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The results and conclusions in this report are based on a series of experiments conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

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

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LIST OF ABBREVIATIONS AND SYMBOLS

<	less than
>	greater than
≤	less than or equal to
≥	more than or equal to
%	per cent
°Brix	degree Brix
°C	degree Celsius
γ	gamma
µl	microlitre
µm	micromoles
µM	micromolar
AACC	American Association of Cereal Scientists
ABA	abscisic acid
ABU	arbitrary unit
ACSO	*S*-alk(en)yl-L-cysteine sulphoxide
Al	aluminium
ANOVA	analysis of variance
AOAC	Association of Analytical Communities
B	boron
CA	controlled atmosphere
ca	approximately
Ca	calcium
CaCl	calcium chloride
CEPA	2- chloroethylphosphonic acid
CO_2	carbon dioxide
cpm	counts per minute
Cu	copper
cv	cultivar
DNPH	2,4-dinitrophenylhydrazine HCl
DW	dry weight
EST	expressed sequence tag
Fe	iron

FW	fresh weight
g	gram
^{3}H	tritium
h	hours
ha	hectare
HCl	hydrochloric acid
ICP-AES	inductively coupled plasma – atomic emission spectroscopy
K	potassium
kg	kilogram
kN	kiloNewton
kPa	kiloPascal
l	litre
LSD	least significant difference
M	Molar
m	metre
MCSO	methyl *S*-alk(en)yl-L-cysteine sulphoxide
mg	milligram
Mg	magnesium
MH	maleic hydrazide
min	minute
ml	millilitre
mm	millimetre
mM	millimolar
Mn	manganese
MW	molecular weight
N_2	nitrogen
Na	sodium
$NaBH_4$	sodium borohydride
Na_2HPO_4	di-sodium hydrogen orthophosphate
NaH_2PO_4	sodium di-hydrogen orthophosphate
$(NH_4)_2SO_4$	ammonium sulphate
NaOH	sodium hydroxide
ng	nanogram
N_2O	nitrous oxide

O_2	oxygen
P	phosphorus
PAHBAH	*p*-hydroxybenzoic acid hydrazide
PBS	phosphate buffered saline
PCSO	propyl *S*-alk(en)yl-L-cysteine sulphoxide
pg	picogram
PGR	plant growth regulator
ppm	parts per million
PRENCSO	1-propenyl *S*-alk(en)yl-L-cysteine sulphoxide
PTM	primary thickening meristem
PVP	polyvinylpyrrolidone
Q_{10}	increase in the respiration rate produced by raising the temperature by 10°C
RH	relative humidity
RNA	ribonucleic acid
rpm	revolutions per minute
RQ	respiratory quotient
S	sulphur
TCA	trichloroacetic acid
TLC	thin layer chromatography
TSS	total soluble solids
TTC	2,3,5-triphenyl tetrazolium chloride
v/v	volume by volume
w/w	weight by weight
WSC	water soluble carbohydrate
Zn	zinc

GROWERS SUMMARY

CP 20

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

Background and expected deliverables

Extended storage life of UK onions currently depends on the use of maleic hydrazide (MH) to suppress the growth of the sprout in store. Concerns over residues have led to retailer pressure to reduce or eliminate MH treatment. This could have a serious effect on 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 apical and floral meristems being mitotically active. 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 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 PGR in onion cultivars with differing storage potential and the consequential changes in significant storage compounds.

The expected deliverables from this project are:

- 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 were profiled in order to determine the significant changes that lead to the breaking of bulb dormancy and the onset of sprouting. The following cultivars were used:

- Renate Long storage potential
- Ailsa Craig Intermediate storage potential
- SSI Short storage potential

The onions were grown according to current commercial practice, and stored in controlled atmosphere conditions (CA; 5% O_2, 3%CO_2, 2ºC). Results show that the concentration of ABA measured before storage was greatest in onion cv. Renate bulbs, less in onion cv. Ailsa Craig bulbs and least in onion cv. SS1 bulbs (on a fresh weight basis). An increase in sprout growth coincided with a decrease in ABA concentration.

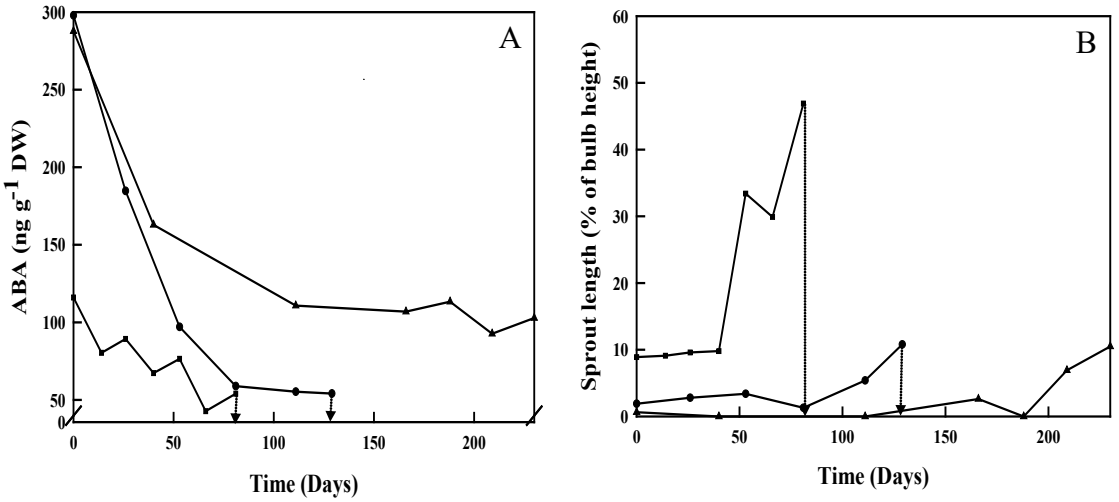

Figure 1. Changes in A: ABA concentration and B: sprout length of onion bulbs cv. Renate (▲), Ailsa Craig (●) and SS1 (■) over time in controlled atmosphere storage. Drop down arrows indicate the last sample taken.

Other changes occurring in the bulb during CA storage

- **Pungency** (as measured by pyruvate concentration)
    - Increased overall in onion cv. Renate and cv. SS1 bulbs.
    - Decreased overall in onion cv. Ailsa Craig bulbs.
- **Fructan** (a storage carbohydrate)
    - Decreased overall in onion cv. Renate and cv. Ailsa Craig bulbs.
    - No net change in onion cv. SS1 bulbs.
    - A decrease in fructan concentration preceded an increase in sprout length.
- **Firmness**
    - Decreased over storage in all cultivars.

Financial benefits

- There are no financial benefits for growers at this stage, however possible benefits for growers in the future could be protocols and markers for the extension of storage life.

Action points for growers

- There are no recommended changes to current grower practice.

ABSTRACT

Dormancy in onion bulbs consists of a true dormant period where sprouting and rooting are not induced, followed by a period of physiological changes preparing the bulb for growth. The true dormant period is short, and appears to be unrelated to storage potential. The internal sprout growth rate is a major determinant of storage life.

Several plant growth regulators (PGRs) have been detected in onions. Abscisic acid (ABA) concentration has been positively correlated with days to external sprouting. A peak concentration occurs at harvest, gradually declining until sprout emergence. Little is known of the relative concentrations of PGRs such as ABA in bulbs with different storage potentials, or the effect of controlled atmosphere (CA) storage on these factors. Understanding the role of PGRs in onion dormancy may produce superior markers for storage potential, breeding targets and manipulation using horticultural practices.

ABA, pyruvate, fructan and firmness were measured in bulbs of short, intermediate and long-storing onion cultivars; SS1, Ailsa Craig and Renate respectively. Onions were treated at the time of drilling with additional sulphur (100 kg ha^{-1}) and/or calcium (300 kg ha^{-1}) in four combinations including a negative control.

It was shown that the ABA concentration in onion bulbs of all cultivars declined exponentially during CA storage, with the greatest decrease occurring within the first 80 days. The pattern of decline was similar for each cultivar; however, onion bulbs

cv. SS1 had the lowest initial ABA concentration. If the concentration of ABA at harvest in the bulbs of short storing cultivars could be increased then storage life may be extended.

Onion cv. Renate, Ailsa Craig and SS1 bulbs were characterised respectively by high, intermediate and low concentrations of pyruvate, fructan and total soluble solids (TSS). Fructan concentration at harvest was positively correlated with storage life. TSS concentration reached a peak between four and six weeks of storage in all cultivars.

Firmness declined steadily throughout the storage period, although dry weight remained constant. Pungency, as measured by pyruvate concentration, increased in cv. Renate and SS1 bulbs, and decreased in cv. Ailsa Craig bulbs. This may have implications for growers holding onions in CA storage. Onion cv. Renate bulbs treated with additional sulphur were more pungent, but no other consistent significant effects of the calcium and sulphur treatments were observed.

Data for bulbs just prior to sprouting was limited due to the high incidence of pathogen attack that occurred during CA storage, thus disease generally became the limiting factor in storage life rather than sprouting.

CHAPTER ONE: GENERAL INTRODUCTION

1.1 Background

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 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.

1.2 Aims

This study aims to detail the temporal changes in bulb abscisic acid concentration as well as quality characteristics; fructan, pyruvate and total soluble solids concentration, and firmness, which occur in three onion cultivars with different storage potentials.

2.1 Economic importance of the onion crop

Onions grown in the UK are produced for human consumption. Onions, leeks and shallots 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 an area of almost 9000 hectares harvested in the UK in 2003, accounting for 0.71% of worldwide production (Table 1).

Table 1 The area of onions[1] harvested, the yield (100g per hectare) and production (tonnes) from 2000 to 2003 in the UK and worldwide (FAOSTAT, 2004).

	Area Harvested (Ha)		Yield (100g/Ha)		Production (Tonne)	
Year	UK	World	UK	World	UK	World
2000	9100	2 835 470	431 538	170 342	392 700	48 300 086
2001	8600	2 895 400	435 930	172 328	394 900	49 895 771
2002	8390	2 940 511	337 783	175 130	283 400	51 497 210
2003	8730	3 011 081	430 355	174 511	375 700	52 546 545

[1]Onions harvested at a mature stage, not dehydrated.

The quantity of onions imported to the UK has increased approximately 1.7-fold, and the value 1.9-fold, between 2000 and 2002 (Table 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 that UK growers can compete at this time of year, so that this valuable market share is not lost to imports.

Table 2. The quantity (tonnes) and value ($1000) of onions imported to the UK, and UK onion exports (FAOSTAT, 2004).

	Imports		Exports	
Year	Quantity (Tonnes)	Value ($1000)	Quantity (Tonnes)	Value ($1000)
2000	156718	50557	5406	2992
2001	231504	74029	4074	3199
2002	263379	95296	5284	4326

2.1.1 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 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.

2.2 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 or sets. An onion bulb is a storage organ, consisting of foliage leaf bases and swollen, bladeless inner sheathes (Fig. 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. A wide range of adaptations to photoperiod and temperature exist, indicating intense selection of cultivars within a range of environments.

2.2.1 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 swell the most to form the storage tissue. As the bulb matures, two or three foliage leaf initials are laid down at the apex. These initials elongate to produce leaf blades in the following season when the bulb sprouts.

2.2.2 Photoperiod and temperature

Bulb formation occurs when onion plants are exposed to critical daylengths at a minimum temperature (Lancaster *et al.*, 1996). In temperate regions, daylength is the most important factor affecting bulb formation (Brewster, 1977a; Wright and Sobeih, 1986). If the critical thermal time is reached before critical daylength then bulbing is delayed and subsequent bulbs have larger diameters and more leaves. Conversely, the critical daylength can be reached before threshold thermal time is met, and in this case bulbing is delayed until the thermal time requirements have been met (Lancaster *et al.*, 1996). Day length, temperature and light spectral quality interact with the photoperiod (Brewster, 1990). Short-day onions form bulbs under short photoperiods at low latitudes; however their behaviour is typical of other onions in that bulbing accelerates with increased photoperiod. Far red light, and to a lesser extent blue light, promote bulbing, whereas red light inhibits it. The photoperiod required to induce bulbing can be reduced by a lower red to far-red ratio indicating the involvement of the phytochrome in a high irradiance reaction (Kahane *et al.*, 1992). Temperature (including night temperature) is positively correlated with the rate of bulb development in an inductive photoperiod (Brewster, 1990; Wheeler *et al.*, 1998). A decrease in the critical daylength necessary for bulb initiation has been associated with an increase in temperature (Brewster, 1977a).

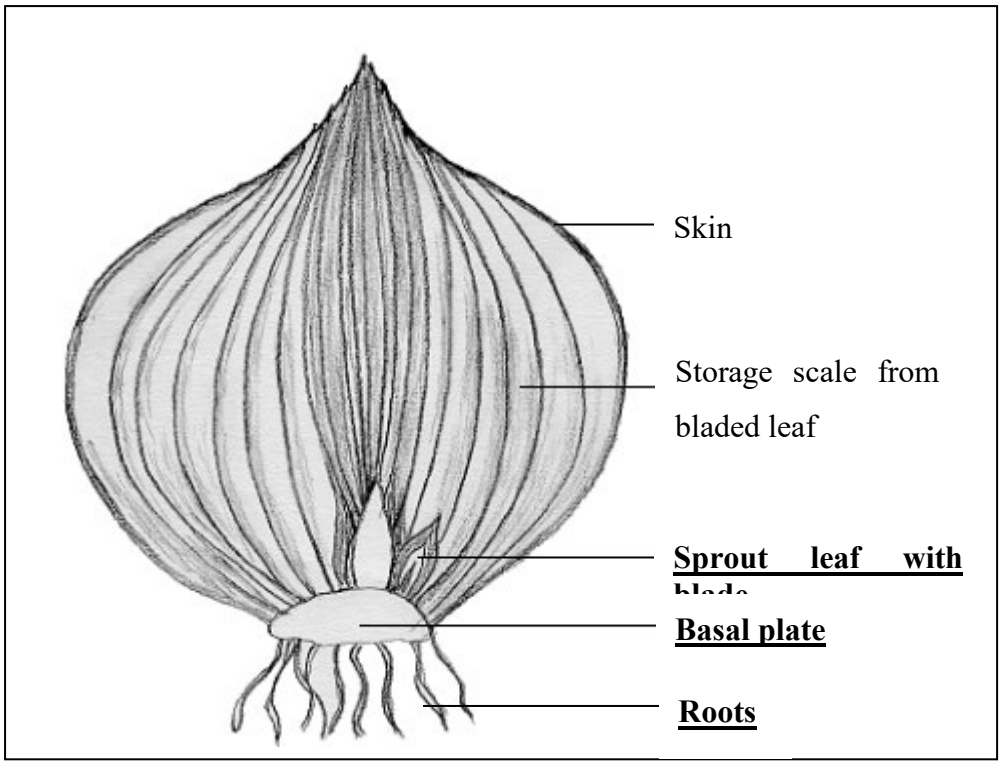

Figure 1. Annotated diagram of a cross-section of an onion bulb.

2.3 Dormancy

In onion bulbs a dormant period, when sprouting and rooting are not induced despite favourable conditions, is followed by a period when internal changes occur. These prepare the bulb for breaking of dormancy and subsequent growth. The bulb then 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 (Fig. 2). The growth rate of the sprout

inside the bulb is known to vary according to cultivar and storage regime, and is a major factor in determining the storage life of onions.

Figure 2. Cross sections of: A – an onion bulb with no sprout and B – an onion bulb with an external sprout.

2.3.1 Root dormancy

Bulbs with roots sprout earlier in dry storage than those where the roots have been removed. Therefore the root system may provide substances that promote sprout growth or elongation. Differences between cultivars in time to sprouting in store are more pronounced in de-rooted bulbs than in rooted bulbs (Miedema and Kamminga, 1994). 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 promotes sprouting and may do so by facilitating gas exchange and promoting respiration.

2.4 Biochemical changes during storage and sprouting

Onion cultivars suitable for growing in temperate regions such as the UK require long days for bulbing to initiate (Carter *et al.*, 1999). As such, the summer crop must be stored over the winter.

Many biochemical characteristics change during storage including water content, and the concentration of flavour compounds, organic acids, carbohydrates, minerals, plant growth regulators and volatile compounds. Changes in these characteristics 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 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.

2.4.1 Flavour precursors and pungency

Onions are eaten for their unique taste and the health giving properties of their flavour compounds (Randle, 1997; Griffiths *et al.*, 2002). These flavour compounds could have evolved from a survival mechanism: grazing animals may learn to avoid strong tasting plants (Havey, 1999), or for storage and transport of carbon, nitrogen and sulphur (Jones *et al.*, 2004). Many flavour compounds contain sulphur. Sulphate ions are taken up by the roots. The sulphate is reduced to sulphite and assimilated into cysteine. The tripeptide glutathione is formed and converted into S-2-carboxypropyl

glutathione which is metabolised through many gamma glutamyl peptides, terminating in *S*-alk(en)yl-L-cysteine sulphoxide (ACSO) synthesis (Block, 1992; Kopsell and Randle, 1997). ACSOs make up between one and five percent of the dry mass of an onion bulb (Jones *et al.*, 2004), representing major biosynthetic activity, and are responsible for the characteristic flavour of onions. Intact onion cells have no flavour. When cells are disrupted the vacuolar enzyme alliinase (*S*-alk(en)yl-L-cysteine sulphoxide lyase) hydrolyses flavour precursors *S*-alk(en)yl-L-sulphoxides (ACSOs) present in the cytoplasm. The products of this reaction are pyruvate, ammonia and unstable alk(en)yl sulphenic acids, which spontaneously rearrange into thiosulphinates that contribute to perceived flavour (Uddin and MacTavish, 2003) (Fig. 3). There are three main ACSOs in onion – methyl (MCSO), propyl (PCSO) and 1-propenyl (PRENCSO). 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).

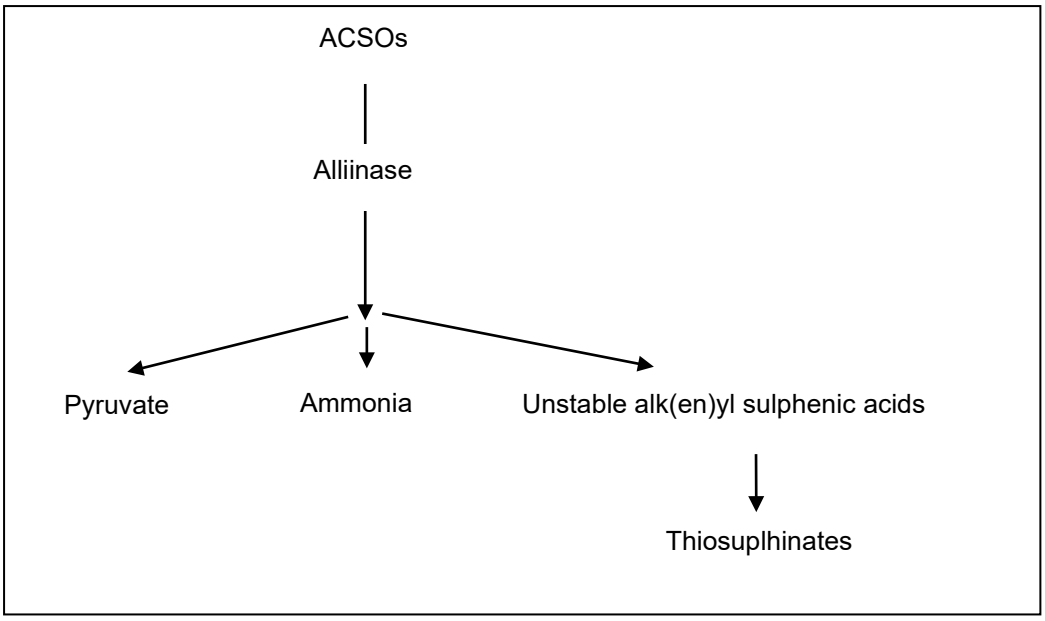

Figure 3. Simplified pathway of flavour production in onion bulb tissue.

