over time as fructans are enzymatically hydrolysed to fructose (Hurst *et al.*, 1985). Fructan content in onion bulbs has been shown to decrease during refrigerated ambient atmosphere storage (Suzuki and Cutliffe, 1989; Pak *et al.*, 1995; Ernst *et al.*, 1998; Benkeblia *et al.*, 2000), or low oxygen storage (Ernst *et al.*, 2003). However, in the experiments reported here, bulb fructan concentration only decreased significantly in cv. Renate bulbs. High fructan concentration may protect against tissue damage caused by anaerobic respiration under low oxygen conditions (Ernst *et al.*, 2003). Onion cv. Renate bulbs have a high fructan concentration, and would, therefore, have been well suited to low oxygen conditions, and, thus, some hydrolysis of fructans to simple sugars could have occurred. The different patterns of change in fructan concentration could be due to a different response of each cultivar to CA conditions.

Information on changes in parameters such as carbohydrates, fructans and pungency in different onion cultivars is relatively abundant, if sometimes conflicting; but few detailed studies on the changes and effects of plant growth regulators on the storage life of onions have been conducted. Nevertheless, postharvest decrease in ABA concentration in a relative of onion, *A. wakegi*, has been correlated with a loss of

dormancy (Yamazaki *et al.*, 1995; Yamazaki *et al.*, 1999a; Yamazaki *et al.*, 1999b; Yamazaki *et al.*, 2002).

The bulb ABA concentration declined exponentially during storage in all three onion cultivars studied here (Figure 3.3). A rapid initial decline in ABA concentration was not observed in bulbs of cv. SS1, and the exponential function fitted the data for cv. SS1 less well than for cvs. Ailsa Craig and Renate. Bulbs of cv. SS1 were harvested 14 days earlier than bulbs of cvs. Ailsa Craig and Renate, but were placed into storage at the same time. Therefore, a rapid decline in ABA concentration may have occurred during the period between harvesting and the beginning of storage. The half life of ABA in bulbs of cvs. Renate, Ailsa Craig and SS1 was calculated to be approximately 26 days, providing further evidence that a significant decrease in ABA concentration could have occurred in SS1 in those first 14 days. Assuming an exponential decrease in the ABA concentration with the parameters listed in Table 3.4, the data can be extrapolated to estimate the ABA concentration in bulbs cv. SS1 14 days prior to the first measurement as 136 ng g<sup>-1</sup> DW based on the individual regression, and 149 ng g<sup>-1</sup> DW based on the grouped regression. These estimated data should be treated with caution; however, these estimated ABA concentrations for bulbs cv. SS1 are approximately 2-fold less than that present in cvs. Ailsa Craig and Renate at day 0.

The maximum and minimum ABA concentrations recorded in this investigation on lyophilised samples were converted to concentrations on a fresh weight basis, and can be compared to those reported by other authors (Table 3.1; Table 3.3). Matsubara and Kimura (1991) reported that the ABA concentration in the outer enlarged leaf of onion bulbs decreased rapidly from *ca.* 9 ng g<sup>-1</sup> to *ca.* 1 ng g<sup>-1</sup> FW after one month of ambient storage under shelter in Japan. This is comparable to the maximum and minimum ABA concentrations found in onion bulbs cv. SS1 (Table 3.3). Yamazaki *et al.* (1995) observed a slight increase in the ABA concentration in *A. wakegi* cv. Kiharabansei No. 1 in ambient storage from 11 ng g<sup>-1</sup> FW to 13 ng g<sup>-1</sup> FW in the first two weeks after harvest, followed by a rapid decrease to 6 ng g<sup>-1</sup> FW over the next four weeks. Yamazaki *et al.* (1999b) and Yamazaki *et al.* (2002) reported a maximum concentration of *ca.* 33 ng g<sup>-1</sup> FW in *A. wakegi* cv. Kiharabansei No. 1 two weeks after harvest, which decreased to

*ca.* 10 ng g<sup>-1</sup> FW after four weeks. This decline is closer to the ABA concentration observed in onion cvs. Ailsa Craig and Renate bulbs recorded in the present study (Figure 3.3). The ABA concentration on a fresh weight basis accentuated the differences between cultivars; (Table 3.3) the maximum concentration in bulbs of cv. Renate was 1.4 and 5.2-fold greater than that in bulbs of cvs. Ailsa Craig and SS1, respectively.

ABA concentration (DW) at day 0 was least in bulbs cv. SS1, the shortest storing cultivar, and was *ca.* 2.5-fold greater in bulbs cvs. Ailsa Craig and Renate. In this experiment, decreasing ABA concentration appeared to be related to an increase in sprouting. Sprouting in bulbs of cvs. SS1 and Ailsa Craig seemed to occur at a lower bulb ABA concentration (*ca.* 75 ng g<sup>-1</sup> DW and 65 ng g<sup>-1</sup> DW, respectively) than in bulbs of cv. Renate (*ca.* 115 ng g<sup>-1</sup> DW). The disparity in minimum ABA concentration between cultivars may indicate that bulbs of cvs. Renate, Ailsa Craig and SS1 have different threshold ABA concentrations for the onset of sprouting. This does not necessarily indicate the presence of a biological connection between ABA concentration and sprouting, although an *in vitro* study has shown that treatment of basal, equatorial and near apical onion bulb tissue with 10<sup>-4</sup> M ABA inhibited nucleolar activation, which supports a role for ABA in inhibition of cell elongation (Karagiannis and Pappelis, 1994). The control of cell elongation is important in the storage life onions as cell division is not closely linked to dormancy (Carter *et al.*, 1999). Diminution in ABA concentration was likely to be caused by degradation of the compound, rather than movement within the bulb, as the regulation of physiological processes is at the primary level of *de novo* synthesis of relevant enzymes for ABA synthesis rather than redistribution (Finkelstein and Rock, 2002).

### 3.6 Conclusion

Onion bulb abscisic acid (ABA) concentration decreased exponentially during storage, and was concomitant to the decline in storage potential. The similarity in the half lives for all of the cultivars provides evidence that it is not necessarily the rate of

decline in ABA concentration that is important. Onion bulb ABA concentration at harvest (measured on a fresh weight basis) may prove to be an indicator of storage life. The ABA concentration at harvest (DW) may be indicative of a greater difference in sprouting during storage between cv. SS1 and the other cultivars than between cv. Renate and cv. Ailsa Craig.

Maximising onion bulb ABA concentration prior to storage, and/or inhibition of the degradation of ABA by cultural, environmental or genetic manipulation may delay sprouting and extend storage life. It follows that prolonging the storage life of short-storing cultivars (e.g. cv. SS1) through elevating ABA concentration and/or delaying the rate of decline in ABA concentration might help increase the period for supply of these onions.

## 4.0 CHAPTER FOUR

### **Neither pre- nor postharvest application of exogenous abscisic acid (ABA) or an ABA analogue affects endogenous ABA concentration of onion bulbs.**

#### **4.1 Abstract**

Bulb abscisic acid (ABA) concentration has been shown to decrease in stored onions, and onset of sprouting to occur at minimal ABA concentration. It was postulated that increasing pre-storage bulb ABA concentration could increase storage life by inhibiting sprout growth. Chemical analogues of ABA that have enhanced biological activity and that resist degradation are available and are becoming commercially viable. Exogenous ABA and an ABA analogue (8'-methylene ABA methyl ester; PBI-365) were applied as a pre-harvest foliar spray to six onion cultivars (*viz.* Carlos, Dinero, Hysam, Red Baron, Renate and SS1) with contrasting storage potential and as a postharvest bulb soak to one cultivar (*viz.* Hysam). Quality markers including pyruvate, total soluble solids and firmness were monitored during storage. Neither method increased endogenous bulb ABA concentration. Bulb ABA concentration decreased during storage and onset of sprouting occurred at a minimal ABA concentration. This was followed by a subsequent increase in ABA concentration as sprout growth continued. No straightforward relationship between ABA and carbohydrate metabolism could be determined, it was therefore postulated that ABA either plays a role in the regulation of cell elongation rather than cell division, or that minimal ABA concentration could be a trigger for remobilisation of carbohydrates to provide energy for the growing sprout. Total soluble solids (%) concentration was a poor indicator of total simple sugars (fructose + glucose + sucrose). The results suggest that change in refractive index was largely due to simple sugars in low dry matter cultivars, and to fructans in high dry matter cultivars.

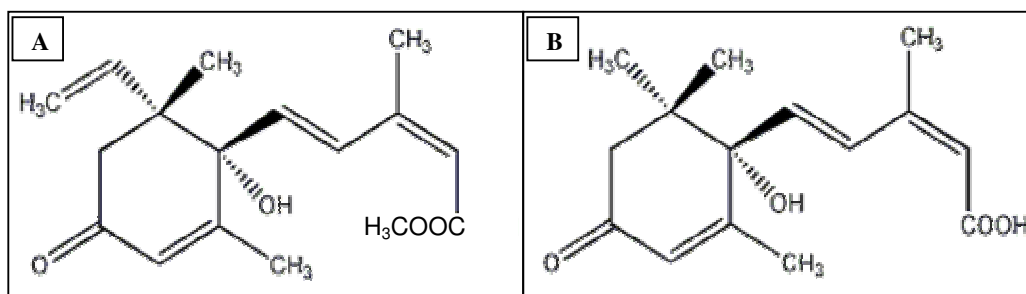
## 4.2 Introduction

Abscisic acid (ABA) has been associated with dormancy in onions. The ABA concentration in onion bulbs decreased throughout controlled atmosphere storage (Chapter 3; Chope *et al.*, 2006), and minimal ABA concentration was associated with the onset of sprouting in onion cvs. Renate, Ailsa Craig and SS1 bulbs. The ABA concentration in *Allium wakegi*, a relative of onion, reached a maximum shortly after harvest (Yamazaki *et al.*, 2002), and the concentration of free endogenous ABA in the bulb basal leaf sheath immediately after harvest was positively correlated with the number of days to sprouting (Yamazaki *et al.*, 2002). Furthermore, plants of *Allium wakegi* that received fluridone (an inhibitor of ABA biosynthesis) as an aqueous soil treatment had reduced amounts of ABA in all parts of the plant and the application of 25 or 125  $\mu\text{M}$  fluridone decreased the number of days to sprouting from 45 days to 29 and 15 days, respectively (Yamazaki *et al.*, 1999a). Cell division is not closely linked with dormancy (Carter *et al.*, 1999), so the control of cell elongation, rather than cell division, is important in suppression of sprout growth. There is evidence that ABA inhibits cell elongation (Karagiannis and Pappelis, 1994), which provides further support for the hypothesis proposed in Chapter 3 that increasing the concentration of ABA in the crop before storage or decreasing the rate of degradation of ABA during storage could prolong storage potential by suppressing sprout growth.

If ABA concentration is linked with control of dormancy then it is also likely that it is linked with carbohydrate metabolism, as carbon and nitrogen containing compounds must be remobilised to provide energy for the growing sprout. Although the postharvest changes in carbohydrate concentrations in onion bulbs have already been studied in detail (Table 6.1), no data on the relationship between ABA and carbohydrates are available. Onion bulb quality characteristics must be monitored to ensure that product standard is not compromised.

Abscisic acid is costly to purchase and is light-sensitive, and therefore chemical analogues of ABA that have similar biological activity, but are less expensive to produce, and not degraded as readily are desirable. Analogues of ABA that are resistant to degradation could extend the storage life of onions and help elucidate the role of ABA in sprout suppression. 8'-methylene ABA methyl ester

(PBI-365) is a chemical analogue of ABA which has been shown to act similarly to ABA in inducing stomatal closure in cut roses (Pompodakis and Joyce, 2003). 8'-methylene ABA methyl ester is chemically identical to ABA apart from the presence of a methylene group at the 8' position, and the presence of an ester group instead of the carboxylic acid group (Figure 4.1). The presence of a methyl group at the 8C position of the ABA molecule has been shown to sterically hinder binding of the molecule with ABA-8'-hydroxylase, thus resisting degradation (Abrams *et al.*, 1997). 8'-methylene ABA methyl ester possesses this methyl group and so should also resist degradation.



**Figure 4.1.** A. 8'-methylene abscisic acid methyl ester. B. (+)-abscisic acid.

Current understanding is that ABA is synthesised in the leaves of the onion plant and translocated to the bulb via the phloem (Hartung *et al.*, 2002) as it reaches maturity. It therefore follows that a foliar application of exogenous ABA may also be translocated in this manner. Maleic hydrazide is known to have high phloem mobility (Price *et al.*, 1975). It is applied to the crop prior to harvest, and can be subsequently detected within the bulb (Pesticide Residue Committee, 2006). This suggests that the timing of MH application could also be suitable for treatment with the hormone. Increasing the amount of endogenous ABA could also be attempted by dipping harvested bulbs in solutions of the hormone or analogue prior to storage. This method may be more efficient as there is direct contact with the bulb.

This experiment investigated the effects of the application of ABA and an ABA analogue (8'-methylene ABA methyl ester; PBI-365) as pre-harvest foliar field treatments or postharvest dip treatments on the physical and biochemical changes



occurring during storage in onion bulbs of various cultivars with differing storage potential.

### 4.3 Materials and methods

#### 4.3.1 Plant material

Six onion (*Allium cepa* L.) cultivars viz. SS1, Carlos, Dinaro, Renate, Red Baron and Hysam were spring-drilled on 23 March 2004 in four rows per bed using a tape seeder at a rate of 18 seeds m<sup>-1</sup> and grown in coarse loamy soil at Warwick HRI (Warks., UK) using standard agronomic practices. The cultivars were selected according to storage potential (NIAB, 2000) and can be divided into long (Renate, Hysam, Red Baron), medium (Carlos, Dinaro) and short storing (SS1) cultivars (Table 4.1).

**Table 4.1.** The estimated storage life (months) of onion cvs. Carlos, Dinaro, Hysam, Red Baron, Renate and SS1 (Tim Crowther, Warwick HRI, pers. comm.).

Storage temperature (°C)	Cultivar					
	Carlos	Dinaro	Hysam	Red Baron	Renate	SS1
4	4-5	4-5	6	5-6	6	1-2
12	2-3	2-3	2-3	2	2-3	0.5-1
20	2	2	1-2	1	1-2	0-0.5

#### 4.3.2 Experiment 1 – Pre-harvest foliar spray method

The following treatments (Table 4.2) were prepared and applied to the crop on 17 August 2004 using a knapsack sprayer with three flat fan tip standard 110° nozzles (B.C.P.C. nozzle code F110/1.60/3; Lurmark, Cambs., UK).

**Table 4.2.** Treatments applied to the onion plants in the field.

Treatment	Application rate (l ha <sup>-1</sup> )	Plot area (m <sup>2</sup> )	Total volume applied (l)
ABA <sup>a</sup> (10 <sup>-4</sup> M)	550	126	6.93
PBI-365 <sup>a</sup> (10 <sup>-4</sup> M)	314.2	126	3.95
Li-700 (0.5% v/v)	550	126	6.93
Water	550	126	6.93

<sup>a</sup> aqueous solution including 0.5% (v/v) Li-700

Since ABA is photosensitive, the treatments were applied at dusk. The leaves of onion plants have a thick, waxy cuticle which represents a barrier to any chemical applied to them. An adjuvant (Li-700, Loveland Industries Ltd., Cambs., UK) was used to aid the movement of ABA and PBI-365 (8'-methylene ABA methyl ester) into the leaf mesophyll tissue. Abscisic acid penetrates the leaf cuticle more effectively at a pH below the pKa (pH 4.8) (Blumenfield and Bukovac, 1972). Li-700 is a penetrating, translocating and acidifying adjuvant and a 0.5% (w/w) solution is pH 4.05. All treatments (except the water control) contained 0.5% (v/v) Li-700. A 2 m buffer zone was left between treatment plots. The weather conditions in the week following the treatment were recorded (Table 4.3).

**Table 4.3.** Weather conditions in the week following pre-harvest treatment.

Date	Maximum temp. (°C)	Minimum temp. (°C)	Sunshine (hours)	Wind speed	Rainfall (mm)
17/08/2004	24.6	15.4	0.0	Moderate	1.2
18/08/2004	22.2	16.5	3.8	Gentle	1.5
19/08/2004	23.4	12.1	9.6	Fresh	0.0
20/08/2004	21.7	12.5	4.8	Moderate	10.1
21/08/2004	20.0	10.8	11.4	Light	0.0
22/08/2004	21.3	9.8	4.8	Gentle	18.3
23/08/2004	20.9	13.1	4.3	Gentle	7.4
24/08/2004	20.6	13.5	4.4	Gentle	1.1

#### 4.3.3 *Harvest procedure*

Onion bulbs were forked on 31 August 2004, and lifted and topped on 3 September 2004. On 6 September 2004 the onion bulbs were loaded into standard 25 kg plastic nets and dried in bin driers with hot air (*ca.* 30°C) for ten days, after which ambient air was blown through the crop for two weeks as per standard practice in the UK. The dry aerial parts and roots were removed and any diseased or damaged bulbs discarded prior to storage.

#### 4.3.4 *Experiment 2 - Postharvest bulb dip method*

Harvested onion cv. Hysam bulbs were soaked in aqueous solutions of  $10^{-4}$  M ABA,  $10^{-4}$  M PBI-365 or water (control). Both ABA and PBI-365 were dissolved in 2.5 ml ethanol and then made up to 2.5 l with sterile distilled water, and these solutions also contained 0.5% Li-700. Bulbs were then placed into the bin driers along with the rest of the harvest.

#### 4.3.5 *Storage regime and experimental design*

Bulbs were stored in air at 4°C, 12°C or 20°C. Relative humidity was not measured. The experiment was conducted as a completely randomised design. Bulbs were removed from storage at regular intervals. For experiment 1 (pre-harvest foliar spray), samples were taken after 0, 53, 109, 186, 215 and 243 days storage at 4°C, after 0, 26, 39, 53, 81 and 109 days storage at 12°C, and after 0, 7, 26, 39, 67 and 98 days storage at 20°C. Five bulbs were sampled at each time point for each treatment and cultivar for sprout length, pyruvate, total soluble solids (TSS), ABA and firmness measurements, and three bulbs of cvs. Renate, Red Baron and SS1 for fructose, glucose, sucrose and fructan concentration. For experiment 2 (postharvest bulb dip), bulbs were sampled after 0, 53, 186 and 215 days storage at 4°C, after 0, 26, 81 and 109 days storage at 12°C, and after 0, 7, 26 and 67 days storage at 20°C with three

bulbs per treatment and per time point. Carbohydrate concentrations were not measured in these samples.

#### 4.3.6 *Sample preparation*

Onion bulbs were sliced vertically in half from the neck to the base. A *ca.* 5 mm thick equatorial slice was cut from the middle of each half. Juice was expressed from the equatorial slice using a hand-operated press (Randle and Bussard, 1993) and allowed to stand for 15 min. A sample of juice was collected in a 1.5 ml Eppendorf tube and frozen at -20°C for pyruvate and TSS measurements. In addition, a section was cut from a longitudinal wedge of the bulb and was snap-frozen in liquid nitrogen and kept at -40°C until the sample was lyophilised (Edwards Super Modulo, Sussex, UK) for use in fructan, ABA and NSC assays.

#### 4.3.7 *Physical assessments*

All physical measurements were carried out as described in Chapter 3. Briefly, dry weight measurements were made on lyophilised samples. Incidence of sprouting was recorded and expressed as the height of the first green leaves appearing inside the bulb as a percentage of the bulb height. Bulb firmness ( $\text{N mm}^{-1}$ ) was measured using an Instron Series IX materials testing machine (Instron, Bucks., UK) according to the method of Lancaster *et al.* (2001) with slight modifications.

#### 4.3.8 *Biochemical assessments*

The concentrations of ABA, pyruvate and TSS were measured as described in Chapter 3. Briefly, ABA was measured by radioimmunoassay (RIA), and pyruvate concentration by colorimetric assay. Total soluble solid content (%) was measured using a digital hand held refractometer (Palette 100, Atago Co. Ltd., Tokyo, Japan). Non-structural carbohydrates (NSC) were quantified using HPLC as described below.

#### 4.3.8.1 NSC extraction

Onion bulbs were extracted according to O'Donoghue *et al.* (2004) with slight modifications (F. Davis and L. A. Terry, Cranfield University, unpublished method). Freeze-dried onion powder (150 mg) was combined with 3 ml of 62.5:37.5 HPLC grade methanol: water (v/v) and mixed well. This method was adopted in preference to the ethanol reflux method (Kahane *et al.*, 2001) due to better recovery of target analytes (F. Davis and L. A. Terry, Cranfield University, unpublished data). Vials of the slurry were placed in a shaking water bath at 55°C for 15 mins. They were removed briefly and shaken for 20 s every 5 min to prevent layering, and then left to cool. The cooled samples were filtered through a 0.2 µm Millex-GV syringe driven filter unit (Millipore Corporation, MA, USA) and stored at -40°C until required.

#### 4.3.8.2 NSC quantification

Non-structural carbohydrates were then quantified according to O'Donoghue *et al.* (2004) with modifications using a HPLC system comprising a P580 pump and GINA 50 autosampler (Dionex, CA, USA). Extracts were diluted 1:10 (v/v) with HPLC grade water immediately before analysis. The diluted extract (20 µl) was injected into a Rezex RCM monosaccharide Ca<sup>+</sup> size exclusion column of 300 mm x 7.8 mm diameter, 8 µm particle size (Phenomenex, CA, USA; Part no. 00H-0130-K0) with a Carbo-Ca<sup>2+</sup> security guard cartridge of 4 mm x 3mm diameter (Phenomenex; Part no. AJ0-4493). The mobile phase was HPLC grade water at a flow rate of 0.6 ml min<sup>-1</sup>. Column temperature was held at 75°C using a Dionex STH column thermostat. Eluted carbohydrates were monitored by an evaporative light scattering detector (ELSD 2420, Waters, MA, USA) connected to the Dionex system using a UCI-50 universal chromatography interface. The presence and abundance of fructose, glucose and sucrose were automatically calculated against external standards using Chromeleon version 4.6 software (Dionex). Assays were performed in triplicate.

#### 4.3.9 Statistical analysis

The data was analysed using analysis of variance (ANOVA) tests for each temperature separately. All statistical analyses were performed using Genstat for Windows Version 7.1.0.198 (VSN International Ltd., Herts., UK). Least significant difference values (LSD;  $P = 0.05$ ) were calculated for means separation using critical values of  $t$  for two-tailed tests. Data for sprout growth (Experiment 1) and ABA concentration (Experiments 1 and 2) were subjected to a log transformation to satisfy the assumptions of ANOVA. Statistical comparisons were made using the transformed means before they were back-transformed to arithmetic numbers for presentation and discussion. Tests for correlations between variables were made using Pearson's Rank Correlation. Correlations are presented with the Pearson's Correlation Coefficient ( $r$ ) and  $P$  value based on a two-tailed test.

## 4.4 Results

### 4.4.1 Experiment 1 - Consequences of a pre-harvest foliar spray of ABA or PBI-365

The onion bulbs in this experiment were exposed to four pre-harvest treatments: the application of  $10^{-4}$  M ABA in 0.5% (v/v) Li-700,  $10^{-4}$  M PBI-365 in 0.5% Li-700, 0.5% (v/v) Li-700 or water and three storage temperatures: 4°C, 12°C or 20°C. There was no consistent effect of pre-harvest treatment on any of the parameters measured. The main effects identified by ANOVA were due to cultivar and storage time. Therefore, the relationship between temporal changes in quality characteristics, bulb ABA concentration and bulb NSC concentration and the onset of sprout growth are presented.

#### 4.4.1.1 Pre-storage cultivar characteristics

Each cultivar was ranked according to the mean pre-storage ABA concentration, and the quality characteristics of dry weight, TSS, pungency and firmness (Table 4.4). Onions cv. SS1 consistently and significantly occupied the lowest rank, dividing the cultivars into two distinct groups – cvs. Carlos, Dinaro, Hysam, Red Baron and Renate constituting one, and cv. SS1 forming the second. Dry weight was positively correlated with TSS concentration (0.668;  $P<0.001$ ), and weakly with firmness (0.400,  $P<0.001$ ) and pyruvate concentration (0.278,  $P=0.002$ ). The TSS concentration was positively correlated with firmness (0.525,  $P<0.001$ ) and weakly with pyruvate concentration (0.429,  $P<0.001$ ). Firmness was also positively but weakly correlated with pyruvate concentration (0.203,  $P=0.03$ ). To summarise, bulbs with a high dry weight tended to be firm and to have high TSS and pyruvate concentrations. Before storage, there was a main effect of cultivar on ABA concentration ( $P<0.001$ ).

The three cultivars (Renate, Red Baron and SS1) that were analysed for NSCs were also ranked according to the mean pre-storage concentrations (Table 4.5). The pre-storage mean sucrose concentration was significantly greater ( $P<0.001$ ) in cvs. Red Baron and Renate than cv. SS1. Conversely, pre-storage fructose and glucose concentrations were significantly greater ( $P<0.001$ ) in cv. SS1 than cv. Renate, and least in cv. Red Baron. Before storage, onions cv. SS1 had the greatest mean concentration of total sugars ( $P<0.001$ ), followed by Renate and finally Red Baron. Onions cv. Red Baron had the highest mean total fructan concentration ( $P>0.001$ ), followed by cvs. Renate and SS1.

**Table 4.4.** The ranking of the cultivars Carlos, Dinaro, Hysam, Red Baron, Renate and SS1 according to mean pre-storage measurements (n=20) of dry weight, total soluble solids, pungency, firmness and abscisic acid.

Rank	Dry weight (mg g <sup>-1</sup> FW)		Total soluble solids (%)		Pyruvate (μmol g <sup>-1</sup> FW)		Firmness (N mm <sup>-1</sup> )		Abscisic acid (ng g <sup>-1</sup> DW)	
	Cultivar	Value	Cultivar	Value	Cultivar	Value	Cultivar	Value	Cultivar	Value
1	Red Baron	140.1 <sup>a</sup>	Red Baron	10.21 <sup>a</sup>	Dinaro	6.41 <sup>a</sup>	Renate	39.64 <sup>a</sup>	Renate	215.28 (2.333 <sup>a</sup> ) <sup>1</sup>
2	Carlos	125.9 <sup>b</sup>	Dinaro	9.36 <sup>b</sup>	Carlos	6.36 <sup>a</sup>	Dinaro	36.74 <sup>ab</sup>	Dinaro	191.87 (2.283 <sup>a</sup> )
3	Dinaro	125.6 <sup>b</sup>	Renate	9.31 <sup>b</sup>	Hysam	5.32 <sup>b</sup>	Red Baron	36.51 <sup>ab</sup>	Hysam	178.24 (2.251 <sup>ab</sup> )
4	Hysam	125.1 <sup>b</sup>	Carlos	9.06 <sup>b</sup>	Red Baron	5.04 <sup>b</sup>	Hysam	36.19 <sup>ab</sup>	Red Baron	132.74 (2.123 <sup>bc</sup> )
5	Renate	118.7 <sup>c</sup>	Hysam	9.00 <sup>b</sup>	Renate	5.02 <sup>b</sup>	Carlos	33.73 <sup>b</sup>	Carlos	129.42 (2.112 <sup>c</sup> )
6	SS1	73.0 <sup>d</sup>	SS1	4.77 <sup>c</sup>	SS1	3.68 <sup>c</sup>	SS1	16.36 <sup>c</sup>	SS1	110.41 (2.043 <sup>c</sup> )
LSD <sub>(P=0.05)</sub>		9.45	0.6067		0.711		6.154		0.1284 <sup>2</sup>	

<sup>abcd</sup> Different letters of superscript indicate significant difference ( $P>0.05$ ). <sup>1</sup> Values in brackets are log transformed means, back transformed means are shown above.

<sup>2</sup> LSD for log transformed means.



**Table 4.5.** The ranking of the cultivars Red Baron, Renate and SS1 according to mean pre-storage measurements (n=12) of glucose, sucrose, fructose and fructan concentration.

Rank	Glucose		Sucrose		Fructose		Total sugars (F+G+S)		Fructan	
	(mg g <sup>-1</sup> DW)		(mg g <sup>-1</sup> DW)		(mg g <sup>-1</sup> DW)		(mg g <sup>-1</sup> DW)		(mg g <sup>-1</sup> DW)	
	Cultivar	Value	Cultivar	Value	Cultivar	Value	Cultivar	Value	Cultivar	Value
1	SS1	286.1 <sup>a</sup>	Renate	169.0 <sup>a</sup>	SS1	276.9 <sup>a</sup>	SS1	643 <sup>a</sup>	Red Baron	340.3 <sup>a</sup>
2	Renate	175.8 <sup>b</sup>	Red Baron	153.4 <sup>a</sup>	Renate	65.3 <sup>b</sup>	Renate	410 <sup>b</sup>	Renate	220.7 <sup>b</sup>
3	Red Baron	159.4 <sup>b</sup>	SS1	79.5 <sup>b</sup>	Red Baron	33.3 <sup>c</sup>	Red Baron	346 <sup>c</sup>	SS1	19.8 <sup>c</sup>
LSD ( <i>P</i> =0.05)		21.85		42.81		18.84		54.3		35.11

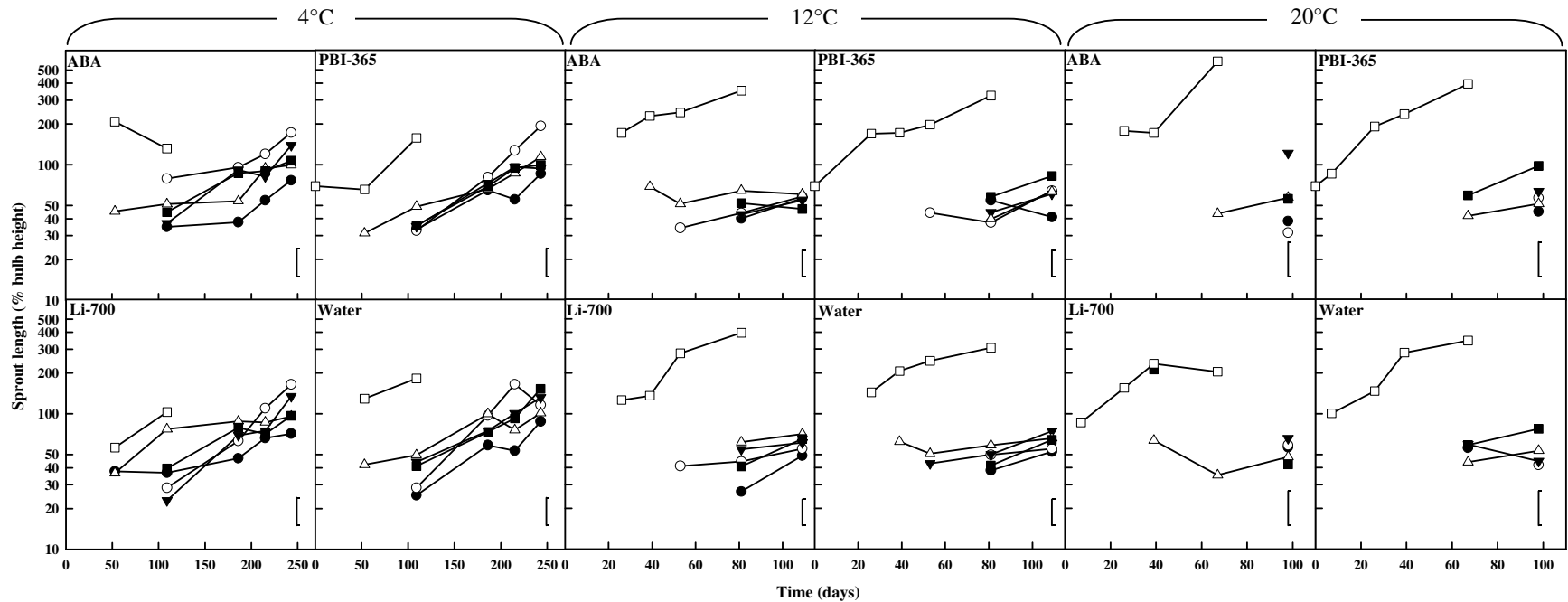
<sup>abc</sup> Different letters of superscript indicate significant difference (*P*>0.05)

#### 4.4.1.2 Sprout growth

Analysis of variance tests were performed on data for sprout length measured as a percentage of bulb height. Binomial regression analysis was also performed on the proportion of analysed bulbs that had begun to sprout, and allowed the estimation of the time when 50% of the bulbs from each sample had sprouted. As there were no consistent differences between the treatments, the mean for each cultivar at each storage temperature was estimated from the binomial curves (Table 4.6). Sprout length increased during storage at all temperatures ( $P < 0.001$ ; Figure 4.2).

**Table 4.6.** The time (days) taken for 50% of the bulbs to sprout at each storage temperature.

Storage temperature (°C)	Cultivar					
	Carlos	Dinaro	Hysam	Red Baron	Renate	SS1
4	105	102	107	50	99	30
12	82	75	77	65	76	10
20	100	90	65	45	10	7



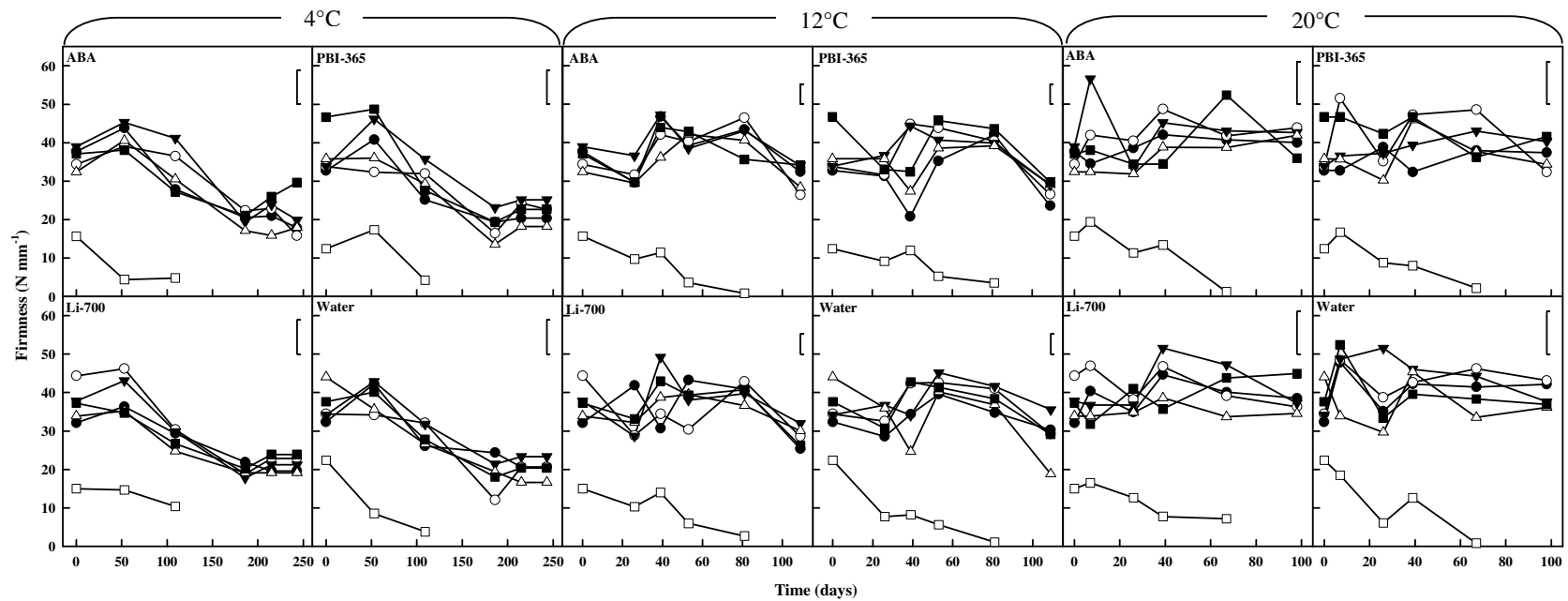
**Figure 4.2.** Sprout length of onion cvs. Carlos (●), Dinaro (○), Hysam (▼), Red Baron (▽), Renate (■) and SS1 (□) treated with a pre-harvest spray of  $10^{-4}$  M ABA in 0.5% (v/v) Li-700,  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 109 days or 20°C for 98 days ( $n=5$ ). LSD bars ( $P=0.05$ ) are shown based on 321, 193 and 120 d.f for 4°C, 12°C and 20°C respectively.

#### 4.4.1.3 Firmness

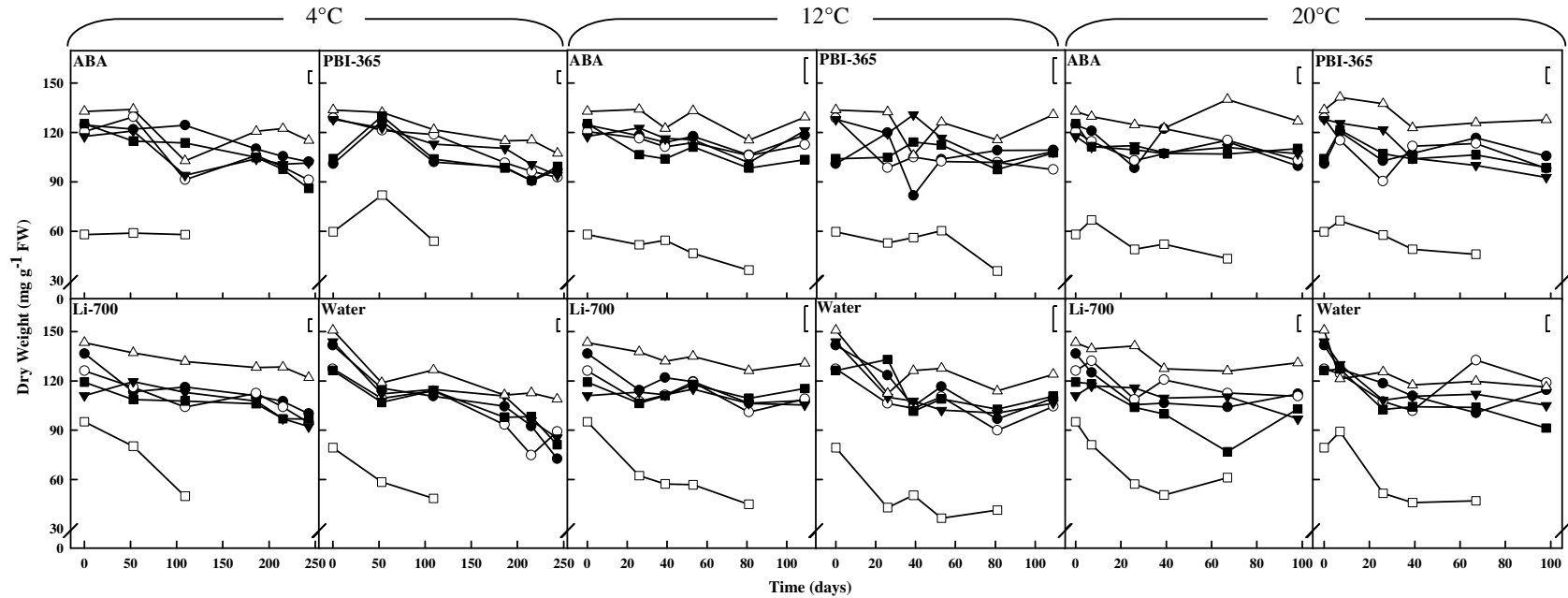
In onion bulbs stored at 4°C and 12°C, firmness decreased with time in storage ( $P < 0.001$ ) (Figure 4.3). At 20°C, there was also a significant effect of storage time ( $P < 0.001$ ), however this was only due to the decrease in firmness of cv. SS1, as firmness did not change in other cultivars.

#### 4.4.1.4 Dry weight

The dry weight of onion bulbs decreased during storage at all temperatures (4°C, 12°C and 20°C;  $P < 0.001$ ; Figure 4.4). In all cases the main cause of the variation was due to differences between cultivars, with increasingly smaller effects of day and treatment.



**Figure 4.3.** Firmness ( $\text{N mm}^{-1}$ ) of onion cvs. Carlos (●), Dinaro (○), Hysam (▼), Red Baron (▽), Renate (■) and SS1 (□) treated with a pre-harvest spray of  $10^{-4}$  M ABA in 0.5% (v/v) Li-700,  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 109 days or 20°C for 98 days ( $n=5$ ). LSD bars ( $P=0.05$ ) are shown based on 526, 556 and 551 d.f for 4°C, 12°C and 20°C respectively.



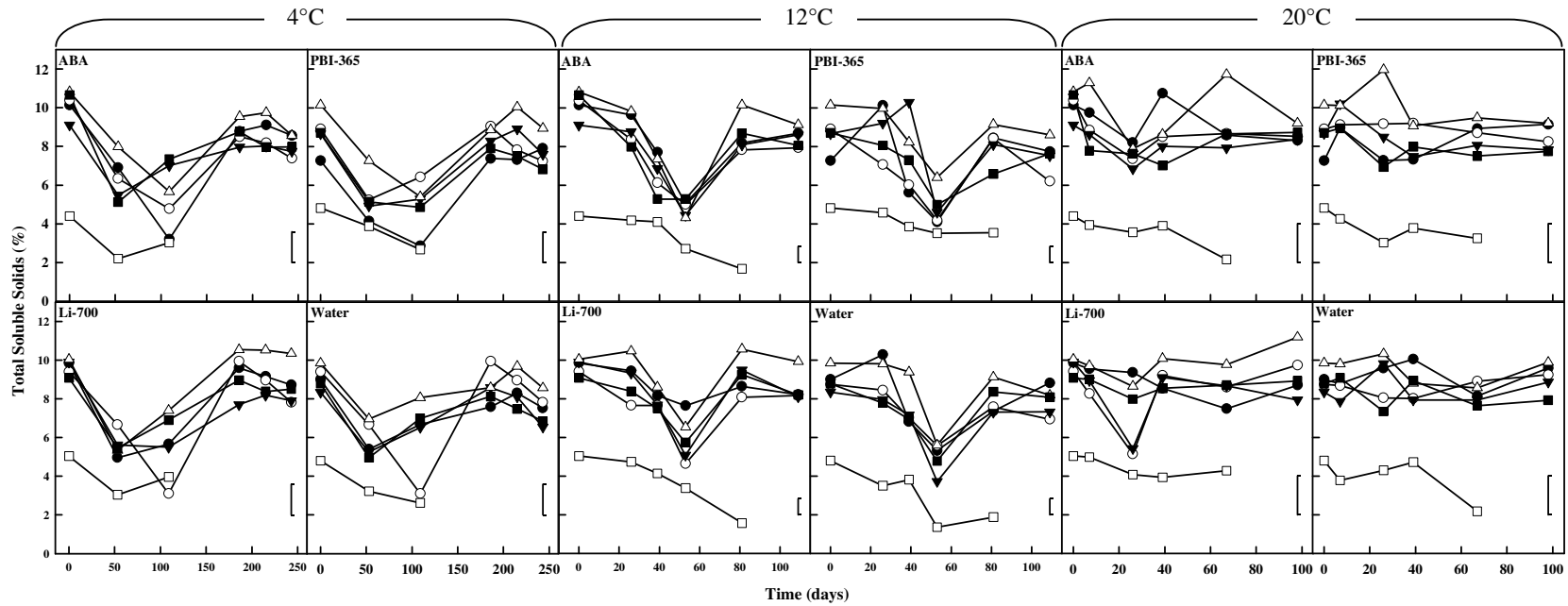
**Figure 4.4.** Dry weight of onion cvs. Carlos (●), Dinaro (○), Hysam (▼), Red Baron (Δ), Renate (■) and SS1 (□) treated with a pre-harvest spray of  $10^{-4}$  M ABA in 0.5% (v/v) Li-700,  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 109 days or 20°C for 98 days (n=5). LSD bars ( $P=0.05$ ) are shown based on 525, 558 and 555 d.f for 4°C, 12°C and 20°C respectively.

#### 4.4.1.5 Total soluble solids

The general trend for the change in TSS concentration during storage at 4°C was for a decrease from the beginning of storage until day 53 to 109, followed by a subsequent increase, resulting in no net change ( $P<0.001$ ; Figure 4.5). In onion bulbs stored at 12°C, TSS concentration decreased to a minimum in the middle of storage (day 53) and then increased again towards the end of storage ( $P<0.001$ ). During storage at 20°C, TSS concentration slightly decreased over time, with a small decrease in the middle of the storage period, followed by a smaller increase ( $P<0.001$ ). Throughout storage at all temperatures there was a main effect of cultivar ( $P<0.001$ ), with cv. Red Baron having the highest TSS concentration, and cv. SS1 the lowest.

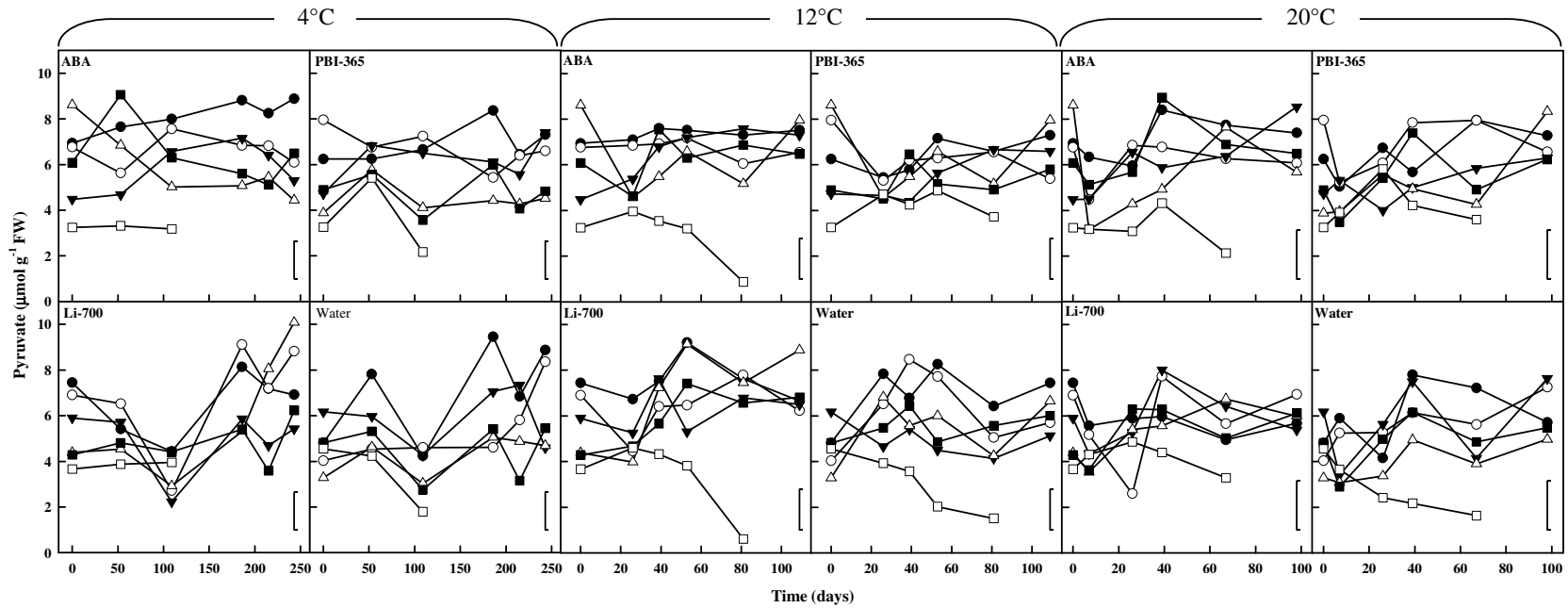
#### 4.4.1.6 Pyruvate concentration

Pyruvate concentration changed during storage at all temperatures ( $P<0.001$ ), and there was a net increase in mean pyruvate concentration during the storage period (Figure 4.6). However the pungency of bulbs cv. SS1 stored at 12°C or 20°C decreased, while that of bulbs cv. Red Baron increased. The pyruvate concentration of onion bulbs stored at 20°C reached a maximum in the middle of storage (day 39) in bulbs of cvs. Carlos, Renate, Dinaro and Hysam.



**Figure 4.5.** Total soluble solids concentration of onion cvs. Carlos (●), Dinaro (○), Hysam (▼), Red Baron (△), Renate (■) and SS1 (□) treated with a pre-harvest spray of  $10^{-4}$  M ABA in 0.5% (v/v) Li-700,  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 109 days or 20°C for 98 days (n=5). LSD bars ( $P=0.05$ ) are shown based on 526, 558 and 557 d.f. for 4°C, 12°C and 20°C respectively.





**Figure 4.6.** Pyruvate concentration of onion cvs. Carlos (●), Dinaro (○), Hysam (▼), Red Baron (△), Renate (■) and SS1 (□) treated with a pre-harvest spray of  $10^{-4}$  M ABA in 0.5% (v/v) Li-700,  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 109 days or 20°C for 98 days (n=5). LSD bars ( $P=0.05$ ) are shown based on 526, 559 and 557 d.f. for 4°C, 12°C and 20°C respectively.

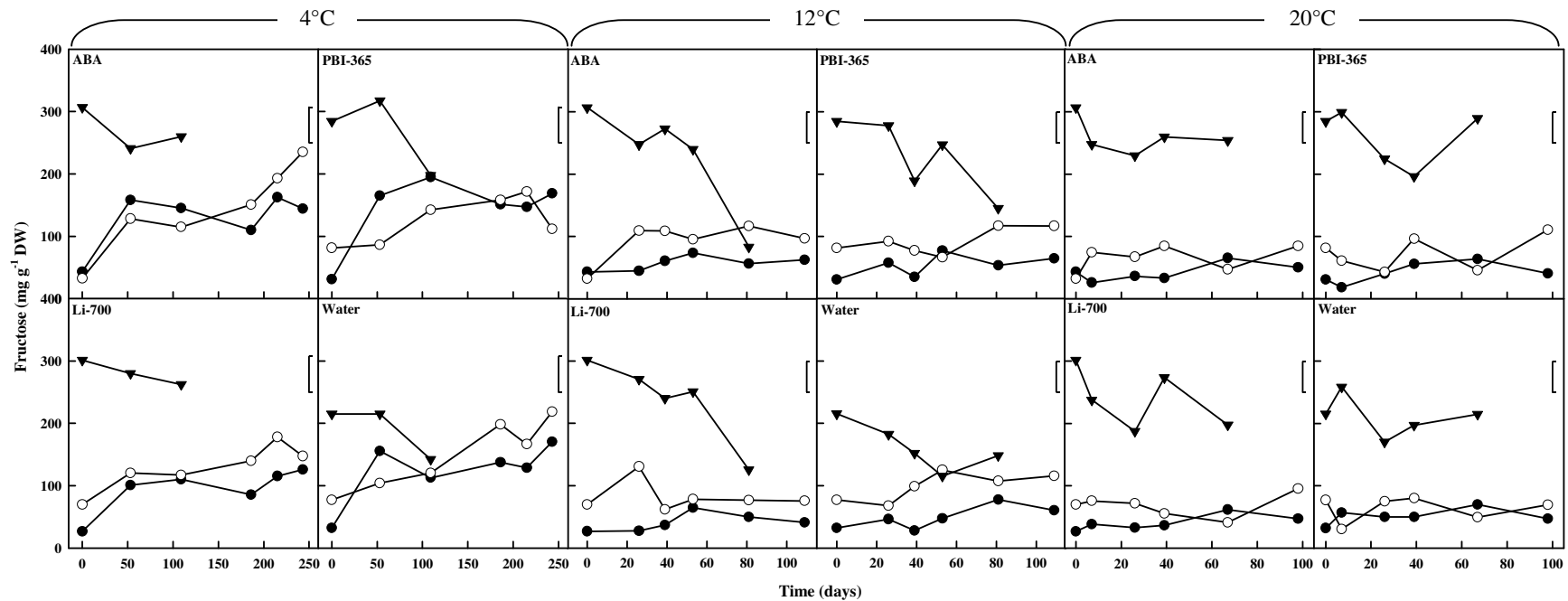
#### 4.4.1.7 Non-structural carbohydrates

Non-structural carbohydrates (fructose, glucose, sucrose and total fructans) were measured in three of the six cultivars; Red Baron, Renate and SS1. The relative amounts of individual carbohydrates varied between these cultivars.

