# FINAL REPORT

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

## CP 20

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**CP 20**

# Understanding the mechanisms behind onion bulb dormancy in relation to the potential for improved onion storage

## Headline

- Minimal abscisic acid (ABA; an endogenous plant growth regulator) is related to increase sprout growth.
- Onion bulb ABA concentration cannot be increased by foliar sprays in the field.
- Current curing practice using extended periods of exposure to high temperature could deplete ABA levels and have a negative impact on onion storage life.
- An ethylene perception inhibitor (1-MCP) reduces sprout growth in onions cv. SS1 stored at 12°C.

## Background and expected deliverables

Extended storage life of UK onions currently depends on the use of maleic hydrazide (MH) to suppress sprout growth in store. Concerns over residues have led to retailer pressure to reduce or eliminate MH treatment. This could seriously affect UK onion growers, who will find it increasingly difficult to supply onions from March onwards, and will lose market share to imports.

Dormancy is the result of two physiological processes; dormancy induction and sprout suppression. The true dormancy period is short and appears to be independent of storage life. During this period substantive sprout growth does not occur. Thereafter, suppression of sprout growth maintains apparent dormancy despite the occurrence of mitotic activity in the apical and floral meristems. Much of the work on improving onion storage potential has concentrated on breeding, cultural practice and storage environment, with little attention to the physiological processes behind onion bulb dormancy.

Abscisic acid (ABA) is a plant growth regulator that has been associated with the control of dormancy in onion bulbs. ABA concentration in onion and *Allium wakegi* (an *A. cepa* x *A. fisulosum* hybrid) has been shown to increase during bulb development, peak at maturation and then fall during dormancy. ABA is also involved in seed and bud dormancy in other plant species and in the control of water uptake. Little is known of the timing of appearance, and levels of this plant growth regulator (PGR) in onion cultivars with differing storage potential and the consequential changes in significant storage compounds.

The expected deliverables from this project were:

- An understanding of the role and effects of ABA in storage potential of onions.
- Potential identification of superior markers for storage condition.

**Summary of the project and main conclusions**

**The role of ABA in onion bulb dormancy**

The changes in ABA during storage of onion bulbs with different storage potentials (*viz*. Renate - long, Ailsa Craig - intermediate and SS1 - short) were profiled in order to determine the significant changes that lead to the breaking of bulb dormancy and the onset of sprouting. The onions were stored in controlled atmosphere (CA) conditions (5% $O_2$, 3%$CO_2$; 2ºC). Bulb ABA concentration measured before storage was greatest in onion cv. Renate and Ailsa Craig bulbs, and least in onion cv. SS1 bulbs (on a fresh weight basis). Bulb ABA concentration declined exponentially during storage at the same rate in each cultivar. Sprouting occurred at a minimal ABA concentration (*ca*. 50-120 ng $g^{-1}$ DW) (Figure 1). It was proposed that extended periods of high concentrations of ABA may delay sprouting.

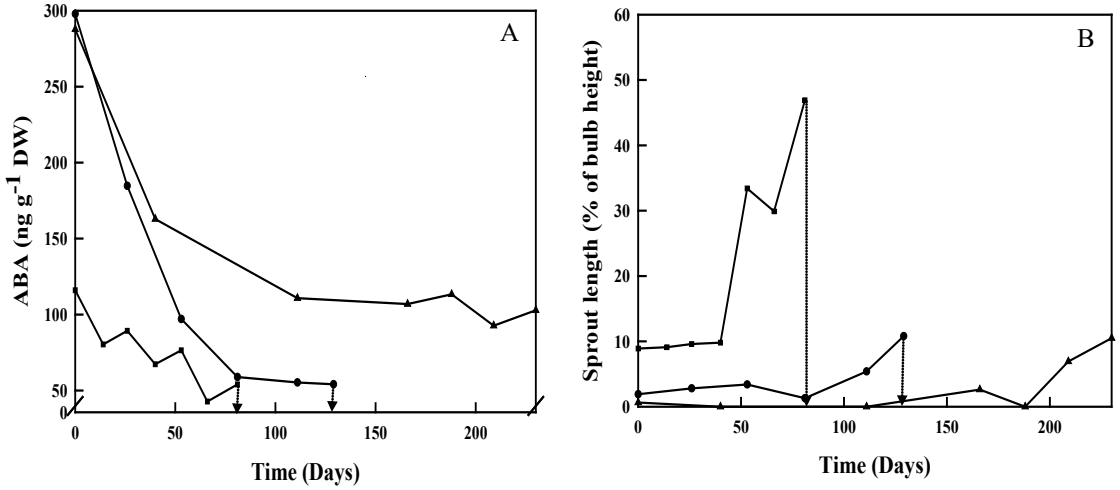


**Figure 1**. Changes in A: ABA concentration and B: sprout length of onion bulbs cv. Renate (▲), Ailsa Craig (●) and SS1 (■) over time in controlled atmosphere storage. Drop down arrows indicate the last sample taken.

An ABA analogue (PBI-365) and exogenous ABA, were applied as preharvest foliar sprays to a range on onion cultivars with contrasting storage potential (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 $g^{-1}$ DW). After the onset of sprouting, ABA concentration increased again, probably due to synthesis by the sprout.

**Effect of the transition between controlled atmosphere and air storage**

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 % $CO_2$, 5 % $O_2$; 2°C) and air storage in three onion cultivars (*viz*. Renate, Carlos and SS1) were investigated. The respiration rate of the short storing cultivar, SS1, was greatest by *ca*. 0.5-fold. Respiration rate 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 (Figure 2A).

Bulb ABA concentration decreased significantly between the time of harvest and after curing (Figure 2B). Therefore, the current practice of curing onions for extended periods at high temperatures could be reducing bulb ABA concentration, and therefore storage life.

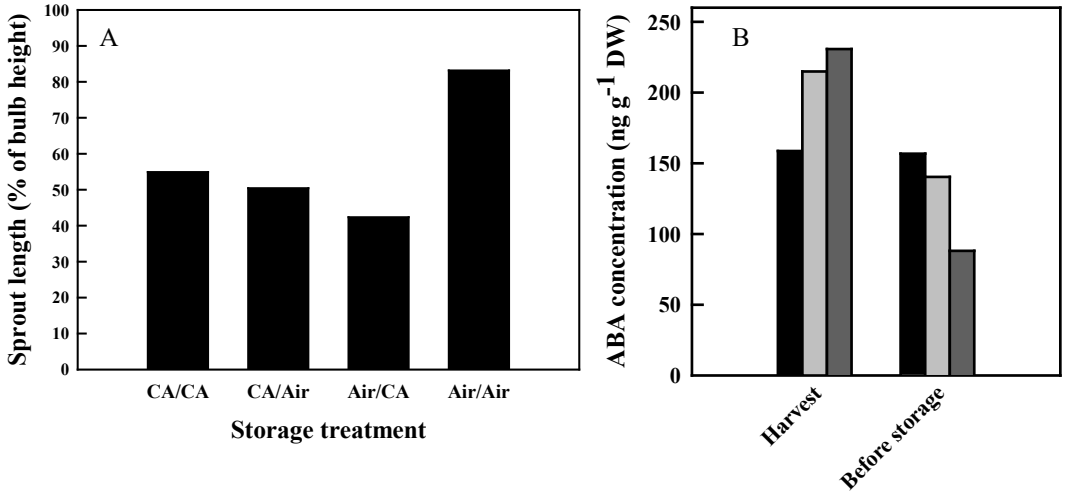


**Figure 2.** A. The sprout length of onion cv. SS1 expressed as a percentage of bulb height after storage in; CA for 42 days (CA/CA), CA for 21 days followed by air for 21 days (CA/Air), air for 21 days followed by CA for 21 days (Air/CA) and air for 42 days (Air/Air). B. The ABA concentration of onions cv. Carlos (▬), Renate (▭) and SS1 (▬) measured at harvest and before storage (day 0).

**The effect of 1-methylcyclopropene on sprout growth in onion cv. SS1 bulbs**

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. Sprout growth was reduced in onions treated with 1-MCP and stored at 4 or 12°C, but not at 20°C (Figure 3). 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 (Figure 4).

It appeared that 1-MCP reduced carbohydrate metabolism. 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. Continual long-term exposure to ethylene (10 µl $l^{-1}$) 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.

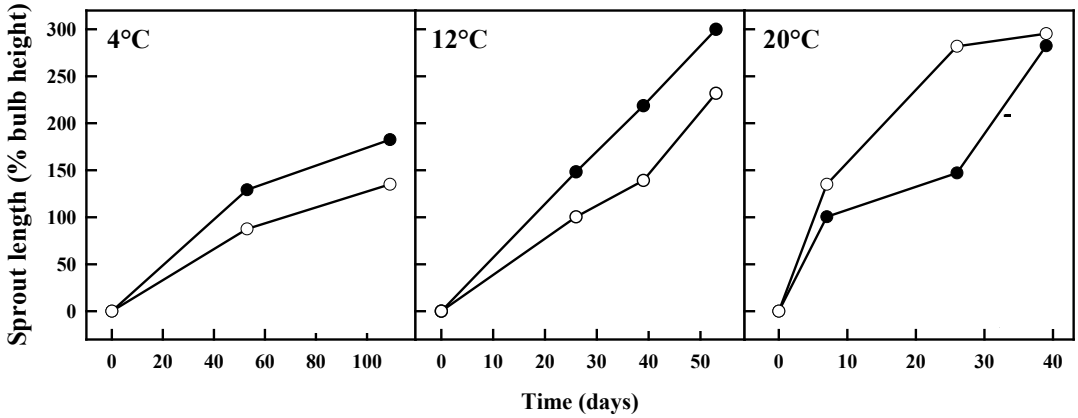


**Figure 3.** Sprout length in onions treated with 1 µl $l^{-1}$ 1-MCP at 20ºC for 24 hours (open symbols) and untreated onions (controls, closed symbols) stored at 4ºC for 109 days, 12ºC for 53 days or 20ºC for 39 days.

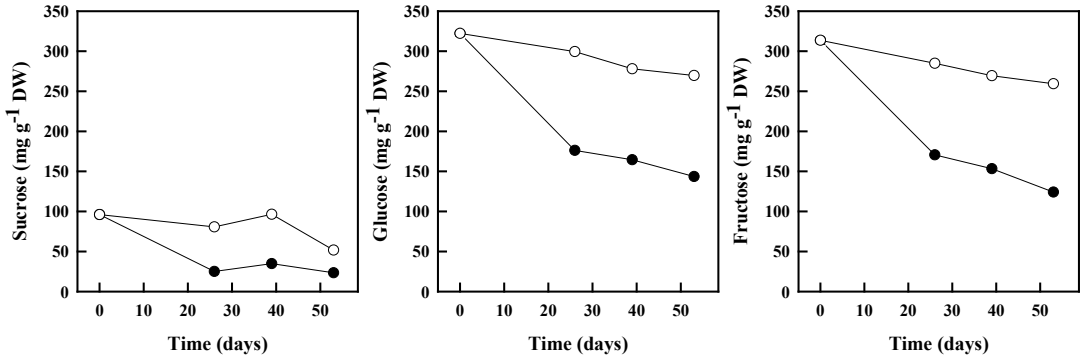

**Figure 4.** Changes in sucrose, glucose and fructose concentrations during storage in untreated onions (controls, closed symbols) and in onions treated with 1 µl $l^{-1}$ 1-MCP at 20ºC for 24 hours (open symbols) prior to storage at 12ºC.

**Financial benefits**

- There are no financial benefits for growers at this stage; however possible benefits for growers in the future are detailed below.

In light of rising energy costs (the cost of commercial electricity increased by *ca* 50% and gas by *ca*. 65% between 2005 and 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 for the curing and drying procedure is based on a method developed in the 1970s. 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. 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; therefore ABA concentration would be a very useful parameter to monitor during different curing and drying regimes. This could lead to breeding of improved onion cultivars that perform better under more efficient curing regimes.

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 using six weeks continuous CA storage. Further investigation into the effects of delaying the start of CA storage has the potential to reduce the costs associated with this method while not compromising on effectiveness.

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. In addition, the concentration of simple sugars in onions cv. SS1 treated with 1-MCP was elevated, implying that that 1-MCP treatment has a positive impact on flavour in this cultivar. Therefore, further investigation should be made into the utilisation of this the use of 1-MCP to manipulate sugar content.

**Action points for growers**

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

However, the results from this project have formed the foundation of a recent successful application to DEFRA HortLink entitled 'HL0181 – Sustaining UK fresh onion supply be improving consumer acceptability, quality and availability'. This project will investigate the effects of reducing the temperature used in the curing process, and the optimisation of novel postharvest treatements, on bulb quality and storability.

# 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 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*[1]

- 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.[2]
- To relate the observations made to the potential to influence onion storage life by changes in horticultural practices.

## 1.3 Structure of the science section

The science section 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.

[1] Objectives revised under agreement.
[2] Additional objective.

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 study were stored at a range of temperatures (4, 12 or 20ºC) to ensure that sprout growth would progress fully in all cultivars allowing measurements to be taken from bulbs at all physiological stages.

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.

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.

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.

# 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[1] harvested (1000 Ha), the yield (100 g $Ha^{-1}$) and production (tonnes) from 2000 to 2005 in the UK and worldwide (FAOSTAT, 2006).

| | Area Harvested (Ha) | | Yield (100g $Ha^{-1}$) | | Production (Tonne) | |
|---|---|---|---|---|---|---|
| Year | 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 |

[1]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[1]) of onions imported to, and exported from, the UK (FAOSTAT, 2006).

| | Imports | | Exports | |
|---|---|---|---|---|
| Year | 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 |

[1] 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.

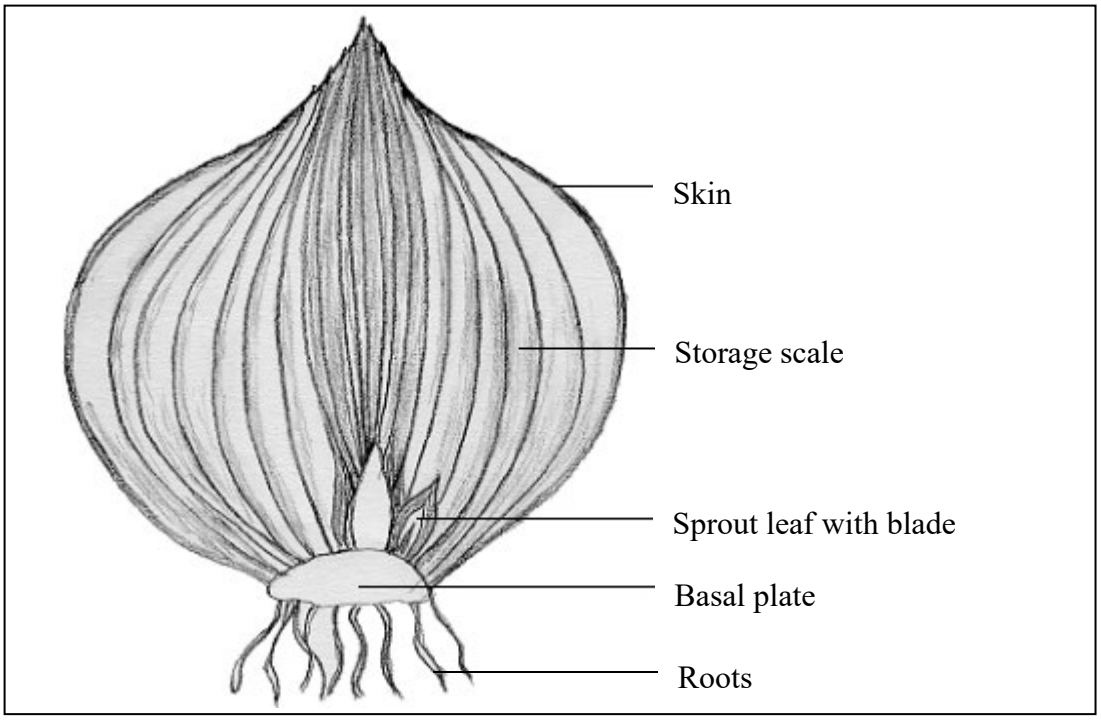


**Figure 2.1.** Annotated diagram of a cross-section of an onion bulb.

### *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.

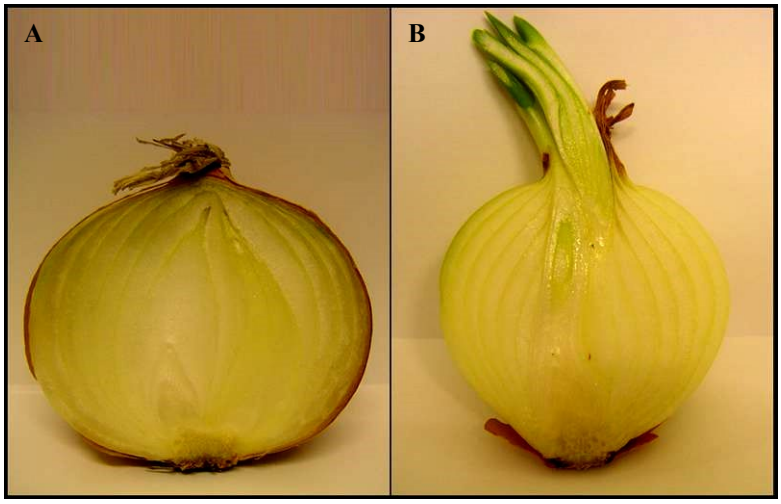


**Figure 2.2.** Cross-sections of an onion cv. Renate bulb: A – with no sprout and B – with an external sprout.

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).

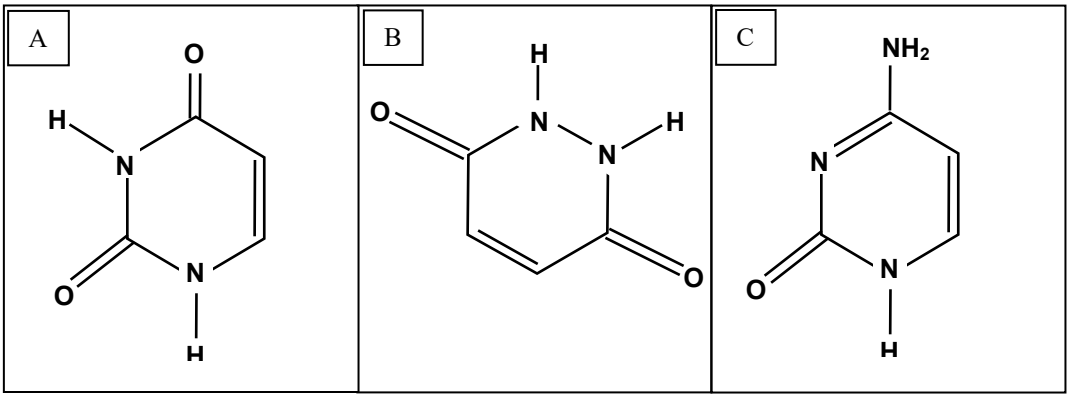


**Figure 2.3.** Chemical structure of: A – Uracil, B – Maleic hydrazide, C – Cytosine.

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 30ºC. After three to five days the temperature is lowered to 24ºC 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 ($^{60}$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 γ-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 $^{60}Co$ source at a dose of 50 Gy, and stored in warehouse conditions (6 to 32°C and 40-50% RH) for *ca*. 43 weeks (Croci *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 ($N_2O$) 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 $N_2O$ and subsequently stored at 18±0.1ºC and 65±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 10-20ºC for most cultivars, with some cultivars displaying a sharp optimum while others have a broader range. Moisture loss is greater at temperature ranges <10ºC and >27ºC.

In developed temperate countries, such as the UK, onions are kept in large, specialised stores. Ventilation is forced, and temperature is usually maintained around 5ºC, but can be as low as -1ºC. 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 25-30ºC 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 35ºC significantly reduced the number of days to sprouting in dry storage at 15ºC, when compared to those exposed to postharvest temperature treatments of 15 and 25ºC, 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% $CO_2$ and 3% $O_2$ 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 γ-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 (PRENCSO) cysteine sulphoxide. PRENCSO 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.