Some ACSOs in onion are bound to glutamic acid as gamma glutamyl peptides and are not susceptible to the action of endogenous alliinase (Hanum *et al.*, 1995). Gamma glutamyl transpeptidase, in combination with exogenous alliinase, enhanced pyruvate production in macerated yellow sweet onions (e.g. cv. Vidalia) and onions cv. Soartan Banner. Addition of exogenous alliinase to macerated onion tissue increased the pyruvate production slightly, implying that the supply of endogenous alliinase may be the limiting factor in the reaction in onion tissue.

The amount of enzymatically produced pyruvate in onions is positively correlated with pungency and selection for less enzymatically produced pyruvate results in a milder onion (Havey and Randle, 1996; Havey, 1999). Relative pungency is dependent on 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). The composition and concentration of ACSOs

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

2.4.1.1 Relationship between pungency and pyruvate content

A highly significant correlation exists between both total pyruvic acid concentration and enzymatically produced pyruvic acid, with threshold olfactory perception (Schwimmer and Weston, 1961; Schwimmer and Guadagni, 1962). Threshold perception was defined as the minimum concentration of onion juice in water that could be detected by 70% of the judges. This demonstrated that the same enzyme system that produces volatile odour compounds when the cells of onion were disrupted, also gives rise to pyruvic acid, and that pyruvic acid concentration is a good indicator of pungency.

2.4.1.2 Sulphur nutrition

The application of additional sulphur in the range of 20-22.4 kg ha^{-1} to onions in the field did not affect the concentration of pyruvic acid in onion bulbs (Hamilton *et al.*, 1998; Abbey, 2003). However, additional sulphur treatments did affect bulb sulphur content and pungency in onion bulbs glasshouses (Hamilton *et al.*, 1998; Abbey, 2003) but there was only poor correlation between enzymatically produced pyruvate

and sulphur content, indicating that not all of the sulphur is assimilated into flavour precursors.

2.4.2 Carbohydrates

Water-soluble carbohydrates (WSC) 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). Changes in carbohydrates over the storage period have been summarised in Table 3.

Table 3. Summary of carbohydrate composition of postharvest onion bulbs and changes during storage at different temperatures.

Carbohydrate	Cultivar	Storage temperature	Storage duration	Effect	Reference
Soluble sugars: Sucrose Glucose Fructose	Cream Gold Syn.[1] Pukekohe Longkeeper	NA[2]	Recently harvested	⇑ Fructan towards the centre of the bulb. ⇓ Fructose towards the centre of the bulb. DP[3] inversely correlated with concentration. Glucose and sucrose were similar in all leaf bases.	Darbyshire and Henry (1978)
Total insoluble sugars Soluble sugars: Sucrose Glucose Fructose	Rijnsberger Robusta	4˚C	6 months	⇔ Insoluble sugars before and during sprouting. ⇔ Total soluble sugars before sprouting. ⇓ Total soluble sugars during sprouting. ⇔ Sucrose in inner scales. ⇑ Sucrose in outer scales. ⇑ Fructose over storage. ⇓ Higher sugars over storage.	Rutherford and Whittle (1982)

Sucrose Glucose Fructose Fructan	Variety of cultivars (n=8)	6-10˚C	2 months	⇓ Fructans over storage ⇑ Sucrose over storage	Suzuki and Cutliffe (1989)
Sucrose Glucose Fructose	Sentinel	O˚C 15˚C 30˚C Range of RH	20 weeks	RH had no effect on the carbohydrates measured. ⇑ Fructose at 0˚C and 15˚C. ⇑ Glucose in first 5 weeks at 0˚C and 15˚C. ⇓ Glucose after 5 weeks at 0˚C and 15˚C and from harvest at 30˚C. ⇑ Total sugars up to 5 weeks. ⇓ Total sugars after 5 weeks. ⇑ Fructose and glucose towards bulb centre.	Salama *et al*. (1990)

Sucrose Glucose Fructose Fructan[4]	Hysam Hystar Centurian	16˚C	15 weeks	Inner and outer scales: ⇔ Glucose and sucrose. ⇓ Fructans over storage. ⇑ Fructose 4-fold. Bulb base: ⇑ Fructans. ⇔ Sucrose, glucose and fructose.	Pak *et al.* (1995)
Fructan	Golden Bear Southport White Globe Stuttgarter Riesen Rolander Karato Super Bear	Unspecified	3 months	⇓ Total fructans during storage.	Ernst *et al*. (1998)
Glucose Soluble sugars	Rouge Amposta	18˚C	8 weeks	⇔Total soluble sugars ⇓ Glucose in inner bud	Benkeblia and Selselet-Attou (1999)

Fructan Fructo-oligosaccharides	Hysam	Unspecified	Unspecified Shortly after harvest?	High fructan concentration associated with high sucrose concentration and low free fructose concentration. DP inversely correlated with concentration. ⇑ Fructan from bottom to top of bulb ⇑ Fructan and sucrose towards centre of bulb ⇓ Free fructose towards centre of the bulb	Jaime *et al*. (2000)
Soluble sugars: Sucrose Glucose Fructose Fructan	Rouge Amposta	4˚C 10˚C 20˚C	6 months	⇑ Soluble sugars up to 6-8 weeks postharvest. ⇓ Soluble sugars from 6-8 weeks to end of storage. ⇓ Total fructans by 20% at 4˚C. ⇑ Total fructans 2 fold at 10˚C and 20˚C.	Benkeblia *et al*. (2002)
Soluble sugars: Sucrose Glucose Fructose Fructan	Rijnsberger Sherpa	2˚C 21% O_2 1% O_2 0.5% O_2	36 weeks	⇓ WSC with decreasing oxygen concentration. ⇑ Fructan with decreasing oxygen concentration. DP of fructans higher after low oxygen storage than in control.	Ernst *et al*. (2003)

[1] Syn.=Synonym. [2]NA=Not applicable. [3] DP = Degree of polymerisation.[4] Calculated as total carbohydrate minus sucrose, glucose and fructose.

⇓ = Increased; ⇓ = Decreased; ⇔ = No change.

At 15-16˚C, fructose levels increase over the storage period (Salama *et al.*, 1990) and fructan levels decrease (Suzuki and Cutliffe, 1989; Ernst *et al.*, 1998). The decrease in fructan concentration has been shown to begin two weeks prior to harvest in onion cv. Rijnsberger Hysam. This could be 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). Fructose concentration is higher in the outer leaves than the inner leaves (Darbyshire and Henry, 1978; Salama *et al.*, 1990). A maximum soluble sugar concentration occurs between five and eight weeks after harvest (Salama *et al.*, 1990; Benkeblia *et al.*, 2002). Salama *et al*. (1990) found that glucose was higher in the outer leaves than in the inner leaves; however Darbyshire and Henry (1978) and Pak *et al*. (1995) found no difference. 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). This suggests that the sucrose synthase pathway plays a more important role in sucrose degradation than the invertase pathway, as invertase was not detected in the bulb.

Fructan content in onion bulbs tends to decrease during refrigerated ambient atmosphere (Suzuki and Cutliffe, 1989; Pak *et al.*, 1995; Ernst *et al.*, 1998; Benkeblia *et al.*, 2000), low oxygen storage (Ernst *et al.*, 2003). An indication of the importance that fructans have in dormancy is demonstrated by the work of (Yamazaki *et al.*, 2001). The authors found that while total carbohydrate content was similar in dormant and non-dormant *A. wakegi* Araki (a hybrid between Japanese bunching onion, *A. fistulosum*, and shallot, *A. cepa* L. Aggregatum group) cultivars, the contribution of fructans and fructose to total carbohydrates prior to bulb development

was 35% and 30% in the dormant cultivar and 70% and 10% in the non-dormant cultivar respectively.

2.4.2.1 Carbohydrate content and storage life

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 of eight 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 levels of carbohydrate at harvest, as observed by (Wheeler *et al.*, 1998).

2.4.3 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.

2.4.4 Respiration

Staining with the redox reagent 2,3,5-triphenyl tetrazolium chloride (TTC) revealed a changing pattern of respiration activity during storage (Carter *et al.*, 1999). TTC is reduced to an insoluble red formazan derivative in tissues of high metabolic activity. At harvest 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. The Q_{10} of onion cv. Rouge Amposta bulbs O_2 and CO_2 respiration rates are 1.67 and 1.84 respectively, confirming that an increase in temperature increased the respiratory quotient (Benkeblia *et al.*, 2000). The respiratory quotient (RQ) is the ratio of the volume of carbon dioxide expired to the volume of oxygen consumed in a given period of time. 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 dependant on the physiological state of the bulb. The respiration rate of a dormant bulb is greater than that of a sprouting bulb sampled simultaneously.

2.4.5 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 cv. Rijnsberger (long storing) and Lancastrian (short storing) bulbs was measured by Thomas (1969) and Thomas and Isenberg (1972). The following pattern existed; gibberellins (GAs) had a first peak in December, followed by peaks of cytokinins and auxins. High auxin activity persisted as sprouting continued. A second gibberellin peak was accompanied by sprouting in March (Fig. 4). This gibberellin peak was more likely to be an effect of sprouting rather than a cause, as gibberellin activity was low in both non-sprouted and internally sprouted bulbs. The peaks in growth substances are thought to be responsible for; floral initiation under cold conditions (first GA peak), cell multiplication (cytokinins) and the initiation of sprout growth (auxins). The inhibitory substance detected by Thomas and Isenberg (1972) is now widely believed to be abscisic acid. 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).

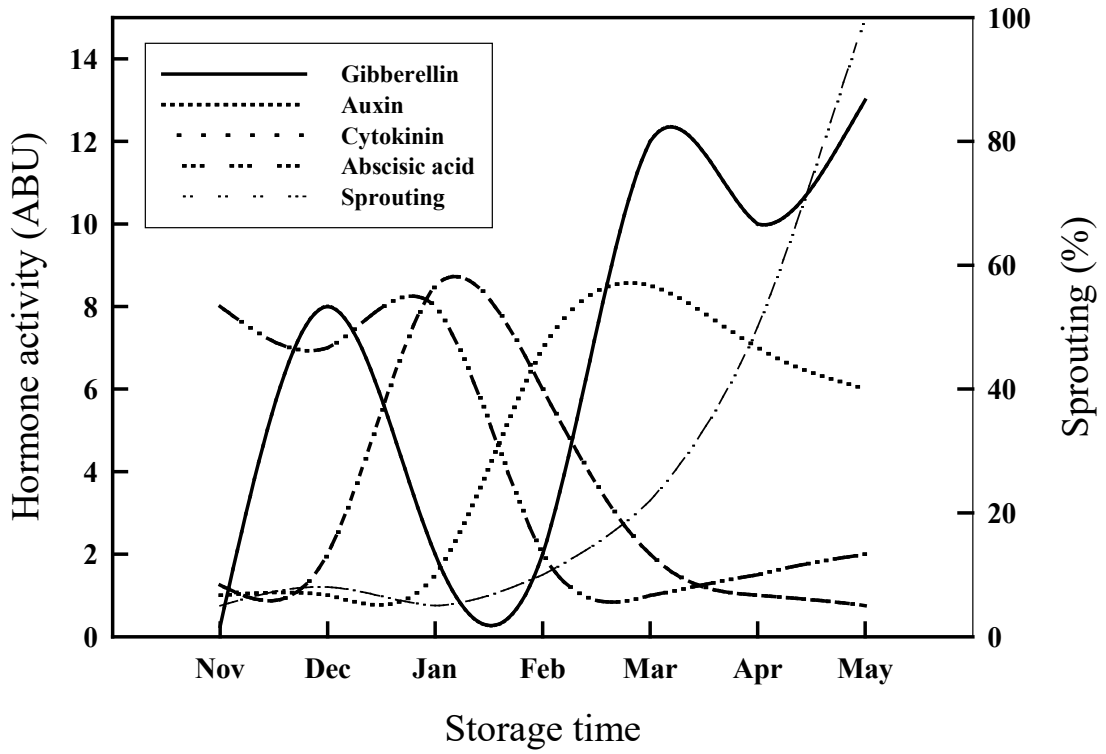

Figure 4. Percentage sprouting and hormone activity of onions cv. Rijnsberger stored at 5-8°C (after Thomas and Isenberg, 1972).

Therefore before an external sprout is visible, there are important internal changes that occur in the apparently dormant onion. 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).

2.4.5.1 Ethylene

Ethylene is a gas that is synthesised in plant tissue (Roberts and Hooley, 1988). It has physiological effects throughout the lifecycle of a plant, including germination,

growth ripening, senescence and abscission. The ability of a plant tissue to respond to or synthesise ethylene varies widely with both species and ontogeny. 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 (1999) found little variation in the ethylene production of 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. 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 at 2 months, 100% at 4 months) than those treated with ethephon alone and controls (50% sprouting at 3 months, 100% at 6 months (Benkeblia and Selselet-Attou, 1999b). 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).

2.4.5.2 Abscisic acid

Abscisic acid (ABA) is a naturally occurring phytohormone. The ABA biosynthesis pathway (Fig. 5) begins in chloroplasts and other plastids with the cleavage of a C_{40} carotenoid precursor to form xanthonin. In the cytoplasm, xanthonin is converted to

ABA via abscisic alcohol (Cutler and Krochko, 1999). ABA has many physiological effects, many to do with 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.

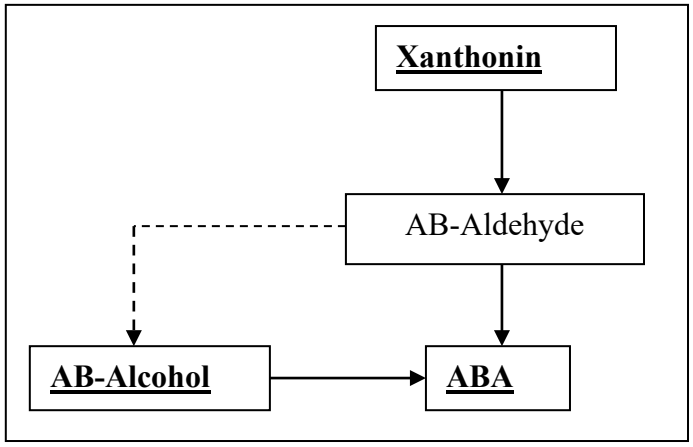

Figure 5. The synthesis of ABA from xanthonin in higher plants. Solid arrows indicate the major pathway, dashed arrows indicate a minor pathway. (AB=abscisic) (Cutler and Krochko, 1999)

Endogenous ABA is found in all onion tissues *viz*. leaf, growing tip, bulb and leaf sheath (Matsubara and Kimura, 1991). ABA accumulates in all tissues during the growing period. The actual concentration of ABA varies according to plant age and tissue type and is mediated by storage temperature regime. At low temperature (5°C) storage ABA concentration in the outer enlarged leaf sheaf of onion bulbs cv. Awaji Chūkodaka has been shown to rapidly decrease from 9 ng g^{-1} FW to 1 ng g^{-1} FW after one month, whereas at high temperature (10°C) storage a rapid decrease occurred a month later. In the inner storage leaf the ABA concentration increased to 10ng g^{-1}

FW at high temperature storage and 7 ng^{-1} g FW at low temperature and then decreased to 1 ng g^{-1} FW within one month and remained at this concentration until sprouting.

2.4.5.2.1 Abscisic acid and bulbing

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 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 peak concentration of 20 ng g^{-1} FW in plants subjected to long day conditions was observed (Yamazaki *et al.*, 1999b). During bulb development ABA concentration increased to 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 relative to control plants (Yamazaki *et al.*, 1999b). The leaf sheaf ratio is the ratio leaf sheath length to

the length the oldest unexpanded leaf, and is a measurement of the development of bulb scales.

2.4.5.2.2 Abscisic acid and dormancy

Abscisic acid has been associated with dormancy in onions. 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 result in increased sprouting 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. ABA concentration reaches 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. However, a crop might put on as much as 15-20% in yield after most of the tops fallen (Smith, B. 2004, pers. comm., 29 July).

The concentration of free endogenous ABA in the bulb basal leaf sheath after harvest is positively correlated with the number of days to sprouting in *Allium wakegi* (Yamazaki *et al.*, 2002). Application of 500 µM exogenous ABA to *A. wakegi* bulbs increased the number of days to sprouting, whilst application of 25 µM fluridone accelerated sprouting. Conjugated ABA changed only slightly during the entire experiment and was therefore unlikely to play a major role. The greatest change in endogenous ABA concentration occurred during the dormant period of the life cycle. Maximum days to sprouting, indicating the deepest dormancy period, occurred one

month before the maximum ABA concentration was recorded. A maximum ABA concentration occurred simultaneously in both harvested and non-harvested bulbs, suggesting that harvesting was not the cause of this peak. However, the maximum ABA concentration was higher in harvested bulbs, suggesting that harvesting may play a role in increasing ABA concentration. The postharvest decrease in ABA concentration corresponded with a significant decrease in the number of days to sprouting, indicating a loss of dormancy. Five weeks after the postharvest maximum, ABA concentration fell to 4 ng g^{-1} FW. Changes in water potential during different stages of dormancy could be the cause for decreasing ABA concentration of *A. wakegi* cv. Kiharabansei No. 1 during storage at ambient temperature (Yamazaki *et al.*, 1995). 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). 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).

2.4.5.2.3 Sensitivity to abscisic acid

Differences in sensitivity to ABA between different tissues, cultivars and life cycle stages may explain the lag in response to the peak in ABA concentration. 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 cv. Kiharabansei No. 1 (26 ng g^{-1} FW) than the cv. Ginoza (33 ng g^{-1} FW) suggesting that cv. Kiharabansei No. 1

responds to ABA at a lower concentration the cv. Ginoza does. Sprouting in *A. wakegi* cv. Ginoza can be delayed by 35 days by soaking in an aqueous solution of 100 μM ABA two weeks after harvest, whereas 1000 μM is required to delay sprouting by 18 days in cv. Kiharabansei No. 1. These results imply that ABA may be an important factor in onion storage life.

2.4.5.2.4 Abscisic acid analogues and inhibitors

The use of ABA analogues eg. 8'-methylene ABA and 8'-methylene ABA methyl ester (Abrams *et al.*, 1997; Churchill *et al.*, 1998; Pompodakis and Joyce, 2003) and inhibitors eg. tetcyclacis (Klein and Hebbe, 2000) of abscisic acid activity can be used to elucidate the importance of ABA in the storage life of onions. This approach is advantageous in that it takes no account of varying sensitivities to, or tissue compartmentalisation of, the phytohormone. ABA analogues have been developed to overcome the difficulties in using ABA for agricultural purposes as it is rapidly metabolised in plants. They also offer a potentially low-cost alternative.

2.4.5.2.5 ABA metabolism

The predominant pathway for ABA metabolism is hydroxylation at the 8’ position to give 8’-hydroxy-ABA, catalysed by the enzyme abscisic acid 8’-hydroxylase. 8’-hydroxy-ABA is unstable and readily converts to phaseic acid (PA), which can be reduced to dihydrophaseic acid (Cutler and Krochko, 1999) (Fig. 6). PA 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.

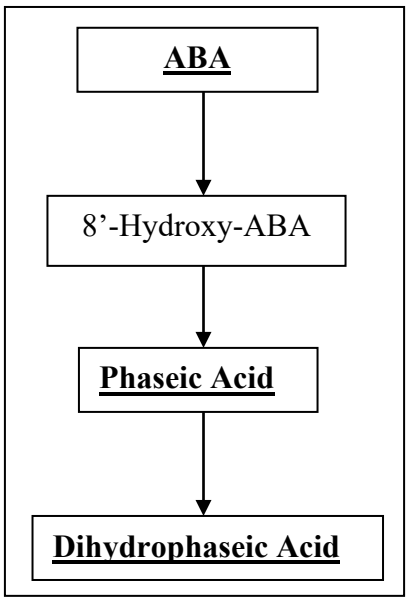

Figure 6. Metabolism of Abscisic Acid (ABA).