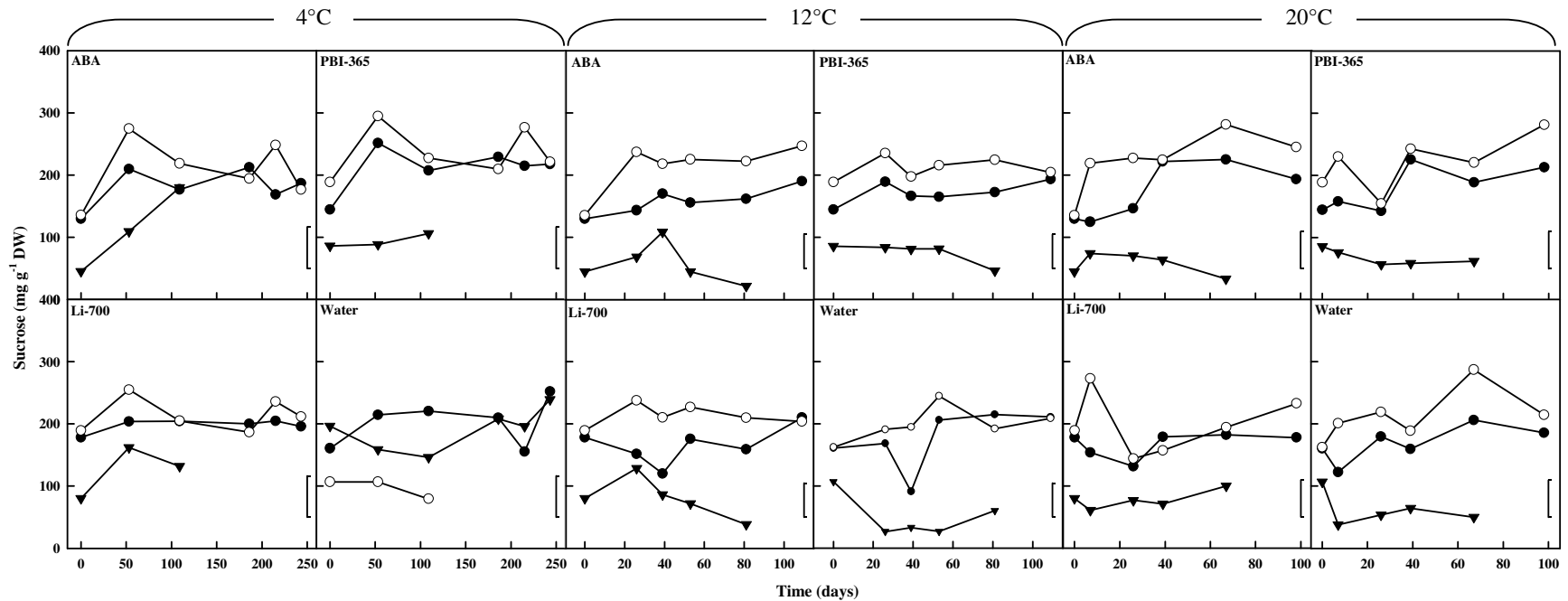
Fructose concentration in onions cv. SS1 stored at 4°C decreased during storage (Figure 4.7). Fructose concentration was stable during storage at 12°C in cvs. Renate and Red Baron, but decreased 2.2-fold in cv. SS1. There was a slight (1.4-fold) increase in fructose concentration in cvs. Renate and Red Baron, and a net decrease (1.2-fold) in cv. SS1, during storage at 20°C. Fructose concentration decreased between day 7 and 26 in cv. SS1 and then increased again until the end of storage.

The concentration of sucrose in onion bulbs cv. Red Baron stored at 4°C remained stable during storage (Figure 4.8), but there was a slight increase in sucrose concentration of onions cv. Renate (from 175.8 to 206.0 mg g<sup>-1</sup> DW) and a decrease in onions cv. SS1 (from 220.3 to 209.4 mg g<sup>-1</sup> DW). When cvs. Renate and Red Baron were stored at 12°C, sucrose concentration generally increased ( $P<0.001$ ) between day 0 and day 26. There was a decrease ( $P<0.001$ ) in sucrose concentration between days 39 and 81 during storage at 12°C in cv. SS1. Sucrose concentration tended to increase ( $P<0.001$ ) during storage at 20°C in cvs. Red Baron and Renate by 1.3- and 1.4-fold, respectively, whereas sucrose concentration in cv. SS1 decreased 1.3-fold during storage, although this was not significant.

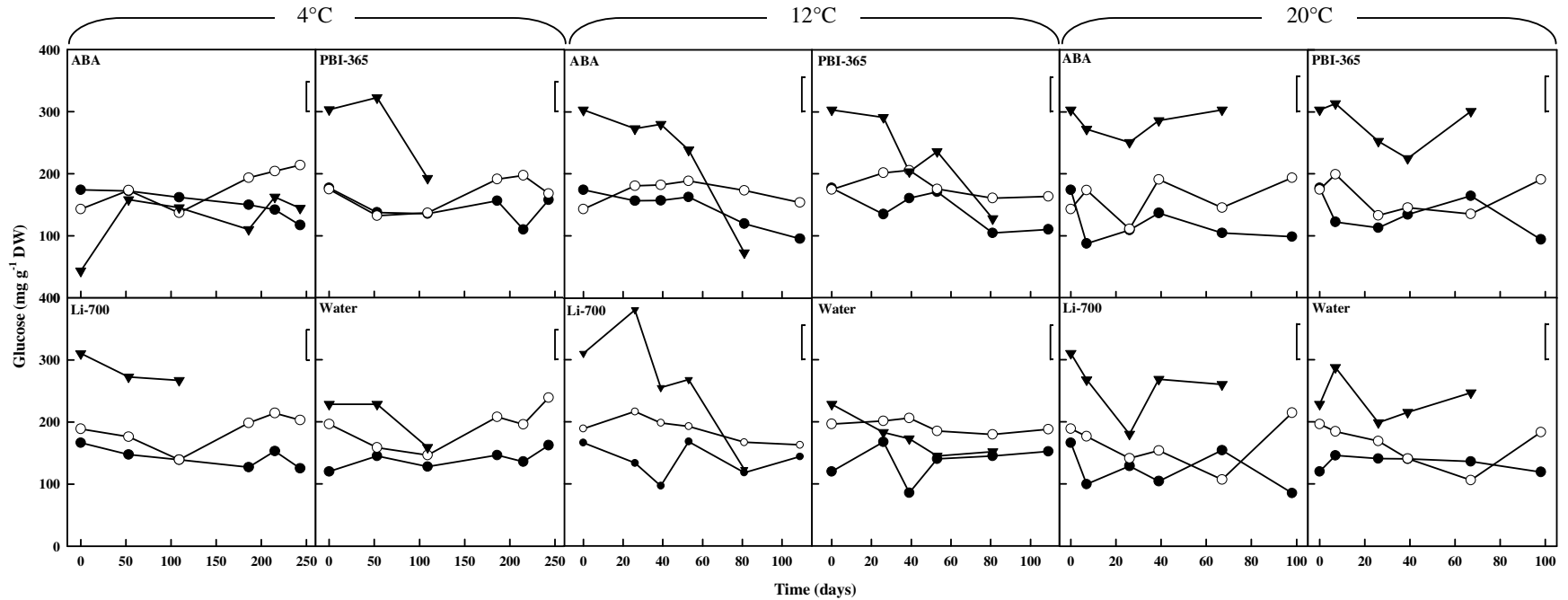
Glucose concentration in onions stored at 4°C changed differently in different cultivars. It decreased with time in cv. SS1, increased in cv. Renate, and was stable in Red Baron (Figure 4.9). The concentration of glucose remained stable in both cvs. Renate and Red Baron stored at 12°C, but decreased 2.4-fold in cv. SS1. Glucose concentration was relatively stable during storage at 20°C in cv. Renate. In cv. Red Baron, glucose concentration decreased between days 0 and 7, then increased between days 7 and 67, before decreasing again between day 67 and day 98. In cv. SS1, glucose concentration decreased between day 7 and 26, and then increased to the end of storage.



**Figure 4.7.** Fructose concentration of onion cvs. Red Baron (●), Renate (○) and SS1 (▼) treated with a pre-harvest spray of  $10^{-4}$  M ABA in 0.5% (v/v) Li-700,  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 109 days or 20°C for 98 days (n=3). LSD bars ( $P=0.05$ ) are shown based on 120, 136 and 132 d.f. for 4°C, 12°C and 20°C respectively.



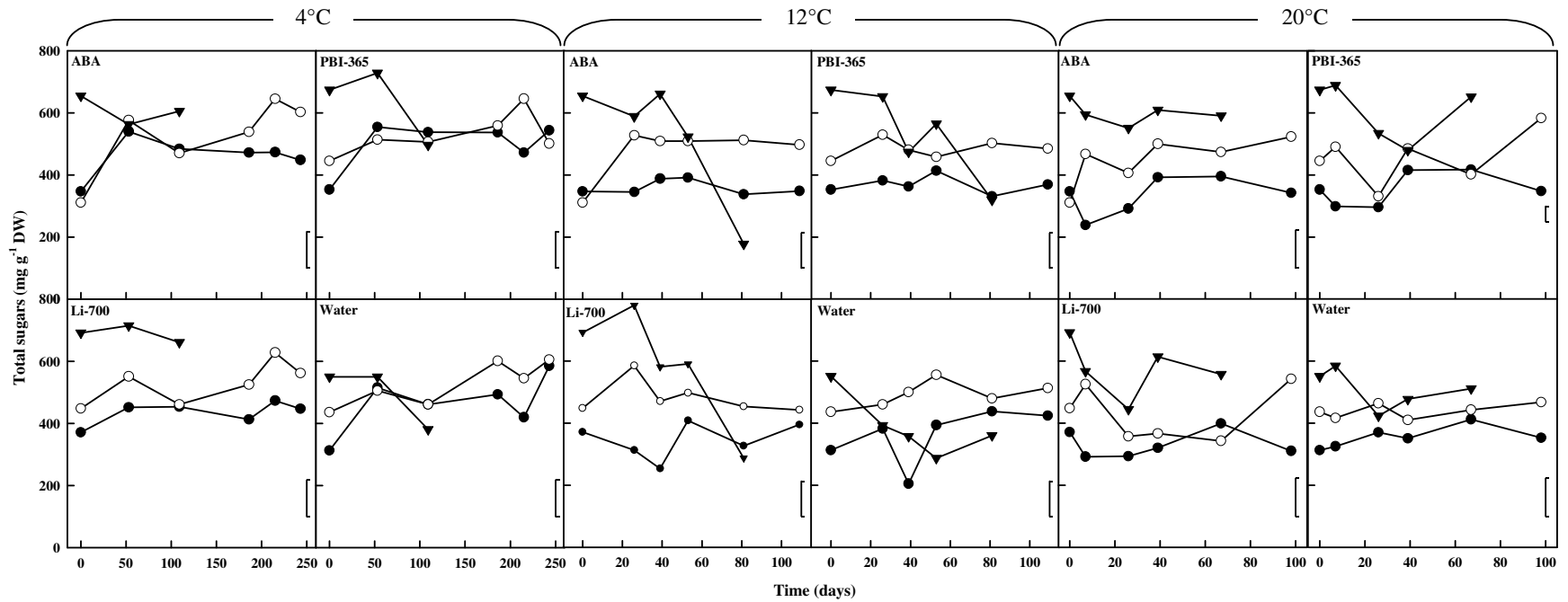
**Figure 4.8.** Sucrose concentration of onion cvs. Red Baron (●), Renate (○) and SS1 (▼) treated with a pre-harvest spray of  $10^{-4}$  M ABA in 0.5% (v/v) Li-700,  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 109 days or 20°C for 98 days (n=3). LSD bars ( $P=0.05$ ) are shown based on 120, 136 and 132 d.f. for 4°C, 12°C and 20°C respectively.



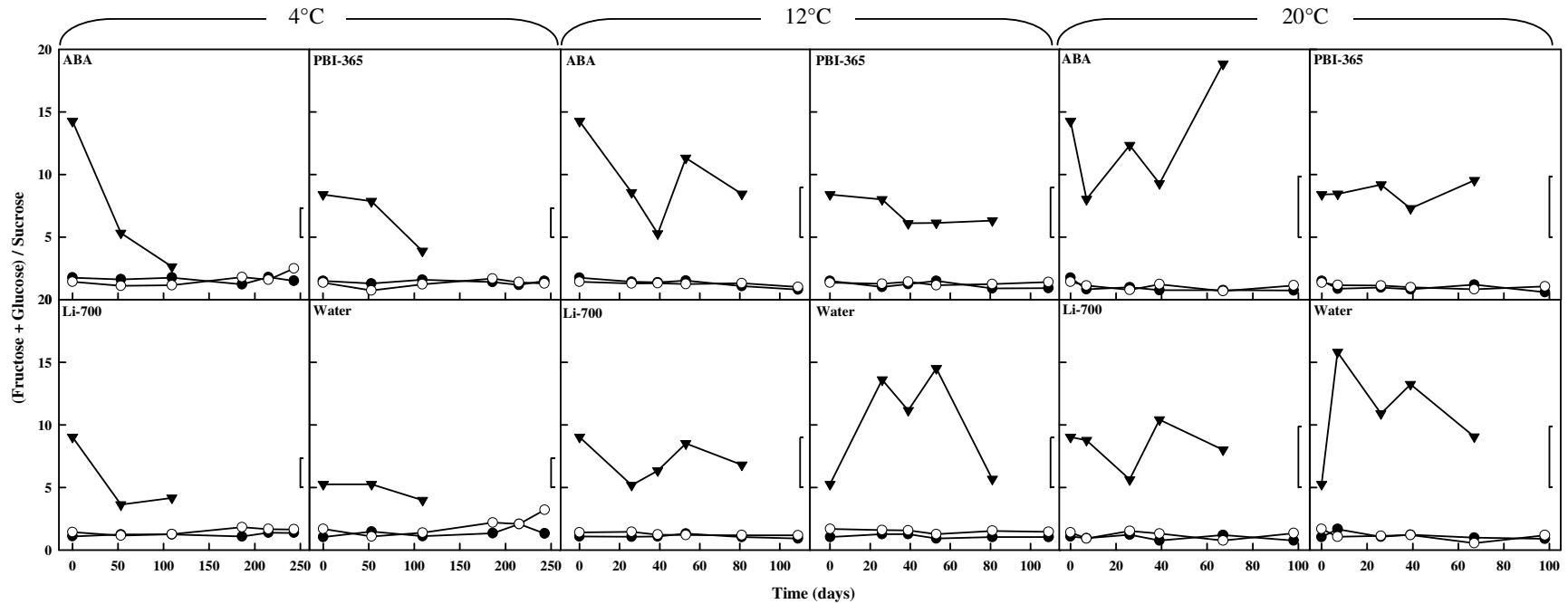
**Figure 4.9.** Glucose concentration of onion cvs. Red Baron (●), Renate (○) and SS1 (▼) treated with a pre-harvest spray of  $10^{-4}$  M ABA in 0.5% (v/v) Li-700,  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 109 days or 20°C for 98 days (n=3). LSD bars ( $P=0.05$ ) are shown based on 120, 136 and 132 d.f. for 4°C, 12°C and 20°C respectively.

Total sugar concentration was calculated as the sum of the fructose, glucose and sucrose concentrations. In general, total sugars increased in Renate and Red Baron during storage at all temperatures, but decreased in cv. SS1 (Figure 4.10). There was a slight increase in total sugar concentration in cvs. Renate and Red Baron, and a 1.9-fold decrease in cv. SS1 stored at 12°C. There was little change in total sugar concentration in cv. Red Baron during storage at 20°C, a net increase ( $P < 0.001$ ) in cv. Renate, but a net decrease in cv. SS1. Total sugar concentration in cv. SS1 decreased between day 0 and day 26, and then increased to the end of storage, day 67. Where a significant correlation between total sugars and TSS existed, it was negative and usually weak for cvs. Renate (4°C and 20°C no correlation; 12°C -0.440  $P > 0.001$ ) and Red Baron (4°C -0.488  $P > 0.001$ ; 12°C -0.330  $P = 0.005$ ; 20°C -0.453  $P > 0.001$ ), and positive for cv. SS1 (4°C and 20°C no correlation; 12°C 0.656  $P > 0.001$ ).

The ratio of monosaccharide to disaccharide content was calculated. There was no change in the ratio of monosaccharide to disaccharide in onions cv. Red Baron and Renate during storage at 4, 12 or 20°C; however the ratio decreased in cv SS1 during storage at 4°C, and changed erratically during storage 12 and 20°C. In general there was a higher ratio of monosaccharide to disaccharide in onions cv. SS1 than cvs. Renate or Red Baron (Figure 4.11).



**Figure 4.10.** Total sugars (glucose + sucrose + fructose) concentration of onion cvs. Red Baron (●), Renate (○) and SS1 (▼) treated with a pre-harvest spray of  $10^{-4}$  M ABA in 0.5% (v/v) Li-700,  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 109 days or 20°C for 98 days (n=3). LSD bars ( $P=0.05$ ) are shown based on 120, 136 and 132 d.f. for 4°C, 12°C and 20°C respectively.



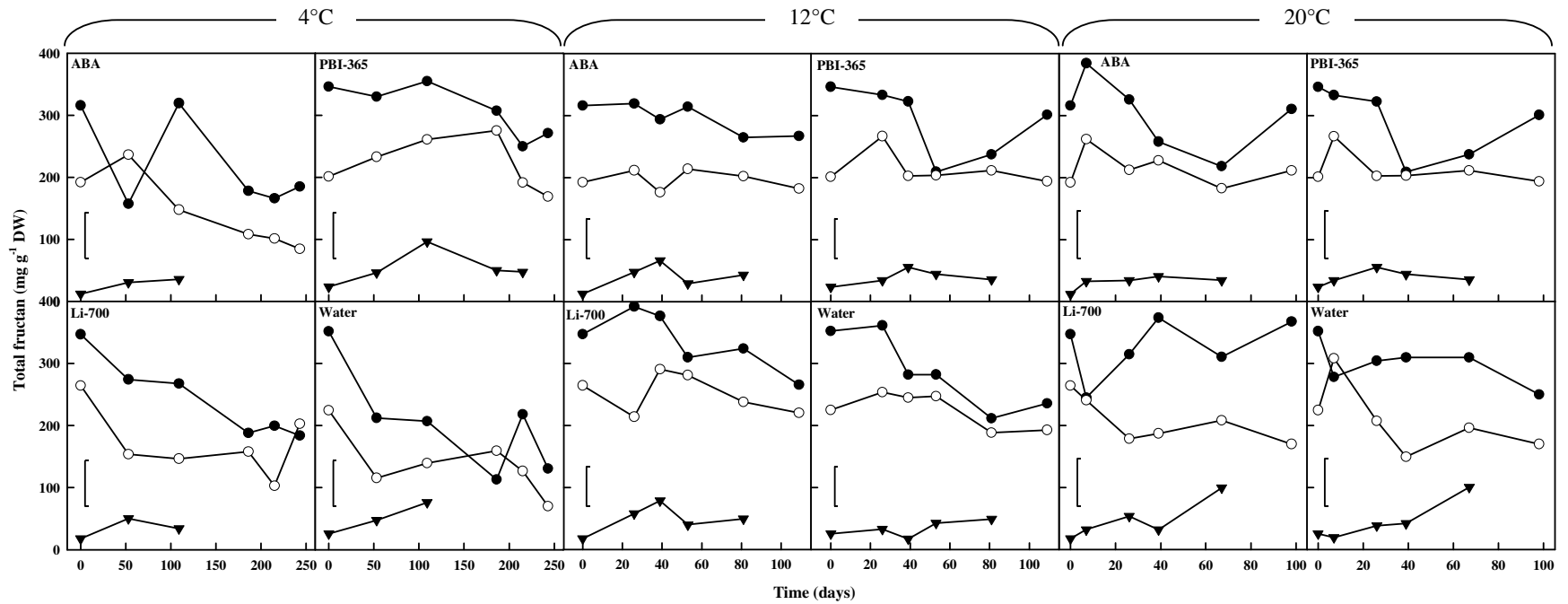
**Figure 4.11.** Ratio of monosaccharide : disaccharide ((fructose+glucose)/sucrose) of onion cvs. Red Baron (●), Renate (○) and SS1 (▼) treated with a pre-harvest spray of  $10^{-4}$  M ABA in 0.5% (v/v) Li-700,  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 109 days or 20°C for 98 days (n=3). LSD bars ( $P=0.05$ ) are shown based on 120, 136 and 132 d.f. for 4°C, 12°C and 20°C respectively.



Fructan concentration decreased in cvs. Red Baron and Renate stored at 4°C, but increased in cv. SS1 (Figure 4.12). There was no significant change in the fructan concentration in cvs. SS1 or Renate during storage at 12°C, although there was a peak at days 39 and 53, respectively, but fructan concentration decreased during storage in Red Baron ( $P<0.001$ ). There was very little change in the fructan concentration in cvs. Renate and Red Baron stored at 20°C, whilst the fructan concentration in cv. SS1 increased.

Fructan concentration was strongly negatively correlated with fructose (4°C -0.805; 12°C -0.812; 20°C -0.818;  $P<0.001$ ) and glucose (4°C -0.591; 12°C -0.503; 20°C -0.705;  $P<0.001$ ) concentration and positively correlated with sucrose concentration (4°C 0.331,  $P=0.002$ ; 12°C 0.565,  $P<0.001$ ; 20°C 0.279,  $P<0.001$ ). Fructose concentration was positively correlated with glucose (4°C 0.795, 12°C 0.790, 20°C 0.856;  $P<0.001$ ) and negatively correlated with sucrose (4°C -0.443, 12°C -0.465, 20°C -0.511;  $P<0.001$ ), and there was a negative correlation between sucrose and glucose (4°C -0.569, 12°C -0.183, 20°C -0.400;  $P<0.001$ ). To summarise, onions containing a high fructan concentration contained less fructose and glucose and more sucrose.

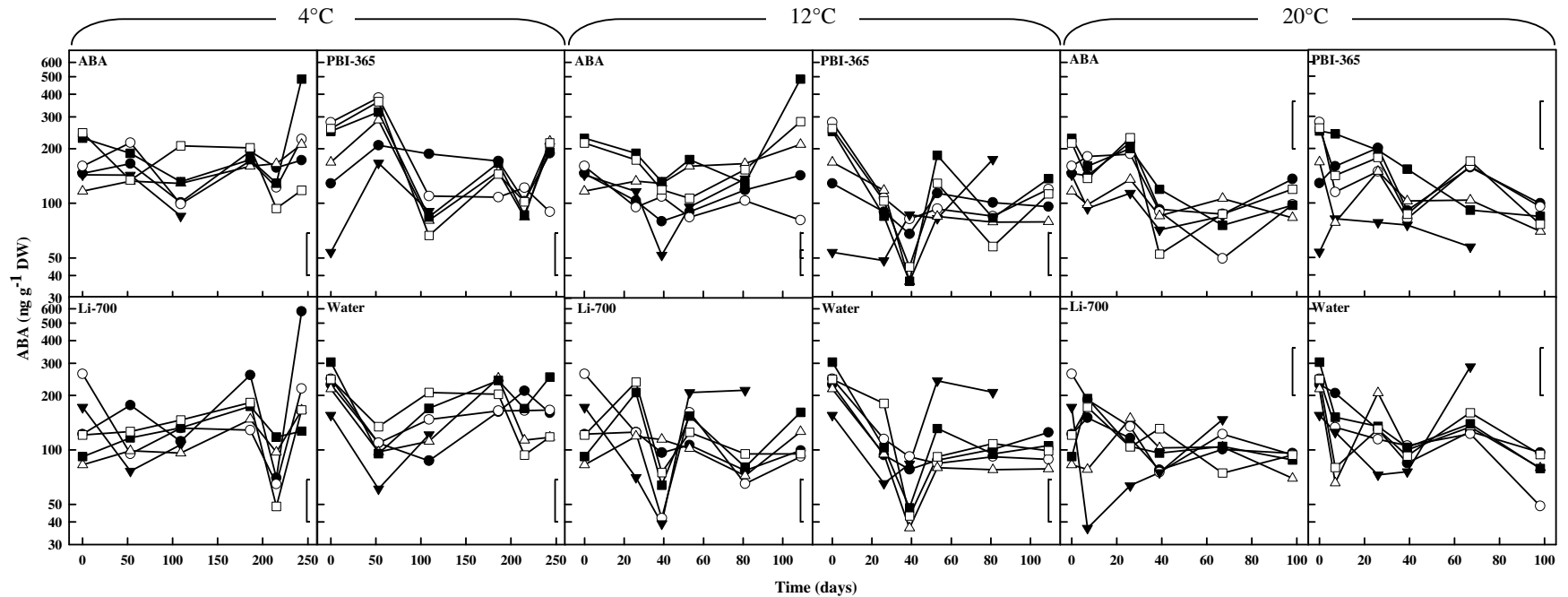
Where a significant correlation existed, the relationship between TSS and fructan concentration was positive in cvs. Renate (4°C 0.232  $P=0.049$ ; 12°C no correlation; 20°C 0.253  $P=0.033$ ) and Red Baron (4°C and 12°C no correlation; 20°C 0.414  $P<0.001$ ) and negative in cv. SS1 (4°C -0.439  $P=0.007$ ; 12°C and 20°C no correlation).



**Figure 4.12.** Total fructan concentration of onion cvs. Red Baron (●), Renate (○) and SS1 (▼) treated with a pre-harvest spray of  $10^{-4}$  M ABA in 0.5% (v/v) Li-700,  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 109 days or 20°C for 98 days (n=3). LSD bars ( $P=0.05$ ) are shown based on 120, 136 and 134 d.f. for 4°C, 12°C and 20°C respectively.

#### 4.4.1.8 Abscisic acid

Bulb ABA concentration decreased during storage ( $P<0.001$ ) at all temperatures (Figure 4.13). There was no consistent effect of pre-harvest treatment on ABA concentration. In most cases, a decrease in ABA concentration was followed by an increase towards the end of the storage period. The increase in ABA concentration was preceded by the time to 50% sprouting. There were no correlations between ABA concentration and sprout growth when analysed over the entire storage period, however, when only the data up until 50% sprouting for each cultivar was analysed, there was a significant negative correlation between ABA concentration and sprout growth at 4°C (-0.193;  $P<0.001$ ), 12°C (-0.164;  $P<0.001$ ) and 20°C (-0.088;  $P=0.021$ ).



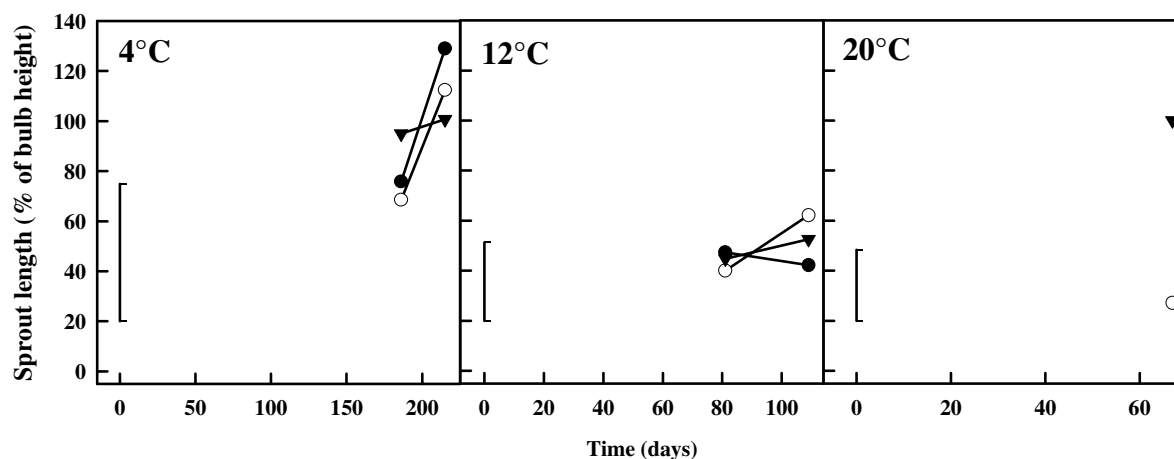
**Figure 4.13.** Abscisic acid concentration of onion cvs. Carlos (●), Dinaro (○), Hysam (▼), Red Baron (Δ), Renate (■) and SS1 (□) treated with a pre-harvest spray of  $10^{-4}$  M ABA in 0.5% (v/v) Li-700,  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 109 days or 20°C for 98 days (n=5). LSD bars ( $P=0.05$ ) are shown based on 524, 556 and 554 d.f for 4°C, 12°C and 20°C respectively.

#### 4.4.2 *Experiment 2- Consequences of postharvest bulb dip into solutions of ABA or PBI-365*

The onion bulbs in this experiment were exposed to three postharvest treatments – the application of  $10^{-4}$  M ABA in 0.5% (v/v) Li-700,  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700 or water, and three storage temperatures – 4°C, 12°C or 20°C. There was no consistent significant effect of postharvest treatment on any of the parameters measured. The main effects identified by ANOVA were due to storage time. Therefore the relationship between temporal changes in quality characteristics and ABA concentration and the onset of sprout growth are presented.

##### 4.4.2.1 *Sprout growth*

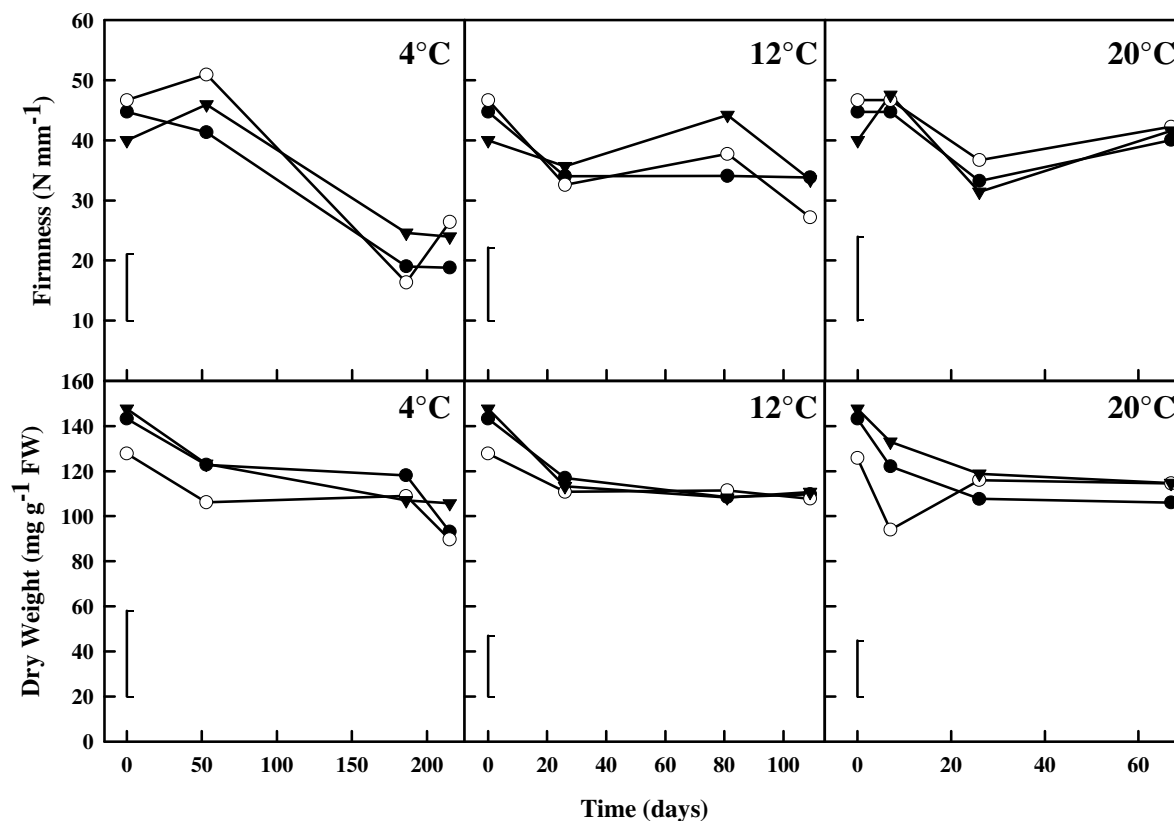
Sprout length increased significantly ( $P < 0.001$ ) during storage at 4°C and sprouting began between day 53 and day 186 (Figure 4.14). Sprout length increased during storage at 12°C ( $P < 0.001$ ) and 20°C ( $P = 0.008$ ).



**Figure 4.14.** Sprout growth of onion cv. Hysam bulbs treated with a postharvest soak in  $10^{-4}$  M ABA in 0.5% (v/v) Li-700 (●),  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700 (▼) or water (○) and stored at 4°C for 215 days, 12°C for 109 days or 20°C for 67 days (n=3). LSD bars ( $P=0.05$ ) are shown based on 39 d.f.

#### 4.4.2.2 Firmness and dry weight

Firmness decreased significantly during storage at all temperatures (4°C  $P<0.001$ , 12°C  $P=0.003$ , 20°C  $P=0.018$ ; Figure 4.15). Dry weight decreased significantly during storage at all temperatures (4°C  $P=0.001$ ; 12°C and 20°C  $P<0.001$ ; Figure 4.15), with significant decreases occurring between day 0 and day 53, and day 183 and day 215 at 4°C, and between day 0 and day 26 at 12°C. Firmness was positively correlated with pyruvate concentration (0.419;  $P=0.047$ ) and TSS (0.504;  $P=0.014$ ) in onions stored at 4°C. There was a positive correlation between TSS and dry weight (0.613,  $P=0.034$ ) in onions stored at 12°C.

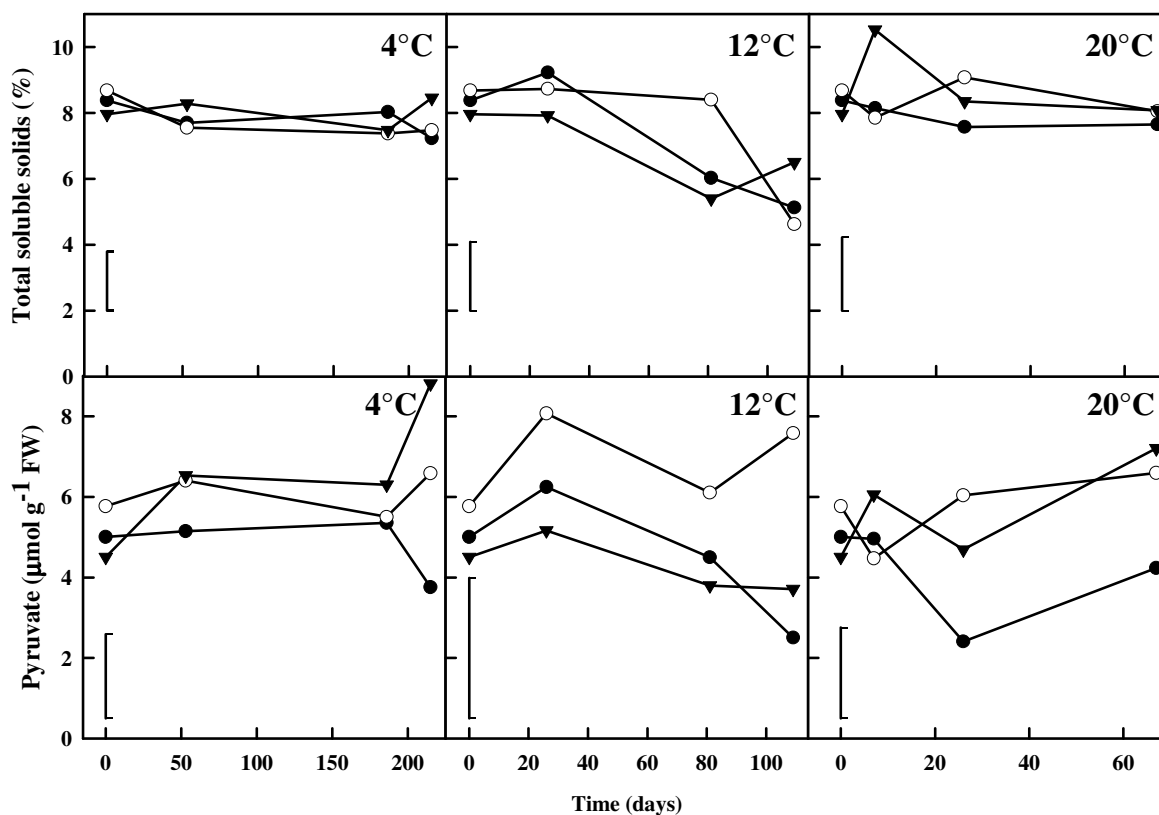


**Figure 4.15.** Firmness and dry weight of onion cv. Hysam bulbs treated with a postharvest soak in  $10^{-4}$  M ABA in 0.5% (v/v) Li-700 (●),  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700 (▼) or water (○) and stored at 4°C for 215 days, 12°C for 109 days or 20°C for 67 days (n=3). LSD bars ( $P=0.05$ ) are shown based on: firmness - 35, 36 and 35 d.f for 4°C, 12°C and 20°C respectively, dry weight - 39 d.f.

#### 4.4.2.3 Total soluble solids and pyruvate concentration

The TSS concentration of onion bulbs stored at 4°C or 20°C did not change during storage or with treatment (Figure 4.16), while TSS changed during storage at 12°C with a significant ( $P<0.001$ ) decrease occurring between days 26 and 81.

Pyruvate concentration did not change significantly during storage at 4°C, 12°C or 20°C (Figure 4.16). Total soluble solids content was positively correlated with pyruvate concentration (0.532,  $P=0.009$ ) in onions stored at 4°C.



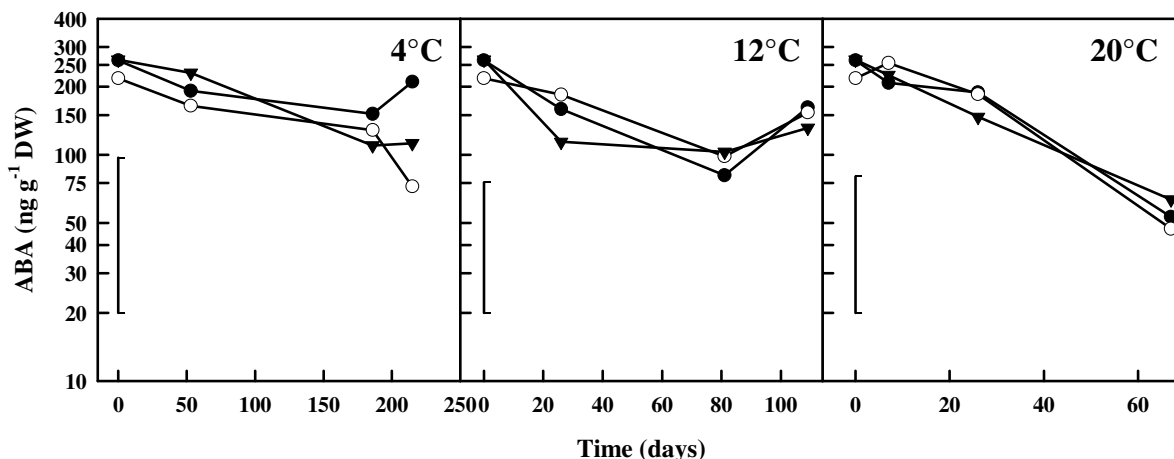
**Figure 4.16.** Total soluble solids and pyruvate concentration of onion cv. Hysam bulbs treated with a postharvest soak in  $10^{-4}$  M ABA in 0.5% (v/v) Li-700 (●),  $10^{-4}$  M PBI-365 in 0.5% (v/v) Li-700 (▼) or water (○) and stored at 4°C for 215 days, 12°C for 109 days or 20°C for 67 days (n=3). LSD bars ( $P=0.05$ ) are shown based on 39 d.f.

#### 4.4.2.4 Abscisic acid

Bulb ABA concentration decreased during storage at 4°C (Figure 4.17) and was negatively correlated with sprout length ( $-0.362$ ;  $P=0.009$ ). Similarly, ABA decreased during storage at 12°C ( $P<0.001$ ), but then increased between day 81 and 109, although this was not significant. Treatment did not have an effect, but there was a weak negative, although non-significant, correlation, between ABA and sprout length ( $-0.148$ ). Again, ABA decreased significantly during storage at 20°C ( $P<0.001$ ) and there was no



difference between treatments. Bulb ABA concentration was negatively, but not significantly, correlated with sprout length (-0.339) during storage at 20°C.



**Figure 4.17.** ABA concentration of onion cv. Hysam bulbs treated with a postharvest soak in 10<sup>-4</sup> M ABA in 0.5% (v/v) Li-700 (●), 10<sup>-4</sup> M PBI-365 in 0.5% (v/v) Li-700 (▼) or water (○) and stored at 4°C for 215 days, 12°C for 109 days or 20°C for 67 days (n=3). LSD bars ( $P=0.05$ ) are shown based on 39, 38 and 39 d.f. for 4°C, 12°C and 20°C respectively.

## 4.5 Discussion

Storage of onion bulbs grown in temperate regions and harvested in the summer can supply the locally-grown product almost all year round. Consumer demand for a residue-free product means that extended storage in the future will have to be accomplished without the use of the synthetic sprout suppressant maleic hydrazide. However, it is essential for the quality of onion bulbs to be maintained throughout storage. The following characteristics can be used to assess onion bulb quality: dry weight, firmness, pyruvate concentration, total soluble solids and carbohydrate concentration. These characteristics differed both between cultivars and during storage at different temperatures (4°C, 12°C and 20°C). By contrast, the ABA and ABA analogue

treatments applied to the onions had no consistent effect on the parameters measured, and the reasons for this are considered.

Firm onions are desirable and firmness was positively correlated with dry weight. Both firmness and dry weight decreased during storage, which was likely to be due to water loss in store. In general, the decrease was most pronounced in onions cv. SS1. Mild (low pungency) onions like cv. SS1 tend to have a thinner outer skin, and also have less outer skin layers than the other cultivars in the current study, allowing water to be lost more easily from the bulb.

Pyruvate concentration is an indicator of the pungency of an onion bulb. Pungency is an important characteristic, particularly for cultivars such as cv. SS1 that are intended to be marketed as mild onions, commanding a price premium, and whose pyruvate concentration must fall below the pre-defined limit of  $4 \mu\text{mol g}^{-1}$  FW (Crowther *et al.*, 2005). An understanding of the factors that affect this parameter and how it changes during storage is therefore desirable. There was a trend for an increase in pyruvate concentration during storage, meaning that onions removed from store will generally be more pungent than freshly harvested bulbs, similarly, pyruvate concentration increased in onion cv. Rouge Amposta increased during 22 weeks storage at  $20^{\circ}\text{C}$ , but there was no net change in those stored at  $4^{\circ}\text{C}$  (Benkeblia, 2000).

As has been previously observed (Chapter 3), a minimum in TSS content occurred around the middle of the storage period, and this was most pronounced in those onions stored at  $4$  and  $12^{\circ}\text{C}$ . The TSS concentration proved to be a poor indicator of total sugar (glucose + sucrose + fructose) concentration, with a positive relationship between TSS and total sugars existing in the low dry matter cultivar (SS1), and a negative relationship in the high dry matter cultivars (Red Baron and Renate). The opposite was true for the relationship with fructans, which indicates that the most likely cause of the change in refractive index was fructans in the high dry matter cultivars, and simple sugars in the low dry matter cultivar.

It has been proposed that the ABA concentration of onion bulbs is linked with storage potential (Chapter 3; Chope *et al.*, 2006). Sprouting in store occurred later in onion bulbs with higher concentrations of ABA before storage and onset of sprouting was

associated with minimal ABA concentration in agreement with results reported in Chapter 3. Similarly, ABA concentration decreased during storage of onions from experiment 1 at all temperatures, followed by a subsequent increase. The subsequent increase, which was not observed in Chapter 3, was thought to be due to synthesis of ABA by the growing sprout. There was no correlation between ABA and sprout length in the onions in experiment 1 (pre-harvest foliar spray). This was probably because the bulbs in this experiment were allowed to form external or advanced sprouts, and explains why ABA was not observed to increase in bulbs from experiment 2 (postharvest bulb soak), and in Chapter 3. Therefore, ABA concentration decreased until the sprout began to grow, when the ABA concentration began to rise. This confounds any linear correlations applied to the data. When the analysis was restricted to data collected up to 50% sprouting, a negative correlation was identified, which, combined with the similar relationship for bulbs cv. Hysam in the postharvest experiment, supports the hypothesis proposed in Chapter 3 that sprouting occurs at minimal ABA concentration. In the current study, the portion of the bulb sampled was a wedge of tissue taken from top to bottom, therefore, the changes observed in ABA concentration were not due to movement vertically within the bulb, but were a result of real changes in the total amount of ABA present.

There were no linear correlations between ABA concentration and non-structural carbohydrate concentration. All energy and nutrients required by the growing sprout in a stored onion must be sourced from within the bulb. A decrease in carbon reserves would therefore be expected to occur in conjunction with an increase in sprout growth. If ABA concentration is not related to the decrease in carbohydrate concentration, but is, as has been shown, correlated with storage life, it is important to consider why this might be. It could be that the carbohydrates measured are not the preferred carbon source for the sprout. Alternative energy sources are other carbon containing compounds such as the flavour precursors, *S*-alk(en)yl cysteine sulphoxides (ACSOs).

An *in vitro* study showed that treatment of basal, equatorial and near apical tissue with  $10^{-4}$  M ABA inhibited nucleolar activation, which supports a role for ABA in inhibition of cell elongation (Karagiannis and Pappelis, 1994). Therefore, ABA could be

involved in sprout growth by cell expansion, rather than cell division, which would not necessarily be expected to have a large effect on remobilisation of carbohydrates. An alternative explanation is that minimal ABA concentration is the trigger for the rate of sprout growth to increase. If this is the case then ABA concentration would not be expected to have a linear relationship with NSC concentration because it is the actual growth of the sprout that requires energy, not the trigger that begins the cascade.

The concentrations of fructans, sucrose, glucose and sucrose were determined in three of the six cultivars from experiment 1. Renate and Red Baron are high dry matter cultivars, and SS1 is a low dry matter cultivar. High dry matter cultivars tend to accumulate fructans during bulb formation which allows limited water uptake, and in this study, fructan was positively correlated with sucrose, and negatively correlated with fructose and glucose. All relationships between sugars are consistent with those reported previously (Jaime *et al.*, 2001).

A decrease in total fructan concentration was coincidental with an increase in fructose concentration in onions of all cultivars stored at 4°C and in cv. Red Baron stored at 20°C. It is probable that the decrease in fructan was accompanied by an increase in fructose due to the metabolism of fructan into fructose and sucrose. The pattern of change in sucrose concentration in onions of all cultivars during storage at 4°C was the inverse of the pattern of change in fructan concentration. This is likely to be due to the conversion of the freed fructose, from metabolism of fructans, into sucrose, which can then be used as energy by the growing sprout.

The time at which an internal sprout had begun to grow in 50% of bulbs of a particular cultivar was extrapolated from the binomial analysis. The time to 50% sprouting was followed by an increase in fructan concentration in onion bulbs cv. SS1 during storage at all temperatures. An increase in fructan concentration during storage in onions cv. SS1 was also observed in Chapter 5, and was attributed to the synthesis of fructans by the growing sprout. However, onset of sprouting was preceded by a decrease in fructan concentration in cvs. Renate and Red Baron stored at all temperatures, after which fructan concentration continued to decrease. Fructans represent a major carbohydrate reserve in onion bulbs cv. Renate and Red Baron, but only constitute a

minor reserve in cv. SS1. The rate of the decrease in fructan concentration was greatest at 4°C and least at 20°C.

A sharp increase in fructose concentration preceded the time to 50% sprouting, followed by little change to the end of storage in onions cv. Red Baron stored at 4°C, however, in onions cv. Renate, fructose increased steadily throughout storage. There was little change in fructose concentration during storage in onions cvs. Renate and Red Baron during storage at 12°C and 20°C. In onions cv. SS1 stored at all temperatures sprouting was followed by a decrease in fructose concentration. This represents the increased importance of free fructose as an energy source for onions cv. SS1 as they do not have fructan reserves to breakdown. A peak in glucose concentration was observed before breaking of dormancy in onions cv. Rouge Amposta stored at 18°C (Benkeblia and Selselet-Attou, 1999b) and this was also observed in onions cv. Renate during storage at 4 and 12°C, but not in cvs. Red Baron or SS1. This suggests that the peak in glucose concentration may be a cultivar-specific phenomenon.

Fructan is depolymerised into fructose, which is then converted to sucrose with the addition of glucose. Therefore, before sprouting occurs, no net loss of fructan would be expected, as when sucrose is abundant it is used as a fructosyl acceptor and so is made into fructan again. However, when sprout growth has begun, sucrose is transported to the sprout where it is metabolised. Therefore, total fructan concentration would decrease in conjunction with an increase in the concentration of fructose and sucrose. Sucrose concentration increased early in the storage period at all temperatures in cv. Renate which is consistent with observations made on onion cv. Tenshin stored at 10 and 20°C (Benkeblia *et al.*, 2005a).

The results of the current study show that the treatments applied to the crop had no consistent effect on the endogenous ABA concentration of onion bulbs. This could have been due to any of a number of reasons. It could be that the exogenous ABA or analogue did not successfully penetrate the leaf cuticle in the case of the pre-harvest treatment, or the outer skins in the case of the postharvest treatment. In field tests, the efficacy of ABA application can be diminished by rain (Zaharia *et al.*, 2005). Four days after the pre-harvest treatment, 10.1 mm of rain fell, and 18.3 mm six days after. This

may have had a detrimental effect on leaf penetration. Sprouting in dormant bulbs of *Allium wakegi* was delayed by 35 days by soaking in an aqueous solution of 100  $\mu\text{M}$  ABA two weeks after harvest, however, endogenous ABA concentration was not measured (Yamazaki *et al.*, 1999b). The concentration of endogenous ABA in bulbs of *Allium wakegi* was increased by soaking in 500  $\mu\text{M}$  ABA (Yamazaki *et al.*, 1999a), but the bulbs were not dried following treatment as bulbs in experiment 2 were. It may have been that the ABA did penetrate the outer leaves, but was depleted during the drying process.

The foliar spray of exogenous ABA may have entered the leaves but was not translocated to the bulb and therefore the concentration of ABA was not greater in the ABA-treated bulbs than the control bulbs. This may be because it was metabolised quickly in the leaf. Finally, the ABA may have entered the bulb, but was metabolised very quickly following entry to the bulb.

Alternative strategies to increase endogenous ABA concentration in onion bulbs using ABA or ABA analogues may be via injection into the bulb tissue (Abdel-Rahman and Isenberg, 1974) or as a root drench under controlled conditions such as a greenhouse. However, these methods are not suitable for commercial practice.

## 4.6 Conclusion

It may be of little benefit to attempt to increase endogenous ABA concentration by the application of exogenous ABA, either pre- or postharvest, unless a more efficient method can be found. The physiology of onion plants means that they present barriers to the influx of exogenous chemicals which must be overcome. This said, the results of this study provide further evidence that ABA plays an important role in onion storage life. A decrease in ABA concentration was correlated with an increase in sprout length; however, no clear relationship between ABA concentration and carbohydrate metabolism was elucidated. This suggests that ABA may play a role in the suppression of cell elongation rather than cell division, and so is not directly related to remobilisation of non-

structural carbohydrates (simple sugars and fructans), or as is perhaps more likely, minimal ABA concentration is a trigger in a cascade that results in an increase in the rate of sprout growth.

Efforts to manipulate ABA concentration in onions should be continued, but it may be more profitable to concentrate efforts to maximise endogenous ABA prior to storage using cultural methods such as adapting the curing and drying practice.

## 5.0 CHAPTER FIVE

### **The effect of 1-methylcyclopropene (1-MCP) on the physical and biochemical characteristics of onion cv. SS1 bulbs during storage**

#### **5.1 Abstract**

There is a paucity of information on the role of ethylene in onion bulb dormancy, and the available literature is conflicting. Onion cv. SS1 bulbs were treated with  $1 \mu\text{l l}^{-1}$  1-methylcyclopropene (1-MCP) for 24hr at 20°C and then stored at 4, 12 or 20°C. Sprout growth was reduced in onions treated with 1-MCP and stored at 4 or 12°C, but not when stored at 20°C. Greater concentrations of sucrose, glucose and fructose were measured in 1-MCP-treated bulbs stored at 12°C as compared with untreated bulbs. Dry weight was also maintained in onions treated with 1-MCP. ABA concentration before storage has previously been shown to be correlated with storage life, but there were no differences in the ABA concentration between 1-MCP-treated and untreated bulbs. It appeared that 1-MCP reduced the rate of carbon utilisation. The mechanism by which this occurred is unknown.

#### **5.2 Introduction**

The storage life of onion bulbs is limited by the rate of elongation of the sprout inside the bulb. Maleic hydrazide, a synthetic sprout suppressant, is used to extend storage life. However, pressure from retailers and consumers to reduce or eliminate chemical residues in food is increasing, and therefore other methods to prolong storage life of bulbs are necessary, particularly for low-pungency cultivars such as SS1 that command a premium price, but have an inherently short storage life. A better



understanding of the mechanisms involved in onion bulb dormancy would assist in the identification of potential targets for manipulation of storage life.

During over-winter storage of onion bulbs a gradual change in the relative composition of plant growth regulators occurs as the concentrations of growth inhibitors drop and those of growth promoters rise. The concentrations of inhibitors in bulbs with internal signs of sprouting are low when compared with the levels in non-sprouting or fully sprouted bulbs (Thomas, 1969). The variation in the concentration of gibberellins, cytokinins, auxins (Thomas, 1969; Thomas and Isenberg, 1972) and abscisic acid (Chapters 3 and 4), have been measured in stored onion bulbs. The peaks in growth substances are thought to be responsible for floral initiation under cold conditions (first gibberellin peak), cell multiplication (cytokinins) and the initiation of sprout growth (auxins). Onion bulb ABA concentration decreased during postharvest storage and onset of sprouting occurred at minimal ABA concentration (Chapters 3 and 4).

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 (1999b) found little variation in ethylene production (range of 4.4 – 4.6 nmol kg<sup>-1</sup> hr<sup>-1</sup>) 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.

Ethylene perception can be blocked using 1-methylcyclopropene (1-MCP), which binds to ethylene binding proteins, thereby preventing ethylene from exerting its effects (Blankenship and Dole, 2003). 1-MCP is approved for food use in several countries, and has been tested on a range of climacteric and non-climacteric fresh produce and cut flowers (Watkins and Miller, 2005; Watkins, 2006).

The aim of this study was to investigate the effect of 1-MCP on sprouting in onions cv. SS1, in order to assess its potential as a treatment to delay sprout growth and/or as a tool to explore the role of ethylene in onion dormancy. The changes in onion bulb ABA concentration, as well as characteristics associated with quality (pyruvate, total soluble solids (TSS) and firmness) and the changes in non-structural carbohydrates (NSC) were measured.