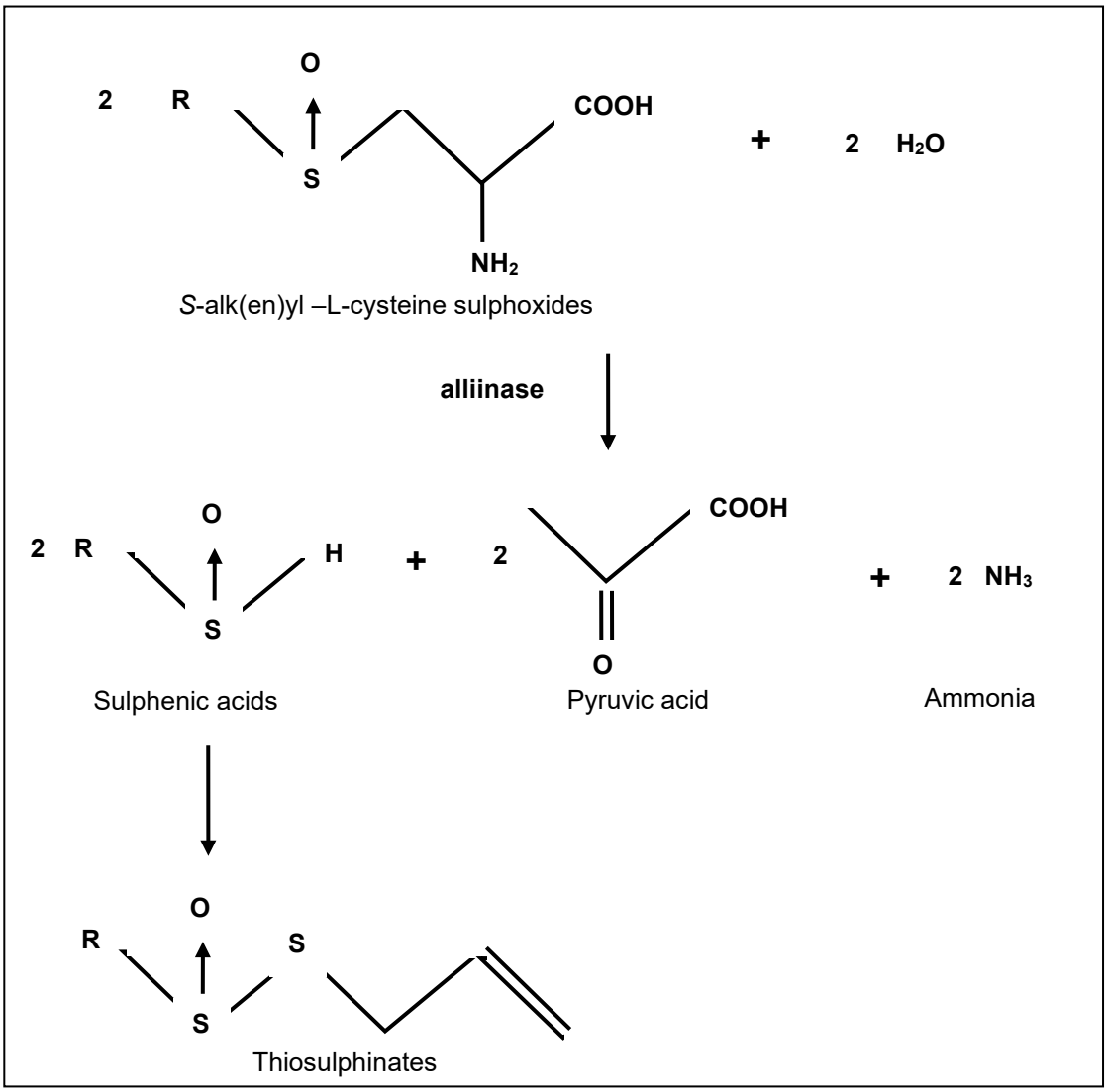


**Figure 2.4.** Simplified pathway of flavour production in onion bulb tissue.

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 15ºC than in those stored at 30ºC, suggesting that hydrolysis of fructans increased at low temperatures (Salama *et al*., 1990). A net increase in total sugars was observed at 0ºC, and a net decrease observed at 15 and 30ºC.

### *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).

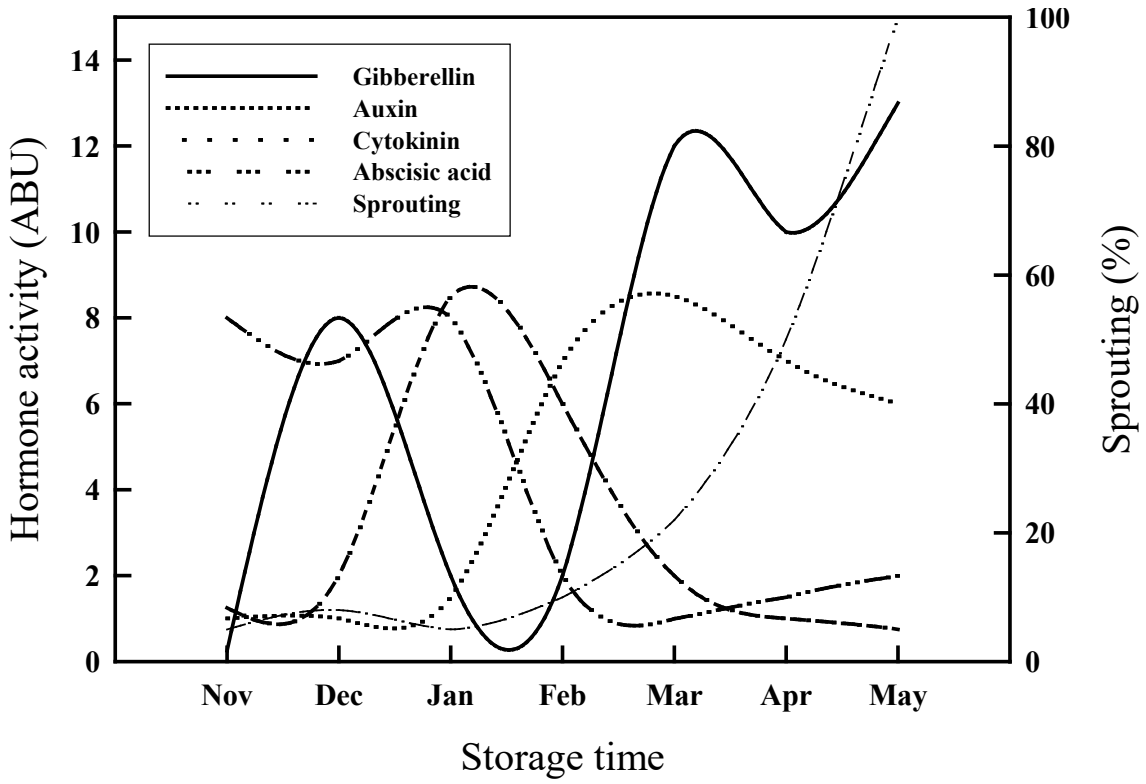


**Figure 2.5.** Percentage sprouting and hormone activity of onions cv. Rijnsberger stored at 5-8°C (Thomas and Isenberg, 1972).

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 $l^{-1}$ 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).

Abscisic acid is a naturally occurring phytohormone. The ABA biosynthesis pathway (Figure 2.6) begins in chloroplasts and other plastids with the cleavage of a $C_{40}$ 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.

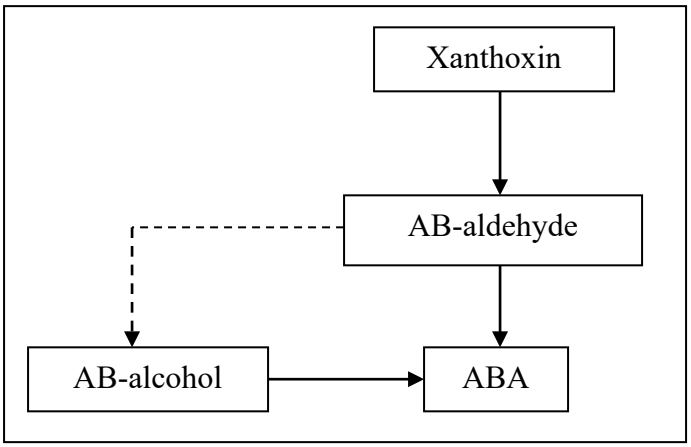


**Figure 2.6.** The synthesis of abscisic acid (ABA) from xanthoxin in higher plants. Solid arrows indicate the major pathway, dashed arrows indicate a minor pathway, AB=abscisic (Cutler and Krochko, 1999).

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.

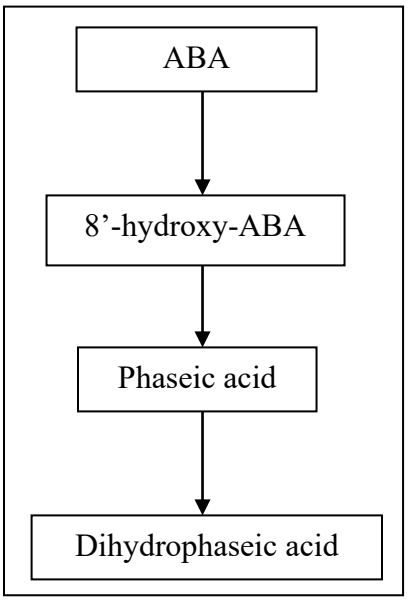


**Figure 2.7.** Metabolism of abscisic acid (ABA).

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 ng $g^{-1}$ FW compared to a maximum concentration of 20 ng $g^{-1}$ 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 ng $g^{-1}$ FW to 13 ng $g^{-1}$ 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 (0.1-5 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% $CO_2$; 5% $O_2$; 2ºC). 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 study 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 $g^{-1}$ FW) | Days in store | Storage conditions | Extraction method | Quantification method | Reference |
|---|---|---|---|---|---|---|
| *A. cepa* 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) |
| *A. wakegi* 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 *et al*. (1995) |
| *A. wakegi* cv. Araki whole bulb tissue | 33 - 10 | 14 - 42 | Hung in plastic nets under an outdoor shelter in Japan | As above | As above | Yamazaki *et al*. (1999b) Yamazaki *et al*. (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 $m^{-1}$ 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 $ha^{-1}$ of sulphur and 300 kg $ha^{-1}$ 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 ($CaCl_2$) 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 % $CO_2$ and 5 % $O_2$; 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% $CO_2$ in $N_2$ (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 $NaBH_4$ solution, which is then neutralised with acetic

acid. The sample is incubated with fructanase 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 $g^{-1}$ 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 $ml^{-1}$) 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 $min^{-1}$. 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-$^{3}$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$^{-1}$ in phosphate buffered saline (PBS; 50 mM $NaH_2PO_4$ adjusted to pH 6.0 with 50 mM $Na_2HPO_4$, and 100 mM NaCl) containing 5 mg ml$^{-1}$ 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$^{-1}$ bovine serum albumin and 4 mg ml$^{-1}$ 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 $^{3}$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 $(NH_4)_2SO_4$ 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 $(NH_4)_2SO_4$ (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 $^{3}$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 $^{3}$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).

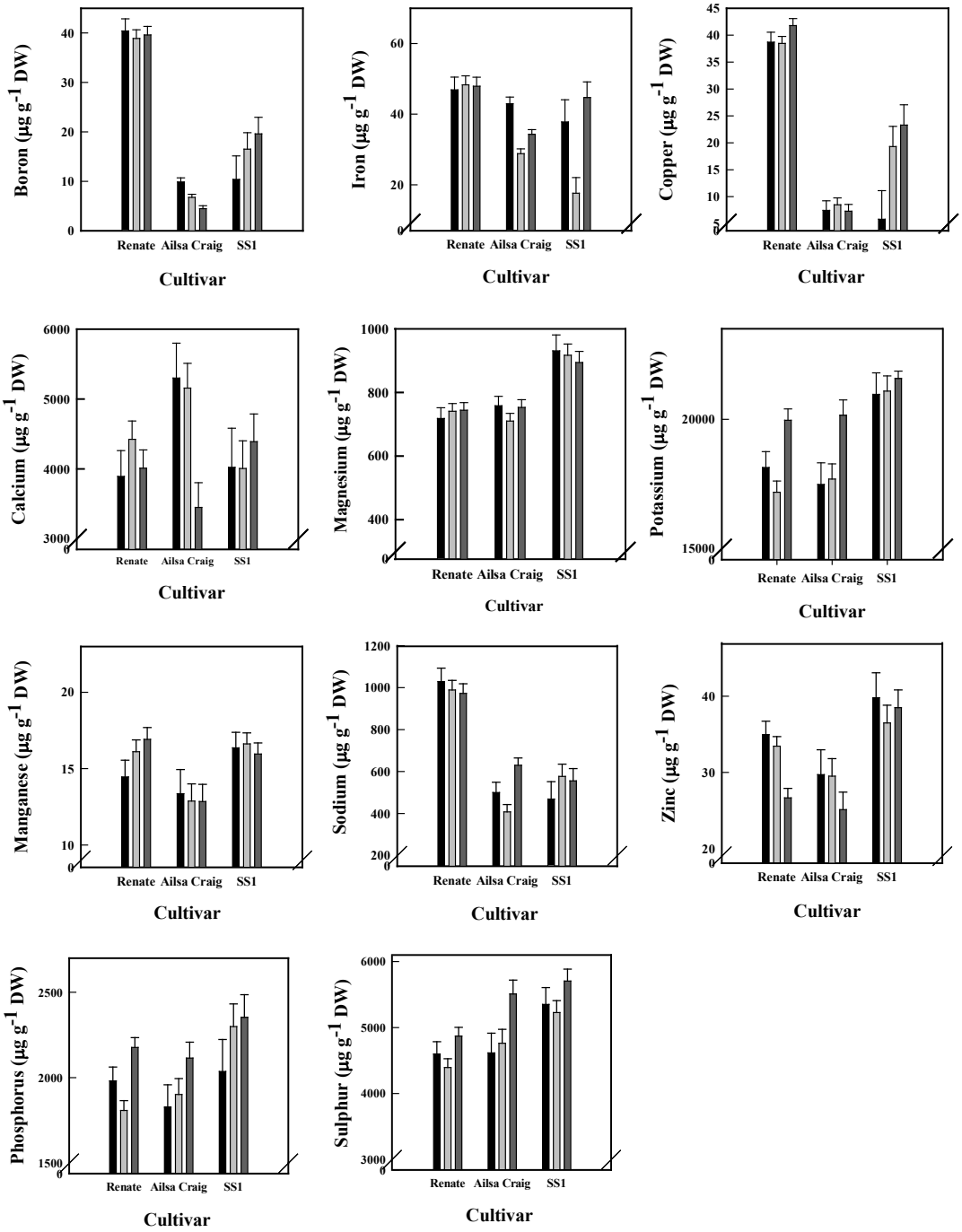


**Figure 3.1.** Elemental content of onion cvs. Renate, Ailsa Craig and SS1 bulbs shortly after harvest (day 0, black bars, n=4), and at early (light grey bars, n=8) and late (dark grey bars, n=8) time points during controlled atmosphere storage (cv. Renate: days 40 and 230, cv. Ailsa Craig: days 81 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[1] | Sweet onion[1] |
| | Concentration (μg $g^{-1}$ 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

[1]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.

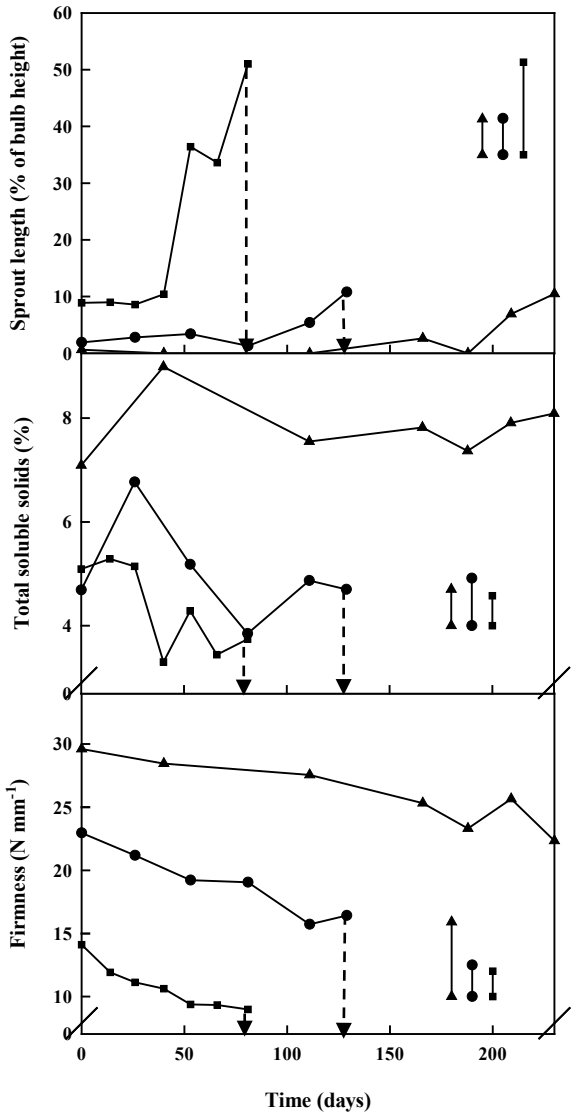


**Figure 3.2.** Changes in the physical parameters; sprout length (expressed as a percentage of bulb height), total soluble solids and firmness (N $mm^{-1}$), in bulbs of onion cvs. Renate (▲), Ailsa Craig (●) and SS1 (■) during controlled atmosphere storage. Drop down arrows indicate the final sample 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 ± 5.83 mg $g^{-1}$ FW, cv. Ailsa Craig: 100 ± 1.84 mg $g^{-1}$ FW and cv. SS1: 71 ± 1.72 mg $g^{-1}$ 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 (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. Alisa 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. Alisa Craig bulbs had a significantly ($P$=0.003) greater pyruvate concentration (6.73 µmol $g^{-1}$ FW) than bulbs of cv. SS1 (3.57 µmol $g^{-1}$ FW).

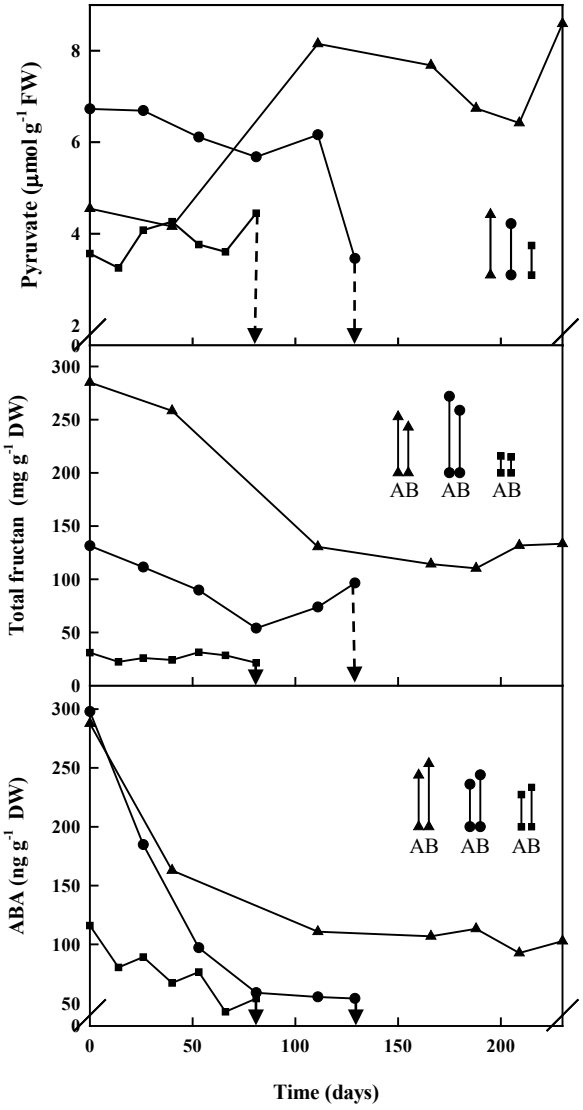


**Figure 3.3**. Changes in the biochemical parameters; pyruvate, total fructan, and ABA concentrations, in bulbs of onion cvs. Renate (▲), Ailsa Craig (●) and SS1 (■) during controlled atmosphere storage. Drop down arrows indicate the final sample taken. LSD bars (*P*=0.05) are shown, symbols correspond 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 ± 20.84 mg $g^{-1}$ DW), followed by cvs. Ailsa Craig (131.3 ± 28.11 mg $g^{-1}$ DW) and SS1 (31.0 ± 7.24 mg $g^{-1}$ 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 ng $g^{-1}$ DW) and cv. Renate (288 ng $g^{-1}$ DW) bulbs, were significantly greater (LSD (*P*=0.05) = 162.1) than that present in onion cv. SS1 bulbs (116 ng $g^{-1}$ 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 (± 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 $g^{-1}$ DW) | | ABA concentration (ng $g^{-1}$ FW)[1] | |
|---|---|---|---|---|
| | Maximum | Minimum | Maximum | Minimum |
| SS1 | 116.0[2] | 42.8[3] | 8.2[2] | 3.0[3] |
| | (13.25) | (9.37) | (4.8) | (1.79) |
| Ailsa Craig | 297.9[2] | 54.1[4] | 29.8[2] | 5.4[4] |
| | (17.23) | (12.18) | (12.8) | (2.3) |
| Renate | 287.7[2] | 92.7[5] | 42.3[2] | 13.6[5] |
| | (21.24) | (15.02) | (22.0) | (7.1) |

[1] Values calculated from mean DW for each cultivar; cv. Renate: 147 ± 5.83 mg $g^{-1}$ FW,
cv. Ailsa Craig: 100 ± 1.84 mg $g^{-1}$ FW and cv. SS1: 71 ± 1.72 mg $g^{-1}$ FW.
[2] Measured at day 0. [3] Measured at day 66. [4] Measured at day 129. [5] 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.

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

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

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

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 (± 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 ± 0.002 | | 0.97 ± 0.003 | 0.98 ± 0.004 | 0.98 ± 0.022 |
| B | 184.6[2] | 262.9[2] | 68.2[2] | 184.7 ± 7.94 | 263.9 ± 14.8 | 75.6 ± 39.5[4] |
| A | 102.0[2] | 39.1[2] | 45.2[2] | 102.9 ± 3.69 | 37.3 ± 12.1 | 35.9 ± 42.9[4] |
| Percentage variance[1] | | 98.0 | | 98.9 | 98.7 | 71.1 |
| Half life[3] (days) | | 26.2 ± 1.90 | | 24.8 ± 3.01 | 27.1 ± 4.13 | 35.0 ± 39.5[4] |

[1]Percentage variance = 100*(1-((residual mean square)/(total mean square))) = adjusted $R^2$ value expressed as a percentage.

[2]Standard error = 9.97.

[3]Half life = log (0.5) / log (R).

[4]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.

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

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 ± 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 $O_2$ (2%) and high $CO_2$ (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 study 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 % $O_2$, 2% $CO_2$ or 2% $O_2$, 8% $CO_2$) (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% $O_2$ than 1.0% $O_2$ (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 $CO_2$ concentration was 10-fold greater in the present study suggesting that the inhibitory effect of high $CO_2$ concentration on respiration was overcome by the high $O_2$ 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 $g^{-1}$ DW based on the individual regression, and 149 ng $g^{-1}$ 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 $g^{-1}$ to *ca*. 1 ng $g^{-1}$ 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 $g^{-1}$ FW to 13 ng $g^{-1}$ FW in the first two weeks after harvest, followed by a rapid decrease to 6 ng $g^{-1}$ FW over the next four weeks. Yamazaki *et al*. (1999b) and Yamazaki *et al*. (2002) reported a maximum concentration of *ca*. 33 ng $g^{-1}$ FW in *A. wakegi* cv. Kiharabansei No. 1 two weeks after harvest, which decreased to *ca*. 10 ng $g^{-1}$ 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 $g^{-1}$ DW and 65 ng $g^{-1}$ DW, respectively) than in bulbs of cv. Renate (*ca*. 115 ng $g^{-1}$ 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 $10^{-4}$ 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, Dinaro, 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 µM 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.