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 and a long photoperiod during bulb maturation. However, the new varieties of less pungent low dry matter onions grown for the fresh market 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.

2.5.1 Pre-harvest factors that affect storage life

Pre-harvest treatment and conditions in the field have a role to play in affecting the storage life. The use of pre-harvest sprout suppressants is discussed in a later in section 2.5.3.

2.5.1.1 Pre-harvest nutrition

Reduced nitrogen fertilisation causes 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. A 75% water deficit in the top 25 cm soil profile reduced postharvest sprouting; however bulbs from all treatments were stored until the same date despite different harvest dates. This meant that although samples were taken simultaneously, the time in storage was not the same. Therefore the reduced sprouting could have been due to the later harvest date and therefore shorter storage time.

Sulphur nutrition not only plays a part in the pungency of the bulb, but also impacts on dry weight and bulb quality. Onions grown in a hydroponic system with high sulphur nutrition are firmer than those grown in low sulphur nutrition, and have higher fresh weight (Lancaster *et al.*, 2001; Sorensen and Grevsen, 2001). As greater bulb dry matter is correlated with improved storage it is possible that sulphur fertilisation can help bulbs to store for longer, although with the increasing demand for less pungent onions it is unlikely that this strategy would be used by growers.

2.5.1.2 Growing season temperature

Storage potential varies between seasons, even though identical cultural, drying and storage regimes are followed, presumably due to the climatic variation between growing seasons (Rutherford and Whittle, 1982). Higher growing season temperatures reduce storage life (Rutherford and Whittle, 1982; Wheeler *et al.*, 1998; Sorensen and Grevsen, 2001). Wheeler *et al*. (1998) concluded that the time until onset of sprouting was not affected by growing season temperature, but that the sprouting rate increased with increasing 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. This method does not allow for any differences that may exist between the 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 accurate.

2.5.1.3 Crop maturity at harvest

The developmental stage of the crop at harvest impacts on both yield and storability. 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 and this encourages pathogen attack. Early harvested bulbs may not be dormant and are therefore 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 early harvested bulbs, dried and stored in the same manner as later harvested bulbs, had lower carbohydrate levels, which were reduced further during sprouting, which occurred earlier.

2.5.1.4 Harvesting process

It is important that physical damage to onion bulbs during harvesting is limited, especially with softer, less pungent onions, because wounding, particularly of the basal plate, causes them to sprout (Miedema, 1994b) and increases storage losses due to rotting (Herold *et al.*, 1998). The mechanism by which this occurs is not known, but is probably not due to cytokinins. Undercutting is usually performed prior to mechanised lifting, often done with modified potato lifters. The aerial parts and roots are removed before onions are stored in bulk; this aids airflow among the bulbs. In temperate countries tops are removed before harvest when a heated, forced air ventilation store is available for immediate curing (Gubb and MacTavish, 2002).

2.5.2 Postharvest factors that affect storage life

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 that act as a barrier against water loss and infection. Curing is complete when the necks have dried out and are tightly closed, and the skins rustle and have an attractive colour. The time this takes depends

on the temperature and relative humidity of the forced ventilating air and the maturation stage of the bulbs. Windrowing is a method of traditional field curing where detached bulbs shaded by their tops are laid on their side to dry for one or two weeks. The direct harvest method is good practice in temperate regions. The bulbs are moved straight to storage after lifting and cured in bulk stores using air at 30-32ºC. After three to five days the temperature is lowered to 27ºC and relative humidity (RH) 70-75% for about twenty days to complete the drying process.

2.5.2.1 Storage conditions

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

2.5.2.1.1 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 (Brewster, 1977b; Miedema, 1994a; Ernst *et al.*, 1999). Different cultivars respond differentially to temperature. 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 (Gubb and MacTavish, 2002).

2.5.2.1.2 High temperature storage

In warm climates, such as the tropics, high temperature storage is a practical option. High temperature storage conditions are a compromise between rotting loss and sprouting loss. Ventilation of storage bins to reduce fluctuations in temperature and humidity reduces the rate of external sprouting, bacterial infection and dehydration over 31 weeks of storage in the red onion cv. Baftain (Brice *et al.*, 1995). High temperature storage conditions are generally 25-30ºC, and 60–75% RH. 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 postharvest high temperature treatments of 30ºC and 35ºC significantly reduced the number of days to sprouting in dry storage at 15ºC, when compared to those treated with 15 and 25ºC, which were not significantly different from one another. 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 (Miedema, 1994a).

2.5.2.1.3 Low temperature storage

In developed temperate countries, onions are kept in large, specialised stores. Ventilation is forced, and temperature is usually maintained around 5ºC, but can be as low as -1ºC. This is the most important storage strategy in the UK.

Short term chilling treatments at 0°C or 9°C for two or three weeks decreased the time to sprouting in onion cv. Rouge Amposta bulbs 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 compared to 20% in the control group. The cold treated bulbs also generally had a lower concentration of soluble sugars (Benkeblia and Selselet-Attou, 1999a). Therefore it is important that the chilling treatment is maintained in order to extend storage life.

2.5.2.1.4 Effect of temperature on carbohydrates

The pattern of changes in total soluble sugar content of onion cv. Rouge Amposta bulbs is similar at 4°C, 10°C and 20°C, with a maximum concentration occurring at approximately seven weeks storage. 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 because of the enzymatic hydrolysis of fructan polymers by depolymerases. The similarity in the pattern of change suggests that the catabolism of carbohydrates is more dependent on physiological stage than temperature (Benkeblia *et al.*, 2002). In contrast, fructose concentration was higher in onion cv. Sentinel bulbs stored at 0 and 15ºC than in those kept at 30ºC, suggesting low temperature hydrolysis of fructans. A net increase in total sugars was observed at 0ºC, and a net decrease observed at 15 and 30ºC (Salama *et al.*, 1990).

2.5.2.2 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 breakdown. Carbon dioxide concentrations above 10% for short-term storage, and 1% for long-term storage, can cause accelerated softening, rooting and a putrid odour CA is also very expensive and therefore reduces the profit margin of the crop.

Quality attributes (colour, texture and flavour) of onions cv. Rijnsberger Sherpa were maintained throughout low oxygen (0.5-1.0% O_2) storage at 2˚C, and this tolerance has been attributed to the presence of fructans (Ernst *et al.*, 2003; Praeger *et al.*, 2003). The fructan and sucrose content of bulbs was found to be greater in those stored at 0.5 and 1% O_2 than in the controls stored at 21% O_2, indicating that the decomposition of carbohydrates is slowed or inhibited by low oxygen levels during cold storage, and accelerated fermentation does not occur in onions as in other plant species, but sugars and fructans are accumulated.

The pyruvate concentration in onion bulbs cv. Hysam decreased after nine weeks of storage at 0.5ºC in the following controlled atmosphere conditions; 2% O_2 and 2% CO_2, and, 2% O_2 and 8% CO2. However, the pyruvate concentration of bulbs in ambient atmosphere storage increased (Uddin and MacTavish, 2003). The decrease

in pyruvate concentration was greater in onions stored at a carbon dioxide concentration of 8%. Although the difference was statistically significant, it may not have been detectable by humans as it was less than 1 µmol - the range of human detection is between 1 and 7 µmol (Uddin and MacTavish, 2003). ACSO content followed the same pattern but the decline was greater than that in pyruvate. The proportion of ACSOs changed over time, and freshly cured onions, ambient atmosphere stored onions and CA stored onions would have different flavour characteristics. Alliinase activity was less in onions stored in atmospheres with a greater proportion of carbon dioxide.

2.5.2.3 Humidity

The relative humidity (RH) of the storage environment is a compromise between keeping levels below that at which pathogens are encouraged and above that at which water is rapidly lost from the bulbs. The outer skins protect against water loss, and they tend to crack and fall off at <55% RH, and pathogen attack is encouraged at >80% RH, therefore RH in the storage environment should be maintained between 55-80%. 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 effect on the properties of onion skins (Hole *et al.*, 2000). This is significant as the ability of onions 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. Relative humidity does not effect the concentration of glucose, fructose and sucrose in onion bulbs cv. Sentinel (Salama *et al.*, 1990).

2.5.2.4 Irradiation

Irradiation is not popular for food use in many countries, but is an effective method of long-term sprout control and reduced 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 0.15 kGy) decreased the respiration rate of onions cv. Rouge Amposta, probably through degeneration of meristematic cells and the death of the sprout caused by the radiation, which slows down the complete respiratory pathway including glycolysis (Benkeblia *et al.*, 2002). After 24 weeks at 20°C, 5% of irradiated bulbs had sprouted, compared with 75% of controls. However, refrigerated storage at 4°C was as effective as ionising radiation in prolonging storage period.

In general ascorbic acid and carbohydrate levels were higher in onion bulbs cv. Valencia sintética 14 irradiated 30 days postharvest with a ^{60}C source and a dose of 50 Gy at 20°C, and stored in warehouse conditions (6 to 32°C and 40-50% RH) for 300 days (Croci *et al.*, 1995). This may have to been due increased ease in extracting these substances because of the treatment or a delay in their metabolism.

2.5.3 Sprout suppressants

Sprout suppressants are chemicals that may be applied to the crop pre- or postharvest and which slow or prevent the growth of the sprout.

2.5.3.1 Maleic hydrazide

Maleic hydrazide (1,2-dihydro-3,6-pyridazinedione) (MH) is a chemical isomer of uracil that is applied as a preharvest spray to inhibit subsequent sprouting of bulbs in store (Fig. 7).

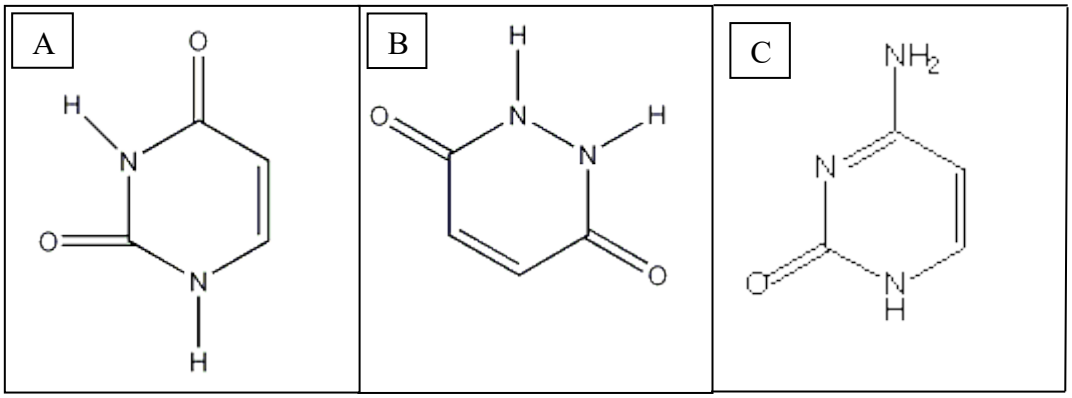

Figure 7. Chemical structure of: A – Uracil. B – Maleic hydrazide. C – Cytosine.

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

MH 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 response to a metabolic inhibitor and is a morphological manifestation of blocked transcription. In this way MH affects the biosynthetic activity of the nucleolus. Maleic hydrazide causes 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. Maleic hydrazide has no effect on sugar and organic acid composition of the bulbs (Salama *et al.*, 1990).

2.5.3.2 Nitrous oxide

Nitrous oxide (N_2O) is similar to carbon dioxide in terms of relative stability and high solubility in water. N_2O 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, and 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 control bulbs, whereas the respiration

rate of bulbs treated for 10 and 15 days was approximately 0.8-fold less than that of the control (Benkeblia and Varoquaux, 2003). Five days treatment decreased rots, whereas ten and fifteen days of treatment increased rots. Total soluble sugars were less in control bulbs. Citric acid increased as a function of N_2O concentration. Succinate, malate, fumerate and oxalate were increased in treated bulbs compared to the control. There was no effect on visible sprouting, but internal sprouting was not assessed. Accumulation of malic acid could be due to its metabolic function during the glycolytic pathway where it is the result of transformation of succinate.

2.5.3.3 Alternative strategies to delay sprouting

There are very few genetic resources for onions. Genomes within the Alliums are large. Onions are diploid (2n=16), with an estimated nuclear genome size of *ca*. 15 290 Mbp per 1C (King *et al.*, 1998). 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 can be assigned (Jones *et al.*, 2004). 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 the 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 would maintain quality over a longer

storage period. Diagnostic tests that could predict sprouting would be beneficial for growers to predict both the storage life and the shelf life of the crop. 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 considerable consumer hostility towards genetically modified crops.

2.5.4 Potential indicators of dormancy

Several parameters that change over the storage period have been considered as indicators of onion bulb dormancy including carbohydrate concentration, pungency, ACSOs - these have been discussed in previous sections. Other potential indicators of dormancy are discussed here.

2.5.4.1 Mitotic activity

Control of cell division in the shoot apex 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 in 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. Expression levels in all parts of the bulb (outer,

middle and inner) decreased from 100 days before harvest until harvest time. The highest initial level was found in the inner bulb, about two thirds less in the outer bulb, and about a third less than that in the mid bulb. Throughout storage, levels remained constantly low in the outer and mid bulbs but levels in the inner bulb peaked at 140–160 days, coinciding with the onset of visible sprouts, and decreased thereafter. A peak in histone 2A appears at the same time point (March to April) in cultivars with varying storage potential, and does not correspond with the developmental stage of the bulbs. The genetic basis of control of sprout leaf elongation is not known, but will play a large part in the determination of storage capacity.

2.5.4.2 Starch in the primary thickening meristem

The primary thickening meristem (PTM) is located near to the apical meristem and is responsible for stem thickening and root initiation (Ernst *et al.*, 1999). A sharp increase in the level of starch in the PTM during storage has been measured in a range of cultivars with different storage lives, but does not consistently interact with sprout growth. Ernst *et al*. (1999) concluded that the increase in starch was indicative of root dormancy rather than shoot dormancy.

2.6 Perspectives

The challenge is to determine what changes are occurring in the onion bulb during storage, with particular reference to those occurring around the time of dormancy breaking to identify potential targets for manipulation to delay sprouting.

The effect of synthetic analogues of abscisic acid on onion dormancy has not been investigated. If synthetic analogues with more potent or longer lived hormonal activity could be successfully applied to onions before or after harvest then the storage period may be extended. Extended periods of high levels of abscisic acid may negate the effects of decreasing sensitivity to ABA.

Once biochemical targets have been identified the further challenge will be molecular work to collect genomic, EST and transcript information on onion as a background to marker assisted breeding programmes or genetic modification.

3.1 Plant material

Onion cvs. SS1, Ailsa Craig and Rijnsberger Renate were drilled on 18/03/2003 and grown in sandy loam soil field at FB Parrish & Son (Bedfordshire, UK) (Fig. 1). The onions were drilled in four rows per bed using a tape seeder at a rate of 35 seeds m^{-2}. 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 from a 3 m length of the middle two rows of a four row x 5 m plot on a 1.8 m wide bed, into standard 25 kg plastic nets and dried with ambient air in bin driers for three weeks. The earlier maturing cv. SS1 was harvested on 19/08/2003 and the cvs. Renate and Ailsa Craig bulbs were harvested on 02/09/2003.

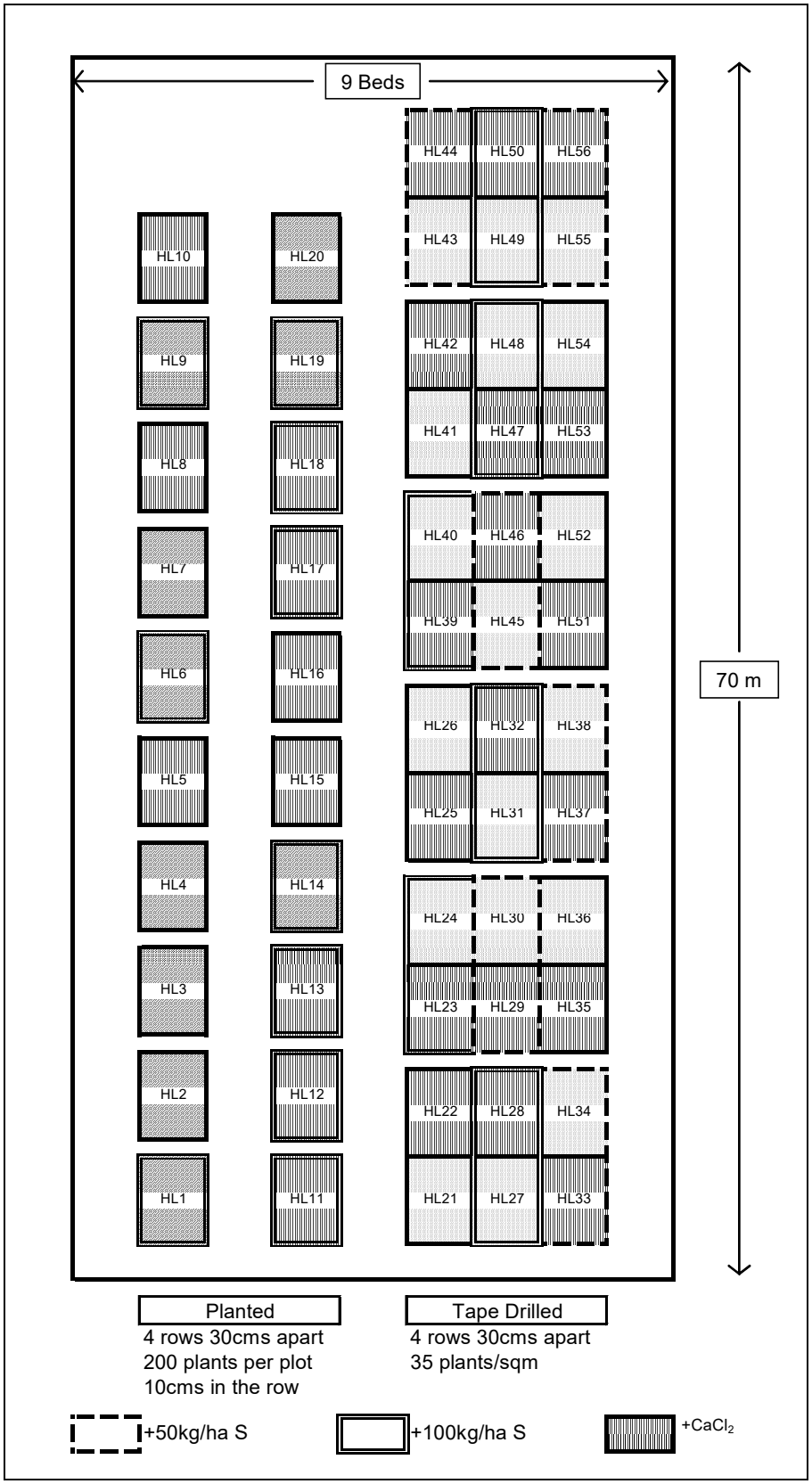

Figure 8. Diagram of the plot layout within the field. Plots shaded in grey were those used in this investigation.

3.2 Storage regime

The dry aerial parts and roots were removed, and any diseased or damaged bulbs discarded prior storage. Bulbs were held under controlled atmosphere (CA; 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 h against 5% CO_2 in N_2 (BOC). Bulbs were stored at 2 ± 1°C inside rigid polypropylene fumigation chambers.

3.3 Measurements

Bulb firmness, sprouting and rooting, total soluble solids and pyruvate measurements were made on each replicate at each outturn during the storage period for all treatments and cultivars. At each outturn, except outturn 0, two sets of three bulbs were sampled. At outturn 0 one set of three bulbs was sampled. Each set of three bulbs for each outturn and treatment were aggregated prior to abscisic acid and fructan analysis; therefore these values represent the mean of the replicates for each outturn, each treatment and each cultivar.