### 5.3 Materials and methods

#### 5.3.1 *Plant material and storage regime*

Onions cv. SS1 were grown from seeds drilled at a rate of 18 seeds m<sup>-1</sup> in March 2004 at Warwick HRI (Warks., UK). Pesticides were applied according to commercial practice. Maleic hydrazide was not applied. Plants were harvested at 80-90% tops down in early September. Onions were placed into 25 kg nets and loaded into bin driers. Hot air (*ca.* 30°C) was blown through the onions for nine days, followed by ambient air for a further two weeks (as per commercial practice). The dry aerial parts and roots were removed, and any diseased or damaged bulbs discarded prior to storage.

#### 5.3.2 *Application of 1-MCP*

Onions bulbs were placed in cardboard trays inside rigid polypropylene fumigation chambers (88 x 59 x 59 cm). A 1-MCP evolving solution was prepared by adding 1.80 g SmartFresh (Rohm and Haas, Philadelphia, USA) to a 50 ml conical flask, and sealing with a SubaSeal (Fisher, Leics., UK), then 20 ml distilled water at 50°C was injected into the flask through the seal (Dauny *et al.*, 2003). The flask was immediately opened and placed in the chamber with the onion bulbs. The chamber was closed with a

moat of water providing an air tight seal. This process achieved initial concentrations of  $0.962 \mu\text{l l}^{-1}$  1-MCP within the chamber. The chamber was kept sealed for 24 hr at  $20^{\circ}\text{C}$ .

### *5.3.3 1-MCP quantification*

The concentration of 1-MCP was quantified by flame ionisation gas chromatography (GC model 8340, EL980 FID and DP800 integrator, Carlo Erba Instruments, Herts., UK). Oven and detector temperatures were set at  $100^{\circ}\text{C}$  and  $250^{\circ}\text{C}$ , respectively. The 2 m x 4 mm stainless steel column was packed with Chromosorb PAW mesh range 80-100, liquid phase OV1701 30% loading (Jones Chromatography, Mid Glamorgan, UK). The carrier gas was helium (British Oxygen Company (BOC) Gases, Surrey, UK) at a flow rate of  $38 \text{ ml min}^{-1}$ . Calibration of 1-MCP was carried out against  $10.7 \mu\text{l l}^{-1}$  isobutane (BOC) (Sisler and Serek, 1997). The concentration of 1-MCP was  $0.962 \mu\text{l l}^{-1}$  after 2 hr.

### *5.3.4 Storage conditions and sampling regime*

Following exposure to 1-MCP, the bulbs were removed from the boxes and stored at three temperatures; 4, 12 or  $20^{\circ}\text{C}$ . Bulbs were removed for sampling before 1-MCP treatment (baseline (day 0),  $n=5$ ). Samples were taken after 53 and 109 days from the  $4^{\circ}\text{C}$  storage treatment, after 26, 39 and 53 days from the  $12^{\circ}\text{C}$  storage treatment and after 7, 26 and 39 days from the  $20^{\circ}\text{C}$  storage treatment. For all samples after the baseline,  $n=5$  for untreated bulbs and  $n=10$  for 1-MCP-treated bulbs.

### 5.3.5 Sample preparation

Juice was expressed from a *ca.* 5 mm equatorial slice using a hand-operated press (Randle and Bussard, 1993) then frozen at -20°C for pyruvate and TSS measurements. A longitudinal section cut from each bulb was snap-frozen in liquid nitrogen and kept at -40°C until the sample was lyophilised (Edwards Super Modulo, Sussex, UK) in preparation for fructan, NSC and ABA assays.

### 5.3.6 Physical assessments

The following physical assessments were made according to the methods reported in Chapter 3: sprout growth, firmness and dry weight. Briefly, sprout growth was recorded and expressed as the height of the first appearing green leaves inside the bulb as a percentage of bulb height; bulb firmness ( $\text{N mm}^{-1}$ ) was measured using an Instron Series IX materials testing machine (Instron, Bucks., UK) according to the method of Lancaster *et al.* (2001) with slight modifications, and dry weight was measured on lyophilised samples.

### 5.3.7 Biochemical assessments

The following biochemical assessments were made: ABA concentration, pyruvate concentration, concentration of NSCs and TSS. Abscisic acid concentration, pyruvate concentration and TSS (%) were measured according to the methods reported in Chapter 3. Briefly, ABA concentration was measured by radioimmunoassay, pyruvate by absorbance assay, fructan by enzyme assay and TSS by the use of a handheld refractometer (Palette 100, Atago Co. Ltd., Tokyo, Japan). Fructose, glucose and sucrose were measured using HPLC as described in Chapter 4.

### 5.3.8 Statistical analyses

All statistical analyses were carried out using Genstat for Windows Version 7.1.0.198 (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 1-MCP treatment at day 0 was the starting point for both treated and control bulbs), plus a factorial combination of storage time and chemical treatment for each temperature separately. 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.

## 5.4 Results

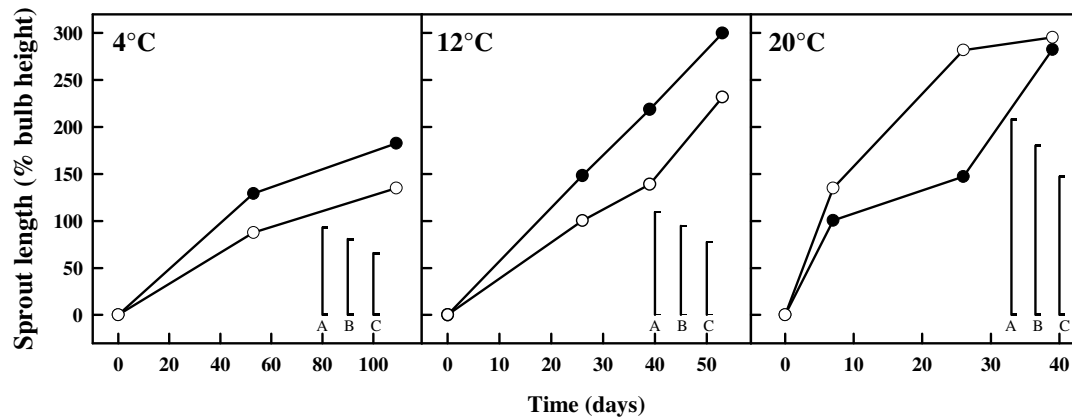
### 5.4.1 Effect of 1-MCP on physical properties of onion bulbs

Treatment with 1-MCP had an effect on the physical properties of onion cv. SS1 bulbs, with the effect varying according to storage temperature.

#### 5.4.1.1 Sprout growth

Sprout length increased with time in both untreated and 1-MCP-treated bulbs stored at 4°C ( $P=0.07$ ), 12°C ( $P<0.001$ ) and 20°C ( $P=0.023$ ) (Figure 5.1). The overall mean sprout length expressed as a percentage of bulb height was smaller ( $P=0.021$ ) in the onions treated with 1-MCP (157% of bulb height) than in the control onions (222% of bulb height) stored at 12°C. Similarly, in onions stored at 4°C, sprout length was also less in bulbs treated with 1-MCP (111% of bulb height) compared to the untreated bulbs (156% of bulb height), however this was not significant ( $P=0.121$ ). Conversely, in onions stored at 20°C, sprout length was greater in bulbs treated with 1-MCP (237% of

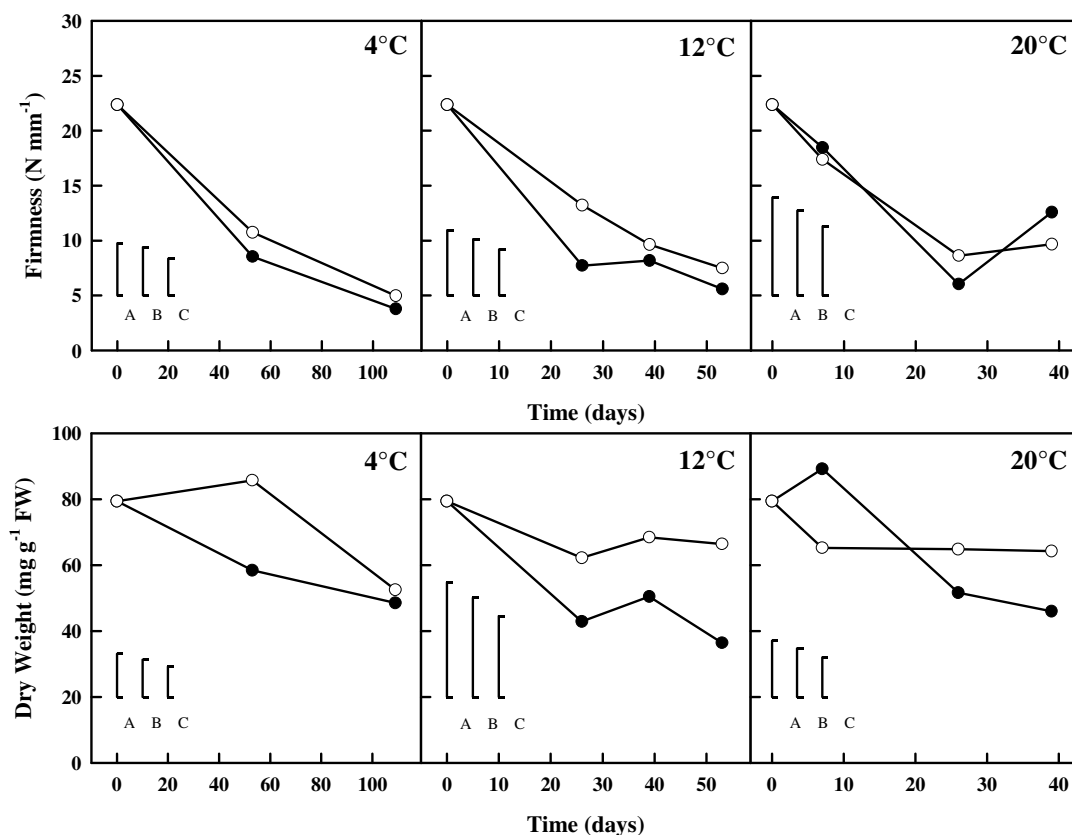
bulb height) compared with the untreated bulbs (177% of bulb height), however this was also not significant ( $P=0.246$ ).



**Figure 5.1.** Sprout length in onions treated with  $1 \mu\text{l l}^{-1}$  1-MCP at  $20^\circ\text{C}$  for 24 hours (open symbols) and untreated onions (controls, closed symbols) stored at  $4^\circ\text{C}$  for 109 days,  $12^\circ\text{C}$  for 53 days or  $20^\circ\text{C}$  for 39 days. Controls  $n=5$ , 1-MCP  $n=10$ . LSD bars ( $P=0.05$ ) A=5,5; B=10,5; C=10,10.

#### 5.4.1.2 Firmness and dry weight

Firmness of onions decreased ( $4^\circ\text{C}$   $P<0.001$ ,  $12^\circ\text{C}$   $P=0.038$ ,  $20^\circ\text{C}$   $P=0.001$ ) under each storage temperature regime (Figure 5.2). Bulbs treated with 1-MCP and stored at  $12^\circ\text{C}$  were 1.4-fold firmer ( $\text{N mm}^{-1}$ ) ( $P=0.051$ ) than untreated bulbs.



**Figure 5.2.** Firmness and dry weight of onions treated with  $1 \mu\text{l l}^{-1}$  1-MCP at  $20^\circ\text{C}$  for 24 hours (open symbols) and untreated onions (controls, closed symbols) stored at  $4^\circ\text{C}$  for 109 days,  $12^\circ\text{C}$  for 53 days or  $20^\circ\text{C}$  for 39 days. Controls  $n=5$ , 1-MCP  $n=10$ . LSD bars ( $P=0.05$ ) A=5,5; B=10,5; C=10,10.

The dry weight of bulbs stored at  $4^\circ\text{C}$  decreased during storage ( $P=0.042$ ), with those bulbs treated with 1-MCP having a greater mean dry weight ( $71.5 \text{ mg g}^{-1} \text{ FW}$ ) than untreated bulbs ( $53.5 \text{ mg g}^{-1} \text{ FW}$ ) ( $P=0.094$ ). This was due to a delayed decrease in dry weight in the 1-MCP-treated bulbs (Figure 5.2). The dry weight of bulbs stored at  $12^\circ\text{C}$  decreased significantly ( $P<0.001$ ) between days 0 and 26, but did not change significantly after that. However, the mean dry weight of 1-MCP-treated bulbs ( $65.7 \text{ mg g}^{-1} \text{ FW}$ ) was greater ( $P<0.001$ ) than that of untreated bulbs ( $43.3 \text{ mg g}^{-1} \text{ FW}$ ). There was a significant ( $P=0.007$ ) decrease in dry weight during storage at  $20^\circ\text{C}$ , and while 1-MCP-treated bulbs maintained a stable dry weight during

storage, that of untreated bulbs decreased, with the greatest decrease occurring between days 7 and 26 ( $P<0.001$ ).

#### 5.4.2 *Effect of 1-MCP on biochemical properties of onion bulbs*

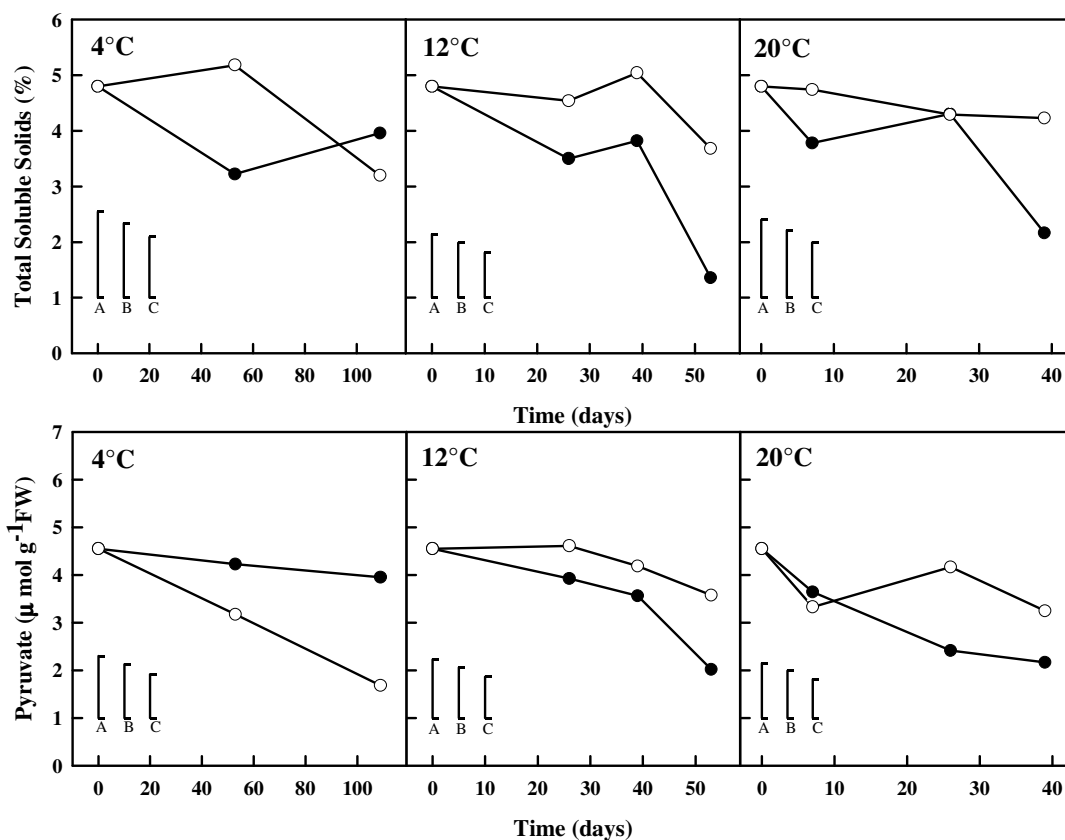
Treatment with 1-MCP affected biochemical properties of onion cv. SS1 bulbs, with the effect, particularly on non-structural carbohydrates, varying according to storage temperature.

##### 5.4.2.1 *Total soluble solids and pungency*

The TSS % decreased over time in onion bulbs stored at 4°C ( $P=0.002$ ) and 12°C ( $P<0.001$ ), and the overall mean was higher (4°C  $P=0.01$ ; 12°C  $P<0.001$ ) in 1-MCP-treated bulbs than untreated bulbs (Figure 5.3). In bulbs stored at 4°C this was mainly due to the observations made at day 53, and mirrors the pattern for changes in dry weight at this temperature. There were no significant main effects or interactions identified by ANOVA on the data for onions stored at 20°C.

The pungency (as determined by pyruvate concentration) of onion bulbs stored at 4°C decreased ( $P<0.001$ ) during storage, but there was no difference between 1-MCP-treated and untreated bulbs (Figure 5.3). Similarly, pyruvate concentration decreased during storage in bulbs stored at 12°C ( $P=0.002$ ), however, 1-MCP-treated bulbs had a greater mean pyruvate concentration than untreated bulbs ( $P=0.003$ ). Pyruvate concentration again decreased significantly ( $P=0.005$ ) between day 0 and day 7 in bulbs stored at 20°C, and mean pyruvate concentration was greater ( $P<0.05$ ) in treated than untreated bulbs.



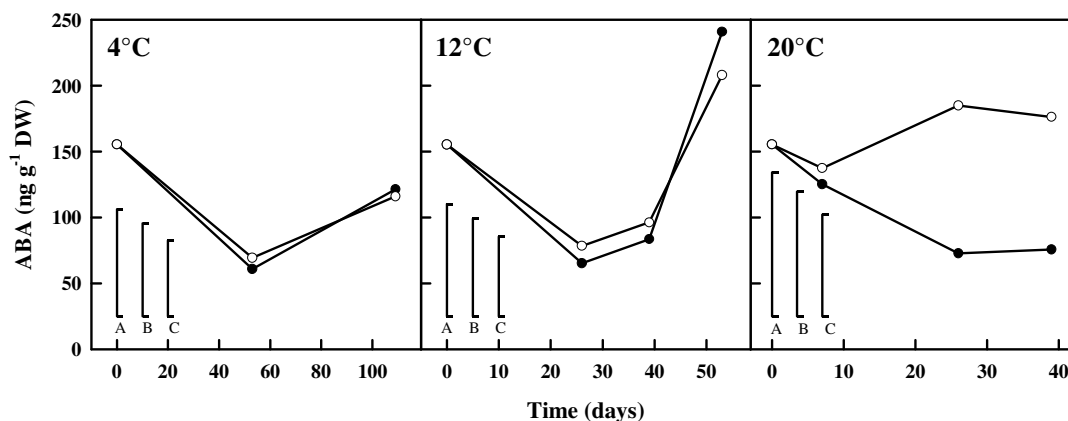


**Figure 5.3.** Total soluble solids (%) and pyruvate concentration of onions treated with  $1 \mu\text{l l}^{-1}$  1-MCP at 20°C for 24 hours (open symbols) and untreated onions (controls, closed symbols) stored at 4°C for 109 days, 12°C for 53 days or 20°C for 39 days. Controls  $n=5$ , 1-MCP  $n=10$ . LSD bars ( $P=0.05$ ) A=5,5; B=10,5; C=10,10.

#### 5.4.2.2 Abscisic acid

In the onions stored at both 4°C and 12°C, ABA concentration changed significantly over time ( $P=0.034$  and  $P<0.001$ , respectively). The concentration of ABA decreased from that present before the 1-MCP treatment (day 0) until the middle of the storage period, before increasing again towards the end of the storage period (Figure 5.4). The pattern of change was similar for both treated and untreated bulbs, and there was no significant difference in the mean ABA concentrations between treated and untreated

bulbs. Onion bulbs stored at 20°C and treated with 1-MCP had a higher ( $P=0.008$ ) overall mean ABA concentration (166 ng g<sup>-1</sup> DW) than untreated bulbs (91 ng g<sup>-1</sup> DW). After day 7, the ABA concentration in 1-MCP-treated bulbs increased, whilst in untreated controls it continued to decline.

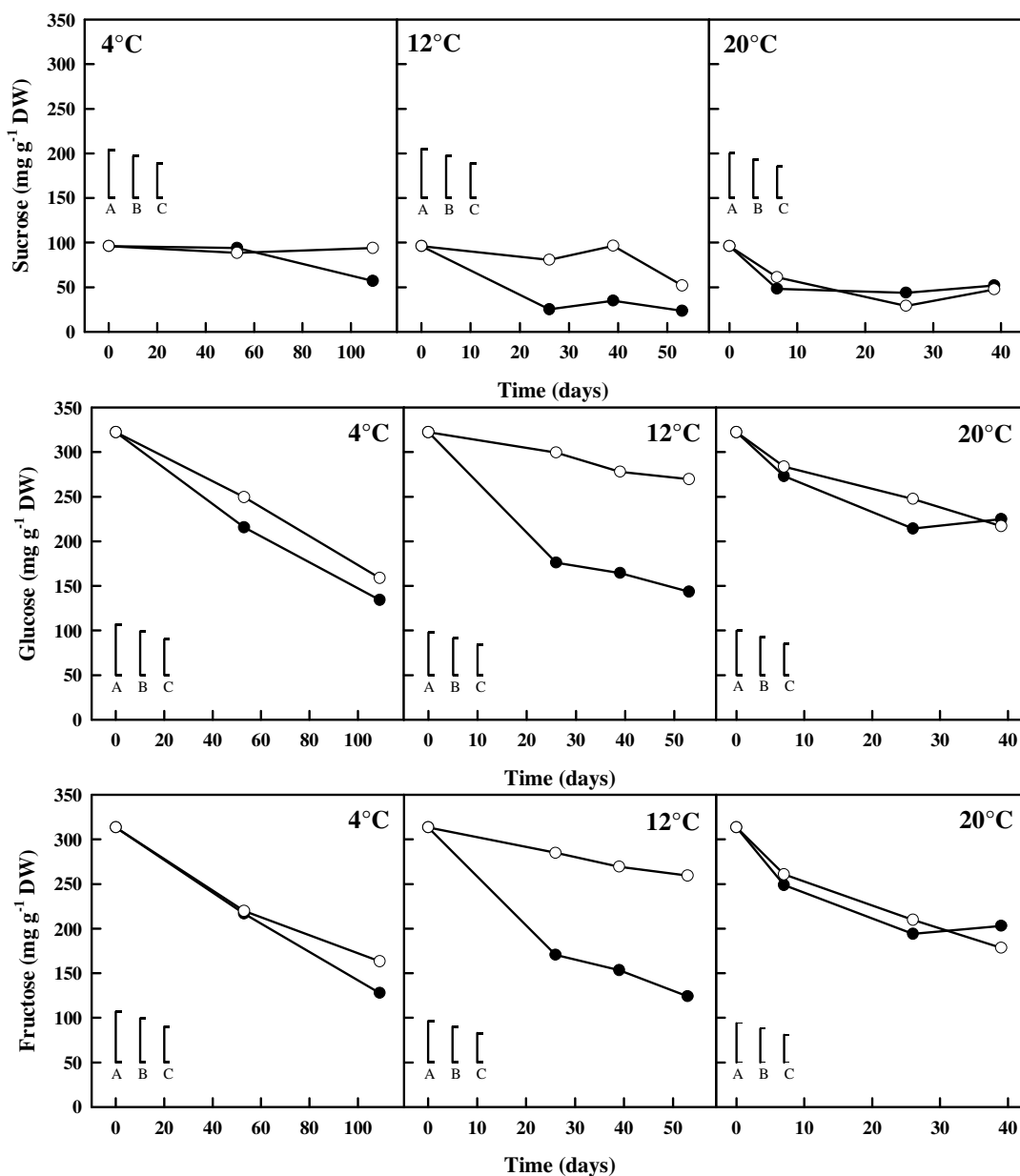


**Figure 5.4.** ABA concentration in onions treated with 1  $\mu\text{l l}^{-1}$  1-MCP at 20°C for 24 hours (open symbols) and untreated onions (controls, closed symbols) stored at 4°C for 109 days, 12°C for 53 days or 20°C for 39 days. Controls  $n=5$ , 1-MCP  $n=10$ . LSD bars ( $P=0.05$ ) A=5,5; B=10,5; C=10,10.

#### 5.4.2.3 Non-structural carbohydrates

Onion bulbs cv. SS1 were characterised by similar concentrations of fructose and glucose, with a comparatively low concentration of sucrose (approximately 2-4 fold less than glucose or fructose), which is in general agreement with Terry *et al.* (2005) and F. Davis and L. A. Terry (Cranfield University, unpublished), using the Kahane *et al.* (2001) or modified O'Donoghue *et al.* (2004)-based extraction methods, respectively. The sucrose concentration decreased between day 0 and all other times in 1-MCP-treated onions stored at 12°C ( $P<0.001$ ) and all bulbs stored at 20°C ( $P=0.011$ ). The only effect of treatment with 1-MCP was observed in onions stored at 12°C, where onions treated with 1-MCP had a greater ( $P<0.001$ ) sucrose concentration than untreated controls

(Figure 5.5). Glucose concentration decreased significantly in onions held at 4°C ( $P<0.001$ ), 12°C ( $P=0.088$ ) and 20°C ( $P<0.001$ ). Glucose concentration was greater ( $P<0.001$ ) in 1-MCP-treated onions stored at 12°C compared with untreated bulbs. Fructose concentration decreased significantly during storage at all three temperatures (4°C  $P<0.001$ ; 12°C  $P=0.061$ ; 20°C  $P<0.001$ ). There were no main effects or interactions between 1-MCP treatment and time.



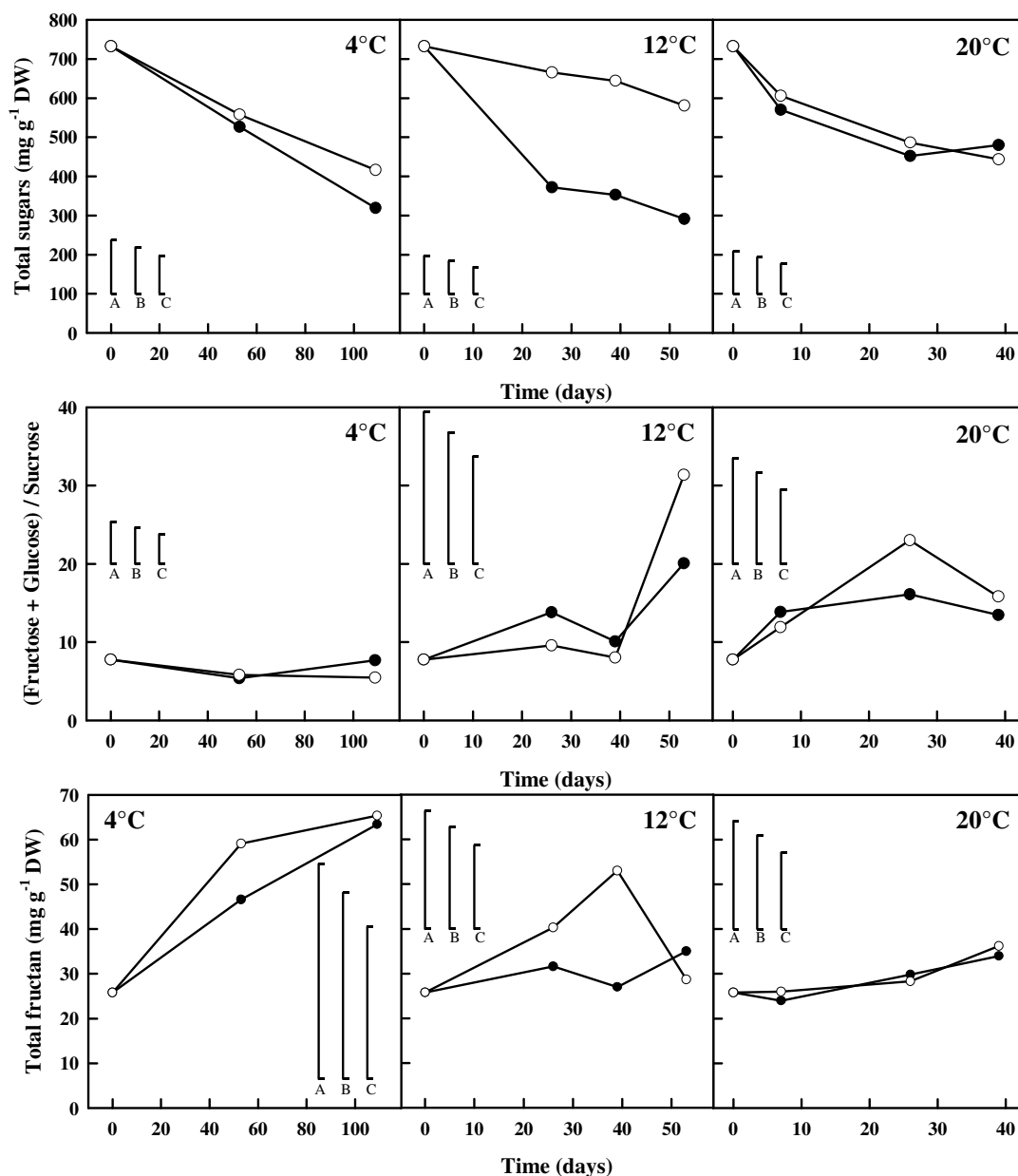
**Figure 5.5.** Sucrose, glucose and fructose concentrations in onions treated with  $1 \mu\text{l l}^{-1}$  1-MCP at  $20^\circ\text{C}$  for 24 hours (open symbols) and untreated onions (controls, closed symbols) stored at  $4^\circ\text{C}$  for 109 days,  $12^\circ\text{C}$  for 53 days or  $20^\circ\text{C}$  for 39 days. Controls  $n=5$ , 1-MCP  $n=10$ . LSD bars ( $P=0.05$ ) A=5,5; B=10,5; C=10,10.

The ratio of monosaccharides (*viz.* fructose and glucose) to disaccharide (sucrose) was calculated to demonstrate how the composition of the soluble sugars varied with time

(Figure 5.6). In onions stored at 4 and 20°C the ratio was not affected by either treatment with 1-MCP or storage time, however in onions held at 12°C there was a significant change ( $P=0.003$ ) in the sugar composition over time, with an increase in the proportion of fructose and glucose compared to sucrose occurring between days 39 and 53 from 10.1 to 20.0 mg g<sup>-1</sup> DW in untreated bulbs and from 8.0 to 31.3 mg g<sup>-1</sup> DW in 1-MCP-treated bulbs.

The total concentration of simple sugars was calculated by adding together the concentrations of fructose, glucose and sucrose (Figure 5.6). The total sugar concentration decreased over time for onions held at each temperature (4°C  $P<0.001$ ; 12°C  $P=0.012$ ; 20°C  $P<0.001$ ), and the overall mean concentration of total sugars was approximately 2-fold greater ( $P<0.001$ ) in 1-MCP-treated onions held at 12°C than the untreated onions.

As reported previously (Chapters 3 and 4) onions cv. SS1 have a far lower fructan concentration than cultivars characterised by higher dry matter and greater pyruvate concentration. The mean fructan concentration in onions stored at 4°C increased between day 0 and all other times ( $P=0.068$ ), but there were no other significant changes in the fructan concentration during storage or due to 1-MCP treatment, however, in general, fructan concentration increased during storage at all temperatures (Figure 5.6).



**Figure 5.6.** Ratio of monosaccharide : disaccharide (fructose+glucose/sucrose), total sugar (sucrose+glucose+fructose) and total fructan concentration in onions treated with 1  $\mu\text{l l}^{-1}$  1-MCP at 20°C for 24 hours (open symbols) and untreated onions (controls, closed symbols) stored at 4°C for 109 days, 12°C for 53 days or 20°C for 39 days. Controls n=5, 1-MCP n=10. LSD bars ( $P=0.05$ ) A=5,5; B=10,5; C=10,10.

## 5.5 Discussion

Treating onion cv. SS1 bulbs with a single exposure of 1-MCP gas at 20°C delayed sprout growth in onions stored at 4 and 12°C, but not at 20°C. This may be because the 1-MCP treatment was not sufficient to counteract the sprout promoting effect of the 20°C storage conditions. In contrast with the results reported here, 1-MCP (1  $\mu\text{l l}^{-1}$ ) had no effect on sprouting (L.A. Terry, Cranfield University, unpublished data) in a high dry matter, high pungency cultivar. The influx of 1-MCP into a tissue is dependent on the chemical composition of that tissue, for example lipid-rich avocados adsorb 1-MCP more readily and retain it for longer periods of time than apples (Dauny *et al.*, 2003). The presence of a physical barrier is also likely to have an effect. The outer skin of low dry matter onions including cv. SS1 is typically thin and consists of only one or two layers, whereas high dry matter cultivars have many layers of thick, tough outer skins that form an effective barrier to water loss. It is possible that while the thin skins of cv. SS1 onions provided little challenge to the influx of 1-MCP gas, the thicker skin of high dry matter cultivars represented a significant obstacle. The delayed sprout growth observed in this study suggests that 1-MCP is able to penetrate the outer skin and inner layers of the onion to act on the meristematic growing point inside the bulb where the sprout is initiated. Both physiological age of the commodity and the temperature at which it is applied can alter the efficacy of 1-MCP treatment (Blankenship and Dole, 2003). The onions used in this study were of a short storing cultivar, therefore, it is likely that they were at a different physiological age or maturity stage than the high dry matter onions used previously (L. A. Terry, Cranfield University, unpublished data).

It has previously been shown that sprouting in onion bulbs occurs when endogenous ABA reaches a minimum concentration (Chapters 3 and 4). There was no difference in the ABA concentration between 1-MCP-treated and untreated bulbs stored at 4 or 12°C, yet sprout growth was delayed. The general trend was for a decrease in ABA concentration between the beginning of storage and the second sampling time, followed by a subsequent increase. Minimum ABA concentration was related to the

onset of sprouting (Figure 5.4), and the subsequent increase is believed to be due to synthesis of ABA by the growing sprout (Chapter 4). However, the lack of difference in ABA concentration between treated and untreated bulbs leads to the conclusion that the mechanism by which 1-MCP delays sprouting is not mediated by ABA. Similarly, an experiment on potatoes demonstrated that ABA concentration temporarily decreased 4-fold after a single 24 hr exposure to  $1.74 \mu\text{mol l}^{-1}$  ethylene. After a few hours the ABA concentration returned to a value similar to that measured before ethylene treatment (Coleman, 1998). Therefore, in the present study it is possible that 1-MCP treatment induced a transient decrease in ABA concentration, which was not detected due to the sampling schedule.

The non-structural carbohydrate profile of the onions in this study was typical of low-pungency cultivars, being characterised by low fructan concentration, ( $20\text{-}70 \text{ mg g}^{-1}$  DW), and high concentrations of monosaccharides compared to disaccharides (Terry *et al.*, 2005). Benkeblia *et al.* (2005a) have suggested that an increase in sucrose concentration acts as a trigger for release from dormancy and the onset of sprouting. However, there was no evidence from the study reported here to support this hypothesis (Figure 5.5) as sucrose concentration remained stable throughout storage. There was little difference in the overall mean sugar compositions between onions stored at different temperatures. This is consistent with Benkeblia *et al.* (2004) who stated that the metabolism of sucrose into glucose and fructose catalysed by the enzyme invertase is largely independent of temperature. The ratio of monosaccharides to disaccharide tended to increase, particularly towards the end of storage (Figure 5.6), explained by the observed decrease in glucose and fructose (Figure 5.5). The decrease in sugar concentration coincided with an increase in sprout length, suggesting that sugars were metabolised to provide energy for the growing sprout.

The concentrations of glucose, sucrose and fructose were greater in onions treated with 1-MCP and stored at  $12^{\circ}\text{C}$ . The sugar concentrations decreased between day 0 and day 26 in untreated bulbs to an amount approximately 2-fold less than that in 1-MCP-treated bulbs (Figure 5.5). Higher levels of fructose and glucose have been positively correlated with greater taste preference in onion (e.g. sweetness and likeability) and thus,



the use of 1-MCP may have implications on final bulb nutritional value and consumer acceptability (F. Davis and L. A. Terry, Cranfield University, unpublished).

Fry colour is an important quality characteristic for potatoes, with a light colour being desirable. Fry colour darkening is caused by the metabolism of starch into reducing sugars, and this process was increased under conditions of continuous ethylene exposure in store which increased the respiration rate (Daniels-Lake *et al.*, 2005). Prange *et al.* (2005) discovered that the application of  $0.9 \mu\text{l l}^{-1}$  1-MCP for 24h at  $9^{\circ}\text{C}$  to potatoes before exposure to continuous ethylene at  $4 \mu\text{l l}^{-1}$  reduced fry colour darkening, but did not interfere with ethylene induced sprout control. These results could lead to the following conclusions. Either, as the authors hypothesise, the effect of 1-MCP is dependent on the target tissue, and the tuber eye tissue of potatoes where the sprout is initiated, is more metabolically active than the cortex, where sugars are metabolised. Therefore, turnover of ethylene binding sites could potentially be more rapid and thus, 1-MCP exerts less effect on the tuber eye tissue. Alternatively 1-MCP might act on pathways other than those concerned with ethylene perception, thus not affecting ethylene-induced inhibition of sprout elongation, but reducing metabolism of sugars, as observed in the current study in onions stored at  $12^{\circ}\text{C}$ . This second theory is further corroborated by Pruski *et al.* (2006), who applied  $4 \mu\text{l l}^{-1}$  1-MCP to seed potatoes for 48 hr followed by continuous exposure to  $4 \mu\text{l l}^{-1}$  ethylene. There was no difference in ethylene-induced sprout control between tubers treated with 1-MCP followed by ethylene or tubers only treated with ethylene. However, following subsequent planting, the average weight of tubers per plant was reduced in 1-MCP-treated potatoes. If it is indeed the case that 1-MCP has an effect mediated by mechanisms other than those pertaining to the inhibition of ethylene perception, this must be taken into consideration when using 1-MCP as a tool to explore ethylene function.

Many authors have reported a decrease in fructan concentration during storage of onions (Rutherford and Whittle, 1982; Suzuki and Cutliffe, 1989; Ernst *et al.*, 1998; Hansen, 1999), although Pak *et al.* (1995) observed an increase in fructan concentration in the bulb base, and Benkeblia *et al.* (2002) reported a 2-fold increase in total fructan concentration in onions cv. Rouge Amposta, stored at 10 and  $20^{\circ}\text{C}$  for 24 weeks by

which time 50 and 75% respectively of the bulbs had sprouted, but a 0.7-fold decrease in onions stored at 4°C where only 5% of bulbs had sprouted. In the study reported here, fructan concentration tended to increase during storage, with a 2-fold increase between the beginning and end of storage at 4°C (109 days), and an increase of approximately 0.75-fold at 12°C and 20°C (53 and 39 days respectively). In the current study, sprout growth had occurred between day 0 and the second sampling date and so the results recorded could reflect the synthesis of fructans by the sprout, as fructan concentration has been shown to increase in the sprout of onions cv. Hysam (Pak *et al.*, 1995). Low dry weight cultivars such as cv. SS1 tend to have low concentrations of fructans, but few studies have detailed the changes in fructan concentration during storage in these onions. It is possible that the synthesis of fructans in the bulb base during storage is greater than the metabolism in the inner and outer scales, resulting in a net increase. It is important to remember that although there are changes occurring in fructan concentration during storage, the concentration of these large carbohydrates are minimal compared with that of the simple sugars, glucose, fructose and sucrose.

Stored onions must be free from green sprouts. Any treatment that reduces sprout growth and extends storage life, but has a detrimental effect on bulb quality is of questionable utility. However, in the onions stored at 12°C, where sprout growth was reduced, there was no negative impact on bulb quality by 1-MCP.

Firmness decreased with time during storage at all temperatures and 1-MCP-treated onions were generally more firm than untreated bulbs. Dry weight ( $\text{mg g}^{-1}$  FW) was also generally greater in 1-MCP-treated than untreated bulbs. Similarly, fresh weight loss in tulip bulbs was promoted by continuous exposure to 0.3 Pa ethylene for 29 days, but this effect was prevented by a pre-treatment with  $1 \mu\text{l l}^{-1}$  1-MCP for 16 hr at 20°C (de Wild *et al.*, 2002). Another important quality characteristic, particularly for mild onions, is low pungency (Abayomi *et al.*, 2006), so it is essential the effect of any treatment on the pyruvate concentration is assessed. Pyruvate concentration decreased over time and the pyruvate concentration of 1-MCP-treated onions stored at 12 and 20°C was only *ca.*  $1 \mu\text{mol g}^{-1}$  FW greater than that of untreated bulbs. Pyruvate concentration at the end of storage did not exceed the value recorded at the beginning of storage.

Onion bulbs are non-climacteric, and have a low sensitivity to ethylene. They produce ethylene slowly and in low concentrations ( $<0.1 \mu\text{l kg}^{-1} \text{FW hr}^{-1}$  at  $20^{\circ}\text{C}$ ) (Suslow, 1998). Reducing the concentration of ethylene that accumulates around produce in store has been used to extend the storage life of other non-climacteric fruits and vegetables such as Chinese cabbage and orange (Wills *et al.*, 1999). It is possible that the storage life of onion may be extended similarly by inhibiting ethylene perception.

The literature on the role of ethylene in onion bulb dormancy is limited. Onion bulbs cv. Rouge Amposta subjected to a combination of a chilling treatment ( $9^{\circ}\text{C}$  for three weeks) and ethephon (a substance that spontaneously degrades to form ethylene) treatment (1 ml of  $100 \text{ mg l}^{-1}$  ethephon solution injected into bulb centre) and stored at  $18^{\circ}\text{C}$  sprouted earlier (50% sprouting at 2 months, 100% at 4 months) than bulbs subjected to ethephon treatment without chilling and controls (no chilling or ethephon) (50% sprouting at 3 months, 100% at 6 months), and sprouting was slightly delayed in bulbs treated with silver thiosulphate solution (an inhibitor of ethylene action) without chilling (50% sprouting at 4 months, 100% at 6 months; Benkeblia and Selselet-Attou, 1999b). It is possible that the effect of chilling treatment reduced the storage life of the bulbs as this had been shown to break dormancy; however, as no bulbs were subjected to chilling alone, this cannot be proven. In another experiment, injection of bulbs with ethephon alone had no effect on sprouting, but when applied in combination with exogenous ABA it partly alleviated the inhibitory effect of ABA on sprouting (Abdel-Rahman and Isenberg, 1974). These results suggest that treatment with ethylene encourages sprouting, and therefore the removal of ethylene from the storage environment, or the blocking of its effects may reduce sprout growth, and supports the findings of the study reported here. Furthermore, sprouting was accelerated in potatoes exposed to ethylene ( $0.02\text{-}20 \mu\text{l l}^{-1}$ ) for 8 to 72 hours, yet continuous exposure to  $2 \mu\text{l l}^{-1}$  ethylene inhibited sprouting. Upon removal from the continuous ethylene treatment sprouting occurred at a rate similar to that of potatoes treated with a single dose of ethylene (Rylski *et al.*, 1974). The authors hypothesised that a single exposure to ethylene diminished the rest or dormant period, but continuous exposure to ethylene inhibited elongation of the sprout. Although there are obvious differences between

onions and potatoes, parallels could perhaps be drawn in the involvement of ethylene in dormancy and inhibition of sprout elongation. Both onions and potatoes produce minimal concentrations of ethylene, but as potatoes responded to ethylene concentrations of  $2 \mu\text{l l}^{-1}$ , the involvement of the accumulation of endogenous ethylene in store cannot be ruled out. Recently, continuous application of ethylene in commercial onion stores has been shown to retard sprout growth (Johnson, 2006). The current study has shown that a single treatment with 1-MCP can reduce sprout growth in onions subsequently stored at 4 or  $12^{\circ}\text{C}$ , but not at  $20^{\circ}\text{C}$ . The inhibition of ethylene perception at the beginning of storage may have slightly prolonged the dormant period, thus delaying sprout growth. The activity of a molecule as an inhibitor depends on the length of time that it remains bound to the receptor (Sisler, 2006), therefore the lack of inhibition of sprout growth observed in onions stored at  $20^{\circ}\text{C}$  could be explained by a reduction in the time 1-MCP molecules spent bound to the ethylene binding protein at this higher temperature, or to increased production of new binding sites, and therefore a reduction in its effectiveness as an inhibitor. It is important to note that ethylene was not measured, nor was exogenous ethylene applied, in the study reported here. It is possible that the mode of action of 1-MCP is not directly due to its inhibition of ethylene perception. It appears that 1-MCP treatment reduced the rate of carbon utilisation in onion cv. SS1 bulbs stored at  $12^{\circ}\text{C}$  by an unknown mechanism.

## 5.6 Conclusion

The dichotomy of the effects of ethylene and 1-MCP in onion provide a case for further investigation and suggest that the interactions of 1-MCP and ethylene on ABA metabolism, carbon utilisation and sprouting in onions is far from simple. It is likely that more than one pathway is involved, and that interactions between cultivar, physiological age and storage temperature occur.

## 6.0 CHAPTER SIX

### **The effect of the transition between controlled atmosphere and air storage on bulbs of onion cultivars SS1, Carlos and Renate.**

#### **6.1 Abstract**

Controlled atmosphere (CA) storage is used to prolong onion storage life; however detrimental effects on shelf-life have been reported. The effect of the transition between CA (5% CO<sub>2</sub>, 3% O<sub>2</sub>) and air (and vice versa) on onion cvs. Renate, Carlos and SS1 in terms of the respiration rate, sprout growth, dry weight, firmness, pungency, total soluble solids, abscisic acid concentration and non-structural carbohydrate composition was assessed. Removal of bulbs from CA storage resulted in an immediate increase in the respiration rate (measured in air), which then reverted to a lower rate following subsequent storage under air conditions for 21 days. In some cultivars, this could be sufficient to trigger the onset of sprouting and thus account for the detrimental effect of CA storage on shelf-life. Delaying the start of CA storage of onions cv. SS1 for 21 days was as effective in suppressing sprout growth as CA storage for 42 days. Further investigation into the use of CA storage in this manner in relation to the optimum time to begin CA conditions could decrease the cost of CA storage without compromising storage life. Abscisic acid concentration has been associated with storage life of onions. There was a significant decrease in the ABA concentration between the time of harvest and the beginning of storage. This is likely to be due to the effects of curing and suggests that curing is having a detrimental effect on storage potential.

#### **6.2 Introduction**

The use of controlled atmosphere (CA) in the storage environment is a ubiquitous method for extending the storage life of fresh produce. However, if misused, off-odours caused by anaerobic respiration can develop when oxygen concentration is not maintained within the tolerance limits of the commodity (Kader,

2004). Controlled atmosphere storage is relatively expensive compared with regular refrigerated air storage, but adds value to the crop by extending the marketing season for the product. An atmosphere consisting of 5% CO<sub>2</sub> and 3% O<sub>2</sub> has been recommended for mild onion cv. Granex bulbs (Smittle, 1988). Low oxygen storage inhibits sprouting of onion bulbs, and reduces weight loss (Yoo and Pike, 1996; Praeger *et al.*, 2003). It can, however, have a detrimental effect on the quality of sweet onions, through an increase in pungency, although this is not as great as the increase observed in air-stored bulbs (Smittle, 1988), and can limit subsequent shelf-life with sprouting accelerating three weeks after removal from CA storage (Sumner, 2000). A similar reduction in shelf-life has been observed in CA-stored, high pungency (long-storing) cultivars (D. O'Connor, Allium and Brassica Centre, pers. comm.).

Water-soluble carbohydrates constitute 60-80% of the dry weight of onion bulbs (Rutherford and Whittle, 1982), and comprise glucose, fructose and sucrose, and a series of oligosaccharides called fructans (Darbyshire and Henry, 1978). Fructose concentration is higher in the outer scales than the inner scales, whilst sucrose and glucose are evenly distributed throughout the scales (Darbyshire and Henry, 1978; Salama *et al.*, 1990; Hansen, 1999). A maximum soluble sugar concentration (fructose + glucose + sucrose) occurs between five and eight weeks after harvest (Salama *et al.*, 1990; Benkeblia *et al.*, 2002). Fructose concentration increases over the storage period (Salama *et al.*, 1990), while fructan concentration in onion bulbs tends to decrease (Suzuki and Cutliffe, 1989; Pak *et al.*, 1995; Ernst *et al.*, 1998; Benkeblia *et al.*, 2000). The decrease in fructan concentration has been shown to begin two weeks prior to harvest in onion cv. Rijnsberger Hysam (Pak *et al.*, 1995). In general, fructans tend to be metabolised during the storage period, accounting for the increase in fructose concentration observed. Soluble sugars are required to provide energy for sprout growth, and so the concentration of soluble sugars decreases concomitant to respiration rate increasing when sprouting occurs (Rutherford and Whittle, 1982). Changes in carbohydrates during onion storage have been summarised in Table 6.1.

**Table 6.1.** Summary of the changes in carbohydrate composition of onion bulbs during storage.

NSC <sup>l</sup> analytes and extraction method	Cultivar	Storage conditions	Storage duration	Effect	Reference
Fructose <sup>b,x</sup> Glucose <sup>a,x</sup> Fructan <sup>b,x</sup>	Cream Gold Syn. <sup>2</sup> Pukekohe Longkeeper <sup>H</sup>	NA <sup>3</sup>	Recently harvested	↑ Fructan towards the centre of the bulb ↓ Fructose towards the centre of the bulb Fructan DP <sup>4</sup> inversely correlated with its concentration Glucose and sucrose similar in all leaf bases	Darbyshire and Henry (1978)
Fructose <sup>x</sup> Glucose <sup>bx</sup> Sucrose <sup>bx</sup>	Rijnsberger Robusta <sup>H</sup>	4°C	24 weeks	↔ Total soluble sugars before sprouting ↓ Total soluble sugars during sprouting ↔ Sucrose in inner scales ↑ Sucrose in outer scales ↑ Fructose over storage	Rutherford and Whittle (1982)
Total carbohydrates <sup>bx</sup>	Granex-Grano <sup>L</sup>	1, 4 or 21°C	24 weeks	↓ Total CHOs <sup>5</sup> at 1°C up to 4 weeks then stable ↑ Total CHOs at 4°C up to 8 weeks ↓ Total CHOs at 21°C to 8 weeks, ↑ to 13 weeks. ↓ to end of storage	Hurst <i>et al.</i> (1985)
Fructose <sup>ay</sup> Glucose <sup>ay</sup> Sucrose <sup>ay</sup> Fructan <sup>dy</sup>	Variety of cultivars (n=8) Range of DM	6-10°C	8 weeks	↓ Fructans over storage ↑ Sucrose over storage	Suzuki and Cutliffe (1989)

Fructose <sup>ex</sup>	Sentinel <sup>L</sup>	0°C	20 weeks	RH had no effect on the carbohydrates measured	Salama <i>et al.</i> (1990)
Glucose <sup>ex</sup>		15°C		↑ Fructose at 0°C and 15°C	
Sucrose <sup>ex</sup>		30°C		↑ Glucose in first 5 weeks at 0°C and 15°C	
		Range of RH		↓ Glucose after 5 weeks at 0°C and 15°C, from harvest at 30°C ↑ Total sugars up to 5 weeks ↓ Total sugars after 5 weeks ↑ Fructose and glucose towards centre of the bulb	
Fructose <sup>ax</sup>	Hysam <sup>H</sup>	16°C	15 weeks	↔ Glucose and sucrose in the inner and outer scales	Pak <i>et al.</i> (1995)
Glucose <sup>ax</sup>	Hystar <sup>H</sup>			↓ Fructans in the inner and outer scales	
Sucrose <sup>ax</sup>	Centurian <sup>H</sup>			↑ Fructose 4-fold in the inner and outer scales	
Fructan <sup>6ax</sup>				↑ Fructans in the bulb base ↔ Sucrose, glucose and fructose in the bulb base	
Glucose <sup>ay</sup>	Valencia sintética <sup>H</sup>	3-32°C	42 weeks	↑ Glucose until 120 days ↓ Glucose after 120 days	Croci <i>et al.</i> (1995)
Fructan <sup>cy</sup>	Variety of cultivars (n=6) Range of DM	Un- specified	12 weeks	↓ Total fructans during storage	Ernst <i>et al.</i> (1998)
Glucose <sup>dy</sup>	Rouge Amposta <sup>H</sup>	18°C	8 weeks	↑ Total soluble sugars (F+G+S) 0.5-fold up to 6 weeks ↓ Total soluble sugars (F+G+S) after 6 weeks ↑ Glucose and fructose preceding sprouting	Benkeblia and Selselet-Attou (1999a)



Sucrose <sup>dx</sup>	Hyduro <sup>H</sup>	1°C	28 weeks	↑ Fructan towards centre of the bulb	Hansen (1999)
Glucose <sup>dx</sup>				↓ Fructose and glucose towards centre of the bulb	
Fructose <sup>dx</sup>				⇔ Sucrose towards centre of the bulb	
Fructan <sup>dx</sup>				↓ Fructan between weeks 10 and 18 of storage ↑ Fructose and sucrose between weeks 3 and 18 of storage	
Fructose <sup>dx</sup>	Hysam <sup>H</sup>	None	Shortly after harvest	High fructan associated with high sucrose and low fructose	Jaime <i>et al.</i> (2000)
Fructan <sup>dx</sup>				Fructan DP inversely correlated with its concentration	
				↑ Fructan from bulb base to top of the bulb	
				↑ Fructan and sucrose towards centre of the bulb ↓ Free fructose towards centre of the bulb	
Fructose <sup>dx</sup>	Variety of cultivars (n=5)	0°C; 60-	24 weeks	↑ Free fructose after 24 weeks	Jaime <i>et al.</i> (2001)
Glucose <sup>dx</sup>	Range of DM	65% RH		⇔ Free glucose and sucrose after 24 weeks	
Sucrose <sup>dx</sup>					
Fructose <sup>dy</sup>	Rouge Amposta <sup>H</sup>	4°C	24 weeks	↑ Soluble sugars up to 6-8 weeks storage.	Benkeblia <i>et al.</i> (2002)
Glucose <sup>dy</sup>		10°C		↓ Soluble sugars from 6-8 weeks to end of storage.	
Sucrose <sup>dy</sup>		20°C		↓ Total fructans by 20% at 4°C.	
Fructan <sup>dx</sup>				↑ Total fructans 2-fold at 10°C and 20°C.	
Fructose <sup>cy</sup>	Rijnsberger Sherpa <sup>H</sup>	2°C	36 weeks	↓ WSC <sup>7</sup> with decreasing oxygen concentration.	Ernst <i>et al.</i> (2003)
Glucose <sup>cy</sup>		21% O <sub>2</sub>		↑ Fructan with decreasing oxygen concentration.	
Sucrose <sup>cy</sup>		1% O <sub>2</sub>		DP of fructans higher after low oxygen storage than in control	
Fructan <sup>cy</sup>		0.5% O <sub>2</sub>			

Fructose <sup>dy</sup>	Rouge Amposta <sup>H</sup>	20°C	8 weeks	↑ Total soluble sugars up to sprouting	Benkeblia and Shiomi (2004)
Glucose <sup>dy</sup>				↓ Total soluble sugars after sprouting	
Sucrose <sup>dy</sup>					
Fructose <sup>dx</sup>	Tenshin <sup>H</sup>	10°C	25 weeks	↑ Fructose, glucose and sucrose up to 4 weeks	Benkeblia <i>et al.</i> (2004)
Glucose <sup>dx</sup>		20°C		↓ Fructose, glucose and sucrose after 4 weeks	
Sucrose <sup>dx</sup>				↑ Fructose, glucose and sucrose gradually after 7 weeks	
Sucrose <sup>dx</sup>	Tenshin <sup>H</sup>	10°C	24 weeks	↑ Sucrose 1.5-fold in first 4 weeks at 10°C	Benkeblia <i>et al.</i> (2005a)
Fructan <sup>dx</sup>		20°C		↑ Sucrose 2-fold in first 4 weeks at 20°C	
				↓ Sucrose 4.4-fold between weeks 4 and 7 at 10°C	
				↓ Sucrose 4-fold between weeks 4 and 7 at 20°C	
				↔ Sucrose between weeks 7 and 18	
				↓ Fructan in first 8 weeks, then stable	
Fructose <sup>dx</sup>	Tenshin <sup>H</sup>	15°C	24 weeks	↑ Fructose after 8 weeks, then stable between 10 and 18 weeks	Benkeblia <i>et al.</i> (2005b)
Fructan <sup>dx</sup>				↓ Fructose after 18 weeks	
				↔ Fructose between 20 and 24 weeks	
				↓ Fructan and total carbohydrates steadily after 5 weeks	

<sup>1</sup>NSC=Non-structural carbohydrates. <sup>2</sup>Syn.=Synonym. <sup>3</sup>NA=Not applicable. <sup>4</sup>DP = Degree of polymerisation. <sup>5</sup>CHOs=Carbohydrates. <sup>6</sup>Calculated as total carbohydrate minus sucrose, glucose and fructose. <sup>7</sup>WSC = Water Soluble Carbohydrates

<sup>a</sup>Colorimetric method. <sup>b</sup>Enzymatic method. <sup>c</sup>High Performance Anion Exchange Chromatography method. <sup>d</sup>HPLC method. <sup>e</sup>GC-FID method <sup>x</sup>Ethanol extraction. <sup>y</sup>Water extraction.