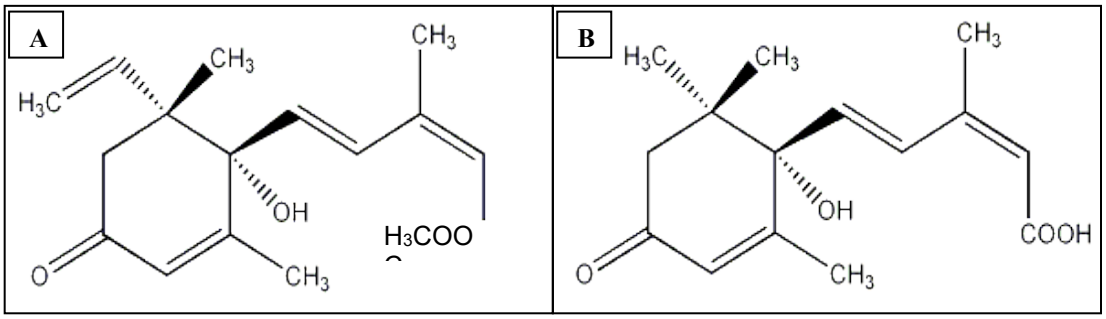


**Figure 4.1.** A. 8'-methylene abscisic acid methyl ester. B. (+)-abscisic acid.

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 study 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 $m^{-1}$ 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 $ha^{-1}$) | Plot area ($m^2$) | Total volume applied (l) |
|---|---|---|---|
| ABA[a] ($10^{-4}$ M) | 550 | 126 | 6.93 |
| PBI-365[a] ($10^{-4}$ M) | 314.2 | 126 | 3.95 |
| Li-700 (0.5% v/v) | 550 | 126 | 6.93 |
| Water | 550 | 126 | 6.93 |

[a] 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. (ºC) | Minimum temp. (ºC) | 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 (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 55ºC 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 -40ºC 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 $Ca^{+}$ 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-$Ca^{2+}$ 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 $min^{-1}$. 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 $g^{-1}$ FW) | | Total soluble solids (%) | | Pyruvate (µmol $g^{-1}$ FW) | | Firmness (N $mm^{-1}$) | | Abscisic acid (ng $g^{-1}$ DW) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Cultivar | Value | Cultivar | Value | Cultivar | Value | Cultivar | Value | Cultivar | Value |
| 1 | Red Baron | 140.1[a] | Red Baron | 10.21[a] | Dinaro | 6.41[a] | Renate | 39.64[a] | Renate | 215.28 (2.333[a])[1] |
| 2 | Carlos | 125.9[b] | Dinaro | 9.36[b] | Carlos | 6.36[a] | Dinaro | 36.74[ab] | Dinaro | 191.87 (2.283[a]) |
| 3 | Dinaro | 125.6[b] | Renate | 9.31[b] | Hysam | 5.32[b] | Red Baron | 36.51[ab] | Hysam | 178.24 (2.251[ab]) |
| 4 | Hysam | 125.1[b] | Carlos | 9.06[b] | Red Baron | 5.04[b] | Hysam | 36.19[ab] | Red Baron | 132.74 (2.123[bc]) |
| 5 | Renate | 118.7[c] | Hysam | 9.00[b] | Renate | 5.02[b] | Carlos | 33.73[b] | Carlos | 129.42 (2.112[c]) |
| 6 | SS1 | 73.0[d] | SS1 | 4.77[c] | SS1 | 3.68[c] | SS1 | 16.36[c] | SS1 | 110.41 (2.043[c]) |
| $LSD_{(P=0.05)}$ | | 9.45 | | 0.6067 | | 0.711 | | 6.154 | | 0.1284[2] |

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

[2] 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 (mg $g^{-1}$ DW) | | Sucrose (mg $g^{-1}$ DW) | | Fructose (mg $g^{-1}$ DW) | | Total sugars (F+G+S) (mg $g^{-1}$ DW) | | Fructan (mg $g^{-1}$ DW) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Cultivar | Value | Cultivar | Value | Cultivar | Value | Cultivar | Value | Cultivar | Value |
| 1 | SS1 | 286.1[a] | Renate | 169.0[a] | SS1 | 276.9[a] | SS1 | 643[a] | Red Baron | 340.3[a] |
| 2 | Renate | 175.8[b] | Red Baron | 153.4[a] | Renate | 65.3[b] | Renate | 410[b] | Renate | 220.7[b] |
| 3 | Red Baron | 159.4[b] | SS1 | 79.5[b] | Red Baron | 33.3[c] | Red Baron | 346[c] | SS1 | 19.8[c] |
| LSD ($P$=0.05) | | 21.85 | | 42.81 | | 18.84 | | 54.3 | | 35.11 |

[abc] 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 (ºC) | 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 |

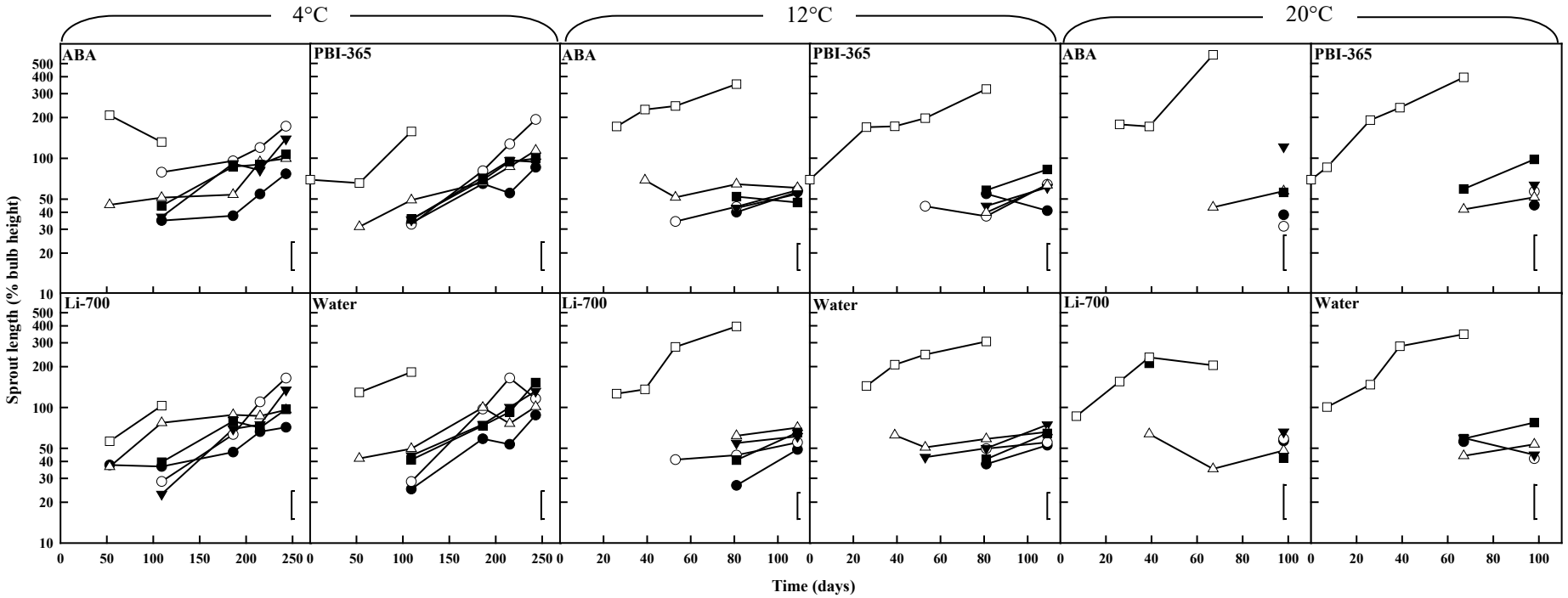


**Figure 4.2.** Sprout length of onion cvs. Carlos (●), Dinaro (○), Hysam (▼), Red Baron (▽), Renate (■) and SS1 (□) treated with a pre-harvest spray of $10^{-4}$ M ABA in 0.5% (v/v) Li-700, $10^{-4}$ M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 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.

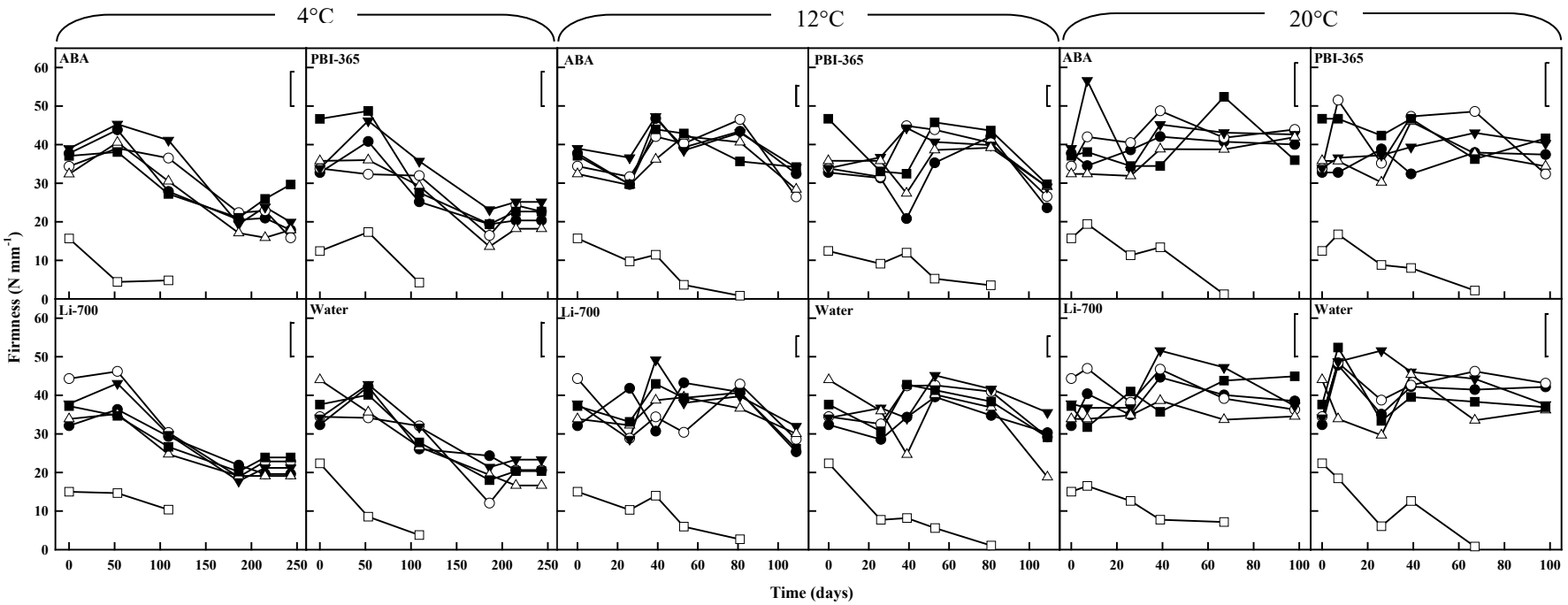


**Figure 4.3.** Firmness (N mm$^{-1}$) of onion cvs. Carlos (●), Dinaro (○), Hysam (▼), Red Baron (▽), Renate (■) and SS1 (□) treated with a pre-harvest spray of $10^{-4}$ M ABA in 0.5% (v/v) Li-700, $10^{-4}$ M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 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.

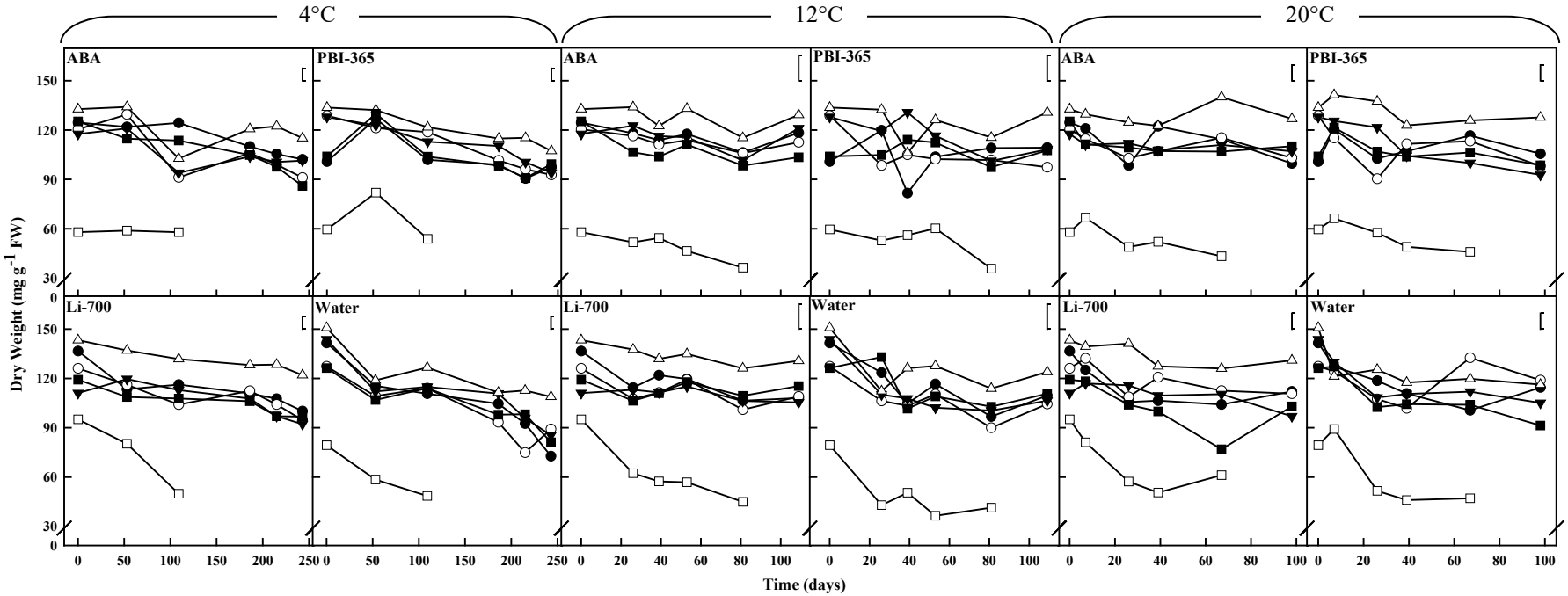


**Figure 4.4.** Dry weight of onion cvs. Carlos (●), Dinaro (○), Hysam (▼), Red Baron (∆), Renate (■) and SS1 (□) treated with a pre-harvest spray of $10^{-4}$ M ABA in 0.5% (v/v) Li-700, $10^{-4}$ M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 109 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.

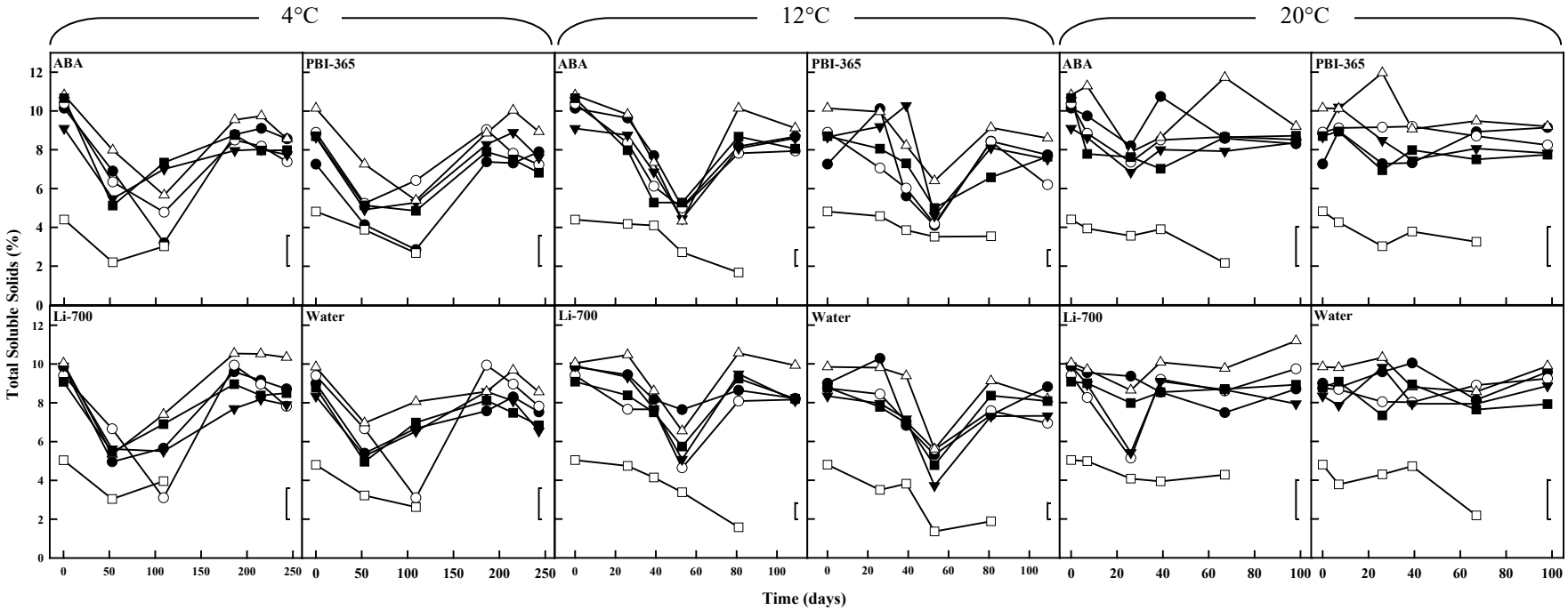


**Figure 4.5.** Total soluble solids concentration of onion cvs. Carlos (●), Dinaro (○), Hysam (▼), Red Baron (Δ), Renate (■) and SS1 (□) treated with a pre-harvest spray of $10^{-4}$ M ABA in 0.5% (v/v) Li-700, $10^{-4}$ M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for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

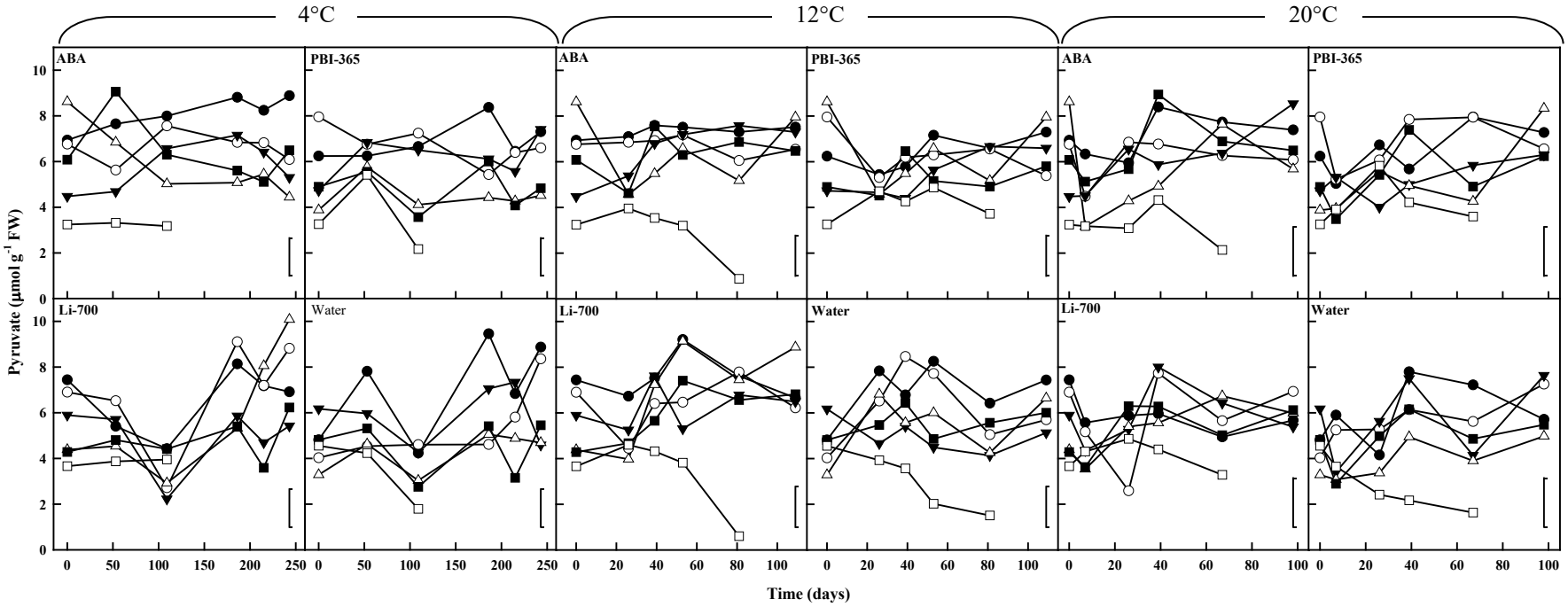


**Figure 4.6.** Pyruvate concentration of onion cvs. Carlos (●), Dinaro (○), Hysam (▼), Red Baron (Δ), Renate (■) and SS1 (□) treated with a pre-harvest spray of $10^{-4}$ M ABA in 0.5% (v/v) Li-700, $10^{-4}$ M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 109 days or 20°C for 98 days (n=5). LSD bars (*P*=0.05) are shown based on 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 4ºC decreased during storage (Figure 4.7). Fructose concentration was stable during storage at 12ºC 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 20ºC. 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 4ºC 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 $g^{-1}$ DW) and a decrease in onions cv. SS1 (from 220.3 to 209.4 mg $g^{-1}$ DW). When cvs. Renate and Red Baron were stored at 12ºC, 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 12ºC in cv. SS1. Sucrose concentration tended to increase ($P$<0.001) during storage at 20ºC 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 4ºC 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 12ºC, but decreased 2.4-fold in cv. SS1. Glucose concentration was relatively stable during storage at 20ºC 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.