3.3.1 Bulb firmness

Onion bulb firmness was assessed according to Lancaster *et al*. (2001) with modifications. A 10 mm flat head probe was mounted onto the crosshead of an

Instron Series IX 4301 Universal Testing Machine (Instron, High Wycombe, 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 per minute. The gradient of the line produced when force was plotted against extension indicated the resistance to pressure, and therefore the firmness of the bulb. The mean of two measurements were used for analysis, with the second measurement taken at a right angle to the first.

3.3.2 Sprouting and rooting

Incidence of sprouting (internal and external), sprout colour and incidence of rooting were recorded.

3.3.3 Total soluble solids

Onion juice samples were extracted by squashing a *ca*. 5 mm equatorial slice in a hand-operated press. Total soluble solids content (TSS; °Brix) of the juice was measured using a digital hand held refractometer (Palette 100, Atago Co. Ltd., Tokyo, Japan).

3.3.4 Dry weight

The percentage dry weight was calculated from the weight loss of samples freeze dried (Edwards Super Modulo, Sussex, UK) for seven days.

3.3.5 Mineral analysis

Mineral concentrations were measured in ground, lyophilised bulb tissue. The samples were ashed and then digested with 1 ml concentrated nitric acid. The intensity of ion response was measured by ICP-AES (Inductively coupled plasma – atomic emission spectroscopy) and concentrations were determined by comparison with external standards. Results were expressed as total ion concentrations.

3.3.6 Pyruvate

Total pyruvate concentration was measured as an indicator of pungency according to an established method (Randle *et al.*, 1993) adapted to a microplate method. Juice was expressed from a *ca.* 5 mm equatorial bulb slice using a hand-operated press and incubated for 15 min to allow the formation of pyruvate from the enzymatic hydrolysis of flavour precursors. A sample of juice was collected in a 1.5 ml Eppendorf tube, frozen at -20°C, and then allowed to thaw. The liquid was separated from the semi-solid matter by centrifugation at 8000 rpm for 4 min (Desaspeed MH-2, Sarstedt, Leicester, UK). 100 µl of the sample (200 µl when analysing SS1) was placed in a 10 ml tube to which 4 ml (3.9 ml when analysing SS1) trichloroacetic acid (TCA) (Sigma, Dorset, UK) solution (1 part 3% TCA to 7 parts deionised water) was added and mixed well. A 30 µl aliquot of the sample solution was added to a microplate well (Cellstar 96 well flat bottom plate with lid, Greiner Bio-One, Gloucester, UK) in quadruplicate. 50 µl of 2,4-dinitrophenylhydrazine in 2N HCl (DNPH) (Spa Contract Synthesis, Coventry, UK) solution (3 parts 0.0125% DNPH to 2 parts deionised water) was added to each well and the microplate was incubated at

37°C for 10 min. After incubation 150 µl of 0.6N NaOH was added to each well and the absorbance of the samples at 450 nm was immediately read on a spectrophotometer (BP808, Biohit, Devon, UK) along with blanks. The concentration of pyruvate in the sample was calculated using a calibration curve constructed using the absorbance of a range of pyruvic acid (Sigma) standards (16, 12, 8, 4, 2 and 1 µm pyruvate ml^{-1}) assayed in eight replicates with each batch.

3.3.7 Abscisic Acid Radioimmunoassay

ABA was quantified according to Quarrie *et al*. (1988) with some modifications. All reagents were purchased from Sigma, Dorset, UK unless otherwise stated. Sample extraction was carried out under conditions of low light intensity as ABA is photosensitive (Dorffling and Tietz, 1983). Ground lyophilised bulb tissue (50 mg) was extracted overnight in 1:20 parts tissue to sterile distilled water 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 UK, Leicestershire, UK) for 10 min at 4°C and the supernatant used. DL-cis, trans-[G-3H] Abscisic acid (Amersham International, Bucks., UK) in 100 % ethanol was diluted 10-fold in sterile distilled water and frozen in 500 µl aliquots. For assaying, this stock solution was diluted further to 4.8 µl ml^{-1} in phosphate buffered saline (PBS) (50mM sodium phosphate - 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 MAC252 (Steve Quarrie, John Innes Centre, Norwich). MAC252 was 1:1000 diluted in PBS containing 5 mg ml^{-1} bovine serum albumin and 4 mg ml^{-1} soluble polyvinylpyrrolidone (PVP; MW 40000) to enhance binding of the

antibody (Quarrie *et al.*, 1988). Incubations were carried out in duplicate. 50 µl sample or ABA standard, 100 µl ^{3}H ABA, 100 µl of MAC252, and 200 µl 100% PBS were added to 2 ml microtubes with push-in caps (Sarstedt, Leicester, UK), and incubated in the dark at 4°C for 45 min. 500 µl saturated ammonium sulphate ($(NH_4)_2SO_4$) was added to precipitate the antibody. 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., Cambridgeshire, UK) to pellet the antibody. The pellet was washed with 1 ml of 50% saturated $(NH_4)_2SO_4$, and centrifuged for 4 min at 8800 rpm (Eppendorf Centrifuge 5413). 100 µl of sterile distilled water was added and left for 10 min before vortexing to resuspend the pellet. 1.2 ml EcoScint H (National Diagnostics, Yorkshire, UK) was added to the tubes 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) and counted on a ^{3}H program in a liquid scintillation counter (LS 6000TA, Beckman Coulter (UK) Ltd., Buckinghamshire, UK). Before counting, the tubes were wiped with a damp cloth to remove any static. Concentrations of ABA were calculated from the radioactivity (counts per minute; cpm) present in the pellets. The calibration curve was produced from two replicates of five ABA standards ranging from 2000 to 62.5 pg per tube assayed with each batch.

3.4 Fructan

Fructan concentration in ground lypohilised bulb tissue was measured using a fructan assay kit (Megazyme, Ireland) according to the manufacturer's method (AOCC

method 999.03, AACC method 32.32). 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 fructose to hydrolyse fructans to fructose and glucose, which are subsequently measured by the PAHBAH (*p*-hydroxybenzoic acid hydrazide) method.

3.4.1 Thin layer chromatography of fructans

Thin layer chromatography (TLC) was used to qualitatively validate the results of the fructan assay. Samples were prepared according to an established method (Pollock and Jones, 1979) with modifications. Ground lyophilised bulb tissue (25 mg) was boiled in 1 ml of 80% ethanol in a screw cap tube for 3 min. After two extractions in 1 ml of boiling sterile distilled water, and one further extraction in 1 ml of boiling 80% ethanol for 3 min, the aqueous and ethanol extracts were pooled and reduced to 1 ml volume by heating to a constant 50°C. A mixture of 50 mg Amberlite Cg 400 (Sigma) and 50 mg Amberlite IR 120 (Sigma) in 15 ml polypropylene tubes was washed with 5 ml sterile distilled water on a tube rotator for 5 min. The water was removed after centrifugation (MSE Mistral 2000, Sanyo) at 3000 rpm for 5 min. The concentrated fructan extracts were diluted to 5 ml with sterile distilled water, and then mixed with the washed Amberlite for 20 min on a tube rotator. The Amberlite was removed by centrifugation (MSE Mistral 2000, Sanyo) at 3000 rpm for 5 min. Sucrose and (d) fructose standards were prepared at 5 mg ml^{-1}.

20 cm x 20 cm Silica Gel 60 TLC plates (Merck, Darmstadt, Germany) were activated by heating to 110°C for 15 min. 6 µl of each standard and 12 µl of each fructan extract were loaded 1.5 cm above the base of the plate. The running solvent was 100 ml 2:1:1 (v/v/v) ethyl acetate : acetic acid : water (L. Trueman, pers. comm.). The plate was developed at room temperature (n=3), sprayed with urea phosphoric acid reagent (Wise *et al.*, 1955) and baked for 30 minutes at 110°C.

3.5 Data analysis

All statistics were carried out using Genstat for Windows Version 7.1.0.198. Treatment means were separated by the least significant difference (LSD) test at p=0.05.

4 CHAPTER FOUR: VALIDATION OF THE ABSICSIC ACID (ABA) RADIOIMMUNOASSAY

4.1 Thin layer chromatography of abscisic acid

TLC was used to investigate the spread of immunoreactivity of samples on the TLC plate compared to that of pure ABA. The concentration of ABA in samples eluted from TLC plates was determined by radioimmunoassay (as described in section 3.3.7), using 50% of the sample volume in each replicate adjusted accordingly with PBS.

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. 20 cm x 20 cm TLC plates (Merck, Darmstadt, Germany) were cleaned by three runs in 100 % methanol followed by two runs in chloroform prior to activation by heating to 120°C for one hour. The running solvent was 100ml of 10:1:1 (v/v/v) isopropanol : ammonia : water (Dorffling and Tietz, 1983). 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% methanol; samples on plate 2 were eluted overnight at 4°C in the dark with 300 µl of 80% methanol, followed by a 6 hour second elution. 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.

The Rf value of ABA in the 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 (Fig 1). Radioimmunoassay 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 Rf value of ABA indicating that the elution step is inefficient.

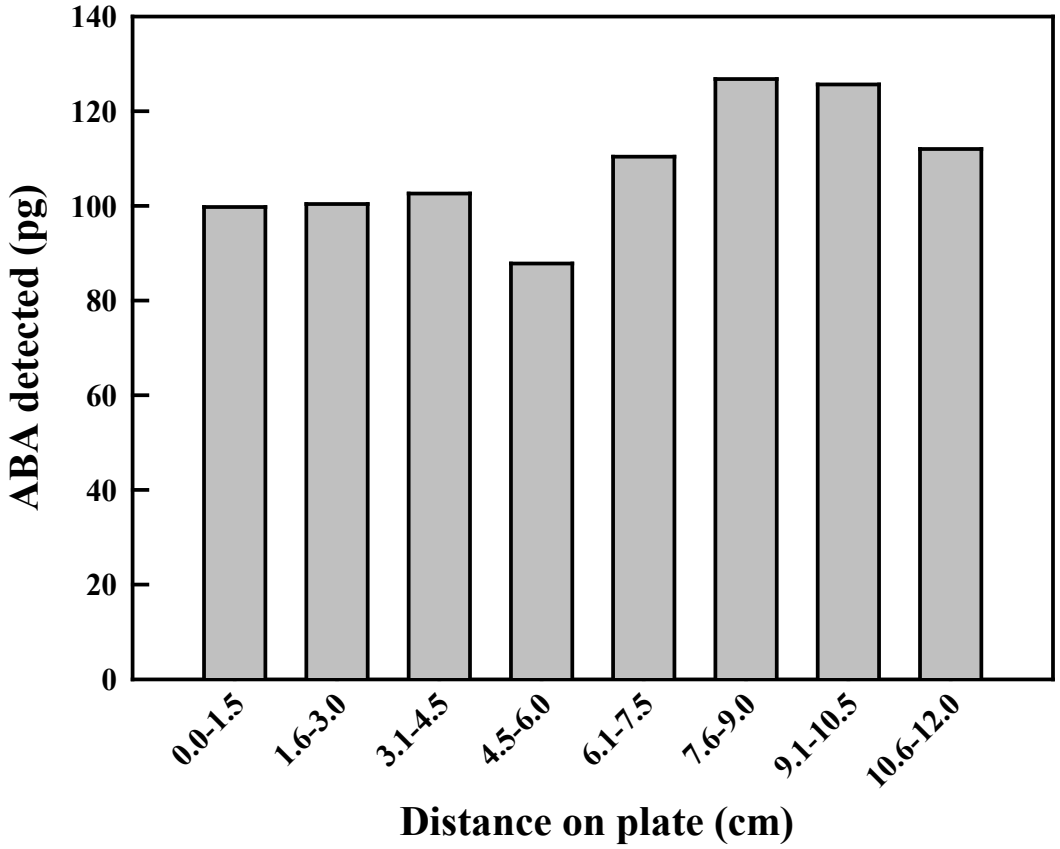

Figure 9. ABA detected by immunoassay in samples eluted from a thin layer chromatogram of crude onion extract dissolved in methanol.

The sensitivity of the assay was investigated by plotting the amount of ABA detected in the samples eluted from the Rf 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) (Fig 2.). ABA detected in the appropriate Rf 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.

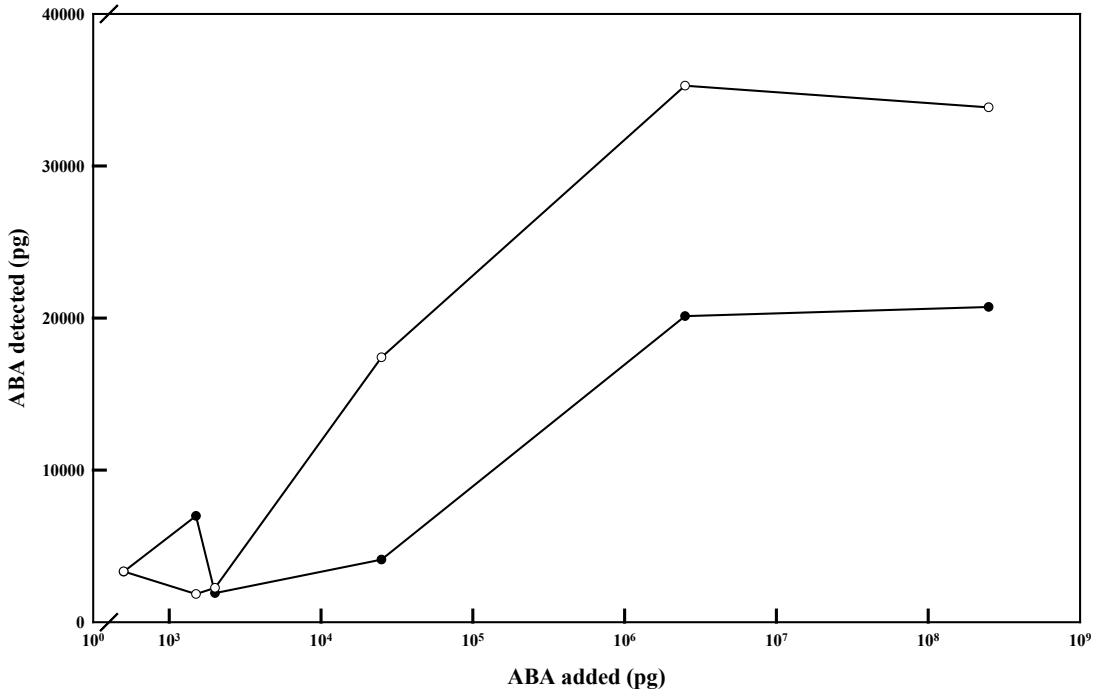

Figure 10. ABA detected in the samples eluted from the appropriate Rf zone of a thin layer chromatogram of known amounts of ABA dissolved in methanol (open symbols) and the mean ABA detected by immunoassay in each zone – the background (closed symbols).

4.2 Spike dilution

A spike dilution 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). Various combinations of cultivar, frozen and lyophilised tissues, water and methanol extractions, and purification processes were used (Appendix I). All initial extractions were performed as in section 3.3.7. Controls were sterile distilled water in place of tissue extract.

Linear regression models using Genstat for Windows version 7.0 were applied to the plots obtained from the spike dilution assays. 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 1).

Table 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).

* $p = <0.001$ ** $p = \leq 0.05$. P values represent the significance of the difference between the intercept of the reference level (control) and each dilution factor. [M] Samples extracted using methanol.

[P] Samples purified following extraction. Samples taken from onion cv. [A] Ailsa Craig or [R] Renate bulb.

All of the lines share 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 the 20 dilution factor. 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.

4.3 Conclusion

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

5 CHAPTER FIVE: RESULTS

5.1 Sprouting

The growth of a green sprout within the bulb indicates the end of the storage period for marketable onion bulbs. Sprout length increased significantly during storage in onion cvs. Renate (p=0.009), Ailsa Craig (p=0.056) and SS1 (p=<0.001) bulbs (Fig. 1). The use of a controlled atmosphere (CA) storage environment extends storage life of onion bulbs, however, the conditions within the storage chambers were humid, and therefore it was often the incidence of disease that ended storage life before sprouting occurred. This was most pronounced in onion cv. SS1 and cv. Ailsa Craig bulbs. No external sprouting was observed.

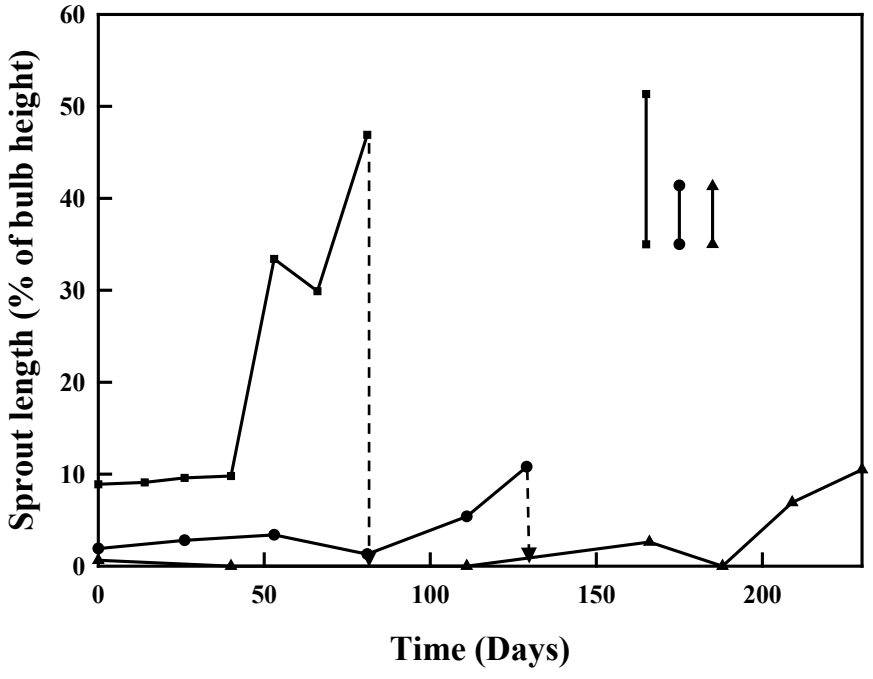

Figure 11. Changes in the sprout length of onions cv. over time in onion bulbs cv. Renate (▲), Ailsa Craig (●) and SS1 (■) in controlled atmosphere storage. LSD bars (p=0.05) are shown, symbols correspond to cultivar. Time zero: n=4, all other times: n=8. Drop down arrows indicate the last sample taken.

5.2 Carbohydrates

5.2.1 Fructan

The fructan content in bulb samples taken prior to CA storage was significantly different (p=<0.001) in each of the three cultivars tested. Bulbs cv. Renate had the highest proportion of fructan (28.50%), followed by cvs. Ailsa Craig (13.13%) and SS1 (3.10%) (s.e. 1.717, LSD p=0.05 = 5.491). Fructan concentration changed significantly (p=<0.001) over time in onion cv. Renate bulbs (Fig. 2) stored under CA at 2°C. This difference was due to the 1.9-fold decrease that occurred between day 40 and day 111. No other differences between the means were significant. The fructan content of cv. Ailsa Craig and SS1 bulbs did not change significantly over time. Onion cvs. Renate and Ailsa Craig bulbs grown with additional calcium contained significantly (p=0.002) less fructan (cv. Renate - 13.16%; cv. Ailsa Craig – 5.93%) than those without (cv. Renate – 18.24%; cv. Ailsa Craig – 11.90%), while onion cv. SS1 bulbs grown with additional calcium contained significantly (p=<0.001) more fructan (3.38%) than those without (1.83%). Onion cv. Renate bulbs grown with additional sulphur contained significantly (p=<0.001) less fructan (18.62%) than those without (12.78%). Sulphur treatment had no effect on the fructan content on onion cv. Ailsa Craig or Renate bulbs.