<sup>H</sup>High dry matter, <sup>L</sup>Low dry matter

↑ = Increased; ↓ = Decreased; ↔ = No change

The biochemical changes that take place in a stored onion bulb are likely to be associated with respiration and the remobilisation of carbohydrates, as all nutrients required for growth of the sprout must come from within the bulb. The concentration of the plant growth regulator abscisic acid (ABA) decreases during storage in onions held under CA (Chapter 3) or ambient atmosphere (Chapter 4) conditions and it has been postulated that a minimal ABA concentration coincides with the onset of sprout growth.

The transition between CA and air is likely to be a cause of stress to the onion bulb. Therefore, elucidating the changes, in terms of the quality, biochemistry and respiration rate, which occur when onion bulbs are moved from CA to air storage environments and vice versa will provide insight into the response of onion bulbs to these conditions.

### **6.3 Materials and methods**

#### *6.3.1 Plant material*

Onion cultivars characterised by high dry matter and high pungency (Renate and Carlos) and low dry matter and low pungency (SS1) were grown from seeds drilled at a rate of 18 seeds m<sup>-1</sup> in March 2005 at Warwick HRI (Warks., UK). Pesticides were applied according to commercial practice. Plants were harvested at 80-90% tops down in early September. Onions were placed into 25 kg nets and loaded into bin driers. For curing and drying, hot air (*ca.* 30°C) was blown through the onions for nine days, followed by ambient air for a further two weeks (as per commercial practice). The dry aerial parts and roots were removed, and any diseased or damaged bulbs discarded prior to storage.

#### *6.3.2 Controlled atmosphere transition treatment*

Onions cvs. Renate, Carlos and SS1 bulbs (n=180) were stored at 2°C under controlled atmosphere (CA; 5% CO<sub>2</sub> and 3% O<sub>2</sub>) or air (*ca.* 0.003% CO<sub>2</sub> and 21% O<sub>2</sub>)

conditions (Chope *et al.*, 2006). Atmospheres were maintained using an Oxystat 2 CA system, attached to an Oxystat 2002 Controller, and Type 770 fruit store analyser (David Bishop Instruments, Sussex, UK). This system was self-calibrating every 24 hr against 5% CO<sub>2</sub>, 95% N<sub>2</sub> (British Oxygen Co., Surrey, UK). Bulbs were stored at 2±1°C in cardboard trays inside rigid polypropylene fumigation chambers (88 x 59 x 59 cm) for a total of six weeks. Relative humidity inside the chambers was not controlled, but was measured using data loggers (TinyTalk TK-0302, Gemini Data Loggers, W. Sussex, UK) and was recorded as 95-100% throughout storage (data not shown). No root growth was observed.

### 6.3.3 Experimental design

The experiment was considered to be arranged as a completely randomised design with the assumption that the storage chambers were identical and that the samples for each treatment combination were selected randomly from the harvested onions for each cultivar. Treatments comprised all combinations of three cultivars (Carlos, Renate and SS1) and eight storage treatments. The storage treatments included two baseline controls; at the time of harvest (n=12; respiration rate not measured), and immediately before storage (n=16; respiration rate n=8). The other storage treatments were; assessment after 21 days storage in either CA or air (n=8; respiration rate n=4), and assessment after 42 days storage for bulbs stored under four different regimes (all combinations of either CA or air storage for the first 21 days, and CA or air storage for the last 21 days – i.e. some bulbs stored in the same conditions throughout, and some swapped from one storage regime to the other after 21 days; n=4; respiration rate n=2). Respiration rate was calculated from the rate of CO<sub>2</sub> production by *ca.* 4-5 bulbs (n=48 per cultivar). All other assays were carried out on individual bulbs (n=60 per cultivar).

### 6.3.4 Sample preparation

Juice was expressed from a *ca.* 5 mm equatorial slice using a hand-operated press (Randle and Bussard, 1993) then frozen at -20°C for pyruvate and total soluble

solids (TSS) measurements. A longitudinal wedge was cut from each bulb and was snap-frozen in liquid nitrogen and kept at  $-40^{\circ}\text{C}$  until the sample was lyophilised (Edwards Super Modulo, Sussex, UK) for use in non-structural carbohydrate (including fructan), and ABA assays.

### 6.3.5 Physical assessments

The following physical assessments were made: sprout growth, firmness, dry weight and respiration rate. Sprout growth, firmness and dry weight were measured according to the methods reported in Chapter 3. Briefly, sprout growth was recorded and expressed as the height of the first appearing green leaves inside the bulb as a percentage of the bulb height, firmness ( $\text{N mm}^{-1}$ ) was measured using an Instron Series IX materials testing machine (Instron, Bucks., UK) according to the method of Lancaster *et al.* (2001) with slight modifications, and dry weight was measured on lyophilised samples. Respiration rate was measured using gas chromatography as described below.

#### 6.3.5.1 Measurement of respiration rate

The respiration rate was assessed as the rate of  $\text{CO}_2$  production under air conditions at  $5^{\circ}\text{C}$ . For respiration measurement, 4-5 onions were removed from the polypropylene storage chambers and the weight recorded. They were immediately placed into 2 l jars with air tight lids fitted with a septum and the jars were sealed for *ca.* 2 hr. After this incubation period, gas samples were removed with repeated full withdrawal-injection displacements of a 30 ml plastic syringe. The gas sample was immediately analysed for  $\text{CO}_2$  using gas chromatography (GC model 8340, DP800 integrator, Carlo Erba Instruments, Herts., UK) with hot wire detection (Budu and Joyce, 2003). The hot wire detector was operated at  $120^{\circ}\text{C}$  and the oven at  $80^{\circ}\text{C}$ . The 2 m long by 4 mm column was packed with 60-80 mesh size Porapak Q (Jones Chromatography, Mid Glamorgan, UK). The GC was calibrated with 10%  $\text{CO}_2$  (10%  $\text{CO}_2$ , 2%  $\text{O}_2$ , 88%  $\text{N}_2$ ; Certified Standard from British Oxygen Co.). Respiration rate was calculated as  $\text{mmol CO}_2 \text{ kg}^{-1} \text{ hr}^{-1}$ .

### 6.3.6 Biochemical assessments

The following biochemical assessments were made: ABA concentration, pyruvate concentration, concentration of non-structural carbohydrates and TSS. The ABA concentration, pyruvate concentration and TSS (%) were measured according to the methods reported in Chapter 3. Briefly, ABA was measured by radioimmunoassay, pyruvate by absorbance assay, total fructan by enzyme assay and TSS by the use of a handheld refractometer (Palette 100, Atago Co. Ltd., Tokyo, Japan). Fructose, glucose and sucrose were measured using HPLC as described in Chapter 4.

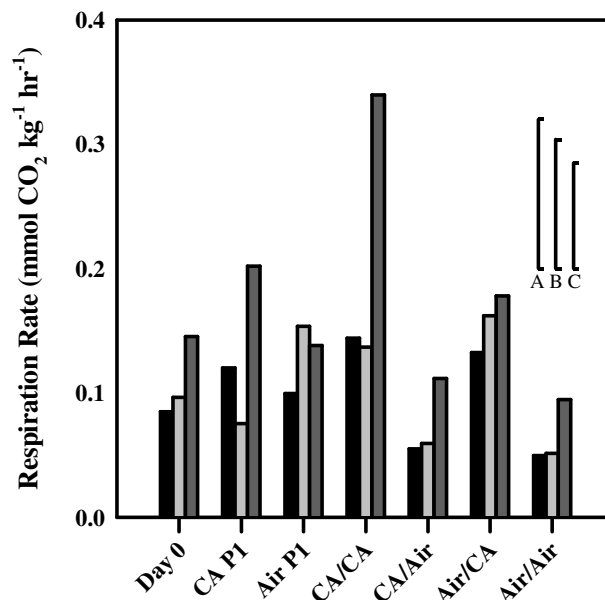
### 6.3.7 Statistical analyses

All statistical analyses were carried out using Genstat for Windows Version 7.1.0.198 (VSN International Ltd., Herts., UK). Analysis of variance was performed on the data as specified in the experimental design section, extracting information about the differences between cultivars and storage regime/times, and the interactions between these factors. A nested factorial treatment structure allowed the separate assessment of the differences between the baseline treatments and the stored treatments, between different timings of sampling (21 or 42 days), between different storage regimes (CA or air), the different combinations of storage regimes, and the interactions between these factors. Least significant difference values (LSD;  $P=0.05$ ) were calculated for comparison of appropriate treatment means, using critical values of  $t$  for two-tailed tests.

## 6.4 Results

### 6.4.1 *Effect of the transition between CA and air storage on physical characteristics of onion bulbs*

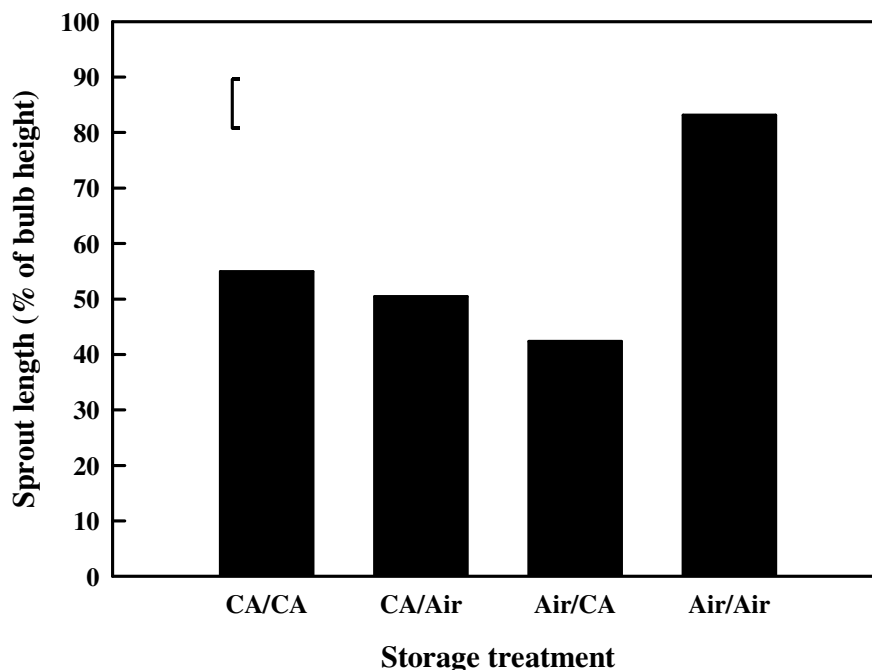
The respiration rates for onion cvs. Carlos and Renate were significantly ( $P < 0.001$ ) greater than SS1 (Carlos 0.0999, Renate 0.1045 and SS1 0.1655 mmol CO<sub>2</sub> kg<sup>-1</sup> hr<sup>-1</sup>). There was a significant effect of the storage conditions in the first 21 days in cv. SS1, where onion bulbs that had been stored in CA had a significantly ( $P = 0.023$ ) higher respiration rate (0.2137) than those stored in air (0.1372 mmol CO<sub>2</sub> kg<sup>-1</sup> hr<sup>-1</sup>) (Figure 6.1). Respiration rate was measured at the end of the storage period. At this time point there was a main interaction of the storage treatment for days 21 to 42, where irrespective of the storage treatment applied in days 0 to 21, onions removed from CA storage had a greater ( $P < 0.001$ ) respiration rate (when measured in air) (0.1822) than those removed from air storage (0.0702 mmol CO<sub>2</sub> kg<sup>-1</sup> hr<sup>-1</sup>).



**Figure 6.1.** The respiration rate of onions cvs. Carlos (■), Renate (▒) and SS1 (■) recorded before storage (Day 0), after 21 days storage in CA (CA P1) or air (Air P1) and after CA for 42 days (CA/CA), CA for 21 days followed by air for 21 days (CA/Air), air for 21 days followed by CA for 21 days (Air/CA) and air for 42 days (Air/Air). Day 0 n=8, CA P1 and Air P1 n=4; CA/CA, CA/Air, Air/CA and Air/Air n=2. LSD bars ( $P=0.05$ ) A=2,2; B=4,2; C=4,4 are shown.

No sprout growth was observed in onions cv. Carlos during the entire storage period, however, sprouting (sprout length 8.2 % of bulb height) was observed in onions cv. Renate after 42 days under air conditions. Sprout growth had occurred after 42 days in all treatments for cv. SS1 (Figure 6.2). Sprout growth was greatest ( $P<0.001$ ) in onions cv. SS1 stored in air for 42 days (83.17% of bulb height) and least in those stored in air followed by CA (42.35% of bulb height). There was no significant difference between those stored for 42 days in CA or those stored in CA followed by air.





**Figure 6.2.** The sprout length of onion cv. SS1 expressed as a percentage of bulb height after storage in; CA for 42 days (CA/CA), CA for 21 days followed by air for 21 days (CA/Air), air for 21 days followed by CA for 21 days (Air/CA) and air for 42 days (Air/Air); n=4. LSD bar ( $P=0.05$ ) is shown.

There was a main effect of cultivar on firmness. Onion cvs. Carlos and Renate, 51.03 and 55.33 N mm<sup>-1</sup>, respectively, were significantly ( $P<0.001$ ) firmer than onions cv. SS1 at 26.31 N mm<sup>-1</sup> (Table 2). Firmness decreased significantly during storage ( $P=0.05$ ) in onions cv. SS1 between days 0 and 42. There were no differences in firmness between different storage treatments.

There was also a main effect of cultivar on dry weight. The dry weight of onion cvs. Carlos and Renate, 139.72 mg g<sup>-1</sup> FW and 128.79 mg g<sup>-1</sup> FW, respectively, was greater ( $P<0.001$ ) than that of onions cv. SS1, 71.11 mg g<sup>-1</sup> FW. There were no main effects or interactions of storage treatment (Table 6.2).

**Table 6.2.** The firmness ( $\text{N mm}^{-1}$ ) and dry weight ( $\text{mg g}^{-1}$  FW) of onion cvs. Carlos, Renate and SS1 measured at harvest, before storage (Day 0), after 21 days storage in CA (CA P1) or air (Air P1) and after CA for 42 days (CA/CA), CA for 21 days followed by air for 21 days (CA/Air), air for 21 days followed by CA for 21 days (Air/CA) and air for 42 days (Air/Air). Firmness LSD ( $P=0.05$ ) 12,16=5.903; 8,4=9.466. Dry weight LSD ( $P=0.05$ ) 12,16=7.941; 8,4=12.733.

	Cultivar	Storage Treatment							
		Harvest (n=12)	Day 0 (n=16)	CA P1 Day 21 (n=8)	Air P1 Day 21 (n=8)	CA/CA Day 42 (n=4)	CA/Air Day 42 (n=4)	Air/CA Day 42 (n=4)	Air/Air Day 42 (n=4)
Firmness ( $\text{N mm}^{-1}$ )	Carlos	47.54	51.03	51.50	49.52	48.49	42.88	50.19	50.63
	Renate	49.55	55.33	52.44	49.71	52.14	55.36	47.51	48.16
	SS1	27.04	26.31	24.45	20.81	20.39	12.09	15.54	17.83
	LSD <sub>0.05</sub>	6.310	5.465	7.723		10.930			
Dry Weight ( $\text{mg g}^{-1}$ FW)	Carlos	133.72	139.08	139.09	147.01	144.11	141.82	140.23	139.99
	Renate	126.86	126.86	125.15	133.49	128.94	130.05	134.44	133.14
	SS1	58.08	69.31	78.53	77.35	77.56	82.86	70.88	72.14
	LSD <sub>0.05</sub>	8.489	7.352	10.397		14.704			

#### 6.4.2 *Effect of the transition between CA and air storage on biochemical characteristics of onion bulbs*

There was a main effect on cultivar on TSS. The TSS % was significantly different ( $P<0.001$ ) between cultivars, and can be ranked in order from highest to lowest: Carlos (11.71)>Renate (10.26)>SS1 (5.473 %). At day 21, onions cv. Carlos stored under CA conditions had a lower TSS % ( $P>0.001$ ) than those stored under air conditions; this situation was reversed at day 42 (Table 6.3). The TSS % of onions cv. SS1 stored for 42 days in air had the highest TSS concentration ( $P>0.001$ ), while the opposite was true for onions cv. Renate.

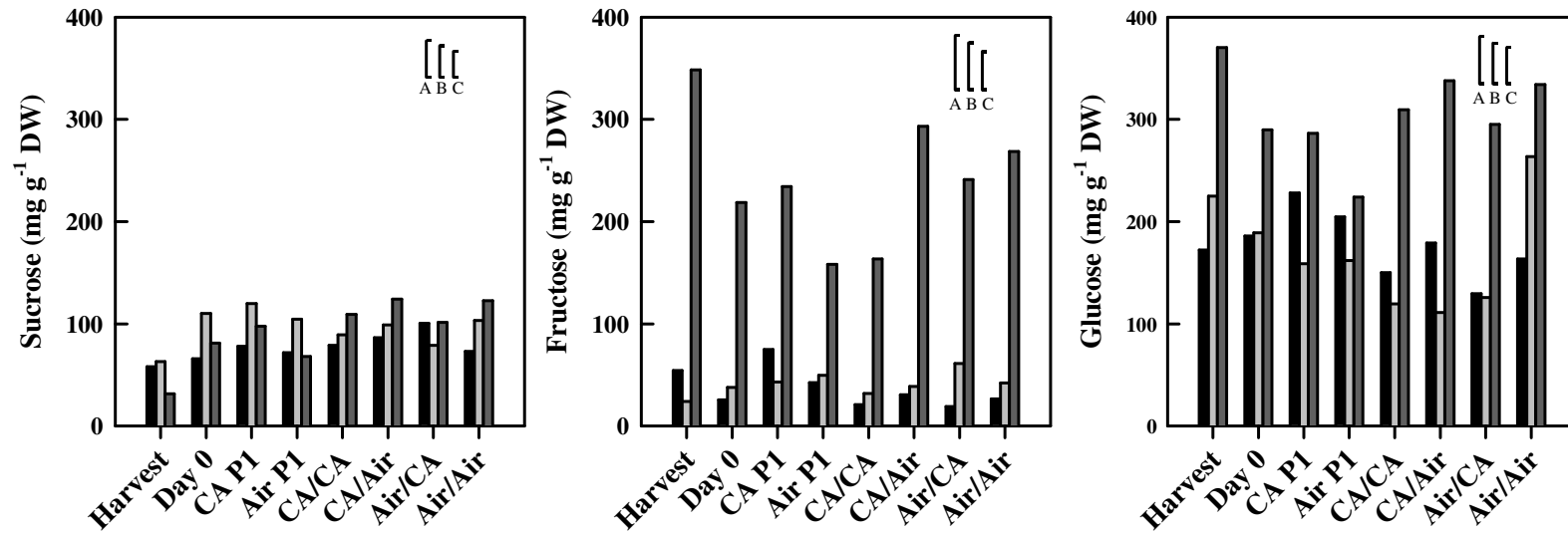
There was a significant ( $P<0.001$ ) main effect of cultivar in terms of pyruvate concentration (Table 6.3), with Carlos having the highest pyruvate concentration (8.39) followed by Renate (7.83) and SS1 (5.00  $\mu\text{mol g}^{-1}$  FW). Onion cv. SS1 bulbs stored under air conditions for the first half of the storage period had a significantly ( $P=0.002$ ) higher mean pyruvate concentration at the end of the storage period than those stored under CA conditions for the first 21 days. There were no other interactions identified by ANOVA in the pyruvate concentration, according to time and storage treatment.

**Table 6.3.** The total soluble solids (TSS) concentration (%) and pyruvate concentration ( $\mu\text{mol g}^{-1}$  FW) of onion cvs. Carlos, Renate and SS1 measured at harvest, before storage (Day 0), after 21 days storage in CA (CA P1) or air (Air P1) and after CA for 42 days (CA/CA), CA for 21 days followed by air for 21 days (CA/Air), air for 21 days followed by CA for 21 days (Air/CA) and air for 42 days (Air/Air). TSS LSD ( $P=0.05$ ) 12,16=0.8538; 8,4=1.3691. Pyruvate LSD ( $P=0.05$ ) 12,16=1.049; 8,4=1.682.

	Cultivar	Storage Treatment							
		Harvest (n=12)	Day 0 (n=16)	CA P1 Day 21 (n=8)	Air P1 Day 21 (n=8)	CA/CA Day 42 (n=4)	CA/Air Day 42 (n=4)	Air/CA Day 42 (n=4)	Air/Air Day 42 (n=4)
Total soluble solids (%)	Carlos	12.033	11.706	11.175	12.463	12.025	10.825	11.775	10.875
	Renate	11.792	10.206	9.375	9.788	10.375	11.100	10.200	7.700
	SS1	5.408	5.156	5.588	5.475	5.200	4.825	4.925	8.175
	LSD <sub>0.05</sub>	0.9127	0.7904	1.178		1.5808			
Pyruvate ( $\mu\text{mol g}^{-1}$ FW)	Carlos	9.57	8.07	8.62	7.58	9.13	9.16	9.06	8.13
	Renate	9.73	6.67	6.80	8.07	7.76	8.07	9.07	6.93
	SS1	4.90	4.30	4.49	6.19	4.30	4.97	5.74	6.74
	LSD <sub>0.05</sub>	0.121	0.971	1.373		1.942			

In cvs. Carlos and Renate, the simple sugar present in the greatest concentration was glucose (range: 125-275; Carlos mean: 183.1; Renate mean: 179.4 mg g<sup>-1</sup> DW), followed by sucrose (range: 50-125; Carlos mean: 71.6; Renate mean: 96.4 mg g<sup>-1</sup> DW) with small amounts of fructose (range: 25-75; Carlos mean: 39.8; Renate mean: 38.8 mg g<sup>-1</sup> DW). The sugar composition of onions cv. SS1 was dominated by glucose and fructose present in high concentrations (range: 150-375; mean glucose: 304.4; mean fructose: 244.7 mg g<sup>-1</sup> DW), with amounts of sucrose comparable with that in Carlos and Renate (range: 25-125; mean: 80.4 mg g<sup>-1</sup> DW). Sucrose concentration increased significantly ( $P<0.001$ ) between harvest and the beginning of storage in cvs. Renate and SS1, by 1.7- and 2.6-fold, respectively (Figure 6.3). The sucrose concentration of all cultivars increased ( $P<0.001$ ) over time between harvest and the end of the storage period.

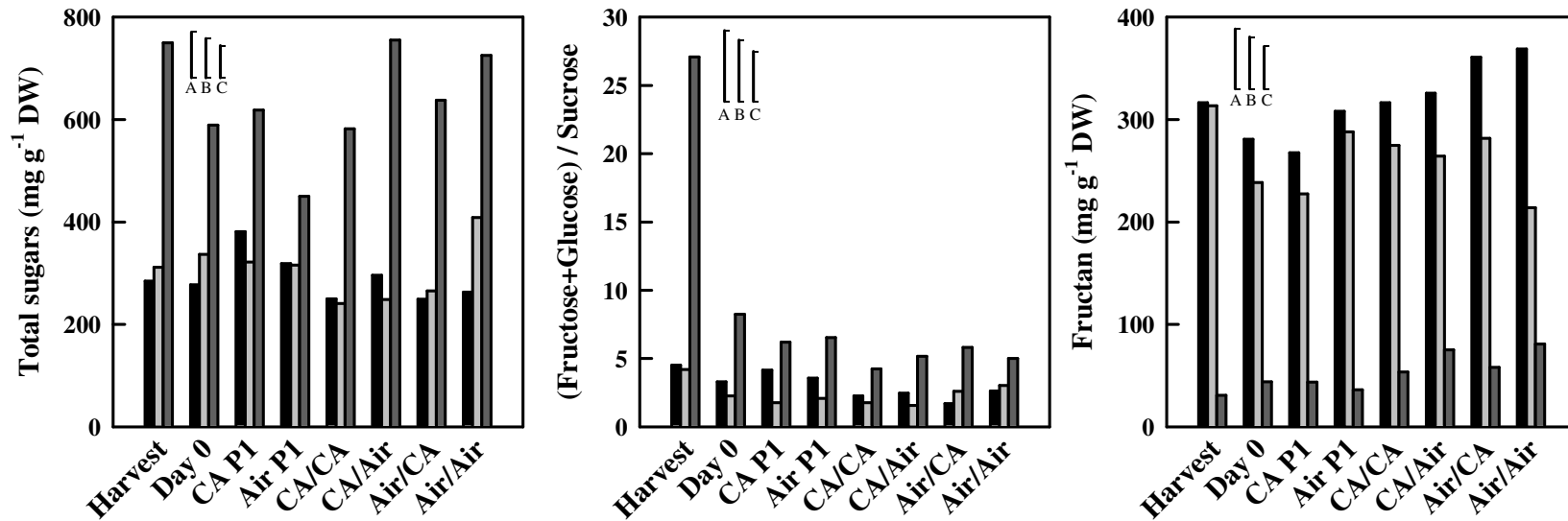
There was a main effect of cultivar on fructose concentration, with onions cv. SS1 containing approximately 8-fold more fructose ( $P<0.001$ ) than cvs. Renate and Carlos. Fructose concentration in onions cv. Carlos and SS1 decreased ( $P<0.001$ ) between harvest and the beginning of storage (Figure 6.3). After 21 days storage in CA conditions the fructose concentration in onions cv. SS1 was significantly higher ( $P=0.0039$ ) than after 21 days in air. A similar pattern was observed in cv. Carlos, although this was not significant. Regardless of the storage conditions for the first 21 days, onions cv. SS1 stored in air between days 21 and 42 had a higher fructose concentration ( $P=0.004$ ) than those stored in CA; again a similar pattern was observed in cv. Carlos but this was not significant. The lowest fructose concentration ( $P=0.05$ ) among onions cv. SS1 was found in those stored in CA conditions for 42 days.



**Figure 6.3.** The sucrose, glucose and fructose concentration of onion cvs. Carlos (■), Renate (□) and SS1 (▒) measured at harvest, before storage (Day 0), after 21 days storage in CA (CA P1) or air (Air P1) and after CA for 42 days (CA/CA), CA for 21 days followed by air for 21 days (CA/Air), air for 21 days followed by CA for 21 days (Air/CA) and air for 42 days (Air/Air). Harvest n=12, Day 0 n=16, CA P1 and Air P1 n=8; CA/CA, CA/Air, Air/CA, Air/Air n=4. LSD bars (P=0.05) A=4,4; B=8,4; C=8,8 are shown.

Glucose concentration varied significantly with cultivar ( $P < 0.001$ ) with onions cv. SS1 having the highest concentration ( $304.4 \text{ mg g}^{-1} \text{ DW}$ ) followed by cvs. Carlos ( $183.1 \text{ mg g}^{-1} \text{ DW}$ ) and Renate ( $179.4 \text{ mg g}^{-1} \text{ DW}$ ). The glucose concentration of both cvs. Renate and SS1 decreased significantly ( $P < 0.001$ ) between harvest and the beginning of storage (Figure 6.3). After 21 days storage in CA conditions onions cv. SS1 had a greater ( $P = 0.003$ ) glucose concentration than those stored in air conditions. The greatest ( $P = 0.003$ ) glucose concentration at the end of storage in onions cv. Renate was in those stored in air conditions for 42 days, being at least 2-fold greater than that recorded for any other storage treatment.

Onions cv. SS1 had a 2-fold greater ( $P < 0.001$ ) mean total sugar (sucrose + glucose + fructose) concentration than cvs. Renate and Carlos (Figure 6.4). The total sugar concentration in onions cv. SS1 decreased significantly ( $P < 0.001$ ) between harvest and the beginning of storage. Whatever the storage treatment in the second half of storage, the mean total sugar concentration at 42 days in onions cv. SS1 stored in CA for the first half of the storage period was greater ( $P < 0.001$ ) than in those stored in air for the first 21 days. The general trend for changes in mean total sugar concentration varied with cultivar, with a peak at day 21 in cv. Renate, no change over storage in cv. Carlos and a decrease until day 21 in cv. SS1 followed by an increase to day 42.



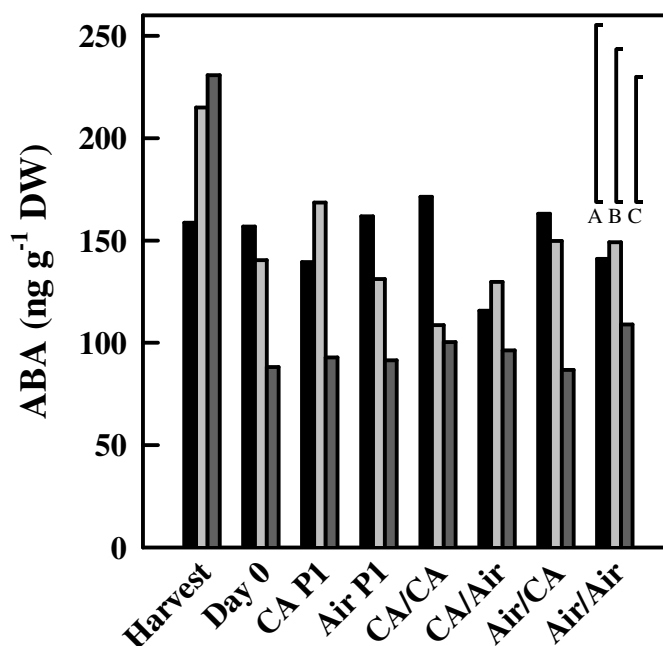
**Figure 6.4.** The total sugar (glucose, sucrose and fructose), monosaccharide to disaccharide ratio, and total fructan concentration of onion cv. Carlos (■), Renate (▒) and SS1 (□) measured at harvest, before storage (Day 0), after 21 days storage in CA (CA P1) or air (Air P1) and after CA for 42 days (CA/CA), CA for 21 days followed by air for 21 days (CA/Air), air for 21 days followed by CA for 21 days (Air/CA) and air for 42 days (Air/Air). Harvest n=12, Day 0 n=16, CA P1 and Air P1 n=8; CA/CA, CA/Air, Air/CA, Air/Air n=4. LSD bars ( $P=0.05$ ) A=4,4; B=8,4; C=8,8 are shown.



The ratio of monosaccharides (fructose and glucose) to disaccharide (sucrose) was calculated. There was a main effect of cultivar ( $P < 0.001$ ) with onions cv. SS1 being dominated by fructose and glucose, and therefore having a much higher mean ratio (10.66) than Carlos (3.41) or Renate (2.55) (Figure 6.4). There was a 3.2-fold decrease ( $P < 0.001$ ) in the ratio between harvest and the beginning of storage in onions cv. SS1. Storage conditions did not have an effect on the mono- to disaccharide ratio.

There was a significant ( $P < 0.001$ ) main effect of cultivar in terms of the fructan concentration (Figure 6.4). The cultivar means were as follows: Carlos (306.3) Renate (264.0) and SS1 ( $46.3 \text{ mg g}^{-1} \text{ DW}$ ). Fructan concentration decreased between harvest and the beginning of storage in cvs. Renate and Carlos. When these cultivars were stored in air conditions for the first 21 days, the fructan concentration was greater ( $P = 0.046$ ) than those stored in CA for the first 21 days, however there was no such difference corresponding with the conditions in the second half of the storage period.

The mean ABA concentration was greatest in cvs. Renate and Carlos than in cv. SS1 ( $P = 0.003$ ). The ABA concentration for each cultivar at the time of harvest was greater in cvs. Renate and SS1 than Carlos, however between harvest and the beginning of storage (i.e. curing period), the ABA concentration decreased 1.5-fold in cv. Renate, and 2.6-fold in SS1, while there was no change in cv. Carlos (Figure 6.5). This meant that the ABA concentration measured before storage was greater in cvs. Carlos and Renate than SS1. There were no interactions between storage treatment and time or cultivar.



**Figure 6.5.** The ABA concentration of onion cvs. Carlos (■), Renate (□) and SS1 (▨) measured at harvest, before storage (Day 0), after 21 days storage in CA (CA P1) or air (Air P1) and after CA for 42 days (CA/CA), CA for 21 days followed by air for 21 days (CA/Air), air for 21 days followed by CA for 21 days (Air/CA) and air for 42 days (Air/Air). Harvest n=12, Day 0 n=16, CA P1 and Air P1 n=8; CA/CA, CA/Air, Air/CA, Air/Air n=4. LSD bars ( $P=0.05$ ) A=4,4; B=8,4; C=8,8 are shown.

## 6.5 Discussion

This study was undertaken to document the changes, both biochemical and physical, that occurred in onions bulbs of different cultivars on the transition between controlled atmosphere and air. The cultivars chosen differed from one another, with Renate and Carlos being firm, pungent bulbs, characterised by high dry weight and TSS %, and bulbs cv. SS1 being less firm with a milder flavour, and low dry weight and TSS %. There are also distinctive NSC profiles that separate cv. SS1 from cvs. Carlos and Renate.

The mean respiration rate of onions cv. SS1 (0.1655) was significantly greater than that of Carlos (0.0966) or Renate (0.1045 mmol CO<sub>2</sub> kg<sup>-1</sup> hr<sup>-1</sup>). The respiration rates of cvs. Carlos and Renate are similar to that reported in the literature for onions cv. Rouge Amposta (high dry matter), stored at 4°C for 6 weeks (0.08 mmol CO<sub>2</sub> kg<sup>-1</sup> hr<sup>-1</sup>) (Benkeblia, 2004). Short-storing onions such as cv. SS1 would be expected to have a higher respiration rate than long-storing cultivars because respiration rate tends to be negatively correlated with storage life. Respiration determines the rate of metabolic processes which in turn have a direct impact on eating quality characteristics such as firmness, sugar content, pungency and overall flavour (Crowther *et al.*, 2005; Terry *et al.*, 2005).

There is evidence to suggest that once onions are removed from CA storage, the shelf-life is limited. Only 5% of onions cv. Walla Walla (low dry matter) stored in air for six months had sprouted after a subsequent shelf-life of 2 weeks at 20°C, compared with 20-55% of onions stored under low oxygen conditions (0.7-1.4%) with 0.05% CO<sub>2</sub> (Sitton *et al.*, 1997). However, some authors have claimed that CA storage extends shelf-life. Onions cv. Sherpa (high dry matter) stored in 1 or 0.5% O<sub>2</sub> with <0.3% CO<sub>2</sub> at 2°C had an improved shelf-life (when tested for 3 weeks at 18°C after 9, 27, and 36 weeks storage) compared with those stored in air (Praeger *et al.*, 2003) and onions cv. Granex stored in 5% CO<sub>2</sub> with 3% O<sub>2</sub> at room temperature or 5°C for 20 weeks, or in 5% CO<sub>2</sub> with 3% O<sub>2</sub> at 1°C for 24 weeks had a greater percentage of marketable bulbs after 2 weeks at 27°C than those stored in air (Smittle, 1988). Only 3 to 10% of onions cv. Rumba (high dry matter) stored in CA (0.5, 1 or 2% O<sub>2</sub> with 3% CO<sub>2</sub> at 0°C) for 36 weeks had sprouted after 3 weeks at 20°C, compared with 40% of air-stored bulbs (Adamicki, 2005). However, in all cases, the air- and CA-stored bulbs were subjected to a shelf-life test after the same storage duration, meaning that there were a lower proportion of marketable bulbs from the air-stored control treatment. The comparison was, therefore, being made between onion bulbs at different maturity stages or depths of dormancy.

In all cultivars in this study, CA conditions during the second period of storage resulted in a greater respiration rate at the end of storage than air for the second period

(Figure 6.1). The respiration rates of all of the onions in this study were measured in air. Therefore, the respiration rate measured for onions removed from CA storage may include the immediate effect of the transition between CA and air. In general, the effect of removing the onions from CA storage was to increase respiration rate on exposure to air. In contrast, when onions (unspecified Japanese cultivar) were exposed to high carbon dioxide concentrations (60% CO<sub>2</sub>, 20% O<sub>2</sub>, 20% N<sub>2</sub>) for 24h and then transferred back to air, there was no significant change in the respiration rate in terms of oxygen uptake (Kubo *et al.*, 1990). In our study, respiration rate was represented by the rate of production of carbon dioxide, so that the higher respiration rates after CA storage could be said to be due to the diffusion of CO<sub>2</sub> out of the onion bulb tissue to equilibrate with the new environment. However, if this was the case, then a higher respiration rate would have also been recorded after 21 days in CA – this was only the true for cv. SS1. The increase in respiration rate may only be a temporary effect, as measurements were taken within 2 hours of the transition. The increase in the respiration rate observed on the transition from CA to air may only be transient, but depending on the cultivar and the developmental stage of the bulbs, particularly of short-storing cultivars, it may be sufficient to trigger the onset of sprout growth.

Onions cv. SS1 stored in CA for the preceding 21 days had smaller sprouts than those stored in air, but respiration rate (measured in air) was higher in those bulbs stored in CA. Similarly, sprouting was observed in onions cv. Renate stored in air for 42 days, yet respiration rate still followed the pattern above. It has been reported that the respiration rate of sprouted onions cv. Rouge Amposta bulbs was 0.06 mmol kg<sup>-1</sup> hr<sup>-1</sup> and 0.08 mmol kg<sup>-1</sup> hr<sup>-1</sup> in unsprouted onions (Benkeblia *et al.*, 2000). This difference is small, and it is therefore possible that the impact of the transition between CA and air was large and masked the respiration increase associated with of sprouting.

Sprout suppression in cv. SS1 was achieved to statistically similar levels by the three combinations of CA/CA, CA/air and air/CA (Figure 6.2). In fact, the most effective treatment was air/CA. This is important, as it suggests that there is potential to delay the use of CA storage for a period of time without compromising sprout suppression, thus not

only reducing monetary costs of storage, but reducing the impact of CA storage on the environment.

When any storage treatment is assessed it is essential that maintenance of bulb quality characteristics is paramount. In this investigation, there was no main effect of the storage treatments on firmness or dry weight (Table 6.2). Similarly, there was no difference in the dry weight of onions cv. Sherpa stored under CA conditions (0.5% O<sub>2</sub> and <0.3% CO<sub>2</sub>) and air-stored controls after 9 weeks, although after 36 weeks the DW of CA-stored onions was greater (Praeger *et al.*, 2003). Pungency of low dry matter onions such as cv. SS1 that are marketed as mild and suitable for consumption raw is an important quality characteristic. Therefore, growers must be aware of any effect that postharvest handling may have on pungency. The effect of storage treatments on pungency varies with cultivar, but has not been clearly defined. Pyruvate concentration in onion bulbs cv. Hysam (high dry matter) decreased after nine weeks storage at 0.5°C in CA conditions (2% O<sub>2</sub>, 2% CO<sub>2</sub> and 2% O<sub>2</sub>, 8% CO<sub>2</sub>) whereas the pyruvate concentration of bulbs cv. Hysam in air storage increased (Uddin and MacTavish, 2003). Pyruvate concentration of onions cv. Granex (low dry matter) increased from 2.60 µmol g<sup>-1</sup> FW over 26 weeks storage to 5.18 in air but only to 3.85 in CA (5% CO<sub>2</sub> and 3% O<sub>2</sub>) at 1°C (Smittle, 1988). Although not statistically different, onions cv. SS1 stored in CA for 42 days had a lower pyruvate concentration than those stored in air for 42 days (Table 6.3). If the storage period was longer then this difference may have widened.

Glucose, sucrose and fructose contribute to the overall taste preference. Glucose concentration has been negatively correlated with perceived bitterness in onion (Terry *et al.*, 2005). Indeed, the concentration of glucose and fructose were correlated with perceived sweetness and likeability as adjudged by taste panellists, whilst sucrose did not appear to affect sweetness (Terry *et al.*, 2005). Non-structural carbohydrate concentrations recorded in this investigation (Figure 6.4) are comparable with other reports (Table 6.1). Total sugars were greater in cv. SS1 (440-780 mg g<sup>-1</sup> DW) than cvs. Carlos and Renate (300-400 mg g<sup>-1</sup> DW) yet the TSS % of onions cvs. Carlos and Renate was consistently *ca.* 2-fold greater than cv. SS1 despite having lower NSC concentrations (Table 6.3). The TSS % was measured on fresh juice

and NSC measured on lyophilised material, but even when total sugars were expressed per fresh weight, the concentration in cv. SS1 was greater than or equal to that in other cultivars. This contradiction shows that TSS concentration is not a good measure of the overall sugar concentration, and therefore should not be used to assess perceived sweetness. The TSS value is likely to be affected by other substances in the onion juice other than simple NSCs, such as fructan, organic acids and proteins.

In the study reported here, sucrose concentration was observed to increase in all cultivars over storage; an effect that has also been noted by Suzuki and Cutliffe (1989). Rutherford and Whittle (1982) stated that total sugars did not change before sprouting, but decreased during sprouting, but Benkeblia and Shiomi (2004) found that total sugars increased up to the time of sprouting. The changes in total sugars during storage in the study reported here varied with cultivar (Figure 6.4). The decrease in total sugar concentration in cv. SS1 between days 0 and 21, followed by an increase between days 21 and 42 is similar to that recorded by Hurst *et al.* (1985) in onions cv. Granex-Grano, a low dry matter cultivar. No sprout growth occurred in cv. Carlos, and there was also no change in total sugar concentration over storage. Increases in the concentrations of glucose and fructose (Benkeblia and Selselet-Attou, 1999a) have been associated with the onset of sprouting, and indeed in the study reported here, the glucose concentration was highest in sprouted bulbs. In general there was no significant effect of treatment on the NSC concentrations measured. This could be because although there were differences in the respiration rates when measured in air, these differences could be due to a stress response rather than the mean respiration rate over storage. Therefore, there may have been little difference in the mean respiration rates of onions stored in CA or air, and correspondingly little difference in the carbon utilisation.

Changes in the sugar composition were associated with the time between harvesting and the beginning of storage, with an increase in sucrose and a decrease in fructose and glucose, suggesting that the current curing process may affect flavour. In particular, the ratio of monosaccharides to disaccharide in onions cv. SS1 changed from 27.08 to 8.24 during this period (Figure 6.4), mainly caused by the reduction in fructose and glucose, rather than the increase in sucrose (Figure 6.3). The implication of the

reduction in monosaccharides is that flavour may be detrimentally affected by curing and that the curing regime should perhaps be adapted to prevent sugar degradation.

Fructans are storage carbohydrates that are metabolised during storage. Typically, larger fructans decrease in concentration with a corresponding increase in fructans with smaller degrees of polymerisation (Benkeblia and Shiomi, 2006). Fructans of all sizes were measured together using the assay employed in this investigation (O'Donoghue *et al.*, 2004), therefore the decrease in the fructan concentration in cvs. Carlos and Renate between harvest and the beginning of storage represents a reduction in total fructan content (Figure 6.4). A decrease in fructan concentration during long-term storage (up to six months) has been observed in many high dry matter cultivars (Ernst *et al.*, 1998; Hansen, 1999; Benkeblia *et al.*, 2002). Fructan concentration did not change over storage in the study reported here, perhaps because the storage period of six weeks was too short for significant metabolism of fructans to occur. In general, the fructan concentration was not affected by CA conditions, which was also the case in onions cv. Granex stored at 3% O<sub>2</sub> and 5% CO<sub>2</sub> (Smittle, 1988). Total fructan concentration measured after 21 days of storage was greater in air-stored bulbs than CA-stored onion cv. Renate bulbs, however, long-term (36 weeks) low oxygen storage of onions cv. Sherpa caused an increase in fructan concentration compared with air-stored controls (Ernst *et al.* 2003; Table 6.1). Ernst *et al.* (2003) postulated that fructans act as protection against the negative effects of low oxygen storage.

Bulb ABA concentration was not affected by the storage treatment, however, it is reported for the first time here that the ABA concentration in onions cv. SS1 significantly decreased, and a smaller decrease in cv. Renate occurred, between harvest and the beginning of storage. This represents the effect of the curing process on the biochemical characteristics of the bulb. Onion bulbs are dried and cured after harvest to tighten the skin around the neck which helps to protect them against pathogen attack (e.g. *Botrytis allii* causing neck rot), and to obtain an attractive skin finish (i.e. caramelisation of non-structural carbohydrates resulting in browner skins). Decreasing ABA concentration has been associated with onset of sprouting in a range of onion cultivars (Chapters 3, 4 and 5), and it was postulated that the ABA concentration before storage was positively

correlated with storage life. There was no change in the ABA concentration of cv. Carlos during this time, and no sprouting was observed in this cultivar. This hypothesis can therefore be refined as ABA concentration at harvest seems to be a better indicator of storage potential. Furthermore, these results strongly indicate that the curing process (hot air, 30°C, for nine days followed by ambient air for 2 weeks) is having an adverse effect on storage life as well as sugar composition, particularly in short-storing cultivars such as SS1.

## 6.6 Conclusion

Removal of bulbs from CA storage resulted in an immediate increase in respiration rate, which then returned to a lower rate after return to air conditions for 21 days. This is likely to represent a stress reaction, and in some cultivars such as the short-storing cv. SS1, could be sufficient to trigger the onset of sprouting and may account for the detrimental effect of CA storage on subsequent shelf-life reported by others. Further investigation into the delayed use of CA storage in terms of the optimum time to commence CA conditions would be beneficial.

The method of curing onion bulbs in the UK has changed little in the last 30 years, and is based on practices determined after experimentation on cultivars commercially important in the 1970s. Ongoing breeding programmes have continued to produce new cultivars, which means that the current practice may not be the most suitable and could in fact have an adverse effect on the storage life. The evidence for this comes from the decline in non-structural carbohydrate and ABA concentrations that were observed between harvesting and the beginning of storage. If best-practice in curing was re-evaluated (i.e reduced temperature and duration), there is the potential to increase storage life and to reduce energy costs.



## 7.0 CHAPTER SEVEN

### General discussion and conclusions

#### 7.1 Discussion

Consumer demand for year-round supply of high quality onion bulbs has led to increasing competition from imports from the Southern Hemisphere during the months from March to September. There is increasing hostility from both consumers and retailers towards the use of synthetic chemicals in the production of food crops that leave a residue in the final product. This pressure to eliminate residues in food means that the future of the use of maleic hydrazide (MH) to suppress sprout growth in stored onions is uncertain. The loss of the principle method of sprout suppression in stored onions currently employed in the UK and other temperate countries will mean that onion growers may lose market share to foreign imports. This has highlighted the need for increasing the understanding of the mechanisms of onion bulb dormancy in order to generate new ideas for markers of storage potential and targets for manipulation in order to extend storage life.

Onion bulb dormancy is composed of two processes; dormancy induction and sprout suppression (Chapter 2, Section 2.3.3). Dormancy induction occurs in the field soon after maturation (Brewster, 1977b). The transition from true dormancy to sprout suppression follows, and it is the maintenance of this subsequent period of sprout suppression that is important in determining storage life. If an onion plant were left to over-winter in the field, once the true dormant period was over, the leaves laid down in the centre of the bulb just before harvest would begin to elongate as soon as environmental conditions were suitable. The same process can occur in stored onions and so it is essential that onions are stored in a dark, well ventilated environment to protect the bulbs from exposure to conditions conducive to sprout growth such as light and moisture. The mechanisms that bring about the breaking of true dormancy are likely

to be initiated by a physiological trigger from inside the bulb, rather than an environmental cue. The state of sprout suppression is likely to be maintained by a combination of physiological processes and environmental cues.

Abscisic acid has been identified as part of the inhibitor complex present in onion bulbs (Tsukamoto *et al.*, 1969) and has been demonstrated to play a functional role in maintenance of dormancy/sprout suppression 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). Application of  $5 \times 10^{-4}$  M (1M = 264.3 g l<sup>-1</sup>) exogenous ABA to *A. wakegi* bulbs increased the number of days to sprouting, whilst application of  $0.25 \times 10^{-4}$  M fluridone accelerated sprouting. Other studies have related a reduction in ABA concentration with loss of dormancy in onion (Matsubara and Kimura, 1991) and *A. wakegi* (Yamazaki *et al.*, 1999a, Yamazaki *et al.*, 1999b, Yamazaki *et al.*, 1995). Soaking excised shoot apices of onion cv. Spartan Banner for one hour in  $ca. 10^{-4}$  M ABA inhibited shoot growth relative to a water-soaked control (Mahotiere *et al.*, 1976). It was postulated that ABA is of importance in controlling dormancy and sprout suppression, and thus storage life, in onion bulbs. To test this hypothesis, novel work to investigate the changes in bulb ABA concentration occurring during storage in onion (*Allium cepa*) cultivars with contrasting storage potential, and changes in other important biochemical and physical characteristics was undertaken in this thesis.

It was initially shown in Chapter 3 that ABA concentration in onion bulbs decreased during controlled atmosphere storage (3% CO<sub>2</sub>, 5% O<sub>2</sub>; 2°C) in onion cultivars representing a range of storage potentials (Figure 3.3), and that the onset of sprouting occurred at minimal ABA concentration, *ca.* 50-120 ng g<sup>-1</sup> DW (Chope *et al.*, 2006). Onion bulbs of the early maturing cultivar (cv. SS1), which were subjected to a longer drying period than the other cultivars in the study, had a lower ABA concentration relative to the other cultivars prior to storage. It is possible that the reduction in ABA concentration was due to the extended exposure to high temperature during the drying period; however it may have been a factor of time. There was no universal threshold ABA concentration at which the onset of sprouting occurred in each cultivar. This could

be a consequence of differences in sensitivity to ABA between cultivars. In dormant (cv. Kiharabansei No. 1) and non-dormant (cv. Ginoza) *A. wakegi* cultivars, ABA concentration increased during bulb development, reaching a maximum shortly after harvest and subsequently decreasing throughout the storage period (Yamazaki *et al.*, 1999a). The maximum ABA concentration was lower in the dormant cultivar (26 ng g<sup>-1</sup> FW) than the non-dormant cultivar (33 ng g<sup>-1</sup> FW) of *A. wakegi* suggesting that the dormant cultivar responded to ABA at a lower concentration than the non-dormant one. In addition, sprouting in the dormant cultivar could be delayed by 35 days by soaking bulbs in an aqueous solution of 10<sup>-5</sup> M ABA two weeks after harvest, whereas 10<sup>-4</sup> M ABA was required to delay sprouting by 18 days in the non-dormant cultivar.