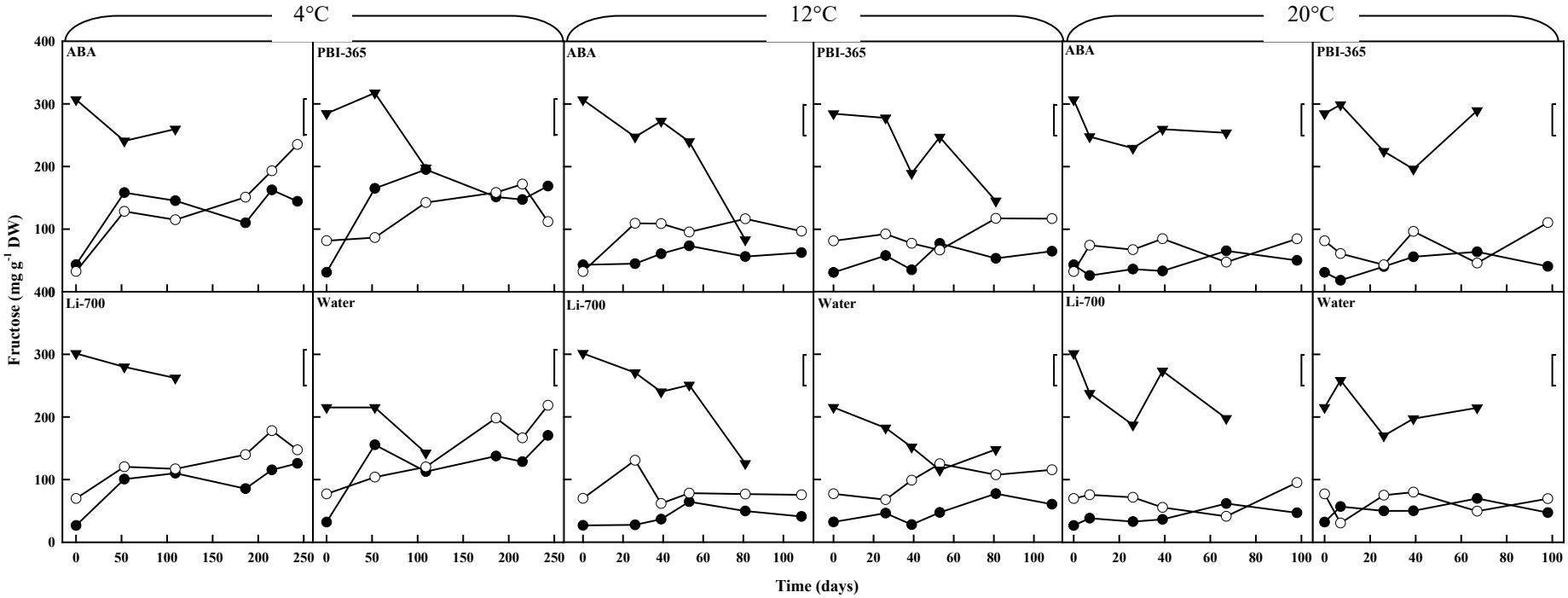


**Figure 4.7.** Fructose concentration of onion cvs. Red Baron (●), Renate (○) and SS1 (▼) treated with a pre-harvest spray of $10^{-4}$ M ABA in 0.5% (v/v) Li-700, $10^{-4}$ M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 109 days or 20°C for 98 days (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.

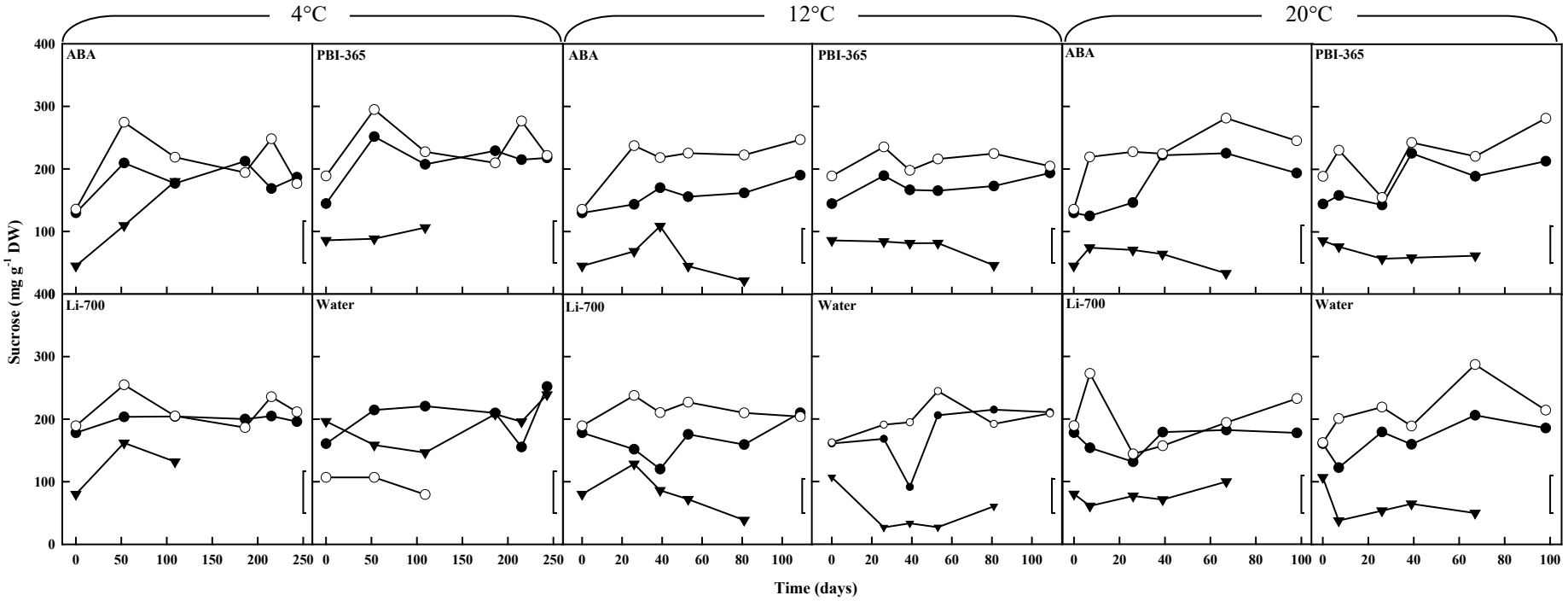


**Figure 4.8.** Sucrose concentration of onion cvs. Red Baron (●), Renate (○) and SS1 (▼) treated with a pre-harvest spray of $10^{-4}$ M ABA in 0.5% (v/v) Li-700, $10^{-4}$ M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 109 days or 20°C for 98 days (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.

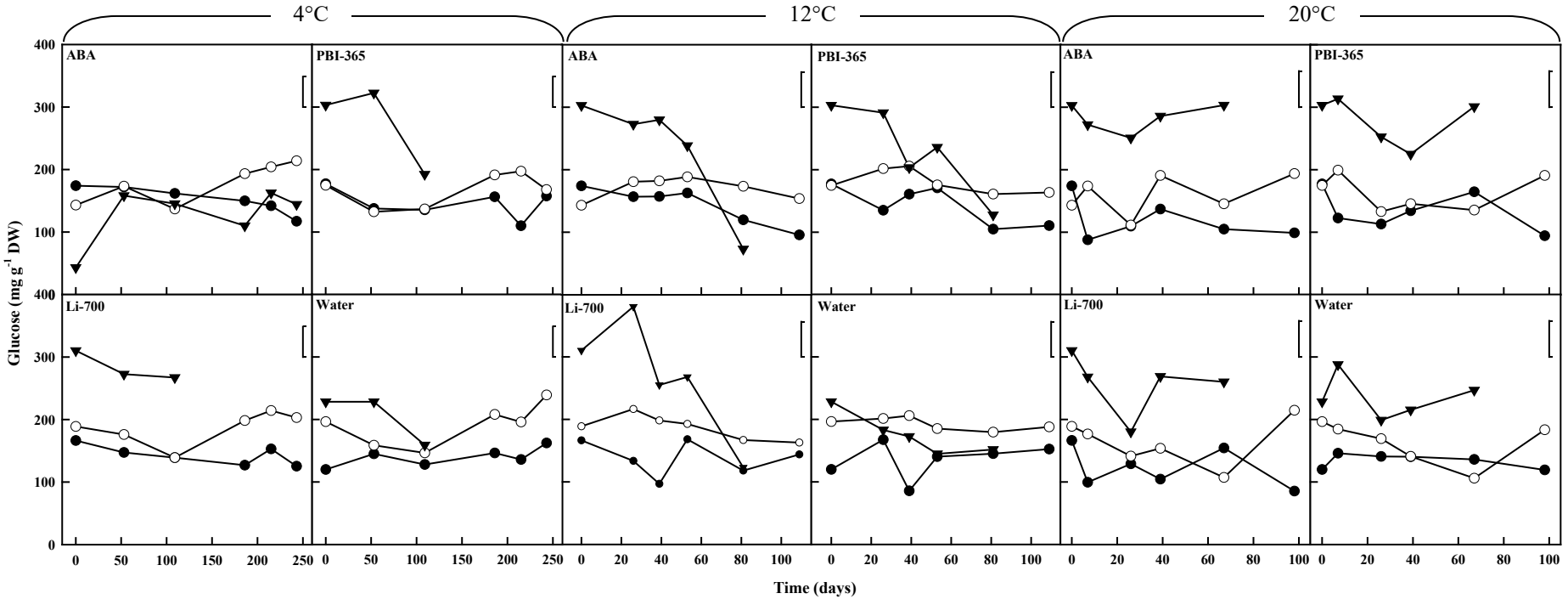


**Figure 4.9.** Glucose concentration of onion cvs. Red Baron (●), Renate (○) and SS1 (▼) treated with a pre-harvest spray of $10^{-4}$ M ABA in 0.5% (v/v) Li-700, $10^{-4}$ M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 109 days or 20°C for 98 days (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 12ºC. There was little change in total sugar concentration in cv. Red Baron during storage at 20ºC, 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 (4ºC and 20ºC no correlation; 12ºC -0.440 $P$>0.001) and Red Baron (4ºC -0.488 $P$>0.001; 12ºC -0.330 $P$=0.005; 20ºC -0.453 $P$>0.001), and positive for cv. SS1 (4ºC and 20ºC no correlation; 12ºC 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 20ºC; however the ratio decreased in cv SS1 during storage at 4ºC, and changed erratically during storage 12 and 20ºC. In general there was a higher ratio of monosaccharide to disaccharide in onions cv. SS1 than cvs. Renate or Red Baron (Figure 4.11).

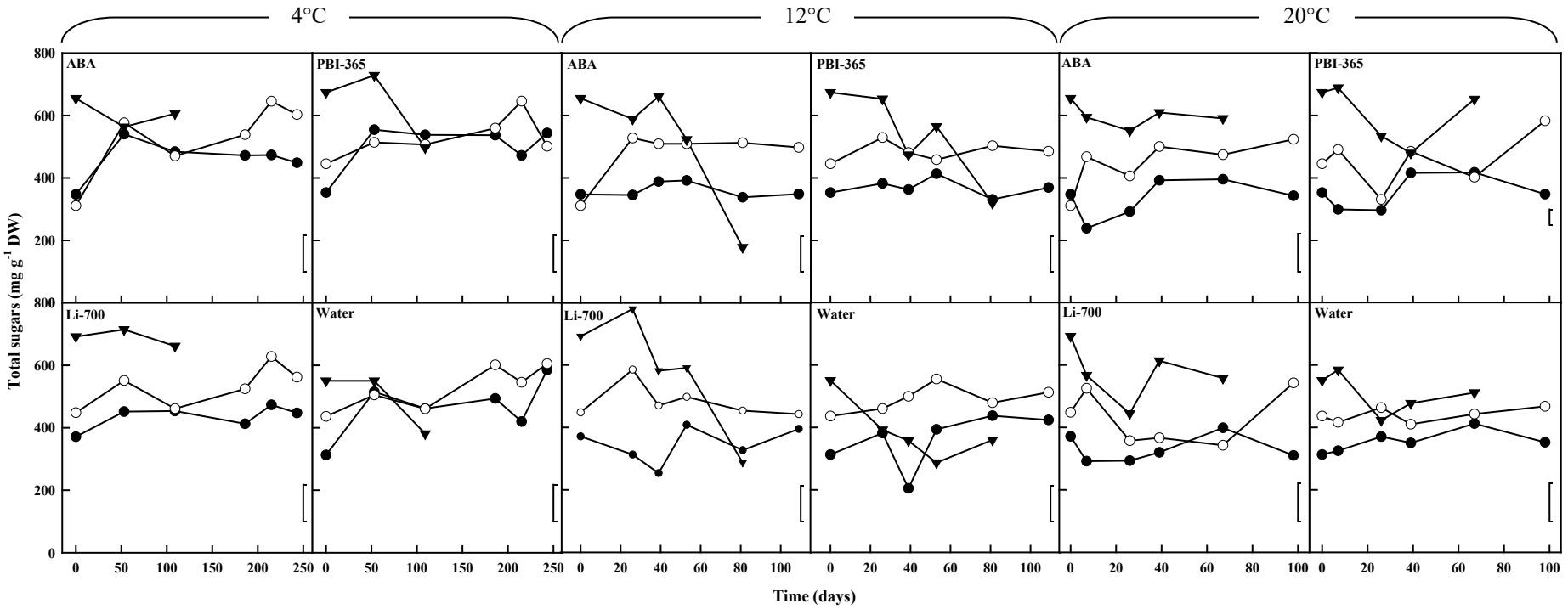


**Figure 4.10.** Total sugars (glucose + sucrose + fructose) concentration of onion cvs. Red Baron (●), Renate (○) and SS1 (▼) treated with a pre-harvest spray of $10^{-4}$ M ABA in 0.5% (v/v) Li-700, $10^{-4}$ M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 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.

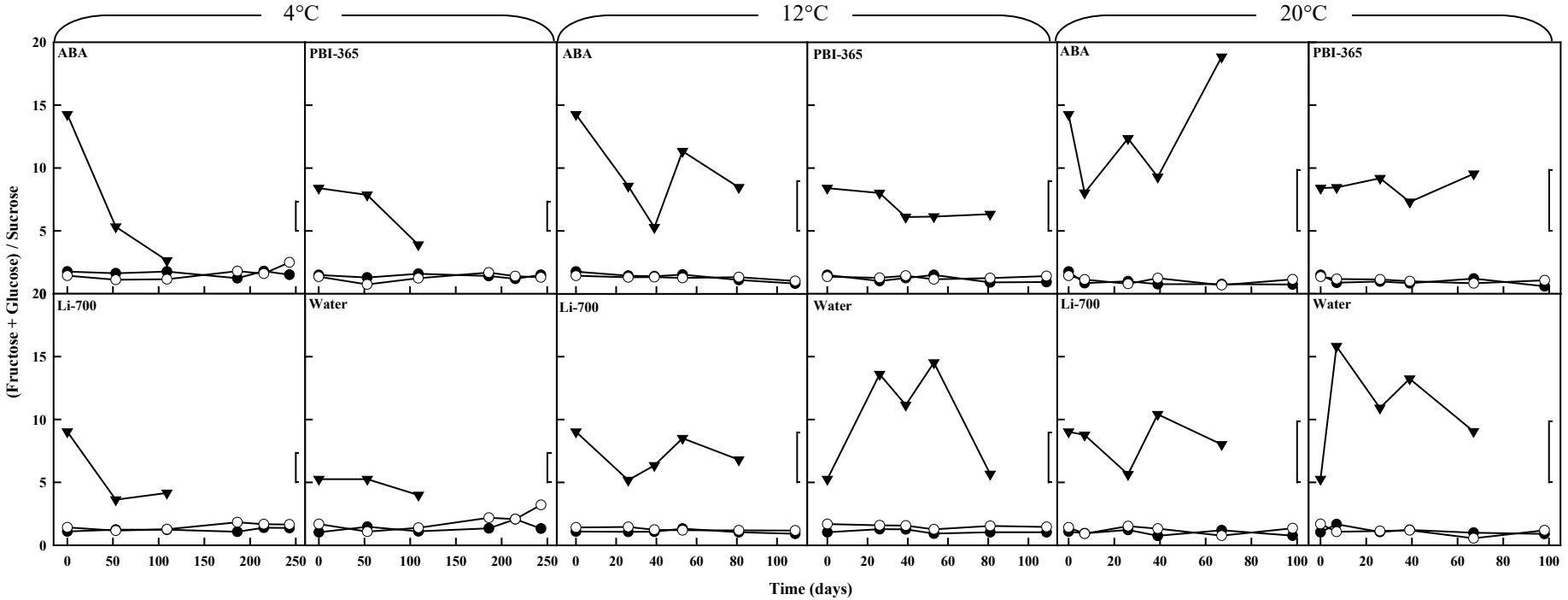


**Figure 4.11.** Ratio of monosaccharide : disaccharide ((fructose+glucose)/sucrose) of onion cvs. Red Baron (●), Renate (○) and SS1 (▼) treated with a pre-harvest spray of $10^{-4}$ M ABA in 0.5% (v/v) Li-700, $10^{-4}$ M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 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 4ºC, 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 12ºC, 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 20ºC, whilst the fructan concentration in cv. SS1 increased.

Fructan concentration was strongly negatively correlated with fructose (4ºC -0.805; 12ºC -0.812; 20ºC -0.818; $P$<0.001) and glucose (4ºC -0.591; 12ºC -0.503; 20ºC -0.705; $P$<0.001) concentration and positively correlated with sucrose concentration (4ºC 0.331, $P$=0.002; 12ºC 0.565, $P$<0.001; 20ºC 0.279, $P$<0.001). Fructose concentration was positively correlated with glucose (4ºC 0.795, 12ºC 0.790, 20ºC 0.856; $P$<0.001) and negatively correlated with sucrose (4ºC -0.443, 12ºC -0.465, 20ºC -0.511; $P$<0.001), and there was a negative correlation between sucrose and glucose (4ºC -0.569, 12ºC -0.183, 20ºC -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 (4ºC 0.232 $P$=0.049; 12ºC no correlation; 20ºC 0.253 $P$=0.033) and Red Baron (4ºC and 12ºC no correlation; 20ºC 0.414 $P$<0.001) and negative in cv. SS1 (4ºC -0.439 $P$=0.007; 12ºC and 20ºC no correlation).

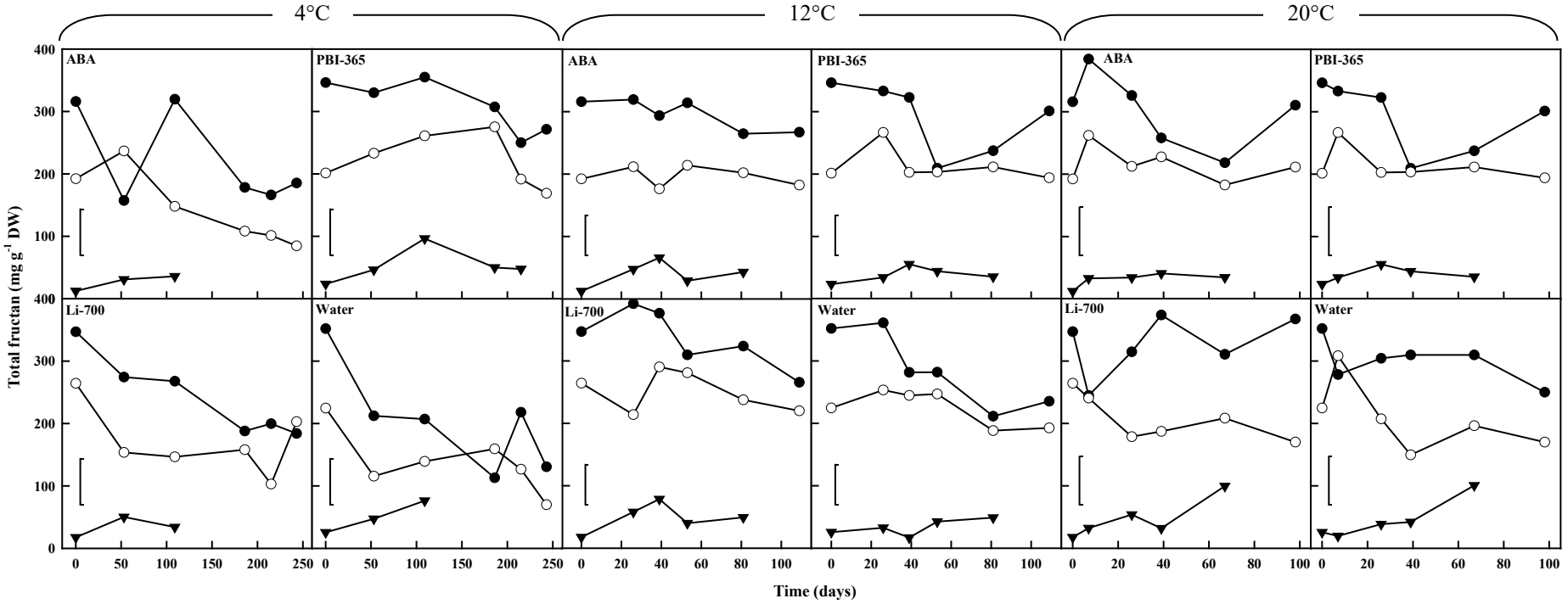


**Figure 4.12.** Total fructan concentration of onion cvs. Red Baron (●), Renate (○) and SS1 (▼) treated with a pre-harvest spray of $10^{-4}$ M ABA in 0.5% (v/v) Li-700, $10^{-4}$ M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 days, 12°C for 109 days or 20°C for 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 4ºC (-0.193; $P$<0.001), 12ºC (-0.164; $P$<0.001) and 20ºC (-0.088; $P$=0.021).