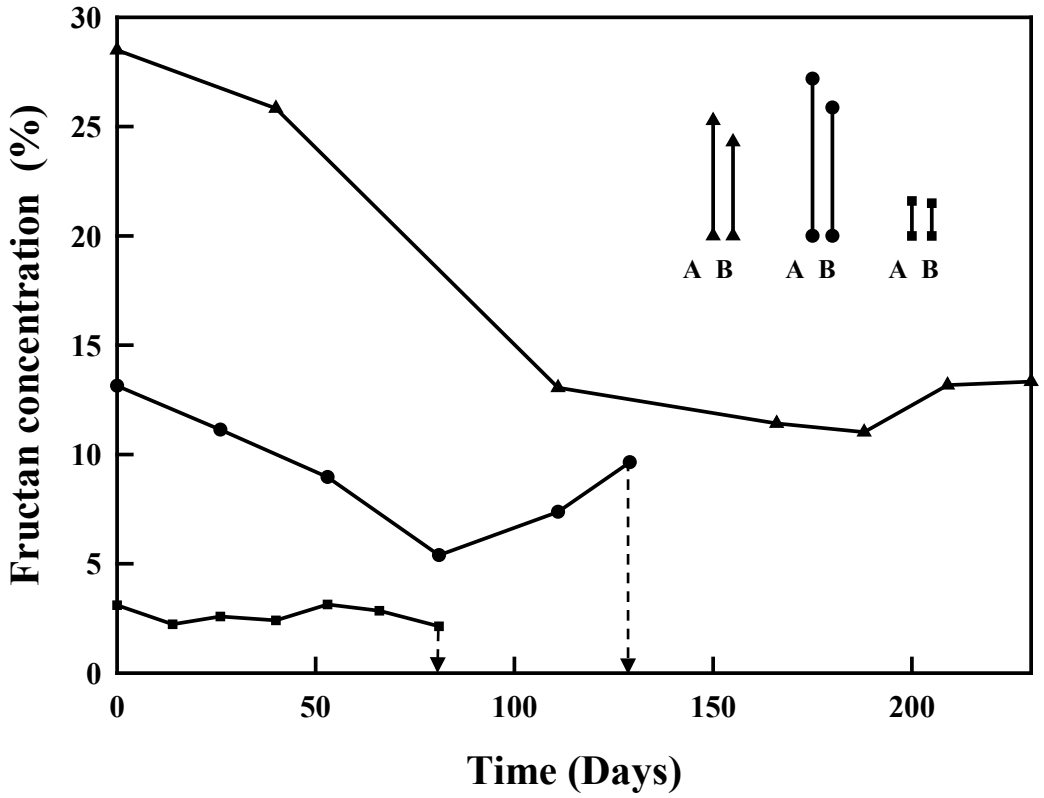

Figure 12. Changes in fructan content by percentage composition of dry weight over time in onion bulbs cv. Renate (▲), Ailsa Craig (●) and SS1 (■) in controlled atmosphere storage. LSD bars (p=0.05) are shown, symbols correspond to cultivar. The LSD bar A is for comparison of time zero with all other times and bar B is for comparison of all times except time zero. Drop down arrows indicate the final sample taken. Time zero: n=4, all other times: n=8.

Fructan extracts from onion cvs. Renate, Ailsa Craig and SS1 bulbs sampled at various times during CA storage were separated by TLC (Fig. 3). There was no difference in the intensity of the colour on the chromatogram over time which indicated that the amount of fructan in the sample did not change over the storage period. Fructans could not be clearly identified in fructan extracts from onion cv. SS1 bulbs, which confirms that the fructan concentration in onion cv. SS1 bulbs was much less than that in other onion cvs. tested.

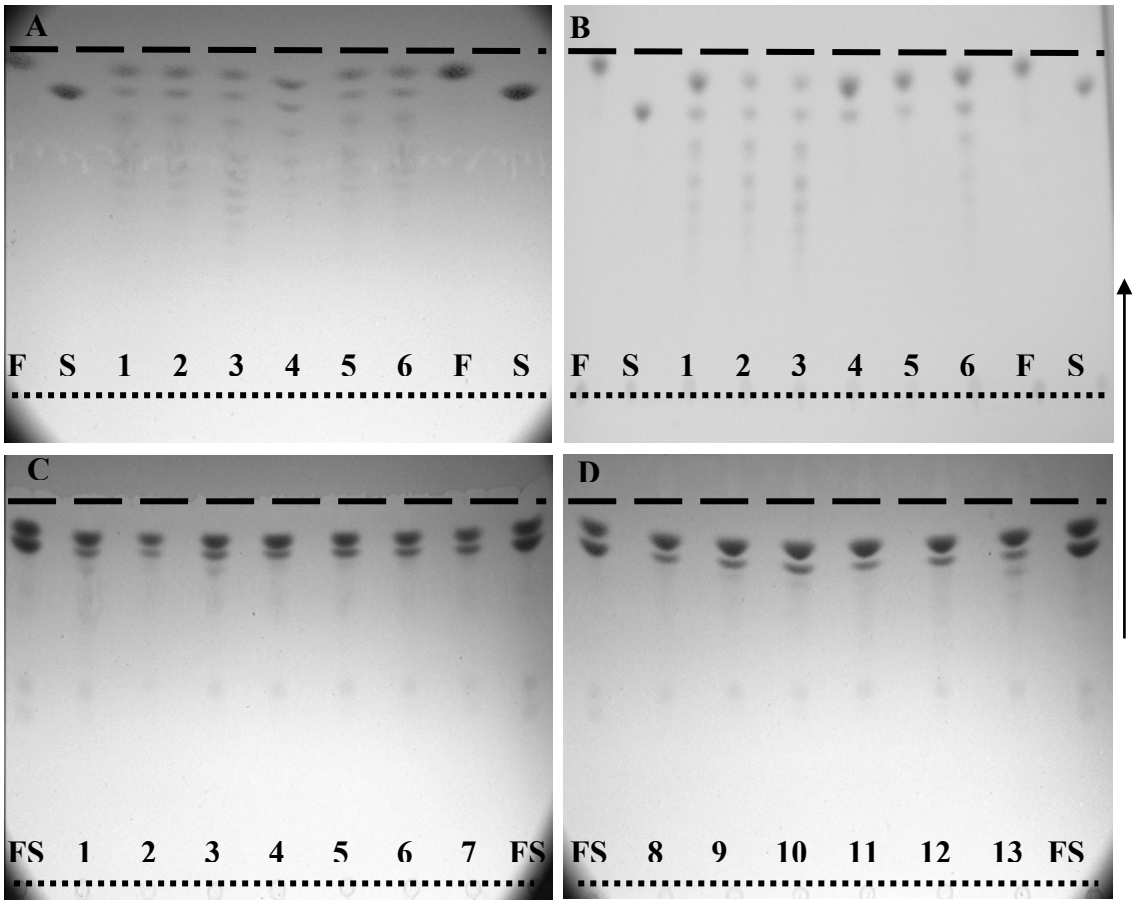

Figure 13. One-dimensional TLC of fructan extracts from onion cvs. Renate, Ailsa Craig and SS1 bulbs (no additional calcium or sulphur treatment) removed from CA storage at different times run in ethyl acetate: acetic acid: water (v/v/v); 2:1:1. F = fructose standard, S – sucrose standard, FS = mixed fructose and sucrose standard. Plate A. cv. Ailsa Craig; lane 1 - time zero, lanes 2 and 3 - day 26, lanes 4 and 5 - day 53, lane 6 - day 81. Plate B. cv. Renate, Ailsa Craig and SS1; lane 1 – Ailsa Craig time zero, lane 2 – Renate day 40, lane 3 – Renate time zero, lane 4 – SS1 time zero, lane 5 – SS1 day 14, lane 6 – Ailsa Craig day 26. Plates C and D. cv. SS1; lane 1 - time zero, lanes 2 and 3 - day 14, lanes 4 and 5 - day 26, lanes 6 and 7 - day 40, lanes 8 and 9 - day 53, lanes 10 and 11- day 66, lanes 12 and 13 - day 81. Dotted line = origin. Dashed line = solvent front. Arrow indicates the direction of the running solvent.

5.2.2 Total soluble solids

Total soluble solid (TSS; °Brix) concentration changed significantly (p=<0.001) over time in onion cvs. SS1 and Ailsa Craig and Renate bulbs (Fig. 4). In all cultivars, a maximum TSS concentration occurred within 40 days of CA storage. There was a difference in the pattern of change in concentration of TSS of onions cv. Renate and the other two cultivars. TSS concentration was much greater in cv. Renate than cv. Ailsa Craig or SS1. No effect of the sulphur and calcium treatments on TSS was observed. Total soluble solids appear to be highly variable over the storage period.

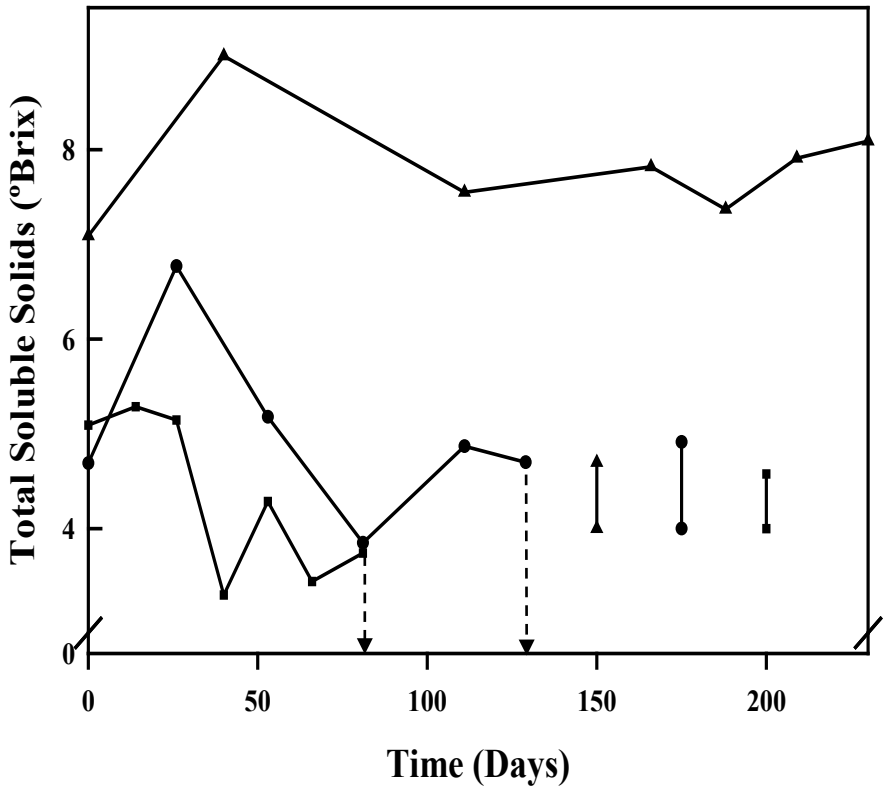

Figure 14. Changes in total soluble solids over time in controlled atmosphere storage in onions cvs. Renate (▲), Ailsa Craig (●) and SS1 (■); n=24. LSD bars (p=0.05) are shown, symbols correspond to cultivar. Drop down arrows indicate the final sample taken.

When analysed together before storage (day 0), TSS concentration was significantly greater in onions cv. Renate than cvs. Ailsa Craig and SS1 (p=<0.004). A net 1.1-fold increase occurred in onions 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 onion cv. SS1 bulbs. When analysed together for days 0, 26, 53 and 81, there was no significant difference in the TSS concentrations in onions cv. Ailsa Craig and SS1, however, a significant interaction between variety and days existed (p=<0.001) indicating a difference in the pattern of change over time. When analysed together at days 0 and 111, mean TSS concentration was significantly higher (p=<0.001) in onion cv. Renate than cv. Ailsa Craig bulbs.

In onion cv. Renate bulbs, TSS concentration reached a maximum after 40 days in storage, but by 111 days of storage had declined to a concentration similar to that recorded before storage (day 0). After 111 days, TSS did not change significantly (p=>0.05) in cv. Renate bulbs for the remainder of the storage period. The maximum TSS concentration in cv. Ailsa Craig was recorded after 26 days of storage, after which it declined, reaching a minimum of 0.8-fold of that present before storage. By 111 days of storage TSS concentration in cv. Ailsa Craig increased to an amount similar to that present before storage (day 0), but did not change significantly (P=>0.05) change after 111 days. In onions cv. SS1, TSS concentration remained constant for 26 days of storage, after which it fell to approximately 0.6-fold of that recorded before storage commenced. The concentration of soluble solids in cv. SS1 then increased 1.3–fold between 40 and 53 days of storage, before returning to a

concentration similar to that recorded at 40 days, and did not significantly change after that.

5.3 Bulb abscisic acid concentration

ABA concentration in tissue taken from the basal half of onion bulbs significantly declined over time for all cultivars (Renate and Ailsa Craig p=<0.001; SS1 p=0.003) (Fig. 5). Bulb ABA concentration declined exponentially over the storage period. The curves for Ailsa Craig and Renate closely fitted an exponential model (p=<0.001), accounting for 98.7% and 98.9% of the variance, respectively. The curve for SS1 also fitted an exponential model (p=0.036) but only 71.7% variance was accounted for. Onion cv. SS1 bulbs grown with additional sulphur had a significantly (p=<0.001) lower ABA concentration – 56.9 ng g^{-1} DW than those without – 87.5 ng g^{-1} DW, conversely the ABA concentration in onion cv. Renate bulbs grown with additional sulphur was significantly (p=0.014) greater – 143.7 ng g^{-1} DW than those without – 112.7 ng g^{-1} DW.

Within the first 81 days of storage, the ABA concentration in onion cvs. Ailsa Craig and SS1 bulbs changed significantly (p=<0.001) differently over time. The initial ABA concentration in onion cv. Ailsa Craig bulbs (298 ng g^{-1} DW) and cv. Renate – (288 ng g^{-1} DW), were significantly greater (p=0.055, LSD (0.05) = 162.1) than that present in onion cv. SS1 bulbs (116 ng g^{-1} DW). At day 0 and 40, the ABA concentration in onions cv. SS1 was significantly (p=<0.001) less than that in cv. Renate, as was the ABA concentration in onions cv. Ailsa Craig (p=0.015) at days 0 and 111. When analysed together for days 0, 26, 53 and 81, cultivar accounted for the

majority of the variation between onions cvs. SS1 and Ailsa Craig (p=<0.001). When analysed together with cvs. Renate (0, 40) or Ailsa Craig (0, 26, 53, 81) ABA concentration in SS1 was significantly lower (p=<0.001).

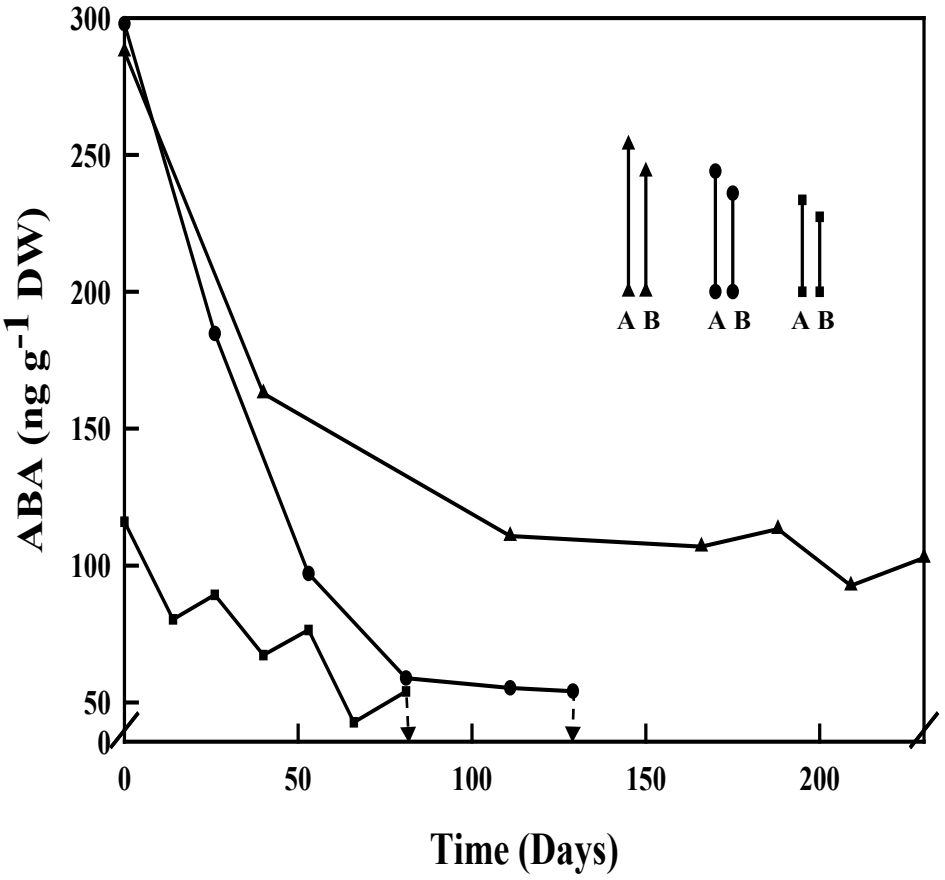

Figure 15. Changes in ABA concentration of onion bulbs cvs. Renate (▲), Ailsa Craig (●) and SS1 (■) over time in controlled atmosphere storage. LSD bars (p=0.05) are shown, symbols correspond to cultivar. The LSD bar A for comparison of time zero with all other times and bar B is for comparison of all times except time zero. Drop down arrows indicate the last sample taken. Time zero: n=4, all other times: n=8.

The relationship between storage time and ABA concentration was described by the negative exponential relationship $y = A + B^{-K\,x}$, where K=-log(R). The minimum ABA concentration (when x=∞) is given by A, and the ABA concentration when x=1

is given by A+B. R denotes the steepness of the decline in ABA concentration. 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 1).

Table 1. The parameters (with standard error) of the exponential curve that described the change in ABA concentration over time in onion bulbs cvs. Renate, Ailsa Craig and SS1.

Cultivar	R	B	A	Percentage variance[1]
Ailsa Craig (n=6)	0.97390 (0.00180)	262.9[2]	39.11[2]	98.0
Renate (n=7)		184.6[2]	102.0[2]	
SS1 (n=7)		68.15[2]	45.21[2]	

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

[2]Standard error = 9.97.

The time taken for the ABA concentration to decrease by half of that present before storage (day 0) ('half life'), A + (B/2) can be calculated using the R parameter using the following equation: half life = log (0.5) / log (R), which is derived from the exponential equation. The half lives can be calculated from each individual regression: 26.72 days (s.e. = 4.11) for onion cv. Ailsa Craig bulbs, 26.32 days (s.e. =

3.39) for cv. Renate and 35.0 days (s.e = 39.5) for cv. SS1. An overall half life can also be calculated from the common R parameter derived from the combined regression analysis: 26.21 days (s.e =1.90).

ABA concentration was measured on a dry weight basis. The mean dry weight percentages of each cultivar are known. The maximum and minimum ABA concentrations recorded were calculated in terms of fresh weight (Table 2). The concentration of ABA in dry tissue would be expected to be greater than that in fresh tissue. ABA concentration determined using fresh bulb tissue during the spike dilution experiment (section 4.2) was much lower than those determined using lyophilised tissue.

Table 2. The maximum and minimum abscisic acid concentrations on a dry weight (with s.e.) and fresh weight basis recorded in onion cvs. SS1, Renate and Ailsa Craig bulbs.