The rate of exponential decline in ABA concentration was similar in three cultivars with contrasting storage potential (cvs. SS1, Renate and Ailsa Craig) (Chapter 3; Chope *et al.*, 2006), and therefore may not be as useful in predicting storage potential as the amount present before storage. Thus, it was suggested in Chapter 3 that increasing the preharvest ABA concentration in short storing cultivars could be a management strategy to delay sprouting in store.

Attempts to increase endogenous ABA concentration were made via application of exogenous ABA and an ABA analogue (8'-methylene ABA methyl ester; PBI-365) either as a preharvest foliar spray or as a postharvest bulb soak (Chapter 4). These experiments failed to increase endogenous ABA concentration, and so it was concluded that these methods of application of ABA or ABA analogues to onion crops in the field or to bulbs after harvest was not practical. Non-structural carbohydrates (fructose, glucose, sucrose and total fructan) were measured in this study. Sprout growth requires energy and would therefore be expected to be associated with changes in carbohydrate metabolism. No straightforward relationships between non-structural carbohydrates (glucose, fructose, sucrose and total fructan) and ABA concentration could be identified in this thesis, leading to the conclusion that minimal ABA concentration could be a trigger for remobilisation of carbohydrates to provide energy for the growing sprout.

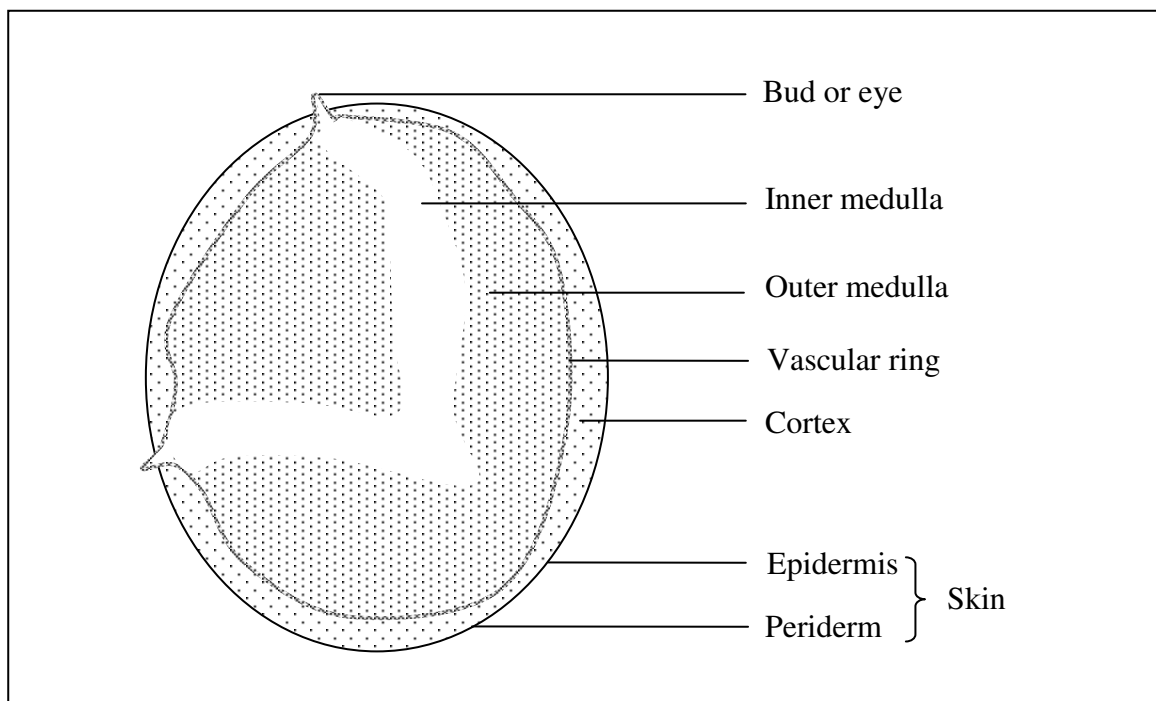
It is important that the quality of onion bulbs is maintained during storage so that a satisfactory product can be delivered to the consumer. A range of quality

measurements can be made, including dry weight, firmness, TSS, and pyruvate concentration. Onion bulb dry weight was found to decrease during long-term storage which is in agreement with the literature (Hurst *et al.*, 1985; Pak *et al.*, 1995; Hansen, 1999). Firmness also decreased during storage (Chapters 3, 4 and 5), and was positively correlated with dry weight (Chapter 4). The decrease in dry weight and firmness was more pronounced in onions cv. SS1, probably due to fewer, thinner, layers of outer skin on onions of this cultivar which provided a less effective barrier to loss of water from the bulb.

Pyruvate concentration is used as an indicator of pungency of onion bulbs (Schwimmer and Weston, 1961; Schwimmer and Guadagni, 1962), and it is particularly important for mild onion cultivars which are marketed as a value-added product. However, onion flavour is complex and not simply determined by pungency; sweetness is also important (Crowther *et al.*, 2005). Pyruvate concentration generally increased during storage, and so it is important that pungency is determined at the time of removal from storage as well as after harvest. The TSS % is commonly used as a measure of sugar content. Low dry matter onions (cv. SS1) tend to have a lower TSS % than high dry matter onions, but contain more glucose, sucrose and fructose, whereas high dry matter onions contain less glucose, sucrose and fructose, and are dominated by fructans. Therefore, TSS % is a very poor indicator of glucose, sucrose and fructose concentration, and consequently, of perceived sweetness (F. Davis and L. A. Terry, Cranfield University, unpublished data).

Recently, investigations have further elucidated the physiological control of dormancy in potatoes (Destefano-Beltrán *et al.*, 2006a; Destefano-Beltrán *et al.*, 2006b) and many parallels can be drawn between conclusions from these studies and the results presented in this thesis. Before comparisons between onion and potato can be made it is important to consider the similarities and differences between the two fresh produce types that may have implications on interpretation. Onions and potatoes both form underground storage organs (bulb and tuber) that undergo a dormant period when no growth can occur, and are important crop species comprising various cultivars displaying a range of potential storage lives. Storage life can be influenced by both pre- and

postharvest factors, and the role of hormones in control of dormancy and sprouting is considered to be of importance. However, onions have a single growing point at the base of the bulb and potatoes have more than one growing point (eye) (Figure 7.1). Where onions have multiple layers of skin, potatoes only have one epidermis which is fused to the cortex. Onion bulbs store fructans whereas potato tubers store starch. Taking into account these similarities and differences between the two crops will allow considered conclusions to be drawn from studies concerning dormancy control.



**Figure 7.1.** Cross-section of a potato tuber.

Links between ABA and dormancy/sprout suppression have been identified in potatoes (Suttle and Hultstrand, 1994; Destefano-Beltrán *et al.*, 2006b). The ABA concentration in inner medulla tissue was greatest in freshly harvested potatoes (350-440 pmol g<sup>-1</sup> FW), and declined during storage to 210-280 pmol g<sup>-1</sup> FW after 24 weeks (Biemelt *et al.*, 2000). The authors found no correlation between free ABA concentration and break of dormancy. However, ABA concentration was only measured after 6 and 24 weeks, so the results can only demonstrate that there is no universal threshold for ABA

concentration in potatoes before sprouting occurs. It is also likely that this is the case in onions, as the minimal ABA concentration reached during storage before the occurrence of sprouting differed with cultivar (Chapters 3, 4, 5 and 6).

It is likely that dormancy and sprout suppression are under the control of a combination of factors, and in onions the role of ethylene in these processes is unknown. The interaction between ABA and ethylene depends on the tissue type and developmental stage. In *Arabidopsis* seeds (Beaudoin *et al.*, 2000) and maize leaves (Voisin *et al.*, 2006), ABA and ethylene act antagonistically, but in *Arabidopsis* roots they work synergistically (Beaudoin *et al.*, 2000). A single dose of the ethylene perception inhibitor 1-MCP (1-methylcyclopropene) was shown to reduce sprout growth in short storage onions cv. SS1 (Chapter 5, Figure 5.1). The ABA concentration in treated and untreated bulbs was not different (Chapter 5, Figure 5.4). However, it was observed that higher concentrations of glucose, fructose and sucrose were maintained in 1-MCP-treated bulbs stored at 12°C (Chapter 5, Figure 5.5). Further investigation of the mechanism by which this occurred would be valuable, particularly since systems to maintain low levels of ethylene in onion stores have recently been marketed as a revolutionary new method to suppress sprout growth in stored onions (Johnson, 2006). The contrast between the use of ethylene to extend storage commercially, and the use of an inhibitor of ethylene perception to extend storage in the studies reported in this thesis demonstrates that there may be a dichotomy in the role that ethylene has to play in onion dormancy.

Potatoes are also low producers of ethylene ( $<0.1 \mu\text{l kg}^{-1} \text{hr}^{-1}$  at 20°C), and have low sensitivity to ethylene (Suslow and Voss, 2002), but continuous (23-33 weeks) exposure to ethylene ( $4 \mu\text{l l}^{-1}$ ) has been shown to be an effective method of sprout control in potatoes (Prange *et al.*, 2005). Increasing the concentration of ethylene to  $40 \mu\text{l l}^{-1}$  increased inhibition of sprout growth, but not enough to justify the additional costs of applying this chemical in a commercial environment (Daniels-Lake *et al.*, 2005). Exposure of stored potatoes to ethylene has the undesired side-effect of darkening the fry colour upon processing. This is caused by an accumulation of fructose and glucose in tubers stored in the presence of ethylene. Fry colour darkening can be prevented by application of 1-MCP ( $0.9 \mu\text{l l}^{-1}$ ) prior to ethylene exposure and at subsequent monthly or

bimonthly intervals (Daniels-Lake *et al.*, 2005). Thus, 1-MCP reduces the rate of ethylene-induced sugar accumulation in potato tubers. This contrasts with results reported in Chapter 5 where 1-MCP ( $1 \mu\text{l l}^{-1}$ ) treated onions stored at  $12^{\circ}\text{C}$  maintained a higher concentration of carbohydrates, probably due to reduced carbohydrate catabolism compared with untreated controls. It would be useful to study the carbohydrate concentration in onion bulbs exposed to continuous ethylene in store. If, as in potatoes, the sugar concentration rises then this could have a positive impact on taste perception and could be used as a marketing tool. Although ethylene can be used as a method of sprout control for potatoes, it reduces the true dormant period (defined by the number of days from planting to shoot emergence) in comparison with control tubers stored in air, or exposed to 1-MCP ( $1 \mu\text{l l}^{-1}$ ; 48 h) at the beginning of storage (Pruski *et al.*, 2006). Taken together, this suggests that ethylene breaks dormancy, but suppresses sprout elongation, and that 1-MCP may act differentially on different tissues, or impact on metabolic pathways other than those concerned with ethylene.

Both ABA and ethylene play a vital role in onion bulb dormancy and if understood in more detail there would be the potential to manipulate these parameters in order to extend storage life. Onions are regarded as non-climacteric. Ripening in climacteric produce, such as bananas, is rapid and under the direct and obvious control of ethylene. The onset of ripening is a defined event and is marked by an increase in respiration and the evolution of ethylene gas. In non-climacteric produce ripening is a continuous but gradual process not related to an obvious or large ethylene burst, therefore, ethylene concentration was not measured in the 1-MCP study (Chapter 5). It would be a logical extension to this work to investigate the effects of 1-MCP on a range of cultivars and to monitor ethylene concentration throughout storage. In addition, measuring equipment with the sufficient resolution to detect ethylene concentrations in the parts per billion to parts per trillion range could yield interesting results since the threshold for ethylene action is thought to be well below  $0.005 \mu\text{l l}^{-1}$  (Wills *et al.*, 1999). The dichotomy between the effect of ethylene and 1-MCP has wider implications for other non-climacteric produce that produce low concentrations of ethylene and have previously been thought to be little affected by its presence in store. Indeed ethylene

concentrations as low as  $0.01 \mu\text{l l}^{-1}$  have been shown to reduce the storage life of Chinese cabbage stored at 0 and  $20^{\circ}\text{C}$ , oranges stored at  $2.5^{\circ}\text{C}$ , and a range of leafy vegetables including broccoli and chives stored at 5 and  $20^{\circ}\text{C}$  (Wills *et al.*, 1999). Similarly by measuring ethylene concentration in the range of parts per billion, the ripening of strawberry fruit *in vivo* has been shown to have distinct patterns of ethylene production and regulation associated with different developmental stages, including an increase in ethylene production towards the end of the ripening process that corresponded with an increase in respiration rate (Ianetta *et al.*, 2006).

Ethylene is involved in the wound response (Ecker, 1995; Wang *et al.*, 2002). Wounding of the basal plate caused onion cv. Balstora and Hyton bulbs to sprout sooner than intact bulbs (Miedema, 1994b), and there is anecdotal evidence that a wounded onion in store will cause those around it to sprout (David O'Connor, Allium and Brassica Centre, pers. comm.). It is possible that this is due to the increased ethylene production caused by the wound response, although other physiological changes are also induced by this type of stress. Perhaps the use of the low dry matter, low pungency cultivar SS1 is the key to the results obtained in Chapter 5: onions cv. SS1 were found to have a lower ABA concentration than any other of the cultivars examined (Chapters 3, 4, 5 & 6), and it could be that this property i.e. low ABA concentration, allowed the onion bulb to respond to 1-MCP. Ethylene production by whole grapefruit was increased four days after treatment with 1-MCP ( $0.05 \mu\text{l l}^{-1} \text{kg}^{-1} \text{hr}^{-1}$ ) compared with untreated controls ( $0.005 \mu\text{l l}^{-1} \text{kg}^{-1} \text{hr}^{-1}$ ) (Mullins *et al.*, 2000). However, the increase in ethylene production was observed soon after 1-MCP treatment and may therefore have been a transient effect. The authors concluded that as ethylene biosynthesis is under negative feedback control, the increase in ethylene concentration was caused by 1-MCP binding to ethylene binding proteins, thus blocking the negative feedback effect of ethylene on its own biosynthesis and leading to uncontrolled ethylene production. If 1-MCP also increases ethylene production in onions, this could explain the sprout inhibiting effect of 1-MCP as although 1-MCP binds to ethylene binding proteins, it is possible that more binding proteins can be produced, thus allowing perception of ethylene (Dauny *et al.*, 2003). Alternatively, 1-MCP could bind to ethylene binding proteins in non-target tissue (i.e. non-meristematic



tissue), causing elevated ethylene biosynthesis in these tissues which was perceived by ethylene binding proteins in the meristematic tissue. The biosynthesis and signal transduction of ethylene is discussed later (Section 7.2).

The effects of ethylene depend on the rate of various metabolic reactions and so keeping produce at low temperatures and low oxygen concentrations will reduce the response (Saltveit, 1999). Carbon dioxide is an inhibitor of ethylene biosynthesis (de Wild *et al.*, 1999). As onion storage life is extended by CA storage, but is also extended by the use of continuous ethylene, it would be advantageous to monitor the levels of ethylene produced by onions in CA storage. It has been shown that the ethylene production of yellow onion bulbs was greater,  $0.14 \mu\text{l kg}^{-1} \text{hr}^{-1}$ , after short-term (<24 hr) exposure to 30% CO<sub>2</sub> and 20% O<sub>2</sub> than air, 10% CO<sub>2</sub> and 20% O<sub>2</sub> or 20% CO<sub>2</sub> and 20% O<sub>2</sub>,  $0.11 \mu\text{l kg}^{-1} \text{hr}^{-1}$  (Pal and Buescher, 1993). The authors concluded that this was probably an early response to physiological injury caused by the very high carbon dioxide concentrations. They detected no difference between air and the 10% and 20% CO<sub>2</sub> atmospheres. However, Kubo *et al.* (1990) found no difference in the ethylene production of onion bulbs (unspecified Japanese cultivar) before and after exposure to 60% CO<sub>2</sub> for 24 hr at 25°C, and recorded both rates as 'trace'. It may be that the use of more sensitive equipment, such as laser photoacoustic spectroscopy (Janetta *et al.*, 2006), to measure the ethylene concentration would reveal small, but significant differences in ethylene production, during postharvest storage of onion bulbs.

Controlled atmosphere is used to extend onion storage life. It is thought this extension is due to a decrease in respiration rate. However, when onions are removed from CA storage, shelf-life can be compromised (David O'Connor, Allium and Brassica Centre, pers. comm.). When onion bulbs are transferred from CA storage into air, respiration rate is increased in comparison with those stored in air continuously (Chapter 6, Figure 6.1), however, the increase in respiration rate was not accompanied by a decrease in sugar concentrations. It was therefore suggested that the increase in respiration rate (measured 2 hr after transition) was a transient response to the stress of being moved from CA into air, and that this may be sufficient to trigger the onset of sprout growth, particularly in short-storing onion, such as cv. SS1. Delaying the start of

controlled atmosphere storage for 3 weeks was as effective at inhibiting sprout growth in onion cv. SS1 bulbs as 6 weeks continual CA storage (Chapter 6, Figure 6.2). The implications of this finding are discussed in Section 7.3. The study reported in Chapter 6 yielded additional results; ABA assays carried out on bulb samples immediately after harvest revealed a significant decrease in ABA concentration between the time of harvest and the beginning of storage (Chapter 6, Figure 6.5). This result strongly suggested that the high temperatures to which the onion bulbs were exposed during the curing period may have been responsible for a decrease in ABA concentration. If the hypothesis holds true that ABA concentration at harvest is correlated with storage life, then current curing techniques could be having a detrimental effect on storage life and/or dormancy. Furthermore, short-term (three weeks) high temperature postharvest treatments of 30-35°C significantly reduced the number of days to sprouting in dry storage at 15°C, when compared to those exposed to postharvest temperature treatments of 15-25°C (Miedema, 1994a).

The question remains as to how to increase the endogenous ABA concentration in the bulb, since it is difficult to achieve this via exogenous application (Chapter 4), and would also negate the issue of whether an exogenously applied hormone has the same effect as the endogenous hormone (Nemhauser *et al.*, 2002). Also known as the drought hormone, ABA concentrations increase during periods of water stress. Irrigation could be withheld prior to bulb maturity, although this method may be impractical as it would have to be balanced with the decrease in yield that would occur as a result, and the occurrence of rainfall cannot be controlled. Drought-stressed onions (75% water deficit in the top 25 cm soil profile) matured earlier with increased dry matter concentration and reduced yield (Sorensen and Grevsen, 2001), but postharvest sprouting was inhibited compared to non-drought stressed bulbs. Bulb ABA concentration was not measured, but the increased storage life could have been due to elevated ABA concentration. Increased salinity and pH also have a role in increasing ABA concentration, but again there are considerations to be made concerning the most desirable growing conditions for the optimum crop yield and quality. This also implies that different soil types are likely to affect ABA concentration in onion bulbs prior to storage. It may be that harvest could be

timed to optimise ABA concentration by testing the ABA concentration throughout growth. However, this is likely to be too costly as the only methods currently available for measuring ABA must be performed by trained personnel in the laboratory. The most likely solution may be to alter the curing practice (Section 7.3).

## 7.2 Recommendations for future experimental work

In the future, the challenge of extending the storage life of onion bulbs without the use of sprout suppressants could be tackled using a genetic or molecular biological approach. This might involve identifying candidate genes involved in quality traits important to both growers and consumers and determining whether these traits could be separated from traits associated with the capacity for long-term storage. If this was possible, then marker assisted breeding programmes could be developed to produce new cultivars with desirable traits, such as low pungency (which is currently linked with poor storage life), which would maintain high quality over a longer storage period.

Genomes within the *Alliums* are large (King *et al.*, 1998). Onions are diploid ( $2n=16$ ), with an estimated nuclear genome size of 15 290-15 797 Mbp per 1C, and a 2C DNA amount of 31.69 - 33.2 pg (Arumuganathan and Earle, 1991; Ricroch and Brown, 1997). This is 6, 16 and 107 times larger than maize, tomato and *Arabidopsis*, respectively (King *et al.*, 1998). The guanine-cytosine (GC) content is a characteristic of a genome which is sometimes used to classify organisms taxonomically. Genes tend to have a higher GC content than the rest of the genome. The GC content of the *A. cepa* genome is *ca.* 37% (Kirk *et al.*, 1970, Ricroch and Brown, 1997). There is a paucity of public genetic information on onion or any other *Allium* crops. Some key genes in the sulphur assimilation pathway have been cloned (McCallum *et al.*, 2002) and recently, a set of over 10 000 onion expressed sequence tags has become available (Kuhl *et al.*, 2004). However, biochemical and molecular investigation are required before the exact function of these genes and the proteins they encode can be assigned (Jones *et al.*, 2004).

Some genetic studies have already been carried out in onions. A low density genetic map has been produced consisting of 116 markers on 12 linkage groups and covering 1064 cM, with an average distance of 9.2 cM between loci (King *et al.*, 1998). Significant positive, genetic and phenotypic correlations have been found among total soluble solids, dry matter, pungency and onion induced anti-platelet activity (Galmarini *et al.*, 2001). More recently, the map has been expanded to include an extra 10 markers and now spans more 1907 cM and all eight chromosomes (Martin *et al.*, 2005). This genetic map has been used with quantitative trait loci (QTL) analysis to identify a locus (*Frc*) that affects bulb fructan content to chromosome 8 (McCallum *et al.*, 2006).

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

There are two possible approaches to producing a microarray chip suitable for this use. Highly detailed genetic information on model plants is available in public databases. Nevertheless, there is a limit to the validity of the application of data from model plants to crop species (Havey, 2004). Genetic resources for other important crop species, such as rice, are also available, however there are important differences between the *Poales* to which rice belongs, and the *Asparagales*, to which onion belongs (Kuhl *et al.*, 2004) which mean that a microarray constructed using sequences from rice would be unlikely to be a total success. The second option would be to construct an onion-specific microarray. There are 11,726 unique sequences available on the TIGR database (The Institute for Genomic Research, 2003), of which 3838 have tentative consensus (TC) status, and have been allocated unique 70-mer oligonucleotide sequence suitable for construction of microarrays (Richmond and Somerville, 2000). This collection of 3838 oligonucleotides could be expanded by mining genomic databases, and adding specific genes related to

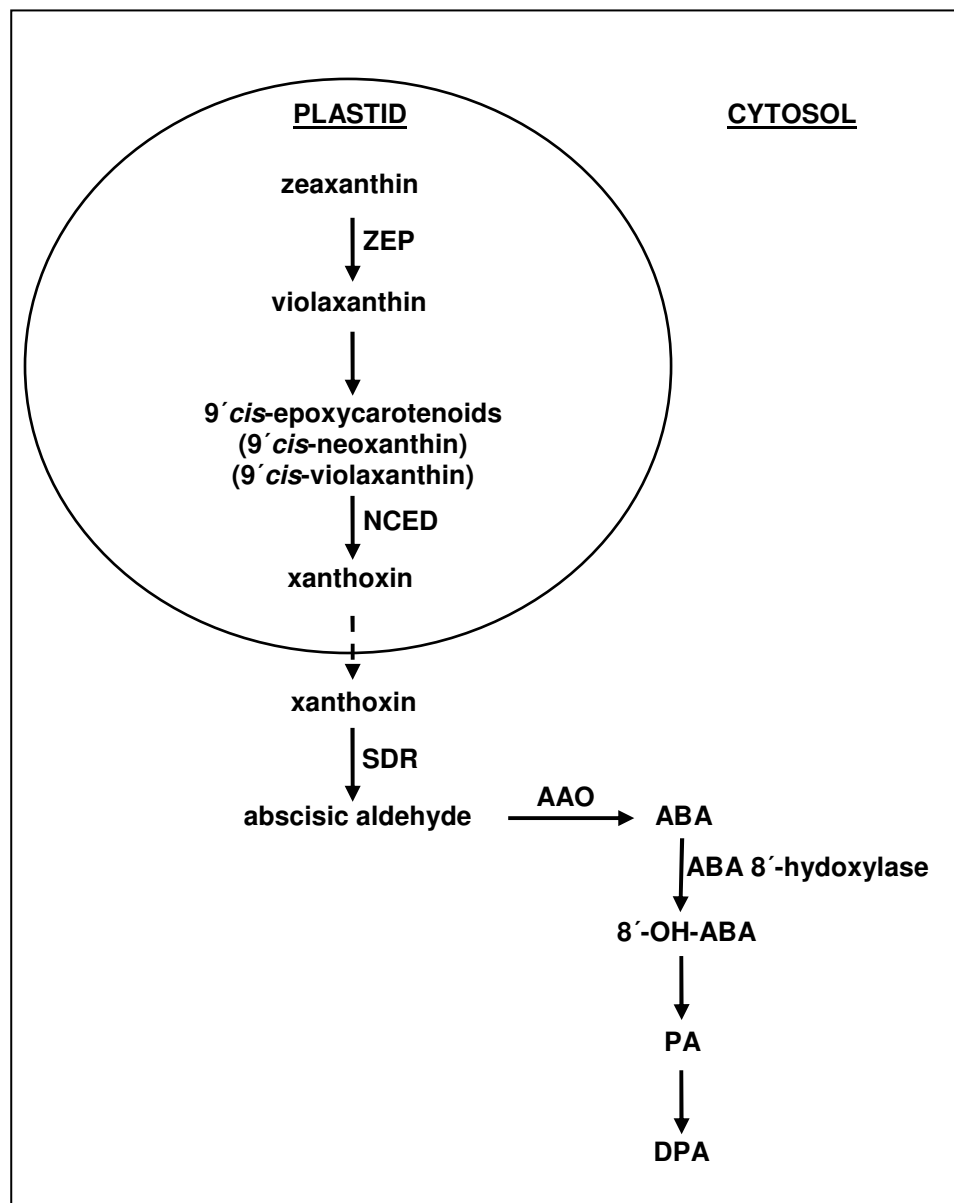
dormancy such as ABA and ethylene metabolism and signaling, and carbohydrate metabolism. In cases where these target genes could not be located on public databases, genes identified by RT-PCR using degenerate primers designed from sequences aligned from other monocotyledonous species could be added. Microarray analysis should be accompanied by detailed metabolomic profiling, as it is likely that post-transcriptional regulation of proteins occurs.

A complex chemical profile including substances such as ABA and ABA metabolites, compounds concerned with sulphur and carbohydrate metabolism as well as the parameters measured in the current study (pyruvate, fructan, fructose, glucose, sucrose, TSS) would allow the application of chemometrics to the gene expression data. Chemometrics is a statistical technique whereby the chemical data collected can be related to the physiological state (Prazen, 2005). In this way it is anticipated that the use of metabolomic profiling in conjunction with microarray technology could identify the factors that are important in the transition from dormancy induction to sprout suppression. The onset of sprouting occurs at different times in different cultivars, and there is also considerable variation within cultivars. In potatoes this issue was overcome with the use of bromoethane (BE) which chemically induced premature breaking of dormancy (Destefano-Beltrán *et al.*, 2006a). If a similar treatment could be applied to onions then this would be advantageous as it would be possible to control when the break of dormancy occurred. However, consideration would have to be made as to whether the chemically-induced dormancy break occurred via the same mechanisms as the natural process.

Potentially, diagnostic microarray chips based on a reduced number of specific sets of genes that were related to specific physiological stages and/or quality traits could be developed. Mass production of these slides would reduce the costs involved. Diagnostic tests that could predict sprouting would be beneficial for growers to predict both the storage life and the shelf life of the crop. The chip could also be used as a replacement in the future for time consuming full biochemical and physical profiling, but microarray analysis would need to be carried out in the laboratory. If sets of genes that were differentially expressed on sprouting were identified then transcription factors that

control the initial stages of meristematic activity could be targets for genetic modification. However, this approach may be problematic, because if re-growth was delayed indefinitely then seeds or sets could not be produced from the modified bulbs. Also there is still considerable consumer hostility towards genetically modified crops.

Abscisic acid has clearly been identified as playing an important role in onion storage (Chapters 3, 4, 5 and 6). Therefore, the mechanisms controlling ABA concentration within the bulb during the different physiological stages in stored onions should be investigated in more detail. The concentration of ABA in any system is a result of a balance between transport, synthesis and catabolism (Taylor *et al.*, 2005; Zaharia *et al.*, 2005). An onion bulb is essentially a closed system, and so transport is unlikely to be a factor. The pathways involved in ABA biosynthesis and catabolism have been discussed in Chapter 2, Section 2.9.6, but in order to understand the molecular mechanisms, the genes and enzymes involved in ABA biosynthesis and catabolism must be studied in more detail (Figure 7.2). The information from *Arabidopsis* in terms of the biosynthetic pathway can be applied to other plant species, as both the pathway and the genes involved are highly conserved in angiosperms (Xiong and Zhu, 2003).



**Figure 7.2.** Summary of the pathways involved in ABA biosynthesis and catabolism. Dashed arrow indicates export of xanthoxin from the plastid to the cytosol. ZEP=zeaxanthin epoxydase, NCED=nine *cis*-epoxycarotenoid dioxygenase; SDR=short chain alcohol dehydrogenase/reductase; AAO=abscisic aldehyde dehydrogenase; ABA=abscisic acid; PA=phaseic acid; DPA=dihydrophaseic acid (Nambara and Marion-Poll, 2005; Taylor *et al.*, 2005).

The first 15C intermediate in the ABA biosynthesis pathway is xanthoxin, whose synthesis begins with the cleavage of a 40C oxygenated carotenoid precursor and ends with the conversion of 9'-*cis*-neoxanthin to xanthoxin which is catalysed by nine-*cis*-epoxycarotenoid dioxygenase (NCED) (Xiong and Zhu, 2003). The formation of xanthoxin takes place within the plastid, from where it is exported into the cytosol. All remaining steps in the pathway occur in the cytoplasm (Taylor *et al.*, 2005). The reaction catalysed by NCED is thought to be the key rate limiting step (Thompson *et al.*, 2000). Early reactions in the pathway to ABA biosynthesis are important to maintain a supply of precursors, so in order to increase ABA biosynthesis in non-photosynthetic tissue, the over-expression of genes controlling the NCED pathway may need to be combined with the over-expression of upstream pathways. A short chain alcohol dehydrogenase/reductase (SDR) catalyses the conversion of xanthoxin to abscisic aldehyde. In *Arabidopsis* seeds SDR is induced by sugar but not by osmotic stress or ABA (Xiong and Zhu, 2003). Nine *NCED* related sequences have been identified in *Arabidopsis*, of which five are probably involved in ABA metabolism (Nambara and Marion-Poll, 2005).

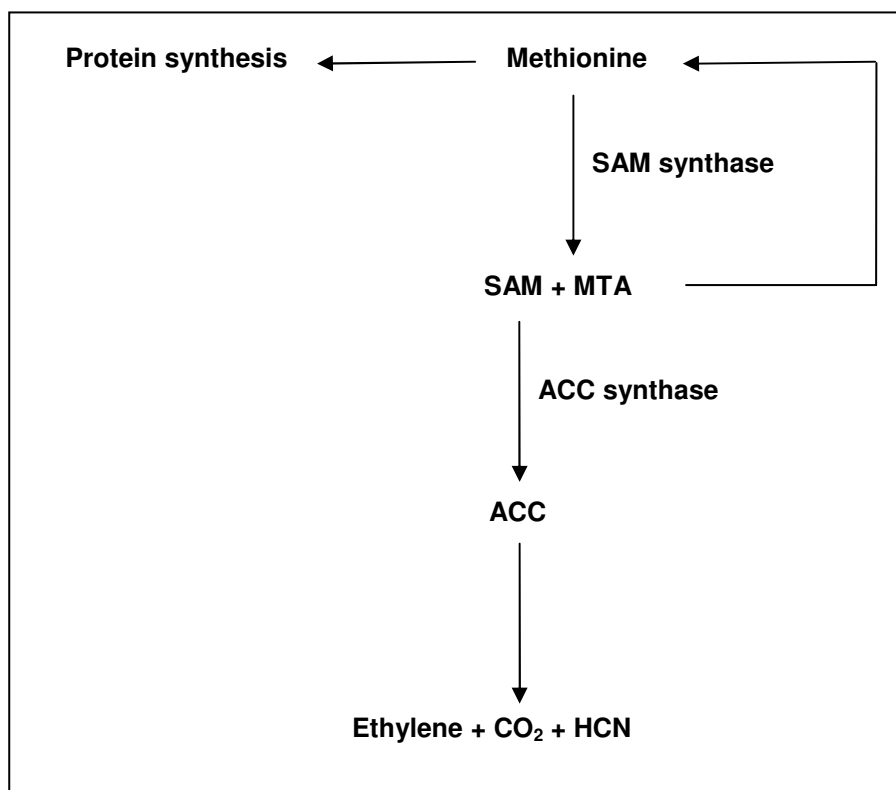
The catabolism of ABA can be split into two categories; hydroxylation and conjugation, of which the former is the most important (Nambara and Marion-Poll, 2005). Hydroxylation is followed by further inactivation, and the major source is hydroxylation at the C8 position. The main ABA catabolites are phaseic acid (PA) and dihydrophaseic acid (DPA). Phaseic acid has minimal biological activity, whereas DPA has none.

In unstressed plants, the vascular tissue is likely to be the main site of ABA biosynthesis, which is then transported to target cells such as stomata (Nambara and Marion-Poll, 2005), or in the case of onions, to the bulb tissue throughout growth. In seeds, dormancy loss is due to a suppression of *de novo* synthesis and activation of catabolism and this is also likely to be the case in bulbs. The genes and proteins involved in ABA biosynthesis are better characterised than those associated with its degradation. Recently, *Arabidopsis thaliana* *CYP707A* genes have been shown to encode (+)-ABA-8'-hydroxylase, which is capable of catalysing the hydroxylation of ABA to 8'-hydroxy



ABA when expressed in *in vitro* cell culture systems (Saito *et al.*, 2004). This multi-gene family exists in many monocotyledons and dicotyledons (Saito *et al.*, 2004). This means that the chances of cloning this gene family from onion, for use in constructing a microarray, will be high.

In light of the results from Chapter 5, further investigation of the genes involved in ethylene metabolism would also be useful. The ethylene biosynthesis pathway is well characterised and is summarised in Figure 7.3. Ethylene is a simple hydrocarbon that is synthesized from methionine. The first committed step in ethylene biosynthesis is the conversion of S-adenosyl-L-methionine (SAM) into 1-amino-cyclopropane-1-carboxylic acid (ACC) catalysed by ACC synthase (Ecker, 1995). This is also thought to be the key rate limiting step. The final stage is the oxidation of ACC by ACC oxidase which results in the production of ethylene along with cyanide and carbon dioxide. Cyanide is detoxified by conversion to  $\beta$ -cyanoalanine by  $\beta$ -cyanoalanine synthase. A pool of methionine is maintained by the conversion of 5'-methylthioadenosine (MTA), produced from the SAM synthase reaction, back into methionine (Wang *et al.*, 2002).



**Figure 7.3.** Simplified pathway of ethylene biosynthesis. SAM=S-adenosyl-L-methionine, MTA=5'-methylthioadenosine, ACC=1-amino-cyclopropane-1-carboxylic acid (Ecker, 1995; Wang *et al.*, 2002).

The ACS (ACC synthase) genes are part of a multi gene family, and many ACS genes from different plant species have been identified and cloned (Wang *et al.*, 2002). Both positive and negative feedback mechanisms for the regulation of ethylene biosynthesis have been identified (Saltveit, 1999). It is likely that the explanation for different feedback mechanisms is due to differential regulation of different genes in the family (Wang *et al.*, 2002). For example, negative feedback occurs in non-climacteric fruit, and positive feedback occurs in climacteric fruit (Saltveit, 1999). Different genes could be expressed in different tissues and at different times, thus allowing for spatial and temporal variation (Wang *et al.*, 2002). There is also evidence of post-transcriptional regulation. This emphasizes the importance of making biochemical measurements, such as ethylene and ABA concentration, in conjunction with genetic analysis. Ethylene

perception is via membrane localized proteins, of which five have been identified in *Arabidopsis thaliana*.

The importance of ABA in onion bulb dormancy has been shown, and the evidence for this would be further strengthened through the use of inhibitors of ABA activity or the use of compounds that inhibit breakdown of ABA. Recently, new inhibitors of ABA degradation have been discovered and developed including competitive inhibitors that are powerful ABA agonists and suicide substrates (Cutler *et al.*, 2000). Unicazone inhibits degradation of ABA 8'-hydroxylase, but also other cytochrome P450 enzymes such as those involved in gibberellin synthesis (Saito *et al.*, 2006). However Araki *et al.* (2006) have developed a non-azole inhibitor of ABA 8'-hydroxylase which has the additional benefits of specificity and having no ABA-like activity (Araki *et al.*, 2006). Tools to investigate the effects of reduced ABA concentration include abamine (ABA biosynthesis inhibitor with an amine moiety) which inhibits NCED by competitive inhibition (Han *et al.*, 2004), and the more potent and specific abamineSG (Kitahata *et al.*, 2006).

As the use of a microarray allows the analysis of many genes at once, and physiological processes are likely to be a result of the interaction of many factors, then it would be useful to also study the genes involved with the metabolism of other plant hormones including auxin, cytokinins and gibberellins (Kuraishi *et al.*, 1989; Mahotiere *et al.*, 1989; Miedema and Kamminga, 1994; Yamazaki *et al.*, 2002). Resources for this type of study are being built up in the form of lists of genes that are up- or down regulated by the application of these hormones (Nemhauser *et al.*, 2006).

### **7.3 Implications for growers**

In light of rising energy costs (the cost of commercial electricity increased by *ca.* 50%, and gas by *ca.* 65% between 2005 and 2006; Department of Trade and Industry, 2006) any reduction in the amount of gas and electricity used in the curing, drying and storage of onions would be desirable to industry. Current UK practice aims to remove

surface moisture within three days of loading the store by heating at 30°C, followed by a further ten days at 24°C (RH not to exceed 75%). The crop is then allowed to cool to approximately 15°C, with ventilation, for a few days until the necks are tight and dry. The temperature can then be slowly reduced until the desired long-term storage temperature is reached. The curing and drying procedure is based on a method developed in the 1970s (Shipway, 1977; O'Connor and Shipway, 1978), and therefore the research that formed the basis of this procedure was carried out on cultivars that are very different from those used today. Thus, it is likely that alterations to current methods, such as a reduction in the temperature and duration of the curing and drying periods would deliver benefits in the form of energy savings and reduced carbon emissions, while still producing onion bulbs of a satisfactory quality standard. As previously mentioned, the concentration of ABA at the beginning of the storage period appeared to be a good marker of storage potential of onion bulbs. Bulb ABA concentration decreased during curing, and therefore ABA concentration would be a very useful parameter to monitor during different curing and drying regimes. This could then lead to the breeding of improved onion cultivars that perform better under more efficient curing regimes.

The role of ethylene in sprouting is complex and of current interest because ethylene generating systems are currently being installed in commercial onion storage facilities (Johnson, 2006). Continual long-term exposure to ethylene (10  $\mu\text{l l}^{-1}$ ) has been shown to be effective in reducing sprouting in stored onion bulbs. This contrasts with the results presented here for onion cv. SS1 bulbs treated with the ethylene inhibitor 1-MCP. The involvement of ethylene in onion bulb dormancy certainly requires further investigation and suggests that there are several mechanisms involved. The introduction of new cultural practices such as altering the curing regime may influence the effect of ethylene on onion quality. While there is a wealth of literature on the role of ethylene in climacteric produce, there is little on that of ethylene (or 1-MCP) in non-climacteric systems. It is recommended that 1-MCP be used as a tool to help elucidate the mechanisms by which continual exposure to ethylene reduces sprout growth in some onion cultivars. It is also likely that this research would have the potential to increase the efficiency and optimise the application of ethylene to the crop either in terms of more

precise timing of application or perhaps using pulsed treatment rather than continual application thus reducing the cost.

The finding that the respiration rate of onion bulbs increased on transfer from CA to air (Chapter 6) has implications for the shelf-life of onions following CA storage. Removal of onions from storage in environments with continual elevated ethylene concentrations may have a similar effect on respiration rate. Monitoring of the respiration rate in onions stored in these conditions and then transferred to air would help to detail how shelf-life is affected following ethylene treatment. It was shown in Chapter 6 that storage of onions cv. SS1 in air for three weeks, followed by three weeks CA storage achieved a level of sprout suppression equal to that achieved using six weeks continuous CA storage. Further investigation into the effects of the delaying the start of CA storage has the potential to reduce the costs associated with this storage method while not compromising on effectiveness.

#### 7.4 Project conclusions

The project objectives were set out in Chapter 1, Section 1.2.2. A brief summary of the conclusions of the project in terms of the objectives is below.

- *To determine if differences exist in the initial concentrations and/or rate of degradation of abscisic acid (ABA) in bulbs of onion cultivars with different storage potential.* There was a difference in the ABA concentration recorded before storage in long and medium-storing onions (cvs. Renate and Ailsa Craig) and short-storing onions (cv. SS1). The rate of degradation during storage was the same for each cultivar (Chapter 3).
- *To determine if the remobilisation of carbohydrates is affected by, and correlated with, the concentration of ABA.* No straightforward relationship between ABA concentration and the remobilisation of NSCs (glucose, sucrose, fructose and total

- fructan) could be identified (Chapters 4, 5 and 6). It was postulated that minimal ABA concentration acted as a trigger for remobilization of carbohydrates.
- To verify the effect, if any, on storage potential of bulb ABA concentration using a chemical analogue of ABA. An analogue of ABA (8'-methylene ABA methyl ester; PBI-365) was applied as a preharvest foliar spray and postharvest bulb soak, however, endogenous bulb ABA concentration was not affected, and there was no consistent effect of treatment on storage life or any of the quality parameters measured.
  - To determine the effect of an ethylene inhibitor on onion storage life. An ethylene perception inhibitor (1-MCP) was applied to onions cv. SS1, and reduced sprout growth when bulbs were subsequently stored at 4 or 12°C (Chapter 5).
  - To determine the effect of the transition between controlled atmosphere storage and air on bulbs of onion cultivars with different storage potential. Respiration rate of three cultivars (*viz.* Renate, Carlos and SS1) increased on removal from CA storage. Glucose concentration was found to be highest in sprouted bulbs cv. SS1 (Chapter 6).
  - To relate the observations made to the potential to influence storage life by changes in horticultural practices. Curing was suggested to have detrimental effects on ABA concentration and therefore also on storage life. Therefore it was recommended that current curing practices be investigated and altered (Chapters 6 and 7). Delaying the start of CA storage of onions cv. SS1 by three weeks was found to be as effective at controlling sprout growth as six weeks continuous CA storage. This has the potential to reduce the costs associated with CA storage (Chapters 6 and 7). Recently, elevated ethylene has been used to prolong the storage life of stored onions. This contrasts with the work on 1-MCP reported here, and so investigation into the mode of action of 1-MCP is recommended, along with further testing of 1-MCP (Chapters 5 and 7).

## CHAPTER EIGHT

### Literature cited

- Abayomi, L. A., Terry, L. A., White, S. F. and Warner, P. J. (2006). Development of a disposable pyruvate biosensor to determine pungency in onions (*Allium cepa* L.). *Biosensors and Bioelectronics*, 21, 2176-2179.
- Abdalla, A. A. and Mann, L. K. (1963). Bulb development in the onion (*Allium cepa* L.) and the effect of storage temperature on bulb rest. *Hilgardia*, 35, 85-112.
- Abdel-Rahman, M. and Isenberg, F. M. R. (1974). The role of exogenous plant growth regulators in the dormancy of onion bulbs. *Journal of Agricultural Science*, 82, 113-116.
- Abrams, S. R., Rose, P. A., Cutler, A. J., Balsevich, J. J., Lei, B. and Walker-Simmons, M. K. (1997). 8'-Methylene abscisic acid. An effective and persistent analogue of abscisic acid. *Plant Physiology*, 114, 89-97.
- Adamicki, F. (2005). Effects of pre-harvest treatments and storage conditions on quality and shelf-life of onions. In: Guangshu, L. (Ed.) *Proceedings of the Fourth International Symposium on Edible Alliaceae*, Beijing, China, 21-26 April 2004, ISHS Acta Horticulturae 688, 26-33.
- Adamicki, F. and Kepka, A. K. (1974). Storage of onions in controlled atmospheres. *Acta Horticulturae*, 38, 53-74.
- ADAS. (1985). *The analysis of agricultural materials, MAFF Reference book 427*. HMSO, London.
- Araki, Y., Miyawaki, A., Miyashita, T., Mizutani, M., Hirai, N. and Todoroki, Y. (2006). A new non-azole inhibitor of ABA 8'-hydroxylase: Effect of the hydroxyl group substituted for geminal methyl groups in the six-membered ring. *Bioorganic and Medicinal Chemistry Letters*, 16, 3302-3305.
- Arumuganathan, K. and Earle, E. D. (1991). Nuclear DNA content of some important plant species. *Plant Molecular Biology Reporter*, 9, 208-218.

- Barrieu, P. and Simonneau, T. (2000). The monoclonal antibody MAC252 does not react with the (-) enantiomer of abscisic acid. *Journal of Experimental Botany*, 51, 305-307.
- Beaudoin, N., Serizet, C., Gosti, F. and Giraudat, J. (2000). Interactions between abscisic acid and ethylene signalling cascades. *Plant Cell*, 12, 1103-1115.
- Benkeblia, N. (2000). Phenylalanine ammonia-lyase, peroxidase, pyruvic acid and total phenolics variations in onion bulbs during long-term storage. *Food Science and Technology - Lebensmittel-Wissenschaft und Technologie*, 33, 112-116.
- Benkeblia, N. (2004). Effect of maleic hydrazide on respiratory parameters of stored onion bulbs (*Allium cepa* L.). *Brazilian Journal of Plant Physiology*, 16, 47-52.
- Benkeblia, N., Onodera, S., Yoshihira, T., Kosaka, S. and Shiomi, N. (2004). Effect of temperature on soluble invertase activity, and glucose, fructose and sucrose status of onion bulbs (*Allium cepa*) in store. *International Journal of Food Science and Nutrition*, 55, 325-331.
- Benkeblia, N., Onodera, S. and Shiomi, N. (2005a). Variation in 1-fructo-exohydrolase (1-FEH) and 1-kestose-hydrolysing (1-KH) activities and fructo-oligosaccharide (FOS) status in onion bulbs. Influence of temperature and storage time. *Journal of the Science of Food and Agriculture*, 85, 227-234.
- Benkeblia, N., Ueno, K., Onodera, S. and Shiomi, N. (2005b). Variation of fructooligosaccharides and their metabolizing enzymes in onion bulb (*Allium cepa* L. cv. Tenshin) during long-term storage. *Journal of Food Science*, 70, S208-S214.
- Benkeblia, N. and Selselet-Attou, G. (1999a). Effects of low temperatures on changes in oligosaccharides, phenolics and peroxidase in inner bud of onion *Allium cepa* L. during break of dormancy. *Acta Agriculturae Scandinavica Section B - Soil and Plant Science*, 49, 98-102.
- Benkeblia, N. and Selselet-Attou, G. (1999b). Role of ethylene on sprouting of onion bulbs (*Allium cepa* L.). *Acta Agriculturae Scandinavica Section B - Soil and Plant Science*, 49, 122-124.
- Benkeblia, N. and Shiomi, N. (2004). Chilling effect on soluble sugars, respiration rate, total phenolics, peroxidase activity and dormancy of onion bulbs. *Scientia Agricola*, 61, 281-285.
- Benkeblia, N. and Shiomi, N. (2006). Hydrolysis kinetic parameters of DP 6, 7, 8, and 9-12 fructooligosaccharides (FOS) of onion bulb tissues. Effect of temperature and storage time. *Journal of Agricultural and Food Chemistry*, 54, 2587-2592.



- Benkeblia, N. and Varoquaux, P. (2003). Effect of nitrous oxide (N<sub>2</sub>O) on respiration rate, soluble sugars and quality attributes of onion bulbs *Allium cepa* cv. Rouge Amposta during storage. *Postharvest Biology and Technology*, 30, 161-168.
- Benkeblia, N., Varoquaux, P., Gouble, B. and Selselet-Attou, G. (2000). Respiratory parameters of onion bulbs (*Allium cepa*) during storage. Effects of ionising radiation and temperature. *Journal of the Science of Food and Agriculture*, 80, 1772-1778.
- Benkeblia, N., Varoquaux, P., Shiomi, N. and Sakai, H. (2002). Storage technology of onion bulbs cv. Rouge Amposta: effects of irradiation, maleic hydrazide and carbamate isopropyl, N-phenyl (CIP) on respiration rate and carbohydrates. *International Journal of Food Science and Technology*, 37, 169-175.
- Biemelt, S., Hajirexaei, M., Hentschel, E. and Sonnewald, U. (2000). Comparative analysis of abscisic acid content and starch degradation during storage of tubers harvested from different potato varieties. *Potato Research*, 43, 371-382.
- Blankenship, S. M. and Dole, J. M. (2003). 1-methylcyclopropene: A review. *Postharvest Biology and Technology*, 28, 1-25.
- Block, E. (1992). The organosulphur chemistry of the genus *Allium* - implications for the organic chemistry of sulphur. *Angewandte Chemie International Edition in English*, 31, 1135-1178.
- Blumenfield, A. and Bukovac, M. J. (1972). Cuticular penetration of abscisic acid. *Planta*, 107, 261-268.
- Brewster, J. L. (1977a). The physiology of the onion. Part 1. *Horticultural Abstracts*, 47, 17-23.
- Brewster, J. L. (1977b). The physiology of the onion. Part 2. *Horticultural Abstracts*, 47, 103-112.
- Brewster, J. L. (1990). Physiology of crop growth and bulbing. In: Rabinowitch, H. D. and Brewster, J. L. (Eds.) *Onions and Allied Crops. Volume 1. Botany, Physiology and Genetics*, CRC Press, Florida.
- Brewster, J. L. (1994). *Onions and other Vegetable Alliums*, CABI Publishing, Wallingford.
- Brice, J. R., Bisbrown, A. J. K. and Curd, L. (1995). Onion storage trials at high ambient temperature in the Republic of Yemen. *Journal of Agricultural and Engineering Research*, 62, 185-192.

- Briggs, W. H. and Goldman, I. L. (2002). Variation in economically and ecologically important traits in onion plant organs during reproductive development. *Plant Cell and Environment*, 25, 1031-1037.
- Budu, A. S. and Joyce, D. C. (2003). Effect of 1-methylcyclopropene on the quality of minimally processed pineapple fruit. *Australian Journal of Experimental Agriculture*, 43, 177-184.
- Carter, C. E., Partis, M. D. and Thomas, B. (1999). The expression of histone 2A in onion (*Allium cepa*) during the onset of dormancy, storage and emergence from dormancy. *New Phytologist*, 143, 461-470.
- Chope, G. A., Terry, L. A. and White, P. J. (2006). Effect of controlled atmosphere storage on abscisic acid concentration and other biochemical attributes of onion bulbs. *Postharvest Biology and Technology*, 39, 233-242.
- Coleman, W. K. (1998). Carbon dioxide, oxygen and ethylene effects on potato tuber dormancy release and sprout growth. *Annals of Botany*, 82, 21-27.
- Coolong, T. W., Kopsell, D. A., Kopsell, D. E. and Randle, W. M. (2004). Nitrogen and sulphur influence nutrient usage and accumulation in onion. *Journal of Plant Nutrition*, 27, 1667-1686.
- Croci, C. A., Banek, S. A. and Curzio, O. A. (1995). Effect of gamma irradiation and extended storage on chemical quality in onion (*Allium cepa* L.). *Food Chemistry*, 54, 151-154.
- Crowther, T., Collin, H. A., Smith, B., Tomsett, A. B., O'Connor, D. and Jones, M. G. (2005). Assessment of the flavour of fresh uncooked onions by taste panels and analysis of flavour precursors, pyruvate and sugars. *Journal of the Science of Food and Agriculture*, 85, 112-120.
- Currah, L. (1981). Onion flowering and seed production. *Scientific Horticulture*, 32, 26-46.
- Cutler, A. J. and Krochko, J. E. (1999). Formation and breakdown of ABA. *Trends in Plant Science*, 4, 472-478.
- Cutler, A. J., Rose, P. A., Squires, T. M., Loewen, M. K., Shaw, A. C., Quail, J. W., Krochko, J. E. and Abrams, S. R. (2000). Inhibitors of abscisic acid 8'-hydroxylase. *Biochemistry*, 39, 13614-13624.
- Dahlhelm, D. and Matjeko, C. (1990). Relations between the physiological state of onions and an effective sprout inhibition by irradiation. *Radiation Physics and Chemistry*, 36, 651-656.