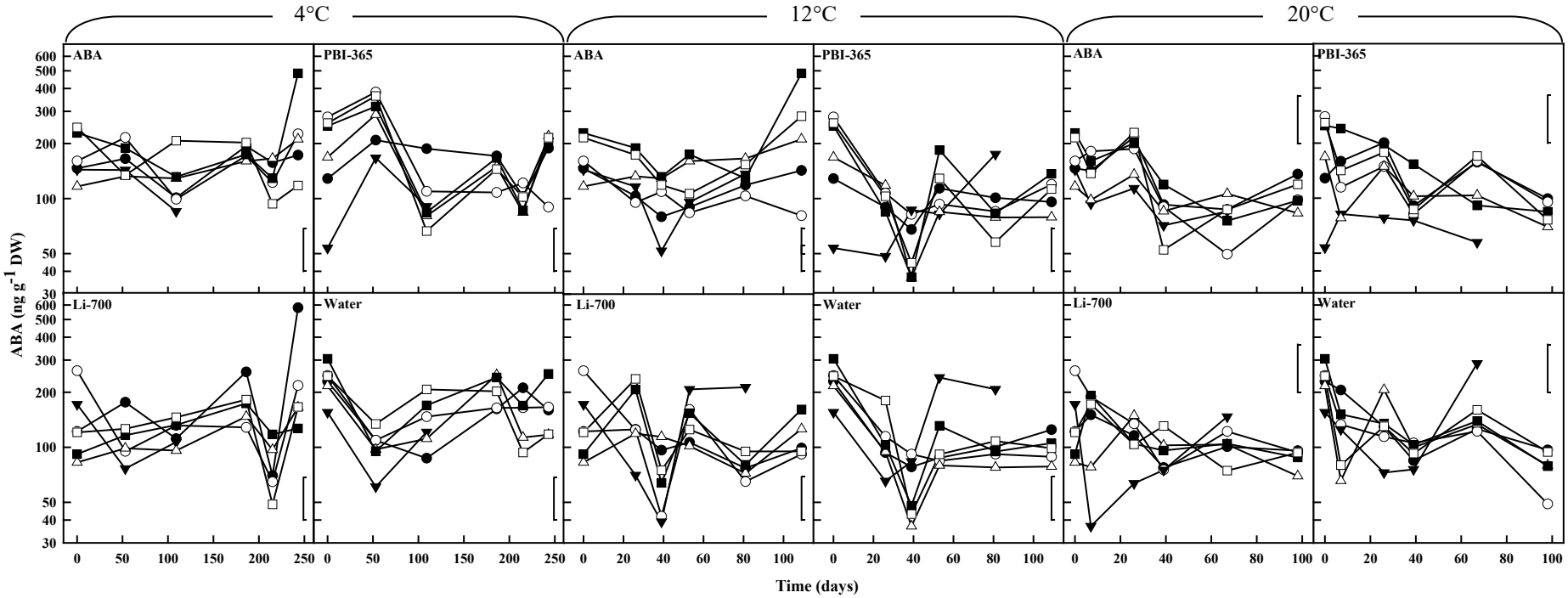


**Figure 4.13.** Abscisic acid concentration of onion cvs. Carlos (●), Dinaro (○), Hysam (▼), Red Baron (Δ), Renate (■) and SS1 (□) treated with a pre-harvest spray of $10^{-4}$ M ABA in 0.5% (v/v) Li-700, $10^{-4}$ M PBI-365 in 0.5% (v/v) Li-700, 0.5% (v/v) Li-700 or water and stored at 4°C for 243 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 4ºC and sprouting began between day 53 and day 186 (Figure 4.14). Sprout length increased during storage at 12ºC ($P$<0.001) and 20ºC ($P$=0.008).

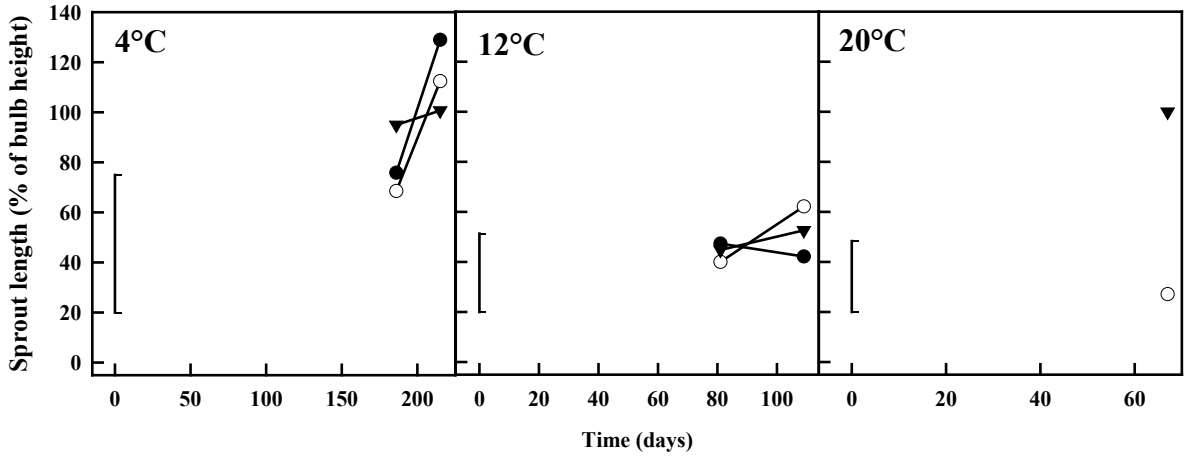


**Figure 4.14.** Sprout growth of onion cv. Hysam bulbs treated with a postharvest soak in $10^{-4}$ M ABA in 0.5% (v/v) Li-700 (●), $10^{-4}$ M PBI-365 in 0.5% (v/v) Li-700 (▼) or water (○) and stored at 4°C for 215 days, 12°C for 109 days or 20°C for 67 days (n=3). LSD bars (*P*=0.05) are shown based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

*4.4.2.2 Firmness and dry weight*

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

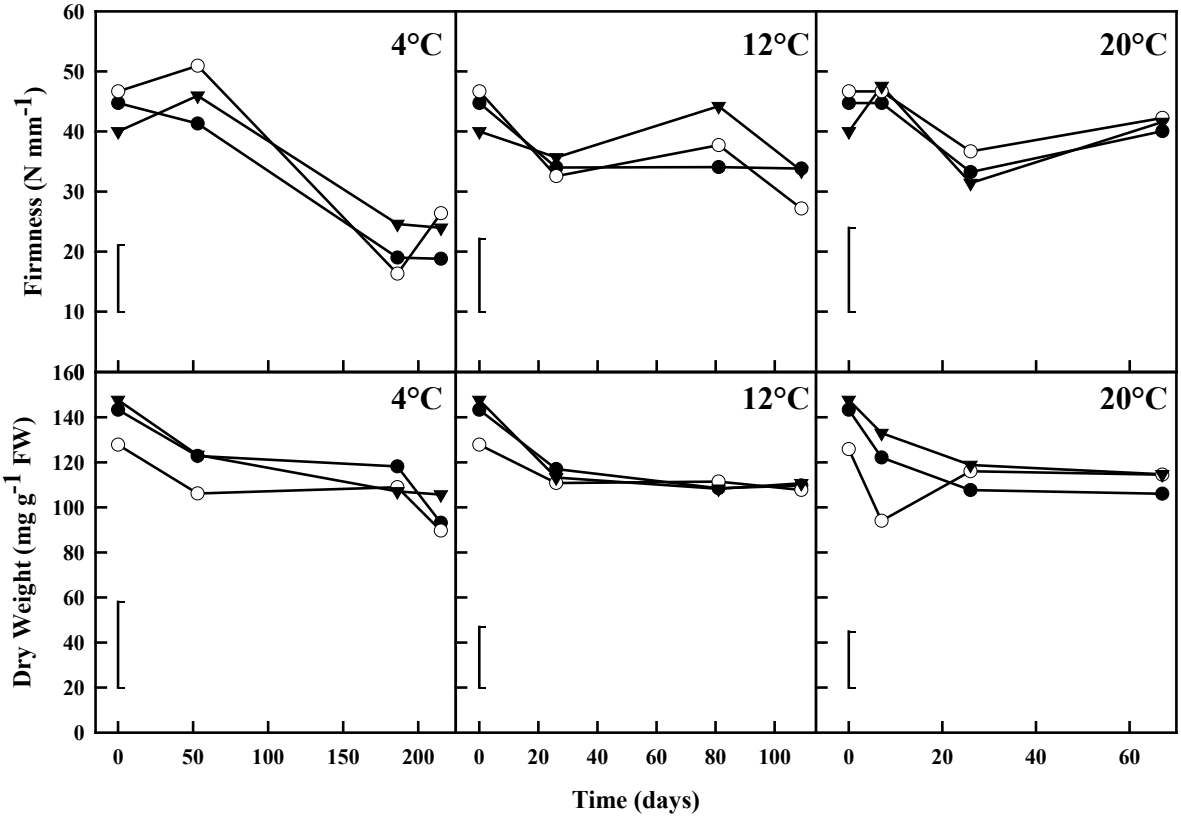


**Figure 4.15.** Firmness and dry weight of onion cv. Hysam bulbs treated with a postharvest soak in $10^{-4}$ M ABA in 0.5% (v/v) Li-700 (●), $10^{-4}$ M PBI-365 in 0.5% (v/v) Li-700 (▼) or water (○) and stored at 4°C for 215 days, 12°C for 109 days or 20°C for 67 days (n=3). LSD bars (*P*=0.05) are 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 4ºC or 20ºC did not change during storage or with treatment (Figure 4.16), while TSS changed during storage at 12ºC with a significant (*P*<0.001) decrease occurring between days 26 and 81.

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

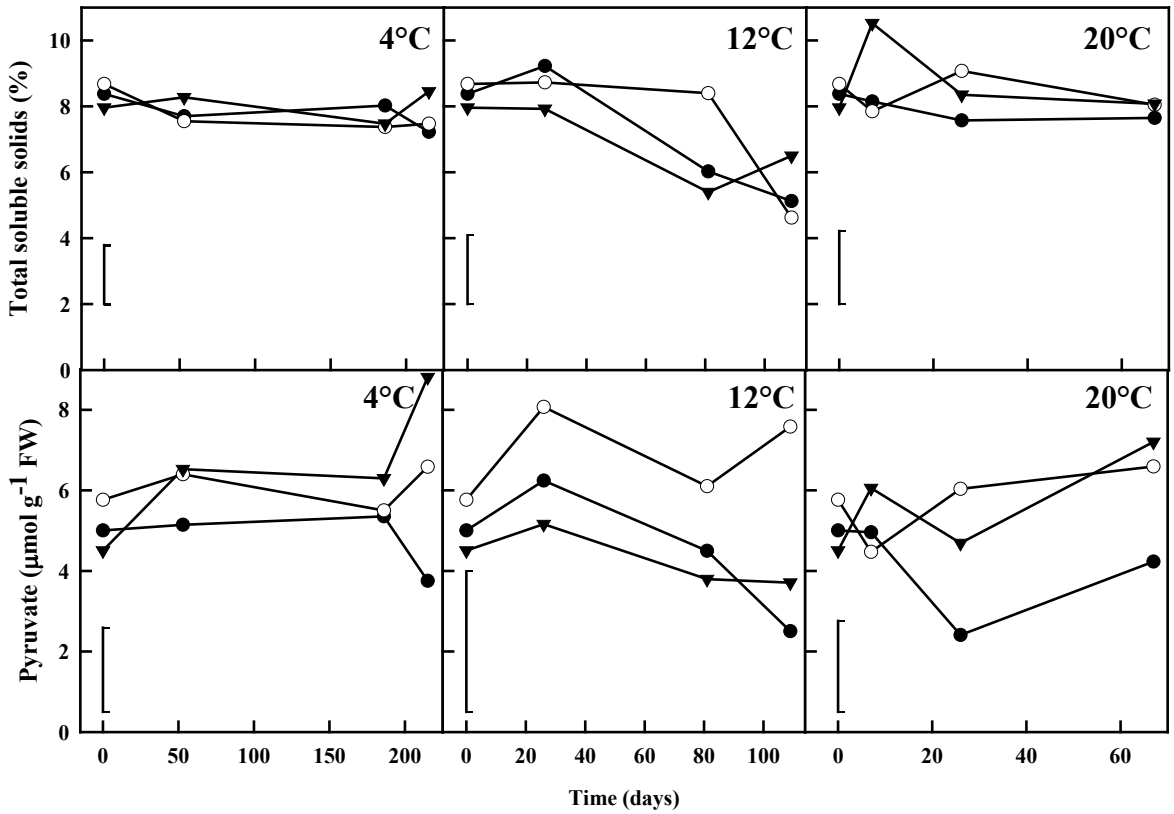


**Figure 4.16.** Total soluble solids and pyruvate concentration of onion cv. Hysam bulbs treated with a postharvest soak in $10^{-4}$ M ABA in 0.5% (v/v) Li-700 (●), $10^{-4}$ M PBI-365 in 0.5% (v/v) Li-700 (▼) or water (○) and stored at 4°C for 215 days, 12°C for 109 days or 20°C for 67 days (n=3). 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 4ºC (Figure 4.17) and was negatively correlated with sprout length (-0.362; *P*=0.009). Similarly, ABA decreased during storage at 12ºC (*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 20ºC (*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 20ºC.

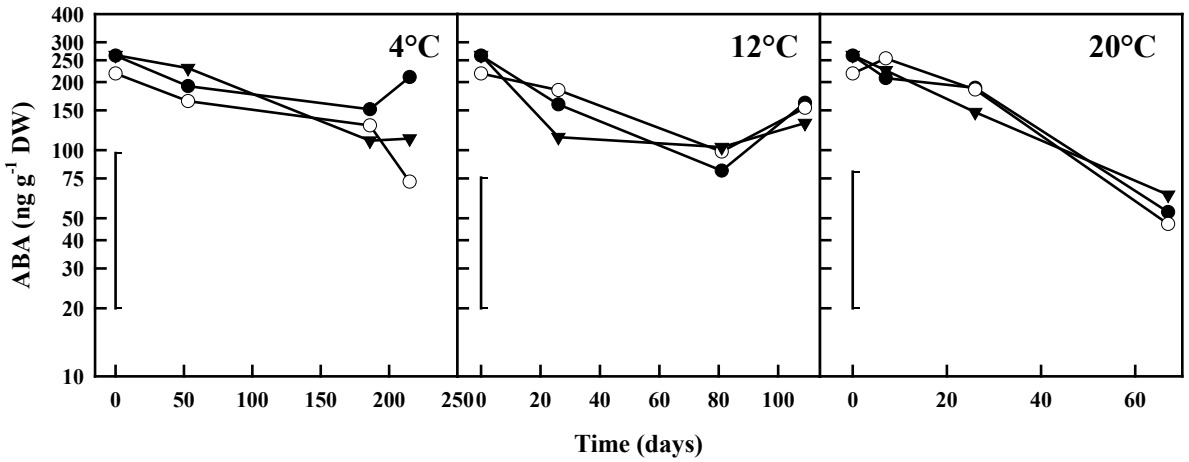


**Figure 4.17.** ABA concentration of onion cv. Hysam bulbs treated with a postharvest soak in $10^{-4}$ M ABA in 0.5% (v/v) Li-700 (●), $10^{-4}$ M PBI-365 in 0.5% (v/v) Li-700 (▼) or water (○) and stored at 4°C for 215 days, 12°C for 109 days or 20°C for 67 days (n=3). LSD bars (*P*=0.05) are shown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

## 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 (4ºC, 12ºC and 20ºC). 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 μmol $g^{-1}$ 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°C, but there was no net change in those stored at 4°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 12ºC. 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 4ºC and in cv. Red Baron stored at 20ºC. 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 4ºC 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 4ºC and least at 20ºC.

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 4ºC, 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 12ºC and 20ºC. 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 18ºC (Benkeblia and Selselet-Attou, 1999b) and this was also observed in onions cv. Renate during

storage at 4 and 12ºC, 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 µM 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 µM 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 µl $l^{-1}$ 1-methylcyclopropene (1-MCP) for 24 hr 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 $kg^{-1}$ $hr^{-1}$) of onion cv. Rouge Amposta bulbs during six months storage at 18°C and 70% RH. The dichotomy between these findings implies that production of ethylene by onion bulbs is likely to be cultivar dependent and that further investigation is required.

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 $m^{-1}$ 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 50ºC 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 µl $l^{-1}$ 1-MCP within the chamber. The chamber was kept sealed for 24 hr at 20ºC.

### *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 100ºC and 250ºC, 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 ml $min^{-1}$. Calibration of 1-MCP was carried out against 10.7 µl $l^{-1}$ isobutane (BOC) (Sisler and Serek, 1997). The concentration of 1-MCP was 0.962 µl $l^{-1}$ 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 20ºC. Bulbs were removed for sampling before 1-MCP treatment (baseline (day 0), n=5). Samples were taken after 53 and 109 days from the 4ºC storage treatment, after 26, 39 and 53 days from the 12ºC storage treatment and after 7, 26 and 39 days from the 20ºC 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 (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 4ºC ($P$=0.07), 12ºC ($P$<0.001) and 20ºC ($P$=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 12ºC. Similarly, in onions stored at 4ºC, 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 20ºC, 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).

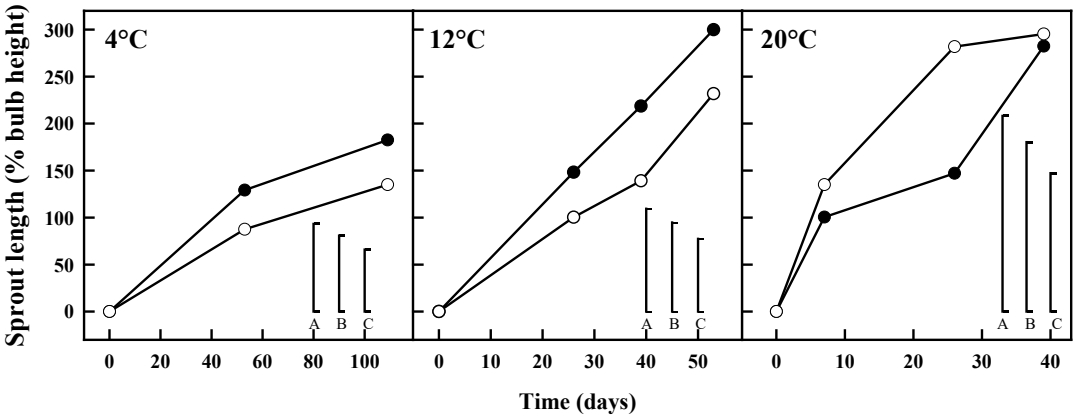


**Figure 5.1.** Sprout length in onions treated with 1 µl $l^{-1}$ 1-MCP at 20ºC for 24 hours (open symbols) and untreated onions (controls, closed symbols) stored at 4ºC for 109 days, 12ºC for 53 days or 20ºC for 39 days. Controls n=5, 1-MCP n=10. LSD bars (*P*=0.05) A=5,5; B=10,5; C=10,10.

*5.4.1.2 Firmness and dry weight*

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

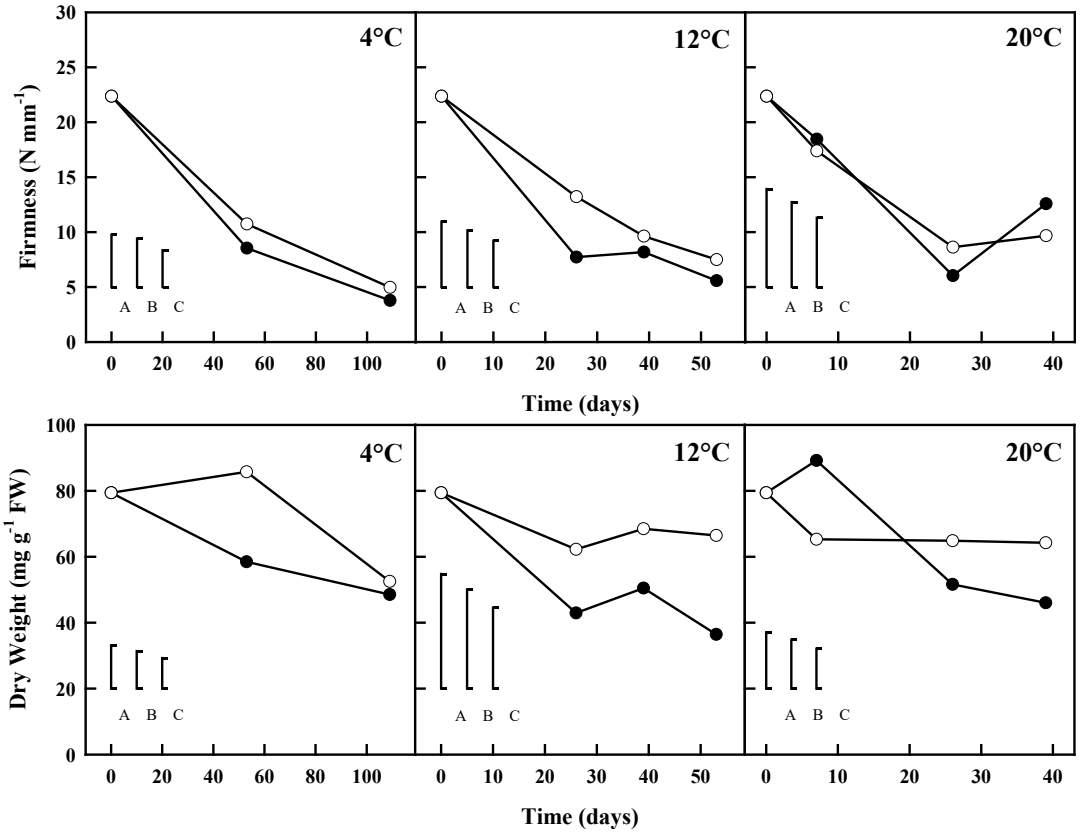


**Figure 5.2.** Firmness and dry weight of onions treated with 1 µl $l^{-1}$ 1-MCP at 20ºC for 24 hours (open symbols) and untreated onions (controls, closed symbols) stored at 4ºC for 109 days, 12ºC for 53 days or 20ºC for 39 days. Controls n=5, 1-MCP n=10. LSD bars (*P*=0.05) A=5,5; B=10,5; C=10,10.