Cultivar	ABA concentration (ng g^{-1} DW)		ABA concentration (ng g^{-1} FW)	
	Maximum	Minimum	Maximum	Minimum
SS1	116.0[1] (13.25)	42.8[2] (9.37)	8.22[1]	3.03[2]
Ailsa Craig	297.9[1] (17.23)	54.1[3] (12.18)	29.89[1]	5.4[3]
Renate	287.7[1] (21.24)	92.7[4] (15.02)	42.26[1]	13.62[4]

[1] Measured at day 0. [2] Measured at day 66. [3] Measured at day 129. [4] Measured at day 209.

5.4 Pyruvate

Pyruvate concentration changed significantly ($p=<0.001$) over time in onion cvs. Ailsa Craig and Renate, and cv. SS1 bulbs ($p=0.001$) (Fig. 6). The pyruvate concentration of onion cvs. Renate and SS1 bulbs showed a net increase over the storage period. In contrast, a net decrease in pyruvate was observed for cv. Ailsa Craig bulbs. A sigmoidal pattern of change in pyruvate concentration occurred in both cvs. Renate and SS1. Two peaks in pyruvate concentration occurred during the storage period, with the first occurring in the middle of storage time – after 40 days for SS1 and after 111 days for Renate – and the second at the end of storage – 81 days for SS1 and 230 days for Renate. The pyruvate concentration of cv. Ailsa Craig bulbs showed a general decline, and the greatest decrease in concentration took place between 111 and 129 days of storage. The net change in pyruvate concentration was a 1.9-fold increase in cv. Renate bulbs during 230 days of storage, a 1.2-fold increase in cv. SS1 bulbs during 81 days of storage and 1.9-fold decrease in cv. Ailsa Craig bulbs during 129 days of storage. Onion cv. Renate bulbs grown with additional sulphur had a significantly ($p=0.028$) higher pyruvate concentration – 7.00 μmol g^{-1}, than those without – 6.21 μmol g^{-1}, conversely onion cv. Ailsa Craig bulbs grown with additional sulphur had a significantly ($p=0.004$) lower pyruvate concentration – 5.30 μmol g^{-1} than those without – 6.31 μmol g^{-1}. A significantly ($p=0.003$) higher pyruvate concentration was observed in onion cv. SS1 bulbs grown with additional calcium – 4.13 μmol g^{-1} than those without – 3.58 μmol g^{-1}.

When analysed together for days 0, 26, 53 and 814, the pattern of change of pyruvate concentration in bulbs cv. Ailsa Craig and cv. SS1 was significantly different

(P=<0.001, ls.d. 0.7476). 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 of 6.73 µmol g^{-1} compared to 3.57 µmol g^{-1} for cv. SS1, and 4.55 µmol g^{-1} for cv. Renate.

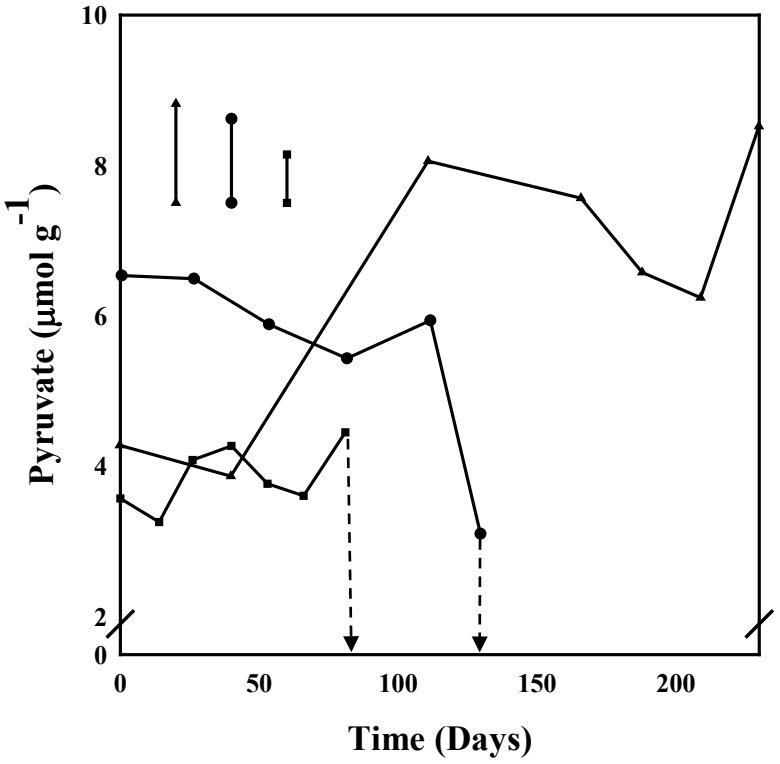

Figure 16. Changes in pyruvate concentration of onion cvs. Renate (▲), Ailsa Craig (●) and SS1 (■) bulbs during controlled atmosphere storage (n=24). The LSD (p=0.05) for each cultivar is represented by bars with corresponding symbols.

5.5 Dry weight

The range of percentage dry weight for each cultivar over the storage period is characteristic of the storage potential. Ranges were 5.5-9.5% for SS1, 7.5-13% for

Ailsa Craig and 7.5-24% for Renate. However, a delay existed between the commencement of storage and the commencement of fresh and dry weight assessments. Measurements began on day 40 for cvs. SS1 and Renate (day 40), and day 53. No effect of the calcium or sulphur treatments applied on percentage dry weight was observed.

Dry weight changed significantly (p=<0.001) over time in onion cv. Renate bulbs. This difference in dry weight over time, however, 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 is likely to be anomalous and not biologically significant. Neither of the other cultivars showed a significant change in dry weight over time. For this reason it is reasonable to use the overall means to represent the differences in dry weight between the cultivars. The mean dry weights were cv. Ailsa Craig: 10.03%, cv. Renate: 14.69%, cv. SS1:7.09 %. These means are significantly different (p=<0.001).

5.6 Firmness

There was a significant difference (p=<0.001; LSD (p=0.05) = 2.818) in the firmness (N mm^{-1}) between onion cvs. Renate, Ailsa Craig and SS1 bulbs when analysed together before storage; cv. Renate bulbs were the most firm, being 1.3-fold firmer than cv. Ailsa Craig bulbs and 2-fold firmer than cv. SS1 bulbs. This pattern persisted throughout storage. The firmness of onion cvs. Ailsa Craig and SS1 bulbs changed significantly over time (p=<0.001). In all cultivars the greatest decrease in firmness occurred between the beginning of controlled atmosphere storage and the first

sampling point. Although the change in the firmness of onion bulbs cv. Renate over time was not significant (p=0.115) a general loss of firmness was observed (Fig. 7). No effect of the calcium or sulphur treatments applied on firmness of the bulbs was observed.

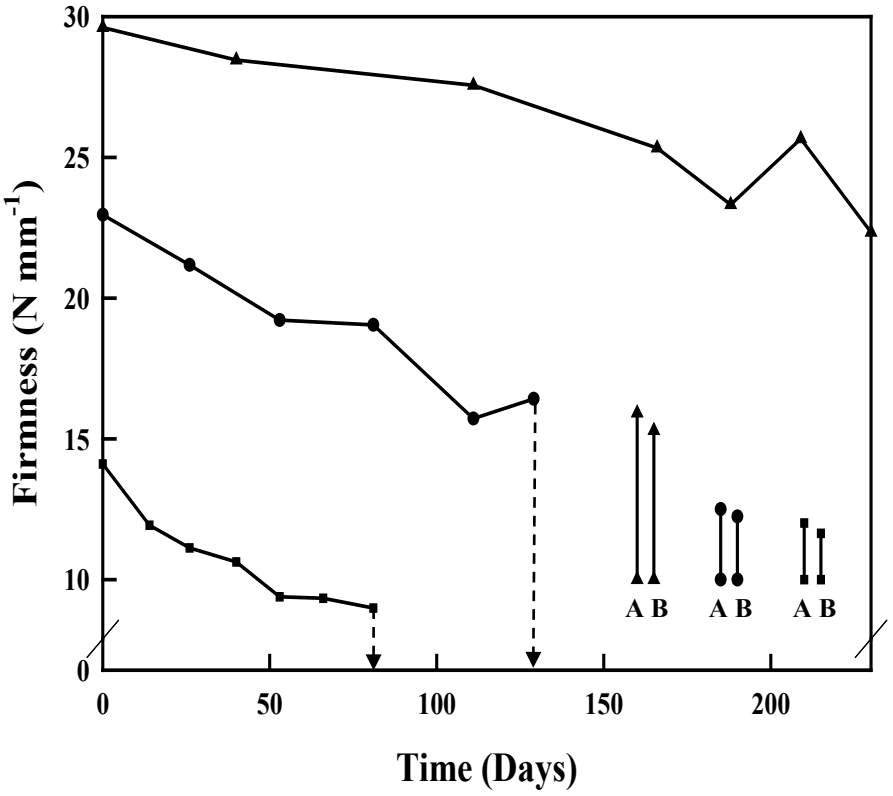

Figure 17. Changes in the firmness (N mm^{-1}) of onion cvs. Renate (▲), Ailsa Craig (●) and SS1 (■) bulbs over time in controlled atmosphere storage. LSD bars (p=0.05) are shown, symbols correspond to cultivar. LSD bar A is for comparison of time zero with all other times and bar B is for comparison all times except time zero. Drop down arrows indicate the final sample taken. Time zero all cultivars: n=16, all other times cv. Ailsa Craig and Renate: n=24; cv. SS1: n=32.

5.7 Mineral Analysis

The mineral content of onion cvs. Renate, Ailsa Craig and SS1 bulbs showed greater variation between cultivars than within cultivars over time (Fig. 8). 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 to which cultivar had the highest and lowest concentration of each element, except that cv. Ailsa Craig did not have the highest concentration of any of the elements measured. There were significant differences ($p=<0.001$) between cultivars in the concentration of boron, copper and sulphur. SS1 showed no variation over time ($p=0.05$). Total sulphur concentration was greatest in cv. SS1, followed by cv. Ailsa Craig and the least in cv. Renate. The bulb calcium and sulphur concentrations were not affected by the calcium and sulphur treatments applied to the crop. In order 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 as before (Table 3).

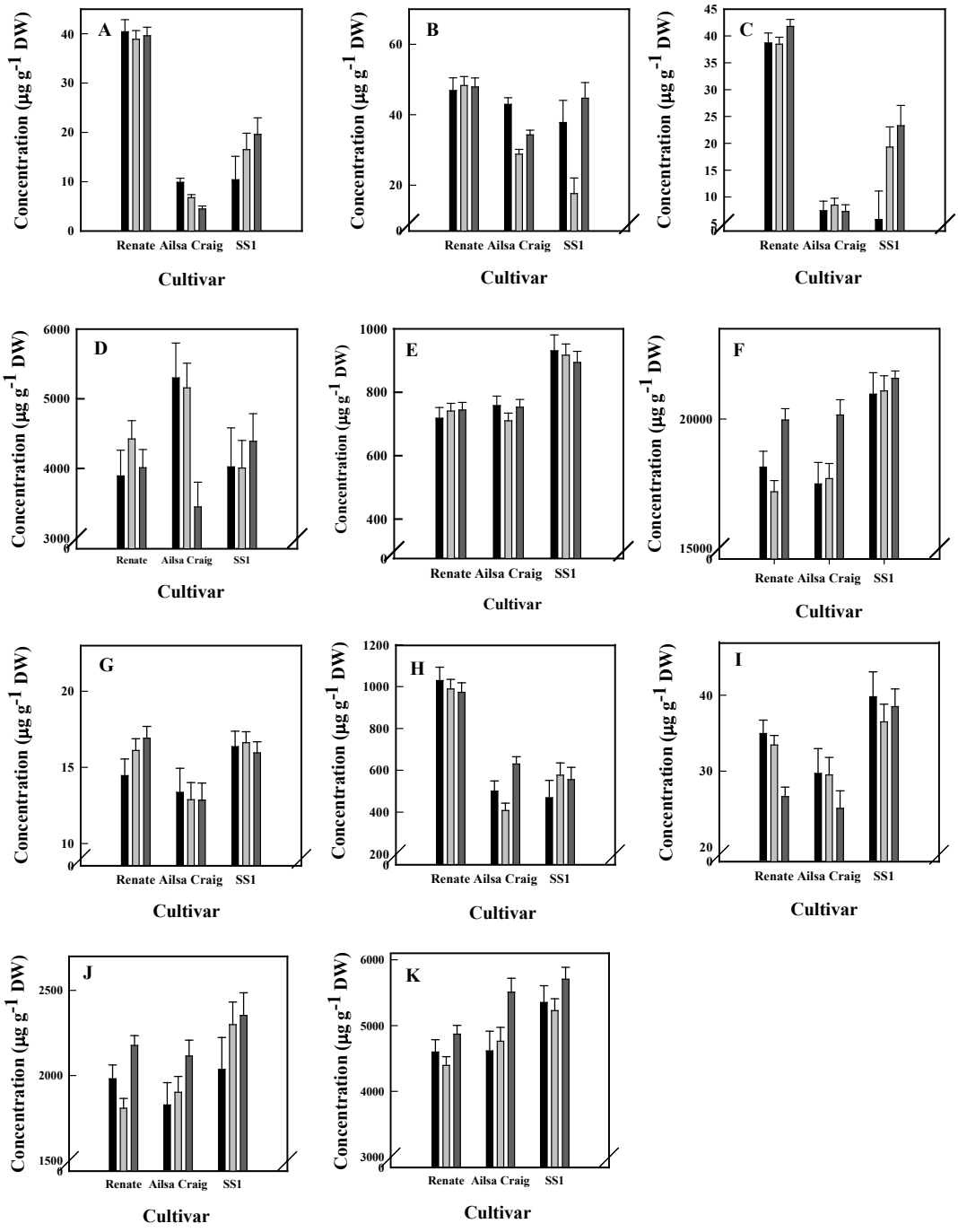

Figure 18. Elemental content of onion cvs. Renate, Ailsa Craig and SS1 bulbs shortly after harvest (black bars, n=4), and at early (light grey bars, n=8) and late (dark grey bars, n=8) time points during controlled atmosphere storage (cv. Renate: days 0, 40 and 230, cv. Ailsa Craig: days 0, 81 and 129, and cv. SS1: days 0, 40 and 81. A – Boron, B- Iron, C – Copper, D – Calcium, E – Magnesium, F – Potassium, G – Manganese, H – Sodium, I – Zinc, J – Phosphorus, K – Sulphur.

Table 3. The mean elemental composition of onion cvs. Renate, Ailsa Craig and SS1 bulbs (n=20; sampled at early, mid and late storage), 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).

6 CHAPTER SIX: DISCUSSION

6.1 Discussion

The study of the physical and biochemical changes that occur in stored onion bulbs, particularly around the period when internal sprouting commences, will help to identify potential targets for manipulation and delay of sprouting. Onion cultivars differ in the time taken for internal sprouting to begin in storage. Differences in physical and biochemical characteristics between cultivars may highlight the important changes that occur prior to sprouting. This study has measured differences in certain physical and biochemical parameters in three onion cultivars with different storage potentials during controlled atmosphere (CA) storage. Abscisic acid has been associated with dormancy in a relative of onion, *A. wakegi* Araki; bulb fructan concentration at harvest has been correlated with storage life. In addition, pyruvate concentration and bulb firmness are important indicators of bulb quality.

CA storage can be used to extend the storage life of onion bulbs and was used in this study to prolong the viable sampling period. However, the atmosphere within the CA chambers was damp, and therefore although sprouting was delayed many bulbs were rendered unsaleable by disease, thus limiting the available data from bulbs just prior to sprouting.

Mature onion bulbs store potential energy in the form of fructans and other carbon containing compounds, which are used to support subsequent sprout growth. A correlation between fructan concentration and storage life was observed in this study.

Fructan concentration measured before the bulbs were placed into storage was greatest in onion cv. Renate bulbs (28.5%), the longest storing cultivar, less in onion cv. Ailsa Craig bulbs (13.13%), the cultivar with intermediate storage life, and least in onion cv. SS1 bulbs (3.10%), the shortest storing cultivar. Other authors have noted a similar correlation between fructan content at harvest and storage life. Total fructan constituted approximately 40% of the dry matter of onion bulbs with intermediate to long storage lives, and between 27-35% of onion bulbs with a short storage life (Suzuki and Cutliffe, 1989). Fructan concentration in long storing onion cv. Pukehohe Longkeeper bulbs was seven-fold that in short storing onion cv. Houston Grano bulbs (O'Donoghue *et al.*, 2004). The fructan concentrations reported here are somewhat less than those stated by Suzuki and Cutliffe (1989); however the authors defined total fructan as comprising fructose, sucrose and fructans, which would have elevated their results.

After *ca*. 33 weeks storage at 2°C, 5% O_2, 3% CO_2 the concentration of fructan in onion bulbs cv. Renate had declined to 13.33%. This is comparable with the concentration of fructan in onion cv. Sherpa bulbs at harvest which was *ca*. 24%, and reduced to *ca*. 10% after 36 weeks storage at 2°C, 1% O_2 , 0.3% CO_2 (Ernst *et al*., 2003). The similarity in fructan concentration between onion cvs. Sherpa and Renate bulbs should be expected as both cultivars are Rijnsberger types. The fructan concentration of onion cv. Sherpa bulbs at 2°C, 1.0% O_2, 0.3% CO_2 only decreased significantly after 27 to 36 weeks of storage, whereas the fructan concentration of onion cv. Renate bulbs in this experiment only decreased significantly between *ca*. 6 and 16 weeks of storage.

Ernst *et al*. (2003) observed that storing onion cv. Sherpa bulbs at 2°C with low oxygen levels reduced the rate of fructan metabolism, with the effect being greater at 0.5% O_2 than 1.0% O_2. The greater oxygen concentration of 5% rather than 1.0% used in this experiment may explain the earlier decrease in fructan concentration in onion cv. Renate bulbs observed in this study, by allowing metabolism of fructans earlier in the storage period. However, the CO_2 concentration was 10-fold greater in this study suggesting that the inhibitory effect of increasing the CO_2 concentration on respiration was overcome by increasing the O_2 concentration. It is proposed that high fructan concentration protects 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 low level hydrolysis of fructans to simple sugars could have occurred.

Fructan concentration would be expected to decrease over time as the enzymatic hydrolysis of fructans to fructose occurs (Hurst *et al.*, 1985). A decrease in fructan concentration has been observed in a variety of cultivars stored at 6-10°C for eight weeks (Suzuki and Cutliffe, 1989), at 16°C for 15 weeks (Pak *et al.*, 1995) and at 4°C for 12 weeks (Ernst *et al.*, 1998) and 24 weeks (Benkeblia *et al.*, 2002). However a significant decrease in fructan concentration over time was only observed in onion cv. Renate bulbs. The concentration in the other two cultivars did not change significantly, although it was observed that fructan concentration in onion cv. Ailsa Craig bulbs decreased until *ca*. 12 weeks of storage, and then increased again. The decrease in fructan concentration in onion cv. Renate and Ailsa Craig bulbs preceded an increase in sprout growth. This is in agreement with the theory that fructans are metabolised to provide energy for the growing sprout (Pak *et al.*, 1995). The different

patterns of change in fructan concentration could be due to a different response of each cultivar to controlled atmosphere conditions.

Dry matter content is the primary characteristic of onion bulb quality, determining the appropriate end use (e.g. as salad, fresh or dehydrated onions), storage life, pungency and firmness. Onions with a dry matter of >15-20% are suitable for dehydration, whereas those <15% dry matter are regarded as better for fresh consumption (Sinclair *et al.*, 1995a). Mild onions, which are eaten raw, generally have dry matters of <10%. Onions at the higher end of acceptable dry matter appropriate for fresh consumption tend to be much firmer and store for longer periods before shoot growth and rots deplete the number of marketable bulbs (Darbyshire and Henry, 1979; Rutherford and Whittle, 1982; Suzuki and Cutliffe, 1989).