- Daniels-Lake, B. J., Prange, R. K., Nowak, J., Asiedu, S. K. and Walsh, J. R. (2005). Sprout development and processing quality changes in potato tubers stored under ethylene: 1. Effects of ethylene concentration. *American Journal of Potato Research*, 82, 389-397.
- Darbyshire, B. and Henry, R. J. (1978). The distribution of fructans in onions. *New Phytologist*, 81, 29-34.
- Darbyshire, B. and Henry, R. J. (1979). The association of fructans with high percentage dry weight in onion cultivars suitable for dehydrating. *Journal of the Science of Food and Agriculture*, 30, 1035-1038.
- Dauny, P. T., Joyce, D. C. and Gamby, C. (2003). 1-Methylcyclopropene influx and efflux in 'Cox' apple and 'Hass' avocado fruit. *Postharvest Biology and Technology*, 29, 101-105.
- DEFRA. (2003). *Family food in 2001/2002*, National Statistics, London.
- Department of Trade and Industry. (2006). *Statistical press release – Energy statistics*. [online]. Government news network. Available at: <http://www.gnn.gov.uk/environment/detail.asp?ReleaseID=210961&NewsAreaID=2>. (Accessed: 10 October 2006).
- Destefano-Beltrán, L., Knauber, D., Huckle, L. and Suttle, J. (2006a). Chemically forced dormancy termination mimics natural dormancy progression in potato tuber meristems by reducing ABA content and modifying expression of genes involved in regulating ABA synthesis and metabolism. *Journal of Experimental Botany*, 57, 2879-2886.
- Destefano-Beltrán, L., Knauber, D., Huckle, L. and Suttle, J. C. (2006b). Effects of postharvest storage and dormancy status on ABA content, metabolism, and expression of genes involved in ABA biosynthesis and metabolism in potato tuber tissues. *Plant Molecular Biology*, 61, 687-697.
- de Wild, H. P. J., Gude, H. and Peppelenbos, H. W. (2002). Carbon dioxide and ethylene interactions in tulip bulbs. *Physiologia Plantarum*, 114, 320-326.
- Dorffling, K. and Tietz, D. (1983). Methods for the detection and estimation of abscisic acid and related compounds. In: Addicott, F. (Ed.) *Abscisic Acid*, Preager Publishers, New York.
- Ecker, J. R. (1995). The ethylene signal transduction pathway in plants. *Science*, 268, 667-675.

- Ernst, M. K., Bufler, G., Röske, M., Ritzkowski, M., Metzger, C. and Liebig, H-P. (1999). Cultivar and temperature effects on starch in the primary thickening meristem of onion bulbs. *Journal of Horticultural Science and Biotechnology*, 74, 588-593.
- Ernst, M. K., Chatterton, N. J., Harrison, P. A. and Matitschka, G. (1998). Characterization of fructan oligomers from species of the genus *Allium* L. *Journal of Plant Physiology*, 153, 53-60.
- Ernst, M. K., Praeger, U. and Weichmann, J. (2003). Effect of low oxygen storage on carbohydrate changes in onion (*Allium cepa* var. *cepa*) bulbs. *European Journal of Horticultural Science*, 68, 59-62.
- FAOSTAT. (2006). *Food and Agriculture Organisation of the United Nations - Agricultural Data (Classic)*. [online]  
Available at: <http://faostat.fao.org/site/395/default>. (Accessed: 19 July 2006).
- Fenwick, G. R. and Hanley, A. B. (1985). The genus *Allium* - Part 1. *CRC Critical Reviews in Food Science and Nutrition*, 22, 199-271.
- Finkelstein, R. R. and Rock, C. D. (2002). Abscisic acid biosynthesis and response. In: Somerville, C.R. and Meyerowitz, E. M. (Eds.) *The Arabidopsis Book*, American Society of Plant Biologists, Rockville, MD, USA.
- Fritsch, R. M. and Friesen, N. (2002). Evolution, domestication and taxonomy. In: Rabinowitch, H. D. and Currah, L. (Eds.) *Allium Crop Science: Recent Advances*. CABI Publishing, Wallingford.
- Galmarini, C. R., Goldman, I. L. and Havey, M. J. (2001). Genetic analyses of correlated solids, flavor, and health-enhancing traits in onion (*Allium cepa* L.). *Molecular Genetics and Genomics*, 265, 543-551.
- Griffiths, G., Trueman, L., Crowther, T., Thomas, B. and Smith, B. (2002). Onions - A global benefit to health. *Phytotherapy Research*, 16, 603-615.
- Gubb, I. and MacTavish, H. S. (2002). Onion pre- and postharvest considerations. In: Rabinowitch, H. D. and Currah, L. (Eds.) *Allium Crop Science: Recent Advances*. CABI Publishing, Wallingford.
- Hamilton, B. K., Yoo, K. S. and Pike, L. M. (1998). Changes in pungency of onions by soil type, sulphur nutrition and bulb maturity. *Scientia Horticulturae*, 74, 249-256.

- Han, S.-Y., Kitahata, N., Sekimata, K., Saito, T., Kobayashi, M., Nakashima, K., Yamaguchi-Shinozaki, K., Shinozaki, K., Yoshida, S. and Asami, T. (2004). A novel inhibitor of 9-cis-epoxycarotenoid dioxygenase in abscisic acid biosynthesis in higher plants. *Plant Physiology*, 135, 1574-1582.
- Hansen, S. L. (1999). Content and composition of dry matter in onion (*Allium cepa* L.) as influenced by developmental stage at time of harvest and long-term storage. *Acta Agriculturae Scandinavica Section B - Soil and Plant Science*, 49, 103-109.
- Hartung, W., Sauter A., and Hose, E. (2002). Abscisic acid in the xylem: where does it come from, where does it go to? *Journal of Experimental Botany*, 53, 27-32
- Havey, M. J. (1999). Advances in new *Alliums*. In: Janick, J. (Ed.) *Perspectives on New Crops and New Uses*, ASHS Press, Alexandria, Virginia.
- Havey, M. J. (2004). Application of genomic technologies to crop plants: opportunities and challenges. *Crop Science*, 44, 1893-1919.
- Havey, M. J. and Randle, W. M. (1996). Combining abilities for yield and bulb quality among long- and intermediate-day open-pollinated onion populations. *Journal of the American Society for Horticultural Science*, 121, 604-608.
- Herold, B., Oberbarnscheidt, B. and Geyer, M. (1998). Mechanical load and its effect on bulb onions due to harvest and post-harvest handling. *Journal of Agricultural and Engineering Research*, 71, 373-383.
- Hole, C. C., Drew, R. L. K. and Gray, D. (2000). Humidity and the mechanical properties of onion skins. *Postharvest Biology and Technology*, 19, 229-237.
- Hurst, W. C., Shewfelt, R. L. and Schuler, G. A. (1985). Shelf-life and quality changes in summer storage onions (*Allium cepa*). *Journal of Food Science*, 50, 761-763.
- Iannetta, P. P. M., Laarhoven, L. J., Medina-Escobar, N., James, E. K., Mcmanus, M. T., Davies, H. V. and Harren, F. J. M. (2006). Ethylene and carbon dioxide production by developing strawberries show a correlative pattern that is indicative of ripening climacteric fruit. *Physiologia Plantarum*, 127, 247-259.
- Imai, S., Tsuge, N., Tomotake, M., Nagatome, Y., Sawada, H., Nagata, T. and Kumagai, H. (2002). Plant biochemistry: An onion enzyme that makes the eyes water. *Nature*, 419, 685.
- Jaime, L., Martinez, F., Martin-Cabrejas, M. A., Molla, E., Lopez-Andreu, F. J., Waldren, K. W. and Esteban, R. M. (2000). Study of total fructan and fructooligosaccharide content in different onion tissues. *Journal of the Science of Food and Agriculture*, 81, 177-182.

- Jaime, L., Martin-Cabrejas, M. A., Molla, E., Lopez-Andreu, F J. and Esteban, R. M. (2001). Effect of storage on fructan and fructooligosaccharide of onion (*Allium cepa* L.). *Journal of Agricultural and Food Chemistry*, 49, 982-988.
- Johnson, J. (2006). Onion storage revolution? *The Vegetable Farmer*, 2, 25-26.
- Jones, M. G., Hughes, J., Tregova, A., Milne, J., Tomsett, A. B. and Colin, H. A. (2004). Biosynthesis of the flavour precursors of onion and garlic. *Journal of Experimental Botany*, 55, 1903-1918.
- Kader, A. A. (2004). Controlled atmosphere storage. In: Gross, K. C., Wang, C. Y. and Saltveit, M. E. (Eds.) *Agricultural Handbook Number 66. The Commercial Storage of Fruits, Vegetables and Florist and Nursery Stocks*. [online]. USDA. Available at: <http://www.ba.ars.usda.gov/hb66/>. (Accessed: 25 May 2005).
- Kahane, R., Delaserve, B. T. and Rancillac, M. (1992). Bulbing in long-day onion (*Allium cepa* L) cultured *in vitro* - comparison between sugar feeding and light induction. *Annals of Botany*, 69, 551-555.
- Kahane, R., Vialle-Guerin, E., Boukema, I., Tzanoudakis, D., Bellamy, C., Chamaux, C. and Kik, C. (2001). Changes in non-structural carbohydrate composition during bulbing in sweet and high-solid onions in field experiments. *Environmental and Experimental Botany*, 45, 73-83.
- Karagiannis, C. S. and Pappelis, A. J. (1994). Effect of abscisic acid, gibberellic acid, indoleacetic acid, and kinetin on selective ribosomal cistron regulation in quiescent and senescent onion leaf base tissue. *Mechanisms of Ageing and Development*, 76, 145-155.
- King, J. J., Bradeen, J. M., Bark, O., McCallum, J. A. and Havey, M. J. (1998). A low-density genetic map of onion reveals a role for tandem duplication in the evolution of an extremely large diploid genome. *Theoretical and Applied Genetics*, 96, 52-62.
- Kirk, J. T. O., Rees, H. and Evans, G. (1970). Base composition of nuclear DNA within genus *Allium*. *Heredity*, 25, 507-512.
- Kitahata, N., Han, S. Y., Noji, N., Saito, T., Kobayashi, M., Nakano, T., Kuchitsu, K., Shinozaki, K., Yoshida, S., Matsumoto, S., Tsujimoto, M. and Asami, T. (2006). A 9-cis-epoxycarotenoid dioxygenase inhibitor for use in the elucidation of abscisic acid action mechanisms. *Bioorganic and Medicinal Chemistry*, 14, 5555-5561.

- Kleinkopf, G. E., Oberg, N. A. and Olsen, N. L. (2003). Sprout inhibition in storage: current status, new chemistries and natural compounds. *American Journal of Potato Research*, 80, 317-327.
- Komochi, S. (1990). Bulb dormancy and storage. In: Rabinowitch, H. D. and Brewster, J. L. (Eds.) *Onions and Allied Crops, I. Botany, Physiology and Genetics*, CRC Press, Florida.
- Ko, S-K., Chany, W-N., Wang, J-F., Cherng, S-J. and Shanmugasundaram, S. (2002). Storage variability among short-day onion cultivars under high temperature and high relative humidity, and its relationship with disease incidence and bulb characteristics. *Journal of the American Society for Horticultural Science*, 127, 848-854.
- Kopsell, D. E. and Randle, W. M. (1997). Short-day onion cultivars differ in bulb selenium and sulphur accumulation which can affect bulb pungency. *Euphytica*, 96, 385-390.
- Kopsell, D. E., Randle, W. M. and Eiteman, M. A. (1999). Changes in the S-alk(en)yl cysteine sulfoxides and their biosynthetic intermediates during onion storage. *Journal of the American Society for Horticultural Science*, 124, 177-183.
- Kubo, Y., Inaba, A. and Nakamura, R. (1990). Respiration and C<sub>2</sub>H<sub>4</sub> production in various harvested crops held in CO<sub>2</sub>-enriched atmospheres. *Journal of the American Society for Horticultural Science*, 115, 975-978.
- Kuhl, J. C., Cheung, F., Yuan, Q., Martin, W., Zewdie, Y., McCallum, J., Catanach, A., Rutherford, P., Sink, K. C., Jenderek, M., Prince, J. P., Town, C. D. and Havey, M. J. (2004). A unique set of 11,108 onion expressed sequence tags reveals expressed sequence and genomic differences between the monocot orders Asparagales and Poales. *The Plant Cell*, 16, 114-125.
- Kuraishi, S., Yamashita, D., Sakurai, N. and Hasegawa, S. (1989). Changes of abscisic acid and auxin as related to dormancy breaking of *Allium wakegi* bulblets by vacuum infiltration and BA treatment. *Journal of Plant Growth Regulation*, 8, 3-9.
- Lancaster, J. E., Farrant, J. and Shaw, M. L. (2001). Sulfur nutrition affects cellular sulfur, dry weight distribution, and bulb quality in onion. *Journal of the American Society for Horticultural Science*, 126, 164-168.
- Lancaster, J. E., Shaw, M. L. and Randle, W. M. (1998). Differential hydrolysis of alk(en)yl cysteine sulfoxides by alliinase in onion macerates: flavour implications. *Journal of the Science of Food and Agriculture*, 78, 367-372.

- Lancaster, J. E., Triggs, C. M., De Ruiter, J. M. and Gandar, P. W. (1996). Bulbing in onions: Photoperiod and temperature requirements and prediction of bulb size and maturity. *Annals of Botany*, 78, 423-430.
- Mahotiere, S., Herner, R. C. and Dennis, F. G. (1976). Effects of applied growth substances on growth of shoot apices excised from onions in rest. *Journal of the American Society for Horticultural Science*, 101, 211-213.
- Marcano, L., Carruyo, I., Del Campo, A. and Montiel, X. (2004). Cytotoxicity and mode of action of maleic hydrazide in root tips of *Allium cepa* L. *Environmental Research*, 94, 221-226.
- Martin, W. J., McCallum, J., Shigyo, M., Jakse, J., Kuhl, J. C., Yamane, N., Pither-Joyce, M., Gokce, A. F., Sink, K. C., Town, C. D. and Havey, M. J. (2005). Genetic mapping of expressed sequences in onion and *in silico* comparisons with rice show scant colinearity. *Molecular Genetics and Genomics*, 274, 197-204.
- Matsubara, S. and Kimura, I. (1991). Changes of ABA content during bulbing and dormancy and *in vitro* bulbing in onion plant. *Journal of the Japanese Society for Horticultural Science*, 59, 757-762.
- Maude, R. B., Shipway, M. R., Presly, A. H. and O'Connor, D. (1984). The effects of direct harvesting and drying systems on the incidence and control of neck rot (*Botrytis allii*) in onions. *Plant Pathology*, 33, 263-268.
- McCallum, J., Clarke, A., Pither-Joyce, M., Shaw, M., Butler, R., Brash, D., Scheffer, J., Sims, I., Van Heusden, S., Shigyo, M. and Havey, M. (2006). Genetic mapping of a major gene affecting onion bulb fructan content. *Theoretical and Applied Genetics*, 112, 958-967.
- McCallum, J., Pither-Joyce, M. and Shaw, M. L. (2002). Sulfur deprivation and genotype affect gene expression and metabolism of onion roots. *Journal of the American Society for Horticultural Science*, 127, 583-589.
- Miedema, P. (1994a). Bulb dormancy in onion. I. The effects of temperature and cultivar on sprouting and rooting. *Journal of Horticultural Science*, 69, 29-39.
- Miedema, P. (1994b). Bulb dormancy in onion. III. The influence of the root system, cytokinin and wounding on sprout emergence. *Journal of Horticultural Science*, 69, 47-52.
- Miedema, P. and Kamminga, G. C. (1994). Bulb dormancy in onion. II. The role of cytokinins in high temperature bulb imposed sprout inhibition. *Journal of Horticultural Science*, 69, 41-45.



- Mullins, E. D., McCollum, T. G. and McDonald, R. E. (2000). Consequences on ethylene metabolism of inactivating the ethylene receptor sites in diseased non-climacteric fruit. *Postharvest Biology and Technology*, 19, 155-164.
- Nambara, E. and Marion-Poll, A. (2005). Abscisic acid biosynthesis and catabolism. *Annual Review of Plant Biology*, 56, 165-185.
- Nemhauser, J. L., Hong, F. X. and Chory, J. (2006). Different plant hormones regulate similar processes through largely nonoverlapping transcriptional responses. *Cell*, 126, 467-475.
- NIAB. (2000). *Vegetable variety handbook*, National Institute of Agricultural Botany.
- O'Connor, D. (1979). *Onion Storage*. Grower Books, London.
- O'Connor, D. B. and Shipway, M. R. (1978). *Bulb onions. The direct lifting/store drying system*. Horticultural Technical Notes, ADAS. (unpublished).
- O'Donoghue, E. M., Somerfield, S. D., Shaw, M., Bendall, M., Hedderly, D., Eason, J. and Sims, I. (2004). Evaluation of carbohydrates in Pukekohe Longkeeper and Grano cultivars of *Allium cepa*. *Journal of Agricultural and Food Chemistry*, 52, 5383-5390.
- Ogata, K. (1961). Physiological studies on the storage of onion bulb. *Bulletin of the University of Osaka Prefecture: Series B: Agriculture and Biology*, 11, 99-120.
- Pak, C., Vanderplas, L. H. W. and Deboer, A. D. (1995). Importance of dormancy and sink strength in sprouting of onions (*Allium cepa*) during storage. *Physiologia Plantarum*, 94, 277-283.
- Pal, R. K. and Buescher, R. W. (1993). Respiration and ethylene evolution of certain fruits and vegetables in response to carbon dioxide in controlled atmosphere storage. *Journal of Food Science and Technology*, 30, 29-32.
- Pesticide Residue Committee. (2006). *Pesticide residues monitoring report 2005. Quarter ended September 2005*. [online]. Available at: [http://www.pesticides.gov.uk/uploadedfiles/Web\\_Assets/PRC/PRC\\_Q3\\_2005.pdf](http://www.pesticides.gov.uk/uploadedfiles/Web_Assets/PRC/PRC_Q3_2005.pdf) (Accessed: 14 August 2006).
- Pompodakis, N. E. and Joyce, D. C. (2003). Abscisic acid analogue effects on the vase life and leaf crisping of cut Baccara roses. *Australian Journal of Experimental Agriculture*, 43, 425-428.

- Praeger, U., Ernst, M. K. and Weichmann, J. (2003). Effects of ultra low oxygen storage on postharvest quality of onion bulbs (*Allium cepa* L. var. *cepa*). *European Journal of Horticultural Science*, 68, 14-19.
- Prange, R. K., Daniels-Lake, B. J., Jeong, J-C. and Binns, M. (2005). Effects of ethylene and 1-methylcyclopropene on potato tuber sprout control and fry color. *American Journal of Potato Research*, 82, 123-128.
- Prazen, .B. (2005). *Chemometrics glossary of terms*. [online]. Chemometrics and Informatics, Jon Wiley and Sons, Ltd. Available at: <http://www.spectroscopynow.com/coi/cda/detail.cda?id=10118&type=EducationFeature&chId=9&page=1>. (Accessed: 2 September 2006).
- Price, C. E., Boatman, S. G. and Boddy, B. J. (1975). The uptake and translocation of 1-methylpyridinium chloride and related model compounds in wheat. *Journal of Experimental Botany*, 26, 521-532.
- Pruski, K., Prange, R. K., Daniels-Lake, B. J., Nowak, J., Astatkie, T. and Ronis, D. H. (2006). Growth-room and field studies with seed tubers treated with ethylene and 1-methylcyclopropene (1-MCP) during storage. *American Journal of Potato Research*, 83, 149-160.
- Quarrie, S. A., Whitford, P. N., Appleford, N. E .J., Wang, T. L., Cook, S. K., Henson, I. E. and Loveys, B. R. (1988). A monoclonal antibody to (S)-abscisic acid: its characterisation and use in a radioimmunoassay for measuring abscisic acid in crude extracts of cereal and lupin leaves. *Planta*, 173, 330-339.
- Randle, W. M. (1992). Sulphur nutrition affects nonstructural water-soluble carbohydrates in onion germplasm. *Hortscience*, 27, 52-55.
- Randle, W. M. (1997). Onion flavour chemistry and factors influencing flavour chemistry. In: Risch, S., J. and Ho, C.-T. (Eds.) *Spices: Flavour chemistry and antioxidant properties*. American Chemical Society, Washington.
- Randle, W. M. and Bussard, M. L. (1993). Streamlining onion pungency analyses. *Hortscience*, 28, 60.
- Randle, W. M., Bussard, M. L. and Warnock, D. F. (1993). Ontogeny and sulphur fertility affect leaf sulphur in short-day onions. *Journal of the American Society for Horticultural Science*, 118, 762-765.

- Randle, W. M., Lancaster, J. E., Shaw, M. L., Sutton, K. H., Hay, R. L. and Bussard, M. L. (1995). Quantifying onion flavor compounds responding to sulfur fertility - sulfur increases levels of alk(en)yl cysteine sulfoxides and biosynthetic intermediates. *Journal of the American Society for Horticultural Science*, 120, 1075-1081.
- Richmond, T. and Somerville, S. (2000). Chasing the dream: plant EST microarrays. *Current Opinion in Plant Biology*, 3, 108-116.
- Ricroch, A. and Brown, S. C. (1997). DNA base composition of *Allium* genomes with different chromosome numbers. *Gene*, 205, 255-260.
- Rosher, P. H., Jones, H. G. and Hedden, P. (1985). Validation of a radioimmunoassay for (+)-abscisic acid in extracts of apple and sweet pepper tissue using high-pressure liquid chromatography and combined gas chromatography-mass spectrometry. *Planta*, 165, 91-99.
- Rutherford, R. and Whittle, R. (1982). The carbohydrate composition of onions during long term cold storage. *Journal of Horticultural Science*, 57, 249-356.
- Rylski, I., Rappaport, L., Pratt, H. K. (1974). Dual effects of ethylene on potato dormancy and sprout growth. *Plant Physiology*, 53, 658-662.
- Saito, S., Hirai, N., Matsumoto, S., Ohigashi, H., Ohta, D., Sakata, K. & Mizutani, M. (2004). Arabidopsis *CYP707As* encode (+)-abscisic acid 8'-hydroxylase, a key enzyme in the oxidative catabolism of abscisic acid. *Plant Physiology*, 134, 1439-1449.
- Saito, S., Okamoto, M., Okamoto, M., Kushiro, T., Koshihara, T., Kamiya, Y., Hirai, N., Todoroki, Y., Sakata, K., Nambara, E. & Mizutani, M. (2006). A plant growth retardant, uniconazole, is a potent inhibitor of ABA catabolism in Arabidopsis. *Bioscience Biotechnology and Biochemistry*, 70, 1731-1739.
- Salama, A. M., Hicks, J. R. and Nock, J. F. (1990). Sugar and organic acid changes in stored onion bulbs treated with maleic hydrazide. *Hortscience*, 25, 1625-1628.
- Saltveit, M. E. (1999). Effect of ethylene on quality of fresh fruits and vegetables. *Postharvest Biology and Technology*, 15, 279-292.
- Schwimmer, S. and Guadagni, D.G. (1962). Relation between olfactory threshold concentration and pyruvic acid content of onion juice. *Journal of Food Science*, 27, 94-97.
- Schwimmer, S. and Weston, W. J. (1961). Enzymatic development of pyruvic acid in onion as a measure of pungency. *Agricultural and Food Chemistry*, 9, 301-304.

- Shipway, M. R. (1977). Dry bulb onions: effects of various handling and drying practices. *Kirton Experimental Horticulture Station: Fourteenth Report 1977*, 53-57.
- Sinclair, P. J., Blakeney, A. B. and Barlow, E. W. R. (1995a). Relationships between bulb dry matter content, soluble solids concentration and non-structural carbohydrate composition in the onion. *Journal of the Science of Food and Agriculture*, 69, 203-209.
- Sinclair, P. J., Neeson, R. J. and Barlow, E. W. R. (1995b). Osmotic potential and soluble solids concentration in onion (*Allium cepa*) bulbs. *Journal of the Science of Food and Agriculture*, 69, 211-214.
- Sisler, E. C. (2006). The discovery and development of compounds counteracting ethylene at the receptor level. *Biotechnology Advances*, 24, 357-367.
- Sisler, E. C. and Serek, M. (1997). Inhibitors of ethylene responses in plants at the receptor level: Recent developments. *Physiologia Plantarum*, 100, 577-582.
- Sitton, J. W., Fellman, J. K., Patterson, M. E. (1997). Effects of low-oxygen and high-carbon dioxide atmospheres on postharvest quality, storage and decay of 'Walla Walla' sweet onions. In: Saltveit, M. E. (Ed.) *Proceedings of the VII International Controlled Atmosphere Research Conference*, Davis, CA, 13-18 July 1997, Postharvest Horticulture Series, 18, 20-25.
- Smittle, D. A. (1988). Evaluation of storage methods for 'Granex' onions. *Journal of the American Society for Horticultural Science*, 133, 877-880.
- Sorensen, J. N. and Grevsen, K. (2001). Sprouting in bulb onions (*Allium cepa* L.) as influenced by nitrogen and water stress. *Journal of Horticultural Science and Biotechnology*, 76, 501-506.
- Sumner, P. E. (2000). *Vidalia onion CA storage requirements*. College of Agricultural and Environmental Sciences, University of Georgia.
- Suslow, T. (1998). *Onion Dry. Recommendations for maintaining postharvest quality*. Department of Vegetable Crops, University of California.
- Suslow, T. V. and Voss, R. (2002). *Potato: (Immature early crop)*. Department of Vegetable Crops, University of California.
- Suttle, J. and Hultstrand, J. F. (1994). Role of endogenous abscisic acid in potato microtuber dormancy. *Plant Physiology*, 105, 891-896.

- Suzuki, M. and Cutliffe, J. (1989). Fructans in onion bulbs in relation to storage life. *Canadian Journal of Plant Science*, 69, 1327-1333.
- Taylor, I. B., Sonneveld, T., Bugg, T. D. H. and Thompson, A. J. (2005). Regulation and manipulation of the biosynthesis of abscisic acid, including the supply of xanthophyll precursors. *Journal of Plant Growth Regulation*, 24, 253-273.
- Terry, L. A., Law, K. A., Hipwood, K. J. and Bellamy, P. H. (2005). Non-structural carbohydrate profiles in onion bulbs influence taste preference. In: *Frutic 05, Information and technology for sustainable fruit and vegetable production*, 12-16 September 2005, Montpellier, France.
- The Institute for Genomic Research. (2003). *TIGR A. cepa Gene Index*. [online]. Available at: [http://www.tigr.org/tigr-scripts/tgi/T\\_index.cgi?species=onion](http://www.tigr.org/tigr-scripts/tgi/T_index.cgi?species=onion). (Accessed: 23 August 2006).
- Thomas, T. H. (1969). The role of growth substances in the regulation of onion bulb dormancy. *Experimental Botany*, 20, 124-137.
- Thomas, T. H. and Isenberg, F. M. R. (1972). Hormone physiology of onion bulbs during dormancy. *Experimental Horticulture*, 23, 48-51.
- Thompson, A. J., Jackson, A. C., Symonds, R. C., Mulholland, B. J., Dadswell, A. R., Blake, P. S., Burbidge, A. and Taylor, I. B. (2000). Ectopic expression of a tomato 9-cis-epoxycarotenoid dioxygenase gene causes over-production of abscisic acid. *Plant Journal*, 23, 363-374.
- Tsukamoto, Y., Fujita, M., Inaba, T. and Asahira, T. (1969). Changes of growth promoting substances and abscisic acid during the dormancy in onion. *Memoirs of the Research Institute for Food Science, Kyoto University*, 30, 24-27.
- Uddin, M. and MacTavish, H. S. (2003). Controlled atmosphere and regular storage-induced changes in S-alk(en)yl-L-cysteine sulfoxides and alliinase activity in onion bulbs (*Allium cepa* L. cv. Hysam). *Postharvest Biology and Technology*, 28, 239-245.
- U.S. Department of Agriculture. (2004). *USDA nutrient database for standard reference. Vol. 2004*. [online]. Available at: <http://www.nal.usda.gov/fnic/foodcomp> (Accessed: 20 October 2004).
- Voisin, A. S., Reidy, B., Parent, B., Rolland, G., Redondo, E., Gerentes, D., Tardieu, F. and Muller, B. (2006). Are ABA, ethylene or their interaction involved in the response of leaf growth to soil water deficit? An analysis using naturally occurring variation or genetic transformation of ABA production in maize. *Plant Cell and Environment*, 29, 1829-1840.

- Wang, K. L. C., Li, H. and Ecker, J. R. (2002). Ethylene biosynthesis and signalling networks. *Plant Cell*, 14, S131-S151.
- Watkins, C. B. (2006). The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. *Biotechnology Advances*, 24, 389-409.
- Watkins, C. B. and Miller, W. B. (2005). *A summary of physiological processes or disorders in fruits, vegetables and ornamental products that are delayed or decreased, increased, or unaffected by application of 1-methylcyclopropene (1-MCP)*. [online]. Department of Horticulture, Cornell University, Ithaca, New York. Available at: <http://www.hort.cornell.edu/departement/faculty/watkins/ethylene/index.htm>. (Accessed: 4 December 2005).
- Wheeler, T. R., Daymond, A. J., Ellis, R. H., Morison, J. I. L. and Hadley, P. (1998). Postharvest sprouting of onion bulbs grown in different temperature and CO<sub>2</sub> environments in the UK. *Journal of Horticultural Science and Biotechnology*, 73, 750-754.
- Wickramasinghe, U. L., Wright, C. J. and Currah, L. (2000). Bulbing responses of two cultivars of red tropical onions to photoperiod, light integral and temperature under controlled growth conditions. *Journal of Horticultural Science and Biotechnology*, 75, 304-311.
- Wills, R. B. H., Ku, V. V. V., Shohet, D. and Kim, G. H. (1999). Importance of low ethylene levels to delay senescence of non-climacteric fruits and vegetables. *Australian Journal of Experimental Agriculture*, 39, 221-224.
- Xiong, L. and Zhu, J-K. (2003). Regulation of abscisic acid biosynthesis. *Plant Physiology*, 133, 29-36.
- Yamazaki, H., Nishijima, T. and Koshioka, M. (1995). Changes in abscisic acid content and water status in bulbs of *Allium wakegi* Araki throughout the year. *Journal of the Japanese Society for Horticultural Science*, 64, 589-598.
- Yamazaki, H., Nishijima, T., Koshioka, M. and Miura, H. (2002). Gibberellins do not act against abscisic acid in the regulation of bulb dormancy of *Allium wakegi* Araki. *Plant Growth Regulation*, 36, 223-229.
- Yamazaki, H., Nishijima, T., Yamato, Y., Koshioka, M. and Miura, H. (1999a). Involvement of abscisic acid (ABA) in bulb dormancy of *Allium wakegi* Araki I. Endogenous levels of ABA in relation to bulb dormancy and effects of exogenous ABA and fluridone. *Plant Growth Regulation*, 29, 189-194.

- Yamazaki, H., Nishijima, T., Yamato, Y., Hamano, M., Koshioka, M. and Miura, H. (1999b). Involvement of abscisic acid in bulb dormancy of *Allium wakegi* Araki. II. A comparison between dormant and nondormant cultivars. *Plant Growth Regulation*, 29, 195-200.
- Yang, S. F. (1969). Ethylene evolution from 2-Chlorophosphonic acid. *Plant Physiology*, 44, 1023-1024.
- Yoo, K. S. and Pike, L. M. (1996). Controlled-atmosphere storage suppresses leaf growth and flowering in onion bulbs. *Hortscience*, 31, 875.
- Zaharia, L. I., Walker-Simmon, M. K., Rodriguez, C. N., and Abrams, S. R. (2005). Chemistry of abscisic acid, abscisic acid catabolites and analogs. *Journal of Plant Growth Regulation*, 24, 274-284.

## A. APPENDIX

### Validation of the abscisic acid radio-immunoassay

#### A.1 Introduction

The radio-immunoassay was validated for onion bulb tissue. Thin layer chromatography of samples and standards was used to investigate the spread of immunoreactivity of samples on the TLC plate compared to that of pure ABA. A spike dilution test was performed as a method of detecting interference in crude onion extracts. A range of serial dilutions of onion bulb extract were spiked with known amounts of ABA to generate a series of plots of ABA detected against ABA added. In the absence of interference the lines generated should be parallel. The absolute values obtained where the plots intercept the y-axis should decrease in concert with the increase, and in proportion with the dilution factor (Roshier *et al.*, 1985).

#### A.2 Materials and methods

##### A.2.1 Experiment 1 - Thin layer chromatography of abscisic acid

Standards and samples were prepared as follows: Plate 1: 20  $\mu\text{l}$  each of  $4 \times 10^4$   $\text{pg ml}^{-1}$  ABA solution and sample (Ailsa Craig, day 0, S-Ca-) were lyophilised and rehydrated with 10  $\mu\text{l}$  of 100 % methanol. Plate 2: 50  $\mu\text{l}$  each of  $5 \times 10^3$ ,  $1 \times 10^4$ ,  $2 \times 10^4$ ,  $4 \times 10^4$ ,  $5 \times 10^5$ ,  $5 \times 10^7$  and  $5 \times 10^9$   $\text{pg ml}^{-1}$  (+)-ABA solution were lyophilised and rehydrated with 15  $\mu\text{l}$  of 100% methanol. The concentration of ABA in samples eluted from TLC plates was determined by radio-immunoassay (as described in Chapter 3, section 3.3.8).



Twenty cm x 20 cm TLC plates (Merck, Darmstadt, Germany) were cleaned by three runs in 100% (v/v) methanol followed by two runs in chloroform prior to activation by heating to 120°C for one hour (Dorffling and Tietz, 1983). The running solvent was 100 ml of 10:1:1 (v/v/v) isopropanol : ammonia : water. The plates were developed (n=1) in the dark. The silica was scraped off the plate in 1.5 cm sections in each lane. Samples on plate 1 were eluted twice in 300 µl of 80% (v/v) methanol (Dorffling and Tietz, 1983); samples on plate 2 were eluted overnight at 4°C in the dark with 300 µl of 80% (v/v) methanol, followed by a 6 hour second elution step. The silica was removed by centrifugation. The supernatant was lyophilised and then rehydrated with 120 µl (plate 1) or 160 µl (plate 2) of sterile distilled water.

### *A.2.2 Spike dilution*

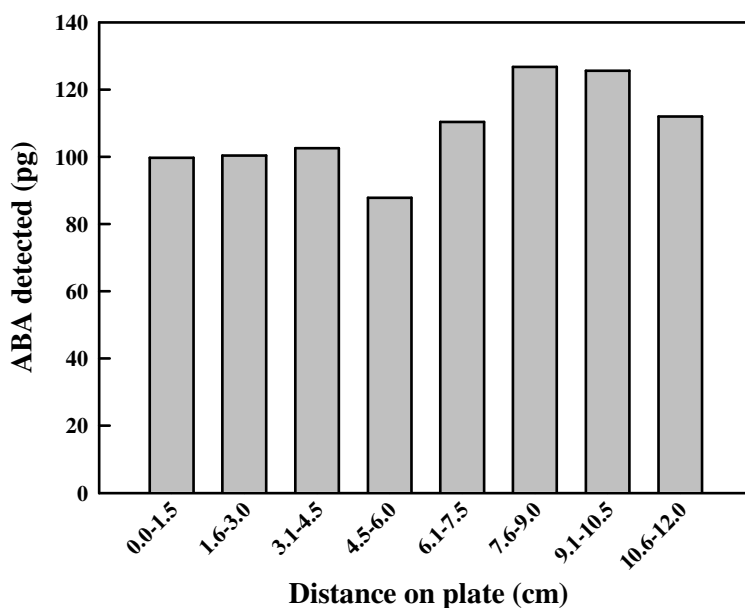
Various combinations of cultivar, frozen and lyophilised tissues, water and methanol extractions, and purification processes were used (Table A.1). All initial extractions were performed as in Chapter 3, section 3.3.8. Controls were sterile distilled water in place of tissue extract. Linear regression models using Genstat for Windows Version 7.1.0.198 (VSN International Ltd., Herts., UK) were applied to the plots of ABA detected against ABA added obtained from the spike dilution assays.

## **A.3 Results and discussion**

### *A.3.1 Experiment 1 – Thin layer chromatography*

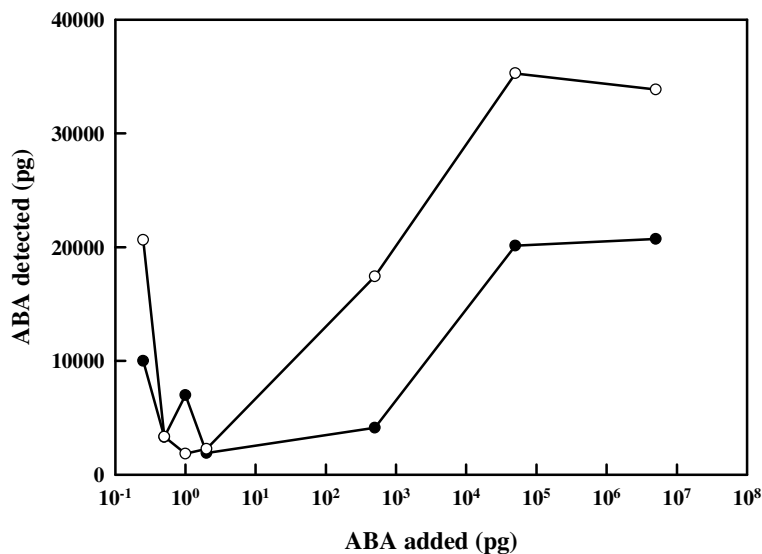
The  $R_f$  value of ABA in the TLC running solvent is 0.6 (Dorffling and Tietz, 1983), therefore the ABA in the sample should have travelled 7.8 cm along the plate. No distinct peak in ABA concentration was observed in the sample eluted from this zone of

plate 1 (Figure A.1). Radio-immunoassay of this crude onion extract detected 573.5 pg ABA per 50  $\mu$ l of extract, therefore *ca.* 230 pg was loaded onto the TLC plate. Approximately 120 pg ABA was detected in the zone covering the  $R_f$  value of ABA indicating that the elution step was inefficient.



**Figure A.1.** ABA detected by radio-immunoassay in samples eluted from a thin layer chromatogram of crude onion extract dissolved in methanol.

The sensitivity of the assay was investigated by plotting the amount of ABA detected in the samples eluted from the  $R_f$  zone on a TLC plate (plate 2) loaded with a range of known amounts of ABA, against the mean ABA detected in each zone eluted from the plate (the background) (Figure A.2). ABA detected in the appropriate  $R_f$  zone can only be distinguished from the background when >1500 pg ABA is loaded onto the plate. This explains why a distinct peak in ABA concentration was not observed in the samples eluted from the crude onion extract thin layer chromatogram, as the relatively tiny amount of ABA in the sample would not have been distinguished from the background.



**Figure A.2.** ABA detected in the samples eluted from the appropriate  $R_f$  zone of a thin layer chromatogram of known amounts of ABA dissolved in methanol (open symbols) and the mean ABA detected by radio-immunoassay in each zone – the background (closed symbols).

### A.3.2 Experiment 2 – Spike dilution

Linear regression analysis of the plots of ABA added against ABA detected from the spike dilution showed that the most appropriate model ( $P < 0.001$ ) was that where all of the lines share the same gradient - 0.8932 (s.e. 0.0238) ABA added / ABA detected, but have different y-intercepts (Table A.1).

**Table A.1.** The y-intercept of each dilution factor and the control from the spike dilution assay, derived from a linear regression model with a common gradient and different y-intercepts.

Assay No.	Dilution factor	Y intercept (pg ABA)	S.E. <sup>1</sup>
1,2,6	Control	24.9	11.2
6	1 <sup>RP</sup>	214.3*	20.5
2	2 <sup>RM</sup>	129.6*	20.5
5	2 <sup>A</sup>	181.6*	22.3
6	2 <sup>RP</sup>	83.1**	20.5
1	3 <sup>R</sup>	260.5*	20.5
5	3 <sup>A</sup>	168.9*	22.3
2	4 <sup>RM</sup>	19.7	20.5
3	4 <sup>A</sup>	26.1	20.5
5	4 <sup>A</sup>	116.8*	22.3
7	5 <sup>A</sup>	111.9*	22.3
3	6 <sup>A</sup>	-2.3	20.5
7	10 <sup>A</sup>	74.6**	22.3
3	14 <sup>A</sup>	-16.4**	20.5
4	20 <sup>LR</sup>	247.7*	20.6
7	20 <sup>A</sup>	70.7**	22.3
1	24 <sup>R</sup>	30.6	20.5
4	300 <sup>LR</sup>	33.3	20.6
4	800 <sup>LR</sup>	34.3	20.6

<sup>1</sup> S.E. = standard error of the difference between the intercept of the reference level (control) and each dilution factor. <sup>L</sup> Samples were lyophilised (all other samples were frozen tissue).

\* p = <0.001, \*\* p = ≤0.05. P values represent the significance of the difference between the intercept of the reference level (control) and each dilution factor.

<sup>M</sup> ABA was extracted from fresh tissue using 100% (v/v) methanol at a ratio of 1ml methanol to 1g of tissue. The sample was lyophilised and then rehydrated with sterile distilled water.

<sup>P</sup> Samples purified following extraction; ABA extract acidified to pH 3 with an equal volume of citrate phosphate buffer. 2 ml of the extract, followed by 5 ml 30% methanol, was loaded onto a reversed phase SepPak C18 Plus cartridge (Waters Ltd., Herts., UK) primed with a strong solvent (5 ml 100% methanol) followed by a weak solvent (5 ml 5% acetic acid) under gravity. The ABA was then eluted from the column with 5 ml 60% methanol. The eluate was lyophilised and rehydrated with sterile distilled water. Samples taken from onion cv. <sup>A</sup> Ailsa Craig or <sup>R</sup> Renate bulb.

All of the lines shared a common gradient, indicating that the assay successfully detected ABA in the sample and that nothing in the onion sample interfered with the measurements. A gradient close to, but less than, one suggests that the assay is not quite 100% efficient. The Y intercept should represent the amount of ABA in 25  $\mu$ l of onion extract. The control intercept should be zero, all other intercepts were greater than the control apart from the two negative intercepts and one other which was not significantly different from the control. The samples with negative intercepts were assayed in the same batch, suggesting that was an experimental error in this batch. The higher the dilution factor, the lower the intercept should be, but the highest intercept was given by dilution factor 20. However, these samples were extracted from dry tissue and as onions have high water content, ABA would be much more concentrated in a dry sample. The methanol extraction used in assay no. 2 seems to have been less efficient at extracting ABA. The Y intercept of dilution factors of  $\geq 24$  were not significantly different from the mean, this may be expected as the initial ABA concentration of a highly diluted sample before the addition of the spike would be negligible and therefore make the intercept very similar to the control.

#### **A.4 Conclusion**

The ABA RIA appeared to be capable of measuring the ABA concentration in crude lyophilised onion extract without the need for further purification of the sample.

## B. APPENDIX

### Statistical tables

#### B.1 ANOVA tables for Chapter Three

**Tables B.1-3.** Effect of preharvest calcium and sulphur treatments, and time during postharvest controlled atmosphere storage on sprout growth in onion cvs. Ailsa Craig, Renate and SS1 (Section 3.4.2, Figure 3.2A).

**Table B.1.** Ailsa Craig.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	5	1761.5	352.3	2.51	0.034
Calcium	1	243	243	1.73	0.191
Sulphur	1	15.9	15.9	0.11	0.737
Day.Calcium	5	1022.1	204.4	1.46	0.21
Day.Sulphur	5	353.5	70.7	0.5	0.773
Calcium.Sulphur	1	39.8	39.8	0.28	0.596
Day.Calcium.Sulphur	5	1155.6	231.1	1.65	0.153
Residual	120	16857.2	140.5		
Total	143	21448.6			

**Table B.2.** Renate.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Days	6		2497.75	416.29	3.73	0.009
Calcium	1		258.44	258.44	2.32	0.141
Sulphur	1		5.77	5.77	0.05	0.822
Days.Calcium	6		856.09	142.68	1.28	0.304
Days.Sulphur	6		978.42	163.07	1.46	0.233
Calcium.Sulphur	1		17.53	17.53	0.16	0.695
Days.Calcium.Sulphur	6		244.65	40.77	0.37	0.893
Residual	138	-2	9790.98	173.91		
Total	165	-2	14632.09			

**Table B.3.** SS1.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Day	6		57604.4	9600.7	10.68	<.001
Calcium	1		97.6	97.6	0.11	0.742
Sulphur	1		1278	1278	1.42	0.235
Day.Calcium	6		3255.4	542.6	0.6	0.727
Day.Sulphur	6		5941.6	990.3	1.1	0.363
Calcium.Sulphur	1		3071	3071	3.42	0.066
Day.Calcium.Sulphur	6		1919.3	319.9	0.36	0.906
Residual	184	-4	165338.2	898.6		
Total	211	-4	234611.5			

**Tables B.4-7.** Effect of preharvest calcium and sulphur treatments, and time during postharvest controlled atmosphere storage on dry weight in onion cvs. Ailsa Craig, Renate and SS1 (Section 3.4.3).

**Table B.4.** All cultivars.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Variety	2		1175.632	587.816	75.54	<.001
Residual	106	-3	824.89	7.782		
Total	108	-3	1964.579			

**Table B.5.** Ailsa Craig.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Days	3		7.6419	2.5473	2.83	0.074
Calcium	1		0.2228	0.2228	0.25	0.626
Sulphur	1		3.1062	3.1062	3.45	0.083
Days.Calcium	3		1.5733	0.5244	0.58	0.636
Days.Sulphur	3		4.6778	1.5593	1.73	0.203
Calcium.Sulphur	1		0.1938	0.1938	0.22	0.649
Days.Calcium.Sulphur	3		1.5361	0.512	0.57	0.644
Residual	15	-1	13.5099	0.9007		
Total	30	-1	31.5995			

**Table B.6.** Renate.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	5	625.335	125.067	25.11	<.001
Calcium	1	0.656	0.656	0.13	0.72
Sulphur	1	0.226	0.226	0.05	0.833
Days.Calcium	5	9.093	1.819	0.37	0.867
Days.Sulphur	5	7.566	1.513	0.3	0.906
Calcium.Sulphur	1	0.096	0.096	0.02	0.891
Days.Calcium.Sulphur	5	4.935	0.987	0.2	0.96
Residual	24	119.524	4.98		
Total	47	767.43			

**Table B.7.** SS1.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Days	3		3.253	1.0843	3.29	0.052
Calcium	1		0.0171	0.0171	0.05	0.823
Sulphur	1		0.456	0.456	1.38	0.259
Days.Calcium	3		0.5955	0.1985	0.6	0.624
Days.Sulphur	3		2.3426	0.7809	2.37	0.114
Calcium.Sulphur	1		0.0496	0.0496	0.15	0.704
Days.Calcium.Sulphur	3		0.1087	0.0362	0.11	0.953
Residual	14	-2	4.6103	0.3293		
Total	29	-2	10.3725			

**Tables B.8-10.** Effect of preharvest calcium and sulphur treatments, and time during postharvest controlled atmosphere storage on total soluble solids in onion cvs. Ailsa Craig, Renate and SS1 (Section 3.4.4, Figure 3.2B).

**Table B.8.** Ailsa Craig.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	5	112.184	22.437	11.3	<.001
Calcium	1	4.731	4.731	2.38	0.125
Sulphur	1	4.445	4.445	2.24	0.137
Days.Calcium	5	8.314	1.663	0.84	0.526
Days.Sulphur	5	18.095	3.619	1.82	0.114
Calcium.Sulphur	1	0.918	0.918	0.46	0.498
Days.Calcium.Sulphur	5	13.396	2.679	1.35	0.249
Residual	120	238.352	1.986		
Total	143	400.434			

**Table B.9.** Renate.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Days	6		53.348	8.891	3.76	0.002
Calcium	1		0.539	0.539	0.23	0.634
Sulphur	1		3.925	3.925	1.66	0.2
Days.Calcium	6		13.328	2.221	0.94	0.469
Days.Sulphur	6		38.945	6.491	2.74	0.015
Calcium.Sulphur	1		0.79	0.79	0.33	0.564
Days.Calcium.Sulphur	6		11.027	1.838	0.78	0.589
Residual	139	-1	328.785	2.365		
Total	166	-1	450.377			

**Table B.10.** SS1.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	6	104.651	17.442	12.06	<.001
Calcium	1	0.001	0.001	0	0.985
Sulphur	1	0.251	0.251	0.17	0.677
Days.Calcium	6	16.251	2.708	1.87	0.09
Days.Sulphur	6	17.78	2.963	2.05	0.063
Calcium.Sulphur	1	1.993	1.993	1.38	0.242
Days.Calcium.Sulphur	6	5.49	0.915	0.63	0.704
Residual	140	202.462	1.446		
Total	167	348.879			



**Tables B.11-14.** Effect of preharvest calcium and sulphur treatments, and time during postharvest controlled atmosphere storage on firmness in onion cvs. Ailsa Craig, Renate and SS1 (Section 3.4.5, Figure 3.2C).

**Table B.11.** All cultivars.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	2	0.0019348	0.0009674	62.63	<.001
Calcium	1	3.024E-05	3.024E-05	1.96	0.17
Sulphur	1	5.47E-06	5.47E-06	0.35	0.556
Cultivar.Calcium	2	7.77E-06	3.88E-06	0.25	0.779
Cultivar.Sulphur	2	0.0000375	1.875E-05	1.21	0.309
Calcium.Sulphur	1	0	0	0	1
Cultivar.Calcium.Sulphur	2	5.87E-06	2.94E-06	0.19	0.828
Residual	36	0.000556	1.545E-05		
Total	47	0.0025776			

**Table B.12.** Ailsa Craig.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	5	0.0007812	0.0001562	13.78	<.001
Calcium	1	1.46E-06	1.46E-06	0.13	0.72
Sulphur	1	5E-08	5E-08	0	0.947
Day.Calcium	5	9.153E-05	1.831E-05	1.61	0.162
Day.Sulphur	5	9.347E-05	1.869E-05	1.65	0.153
Calcium.Sulphur	1	2.97E-06	2.97E-06	0.26	0.61
Day.Calcium.Sulphur	5	6.194E-05	1.239E-05	1.09	0.368
Residual	112	0.0012697	1.134E-05		
Total	135	0.0023024			

**Table B.13.** Renate.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	6	0.0009173	0.0001529	2.15	0.052
Calcium	1	0.0001076	0.0001076	1.51	0.221
Sulphur	1	0.0001772	0.0001772	2.49	0.117
Days.Calcium	6	0.0005534	9.223E-05	1.3	0.263
Days.Sulphur	6	0.0002548	4.247E-05	0.6	0.732
Calcium.Sulphur	1	1.334E-05	1.334E-05	0.19	0.666
Days.Calcium.Sulphur	6	0.0004891	8.151E-05	1.15	0.339
Residual	132	0.0093873	7.112E-05		
Total	159	0.0119001			

**Table B.14.** SS1.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Days	6		4.41E-04	7.35E-05	11.94	<.001
Calcium	1		3.42E-05	3.42E-05	5.55	0.02
Sulphur	1		4.66E-06	4.66E-06	0.76	0.385
Days.Calcium	6		2.65E-05	4.42E-06	0.72	0.636
Days.Sulphur	6		7.23E-05	1.21E-05	1.96	0.074
Calcium.Sulphur	1		1.86E-05	1.86E-05	3.02	0.084
Days.Calcium.Sulphur	6		1.13E-05	1.88E-06	0.31	0.933
Residual	177	-3	1.09E-03	6.16E-06		
Total	204	-3	1.69E-03			

**Tables B.15-18.** Effect of preharvest calcium and sulphur treatments, and time during postharvest controlled atmosphere storage on pyruvate concentration in onion cvs. Ailsa Craig, Renate and SS1 (Section 3.4.6, Figure 3.3A).

**Table B.15.** Pre-storage, all cultivars.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	2	125.344	62.672	19.15	0.003
Calcium	1	5.515	5.515	1.69	0.199
Sulphur	1	14.681	14.681	4.49	0.038
Variety.Calcium	2	13.523	6.761	2.07	0.136
Variety.Sulphur	2	3.995	1.998	0.61	0.546
Calcium.Sulphur	1	0.264	0.264	0.08	0.777
Variety.Calcium.Sulphur	2	1.417	0.709	0.22	0.806
Residual	60	196.375	3.273		
Total	71	361.115			

**Table B.16.** Ailsa Craig.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	5	176.606	35.321	13.42	<.001
Calcium	1	4.827	4.827	1.83	0.178
Sulphur	1	36.875	36.875	14.01	<.001
Days.Calcium	5	38.827	7.765	2.95	0.015
Days.Sulphur	5	42.311	8.462	3.21	0.009
Calcium.Sulphur	1	11.326	11.326	4.3	0.04
Days.Calcium.Sulphur	5	17.633	3.527	1.34	0.252
Residual	120	315.937	2.633		
Total	143	644.342			

**Table B.17.** Renate.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Days	6		424.517	70.753	13.41	<.001
Calcium	1		2.783	2.783	0.53	0.469
Sulphur	1		26.178	26.178	4.96	0.028
Days.Calcium	6		15.227	2.538	0.48	0.822
Days.Sulphur	6		30.653	5.109	0.97	0.449
Calcium.Sulphur	1		4.382	4.382	0.83	0.364
Days.Calcium.Sulphur	6		14.516	2.419	0.46	0.838
Residual	139	-1	733.383	5.276		
Total	166	-1	1251.628			

**Table B.18.** SS1.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	6	26.072	4.345	3.91	0.001
Calcium	1	12.835	12.835	11.55	<.001
Sulphur	1	0.237	0.237	0.21	0.645
Days.Calcium	6	6.459	1.076	0.97	0.449
Days.Sulphur	6	11.149	1.858	1.67	0.132
Calcium.Sulphur	1	0.928	0.928	0.83	0.363
Days.Calcium.Sulphur	6	7.276	1.213	1.09	0.371
Residual	140	155.636	1.112		
Total	167	220.592			

**Tables B.19-22.** Effect of preharvest calcium and sulphur treatments, and time during postharvest controlled atmosphere storage on total fructan concentration in onion cvs. Ailsa Craig, Renate and SS1 (Section 3.4.7, Figure 3.3B).