The dry weight of bulbs stored at 4ºC decreased during storage (*P*=0.042), with those bulbs treated with 1-MCP having a greater mean dry weight (71.5 mg $g^{-1}$ FW) than untreated bulbs (53.5 mg $g^{-1}$ 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 12ºC 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 mg $g^{-1}$ FW) was greater (*P*<0.001) than that of untreated bulbs (43.3 mg $g^{-1}$ FW). There was a significant (*P*=0.007) decrease in dry weight during storage at 20ºC, 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 4ºC ($P$=0.002) and 12ºC ($P$<0.001), and the overall mean was higher (4ºC $P$=0.01; 12ºC P<0.001) in 1-MCP-treated bulbs than untreated bulbs (Fiure 5.3). In bulbs stored at 4ºC 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 20ºC.

The pungency (as determined by pyruvate concentration) of onion bulbs stored at 4ºC 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 20ºC, and mean pyruvate concentration was greater ($P$<0.05) in treated than untreated bulbs.

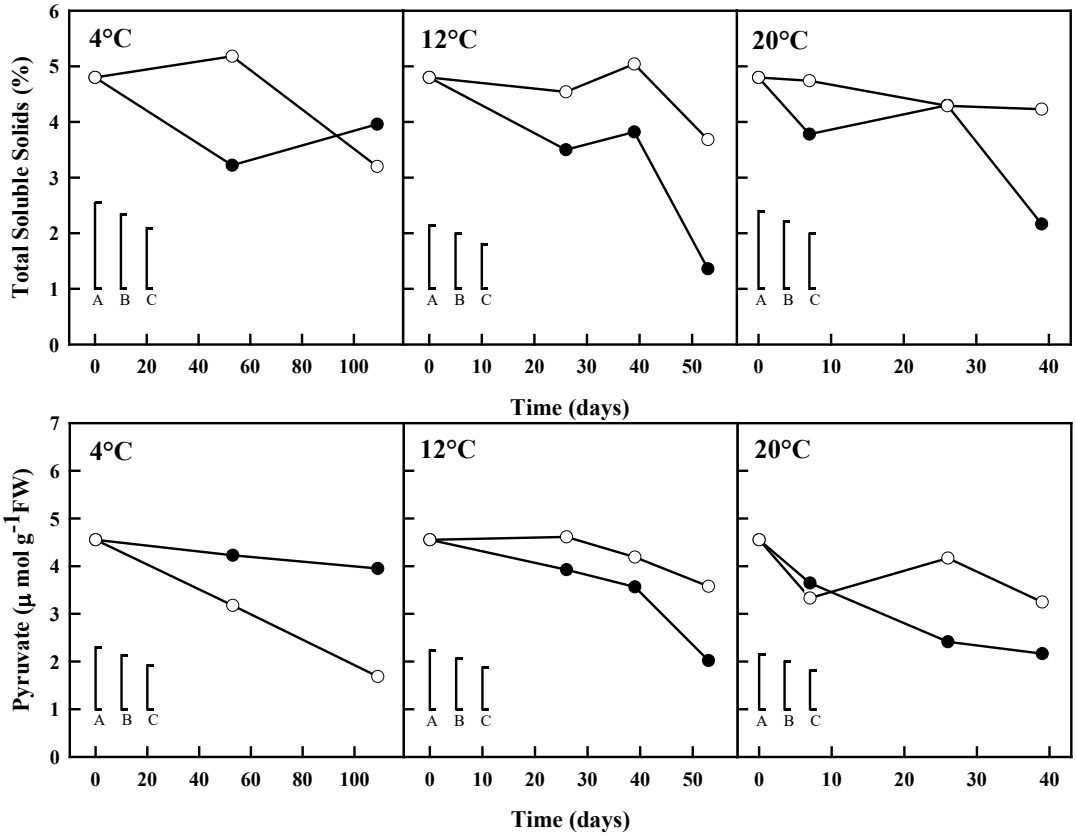


**Figure 5.3.** Total soluble solids (%) and pyruvate concentration of onions treated with 1 µl $l^{-1}$ 1-MCP at 20ºC for 24 hours (open symbols) and untreated onions (controls, closed symbols) stored at 4ºC for 109 days, 12ºC for 53 days or 20ºC for 39 days. Controls n=5, 1-MCP n=10. LSD bars (*P*=0.05)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

### *5.4.2.2 Abscisic acid*

In the onions stored at both 4ºC and 12ºC, 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 20ºC and treated with 1-MCP had a higher (*P*=0.008) overall mean ABA concentration (166 ng $g^{-1}$ DW) than untreated bulbs (91 ng

$g^{-1}$ DW). After day 7, the ABA concentration in 1-MCP-treated bulbs increased, whilst in untreated controls it continued to decline.

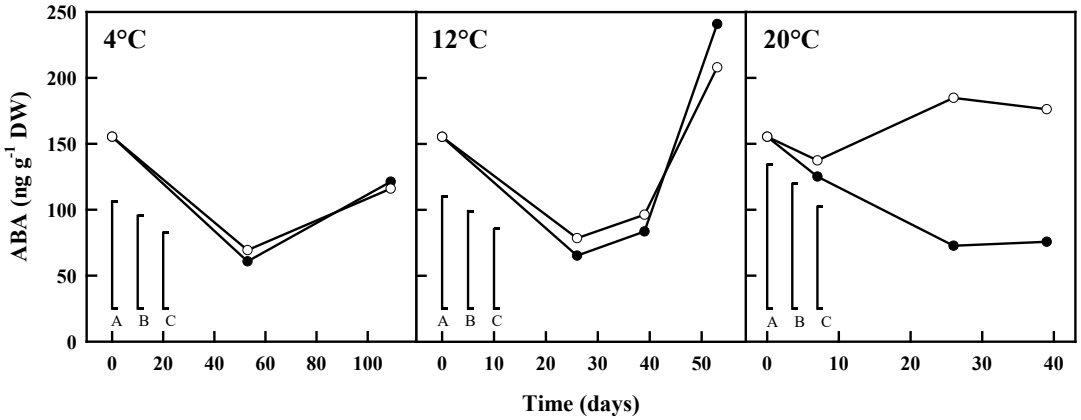


**Figure 5.4.** ABA concentration in onions treated with 1 µl $l^{-1}$ 1-MCP at 20ºC for 24 hours (open symbols) and untreated onions (controls, closed symbols) stored at 4ºC for 109 days, 12ºC for 53 days or 20ºC for 39 days. Controls n=5, 1-MCP n=10. LSD bars (*P*=0.05) A=5,5; B=10,5; C=10,10.

*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 12ºC (*P*<0.001) and all bulbs stored at 20ºC (*P*=0.011). The only effect of treatment with 1-MCP was observed in onions stored at 12ºC, 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 4ºC (*P*<0.001), 12ºC (*P*=0.088) and 20ºC (*P*<0.001). Glucose concentration was greater (*P*<0.001) in 1-MCP-treated onions stored at 12ºC compared with untreated bulbs. Fructose concentration decreased significantly during storage at all three temperatures

(4ºC P<0.001; 12ºC P=0.061; 20ºC P<0.001). There were no main effects or interactions between 1-MCP treatment and time.

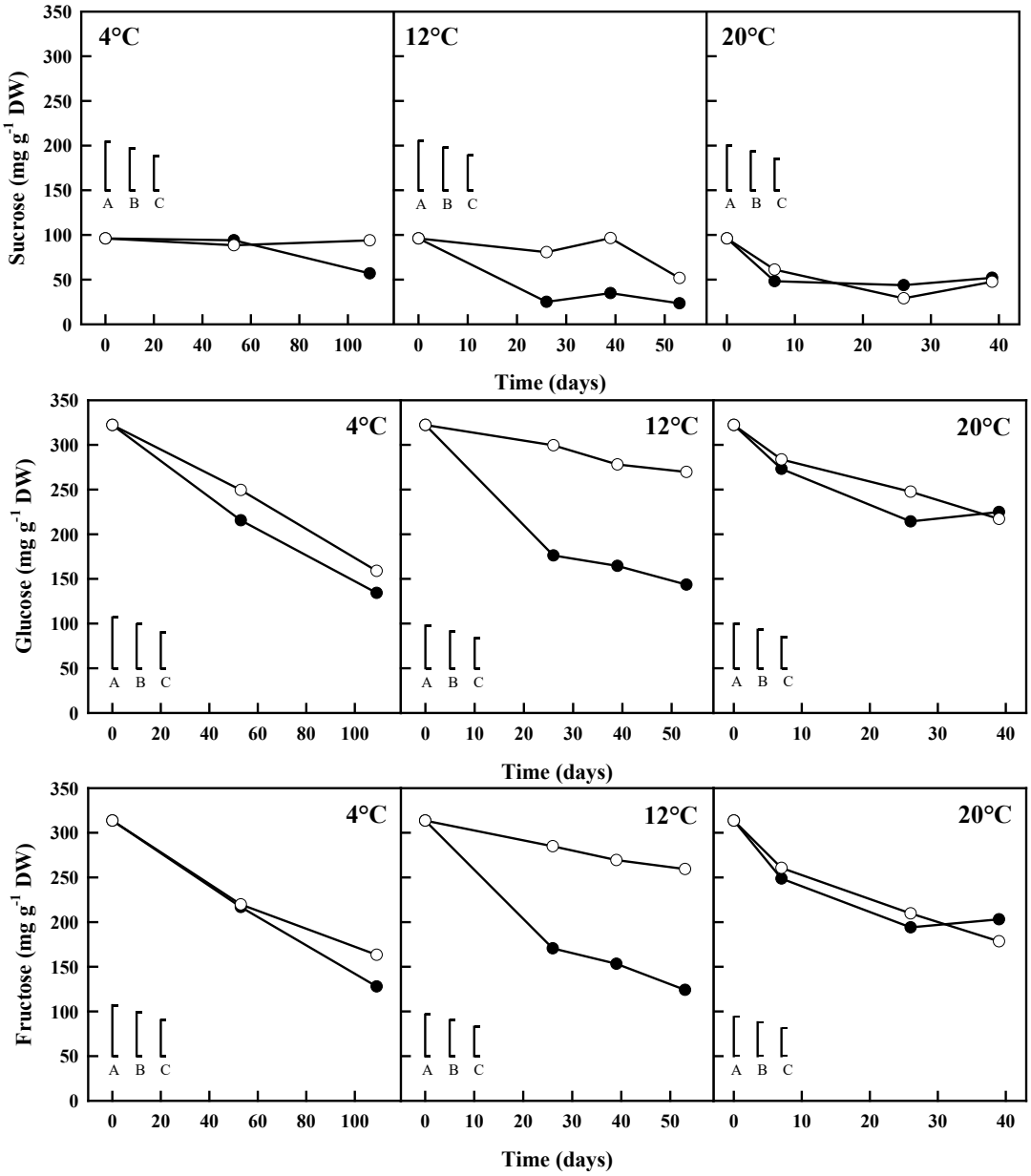


**Figure 5.5.** Sucrose, glucose and fructose concentrations in onions treated with 1 µl $l^{-1}$ 1-MCP at 20ºC for 24 hours (open symbols) and untreated onions (controls, closed symbols) stored at 4ºC for 109 days, 12ºC for 53 days or 20ºC for 39 days. Controls n=5, 1-MCP n=10. LSD bars (*P*=0.05) A=5,5; 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 20ºC the ratio was not affected by either treatment with 1-MCP or storage time, however in onions held at 12ºC 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 $g^{-1}$ DW in untreated bulbs and from 8.0 to 31.3 mg $g^{-1}$ 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 (4ºC $P$<0.001; 12ºC $P$=0.012; 20ºC $P$<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 12ºC 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 4ºC 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).

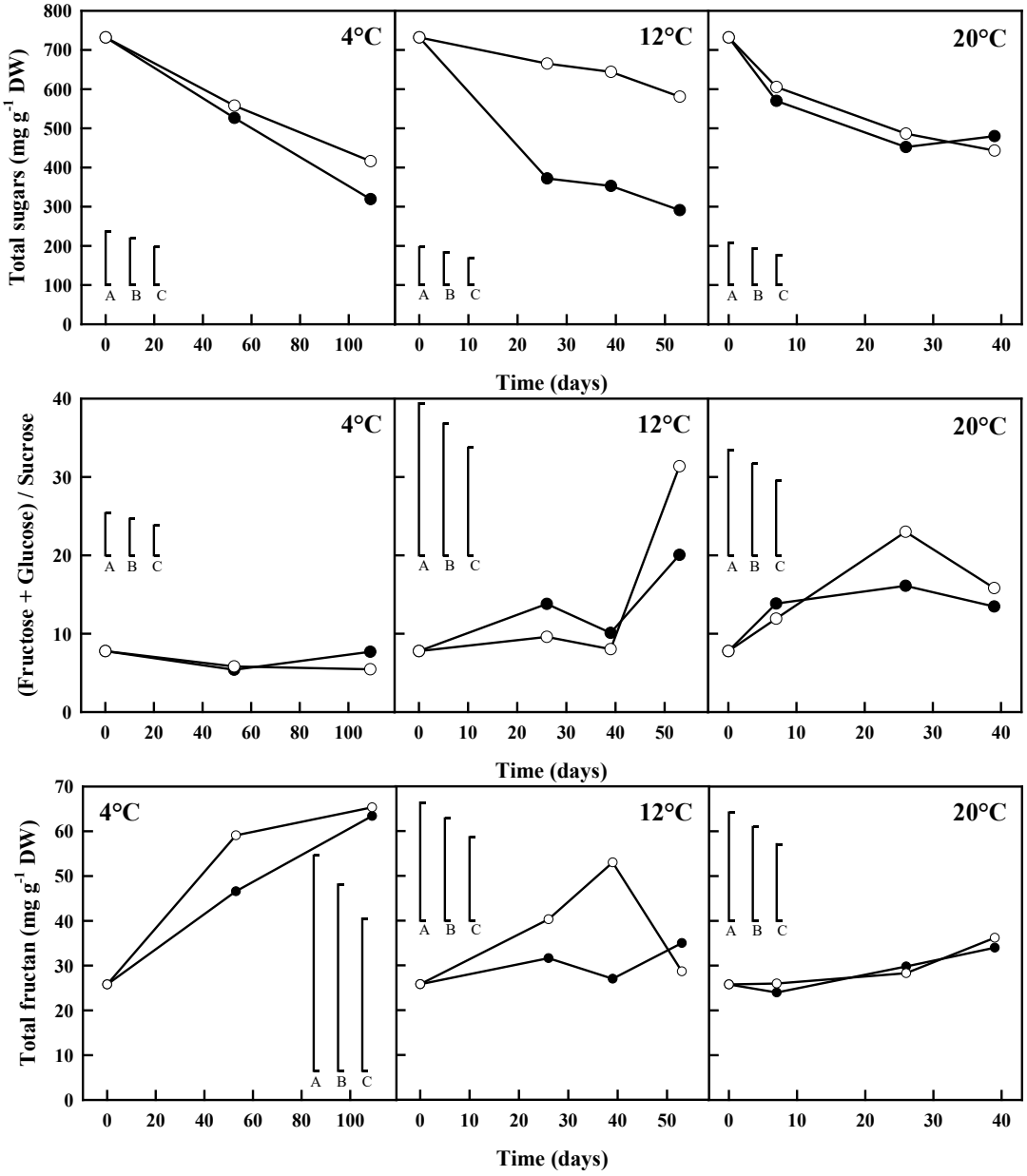


**Figure 5.6.** Ratio of monosaccharide : disaccharide (fructose+glucose/sucrose), total sugar (sucrose+glucose+fructose) and total fructan concentration in onions treated with 1 µl $l^{-1}$ 1-MCP at 20ºC for 24 hours (open symbols) and untreated onions (controls, closed symbols) stored at 4ºC for 109 days, 12ºC for 53 days or 20ºC 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 20ºC delayed sprout growth in onions stored at 4 and 12ºC, but not at 20ºC. This may be because the 1-MCP treatment was not sufficient to counteract the sprout promoting effect of the 20ºC storage conditions. In contrast with the results reported here, 1-MCP (1 µl $l^{-1}$) 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 μmol $l^{-1}$ 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, (20-70 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 sucrose were greater in onions treated with 1-MCP and stored at 12°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 µl $l^{-1}$ 1-MCP for 24h at 9°C to potatoes before exposure to continuous ethylene at 4 µl $l^{-1}$ 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°C. This second theory is further corroborated by Pruski *et al*. (2006), who applied 4 µl $l^{-1}$ 1-MCP to seed potatoes for 48 hr followed by continuous exposure to 4 µl $l^{-1}$ 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°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 12ºC, 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 (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 µl $l^{-1}$ 1-MCP for 16 hr at 20ºC (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 20ºC was only *ca.* 1 µmol $g^{-1}$ 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 µl $kg^{-1}$ FW $hr^{-1}$ at 20°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°C for three weeks) and ethephon (a substance that spontaneously degrades to form ethylene) treatment (1 ml of 100 mg $l^{-1}$ ethephon solution injected into bulb centre) and stored at 18°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 (0.02-20 µl $l^{-1}$) for 8 to 72 hours, yet continuous exposure to 2 µl $l^{-1}$ 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 µl $l^{-1}$, 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°C, but not at 20°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°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 that1-MCP treatment reduced the rate of carbon utilisation in onion cv. SS1 bulbs stored at 12°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% $CO_2$, 3% $O_2$) 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 atmophere 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% $CO_2$ and 3% $O_2$ 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[1] analytes and extraction method | Cultivar | Storage conditions | Storage duration | Effect | Reference |
|---|---|---|---|---|---|
| Fructose[b,x]<br>Glucose[a,x]<br>Fructan[b,x] | Cream Gold Syn.[2]<br>Pukekohe Longkeeper[H] | NA[3] | Recently harvested | ⇑ Fructan towards the centre of the bulb<br>⇓ Fructose towards the centre of the bulb<br>Fructan DP[4] inversely correlated with its concentration<br>Glucose and sucrose similar in all leaf bases | Darbyshire and Henry (1978) |
| Fructose[x]<br>Glucose[bx]<br>Sucrose[bx] | Rijnsberger Robusta[H] | 4˚C | 24 weeks | ⇔ Total soluble sugars before sprouting<br>⇓ Total soluble sugars during sprouting<br>⇔ Sucrose in inner scales<br>⇑ Sucrose in outer scales<br>⇑ Fructose over storage | Rutherford and Whittle (1982) |
| Total carbohydrates[bx] | Granex-Grano[L] | 1, 4 or 21˚C | 24 weeks | ⇓ Total CHOs[5] at 1°C up to 4 weeks then stable<br>⇑ Total CHOs at 4°C up to 8 weeks<br>⇓ Total CHOs at 21°C to 8 weeks, ⇑ to 13 weeks. ⇓ to end of storage | Hurst *et al*. (1985) |
| Fructose[ay]<br>Glucose[ay]<br>Sucrose[ay]<br>Fructan[dy] | Variety of cultivars (n=8)<br>Range of DM | 6-10˚C | 8 weeks | ⇓ Fructans over storage<br>⇑ Sucrose over storage | Suzuki and Cutliffe (1989) |

| | | | | | |
|---|---|---|---|---|---|
| Fructose[ex]<br>Glucose[ex]<br>Sucrose[ex] | Sentinel[L] | 0˚C<br>15˚C<br>30˚C<br>Range of RH | 20 weeks | RH had no effect on the carbohydrates measured<br>⇑ Fructose at 0˚C and 15˚C<br>⇑ Glucose in first 5 weeks at 0˚C and 15˚C<br>⇓ Glucose after 5 weeks at 0˚C and 15˚C, from harvest at 30˚C<br>⇑ Total sugars up to 5 weeks<br>⇓ Total sugars after 5 weeks<br>⇑ Fructose and glucose towards centre of the bulb | Salama *et al*. (1990) |
| Fructose[ax]<br>Glucose[ax]<br>Sucrose[ax]<br>Fructan[6ax] | Hysam[H]<br>Hystar[H]<br>Centurian[H] | 16˚C | 15 weeks | ⇔ Glucose and sucrose in the inner and outer scales<br>⇓ Fructans in the inner and outer scales<br>⇑ Fructose 4-fold in the inner and outer scales<br>⇑ Fructans in the bulb base<br>⇔ Sucrose, glucose and fructose in the bulb base | Pak *et al*. (1995) |
| Glucose[ay] | Valencia sintética[H] | 3-32˚C | 42 weeks | ⇑ Glucose until 120 days<br>⇓ Glucose after 120 days | Croci *et al*. (1995) |
| Fructan[cy] | Variety of cultivars (n=6)<br>Range of DM | Un-specified | 12 weeks | ⇓ Total fructans during storage | Ernst *et al*. (1998) |