Low dry matter onion cultivars tend to accumulate simple sugars such as sucrose, glucose and fructose, whereas high dry matter cultivars tend to accumulate fructans. The polymerisation of fructans reduces osmotic activity and allows accumulation of carbohydrates. Fructans are soluble in water in the vacuole and therefore have a role in osmoregulation. The dry matter of onion cvs. Renate, Ailsa Craig and SS1 bulbs was constant over the storage period. Ernst *et al*. (2003) observed no change in the dry matter of onion cv. Sherpa bulbs stored at 2°C for 36 weeks. The CA storage boxes used water to form a seal, thus creating a damp environment. The more water present in the air surrounding the bulbs the less water that will be lost from them. This explains the stability of the dry weight measurements. In addition, in the majority of occasions, sprouting did not occur and so this would have had an effect.

Onion bulbs cv. Renate had the highest dry matter content, followed by cv. Ailsa Craig and cv. SS1. Similarly, bulbs cv. Renate were the most firm, followed by cv. Ailsa Craig and cv. SS1. Therefore the greater the dry matter content the firmer the bulb. Firmness of bulbs cv. Ailsa Craig and SS1 decreased significantly over the storage period. A decrease in firmness would normally be associated with a decrease in the water content of the bulb. No change in dry weight was detected; therefore the firmness measurement may have been more sensitive and have detected changes in water content that measuring dry weight could not. Another reason for changes in firmness could be variation in the cell wall composition.

A study has classified onion cultivars according to dry matter, total fructan and soluble solids concentration (Table 1) (Jaime *et al.*, 2001). The cultivars used in this investigation can be categorised according to this system. The fructan levels in fresh onions recorded by Jaime *et al*. (2001) were somewhat greater than those in this study, and TSS levels were also consistently greater. The lower TSS concentrations measured in this investigation can be explained by the fact that CA conditions were used, which would have slowed down the respiration rate of the bulbs. The decrease in fructan concentration over the storage period was greater in cv. Renate than cv. Ailsa Craig, thus indicating greater hydrolysis. The constant fructan level in SS1 is consistent with this study.

Table 1. Categorisation of the onion cvs. Renate, Ailsa Craig and SS1 according to Jaime *et al*. (2001).

Cultivar	Fructan content	Fructan hydrolysis	Dry matter	Total soluble solids	Sprouting before 6 months storage
Renate	High	High	High	High	None
Ailsa Craig	Medium	Medium	Medium	Medium	Some
SS1	Low	Low	Low	Low	High

Net TSS concentration did not change over time in onion cvs. Renate and Ailsa Craig bulbs, which is consistent with observations by Rutherford and Whittle (1982) on Rijnsberger onions stored at 4°C. In this experiment a peak in TSS concentration occurred between *ca*. four and six weeks in all cultivars, followed by a general declining trend towards the end of the storage period. This is comparable with the peaks observed by other authors at six to eight weeks at 4, 10 and 20°C (Benkeblia *et al.*, 2002) and at five weeks at 0, 15 and 30°C (Salama *et al.*, 1990), and both of these peaks were followed by a decline to the end of the storage period (24 and 20 weeks, respectively). TSS is defined as the total of all the solids that dissolve in water, including sugar, salts, protein, acids, etc., and the refractometer reading is the sum total of these. It is therefore difficult to relate TSS concentration to sweetness. Onions cv. SS1 are mild, and may therefore be expected to have a high concentration of soluble sugars, but this was not reflected in the TSS concentration. The effect of TSS on osmotic potential can be decreased by the ability to store highly polymerised fructans (Sinclair *et al.*, 1995b). This means that high dry matter bulbs are able to accumulate high TSS concentrations without taking up more water.

ABA is a weak acid (pKa = 4.8), and is mostly uncharged when present in the weakly acidic apoplastic compartment of plants and can easily enter cells across the plasma membrane (Finkelstein and Rock, 2002). The maximum ABA concentration in onion bulbs was recorded at the beginning of storage, and ABA concentration then decreased exponentially throughout storage in the bulbs of all cultivars. A rapid initial decline in ABA concentration was not observed in onion bulbs cv. SS1, and the exponential model fitted this curve less well than for cvs. Ailsa Craig and Renate. This may be explained by the fact that onion cv. SS1 bulbs were harvested two weeks earlier than onion cvs. Ailsa Craig and Renate bulbs, 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. However, it is also possible that ABA concentration in onion cv. SS1 bulbs does not change as dramatically as in cv. Renate and Ailsa Craig bulbs.

The maximum and minimum ABA concentrations recorded in this investigation on lyophilised samples were converted to concentrations on a fresh weight basis. These calculated values (Chapter 5, Table 2) can be compared to those recorded by other authors; Matsubara and Kimura (1991) reported that the ABA concentration in the outer enlarged leaf of onion bulbs (in this investigation, the ABA concentration in a sample of all bulb tissues from the basal half of the bulb was determined) 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 cv. SS1 bulbs. 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*. (1999a) and Yamazaki *et al*. (2002) recorded a maximum concentration of *ca*. 33ng 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 is closer to the ABA concentration in onion cvs. Ailsa Craig and Renate recorded in this experiment. The ABA concentration on a fresh weight basis highlights the differences between cultivars; the maximum concentration in cv. Renate is 1.4-fold that in cv. Ailsa Craig and 5.2-fold that in cv. SS1.

Postharvest decrease in ABA concentration in *Allium 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). ABA concentration (DW) at day 0 was least in onion cv. SS1 bulbs, the shortest storing cultivar, and was *ca*. 2.5-fold greater in onion cvs. Ailsa Craig and Renate bulbs. In this experiment, decreasing ABA concentration appears to be related to an increase in sprouting. Sprouting in onion cv. SS1 bulbs seems to occur at a lower bulb ABA concentration than in onion cv. Ailsa Craig bulbs, which in turn is higher than the concentration at which sprouting begins in onion cv. Renate bulbs. This may indicate that these cultivars have different threshold ABA concentrations for the onset of sprouting. The half life of ABA in Renate and Ailsa Craig was calculated to be approximately 26 days. This means that a significant decrease in ABA concentration could have occurred in SS1 in the extra 14 day delay. The decline in ABA concentration could be caused by enzymatic degradation of ABA, or movement of ABA from the basal area (that which was measured in this experiment) to another part of the bulb. However it is unlikely that the latter is the case as the regulation of physiological processes is at the primary level

of *de novo* synthesis of relevant enzymes rather than redistribution (Finkelstein and Rock, 2002). The molecular control of the ABA degradation pathway is still largely unknown; the genes involved in degradation have not been cloned, and no ABA null or ABA catabolism *Arabidopsis* mutants have been discovered. The phaseic acid pathway is the dominant catabolic pathway (Figure 6, Section 2.4.5.2.5), and is rate limiting. This is thought to be the target for regulation at transcriptional level by water status, and ABA accumulation regulates ABA degradation. ABA synthesis is unregulated in periods of drought stress. Environmental triggers such as water availability and temperature may be involved in regulating the concentration of ABA in an onion bulb left to over-winter in the soil. Onion bulbs in store are maintained in a dry, cold environment with no access to nutrients and so the trigger for regulating ABA concentration is most likely to come from within the bulb.

The range of pyruvate concentrations measured was between 3 and 9 μm g^{-1}, which is within the range of 4-20 μm g^{-1} stated by Schwimmer and Guadagni (1962). Schwimmer and Weston (1961) found that weak onions produced 2-4 μm g^{-1}, intermediate strength onions produced 8-10 μm g^{-1}, and strong onions produced 15-20 μm g^{-1} pyruvate. The cultivars used in this experiment can be ranked using the mean pyruvic acid concentration taken across the entire storage period. Onion cvs. Renate and Ailsa Craig bulbs fall between the weak and intermediate categories, and onion cv. SS1 bulbs belong in the weak category. The difference between the pyruvate concentrations of onion cvs. Renate and Ailsa Craig bulbs was small, but the difference between onion cv. SS1 bulbs and onion cvs. Renate and Ailsa Craig bulbs was large. Onions cv. SS1 are marketed as a mild variety for being eaten raw in salads etc., and so would be expected to have a low pyruvate concentration, and be

less pungent. Onion cv. Renate is a Rijnsberger type, which are fairly pungent, but the overall mean pyruvate concentration does not rank it as an intermediate onion. However, pyruvate concentration in onion cv. Renate bulbs showed the most variation, and the maximum recorded concentration was approximately 8 μm g^{-1} which, according to Schwimmer and Weston (1961), places it in the intermediate category.

The pyruvate concentration of Ailsa Craig bulbs decreased over the storage period. This is consistent with the findings of others who reported a decrease in the pyruvate concentration of onion bulbs cv. Hysam over nine weeks in CA (2% O_2, 2% CO_2 and 2% O_2, 8% CO_2) storage (Uddin and MacTavish, 2003). Pyruvate concentration increased overall in cvs. Renate and SS1. Pyruvate concentration appears to change differently in different cultivars; pyruvate concentration increased in onion cv. Hysam bulbs stored under ambient atmosphere conditions for nine weeks (Uddin and MacTavish, 2003) and in onion cv. Granex-Grano bulbs stored at 4°C for 24 weeks (Hurst *et al.*, 1985) but decreased in various long-day onion cultivars stored at 5°C for 24 weeks (Kopsell and Randle, 1997). Pyruvate is a stable product of the hydrolysis of ACSOs that occurs when onion cells are disrupted; therefore changes in pyruvate concentration over storage are likely to be due to differences in availability of ACSOs. ACSOs may be metabolised to supply carbon and nitrogen to the growing sprout. Another reason for changes in pyruvate concentration is a change in the activity of alliinase, the enzyme that catalyses the hydrolysis of ASCOs to pyruvate and ammonia. It has been shown that the activity of this enzyme is decreased at low oxygen (2%) and high carbon dioxide (8%) concentrations. Differential changes in

the metabolism of ACSOs over storage are likely to be associated with different genotypes.

Sulphur and calcium treatments were applied, at the time of drilling, to the onions in this investigation. The effects of these calcium and sulphur treatments on the parameters measured (including total bulb sulphur and calcium concentrations) were non-significant or inconsistent, and were therefore unlikely to be biologically significant. 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 free sulphur than cv. Ailsa Craig and Renate. This is perhaps surprising as this 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 (Chapter 5, Table 3). 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 contain 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.

7 CHAPTER SEVEN: CONCLUSIONS AND AREAS FOR FUTURE RESEARCH

7.1 Conclusions

Bulb abscisic acid (ABA) concentration was different in cultivars with different storage potentials. The difference in ABA concentration before storage became more apparent when calculated in terms of bulb fresh weight. ABA concentration before storage was positively correlated with storage life. The ABA concentration declined exponentially in bulbs of all cultivars, and this decline was associated with an increase in sprouting. The decrease in ABA concentration was likely to be due to degradation of the compound within the bulb, rather than movement within the bulb. Maximising the bulb ABA concentration prior to storage, or inhibition of the degradation of ABA may delay sprouting and extend storage life. If the storage life of short-storing cultivars e.g. SS1 could be prolonged, thus maximising the window for fresh supply of these onions from the UK, growers would be able to retain market share that would otherwise be lost to imports from the Southern Hemisphere.

Each cultivar behaved differently in terms of the other parameters measured such as pungency, fructan and total soluble solids (TSS) concentration, although there were some common patterns. Each cultivar had a distinct characteristic profile of TSS, fructan, dry weight and sprouting.

Sprouting was preceded by a decrease in fructan concentration in onion cv. Renate and Ailsa Craig bulbs, which fits the theory that fructans act as storage carbohydrates that are metabolised to provide energy for the growing sprout. Further investigation

of the changes in fructan concentration may mean that onset of sprouting could be predicted.

Pungency, as measured by pyruvate concentration, increased in cv. Renate and SS1 bulbs, and decreased in cv. Ailsa Craig bulbs. Pyruvate concentration was particularly variable in onion cv. SS1 and Renate bulbs, following a sigmoidal pattern. Growers holding onions in CA storage should be aware of this, particularly with regard to cv. SS1, as this is marketed as a mild variety.

The method used to measure firmness in this investigation was effective in detecting differences both between cultivars, and over time. Firmness decreased steadily in all cultivars during controlled atmosphere storage, indicating a decrease in quality; however, the reason for this was not clear as dry weight composition did not change over time. It is possible that in this case, measuring firmness was a more sensitive method for detecting water loss from the bulb.

In this investigation, storage conditions were controlled atmosphere, and in many cases the marketable storage life was ended by disease onions before sprout occurred. Therefore the results obtained from this investigation could be enhanced by collecting more data from sprouting bulbs and those just prior to sprouting.

Sulphur treatment increased the pungency of onion bulbs cv. Renate, but sulphur and calcium treatments did not have any other consistent significant effects. This may be partly due to differing capacities for mineral uptake between cultivars, and partly due

to the early application time. The treatments may have been more effective if they had been applied later in the growing season, rather than at the time of drilling.

7.2 Areas for future research

Owing to the differing behaviour of cultivars it will be useful to profile the same changes in a wider range of cultivars to help to discern which are the most important. This will also ensure the reproducibility of the results from the first experiment. It is also important that measurements are taken from bulbs around the time of sprout growth, therefore the use of the same storage environment for all cultivars is not practical and so three different storage temperatures will be used to overcome this problem.

ABA analogues 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 is an 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 (Fig. 1). The methyl group on the 8'-methlyene ABA molecule has been shown to sterically hinder binding of the molecule with ABA-8'-hydroxylase, thus resisting degradation. 8'-methylene ABA methyl ester also possesses this methyl group and so should also resist degradation.

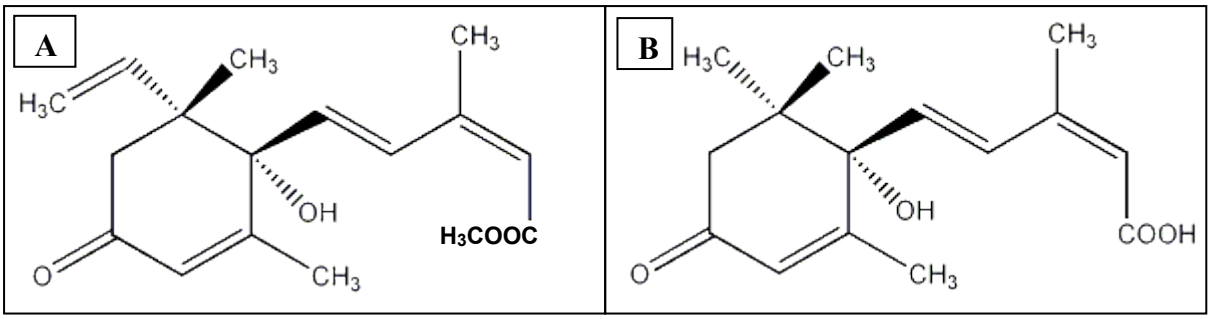

Figure 19. A - 8'-methylene abscisic acid methyl ester, B - (+)-Abscisic acid.

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 (Wills *et al.*, 1999). The effects of ethylene on onion storage life are inconclusive, but the fact that onions produce more ethylene towards the end of the storage period (Abdel-Rahman and Isenberg, 1974) suggests that ethylene may have a role in sprouting. 1-MCP is an inhibitor of ethylene action – it binds to ethylene binding sites and therefore prevents ethylene from binding (Blankenship and Dole, 2003).

Six *A. cepa* cultivars were grown at Warwick HRI, Wellesbourne. The cultivars were chosen according to storage potential (NIAB, 2000) and can be divided into long (Renate, Hysam, Red Baron), medium (Carlos, Dinaro) and short storing (SS1) cultivars. Onions were grown from seeds drilled at a rate of 18 seeds m^{-2} in March 2004. Pesticides were applied according to commercial practice.

Treatments of 10^{-4} M ABA, 10^{-4} PBI-365, Li-700 and a water control were applied prior to plant maturity (*ca*. 80% tops down) in late August (Table 1), as green photosynthesising leaves must be present in order to translocate any substances applied to the plants down into the bulb (Thomas and Isenberg, 1972; Yamazaki *et*

al., 1999). It is essential that all substances applied to the plant are translocated to the bulb, as the roots and aerial parts are removed at harvest. Preharvest treatments were applied in the field using a backpack sprayer with a flat fan tip (B.C.P.C. nozzle code F110/1.60/3). ABA is photosensitive therefore the treatments were applied in conditions of low light intensity. The leaves of onion plants have a thick, waxy cuticle which represents a barrier to any treatment applied to the plants. It has been shown that ABA penetrates the leaf cuticle more effectively at a pH below the pK (pH 4.8) (Blumenfield and Bukovac, 1972). Li-700 (Loveland Industries Ltd., Cambridge, UK) is a penetrating, translocating and acidifying adjuvant. A solution 0.5% w/w of Li-700 is pH 4.05. All treatments (except water control) contained 0.5% Li-700.

Table 1. The application rate and total amount of each treatment applied to the onion crop.

Treatment	**Application rate ($l\ ha^{-1}$)**	**Total amount applied (l)**
10^{-4} ABA plus 0.5% Li-700	550	6.93
10^{-4} PBI-365 plus 0.5% Li-700	314.2	3.95
0.5 % Li-700	550	6.93
Water	550	6.93

The following treatments were also applied to a small plot of onions cv. Renate to investigate the effect of concentration of ABA applied on the bulb ABA concentration at harvest; 10^{-3} M ABA, 10^{-4} M ABA, 10^{-5} M ABA (including 0.5% Li-700), water and 0.5% Li-700. The experiment consisted of four blocks, and five plots (1 m x 4 rows) per block.

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. Diseased bulbs were removed before onions were placed into store.

10^{-4} M ABA, 10^{-4} PBI-365 and a water control were applied to onions cv. Hysam as a soak before curing. Onion bulbs with the roots and aerial parts removed were placed in a plastic tray containing the treatment solution, and the basal part of the bulb was soaked for 24 h in the dark. The bulbs were then dried and stored with the rest of the harvest.

Onions cv. SS1 were exposed to 1.4% 1-MCP (SmartFresh, Rohm and Haas, USA) for 24 h at 20ºC in a sealed polypropylene box. The concentration of 1-MCP was 0.962 ppm after 2 h. The bulbs were then stored with the rest of the harvest.

Onions were stored at three temperatures; 4, 12 and 20ºC. Samples were taken at regular intervals during storage. The following assessments were made: firmness, abscisic acid concentration, fructan concentration, pyruvate concentration, total soluble solids, mineral content, and dry weight. This work is ongoing.

REFERENCES

Abbey, L. (2003). Discrimination of *Allium* headspace volatiles affected by variations in genotype, growing environment and storage using an electronic nose. *PhD Thesis. Institute of Bioscience and Technology, Cranfield University*.

Abdalla, A. A. and Mann, L. K. (1963). Bulb development in the onion (*Allium cepa L*.) and the effect of storage temperature on bulb rest. *Hilgardia* **35**(5), 85-112.

Abdel-Rahman, M. and Isenberg, F. M. R. (1974). The role of exogenous plant growth regulators in the dormancy of onion bulbs. *Journal of Agricultural Science, Cambridge* **82**, 113-116.

Abrams, S. R., Rose, P. A., Cutler, A. J., Balsevich, J. J., Lei, B. and Walker-Simmons, M. K. (1997). 8'-Methylene Abscisic Acid. An effective and persistent analogue of abscisic acid. *Plant Physiology* **114**, 98-97.

Benkeblia, N. (2004). Effect of maleic hydrazide on respiratory parameters of stored onion bulbs (*Allium cepa L*.). *Brazilian Journal of Plant Physiology* **16**(1), 47-52.

Benkeblia, N. and Selselet-Attou, G. (1999a). Effects of low temperatures on changes in oligosaccharides, phenolics and peroxidase in inner bud of onion *Allium cepa* L. during break of dormancy. *Acta Agriculturae Scandinavica Section B-Soil and Plant Science* **49**, 98-102.