**Table B.19.** Pre-storage, all cultivars.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	2	1309.26	654.63	55.54	<.001
Residual	9	106.07	11.79		
Total	11	1415.33			

**Table B.20.** Ailsa Craig.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	5	233.31	46.66	1.48	0.242
Calcium	1	391.81	391.81	12.39	0.002
Sulphur	1	17.01	17.01	0.54	0.472
Days.Calcium	5	53.81	10.76	0.34	0.882
Days.Sulphur	5	40.64	8.13	0.26	0.931
Calcium.Sulphur	1	22.41	22.41	0.71	0.41
Days.Calcium.Sulphur	5	63.24	12.65	0.4	0.843
Residual	20	632.25	31.61		
Total	43	1454.49			

**Table B.21.** Renate.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	6	1951.59	325.27	18.73	<.001
Calcium	1	334.42	334.42	19.25	<.001
Sulphur	1	442.26	442.26	25.46	<.001
Days.Calcium	6	69.95	11.66	0.67	0.674
Days.Sulphur	6	157.12	26.19	1.51	0.218
Calcium.Sulphur	1	330.78	330.78	19.04	<.001
Days.Calcium.Sulphur	6	219.18	36.53	2.1	0.09
Residual	24	416.85	17.37		
Total	51	3922.14			

**Table B.22.** SS1.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Day	6		6.871	1.145	0.55	0.767
Calcium	1		31.389	31.389	14.98	<.001
Sulphur	1		8.289	8.289	3.96	0.059
Day.Calcium	6		15.382	2.564	1.22	0.33
Day.Sulphur	6		18.654	3.109	1.48	0.228
Calcium.Sulphur	1		6.19	6.19	2.95	0.099
Day.Calcium.Sulphur	6		24.727	4.121	1.97	0.112
Residual	23	-1	48.189	2.095		
Total	50	-1	158.441			

**Table B.23-** Effect of preharvest calcium and sulphur treatments, and time during postharvest controlled atmosphere storage on abscisic acid concentration in onion cvs. Ailsa Craig, Renate and SS1 (Section 3.4.8, Figure 3.3C).**Table B.23-28.** Pre-storage, all cultivars.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	2	83588	41794	4.07	0.055
Residual	9	92387	10265		
Total	11	175976			

**Table B.24.** Ailsa Craig.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	5	256930	51386	43.28	<.001
Calcium	1	4067	4067	3.43	0.079
Sulphur	1	53	53	0.04	0.835
Days.Calcium	5	42337	8467	7.13	<.001
Days.Sulphur	5	57347	11469	9.66	<.001
Calcium.Sulphur	1	209	209	0.18	0.679
Days.Calcium.Sulphur	5	3285	657	0.55	0.734
Residual	20	23747	1187		
Total	43	387977			

**Table B.25.** Renate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	6	134489	22415	12.42	<.001
Calcium	1	8	8	0	0.947
Sulphur	1	12563	12563	6.96	0.014
Days.Calcium	6	16079	2680	1.48	0.226
Days.Sulphur	6	7376	1229	0.68	0.667
Calcium.Sulphur	1	6	6	0	0.956
Days.Calcium.Sulphur	6	20485	3414	1.89	0.124
Residual	24	43329	1805		
Total	51	234334			

**Table B.26.** SS1.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Days	6		20024.8	3337.5	4.75	0.003
Calcium	1		2538.2	2538.2	3.62	0.07
Sulphur	1		12155.3	12155.3	17.31	<.001
Days.Calcium	6		12247.3	2041.2	2.91	0.029
Days.Sulphur	6		5900.4	983.4	1.4	0.257
Calcium.Sulphur	1		5379.3	5379.3	7.66	0.011
Days.Calcium.Sulphur	6		12676.8	2112.8	3.01	0.025
Residual	23	-1	16148	702.1		
Total	50	-1	85437.7			

**Table B.27.** Ailsa Craig v SS1.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	51340	51340	40.96	<.001
Days	3	141323	47108	37.58	<.001
Calcium	1	14854	14854	11.85	0.002
Sulphur	1	3032	3032	2.42	0.133
Variety.Days	3	53081	17694	14.12	<.001
Variety.Calcium	1	2086	2086	1.66	0.209
Days.Calcium	3	27627	9209	7.35	0.001
Variety.Sulphur	1	5759	5759	4.59	0.042
Days.Sulphur	3	40083	13361	10.66	<.001
Calcium.Sulphur	1	2546	2546	2.03	0.167
Variety.Days.Calcium	3	10006	3335	2.66	0.071
Variety.Days.Sulphur	3	21026	7009	5.59	0.005
Variety.Calcium.Sulphur	1	2054	2054	1.64	0.213
Days.Calcium.Sulphur	3	7052	2351	1.88	0.161
Variety.Days.Calcium.Sulphur	3	648	216	0.17	0.914
Residual	24	30083	1253		
Total	55	412599			

**Table B.28.** Renate v SS1.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	87900	87900	76.64	<.001
Days	1	40125	40125	34.98	<.001
Calcium	1	1515	1515	1.32	0.284
Sulphur	1	2651	2651	2.31	0.167
Variety.Days	1	7710	7710	6.72	0.032
Variety.Calcium	1	3638	3638	3.17	0.113
Days.Calcium	1	3803	3803	3.32	0.106
Variety.Sulphur	1	8184	8184	7.14	0.028
Days.Sulphur	1	162	162	0.14	0.717
Calcium.Sulphur	1	5528	5528	4.82	0.059
Variety.Days.Calcium	1	387	387	0.34	0.577
Variety.Days.Sulphur	1	306	306	0.27	0.62
Variety.Calcium.Sulphur	1	1187	1187	1.04	0.339
Days.Calcium.Sulphur	1	155	155	0.13	0.723
Variety.Days.Calcium.Sulphur	1	2454	2454	2.14	0.182
Residual	8	9176	1147		
Total	23	174880			

**Table B.29-39.** Effect of preharvest calcium and sulphur treatments, and time during postharvest controlled atmosphere storage on elemental composition in onion cvs. Ailsa Craig, Renate and SS1 (Section 3.4.1, Figure 3.1).**Table B.29.** Boron, all cultivars.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	2	11443.69	5721.85	147.27	<.001
Days	2	8.34	4.17	0.11	0.899
Calcium	1	64.54	64.54	1.66	0.21
Sulphur	1	17.43	17.43	0.45	0.509
Variety.Days	4	303.09	75.77	1.95	0.135
Variety.Calcium	2	84.63	42.31	1.09	0.353
Days.Calcium	2	14.54	7.27	0.19	0.831
Variety.Sulphur	2	2.34	1.17	0.03	0.97
Days.Sulphur	2	973.66	486.83	12.53	<.001
Calcium.Sulphur	1	21.55	21.55	0.55	0.464
Variety.Days.Calcium	4	31.87	7.97	0.21	0.933
Variety.Days.Sulphur	4	1581.52	395.38	10.18	<.001
Variety.Calcium.Sulphur	2	25.8	12.9	0.33	0.721
Days.Calcium.Sulphur	2	20.19	10.09	0.26	0.773
Variety.Days.Calcium.Sulphur	4	18.38	4.6	0.12	0.975
Residual	24	932.44	38.85		
Total	59	15544			

**Table B.30.** Calcium, all cultivars.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Variety	2		1844574	922287	0.99	0.386
Days	2		4091468	2045734	2.2	0.133
Calcium	1		181852	181852	0.2	0.662
Sulphur	1		3644077	3644077	3.91	0.059
Variety.Days	4		13133408	3283352	3.53	0.021
Variety.Calcium	2		2583158	1291579	1.39	0.269
Days.Calcium	2		78166	39083	0.04	0.959
Variety.Sulphur	2		1932761	966381	1.04	0.37
Days.Sulphur	2		1926739	963369	1.03	0.371
Calcium.Sulphur	1		1941150	1941150	2.08	0.162
Variety.Days.Calcium	4		4449664	1112416	1.19	0.339
Variety.Days.Sulphur	4		2936317	734079	0.79	0.544
Variety.Calcium.Sulphur	2		1067050	533525	0.57	0.571
Days.Calcium.Sulphur	2		1494150	747075	0.8	0.46
Variety.Days.Calcium.Sulphur	3	-1	4717217	1572406	1.69	0.196
Residual	24		22344199	931008		
Total	58	-1	65136521			

**Table B.31.** Copper, all cultivars.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Variety	2		10449.98	5224.99	112.53	<.001
Days	2		435.1	217.55	4.69	0.019
Calcium	1		20.65	20.65	0.44	0.511
Sulphur	1		0.08	0.08	0	0.968
Variety.Days	4		474.48	118.62	2.55	0.065
Variety.Calcium	2		14.72	7.36	0.16	0.854
Days.Calcium	2		60.67	30.33	0.65	0.529
Variety.Sulphur	2		3.58	1.79	0.04	0.962
Days.Sulphur	2		1646.36	823.18	17.73	<.001
Calcium.Sulphur	1		21.06	21.06	0.45	0.507
Variety.Days.Calcium	4		85.63	21.41	0.46	0.764
Variety.Days.Sulphur	4		2391.64	597.91	12.88	<.001
Variety.Calcium.Sulphur	2		132.16	66.08	1.42	0.261
Days.Calcium.Sulphur	2		66.93	33.46	0.72	0.497
Variety.Days.Calcium.Sulphur	3	-1	63.24	21.08	0.45	0.717
Residual	24		1114.35	46.43		
Total	58	-1	16878.41			

**Table B.32.** Iron, all cultivars.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Variety	2		2140.66	1070.33	14.36	<.001
Days	2		10.08	5.04	0.07	0.935
Calcium	1		333.58	333.58	4.48	0.045
Sulphur	1		303.25	303.25	4.07	0.055
Variety.Days	4		780.9	195.23	2.62	0.06
Variety.Calcium	2		474.07	237.03	3.18	0.06
Days.Calcium	2		187.25	93.63	1.26	0.303
Variety.Sulphur	2		40.51	20.26	0.27	0.764
Days.Sulphur	2		376.68	188.34	2.53	0.101
Calcium.Sulphur	1		257.42	257.42	3.45	0.075
Variety.Days.Calcium	4		148.27	37.07	0.5	0.738
Variety.Days.Sulphur	4		293.39	73.35	0.98	0.435
Variety.Calcium.Sulphur	2		120.67	60.34	0.81	0.457
Days.Calcium.Sulphur	2		29.59	14.79	0.2	0.821
Variety.Days.Calcium.Sulphur	3	-1	21.61	7.2	0.1	0.961
Residual	24		1789.01	74.54		
Total	58	-1	7073.26			

**Table B.33.** Potassium, all cultivars.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Variety	2		99736242	49868121	21.26	<.001
Days	2		50788708	25394354	10.82	<.001
Calcium	1		1085722	1085722	0.46	0.503
Sulphur	1		1082	1082	0	0.983
Variety.Days	4		14317115	3579279	1.53	0.226
Variety.Calcium	2		30357546	15178773	6.47	0.006
Days.Calcium	2		14473668	7236834	3.08	0.064
Variety.Sulphur	2		454467	227234	0.1	0.908
Days.Sulphur	2		52971391	26485696	11.29	<.001
Calcium.Sulphur	1		10899550	10899550	4.65	0.041
Variety.Days.Calcium	4		14687342	3671836	1.57	0.216
Variety.Days.Sulphur	4		28878813	7219703	3.08	0.035
Variety.Calcium.Sulphur	2		12380655	6190327	2.64	0.092
Days.Calcium.Sulphur	2		8288456	4144228	1.77	0.192
Variety.Days.Calcium.Sulphur	3	-1	10346971	3448990	1.47	0.248
Residual	24		56306509	2346105		
Total	58	-1	403271259			

**Table B.34.** Magnesium, all cultivars.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Variety	2		405817	202909	36.04	<.001
Days	2		978	489	0.09	0.917
Calcium	1		22302	22302	3.96	0.058
Sulphur	1		4661	4661	0.83	0.372
Variety.Days	4		17356	4339	0.77	0.555
Variety.Calcium	2		29760	14880	2.64	0.092
Days.Calcium	2		5271	2635	0.47	0.632
Variety.Sulphur	2		21703	10851	1.93	0.167
Days.Sulphur	2		31067	15534	2.76	0.083
Calcium.Sulphur	1		21496	21496	3.82	0.062
Variety.Days.Calcium	4		5606	1402	0.25	0.907
Variety.Days.Sulphur	4		23268	5817	1.03	0.41
Variety.Calcium.Sulphur	2		3189	1595	0.28	0.756
Days.Calcium.Sulphur	2		1356	678	0.12	0.887
Variety.Days.Calcium.Sulphur	3	-1	8218	2739	0.49	0.695
Residual	24		135104	5629		
Total	58	-1	723793			



**Table B.35.** Manganese, all cultivars.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Variety	2		133.077	66.539	14.28	<.001
Days	2		6.674	3.337	0.72	0.499
Calcium	1		0.174	0.174	0.04	0.848
Sulphur	1		5.871	5.871	1.26	0.273
Variety.Days	4		28.619	7.155	1.54	0.224
Variety.Calcium	2		2.417	1.208	0.26	0.774
Days.Calcium	2		2.628	1.314	0.28	0.757
Variety.Sulphur	2		35	17.5	3.76	0.038
Days.Sulphur	2		29.226	14.613	3.14	0.062
Calcium.Sulphur	1		5.727	5.727	1.23	0.279
Variety.Days.Calcium	4		5.127	1.282	0.28	0.891
Variety.Days.Sulphur	4		31.752	7.938	1.7	0.182
Variety.Calcium.Sulphur	2		20.754	10.377	2.23	0.13
Days.Calcium.Sulphur	2		10.704	5.352	1.15	0.334
Variety.Days.Calcium.Sulphur	3	-1	5.382	1.794	0.39	0.765
Residual	24		111.796	4.658		
Total	58	-1	418.038			

**Table B.36.** Sodium, all cultivars.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Variety	2		2833311	1416656	79.56	<.001
Days	2		49373	24686	1.39	0.269
Calcium	1		21063	21063	1.18	0.288
Sulphur	1		45252	45252	2.54	0.124
Variety.Days	4		188811	47203	2.65	0.058
Variety.Calcium	2		65	32	0	0.998
Days.Calcium	2		41789	20895	1.17	0.326
Variety.Sulphur	2		113652	56826	3.19	0.059
Days.Sulphur	2		211869	105935	5.95	0.008
Calcium.Sulphur	1		3038	3038	0.17	0.683
Variety.Days.Calcium	4		121769	30442	1.71	0.181
Variety.Days.Sulphur	4		226688	56672	3.18	0.031
Variety.Calcium.Sulphur	2		5348	2674	0.15	0.861
Days.Calcium.Sulphur	2		52238	26119	1.47	0.251
Variety.Days.Calcium.Sulphur	3	-1	172702	57567	3.23	0.04
Residual	24		427347	17806		
Total	58	-1	4514028			

**Table B.37.** Phosphorus, all cultivars.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	2	1097061	548530	6.96	0.004
Days	2	790056	395028	5.01	0.015
Calcium	1	232835	232835	2.95	0.099
Sulphur	1	20153	20153	0.26	0.618
Variety.Days	4	326201	81550	1.03	0.41
Variety.Calcium	2	51078	25539	0.32	0.726
Days.Calcium	2	156788	78394	0.99	0.385
Variety.Sulphur	2	183695	91847	1.17	0.329
Days.Sulphur	2	1394608	697304	8.85	0.001
Calcium.Sulphur	1	54131	54131	0.69	0.415
Variety.Days.Calcium	4	119420	29855	0.38	0.821
Variety.Days.Sulphur	4	1187750	296937	3.77	0.016
Variety.Calcium.Sulphur	2	197623	98811	1.25	0.303
Days.Calcium.Sulphur	2	120212	60106	0.76	0.477
Variety.Days.Calcium.Sulphur	4	223224	55806	0.71	0.594
Residual	24	1891359	78807		
Total	59	8046192			

**Table B.38.** Sulphur, all cultivars.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	2	6711232	3355616	13.27	<.001
Days	2	4336825	2168413	8.57	0.002
Calcium	1	1102659	1102659	4.36	0.048
Sulphur	1	4460	4460	0.02	0.895
Variety.Days	4	614981	153745	0.61	0.661
Variety.Calcium	2	302287	151144	0.6	0.558
Days.Calcium	2	503535	251768	1	0.384
Variety.Sulphur	2	1242240	621120	2.46	0.107
Days.Sulphur	2	1098535	549268	2.17	0.136
Calcium.Sulphur	1	454973	454973	1.8	0.192
Variety.Days.Calcium	4	234640	58660	0.23	0.918
Variety.Days.Sulphur	4	335960	83990	0.33	0.854
Variety.Calcium.Sulphur	2	520359	260180	1.03	0.373
Days.Calcium.Sulphur	2	242235	121117	0.48	0.625
Variety.Days.Calcium.Sulphur	4	1953817	488454	1.93	0.138
Residual	24	6069929	252914		
Total	59	25728667			

**Table B.39.** Zinc, all cultivars.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Variety	2		1088.71	544.36	16.56	<.001
Days	2		200.57	100.28	3.05	0.066
Calcium	1		24.96	24.96	0.76	0.392
Sulphur	1		267.44	267.44	8.13	0.009
Variety.Days	4		173.07	43.27	1.32	0.292
Variety.Calcium	2		154.93	77.46	2.36	0.116
Days.Calcium	2		73.59	36.79	1.12	0.343
Variety.Sulphur	2		203.87	101.93	3.1	0.063
Days.Sulphur	2		463.26	231.63	7.04	0.004
Calcium.Sulphur	1		86.64	86.64	2.64	0.118
Variety.Days.Calcium	4		197	49.25	1.5	0.234
Variety.Days.Sulphur	4		129.7	32.43	0.99	0.434
Variety.Calcium.Sulphur	2		354	177	5.38	0.012
Days.Calcium.Sulphur	2		5.79	2.89	0.09	0.916
Variety.Days.Calcium.Sulphur	3	-1	93.43	31.14	0.95	0.433
Residual	24		789.14	32.88		
Total	58	-1	4036.46			

## B.2 Non-linear regression analysis for Chapter Three

**Tables B.40-42.** Non-linear regression analysis of the relationship between ABA concentration and time in controlled atmosphere storage (Section 3.4.8, Table 3.4).

**Table B.40.** Ailsa Craig.

Response variate: ABA  
 Explanatory: Days  
 Fitted Curve:  $A + B*(R^{**}X)$   
 Constraints:  $R < 1$

	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	2	48020.9	24010.5	187.89	<.001
Residual	3	383.4	127.8		
Total	5	48404.3	9680.9		

Percentage variance accounted for 98.7  
 Standard error of observations is estimated to be 11.3

**Table B.41.** Renate.

Response variate: ABA  
 Explanatory: Days  
 Fitted Curve:  $A + B*(R^{**}X)$   
 Constraints:  $R < 1$

	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	2	28411.1	14205.55	271	<.001
Residual	4	209.7	52.42		
Total	6	28620.8	4770.13		

**Table B.42.** SS1.

Response variate: ABA  
 Explanatory: Days  
 Fitted Curve:  $A + B*(R^{**X})$   
 Constraints:  $R < 1$

	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	2	2802.2	1401.1	8.6	0.036
Residual	4	651.6	162.9		
Total	6	3453.8	575.6		

Percentage variance accounted for 71.7

### B.3 ANOVA tables for Chapter Four

**Tables B.43-48.** Effect of pre-harvest (Section 4.4.1.2, Table 4.6, Figure 4.2) or postharvest (Section 4.4.2.1, Figure 4.14) application of exogenous ABA or an ABA analogue, and time during postharvest storage at 4, 12 or 20°C on sprout growth (log transformed values for preharvest application data) in onion cvs. Carlos, Dinaro, Hysam, Red Baron, Renate and SS1.

**Table B.43.** Pre-harvest application - 4°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	5		18.38138	3.67628	135.08	<.001
Day	5		25.25562	5.05112	185.6	<.001
Treatment	3		1.19507	0.39836	14.64	<.001
Cultivar.Day	14	-11	2.31009	0.16501	6.06	<.001
Cultivar.Treatment	15		1.22022	0.08135	2.99	<.001
Day.Treatment	12	-3	1.24182	0.10348	3.8	<.001
Cultivar.Day.Treatment	39	-36	1.3508	0.03464	1.27	0.136
Residual	322	-254	8.76319	0.02721		
Total	415	-304	25.76757			

**Table B.44.** Pre-harvest application - 12°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	5		49.26398	9.8528	413.64	<.001
Day	5		20.91649	4.1833	175.62	<.001
Treatment	3		0.7415	0.24717	10.38	<.001
Cultivar.Day	8	-17	0.93575	0.11697	4.91	<.001
Cultivar.Treatment	15		1.328	0.08853	3.72	<.001
Day.Treatment	12	-3	1.40707	0.11726	4.92	<.001
Cultivar.Day.Treatment	16	-59	0.48475	0.0303	1.27	0.218
Residual	194	-382	4.62104	0.02382		
Total	258	-461	26.28115			

**Table B.45.** Pre-harvest application - 20°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	5		27.32352	5.4647	134.32	<.001
Day	5		27.69724	5.53945	136.15	<.001
Treatment	3		1.06179	0.35393	8.7	<.001
Cultivar.Day	5	-20	5.02802	1.0056	24.72	<.001
Cultivar.Treatment	15		5.00458	0.33364	8.2	<.001
Day.Treatment	11	-4	3.78713	0.34428	8.46	<.001
Cultivar.Day.Treatment	0	-75	0.0537			
Residual	121	-455	4.92292	0.04069		
Total	165	-554	20.95626			

**Table B.46.** Postharvest application - 4°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	3	126326.7	42108.9	66.69	<.001
Treatment	2	272.5	136.3	0.22	0.807
Days.Treatment	6	2831.4	471.9	0.75	0.615
Residual	39	24626.2	631.4		
Total	50	154056.8			

**Table B.47.** Postharvest application - 12°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	3	13930.4	4643.5	17.75	<.001
Treatment	2	728.2	364.1	1.39	0.261
Days.Treatment	6	2669.7	445.0	1.70	0.147
Residual	39	10202.3	261.6		
Total	50	27530.6			

**Table B.48.** Postharvest application - 20°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	3	2767.5	922.5	4.51	0.008
Treatment	2	427.8	213.9	1.05	0.361
Days.Treatment	6	1390.5	231.7	1.13	0.361
Residual	39	7975.1	204.5		
Total	50	12560.9			

**Tables B.49-55.** Effect of pre-harvest (Section 4.4.1.3, Figure 4.3) or postharvest (Section 4.4.2.2, Figure 4.15) application of exogenous ABA or an ABA analogue, and time during postharvest storage at 4, 12 or 20°C on firmness of onion cvs. Carlos, Dinaro, Hysam, Red Baron, Renate and SS1.

**Table B.49.** Pre-harvest application – Pre-storage.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	5	7156.16	1431.23	14.89	<.001
Treatment	3	48.72	16.24	0.17	0.917
Cultivar.Treatment	15	1549.59	103.31	1.07	0.39
Residual	96	9226.46	96.11		
Total	119	17980.94			

**Table B.50.** Pre-harvest application - 4°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Day	5		42267.3	8453.46	165.7	<.001
Cultivar	5		49642.72	9928.54	194.61	<.001
Treatment	3		64.13	21.38	0.42	0.739
Day.Cultivar	22	-3	3007.46	136.7	2.68	<.001
Day.Treatment	15		495.97	33.06	0.65	0.835
Cultivar.Treatment	15		1528.76	101.92	2	0.014
Day.Cultivar.Treatment	66	-9	3826.07	57.97	1.14	0.226
Residual	526	-50	26835.56	51.02		
Total	657	-62	91340.12			

**Table B.51.** Pre-harvest application - 12°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Day	5		10040.21	2008.04	28.93	<.001
Cultivar	5		84172.02	16834.4	242.53	<.001
Treatment	3		244.42	81.47	1.17	0.319
Day.Cultivar	24	-1	6209.23	258.72	3.73	<.001
Day.Treatment	15		1444.39	96.29	1.39	0.148
Cultivar.Treatment	15		976.78	65.12	0.94	0.521
Day.Cultivar.Treatment	72	-3	6553.64	91.02	1.31	0.052
Residual	556	-20	38593.59	69.41		
Total	695	-24	127520.49			

**Table B.52.** Pre-harvest application - 20°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Day	5		2698.3	539.66	6.79	<.001
Cultivar	5		83213.58	16642.72	209.3	<.001
Treatment	3		307.42	102.47	1.29	0.277
Day.Cultivar	24	-1	5822.08	242.59	3.05	<.001
Day.Treatment	15		981.36	65.42	0.82	0.652
Cultivar.Treatment	15		1285.38	85.69	1.08	0.374
Day.Cultivar.Treatment	72	-3	8627.57	119.83	1.51	0.007
Residual	551	-25	43813.87	79.52		
Total	690	-29	130092.56			

**Table B.53.** Postharvest application - 4°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Days	3		6985.44	2328.48	39.46	<.001
Treatment	2		136.07	68.04	1.15	0.327
Days.Treatment	6		429.44	71.57	1.21	0.323
Residual	35	-4	2065.44	59.01		
Total	46	-4	8981.11			

**Table B.54.** Postharvest application - 12°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Days	3		1187.44	395.81	5.55	0.003
Treatment	2		28.82	14.41	0.2	0.818
Days.Treatment	6		432.35	72.06	1.01	0.434
Residual	36	-3	2568.82	71.36		
Total	47	-3	4012.54			

**Table B.55.** Postharvest application - 20°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Days	3		1075.93	358.64	3.8	0.018
Treatment	2		91.68	45.84	0.49	0.619
Days.Treatment	6		110.27	18.38	0.19	0.976
Residual	35	-4	3301.62	94.33		
Total	46	-4	4515.26			

**Tables B.56-62.** Effect of pre-harvest (Section 4.4.1.4, Figure 4.4) or postharvest (Section 4.4.2.2, Figure 4.15) application of exogenous ABA or an ABA analogue, and time during postharvest storage at 4, 12 or 20°C on dry weight of onion cvs. Carlos, Dinaro, Hysam, Red Baron, Renate and SS1.

**Table B.56.** Pre-harvest application – Pre-storage.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	5	536.884	107.377	47.41	<.001
Treatment	3	66.491	22.164	9.79	<.001
Cultivar.Treatment	15	89.094	5.94	2.62	0.002
Residual	96	217.445	2.265		
Total	119	909.914			

**Table B.57.** Pre-harvest application - 4°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Day	5		690.93	138.186	98.9	<.001
Cultivar	5		2758.088	551.618	394.8	<.001
Treatment	3		45.482	15.161	10.85	<.001
Day.Cultivar	22	-3	57.228	2.601	1.86	0.01
Day.Treatment	15		150.758	10.051	7.19	<.001
Cultivar.Treatment	15		91.629	6.109	4.37	<.001
Day.Cultivar.Treatment	66	-9	167.845	2.543	1.82	<.001
Residual	525	-51	733.537	1.397		
Total	656	-63	3263.967			

**Table B.58.** Pre-harvest application - 12°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Day	5		357.488	71.498	47.83	<.001
Cultivar	5		4049.298	809.86	541.78	<.001
Treatment	3		41.737	13.912	9.31	<.001
Day.Cultivar	24	-1	52.651	2.194	1.47	0.071
Day.Treatment	15		77.188	5.146	3.44	<.001
Cultivar.Treatment	15		80.546	5.37	3.59	<.001
Day.Cultivar.Treatment	72	-3	212.508	2.952	1.97	<.001
Residual	558	-18	834.103	1.495		
Total	697	-22	5074.76			

**Table B.59.** Pre-harvest application - 20°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Day	5		411.385	82.277	36.21	<.001
Cultivar	5		3591.427	718.285	316.1	<.001
Treatment	3		31.761	10.587	4.66	0.003
Day.Cultivar	24	-1	93.714	3.905	1.72	0.019
Day.Treatment	15		83.097	5.54	2.44	0.002
Cultivar.Treatment	15		88.541	5.903	2.6	<.001
Day.Cultivar.Treatment	72	-3	208.739	2.899	1.28	0.072
Residual	555	-21	1261.154	2.272		
Total	694	(25)	5024.728			

**Table B.60.** Postharvest application - 4°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	3	132.396	44.132	6.25	0.001
Treatment	2	17.533	8.766	1.24	0.3
Days.Treatment	6	9.506	1.584	0.22	0.966
Residual	39	275.477	7.064		
Total	50	434.912			

**Table B.61.** Postharvest application - 12°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	3	89.25	29.75	8.41	<.001
Treatment	2	4.21	2.105	0.59	0.557
Days.Treatment	6	7.896	1.316	0.37	0.892
Residual	39	137.988	3.538		
Total	50	239.343			

**Table B.62.** Postharvest application - 20°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	3	66.349	22.116	7.31	<.001
Treatment	2	8.879	4.439	1.47	0.243
Days.Treatment	6	9.92	1.653	0.55	0.769
Residual	39	117.931	3.024		
Total	50	203.078			

**Tables B.63-69.** Effect of pre-harvest (Section 4.4.1.5, Figure 4.5) or postharvest (Section 4.4.2.3, Figure 4.16) application of exogenous ABA or an ABA analogue, and time during postharvest storage at 4, 12 or 20°C on total soluble solids content of onion cvs. Carlos, Dinero, Hysam, Red Baron, Renate and SS1.

**Table B.63.** Pre-harvest application – Pre-storage.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	5	374.7294	74.9459	80.22	<.001
Treatment	3	26.8402	8.9467	9.58	<.001
Cultivar.Treatment	15	29.7182	1.9812	2.12	0.015
Residual	96	89.692	0.9343		
Total	119	520.9799			



**Table B.64.** Pre-harvest application - 4°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Day	5		1268.192	253.638	156.86	<.001
Cultivar	5		1156.292	231.258	143.02	<.001
Treatment	3		58.048	19.349	11.97	<.001
Day.Cultivar	22	-3	123.791	5.627	3.48	<.001
Day.Treatment	15		97.007	6.467	4	<.001
Cultivar.Treatment	15		82.113	5.474	3.39	<.001
Day.Cultivar.Treatment	66	-9	183.663	2.783	1.72	<.001
Residual	526	-50	850.511	1.617		
Total	657	-62	3575.993			

**Table B.65.** Pre-harvest application - 12°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Day	5		1095.465	219.093	126.1	<.001
Cultivar	5		1990.653	398.131	229.15	<.001
Treatment	3		51.227	17.076	9.83	<.001
Day.Cultivar	24	-1	221.16	9.215	5.3	<.001
Day.Treatment	15		48.579	3.239	1.86	0.024
Cultivar.Treatment	15		60.364	4.024	2.32	0.003
Day.Cultivar.Treatment	72	-3	176.25	2.448	1.41	0.019
Residual	558	-18	969.499	1.737		
Total	697	-22	4314.539			

**Table B.66.** Pre-harvest application - 20°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Day	5		112.053	22.411	8.51	<.001
Cultivar	5		2524.569	504.914	191.66	<.001
Treatment	3		6.121	2.04	0.77	0.509
Day.Cultivar	24	-1	59.935	2.497	0.95	0.535
Day.Treatment	15		110.487	7.366	2.8	<.001
Cultivar.Treatment	15		52.81	3.521	1.34	0.175
Day.Cultivar.Treatment	72	-3	262.27	3.643	1.38	0.026
Residual	557	-19	1467.371	2.634		
Total	696	(23)	4189.902			

**Table B.67.** Postharvest application - 4°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	3	4.253	1.418	0.91	0.446
Treatment	2	0.429	0.214	0.14	0.872
Days.Treatment	6	6.383	1.064	0.68	0.666
Residual	39	60.933	1.562		
Total	50	71.997			

**Table B.68.** Postharvest application - 12°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	3	85.589	28.53	13.38	<.001
Treatment	2	3.827	1.914	0.9	0.416
Days.Treatment	6	28.504	4.751	2.23	0.061
Residual	39	83.133	2.132		
Total	50	201.053			

**Table B.69.** Postharvest application - 20°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	3	5.069	1.69	0.7	0.556
Treatment	2	4.505	2.252	0.94	0.4
Days.Treatment	6	18.942	3.157	1.31	0.274
Residual	39	93.668	2.402		
Total	50	122.184			

**Tables B.70-76.** Effect of pre-harvest (Section 4.4.1.6, Figure 4.6) or postharvest (Section 4.4.2.3, Figure 4.16) application of exogenous ABA or an ABA analogue, and time during postharvest storage at 4, 12 or 20°C on pyruvate concentration of onion cvs. Carlos, Dinero, Hysam, Red Baron, Renate and SS1.

**Table B.70.** Pre-harvest application – Pre-storage.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	5	102.724	20.545	16.01	<.001
Treatment	3	30.81	10.27	8	<.001
Cultivar.Treatment	15	143.951	9.597	7.48	<.001
Residual	96	123.212	1.283		
Total	119	400.697			

**Table B.71.** Pre-harvest application - 4°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Day	5		248.391	49.678	28.17	<.001
Cultivar	5		696.901	139.38	79.03	<.001
Treatment	3		60.834	20.278	11.5	<.001
Day.Cultivar	22	-3	131.563	5.98	3.39	<.001
Day.Treatment	15		224.365	14.958	8.48	<.001
Cultivar.Treatment	15		233.357	15.557	8.82	<.001
Day.Cultivar.Treatment	66	-9	458.395	6.945	3.94	<.001
Residual	526	-50	927.621	1.764		
Total	657	-62	2830.784			

**Table B.72.** Pre-harvest application - 12°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Day	5		121.153	24.231	11.92	<.001
Cultivar	5		907.863	181.573	89.33	<.001
Treatment	3		53.861	17.954	8.83	<.001
Day.Cultivar	24	-1	213.67	8.903	4.38	<.001
Day.Treatment	15		93.887	6.259	3.08	<.001
Cultivar.Treatment	15		95.684	6.379	3.14	<.001
Day.Cultivar.Treatment	72	-3	386.57	5.369	2.64	<.001
Residual	559	-17	1136.199	2.033		
Total	698	-21	2905.292			

**Table B.73.** Pre-harvest application - 20°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Day	5		277.903	55.581	18.69	<.001
Cultivar	5		541.042	108.208	36.38	<.001
Treatment	3		91.59	30.53	10.26	<.001
Day.Cultivar	24	-1	167.093	6.962	2.34	<.001
Day.Treatment	15		44.563	2.971	1	0.455
Cultivar.Treatment	15		117.893	7.86	2.64	<.001
Day.Cultivar.Treatment	72	-3	441.334	6.13	2.06	<.001
Residual	557	-19	1656.738	2.974		
Total	696	-23	3302.417			

**Table B.74.** Postharvest application - 4°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	3	12.19	4.063	1.9	0.146
Treatment	2	23.536	11.768	5.5	0.008
Days.Treatment	6	38.564	6.427	3	0.016
Residual	39	83.445	2.14		
Total	50	157.734			

**Table B.75.** Postharvest application - 12°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	3	26.418	8.806	1.48	0.235
Treatment	2	64.184	32.092	5.39	0.009
Days.Treatment	6	24.638	4.106	0.69	0.659
Residual	39	232.02	5.949		
Total	50	347.259			

**Table B.76.** Postharvest application - 20°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	3	16.037	5.346	2.15	0.11
Treatment	2	23.534	11.767	4.73	0.015
Days.Treatment	6	32.383	5.397	2.17	0.067
Residual	39	97.119	2.49		
Total	50	169.073			

**Tables B.77-83.** Effect of pre-harvest (Section 4.4.1.8, Figure 4.13) or postharvest (Section 4.4.2.4, Figure 4.17) application of exogenous ABA or an ABA analogue, and time during postharvest storage at 4, 12 or 20°C on ABA concentration (log transformed values) of onion cvs. Carlos, Dinero, Hysam, Red Baron, Renate and SS1.

**Table B.77.** Pre-harvest application – Pre-storage.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	5		1.29814	0.25963	6.2	<.001
Treatment	3		0.94817	0.31606	7.55	<.001
Cultivar.Treatment	15		2.13696	0.14246	3.4	<.001
Residual	95	-1	3.97644	0.04186		
Total	118	-1	8.31377			

**Table B.78.** Pre-harvest application - 4°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	5		3.09795	0.61959	17.75	<.001
Day	5		6.81252	1.3625	39.03	<.001
Treatment	3		0.33244	0.11081	3.17	0.024
Cultivar.Day	24	-1	2.48683	0.10362	2.97	<.001
Cultivar.Treatment	15		1.44231	0.09615	2.75	<.001
Day.Treatment	15		3.24317	0.21621	6.19	<.001
Cultivar.Day.Treatment	72	-3	4.31179	0.05989	1.72	<.001
Residual	554	-22	19.33983	0.03491		
Total	693	-26	39.06553			

**Table B.79.** Pre-harvest application -12°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	5		0.53298	0.1066	2.95	0.012
Day	5		9.59459	1.91892	53.15	<.001
Treatment	3		0.42604	0.14201	3.93	0.009
Cultivar.Day	24	-1	3.39555	0.14148	3.92	<.001
Cultivar.Treatment	15		0.853	0.05687	1.57	0.076
Day.Treatment	15		2.85841	0.19056	5.28	<.001
Cultivar.Day.Treatment	72	-3	6.48551	0.09008	2.49	<.001
Residual	556	-20	20.07489	0.03611		
Total	695	-24	43.89285			

**Table B.80.** Pre-harvest application - 20°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	5		2.62507	0.52501	11.71	<.001
Day	5		5.28595	1.05719	23.57	<.001
Treatment	3		0.91999	0.30666	6.84	<.001
Cultivar.Day	22	-3	1.44693	0.06577	1.47	0.079
Cultivar.Treatment	15		1.37557	0.0917	2.04	0.011
Day.Treatment	15		7.32645	0.48843	10.89	<.001
Cultivar.Day.Treatment	66	-9	6.47004	0.09803	2.19	<.001
Residual	524	-52	23.50302	0.04485		
Total	655	-64	46.53588			

**Table B.81.** Postharvest application - 4°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	3	0.65722	0.21907	5.75	0.002
Treatment	2	0.21475	0.10737	2.82	0.072
Days.Treatment	6	0.30367	0.05061	1.33	0.268
Residual	39	1.48702	0.03813		
Total	50	2.66266			

**Table B.82.** Postharvest application - 12°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Days	3		1.03533	0.34511	12.27	<.001
Treatment	2		0.01765	0.00882	0.31	0.733
Days.Treatment	6		0.14711	0.02452	0.87	0.525
Residual	38	-1	1.06914	0.02814		
Total	49	-1	2.23386			

**Table B.83.** Postharvest application - 12°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	3	3.37413	1.12471	36.97	<.001
Treatment	2	0.0016	0.0008	0.03	0.974
Days.Treatment	6	0.11881	0.0198	0.65	0.689
Residual	39	1.18643	0.03042		
Total	50	4.68097			

**Tables B.84-87.** Effect of pre-harvest (Section 4.4.1.7, Figure 4.8) application of exogenous ABA or an ABA analogue, and time during postharvest storage at 4, 12 or 20°C on sucrose concentration of onion cvs. Red Baron, Renate and SS1.

**Table B.84.** Pre-harvest application – Pre-storage.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	2	54850	27425	14.17	<.001
Treatment	3	11571	3857	1.99	0.142
Cultivar.Treatment	6	4180	697	0.36	0.897
Residual	24	46458	1936		
Total	35	117058			

**Table B.85.** Pre-harvest application - 4°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	2		325929	162965	96.15	<.001
Day	5		94191	18838	11.11	<.001
Treatment	3		13688	4563	2.69	0.049
Cultivar.Day	7	-3	50336	7191	4.24	<.001
Cultivar.Treatment	6		24402	4067	2.4	0.032
Day.Treatment	15		33647	2243	1.32	0.199
Cultivar.Day.Treatment	21	-9	35641	1697	1	0.467
Residual	120	-24	203384	1695		
Total	179	-36	706294			

**Table B.86.** Pre-harvest application - 12°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	2		733291	366645	318.54	<.001
Day	5		28874	5775	5.02	<.001
Treatment	3		5167	1722	1.5	0.218
Cultivar.Day	9	-1	47122	5236	4.55	<.001
Cultivar.Treatment	6		9761	1627	1.41	0.214
Day.Treatment	15		40165	2678	2.33	0.005
Cultivar.Day.Treatment	27	-3	35629	1320	1.15	0.298
Residual	136	-8	156537	1151		
Total	203	-12	1011370			

**Table B.87.** Pre-harvest application -20°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	2		764670	382335	280.2	<.001
Day	5		60444	12089	8.86	<.001
Treatment	3		3522	1174	0.86	0.463
Cultivar.Day	9	-1	56496	6277	4.6	<.001
Cultivar.Treatment	6		9568	1595	1.17	0.327
Day.Treatment	15		54365	3624	2.66	0.002
Cultivar.Day.Treatment	27	-3	50276	1862	1.36	0.128
Residual	132	-12	180115	1365		
Total	199	-16	1112576			

**Tables B.88-91.** Effect of pre-harvest (Section 4.4.1.7, Figure 4.9) application of exogenous ABA or an ABA analogue, and time during postharvest storage at 4, 12 or 20°C on glucose concentration of onion cvs. Red Baron, Renate and SS1.

**Table B.88.** Pre-harvest application – Pre-storage.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	2	113907.6	56953.8	84.73	<.001
Treatment	3	8946.3	2982.1	4.44	0.013
Cultivar.Treatment	6	15955.2	2659.2	3.96	0.007
Residual	24	16132.9	672.2		
Total	35	154942			

**Table B.89.** Pre-harvest application - 4°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	2		320335	160167.5	177.18	<.001
Day	5		44810.5	8962.1	9.91	<.001
Treatment	3		11035.3	3678.4	4.07	0.009
Cultivar.Day	7	-3	58574.3	8367.8	9.26	<.001
Cultivar.Treatment	6		39303.7	6550.6	7.25	<.001
Day.Treatment	15		21313.7	1420.9	1.57	0.092
Cultivar.Day.Treatment	21	-9	38325.1	1825	2.02	0.01
Residual	120	-24	108480.3	904		
Total	179	-36	549842.3			

**Table B.90.** Pre-harvest application - 12°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	2		254427	127214	105.3	<.001
Day	5		133473	26695	22.1	<.001
Treatment	3		22628	7543	6.24	<.001
Cultivar.Day	9	-1	119556	13284	11	<.001
Cultivar.Treatment	6		54967	9161	7.58	<.001
Day.Treatment	15		44461	2964	2.45	0.003
Cultivar.Day.Treatment	27	-3	62077	2299	1.9	0.009
Residual	136	-8	164297	1208		
Total	203	-12	836391			

**Table B.91.** Pre-harvest application - 20°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	2		725867	362933	282.84	<.001
Day	5		44185	8837	6.89	<.001
Treatment	3		6157	2052	1.6	0.193
Cultivar.Day	9	-1	66768	7419	5.78	<.001
Cultivar.Treatment	6		22714	3786	2.95	0.01
Day.Treatment	15		29170	1945	1.52	0.108
Cultivar.Day.Treatment	27	-3	39188	1451	1.13	0.315
Residual	132	-12	169378	1283		
Total	199	-16	970976			

**Tables B.92-95.** Effect of pre-harvest (Section 4.4.1.7, Figure 4.7) application of exogenous ABA or an ABA analogue, and time during postharvest storage at 4, 12 or 20°C on fructose concentration of onion cvs. Red Baron, Renate and SS1.

**Table B.92.** Pre-harvest application – Pre-storage.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	2	420656.8	210328.4	420.53	<.001
Treatment	3	3608.9	1203	2.41	0.092
Cultivar.Treatment	6	17411.2	2901.9	5.8	<.001
Residual	24	12003.6	500.1		
Total	35	453680.5			

**Table B.93.** Pre-harvest application - 4°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	2		685931	342965	272.61	<.001
Day	5		105439	21088	16.76	<.001
Treatment	3		12583	4194	3.33	0.022
Cultivar.Day	7	-3	159039	22720	18.06	<.001
Cultivar.Treatment	6		63411	10569	8.4	<.001
Day.Treatment	15		44058	2937	2.33	0.006
Cultivar.Day.Treatment	21	-9	43491	2071	1.65	0.05
Residual	120	-24	150972	1258		
Total	179	-36	996723			

**Table B.94.** Pre-harvest application - 12°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	2		1080149.9	540075	588.06	<.001
Day	5		28340.5	5668.1	6.17	<.001
Treatment	3		18335.8	6111.9	6.65	<.001
Cultivar.Day	9	-1	147458	16384.2	17.84	<.001
Cultivar.Treatment	6		50825.9	8471	9.22	<.001
Day.Treatment	15		32005.8	2133.7	2.32	0.006
Cultivar.Day.Treatment	27	-3	49352.2	1827.9	1.99	0.006
Residual	136	-8	124901.9	918.4		
Total	203	-12	1380055			

**Table B.95.** Pre-harvest application - 20°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	2		1732032.6	866016.3	928.6	<.001
Day	5		16871.4	3374.3	3.62	0.004
Treatment	3		9317.4	3105.8	3.33	0.022
Cultivar.Day	9	-1	46141.6	5126.8	5.5	<.001
Cultivar.Treatment	6		24044.5	4007.4	4.3	<.001
Day.Treatment	15		8777.8	585.2	0.63	0.848
Cultivar.Day.Treatment	27	-3	47209.7	1748.5	1.87	0.011
Residual	132	-12	123103.8	932.6		
Total	199	-16	1686087.7			

**Table B.96-99.** Effect of pre-harvest (Section 4.4.1.7, Figure 4.1.1) application of exogenous ABA or an ABA analogue, and time during postharvest storage at 4, 12 or 20°C on the ratio of monosaccharide to disaccharide in onion cvs. Red Baron, Renate and SS1.**Table B.96.** Pre-harvest application – Pre-storage.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	2	489.62	244.81	35.28	<.001
Treatment	3	46.709	15.57	2.24	0.109
Cultivar.Treatment	6	80.009	13.335	1.92	0.118
Residual	24	166.553	6.94		
Total	35	782.89			

**Table B.97.** Pre-harvest application - 4°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	2		770.691	385.345	183.3	<.001
Day	5		72.254	14.451	6.87	<.001
Treatment	3		14.591	4.864	2.31	0.079
Cultivar.Day	7	-3	161.282	23.04	10.96	<.001
Cultivar.Treatment	6		31.998	5.333	2.54	0.024
Day.Treatment	15		46.917	3.128	1.49	0.12
Cultivar.Day.Treatment	21	-9	87.102	4.148	1.97	0.012
Residual	120	-24	252.266	2.102		
Total	179	-36	1247.535			

**Table B.98.** Pre-harvest application - 12°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	2		2455.134	1227.567	203.23	<.001
Day	5		36.209	7.242	1.2	0.313
Treatment	3		51.629	17.21	2.85	0.04
Cultivar.Day	9	-1	59.528	6.614	1.1	0.371
Cultivar.Treatment	6		86.111	14.352	2.38	0.033
Day.Treatment	15		107.487	7.166	1.19	0.29
Cultivar.Day.Treatment	27	-3	212.056	7.854	1.3	0.166
Residual	136	-8	821.487	6.04		
Total	203	-12	3537.305			



**Table B.99.** Pre-harvest application - 20°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	2		3872.875	1936.437	213.22	<.001
Day	5		4.835	0.967	0.11	0.991
Treatment	3		70.123	23.374	2.57	0.057
Cultivar.Day	9	-1	33.542	3.727	0.41	0.928
Cultivar.Treatment	6		145.728	24.288	2.67	0.018
Day.Treatment	15		151.185	10.079	1.11	0.354
Cultivar.Day.Treatment	27	-3	282.391	10.459	1.15	0.293
Residual	132	-12	1198.785	9.082		
Total	199	-16	5200.208			

**Tables B.100-103.** Effect of pre-harvest (Section 4.4.1.7, Figure 4.10) application of exogenous ABA or an ABA analogue, and time during postharvest storage at 4, 12 or 20°C on total sugar concentration of onion cvs. Red Baron, Renate and SS1.**Table B.100.** Pre-harvest application - Pre-storage.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	2	583942	291971	70.34	<.001
Treatment	3	35082	11694	2.82	0.061
Cultivar.Treatment	6	45744	7624	1.84	0.134
Residual	24	99621	4151		
Total	35	764389			

**Table B.101.** Pre-harvest application - 4°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	2		753348	376674	71.04	<.001
Day	5		315669	63134	11.91	<.001
Treatment	3		69056	23019	4.34	0.006
Cultivar.Day	7	-3	328085	46869	8.84	<.001
Cultivar.Treatment	6		172512	28752	5.42	<.001
Day.Treatment	15		165006	11000	2.07	0.016
Cultivar.Day.Treatment	21	-9	162518	7739	1.46	0.105
Residual	120	-24	636301	5303		
Total	179	-36	2232816			

**Table B.102.** Pre-harvest application - 12°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	2		957525	478763	97.47	<.001
Day	5		295044	59009	12.01	<.001
Treatment	3		99156	33052	6.73	<.001
Cultivar.Day	9	-1	790890	87877	17.89	<.001
Cultivar.Treatment	6		260242	43374	8.83	<.001
Day.Treatment	15		243112	16207	3.3	<.001
Cultivar.Day.Treatment	27	-3	306526	11353	2.31	<.001
Residual	136	-8	668036	4912		
Total	203	-12	3513190			

**Table B.103.** Pre-harvest application - 20°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	2		2033163	1016581	177.78	<.001
Day	5		191829	38366	6.71	<.001
Treatment	3		51821	17274	3.02	0.032
Cultivar.Day	9	-1	261028	29003	5.07	<.001
Cultivar.Treatment	6		86818	14470	2.53	0.024
Day.Treatment	15		114481	7632	1.33	0.19
Cultivar.Day.Treatment	27	-3	195984	7259	1.27	0.189
Residual	132	-12	754791	5718		
Total	199	-16	3200907			

**Tables B.104-107.** Effect of pre-harvest (Section 4.4.1.7, Figure 4.12) application of exogenous ABA or an ABA analogue, and time during postharvest storage at 4, 12 or 20°C on total fructan concentration of onion cvs. Red Baron, Renate and SS1.

**Table B.104.** Pre-harvest application - Pre-storage.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	2	629498	314749	181.24	<.001
Treatment	3	6493	2164	1.25	0.315
Cultivar.Treatment	6	5560	927	0.53	0.777
Residual	24	41680	1737		
Total	35	683231			

**Table B.105.** Pre-harvest application - 4°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	2		1394673	697337	337.9	<.001
Day	5		249568	49914	24.19	<.001
Treatment	3		18509	6170	2.99	0.034
Cultivar.Day	7	-3	149086	21298	10.32	<.001
Cultivar.Treatment	6		24852	4142	2.01	0.07
Day.Treatment	15		68498	4567	2.21	0.009
Cultivar.Day.Treatment	21	-9	92661	4412	2.14	0.006
Residual	120	-24	247647	2064		
Total	179	-36	1636260			

**Table B.106.** Pre-harvest application - 12°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	2		2785419	1392710	891.91	<.001
Day	5		105098	21020	13.46	<.001
Treatment	3		35517	11839	7.58	<.001
Cultivar.Day	9	-1	54886	6098	3.91	<.001
Cultivar.Treatment	6		19251	3209	2.05	0.063
Day.Treatment	15		33006	2200	1.41	0.151
Cultivar.Day.Treatment	27	-3	32658	1210	0.77	0.777
Residual	136	-8	212363	1561		
Total	203	-12	2802117			

**Table B.107.** Pre-harvest application - 20°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	2		2640903	1320451	583.39	<.001
Day	5		26009	5202	2.3	0.049
Treatment	3		6006	2002	0.88	0.451
Cultivar.Day	9	-1	84373	9375	4.14	<.001
Cultivar.Treatment	6		8801	1467	0.65	0.692
Day.Treatment	15		66974	4465	1.97	0.022
Cultivar.Day.Treatment	27	-3	95387	3533	1.56	0.052
Residual	134	-10	303296	2263		
Total	201	-14	2882516			

## B.4 Binomial regression analysis for Chapter Four.

**Tables B.107-109.** Binomial regression analysis of the proportion of bulbs sprouted (Section 4.4.1.2, Table 4.10)**Table B.107.** Pre-harvest application - 4°C.

Response variate: No\_sprouted  
 Binomial totals: No\_assessed  
 Distribution: Binomial  
 Link function: Logit  
 Fitted terms: Constant + Day + Cultivar + Treatment + Day.Cultivar + Day.Treatment + Cultivar.Treatment + Day.Cultivar.Treatment

### Summary of analysis

Source	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
Regression	47	707.13	15.0452	15.05	<.001
Residual	88	41.71	0.474		
Total	135	748.84	5.547		

Dispersion parameter is fixed at 1.00.

**Table B.108.** Pre-harvest application - 12°C.

Response variate: No\_sprouted  
 Binomial totals: No\_assessed  
 Distribution: Binomial  
 Link function: Logit  
 Fitted terms: Constant + Day + Cultivar + Treatment + Day.Cultivar + Day.Treatment + Cultivar.Treatment + Day.Cultivar.Treatment

### Summary of analysis

Source	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
Regression	47	692.31	14.7299	14.73	<.001
Residual	92	48.74	0.5298		
Total	139	741.05	5.3313		

Dispersion parameter is fixed at 1.00.

**Table B.109.** Pre-harvest application - 20°C.

Response variate: No\_sprouted  
 Binomial totals: No\_assessed  
 Distribution: Binomial  
 Link function: Logit  
 Fitted terms: Constant + Day + Cultivar + Treatment + Day.Cultivar + Day.Treatment + Cultivar.Treatment + Day.Cultivar.Treatment

Summary of analysis

Source	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
Regression	47	571.64	12.1626	12.16	<.001
Residual	88	46.04	0.5232		
Total	135	617.68	4.5754		

Dispersion parameter is fixed at 1.00.