| | | | | | |
|---|---|---|---|---|---|
| Glucose[dy]<br>Soluble sugars[dy] | Rouge Amposta[H] | 18˚C | 8 weeks | ⇑ Total soluble sugars (F+G+S) 0.5-fold up to 6 weeks<br>⇓ Total soluble sugars (F+G+S) after 6 weeks<br>⇑ Glucose and fructose preceding sprouting | Benkeblia and Selselet-Attou (1999a) |
| Sucrose[dx]<br>Glucose[dx]<br>Fructose[dx]<br>Fructan[dx] | Hyduro[H] | 1°C | 28 weeks | ⇑ Fructan towards centre of the bulb<br>⇓ Fructose and glucose towards centre of the bulb<br>⇔ Sucrose towards centre of the bulb<br>⇓ Fructan between weeks 10 and 18 of storage<br>⇑ Fructose and sucrose between weeks 3 and 18 of storage | Hansen (1999) |
| Fructose[dx]<br>Fructan[dx] | Hysam[H] | None | Shortly after harvest | High fructan associated with high sucrose and low fructose<br>Fructan DP inversely correlated with its concentration<br>⇑ Fructan from bulb base to top of the bulb<br>⇑ Fructan and sucrose towards centre of the bulb<br>⇓ Free fructose towards centre of the bulb | Jaime *et al*. (2000) |
| Fructose[dx]<br>Glucose[dx]<br>Sucrose[dx] | Variety of cultivars (n=5)<br>Range of DM | 0˚C; 60-65% RH | 24 weeks | ⇑ Free fructose after 24 weeks<br>⇔ Free glucose and sucrose after 24 weeks | Jaime *et al*. (2001) |

| | | | | | |
|---|---|---|---|---|---|
| Fructose$^{dy}$<br>Glucose$^{dy}$<br>Sucrose$^{dy}$<br>Fructan$^{dx}$ | Rouge Amposta$^{H}$ | 4˚C<br>10˚C<br>20˚C | 24 weeks | ⇑ Soluble sugars up to 6-8 weeks storage.<br>⇓ Soluble sugars from 6-8 weeks to end of storage.<br>⇓ Total fructans by 20% at 4˚C.<br>⇑ Total fructans 2-fold at 10˚C and 20˚C. | Benkeblia *et al*. (2002) |
| Fructose$^{cy}$<br>Glucose$^{cy}$<br>Sucrose$^{cy}$<br>Fructan$^{cy}$ | Rijnsberger Sherpa$^{H}$ | 2˚C<br>21% $O_2$<br>1% $O_2$<br>0.5% $O_2$ | 36 weeks | ⇓ WSC$^{7}$ with decreasing oxygen concentration.<br>⇑ Fructan with decreasing oxygen concentration.<br>DP of fructans higher after low oxygen storage than in control | Ernst *et al*. (2003) |
| Fructose$^{dy}$<br>Glucose$^{dy}$<br>Sucrose$^{dy}$ | Rouge Amposta$^{H}$ | 20˚C | 8 weeks | ⇑ Total soluble sugars up to sprouting<br>⇓ Total soluble sugars after sprouting | Benkeblia and Shiomi (2004) |
| Fructose$^{dx}$<br>Glucose$^{dx}$<br>Sucrose$^{dx}$ | Tenshin$^{H}$ | 10˚C<br>20˚C | 25 weeks | ⇑ Fructose, glucose and sucrose up to 4 weeks<br>⇓ Fructose, glucose and sucrose after 4 weeks<br>⇑ Fructose, glucose and sucrose gradually after 7 weeks | Benkeblia *et al*. (2004) |
| Sucrose$^{dx}$<br>Fructan$^{dx}$ | Tenshin$^{H}$ | 10˚C<br>20˚C | 24 weeks | ⇑ Sucrose 1.5-fold in first 4 weeks at 10˚C<br>⇑ Sucrose 2-fold in first 4 weeks at 20˚C<br>⇓ Sucrose 4.4-fold between weeks 4 and 7 at 10˚C<br>⇓ Sucrose 4-fold between weeks 4 and 7 at 20˚C<br>⇔ Sucrose between weeks 7 and 18<br>⇓ Fructan in first 8 weeks, then stable | Benkeblia *et al*. (2005a) |

| | | | | | |
|---|---|---|---|---|---|
| Fructose[dx]<br>Fructan[dx] | Tenshin[H] | 15˚C | 24 weeks | ⇑ Fructose after 8 weeks, then stable between 10 and 18 weeks<br>⇓ Fructose after 18 weeks<br>⇔ Fructose between 20 and 24 weeks<br>⇓ Fructan and total carbohydrates steadily after 5 weeks | Benkeblia *et al*. (2005b) |

[1]NSC=Non-structural carbohydrates. [2] Syn.=Synonym. [3]NA=Not applicable. [4] DP = Degree of polymerisation.[5] CHOs=Carbohydrates.

[6]Calculated as total carbohydrate minus sucrose, glucose and fructose. [7]WSC = Water Soluble Carbohydrates

[a]Colorimetric method. [b]Enzymatic method. [c]High Performance Anion Exchange Chromatography method. [d]HPLC method. [d]GC-FID method

[x]Ethanol extraction. [y]Water extraction.

[H]High dry matter, [L]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 $m^{-1}$ 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% $CO_2$ and 3% $O_2$) or air (*ca*. 0.003% $CO_2$ and 21% $O_2$) 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% $CO_2$, 95% $N_2$ (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 $CO_2$ 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°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 (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 $CO_2$ production under air conditions at 5ºC. 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 $CO_2$ 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 120ºC and the oven at 80ºC. 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% $CO_2$ (10% $CO_2$, 2% $O_2$, 88% $N_2$; Certified Standard from British Oxygen Co.). Respiration rate was calculated as mmol $CO_2$ $kg^{-1}$ $hr^{-1}$.

*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 $CO_2$ $kg^{-1}$ $hr^{-1}$). 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 $CO_2$ $kg^{-1}$ $hr^{-1}$) (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 $CO_2$ $kg^{-1}$ $hr^{-1}$).

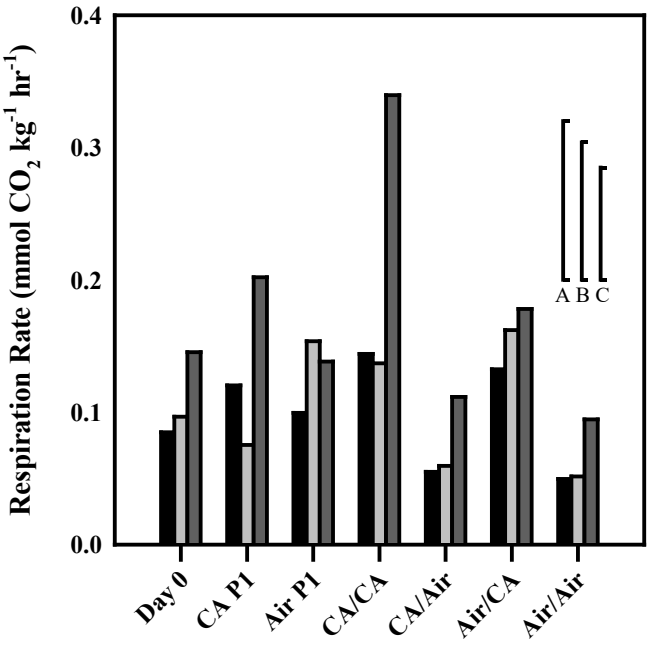


**Figure 6.1.** The respiration rate of onions cvs. Carlos (▬), Renate (▭) and SS1 (▭) recorded before storage (Day 0), after 21 days storage in CA (CA P1) or air (Air P1) and after CA for 42 days (CA/CA), CA for 21 days followed by air for 21 days (CA/Air), air for 21 days followed by CA for 21 days (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.

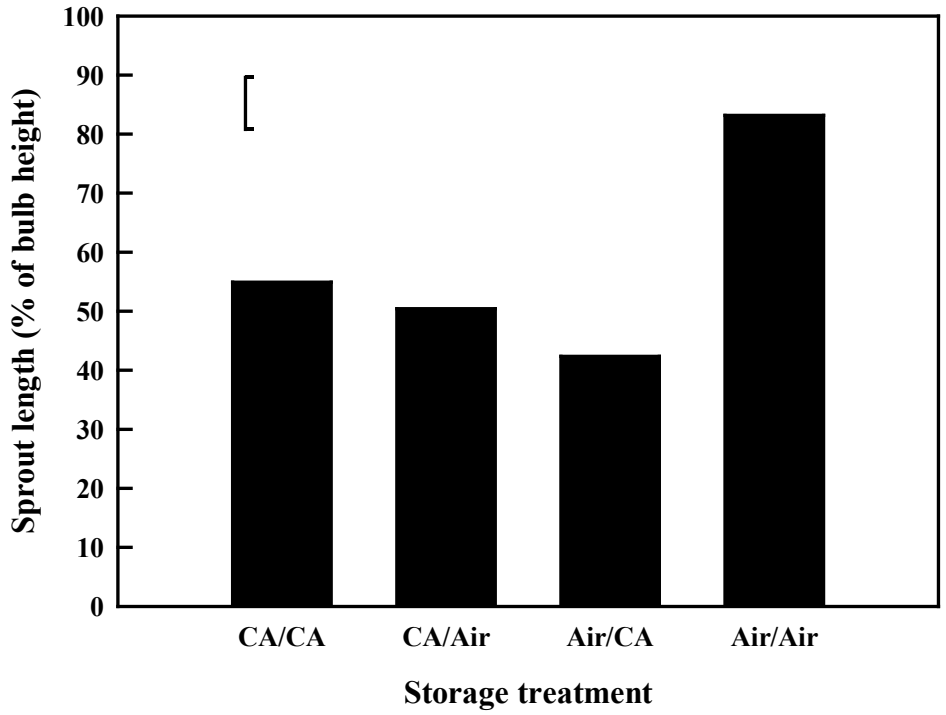


**Figure 6.2.** The sprout length of onion cv. SS1 expressed as a percentage of bulb height after storage in; CA for 42 days (CA/CA), CA for 21 days followed by air for 21 days (CA/Air), air for 21 days followed by CA for 21 days (Air/CA) and air for 42 days (Air/Air); n=4. LSD bar (*P*=0.05) is shown.

There was a main effect of cultivar on firmness. Onion cvs. Carlos and Renate, 51.03 and 55.33 N $mm^{-1}$, respectively, were significantly (*P*<0.001) firmer than onions cv. SS1 at 26.31 N $mm^{-1}$ (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 $g^{-1}$ FW and 128.79 mg $g^{-1}$ FW, respectively, was greater (*P*<0.001) than that of onions cv. SS1, 71.11 mg $g^{-1}$ FW. There were no main effects or interactions of storage treatment (Table 6.2).

**Table 6.2.** The firmness (N $mm^{-1}$) and dry weight (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.

| | | Storage Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Harvest | Day 0 | CA P1 Day 21 | Air P1 Day 21 | CA/CA Day 42 | CA/Air Day 42 | Air/CA Day 42 | Air/Air Day 42 |
| | Cultivar | (n=12) | (n=16) | (n=8) | (n=8) | (n=4) | (n=4) | (n=4) | (n=4) |
| **Firmness (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 |
| | **$LSD_{0.05}$** | 6.310 | 5.465 | 7.723 | | | 10.930 | | |
| **Dry Weight (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 |
| | **$LSD_{0.05}$** | 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 µmol $g^{-1}$ 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 (µmol $g^{-1}$ 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.

| | | Storage Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | CA P1 | Air P1 | CA/CA | CA/Air | Air/CA | Air/Air |
| | | Harvest | Day 0 | Day 21 | Day 21 | Day 42 | Day 42 | Day 42 | Day 42 |
| | Cultivar | (n=12) | (n=16) | (n=8) | (n=8) | (n=4) | (n=4) | (n=4) | (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 |
| | **$LSD_{0.05}$** | 0.9127 | 0.7904 | 1.178 | | | 1.5808 | | |
| **Pyruvate (µmol $g^{-1}$ 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 |
| | **$LSD_{0.05}$** | 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 $g^{-1}$ DW), followed by sucrose (range: 50-125; Carlos mean: 71.6; Renate mean: 96.4 mg $g^{-1}$ DW) with small amounts of fructose (range: 25-75; Carlos mean: 39.8; Renate mean: 38.8 mg $g^{-1}$ 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 $g^{-1}$ DW), with amounts of sucrose comparable with that in Carlos and Renate (range: 25-125; mean: 80.4 mg $g^{-1}$ 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.

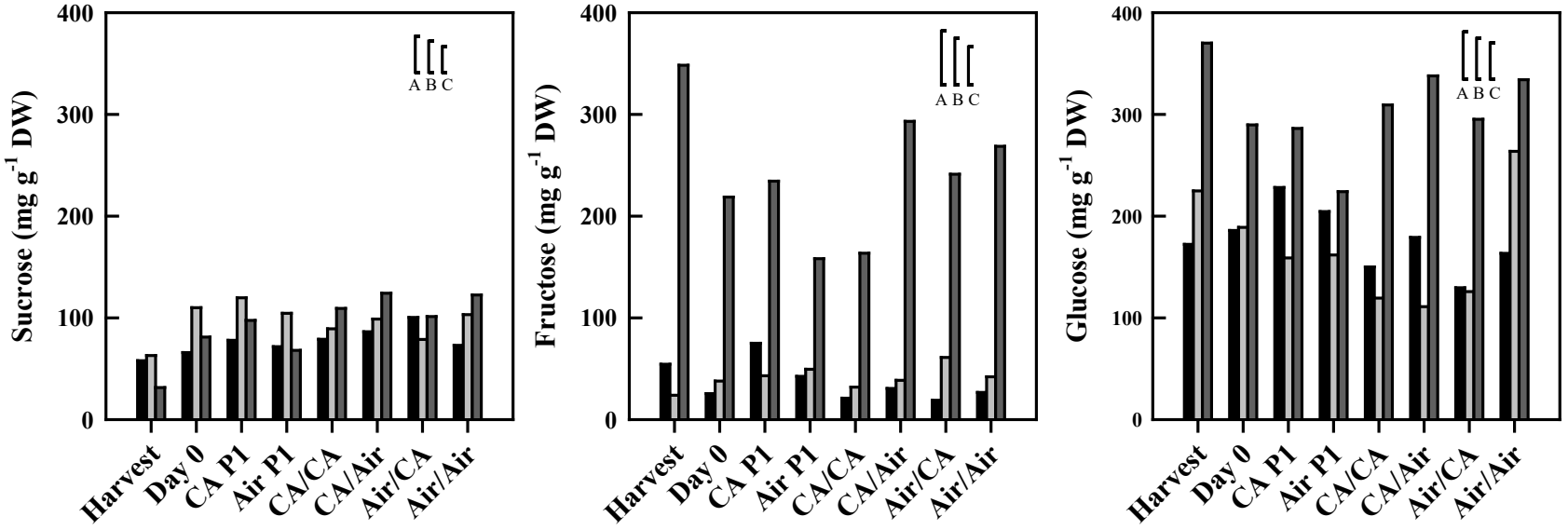


**Figure 6.3.** The sucrose, glucose and fructose concentration of onion cvs. Carlos ( ), Renate ( ) and SS1 ( ) measured at harvest, before storage (Day 0), after 21 days storage in CA (CA P1) or air (Air P1) and after CA for 42 days (CA/CA), CA for 21 days followed by air for 21 days (CA/Air), air 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 mg $g^{-1}$ DW) followed by cvs. Carlos (183.1 mg $g^{-1}$ DW) and Renate (179.4 mg $g^{-1}$ 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.

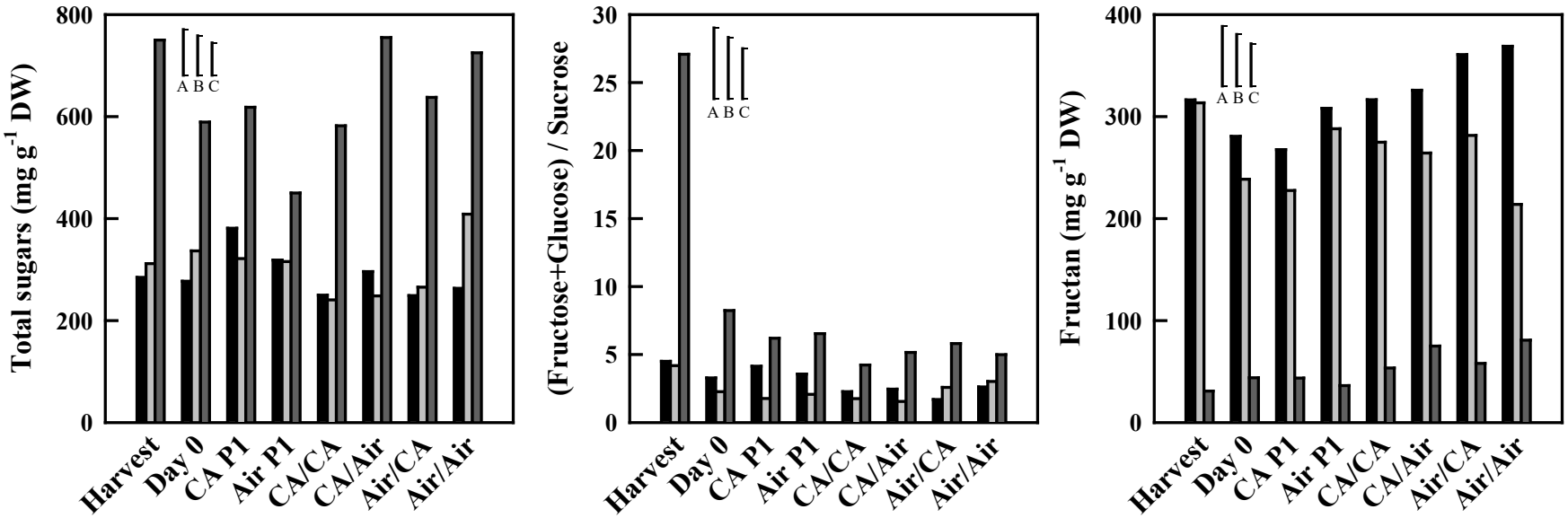


**Figure 6.4.** The total sugar (glucose, sucrose and fructose), monosaccharide to disaccharide ratio, and total fructan concentration of onion cv. Carlos (▬), Renate (▭) and SS1 (▬) measured at harvest, before storage (Day 0), after 21 days storage in CA (CA P1) or air (Air P1) and after CA for 42 days (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 mg $g^{-1}$ 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.

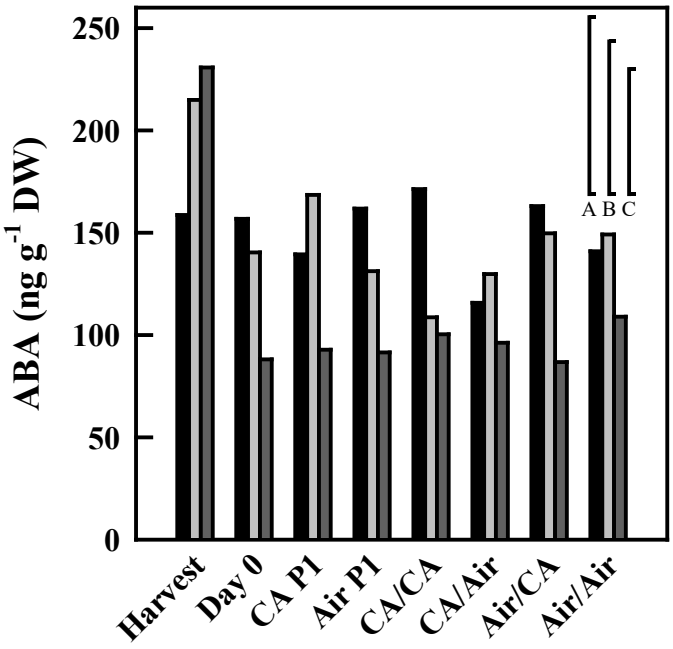


**Figure 6.5.** The ABA concentration of onion cvs. Carlos ( ), Renate ( ) and SS1 ( ) measured at harvest, before storage (Day 0), after 21 days storage in CA (CA P1) or air (Air P1) and after CA for 42 days (CA/CA), CA for 21 days followed by air for 21 days (CA/Air), air for 21 days followed by CA 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 $CO_2$ $kg^{-1}$ $hr^{-1}$). 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 4ºC for 6 weeks (0.08 mmol $CO_2$ $kg^{-1}$ $hr^{-1}$) (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 20ºC, compared with 20-55% of onions stored under low oxygen conditions (0.7-1.4%) with 0.05% $CO_2$ (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% $O_2$ with <0.3% $CO_2$ at 2ºC had an improved shelf-life (when tested for 3 weeks at 18ºC after 9, 27, and 36 weeks storage) compared with those stored in air (Praeger *et al*., 2003) and onions cv. Granex stored in 5% $CO_2$ with 3% $O_2$ at room temperature or 5ºC for 20 weeks, or in 5% $CO_2$ with 3% $O_2$ at 1ºC for 24 weeks had a greater percentage of marketable bulbs after 2 weeks at 27ºC 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% $O_2$ with 3% $CO_2$ at 0ºC) for 36 weeks had sprouted after 3 weeks at 20ºC, 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% $CO_2$, 20% $O_2$, 20% $N_2$) 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 $CO_2$ 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 $kg^{-1}$ $hr^{-1}$ and 0.08 mmol $kg^{-1}$ $hr^{-1}$ 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% $O_2$ and <0.3% $CO_2$) 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 0.5ºC in CA conditions (2% $O_2$, 2% $CO_2$ and 2% $O_2$, 8% $CO_2$) 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 $g^{-1}$ FW over 26 weeks storage to 5.18 in air but only to 3.85 in CA (5% $CO_2$ and 3% $O_2$) 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 $g^{-1}$ DW) than cvs. Carlos and Renate (300-400 mg $g^{-1}$ 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% $O_2$ and 5% $CO_2$ (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, 30ºC, 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 x $10^{-4}$ M (1M = 264.3 g $l^{-1}$) exogenous ABA to *A. wakegi* bulbs increased the number of days to sprouting, whilst application of 0.25 x $10^{-4}$ 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% $CO_2$, 5% $O_2$; 2ºC) 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 $g^{-1}$ 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 $g^{-1}$ FW) than the non-dormant cultivar (33 ng $g^{-1}$ 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 $10^{-5}$ M ABA two weeks after harvest, whereas $10^{-4}$ 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, suctose 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 an 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.

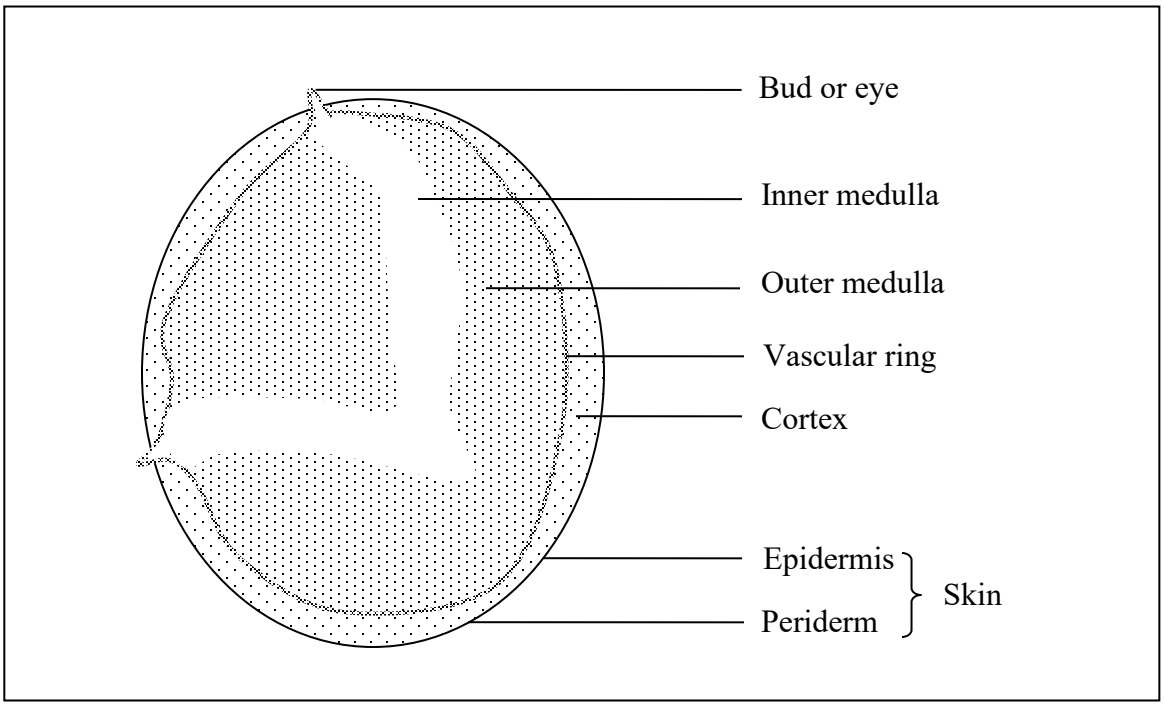


**Figure 7.1.** Cross-section of a potato tuber.

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 $g^{-1}$ FW), and declined during storage to 210-280 pmol $g^{-1}$ 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 *Arapidopsis* 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 µl $kg^{-1}$ $hr^{-1}$ at 20ºC), and have low sensitivity to ethylene (Suslow and Voss, 2002), but continuous (23-33 weeks) exposure to ethylene (4 µl $l^{-1}$) has been shown to be an effective method of sprout control in potatoes (Prange *et al*., 2005). Increasing the concentration of ethylene to 40 µl $l^{-1}$ 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 µl $l^{-1}$) 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 µl $l^{-1}$) treated onions stored at 12°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 onions 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 µl $l^{-1}$; 48 hr) 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 extend to 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 µl $l^{-1}$ (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 µl $l^{-1}$ have been shown to reduce the storage life of Chinese cabbage stored at 0 and 20°C, oranges stored at 2.5°C, and a range of leafy vegetables including broccoli and chives stored at 5 and 20°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 µl $l^{-1}$ $kg^{-1}$ $hr^{-1}$) compared with untreated controls (0.005 µl $l^{-1}$ $kg^{-1}$ $hr^{-1}$) (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 is 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 onions bulbs was greater, 0.14 µl $kg^{-1}$ $hr^{-1}$, after short-term (<24 hr) exposure to 30% $CO_2$ and 20% $O_2$ than air, 10% $CO_2$ and 20% $O_2$ or 20% $CO_2$ and 20% $O_2$, 0.11 µl $kg^{-1}$ $hr^{-1}$ (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% $CO_2$ 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% $CO_2$ 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 (Ianetta *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. Is 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 30-35ºC significantly reduced the number of days to sprouting in dry storage at 15ºC, when compared to those exposed to postharvest temperature treatments of 15-25ºC (Miedema, 1994a).

The question remains as to how to increase the endogenous ABA concentration in the bulb, since it is difficult 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 *Arapidopsis* 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).

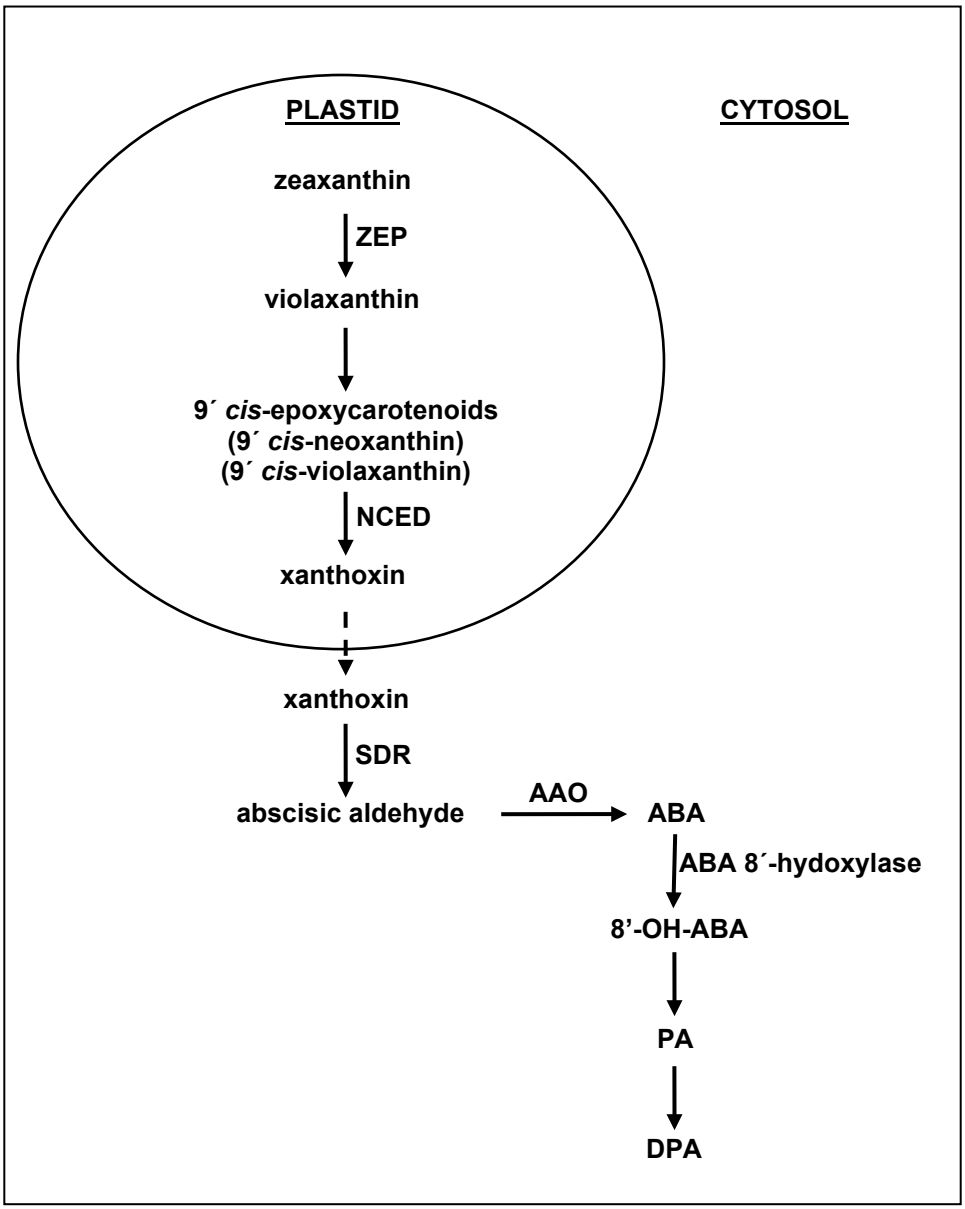


**Figure 7.2.** Summary of the pathways involved in ABA biosynthesis and catabolism. Dashed arrow indicates export of xanthoxin from the plastid to the cytosol. ZEP=zeaxanthin epoxydase, NCED=nine *cis*-epoxycarotenoid dioxygenase; SDR=short chain alcohol dehydrogenase/reductase; AAO=abscisic aldehyde 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 *Arapidopsis*, 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 thalinana 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 β-cyanoalanine by β-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).

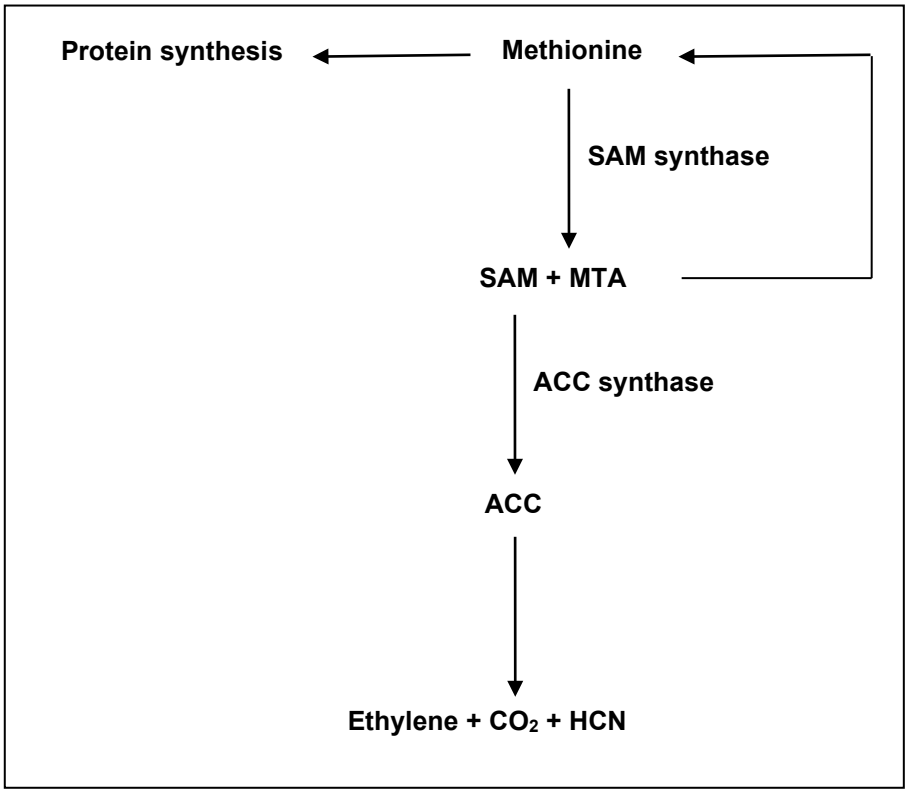


**Figure 7.3.** Simplified pathway of ethylene biosynthesis. SAM=S-adenosyl-L-methionine, MTA=5'-methylthioadenosine, ACC=1-amino-cyclopropane-1-carboxylic acid (Ecker, 1995; Wang *et al*., 2002).

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). Unicazole 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 30ºC, followed by a further ten days at 24ºC (RH not to exceed 75%). The crop is then allowed to cool to approximately 15ºC, 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 µl $l^{-1}$) 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).

## TECHNOLOGY TRANSFER

Results from this project have been presented, published and submitted for publication as follows:

Articles in grower publications:

- Chope, G. A. (2004). Better onions in store. *HDC News*, July.
- Chope, G. A. (2005). Storing onions: the physiology of dormancy. *The Vegetable Farmer*, November, 25-26.

Presentations at grower events:

- Onion and Carrot Conference, Cambridge, November 2003
- Onion and Carrot Conference, Peterborough, November 2007

Presentations at scientific conferences:

- 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.
- 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 21st Century. 15-17 December 2004, Oxford.

Publications in scientific journals:

- 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.
- 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.

- 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.
- 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.

The results from this project have formed the foundation of a recent successful application to DEFRA HortLINK entitled 'HL0182 - Sustaining UK fresh onion supply by improving consumer acceptability, quality and availability'.

## GLOSSARY

| | |
|---|---|
| < | 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 | *S*-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 |
| *ca.* | approximately |
| Ca | calcium |
| $CaCl_2$ | calcium chloride |
| CEPA | 2- chloroethylphosphonic acid |
| CHO | carbohydrate |
| cm | centimetre |

| | |
|---|---|
| cM | centi Morgan |
| Co | Cobalt |
| $CO_2$ | carbon dioxide |
| cv. | cultivar |
| DEFRA | Department for Environment Food and Rural Affairs |
| *de novo* | 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 |
| *et al.* | 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 |
| $^3H$ | tritium |
| ha | hectare |
| HCl | hydrochloric acid |
| HDC | Horticultural Development Council |
| HPLC | High Performance Liquid Chromatography |
| ICP-AES | Inductively Coupled Plasma – Atomic Emission Spectroscopy |
| *in vitro* | outside a living organism |
| *in vivo* | 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 *S*-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 |
| $N_2$ | nitrogen |
| NA | not applicable |
| $NaBH_4$ | sodium borohydride |
| NaCl | sodium chloride |
| $Na_2HPO_4$ | di-sodium hydrogen orthophosphate |
| $NaH_2PO_4$ | sodium di-hydrogen orthophosphate |
| NaOH | sodium hydroxide |
| NCED | nine *cis*-epoxycarotenoid dioxygenase |
| ng | nanogram |
| $(NH_4)_2SO_4$ | ammonium sulphate |
| NSC | non-structural carbohydrate |
| nm | nanometre |
| nmol | nanomole |
| $N_2O$ | nitrous oxide |
| $O_2$ | oxygen |
| *P* | probabiltity |

| | |
|---|---|
| PA | phaseic acid |
| PAHBAH | *p*-hydroxybenzoic acid hydrazide |
| PBI-365 | 8'-methylene ABA methyl ester |
| PBS | phosphate buffered saline |
| PCSO | propyl *S*-alk(en)yl-L-cysteine sulphoxide |
| pg | picogram |
| PRENCSO | 1-propenyl *S*-alk(en)yl-L-cysteine sulphoxide |
| $Q_{10}$ | 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 | S-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 |
| *viz.* | namely |
| v/v | volume by volume |
| w/w | weight by weight |
| WSC | water soluble carbohydrate |
| ZEP | zeaxanthin epoxydase |

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# 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 immuno-reactivity 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 (Rosher *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 µl each of 4 x $10^4$ pg $ml^{-1}$ ABA solution and sample (Ailsa Craig, day 0, S-Ca-) were lyophilised and rehydrated with 10 µl of 100 % methanol. Plate 2: 50 µl each of 5 x $10^3$, 1 x $10^4$, 2 x $10^4$, 4 x $10^4$, 5 x $10^5$, 5 x $10^7$ and 5 x $10^9$ pg $ml^{-1}$ (+)-ABA solution were lyophilised and rehydrated with 15 µl 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, 1893); 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 µl 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.

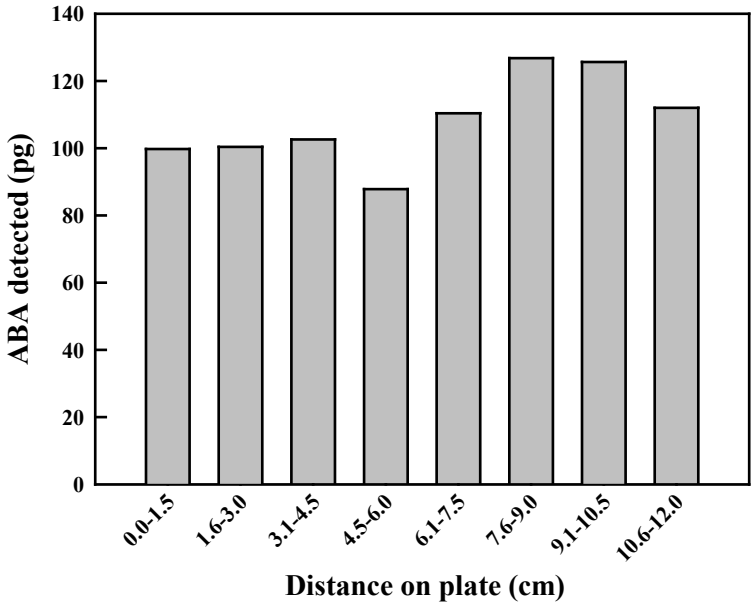


**Figure A.1.** ABA detected by radio-immunoassay in samples eluted from a thin layer chromatogram of crude onion extract dissolved in methanol.

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.

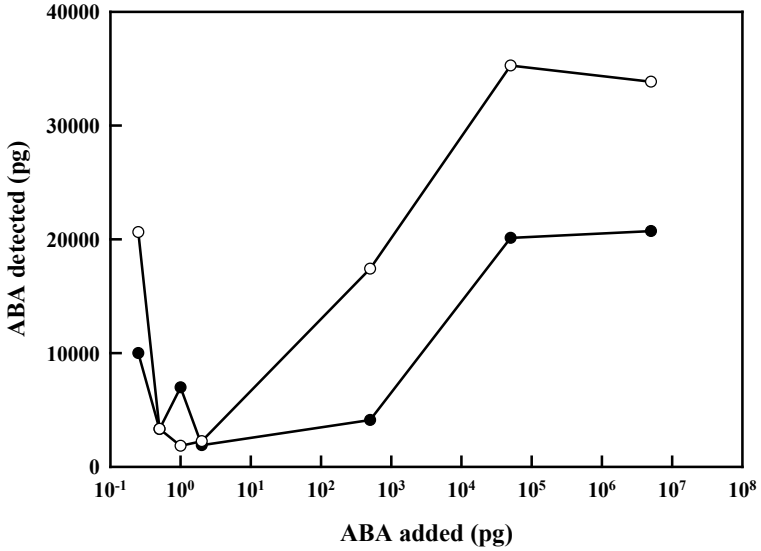


**Figure A.2.** ABA detected in the samples eluted from the appropriate $R_f$ zone of a thin layer chromatogram of known amounts of ABA dissolved in methanol (open symbols) and the mean ABA detected by radio-immunoassay in each zone – the background (closed symbols).

*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.[1] |
|---|---|---|---|
| 1,2,6 | Control | 24.9 | 11.2 |
| 6 | $1^{RP}$ | 214.3* | 20.5 |
| 2 | $2^{RM}$ | 129.6* | 20.5 |
| 5 | $2^{A}$ | 181.6* | 22.3 |
| 6 | $2^{RP}$ | 83.1** | 20.5 |
| 1 | $3^{R}$ | 260.5* | 20.5 |
| 5 | $3^{A}$ | 168.9* | 22.3 |
| 2 | $4^{RM}$ | 19.7 | 20.5 |
| 3 | $4^{A}$ | 26.1 | 20.5 |
| 5 | $4^{A}$ | 116.8* | 22.3 |
| 7 | $5^{A}$ | 111.9* | 22.3 |
| 3 | $6^{A}$ | -2.3 | 20.5 |
| 7 | $10^{A}$ | 74.6** | 22.3 |
| 3 | $14^{A}$ | -16.4** | 20.5 |
| 4 | $20^{LR}$ | 247.7* | 20.6 |
| 7 | $20^{A}$ | 70.7** | 22.3 |
| 1 | $24^{R}$ | 30.6 | 20.5 |
| 4 | $300^{LR}$ | 33.3 | 20.6 |
| 4 | $800^{LR}$ | 34.3 | 20.6 |

[1] S.E. = standard error of the difference between the intercept of the reference level (control) and each dilution factor. [L] 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.

[M] 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.

[P] 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. [A] Ailsa Craig or [R] 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 µl 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 ≥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 20ºC 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 20ºC 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 20ºC 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 20ºC on total soluble solids content of onion cvs. Carlos, Dinaro, 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 20ºC on pyruvate concentration of onion cvs. Carlos, Dinaro, 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 20ºC on ABA concentration (log transformed values) of onion cvs. Carlos, Dinaro, 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 20ºC 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 20ºC 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 20ºC 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 20ºC 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 20ºC 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 20ºC 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 | TSS | ABA | Fructan | Fructose | Glucose | Sucrose | Total sugars |
|---|---|---|---|---|---|---|---|---|
| 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 |

**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 | TSS | ABA | Fructan | Fructose | Glucose | Sucrose | Total sugars |
|---|---|---|---|---|---|---|---|---|
| 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 |

**Table B.114.** Experiment 1: Pre-harvest application – cv. SS1, 12°C (Sections 4.4.1.7 and 4.4.1.8).

| | Sprout length | TSS | ABA | Fructan | Fructose | Glucose | Sucrose | Total sugars |
|---|---|---|---|---|---|---|---|---|
| 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 |

**Table B.115.** Experiment 1: Pre-harvest application – cv. Renate, 12°C (Sections 4.4.1.7 and 4.4.1.8).

| | Sprout length | TSS | ABA | Fructan | Fructose | Glucose | Sucrose | Total sugars |
|---|---|---|---|---|---|---|---|---|
| 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 |

**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: Preiharvest 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 20ºC 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 20ºC 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 20ºC 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 20ºC 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 20ºC 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 20ºC 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 20ºC 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 20ºC 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 20ºC 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 20ºC 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 20ºC 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 20ºC 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 Biol. Technol. 39, 233-242.

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## Effect of controlled atmosphere storage on abscisic acid concentration and other biochemical attributes of onion bulbs

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**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 (3.03 kPa $CO_2$; 5.05 kPa $O_2$; 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. 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.

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.


*Keywords:* ABA; *Allium cepa* L.; Firmness; Fructan; Pyruvate; Sprouting