## B.5 Correlation matrices for Chapter Four

**Table B.110.** Experiment 1: Pre-harvest application – Pre-storage, all cultivars (Carlos, Dinaro, Hysam, Red Baron, Renate,SS1), 4°C (Section 4.4.1.1)

Dry weight	1					
ABA	0.157	1				
TSS	0.668	0.055	1			
Firmness	0.4	0.174	0.525	1		
Pyruvate	0.278	-0.042	0.429	0.3	1	
	Dry weight	ABA	TSS	Firmness	Pyruvate	

**Table B.111.** Experiment 1: Pre-harvest application - cv. SS1, 4°C (Sections 4.4.1.7 and 4.4.1.8).

Sprout length	1							
TSS	-0.65	1						
ABA	-0.248	0.068	1					
Fructan	0.481	-0.439	-0.047	1				
Fructose	-0.605	0.237	0.136	-0.57	1			
Glucose	-0.66	0.457	0.116	-0.603	0.814	1		
Sucrose	-0.132	-0.076	-0.335	0.016	0.163	-0.143	1	
Total sugars	-0.66	0.304	-0.01	-0.558	0.936	0.817	0.4	1
	Sprout length	TSS	ABA	Fructan	Fructose	Glucose	Sucrose	Total sugars

**Table B.112.** Experiment 1: Pre-harvest application – cv. Renate, 4°C (Sections 4.4.1.7 and 4.4.1.8).

Sprout length	1							
TSS	0.092	1						
ABA	-0.271	0.117	1					
Fructan	-0.499	0.232	0.323	1				
Fructose	0.716	-0.19	-0.217	-0.564	1			
Glucose	0.547	0.207	0.024	-0.173	0.651	1		
Sucrose	-0.163	-0.337	-0.016	-0.014	-0.003	-0.146	1	
Total sugars	0.532	-0.22	-0.127	-0.402	0.824	0.67	0.493	1
	Sprout length	TSS	ABA	Fructan	Fructose	Glucose	Sucrose	Total sugars

**Table B.113.** Experiment 1: Pre-harvest application – cv. Red Baron, 4°C (Sections 4.4.1.7 and 4.4.1.8).

Sprout length	1							
TSS	0.027	1						
ABA	0.143	0.078	1					
Fructan	-0.482	0.203	-0.179	1				
Fructose	0.373	-0.363	0.009	-0.627	1			
Glucose	-0.115	-0.237	-0.081	-0.106	-0.071	1		
Sucrose	0.153	-0.322	0.056	-0.463	0.384	-0.105	1	
Total sugars	0.272	-0.488	0.006	-0.68	0.804	0.272	0.739	1
	Sprout length	TSS	ABA	Fructan	Fructose	Glucose	Sucrose	Total sugars

**Table B.114.** Experiment 1: Pre-harvest application – cv. SS1, 12°C (Sections 4.4.1.7 and 4.4.1.8).

Sprout length	1							
TSS	-0.579	1						
ABA	0.446	-0.406	1					
Fructan	-0.057	0.13	-0.318	1				
Fructose	-0.707	0.573	-0.532	-0.003	1			
Glucose	-0.697	0.605	-0.528	0.09	0.886	1		
Sucrose	-0.536	0.588	-0.436	0.456	0.452	0.541	1	
Total sugars	-0.745	0.656	-0.57	0.14	0.94	0.967	0.664	1
	Sprout length	TSS	ABA	Fructan	Fructose	Glucose	Sucrose	Total sugars

**Table B.115.** Experiment 1: Pre-harvest application – cv. Renate, 12°C (Sections 4.4.1.7 and 4.4.1.8).

Sprout length	1							
TSS	0.183	1						
ABA	-0.114	0.297	1					
Fructan	-0.374	-0.017	0.014	1				
Fructose	0.278	-0.321	-0.111	-0.539	1			
Glucose	-0.343	-0.163	0.01	0.213	0.294	1		
Sucrose	0.049	-0.414	-0.262	-0.034	0.403	0.117	1.000	
Total sugars	0.039	-0.44	-0.198	-0.192	0.786	0.538	0.8	1
	Sprout length	TSS	ABA	Fructan	Fructose	Glucose	Sucrose	Total sugars

**Table B.116.** Experiment 1: Pre-harvest application – cv. Red Baron, 12°C (Sections 4.4.1.7 and 4.4.1.8).

Sprout length	1							
TSS	-0.056	1						
ABA	-0.177	0.092	1					
Fructan	-0.55	0.109	0.148	1				
Fructose	0.325	-0.379	-0.019	-0.467	1			
Glucose	-0.203	-0.158	-0.027	-0.055	0.17	1		
Sucrose	0.376	-0.205	-0.101	-0.438	0.293	0.142	1	
Total sugars	0.216	-0.33	-0.079	-0.438	0.594	0.68	0.752	1
	Sprout length	TSS	ABA	Fructan	Fructose	Glucose	Sucrose	Total sugars

**Table B.117.** Experiment 1: Pre-harvest application – cv. SS1, 20°C (Sections 4.4.1.7 and 4.4.1.8).

Sprout length	1							
TSS	-0.376	1						
ABA	-0.006	-0.114	1					
Fructan	0.194	0.062	0.043	1				
Fructose	-0.308	0.1	-0.103	-0.412	1			
Glucose	-0.222	0.094	-0.162	-0.276	0.891	1		
Sucrose	-0.235	0.461	0.01	0.304	-0.059	-0.083	1	
Total sugars	-0.346	0.257	-0.127	-0.237	0.917	0.906	0.279	1
Sprout length	TSS	ABA	Fructan	Fructose	Glucose	Sucrose	Total sugars	

**Table B.118.** Experiment 1: Pre-harvest application – cv. Renate, 20°C (Sections 4.4.1.7 and 4.4.1.8).

Sprout length	1							
TSS	-0.151	1						
ABA	-0.199	0.13	1					
Fructan	-0.308	0.253	0.297	1				
Fructose	0.088	-0.21	-0.106	-0.252	1			
Glucose	0.069	0.117	0.046	0.23	0.483	1		
Sucrose	0.18	-0.213	-0.202	-0.068	0.341	0.114	1	
Total sugars	0.17	-0.156	-0.142	-0.033	0.745	0.64	10.784	1
Sprout length	TSS	ABA	Fructan	Fructose	Glucose	Sucrose	Total sugars	

**Table B.119.** Experiment 1: Preharvest application – cv. Red Baron, 20°C (Sections 4.4.1.7 and 4.4.1.8).

Sprout length	1							
TSS	0.012	1						
ABA	-0.149	0.059	1					
Fructan	-0.083	0.414	0.124	1				
Fructose	0.221	-0.303	-0.062	-0.569	1			
Glucose	-0.207	-0.249	-0.159	-0.42	0.295	1		
Sucrose	0.334	-0.422	-0.234	-0.59	0.396	0.17	1	
Total sugars	0.127	-0.453	-0.234	-0.713	0.657	0.733	0.75	1
Sprout length	TSS	ABA	Fructan	Fructose	Glucose	Sucrose	Total sugars	

**Table B.120.** Experiment 1: Pre-harvest application – up to 50% sprouting, cv. SS1, 4°C (Section 4.4.1.8).

Sprout length	1							
TSS	-0.551	1						
ABA	-0.375	0.158	1					
Fructan	0.194	-0.212	-0.269	1				
Fructose	-0.515	0.069	0.25	-0.274	1			
Glucose	-0.563	0.228	0.15	-0.38	0.88	1		
Sucrose	-0.138	-0.092	-0.254	0.368	-0.089	-0.169	1	
Total sugars	-0.593	0.105	0.088	-0.162	0.892	0.855	0.31	1
Sprout length	TSS	ABA	Fructan	Fructose	Glucose	Sucrose	Total sugars	

**Table B.121.** Experiment 1: Pre-harvest application – up to 50% sprouting, cv. Renate, 4°C (Section 4.4.1.8).

Sprout length	1							
TSS	-0.284	1						
ABA	-0.363	0.152	1					
Fructan	-0.306	0.369	0.279	1				
Fructose	0.463	-0.658	-0.229	-0.301	1			
Glucose	-0.386	0.193	0.314	0.463	0.053	1		
Sucrose	-0.03	-0.667	0.047	-0.186	0.373	-0.004	1	
Total sugars	0.067	-0.674	0.03	-0.102	0.733	0.368	0.818	1
Sprout length	TSS	ABA	Fructan	Fructose	Glucose	Sucrose	Total sugars	

**Table B.122.** Experiment 1: Pre-harvest application – up to 50% sprouting, cv. Red Baron, 4°C (Section 4.4.1.8).

Sprout length	1							
TSS	-0.282	1						
ABA	-0.06	-0.004	1					
Fructan	-0.419	0.456	-0.123	1				
Fructose	0.52	-0.427	0.162	-0.859	1			
Glucose	-0.27	0.02	-0.234	-0.1	-0.125	1		
Sucrose	-0.099	-0.653	0.05	-0.536	0.528	-0.047	1	
Total sugars	0.168	-0.593	0.048	-0.823	0.831	0.218	0.829	1
Sprout length	TSS	ABA	Fructan	Fructose	Glucose	Sucrose	Total sugars	

**Table B.123.** Experiment 1: Pre-harvest application – up to 50% sprouting, cv. SS1, 12°C (Section 4.4.1.8).

Sprout length	1							
TSS	-0.443	1						
ABA	-0.556	-0.062	1					
Fructan	0.472	0.125	-0.421	1				
Fructose	-0.374	0.285	0.197	-0.151	1			
Glucose	-0.186	0.291	-0.015	0.289	0.497	1		
Sucrose	-0.149	0.471	-0.187	0.331	-0.181	0.187	1	
Total sugars	-0.329	0.484	-0.001	0.247	0.652	0.9	0.434	1
Sprout length	TSS	ABA	Fructan	Fructose	Glucose	Sucrose	Total sugars	

**Table B.124.** Experiment 1: Pre-harvest application – up to 50% sprouting, cv. Renate, 12°C (Section 4.4.1.8).

Sprout length	1							
TSS	0.121	1						
ABA	-0.078	0.33	1					
Fructan	-0.26	-0.001	-0.015	1				
Fructose	0.258	-0.348	-0.104	-0.493	1			
Glucose	0.276	-0.133	-0.017	0.156	0.35	1		
Sucrose	0.071	-0.447	-0.258	0.055	0.389	0.185	1	
Total sugars	0.067	-0.454	-0.199	-0.14	0.784	0.587	0.803	1
Sprout length	TSS	ABA	Fructan	Fructose	Glucose	Sucrose	Total sugars	

**Table B.125.** Experiment 1: Pre-harvest application – up to 50% sprouting, cv. Red Baron, 12°C (Section 4.4.1.8).

Sprout length	1								
TSS	-0.143	1							
ABA	-0.215	0.142	1						
Fructan	-0.472	0.099	0.196	1					
Fructose	0.347	-0.362	-0.076	-0.465	1				
Glucose	-0.146	-0.142	-0.107	-0.084	0.262	1			
Sucrose	0.239	-0.21	-0.118	-0.349	0.325	0.213	1		
Total sugars	0.172	-0.307	-0.145	-0.389	0.64	0.713	0.77	1	
	Sprout length	TSS	ABA	Fructan	Fructose	Glucose	Sucrose	Total sugars	

**Table B.126.** Experiment 1: Pre-harvest application – up to 50% sprouting, cv. SS1, 20°C (Section 4.4.1.8).

Sprout length	1								
TSS	-0.166	1							
ABA	-0.62	0.008	1						
Fructan	0.165	0.226	-0.227	1					
Fructose	-0.2	-0.05	0.181	-0.132	1				
Glucose	-0.08	-0.102	-0.02	-0.139	0.932	1			
Sucrose	-0.381	0.332	0.13	-0.024	-0.208	-0.246	1		
Total sugars	-0.306	0.062	0.142	-0.149	0.907	0.888	0.185	1	
	Sprout length	TSS	ABA	Fructan	Fructose	Glucose	Sucrose	Total sugars	

**Table B.127.** Experiment 1: Pre-harvest application – up to 50% sprouting, cv. Renate, 20°C (Section 4.4.1.8).

Sprout length	1								
TSS	-0.151	1							
ABA	-0.199	0.13	1						
Fructan	-0.308	0.253	0.297	1					
Fructose	0.088	-0.21	-0.106	-0.252	1				
Glucose	0.069	0.117	0.046	0.23	0.483	1			
Sucrose	0.18	-0.213	-0.202	-0.068	0.341	0.114	1		
Total sugars	0.17	-0.156	-0.142	-0.033	0.745	0.641	0.784	1	
	Sprout length	TSS	ABA	Fructan	Fructose	Glucose	Sucrose	Total sugars	

**Table B.128.** Experiment 1: Pre-harvest application – up to 50% sprouting, cv. Red Baron, 20°C (Section 4.4.1.8).

Sprout length	1								
TSS	-0.055	1							
ABA	-0.13	0.079	1						
Fructan	-0.181	0.387	0.145	1					
Fructose	0.372	-0.29	-0.072	-0.58	1				
Glucose	0.037	-0.195	-0.225	-0.358	0.32	1			
Sucrose	0.328	-0.428	-0.255	-0.594	0.38	0.21	1		
Total sugars	0.307	-0.421	-0.279	-0.682	0.666	0.733	0.769	1	
	Sprout length	TSS	ABA	Fructan	Fructose	Glucose	Sucrose	Total sugars	

**Table B.129.** Experiment 1: Pre-harvest application – all cultivars (Red Baron, Renate, SS1), 4°C (Section 4.4.1.7).

Fructan	1								
Fructose	-0.805	1							
Glucose	-0.591	0.795	1						
Sucrose	0.331	-0.443	-0.569	1					
Total sugars	-0.701	0.883	0.766	-0.053	1				
	Fructan	Fructose	Glucose	Sucrose	Total sugars				



**Table B.130.** Experiment 1: Pre-harvest application – all cultivars (Red Baron, Renate, SS1), 12°C (Section 4.4.1.7).

Fructan	1				
Fructose	-0.812	1			
Glucose	-0.503	0.79	1		
Sucrose	0.565	-0.465	-0.183	1	
Total sugars	-0.461	0.773	0.891	0.144	1
	Fructan	Fructose	Glucose	Sucrose	Total sugars

**Table B.131.** Experiment 1: Pre-harvest application – all cultivars (Red Baron, Renate, SS1), 20°C (Section 4.4.1.7).

Fructan	1				
Fructose	-0.849	1			
Glucose	-0.743	0.868	1		
Sucrose	0.495	-0.631	-0.5	1	
Total sugars	-0.73	0.827	0.881	-0.14	1
	Fructan	Fructose	Glucose	Sucrose	Total sugars

**Table B.132.** Experiment 2: Postharvest application (cv. Hysam) - 4°C (Sections 4.4.2.2, 4.4.2.3 and 4.4.2.4).

Firmness	1					
Dry weight	-0.149	1				
Pyruvate	0.419	0.1	1			
Sprout length	0.132	-0.629	-0.083	1		
TSS	0.083	0.504	0.532	-0.373	1	
ABA	0.135	0.087	0.2	-0.362	-0.006	1
	Firmness	Dry weight	Pyruvate	Sprout length	TSS	ABA

**Table B.133.** Experiment 2: Postharvest application (cv. Hysam) - 12°C (Sections 4.4.2.2, 4.4.2.3 and 4.4.2.4).

Firmness	1					
Dry weight	0.169	1				
Pyruvate	-0.137	0.189	1			
Sprout length	-0.459	-0.522	0.112	1		
TSS	0.358	0.613	0.149	-0.415	1	
ABA	0.142	0.574	0.222	-0.148	0.128	1
	Firmness	Dry weight	Pyruvate	Sprout length	TSS	ABA

**Table B.134.** Experiment 2: Postharvest application (cv. Hysam) - 20°C (Sections 4.4.2.2, 4.4.2.3 and 4.4.2.4).

Firmness	1					
Dry weight	-0.39	1				
Pyruvate	-0.695	-0.37	1			
Sprout length	-0.343	-0.27	0.661	1		
TSS	-0.718	0.079	0.539	0.05	1	
ABA	-0.392	0.016	0.375	-0.271	0.116	1
	Firmness	Dry weight	Pyruvate	Sprout length	TSS	ABA

## B.5 ANOVA tables for Chapter Five

**Tables B.135-137.** Effect of pre-harvest application of 1-MCP, and time during postharvest storage at 4, 12 or 20°C on sprout growth in onions cv. SS1 (Section 5.4.1.1, Figure 5.1).

**Table B.135.** 4°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	68184	68184	13.08	0.001
Baseline.Time	1	18343	18343	3.52	0.07
Baseline.Treatment	1	13256	13256	2.54	0.121
Baseline.Time.Treatment	1	58	58	0.01	0.917
Residual	30	156344	5211		
Total	34	256185			

**Table B.136.** 12°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		143824	143824	19.66	<.001
Baseline.Time	2		147171	73585	10.06	<.001
Baseline.Treatment	1		42528	42528	5.81	0.021
Baseline.Time.Treatment	2		1703	852	0.12	0.89
Residual	39	-4	285344	7317		
Total	45	-4	598802			

**Table B.137.** 20°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	212182	212182	7.96	0.007
Baseline.Time	2	219093	109547	4.11	0.023
Baseline.Treatment	1	36841	36841	1.38	0.246
Baseline.Time.Treatment	2	28178	14089	0.53	0.593
Residual	43	1145808	26647		
Total	49	1642102			

**Tables B.138-140.** Effect of pre-harvest application of 1-MCP, and time during postharvest storage at 4, 12 or 20°C on firmness of onions cv. SS1 (Section 5.4.1.2, Figure 5.2).

**Table B.138.** 4°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	973.31	973.31	71.99	<.001
Baseline.Time	1	221.41	221.41	16.38	<.001
Baseline.Treatment	1	19.15	19.15	1.42	0.243
Baseline.Time.Treatment	1	1.7	1.7	0.13	0.725
Residual	30	405.6	13.52		
Total	34	1621.17			

**Table B.139.** 12°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		787.36	787.36	36.59	<.001
Baseline.Time	2		153.65	76.82	3.57	0.038
Baseline.Treatment	1		87.39	87.39	4.06	0.051
Baseline.Time.Treatment	2		32.6	16.3	0.76	0.476
Residual	38	-5	817.69	21.52		
Total	44	-5	1831.82			

**Table B.140.** 20°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	478.23	478.23	9.78	0.003
Baseline.Time	2	791.81	395.9	8.09	0.001
Baseline.Treatment	1	2.15	2.15	0.04	0.835
Baseline.Time.Treatment	2	52.26	26.13	0.53	0.59
Residual	43	2103.1	48.91		
Total	49	3427.53			

**Tables B.141-143.** Effect of pre-harvest application of 1-MCP, and time during postharvest storage at 4, 12 or 20°C on dry weight of onions cv. SS1 (Section 5.4.1.2, Figure 5.2).

**Table B.141.** 4°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		8.281	8.281	1.15	0.293
Baseline.Time	1		32.592	32.592	4.52	0.042
Baseline.Treatment	1		21.616	21.616	3	0.094
Baseline.Time.Treatment	1		4.455	4.455	0.62	0.438
Residual	29	-1	209.04	7.208		
Total	33	-1	275.13			

**Table B.142.** 12°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		20.142	20.142	19.16	<.001
Baseline.Time	2		4.095	2.047	1.95	0.156
Baseline.Treatment	1		50.321	50.321	47.88	<.001
Baseline.Time.Treatment	2		2.89	1.445	1.37	0.265
Residual	39	-4	40.988	1.051		
Total	45	-4	116.833			

**Table B.143.** 20°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	10.73	10.73	5.96	0.019
Baseline.Time	2	19.857	9.928	5.52	0.007
Baseline.Treatment	1	0.635	0.635	0.35	0.556
Baseline.Time.Treatment	2	35.3	17.65	9.81	<.001
Residual	43	77.394	1.8		
Total	49	143.916			

**Tables B.144-146.** Effect of pre-harvest application of 1-MCP, and time during postharvest storage at 4, 12 or 20°C on total soluble solids content of onions cv. SS1 (Section 5.4.2.1, Figure 5.3).

**Table B.144.** 4°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	4.576	4.576	3.2	0.084
Baseline.Time	1	17.328	17.328	12.13	0.002
Baseline.Treatment	1	10.753	10.753	7.52	0.01
Baseline.Time.Treatment	1	3.174	3.174	2.22	0.147
Residual	30	42.872	1.429		
Total	34	78.703			

**Table B.145.** 12°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		3.5476	3.5476	4.47	0.041
Baseline.Time	2		24.0624	12.0312	15.16	<.001
Baseline.Treatment	1		23.3528	23.3528	29.42	<.001
Baseline.Time.Treatment	2		3.2247	1.6123	2.03	0.145
Residual	39	-4	30.9564	0.7938		
Total	45	-4	84.7133			

**Table B.146.** 20°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	0.836	0.836	0.7	0.409
Baseline.Time	2	0.134	0.067	0.06	0.946
Baseline.Treatment	1	0.235	0.235	0.2	0.66
Baseline.Time.Treatment	2	3.638	1.819	1.51	0.231
Residual	43	51.63	1.201		
Total	49	56.473			

**Tables B.147-149.** Effect of pre-harvest application of 1-MCP, and time during postharvest storage at 4, 12 or 20°C on pyruvate concentration of onions cv. SS1 (Section 5.4.2.1, Figure 5.3).

**Table B.147.** 4°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	15.98	15.98	15.77	<.001
Baseline.Time	1	24.498	24.498	24.17	<.001
Baseline.Treatment	1	2.235	2.235	2.21	0.148
Baseline.Time.Treatment	1	1.492	1.492	1.47	0.235
Residual	30	30.405	1.014		
Total	34	74.61			

**Table B.148.** 12°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		2.4977	2.4977	2.71	0.108
Baseline.Time	2		13.8547	6.9274	7.51	0.002
Baseline.Treatment	1		9.1677	9.1677	9.94	0.003
Baseline.Time.Treatment	2		1.8188	0.9094	0.99	0.382
Residual	39	-4	35.9757	0.9225		
Total	45	-4	62.9171			

**Table B.149.** 20°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	7.0513	7.0513	8.69	0.005
Baseline.Time	2	4.0497	2.0249	2.5	0.094
Baseline.Treatment	1	7.0616	7.0616	8.71	0.005
Baseline.Time.Treatment	2	7.4311	3.7156	4.58	0.016
Residual	43	34.8807	0.8112		
Total	49	60.4744			

**Tables B.150-152.** Effect of pre-harvest application of 1-MCP, and time during postharvest storage at 4, 12 or 20°C on abscisic acid concentration of onions cv. SS1 (Section 5.4.2.2, Figure 5.4).

**Table B.150.** 4°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		17144	17144	4.33	0.046
Baseline.Time	1		19662	19662	4.96	0.034
Baseline.Treatment	1		18	18	0	0.947
Baseline.Time.Treatment	1		320	320	0.08	0.778
Residual	29	-1	114850	3960		
Total	33	-1	151769			

**Table B.151.** 12°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		3279	3279	0.73	0.397
Baseline.Time	2		186991	93495	20.92	<.001
Baseline.Treatment	1		54	54	0.01	0.913
Baseline.Time.Treatment	2		4684	2342	0.52	0.596
Residual	39	-4	174301	4469		
Total	45	-4	343683			

**Table B.152.** 20°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	907	907	0.12	0.727
Baseline.Time	2	1562	781	0.11	0.899
Baseline.Treatment	1	56190	56190	7.66	0.008
Baseline.Time.Treatment	2	19862	9931	1.35	0.269
Residual	43	315366	7334		
Total	49	393887			

**Tables B.153-155.** Effect of pre-harvest application of 1-MCP, and time during postharvest storage at 4, 12 or 20°C on sucrose concentration of onions cv. SS1 (Section 5.4.2.3, Figure 5.5).

**Table B.153.** 4°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		445	445	0.25	0.619
Baseline.Time	1		571	571	0.32	0.574
Baseline.Treatment	1		1642	1642	0.93	0.342
Baseline.Time.Treatment	1		3012	3012	1.71	0.201
Residual	29	-1	51110	1762		
Total	33	-1	56736			

**Table B.154.** 12°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		5826	5826	3.15	0.084
Baseline.Time	2		8547	4274	2.31	0.112
Baseline.Treatment	1		23551	23551	12.74	<.001
Baseline.Time.Treatment	2		2099	1049	0.57	0.571
Residual	39	-4	72074	1848		
Total	45	-4	111482			

**Table B.155.** 20°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		11034	11034	7.19	0.011
Baseline.Time	2		4052	2026	1.32	0.278
Baseline.Treatment	1		43	43	0.03	0.867
Baseline.Time.Treatment	2		1304	652	0.42	0.657
Residual	41	-2	62935	1535		
Total	47	-2	79178			

**Tables B.156-158.** Effect of pre-harvest application of 1-MCP, and time during postharvest storage at 4, 12 or 20°C on glucose concentration of onions cv. SS1 (Section 5.4.2.3, Figure 5.5).

**Table B.156.** 4°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		70091	70091	36.29	<.001
Baseline.Time	1		57418	57418	29.73	<.001
Baseline.Treatment	1		5702	5702	2.95	0.096
Baseline.Time.Treatment	1		140	140	0.07	0.789
Residual	29	-1	56011	1931		
Total	33	-1	186382			

**Table B.157.** 12°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		28961	28961	20.84	<.001
Baseline.Time	2		7185	3592	2.59	0.088
Baseline.Treatment	1		146502	146502	105.44	<.001
Baseline.Time.Treatment	2		294	147	0.11	0.9
Residual	39	-4	54187	1389		
Total	45	-4	235464			

**Table B.158.** 20°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		26608	26608	17.55	<.001
Baseline.Time	2		29209	14605	9.63	<.001
Baseline.Treatment	1		1440	1440	0.95	0.335
Baseline.Time.Treatment	2		2820	1410	0.93	0.403
Residual	41	-2	62165	1516		
Total	47	-2	120299			

**Tables B.159-161.** Effect of pre-harvest application of 1-MCP, and time during postharvest storage at 4, 12 or 20°C on fructose concentration of onions cv. SS1 (Section 5.4.2.3, Figure 5.5).

**Table B.159.** 4°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		70621	70621	36.4	<.001
Baseline.Time	1		33865	33865	17.46	<.001
Baseline.Treatment	1		2455	2455	1.27	0.27
Baseline.Time.Treatment	1		1757	1757	0.91	0.349
Residual	29	-1	56257	1940		
Total	33	-1	163298			

**Table B.160.** 12°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		30982	30982	23.48	<.001
Baseline.Time	2		7945	3972	3.01	0.061
Baseline.Treatment	1		148502	148502	112.55	<.001
Baseline.Time.Treatment	2		897	449	0.34	0.714
Residual	39	-4	51458	1319		
Total	45	-4	237971			

**Table B.161.** 20°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		42846	42846	36.55	<.001
Baseline.Time	2		39719	19859	16.94	<.001
Baseline.Treatment	1		9	9	0.01	0.929
Baseline.Time.Treatment	2		3309	1655	1.41	0.255
Residual	41	-2	48069	1172		
Total	47	-2	131406			

**Tables B.162-164.** Effect of pre-harvest application of 1-MCP, and time during postharvest storage at 4, 12 or 20°C on the ratio of monosaccharide to disaccharide in onions cv. SS1 (Section 5.4.2.3, Figure 5.6).

**Table B.162.** 4°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		14.52	14.52	0.84	0.366
Baseline.Time	1		2.08	2.08	0.12	0.731
Baseline.Treatment	1		5.56	5.56	0.32	0.574
Baseline.Time.Treatment	1		11.76	11.76	0.68	0.416
Residual	29	-1	500.29	17.25		
Total	33	-1	533.65			

**Table B.163.** 12°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		287	287	1.25	0.271
Baseline.Time	2		3179.1	1589.5	6.91	0.003
Baseline.Treatment	1		27.8	27.8	0.12	0.73
Baseline.Time.Treatment	2		469.9	234.9	1.02	0.369
Residual	39	-4	8968.4	230		
Total	45	-4	11770.5			

**Table B.164.** 20°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		311.5	311.5	2.8	0.102
Baseline.Time	2		523.3	261.7	2.36	0.108
Baseline.Treatment	1		59.3	59.3	0.53	0.469
Baseline.Time.Treatment	2		129.6	64.8	0.58	0.563
Residual	41	-2	4555	111.1		
Total	47	-2	5555.3			

**Tables B.165-167.** Effect of pre-harvest application of 1-MCP, and time during postharvest storage at 4, 12 or 20°C on total sugar concentration of onions cv. SS1 (Section 5.4.2.3, Figure 5.6).

**Table B.165.** 4°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		304248	304248	27.16	<.001
Baseline.Time	1		200286	200286	17.88	<.001
Baseline.Treatment	1		27417	27417	2.45	0.129
Baseline.Time.Treatment	1		7218	7218	0.64	0.429
Residual	29	-1	324863	11202		
Total	33	-1	856161			

**Table B.166.** 12°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		178567	178567	31.28	<.001
Baseline.Time	2		56366	28183	4.94	0.012
Baseline.Treatment	1		849109	849109	148.76	<.001
Baseline.Time.Treatment	2		23	11	0	0.998
Residual	39	-4	222609	5708		
Total	45	-4	1303299			

**Table B.167.** 20°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		225774	225774	31.59	<.001
Baseline.Time	2		167874	83937	11.74	<.001
Baseline.Treatment	1		1185	1185	0.17	0.686
Baseline.Time.Treatment	2		11470	5735	0.8	0.455
Residual	41	-2	293063	7148		
Total	47	-2	687628			

**Tables B.168-170.** Effect of pre-harvest application of 1-MCP, and time during postharvest storage at 4, 12 or 20°C on total fructan concentration of onions cv. SS1 (Section 5.4.2.3, Figure 5.6).

**Table B.168.** 4°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		4957	4957	3.59	0.068
Baseline.Time	1		714	714	0.52	0.478
Baseline.Treatment	1		347	347	0.25	0.62
Baseline.Time.Treatment	1		186	186	0.13	0.716
Residual	29	-1	40056	1381		
Total	33	-1	46150			



**Table B.169.** 12°C.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Baseline	1		617	617	1.45	0.235
Baseline.Time	2		1374.6	687.3	1.62	0.211
Baseline.Treatment	1		895.8	895.8	2.11	0.154
Baseline.Time.Treatment	2		1742.3	871.2	2.05	0.142
Residual	39	-4	16551.4	424.4		
Total	45	-4	20930			

**Table B.170.** 20°C.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	74.4	74.4	0.21	0.652
Baseline.Time	2	795.7	397.9	1.11	0.34
Baseline.Treatment	1	8.1	8.1	0.02	0.881
Baseline.Time.Treatment	2	28.9	14.4	0.04	0.961
Residual	43	15475.3	359.9		
Total	49	16382.4			

## B.6 ANOVA tables for Chapter Six

**Table B.171.** Effect of the transition between CA and air storage on respiration rate of onion cvs. Renate, Carlos and SS1 (Section 6.4.1, Figure 6.1).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	2	0.068092	0.034046	9.56	<.001
Baseline	1	0.00636	0.00636	1.79	0.187
Cultivar.Baseline	2	0.000924	0.000462	0.13	0.879
Baseline.Time	1	0.000336	0.000336	0.09	0.76
Baseline.P1	1	0.003024	0.003024	0.85	0.361
Cultivar.Baseline.Time	2	0.001558	0.000779	0.22	0.804
Cultivar.Baseline.P1	2	0.028809	0.014405	4.05	0.023
Baseline.Time.P1	1	0.00231	0.00231	0.65	0.424
Baseline.Time.P2	1	0.075152	0.075152	21.1	<.001
Cultivar.Baseline.Time.P1	2	0.00339	0.001695	0.48	0.624
Cultivar.Baseline.Time.P2	2	0.005828	0.002914	0.82	0.447
Baseline.Time.P1.P2	1	0.002301	0.002301	0.65	0.425
Cultivar.Baseline.Time.P1.P2	2	0.008702	0.004351	1.22	0.303
Residual	51	0.181605	0.003561		
Total	71	0.388392			

**Table B.172.** Effect of the transition between CA and air storage on sprout growth of onion cvs. Renate, Carlos and SS1 (Section 6.4.1, Figure 6.2).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	2	9125.97	4562.98	115.35	<.001
Baseline	2	4467.89	2233.95	56.47	<.001
Cultivar.Baseline	4	7985.22	1996.31	50.46	<.001
Baseline.Time	1	9574.06	9574.06	242.02	<.001
Baseline.P1	1	140.21	140.21	3.54	0.062
Cultivar.Baseline.Time	2	17111.19	8555.6	216.28	<.001
Cultivar.Baseline.P1	2	102.36	51.18	1.29	0.277
Baseline.Time.P1	1	140.21	140.21	3.54	0.062
Baseline.Time.P2	1	679.24	679.24	17.17	<.001
Cultivar.Baseline.Time.P1	2	102.36	51.18	1.29	0.277
Cultivar.Baseline.Time.P2	2	717.58	358.79	9.07	<.001
Baseline.Time.P1.P2	1	977	977	24.7	<.001
Cultivar.Baseline.Time.P1.P2	2	1154.32	577.16	14.59	<.001
Residual	156	6171.12	39.56		
Total	179	58448.75			

**Table B.173.** Effect of the transition between CA and air storage on firmness of onion cvs. Renate, Carlos and SS1 (Section 6.4.1, Table 6.2).

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	2		31029.02	15514.51	254.45	<.001
Baseline	1	-1	588.74	588.74	9.66	0.002
Cultivar.Baseline	2	-2	531.53	265.76	4.36	0.015
Baseline.Time	1		207.78	207.78	3.41	0.067
Baseline.P1	1		109.17	109.17	1.79	0.183
Cultivar.Baseline.Time	2		141.34	70.67	1.16	0.317
Cultivar.Baseline.P1	2		143.02	71.51	1.17	0.313
Baseline.Time.P1	1		10.11	10.11	0.17	0.685
Baseline.Time.P2	1		0.17	0.17	0	0.958
Cultivar.Baseline.Time.P1	2		96.43	48.22	0.79	0.456
Cultivar.Baseline.Time.P2	2		47.28	23.64	0.39	0.679
Baseline.Time.P1.P2	1		70.83	70.83	1.16	0.283
Cultivar.Baseline.Time.P1.P2	2		89.03	44.51	0.73	0.484
Residual	122	-34	7438.59	60.97		
Total	142	-37	36656.81			

**Table B.174.** Effect of the transition between CA and air storage on dry weight of onion cvs. Renate, Carlos and SS1 (Section 6.4.1, Table 6.2).

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	2		1630.765	815.383	735.82	<.001
Baseline	2		29.364	14.682	13.25	<.001
Cultivar.Baseline	4		10.694	2.674	2.41	0.051
Baseline.Time	1		0.043	0.043	0.04	0.843
Baseline.P1	1		0.408	0.408	0.37	0.545
Cultivar.Baseline.Time	2		0.916	0.458	0.41	0.662
Cultivar.Baseline.P1	2		5.25	2.625	2.37	0.097
Baseline.Time.P1	1		3.327	3.327	3	0.085
Baseline.Time.P2	1		0.049	0.049	0.04	0.834
Cultivar.Baseline.Time.P1	2		0.452	0.226	0.2	0.816
Cultivar.Baseline.Time.P2	2		0.446	0.223	0.2	0.818
Baseline.Time.P1.P2	1		0.064	0.064	0.06	0.81
Cultivar.Baseline.Time.P1.P2	2		0.198	0.099	0.09	0.914
Residual	155	-1	171.76	1.108		
Total	178	-1	1851.863			

**Table B.175.** Effect of the transition between CA and air storage on total soluble solids content of onion cvs. Renate, Carlos and SS1 (Section 6.4.2, Table 6.3).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	2	1279.239	639.62	499.34	<.001
Baseline	2	15.999	7.999	6.24	0.002
Cultivar.Baseline	4	26.185	6.546	5.11	<.001
Baseline.Time	1	0.013	0.013	0.01	0.921
Baseline.P1	1	1.021	1.021	0.8	0.373
Cultivar.Baseline.Time	2	2.614	1.307	1.02	0.363
Cultivar.Baseline.P1	2	9.642	4.821	3.76	0.025
Baseline.Time.P1	1	2.503	2.503	1.95	0.164
Baseline.Time.P2	1	0.333	0.333	0.26	0.611
Cultivar.Baseline.Time.P1	2	16.473	8.236	6.43	0.002
Cultivar.Baseline.Time.P2	2	15.493	7.746	6.05	0.003
Baseline.Time.P1.P2	1	0.163	0.163	0.13	0.722
Cultivar.Baseline.Time.P1.P2	2	23.468	11.734	9.16	<.001
Residual	156	199.824	1.281		
Total	179	1592.97			

**Table B.176.** Effect of the transition between CA and air storage on pyruvate concentration of onion cvs. Renate, Carlos and SS1 (Section 6.4.2, Table 6.3).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	2	429.837	214.919	111.19	<.001
Baseline	2	61.733	30.867	15.97	<.001
Cultivar.Baseline	4	33.141	8.285	4.29	0.003
Baseline.Time	1	5.069	5.069	2.62	0.107
Baseline.P1	1	6.253	6.253	3.23	0.074
Cultivar.Baseline.Time	2	1.827	0.914	0.47	0.624
Cultivar.Baseline.P1	2	24.306	12.153	6.29	0.002
Baseline.Time.P1	1	0.416	0.416	0.22	0.643
Baseline.Time.P2	1	0.373	0.373	0.19	0.661
Cultivar.Baseline.Time.P1	2	2.899	1.45	0.75	0.474
Cultivar.Baseline.Time.P2	2	6.545	3.272	1.69	0.187
Baseline.Time.P1.P2	1	3.137	3.137	1.62	0.205
Cultivar.Baseline.Time.P1.P2	2	3.871	1.935	1	0.37
Residual	156	301.526	1.933		
Total	179	880.934			

**Table B.177.** Effect of the transition between CA and air storage on sucrose concentration of onion cvs. Renate, Carlos and SS1 (Section 6.4.3, Figure 6.3).

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	2		18927.7	9463.8	14.64	<.001
Baseline	2		48255.7	24127.8	37.32	<.001
Cultivar.Baseline	4		13330.1	3332.5	5.15	<.001
Baseline.Time	1		1267	1267	1.96	0.164
Baseline.P1	1		1987	1987	3.07	0.082
Cultivar.Baseline.Time	2		10523.6	5261.8	8.14	<.001
Cultivar.Baseline.P1	2		1022	511	0.79	0.455
Baseline.Time.P1	1		1484.1	1484.1	2.3	0.132
Baseline.Time.P2	1		837.6	837.6	1.3	0.257
Cultivar.Baseline.Time.P1	2		240.3	120.2	0.19	0.831
Cultivar.Baseline.Time.P2	2		2013.8	1006.9	1.56	0.214
Baseline.Time.P1.P2	1		59.6	59.6	0.09	0.762
Cultivar.Baseline.Time.P1.P2	2		1402.5	701.3	1.08	0.341
Residual	154	-2	99558.1	646.5		
Total	177	-2	200263.2			

**Table B.178.** Effect of the transition between CA and air storage on fructose concentration of onion cvs. Renate, Carlos and SS1 (Section 6.4.2, Figure 6.3).

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	2		1687710	843855	582.07	<.001
Baseline	2		55330	27665	19.08	<.001
Cultivar.Baseline	4		115411	28853	19.9	<.001
Baseline.Time	1		169	169	0.12	0.733
Baseline.P1	1		2612	2612	1.8	0.181
Cultivar.Baseline.Time	2		25863	12932	8.92	<.001
Cultivar.Baseline.P1	2		5870	2935	2.02	0.136
Baseline.Time.P1	1		13531	13531	9.33	0.003
Baseline.Time.P2	1		8760	8760	6.04	0.015
Cultivar.Baseline.Time.P1	2		9572	4786	3.3	0.039
Cultivar.Baseline.Time.P2	2		16374	8187	5.65	0.004
Baseline.Time.P1.P2	1		5621	5621	3.88	0.051
Cultivar.Baseline.Time.P1.P2	2		5491	2746	1.89	0.154
Residual	154	-2	223262	1450		
Total	177	-2	2163626			

**Table B.179.** Effect of the transition between CA and air storage on glucose concentration of onion cvs. Renate, Carlos and SS1 (Section 6.4.2, Figure 6.3).

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	2		606627	303314	277.05	<.001
Baseline	2		54127	27063	24.72	<.001
Cultivar.Baseline	4		53786	13447	12.28	<.001
Baseline.Time	1		13	13	0.01	0.915
Baseline.P1	1		620	620	0.57	0.453
Cultivar.Baseline.Time	2		62516	31258	28.55	<.001
Cultivar.Baseline.P1	2		26569	13285	12.13	<.001
Baseline.Time.P1	1		12244	12244	11.18	0.001
Baseline.Time.P2	1		22518	22518	20.57	<.001
Cultivar.Baseline.Time.P1	2		5238	2619	2.39	0.095
Cultivar.Baseline.Time.P2	2		2788	1394	1.27	0.283
Baseline.Time.P1.P2	1		8669	8669	7.92	0.006
Cultivar.Baseline.Time.P1.P2	2		12887	6443	5.89	0.003
Residual	154	-2	168598	1095		
Total	177	-2	1024754			

**Table B.180.** Effect of the transition between CA and air storage on the ratio of monosaccharide: disaccharides in onion cvs. Renate, Carlos and SS1 (Section 6.4.2, Figure 6.4).

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	2		2381.47	1190.74	86.05	<.001
Baseline	2		1862.48	931.24	67.3	<.001
Cultivar.Baseline	4		2308.76	577.19	41.71	<.001
Baseline.Time	1		18.22	18.22	1.32	0.253
Baseline.P1	1		2	2	0.14	0.704
Cultivar.Baseline.Time	2		16.97	8.49	0.61	0.543
Cultivar.Baseline.P1	2		5.58	2.79	0.2	0.818
Baseline.Time.P1	1		1.67	1.67	0.12	0.729
Baseline.Time.P2	1		0.68	0.68	0.05	0.824
Cultivar.Baseline.Time.P1	2		0.32	0.16	0.01	0.989
Cultivar.Baseline.Time.P2	2		0.61	0.31	0.02	0.978
Baseline.Time.P1.P2	1		0.04	0.04	0	0.956
Cultivar.Baseline.Time.P1.P2	2		3.89	1.95	0.14	0.869
Residual	154	-2	2130.9	13.84		
Total	177	-2	8715.74			

**Table B.181.** Effect of the transition between CA and air storage on total sugar concentration in onion cvs. Renate, Carlos and SS1 (Section 6.4.2, Figure 6.4).

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	2		4233779	2116890	519.9	<.001
Baseline	2		58119	29060	7.14	0.001
Cultivar.Baseline	4		183854	45964	11.29	<.001
Baseline.Time	1		2027	2027	0.5	0.481
Baseline.P1	1		14538	14538	3.57	0.061
Cultivar.Baseline.Time	2		221267	110634	27.17	<.001
Cultivar.Baseline.P1	2		61177	30588	7.51	<.001
Baseline.Time.P1	1		70495	70495	17.31	<.001
Baseline.Time.P2	1		74299	74299	18.25	<.001
Cultivar.Baseline.Time.P1	2		18602	9301	2.28	0.105
Cultivar.Baseline.Time.P2	2		20146	10073	2.47	0.088
Baseline.Time.P1.P2	1		109	109	0.03	0.87
Cultivar.Baseline.Time.P1.P2	2		26642	13321	3.27	0.041
Residual	154	-2	627045	4072		
Total	177	-2	5574303			

**Table B.182.** Effect of the transition between CA and air storage on total fructan concentration in onion cvs. Renate, Carlos and SS1 (Section 6.4.2, Figure 6.4).

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	2		2334507	1167254	669.66	<.001
Baseline	2		24104	12052	6.91	0.001
Cultivar.Baseline	4		35601	8900	5.11	<.001
Baseline.Time	1		18315	18315	10.51	0.001
Baseline.P1	1		9680	9680	5.55	0.02
Cultivar.Baseline.Time	2		11833	5917	3.39	0.036
Cultivar.Baseline.P1	2		7468	3734	2.14	0.121
Baseline.Time.P1	1		2970	2970	1.7	0.194
Baseline.Time.P2	1		94	94	0.05	0.817
Cultivar.Baseline.Time.P1	2		10933	5466	3.14	0.046
Cultivar.Baseline.Time.P2	2		8285	4143	2.38	0.096
Baseline.Time.P1.P2	1		1093	1093	0.63	0.43
Cultivar.Baseline.Time.P1.P2	2		2188	1094	0.63	0.535
Residual	155	-1	270172	1743		
Total	178	-1	2725545			


**Table B.183.** Effect of the transition between CA and air storage on abscisic acid concentration in onion cvs. Renate, Carlos and SS1 (Section 6.4.2, Figure 6.5).

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cultivar	2		47231	23616	6.16	0.003
Baseline	2		152482	76241	19.9	<.001
Cultivar.Baseline	4		83495	20874	5.45	<.001
Baseline.Time	1		416	416	0.11	0.742
Baseline.P1	1		322	322	0.08	0.772
Cultivar.Baseline.Time	2		1846	923	0.24	0.786
Cultivar.Baseline.P1	2		1679	839	0.22	0.804
Baseline.Time.P1	1		1964	1964	0.51	0.475
Baseline.Time.P2	1		505	505	0.13	0.717
Cultivar.Baseline.Time.P1	2		7482	3741	0.98	0.379
Cultivar.Baseline.Time.P2	2		6289	3144	0.82	0.442
Baseline.Time.P1.P2	1		480	480	0.13	0.724
Cultivar.Baseline.Time.P1.P2	2		1808	904	0.24	0.79
Residual	155	-1	593787	3831		
Total	178	-1	894609			

## C. APPENDIX



### Published literature.

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



 ELSEVIER	Available online at <a href="http://www.sciencedirect.com">www.sciencedirect.com</a> <b>SCIENCE @ DIRECT®</b> Postharvest Biology and Technology 39 (2006) 233–242	<b>Postharvest Biology and Technology</b> <a href="http://www.elsevier.com/locate/postharvbio">www.elsevier.com/locate/postharvbio</a>
<h3 style="margin: 0;">Effect of controlled atmosphere storage on abscisic acid concentration and other biochemical attributes of onion bulbs</h3> <p style="margin: 0;">Gemma A. Chope<sup>a,b</sup>, Leon A. Terry<sup>a,*</sup>, Philip J. White<sup>b</sup></p> <p style="margin: 0;"><sup>a</sup> Plant Science Laboratory, Cranfield University, Silsoe, Bedfordshire MK45 4DT, UK  <sup>b</sup> Warwick HRI, Wellesbourne, Warwick CV35 9EF, UK</p> <p style="margin: 0;">Received 17 May 2005; accepted 18 October 2005</p>		
<b>Abstract</b>		
<p>Onion bulbs (<i>Allium cepa</i> L.) of cultivars with long-, medium- and short-storage lives, viz. Renate, Ailsa Craig and SS1, respectively, were stored in controlled atmosphere (CA) conditions (3.03 kPa CO<sub>2</sub>; 5.05 kPa O<sub>2</sub>; 2 °C). Bulb abscisic acid (ABA) concentration, pyruvate, fructans, total soluble solids (TSS) and firmness were measured throughout storage.</p> <p>In all cultivars, bulb ABA concentration declined exponentially during storage. The greatest decrease in ABA concentration occurred during the first 80 days of storage. Although the pattern of decline was similar for the long-, medium- and short-storing onion bulbs, onion cv. SS1 bulbs had the lowest initial ABA concentration. Onion bulb ABA concentration at harvest (measured on a fresh weight basis) may prove to be a better indicator of storage life. The ABA concentration at harvest (DW) may be indicative of a greater difference in sprouting during storage between cv. SS1 and the other cultivars than between cvs. Renate and Ailsa Craig.</p> <p>It is hypothesised that the storage potential of bulbs of different onion cultivars is inversely related to the time at which they reach a minimal ABA content. Thus, the storage life of short-storing cultivars (e.g. cv. SS1) might be prolonged by slowing the decline in ABA concentration. This could help extend the period for supplying these onions from temperate regions. Onion bulbs of cvs. Renate, Ailsa Craig and SS1 were characterised by high, intermediate and low concentrations of pyruvate, fructan and total soluble solids, respectively.</p> <p>© 2005 Elsevier B.V. All rights reserved.</p>		
<p><i>Keywords:</i> ABA; <i>Allium cepa</i> L.; Firmness; Fructan; Pyruvate; Sprouting</p>		



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

POSTEC-8572; No. of Pages 10	<b>ARTICLE IN PRESS</b>	
 ELSEVIER	Available online at <a href="http://www.sciencedirect.com">www.sciencedirect.com</a>	 <b>Postharvest Biology and Technology</b>
	Postharvest Biology and Technology xxx (2007) xxx–xxx	<a href="http://www.elsevier.com/locate/postharvbio">www.elsevier.com/locate/postharvbio</a>
<h2>The effect of 1-methylcyclopropene (1-MCP) on the physical and biochemical characteristics of onion cv. SS1 bulbs during storage</h2>		
<p>Gemma A. Chope<sup>a,b</sup>, Leon A. Terry<sup>a,*</sup>, Philip J. White<sup>c</sup></p>		
<p><sup>a</sup> Plant Science Laboratory, Cranfield University, Silsoe, Bedfordshire MK45 4DT, UK</p>		
<p><sup>b</sup> Warwick HRI, Wellesbourne, Warwickshire CV35 9EF, UK</p>		
<p><sup>c</sup> Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK</p>		
<p>Received 10 August 2006; accepted 25 November 2006</p>		
<b>Abstract</b>		
<p>There is a paucity of information on the role of ethylene in onion bulb dormancy, and the available literature is conflicting. Onion cv. SS1 bulbs were treated with <math>1 \mu\text{l}^{-1}</math> 1-MCP for 24 h at 20 °C and then stored at 4, 12 or 20 °C. Sprout growth was reduced in onions treated with 1-MCP and stored at 4 or 12 °C, but not when stored at 20 °C. Greater concentrations of sucrose, glucose and fructose were measured in 1-MCP treated bulbs stored at 12 °C as compared with untreated bulbs. Dry weight was also maintained in onions treated with 1-MCP. Abscisic acid (ABA) concentration before storage has previously been shown to be correlated with storage life, but there were no differences in the ABA concentration between 1-MCP treated and untreated bulbs. It appeared that 1-MCP reduced the rate of carbon utilisation. The mechanism by which this occurred is unknown although it is unlikely to be mediated by ABA.</p>		
<p>© 2006 Elsevier B.V. All rights reserved.</p>		
<p><b>Keywords:</b> Abscisic acid; <i>Allium cepa</i> L.; Ethylene; Non-structural carbohydrates; Sprouting</p>		

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

+Model POSTEC-8595; No. of Pages 12	<b>ARTICLE IN PRESS</b>	
 <b>ELSEVIER</b>	Available online at <a href="http://www.sciencedirect.com">www.sciencedirect.com</a>	 <b>ScienceDirect</b> <hr/> <b>Postharvest Biology and Technology</b> <hr/> <a href="http://www.elsevier.com/locate/postharvbio">www.elsevier.com/locate/postharvbio</a>
Postharvest Biology and Technology xxx (2007) xxx–xxx		
<h2>The effect of the transition between controlled atmosphere and regular atmosphere storage on bulbs of onion cultivars SS1, Carlos and Renate</h2>		
<p>Gemma A. Chope<sup>a,b</sup>, Leon A. Terry<sup>a,*</sup>, Philip J. White<sup>c</sup></p>		
<p><sup>a</sup> Plant Science Laboratory, Cranfield University, Silsoe, Bedfordshire MK45 4DT, UK</p>		
<p><sup>b</sup> Warwick HRI, Wellesbourne, Warwick CV35 9EF, UK</p>		
<p><sup>c</sup> Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK</p>		
<p>Received 10 August 2006; accepted 22 December 2006</p>		
<p><b>Abstract</b></p>		
<p>Controlled atmosphere storage (CA) is used to increase the storage life of onions; however detrimental effects on the shelf-life have been reported. The effect of the transition between CA (5 kPa CO<sub>2</sub>, 3 kPa O<sub>2</sub>) and air (and vice versa) on onion cvs. Renate, Carlos and SS1 in terms of the respiration rate, sprout growth, dry weight, firmness, pungency, total soluble solids, abscisic acid concentration and non-structural carbohydrate composition was assessed. Removal of bulbs from CA storage resulted in an immediate increase in the respiration rate (measured in air), which then reverted to a lower rate following subsequent storage under air conditions for 21 days. In some cultivars, this could be sufficient to trigger the onset of sprouting and thus account for the detrimental effect of CA storage on shelf-life. Delaying the start of CA storage of onions cv. SS1 for 21 days was as effective in suppressing sprout growth as CA storage for 42 days. Further investigation into the use of CA storage in this manner with relation to the optimum time to begin CA conditions could decrease the cost of CA storage without compromising storage life. Abscisic acid concentration has been associated with storage life of onions. There was a significant decrease in the bulb ABA concentration between the time of harvest and the beginning of storage. This is likely to be due to the effects of curing and suggests that current curing practices are having a detrimental effect on storage potential.</p> <p>© 2007 Elsevier B.V. All rights reserved.</p>		
<p><i>Keywords:</i> Abscisic acid; <i>Allium cepa</i> L.; Non-structural carbohydrates; Respiration rate</p>		