Benkeblia, N. and Selselet-Attou, G. (1999b). Role of ethylene on sprouting of onion bulbs (*Allium cepa* L.*). Acta Agriculturae Scandinavica Section B-Soil and Plant Science* **49**, 122-124.

Benkeblia, N. and Varoquaux, P. (2003). Effect of nitrous oxide (N2O) on respiration rate, soluble sugars and quality attributes of onion bulbs *Allium cepa* cv. Rouge Amposta during storage. *Postharvest Biology and Technology* **30**(2), 161-168.

Benkeblia, N., Varoquaux, P., Gouble, B. and Selselet-Attou, G. (2000). Respiratory parameters of onion bulbs (*Allium cepa*) during storage. Effects of ionising radiation and temperature. *Journal of the Science of Food and* Agriculture **80**(12), 1772-1778.

Benkeblia, N., Varoquaux, P., Shiomi, N. and Sakai, H. (2002). Storage technology of onion bulbs cv. Rouge Amposta: effects of irradiation, maleic hydrazide and carbamate isopropyl, N-phenyl (CIP) on respiration rate and carbohydrates. *International Journal of Food Science and Technology* **37**(2), 169-175.

Blankenship, S. M. and Dole, J. M. (2003). 1-methylcyclopropene: a review. *Postharvest Biology and Technology* **28**, 1-25.

Blumenfield, A. and Bukovac, M. J. (1972). Cuticular penetration of abscisic acid. *Planta* 107, 261-268.

Block, E. (1992). The organosulphur chemistry of the genus *Allium* - implications for the organic chemistry of sulphur. *Angewandte Chemie International Edition in English* **31**, 1135-1178.

Brewster, J. L. (1977a). The physiology of the onion. Part 1. *Horticultural Abstracts* **47**(1), 17-23.

Brewster, J. L. (1977b). The physiology of the onion. Part 2. *Horticultural Abstracts,* **47**(2), 103-112.

Brewster, J. L. (1990). Physiology of crop growth and bulbing. In *Onions and allied crops. Volume 1: Botany, physiology and genetics.* Edited by Rabinowitch, H. D. and Brewster, J. L. CRC Press, Florida.

Brewster, J. L. (1994). *Onions and other vegetable Alliums*. CABI Publishing, Wallingford.

Brice, J. R., Bisbrown, A. J. K. and Curd, L. (1995). Onion storage trials at high ambient temperature in the Republic of Yemen. *Journal of Agricultural and Engineering Research* **62**, 185-192.

Carter, C. E., Partis, M. D. and Thomas, B. (1999). The expression of histone 2A in onion (*Allium cepa*) during the onset of dormancy, storage and emergence from dormancy. *New Phytologist* **143**(3), 461-470.

Churchill, G., Reaney, M., Abrams, S. R. and Gusta, L. (1998). Effects of abscisic acid and abscisic acid analogues on the induction of freezing tolerance of winter rye (Secale cereale L.) seedlings. *Plant Growth Regulation* **25**, 35-45.

Coolong, T. W., Kopsell, D. A., Kopsell, D. E. and Randle, W. M. (2004). Nitrogen and sulphur influence nutrient usage and accumulation in onion. J*ournal of Plant Nutrition* **27**(9), 1667-1686.

Croci, C. A., Banek, S. A. and Curzio, O. A. (1995). Effect of gamma-irradiation and extended storage on chemical quality in onion (*Allium Cepa* L). *Food Chemistry* **54**(2), 151-154.

Cutler, A. J. and Krochko, J. E. (1999). Formation and breakdown of ABA. *Trends in Plant Science* **4**(12), 472-478.

Darbyshire, B. and Henry, R. J. (1978). The distribution of fructans in onions. *New Phytologist* **81**, 29-34.

DEFRA (2003). *Family food in 2001/2002*. National Statistics.

Dorffling, K. and Tietz, D. (1983). Methods for the detection and estimation of abscisic acid and related compounds. In *Abscisic acid*. pp. 23-77. Edited by Addicott, F. T. Praeger Publishers, New York.

Ernst, M. K., Bufler, G., Röske, M., Ritzkowski, M., Metzger, C. and Liebig, H.-P. (1999). Cultivar and temperature effects on starch in the primary thickening meristem of onion bulbs. *Journal of Horticultural Science and Biotechnology* **74**(5), 588-593.

Ernst, M. K., Chatterton, N. J., Harrison, P. A. and Matitschka, G. (1998). Characterization on fructan oligomers from species of the genus *Allium* L. *Journal of Plant Physiology* **153**, 53-60.

Ernst, M. K., Praeger, U. and Weichmann, J. (2003). Effect of low oxygen storage on carbohydrate changes in onion (*Allium cepa* var. *cepa*) bulbs. *European Journal of Horticultural Science* **68**(2), 59-62.

FAOSTAT (2004). Food and Agriculture Organisation of the United Nations. Agricultural Data. [WWW] Date accessed: 19th May 2004. http://apps.fao.org/faostat/form?collection=CBD.CropsAndProducts&Domain=CBD&servlet=1&hasbulk=0&version=ext&language=EN

Finkelstein, R. R. and Rock, C. D. (2002). Abscisic acid biosynthesis and response. The Arabidopsis Book, 1-48.

Griffiths, G., Trueman, L., Crowther, T., Thomas, B. and Smith, B. (2002). Onions - A global benefit to health. *Phytotherapy Research* **16**(7), 603-615.

Gubb, I. and MacTavish, H. S. (2002). Onion pre- and postharvest considerations In *Allium crop science: Recent advances,* pp. 233-266. Edited by Rabinowitch, H. D. and Currah, L. CABI Publishing, Wallingford,

Hamilton, B. K., Yoo, K. S. and Pike, L. M. (1998). Changes in pungency of onions by soil type, sulphur nutrition and bulb maturity. *Scientia Horticulturae* **74**, 249-256.

Hanum, T., Sinha, N. K., Guyer, G. E. and Cash, J. N. (1995). Pyruvate and flavor development in macerated onions (Allium cepa L.) by gamma-glutamyl transpeptidase and exogenous C-S lyase. *Food Chemistry* **54**, 183-188.

Havey, M. J. (1999). Advances in new *Alliums* In *Perspectives on new crops and new uses,* Edited by Janick, J. ASHS Press, Alexandria, V.A.

Havey, M. J. and Randle, W. M. (1996). Combining abilities for yield and bulb quality among long- and intermediate-day open-pollinated onion populations. *Journal of the American Society for Horticultural Science* **121**(4), 604-608.

Herold, B., Oberbarnscheidt, B. and Geyer, M. (1998). Mechanical load and its effect on bulb onions due to harvest and post-harvest handling. *Journal of Agricultural and Engineering Research* **71**, 373-383.

Hole, C. C., Drew, R. L. K. and Gray, D. (2000). Humidity and the mechanical properties of onion skins. *Postharvest Biology and Technology* **19**, 229-237.

Hurst, W. C., Shewfelt, R. L. and Schuler, G. A. (1985). Shelf-life and quality changes in summer storage onions (*Allium cepa*). *Journal of Food Science* **50**, 761-763.

Imai, S., Tsuge, N., Tomotake, M., Nagatome, Y., Sawada, H., Nagata, T. and Kumagai, H. (2002). Plant biochemistry: An onion enzyme that makes the eyes water. *Nature* **419**(6908), 685.

Jaime, L., Martin-Cabrejas, M. A., Molla, E., Lopez-Andreu, F. J. and Esteban, R. M. (2001). Effect of storage on fructan and fructooligosaccharide of onion (*Allium cepa* L.). *Journal of Agricultural and Food Chemistry* **49**(2), 982-988.

Jones, M. G., Hughes, J., Tregova, A., Milne, J., Tomsett, A. B. and Colin, H. A. (2004). Biosynthesis of the flavour precursors of onion and garlic. *Journal of Experimental Botany* **55**(404), 1903-1918.

Kahane, R., Delaserve, B. T. and Rancillac, M. (1992). Bulbing in long-day onion (*Allium cepa* L) cultured *in vitro* - comparison between sugar feeding and light induction. *Annals of Botany* **69**(6), 551-555.

Karagiannis, C. S. and Pappelis, A. J. (1994). Effect of abscisic acid, gibberellic acid, indoleacetic acid, and kinetin on selective ribosomal cistron regulation in quiescent and senescent onion leaf base tissue. *Mechanisms of Ageing and Development* **76**(2-3), 145-155.

King, J. J., Bradeen, J. M., Bark, O., McCallum, J. A. and Havey, M. J. (1998). A low-density genetic map of onion reveals a role for tandem duplication in the evolution of an extremely large diploid genome. *Theoretical and Applied Genetics*, **96**(1), 52-62.

Klein, J. D. and Hebbe, Y. (2000). Tetcyclacis and abscisic acid differentially affect growth of wheat (*Triticum aestivum* L.) seedlings isogenic for reduced-height genes. *Crop Science* **40**, 1702-1705.

Kleinkopf, G. E., Oberg, N. A. and Olsen, N. L. (2003). Sprout inhibition in storage: current status, new chemistries ad natural compounds. *American Journal of Potato Research* **80**, 317-327.

Kopsell, D. E. and Randle, W. M. (1997). Short-day onion cultivars differ in bulb selenium and sulphur accumulation which can affect bulb pungency. *Euphytica* **96**, 385-390.

Kopsell, D. E., Randle, W. M. and Eiteman, M. A. (1999). Changes in the S-alk(en)yl cysteine sulfoxides and their biosynthetic intermediates during onion storage. *Journal of the American Society for Horticultural Science* **124**(2), 177-183.

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

Lancaster, J. E., Farrant, J. and Shaw, M. L. (2001). Sulphur nutrition affects cellular sulphur, dry weight distribution, and bulb quality in onion. *Journal of the American Society for Horticultural Science* **126**(2), 164-168.

Lancaster, J. E., Shaw, M. L. and Randle, W. M. (1998). Differential hydrolysis of alk(en)yl cysteine sulphoxides by alliinase in onion macerates: flavour implications. *Journal of the Science of Food and Agriculture* **78**, 367-372.

Lancaster, J. E., Triggs, C. M., De Ruiter, J. M. and Gandar, P. W. (1996). Bulbing in onions: photoperiod and temperature requirements and prediction of bulb size and maturity. *Annals of Botany* **78**(4), 423-430.

Marcano, L., Carruyo, I., Del Campo, A. and Montiel, X. (2004). Cytotoxicity and mode of action of maleic hydrazide in root tips of Allium *cepa* L. *Environmental Research* **94**(2), 221-226.

Matsubara, S. and Kimura, I. (1991). Changes of ABA content during bulbing and dormancy and *in vitro* bulbing in onion plants. *Journal of the Japanese Society for Horticultural Science* **59**(4), 757-762.

McCallum, J., Pither-Joyce, M. and Shaw, M. L. (2002). Sulphur deprivation and genotype affect gene expression and metabolism of onion roots. *Journal of the American Society for Horticultural Science* **127**(4), 583-589.

Miedema, P. (1994a). Bulb dormancy in onion. I. The effects of temperature and cultivar on sprouting and rooting. *Journal of Horticultural Science* **69**(1), 29-39.

Miedema, P. (1994b). Bulb dormancy in onion. III. The influence of the root system, cytokinin and wounding on sprout emergence. *Journal of Horticultural Science* **69**(1), 47-52.

Miedema, P. and Kamminga, G. C. (1994). Bulb dormancy in onion. II. The role of cytokinins in high-temperature bulb imposed sprout inhibition. *Journal of Horticultural Science* **69**(1), 41-45.

NIAB (2000). *Vegetable Variety Handbook*, National Institute of Agricultural Botany.

O'Connor, D. (1979). *Onion Storage*, Grower Books, London.

O'Donoghue, E. M., Somerfield, S. D., Shaw, M., Bendall, M., Hedderly, D., Eason, J. and Sims, I. (2004). Evaluation of carbohydrates in Pukekohe Longkeeper and Grano cultivars of *Allium cepa*. *Journal of Agricultural and Food Chemistry* **52**(17), 5383-5390.

Pak, C., Vanderplas, L. H. W. and Deboer, A. D. (1995). Importance of dormancy and sink strength in sprouting of onions (*Allium cepa*) during storage. *Physiologia Plantarum* **94**(2), 277-283.

Pollock, C. J. and Jones, T. (1979). Seasonal patterns of fructan metabolism in forage grasses. *New Phytologist* **83**, 9-15.

Pompodakis, N. E. and Joyce, D. C. (2003). Abscisic acid analogue effects on the vase life and leaf crisping of cut Baccara roses. *Australian Journal of Experimental Agriculture* **43**, 425-428.

Praeger, U., Ernst, M. K. and Weichmann, J. (2003). Effects of ultra low oxygen storage on postharvest quality of onion bulbs (*Allium cepa* L. var. c*epa*). *European Journal of Horticultural Science* **68**(1), 14-19.

Quarrie, S. A., Whitford, P. N., Appleford, N. E. J., Wang, T. L., Cook, S. K., Henson, I. E. and Loveys, B. R. (1988). A monoclonal antibody to (S)-abscisic acid: its characterisation and use in a radioimmunoassay for measuring abscisic acid in crude extracts of cereal and lupin leaves. *Planta* **173**, 330-339.

Randle, W. M. (1992). Sulphur nutrition affects nonstructural water-soluble carbohydrates in onion germplasm. *Hortscience* **27**(1), 52-55.

Randle, W. M. (1997). Onion flavour chemistry and factors influencing flavour chemistry. In *Spices: Flavour chemistry and antioxidant properties*. Edited by Risch, S., J. and Ho, C.-T. American Chemical Society.

Randle, W. M., Bussard, M. L. and Warnock, D. F. (1993). Ontogeny and sulphur fertility affect leaf sulphur in short-day onions. *Journal of the American Society for Horticultural Science* **118**(6), 762-765.

Roberts, J. A. and Hooley, R. (1988). *Plant growth regulators*, Chapman and Hall, New York.

Rosher, P. H., Jones, H. G. and Hedden, P. (1985). Validation of a radioimmunoassay for (+)-abscisic acid in extracts of apple and sweet pepper tissue using high-pressure liquid chromatography and combined gas chromatography-mass spectrometry. *Planta* **165**, 91-99.

Rutherford, R. and Whittle, R. (1982). The carbohydrate composition of onions during long term cold storage. *Journal of Horticultural Science* **57**(3), 249-356.

Salama, A. M., Hicks, J. R. and Nock, J. F. (1990). Sugar and organic acid changes in stored onion bulbs treated with maleic hydrazide. *Hortscience* **25**(12), 1625-1628.

Schwimmer, S. and Guadagni, D. G. (1962). Relation between olfactory threshold concentration and pyruvic acid content of onion juice. *Journal of Food Science* **27**, 94.97.

Schwimmer, S. and Weston, W. J. (1961). Enzymatic development of pyruvic acid in onion as a measure of pungency. *Agricultural and Food Chemistry* **9**(4), 301-304.

Sinclair, P. J., Blakeney, A. B. and Barlow, E. W. R. (1995a). Relationships between bulb dry matter content, soluble solids concentration and non-structural carbohydrate composition in the onion. *Journal of the Science of Food and Agriculture* **69**, 203-209.

Sinclair, P. J., Neeson, R. J. and Barlow, E. W. R. (1995b). Osmotic potential and soluble solids concentration in onion (*Allium cepa*) bulbs. *Journal of the Science of Food and Agriculture* **69**, 211-214.

Smittle, D. A. (1988). Evaluation of storage methods for 'Granex' onions. *Journal of the American Society for Horticultural Science* **133**, 877-880.

Sorensen, J. N. and Grevsen, K. (2001). Sprouting in bulb onions (*Allium cepa* L.) as influenced by nitrogen and water stress. *Journal of Horticultural Science and Biotechnology* **76**(4), 501-506.

Suzuki, M. and Cutliffe, J. (1989). Fructans in onion bulbs in relation to storage life. *Canadian Journal of Plant Science* **69**, 1327-1333.

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

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

Uddin, M. and MacTavish, H. S. (2003). Controlled atmosphere and regular storage-induced changes in S- alk(en)yl-L-cysteine sulphoxides and alliinase activity in onion bulbs (*Allium cepa* L. cv. Hysam). *Postharvest Biology and Technology* **28**(2), 239-245.

U.S. Department of Agriculture (2004). USDA nutrient database for standard reference. Vol. 2004. [WWW] Date accessed: 20.10.2004. http://www.nal.usda.gov/fnic/foodcomp

Wheeler, T. R., Daymond, A. J., Ellis, R. H., Morison, J. I. L. and Hadley, P. (1998). Postharvest sprouting of onion bulbs grown in different temperature and CO_2 environments in the UK. *Journal of Horticultural Science & Biotechnology* **73**(6), 750-754.

Wills, R. B. H., Ku, V. V. V., Shohet, D. and Kim, G. H. (1999). Importance of low ethylene levels to delay senescence of non-climacteric fruits and vegetables. *Australian Journal of Experimental Agriculture* **39**, 221-224.

Wise, C. S., Dimler, R. J., Davis, H. A. and Rist, C. E. (1955). Determination of easily hydrolysable fructose units in dextran preparations. *Analytical Chemistry* **27**, 33-36.

Wright, C. J. and Sobeih, W. Y. (1986). The photoperiodic regulation of bulbing in onions (*Allium cepa* L.). I. Effects of irradiance. *Journal of Horticultural Science* **61**(3), 331-335.

Yamazaki, H., Nishijima, T. and Koshioka, M. (1995). Changes in abscisic acid content and water status in bulbs of *Allium wakegi arak*i throughout the year. *Journal of the Japanese Society for Horticultural Science* **64**(3), 589-598.

Yamazaki, H., Nishijima, T., Koshioka, M. and Miura, H. (2001). Changes in carbohydrate concentrations in basal leaf sheaths of *Allium x wakegi Araki* in relation to bulb development and dormancy. *Journal of the Japanese Society for Horticultural* Science **70**(3), 353-359.

Yamazaki, H., Nishijima, T., Koshioka, M. and Miura, H. (2002). Gibberellins do not act against abscisic acid in the regulation of bulb dormancy of *Allium wakegi Araki*. *Plant Growth Regulation* **36**(3), 223-229.

Yamazaki, H., Nishijima, T., Yamato, Y., Hamano, M., Koshioka, M. and Miura, H. (1999a). Involvement of abscisic acid in bulb dormancy of *Allium wakegi Araki*. II. A comparison between dormant and nondormant cultivars. *Plant Growth Regulation* **29**(3), 195-200.

Yamazaki, H., Nishijima, T., Yamato, Y., Koshioka, M. and Miura, H. (1999b). Involvement of abscisic acid (ABA) in bulb dormancy of *Allium wakegi Araki* I. Endogenous levels of ABA in relation to bulb dormancy and effects of exogenous ABA and fluridone. *Plant Growth Regulation* **29**(3), 189-194.

Yang, S. F. (1969). Ethylene evolution from 2-Chlorophosphonic acid. *Plant Physiology* **44**, 1023-1024.

APPENDICES

Appendix I.

Abscisic acid radioimmunoassay evaluation – spike dilution details.

Table 4. The extraction method and dilution factor of the onion bulb samples used in the spike dilution. All samples were from time zero and the control treatment (S-Ca-).

Assay No.	Dilution factor	Cultivar
1,2,6	Control	
6	1[P]	Renate
2	2[M]	Renate
5	2	Ailsa Craig
6	2[P]	Renate
1	3	Renate
5	3	Ailsa Craig
2	4[M]	Renate
3	4	Ailsa Craig
5	4	Ailsa Craig
7	5	Ailsa Craig
3	6	Ailsa Craig
7	10	Ailsa Craig
3	14	Ailsa Craig
4	20	Renate[L]
7	20	Ailsa Craig
1	24	Renate
4	300	Renate[L]
4	80	Renate[L]

[L] = Lyophilised tissue, all other samples were frozen.
[M] = ABA was extracted from fresh tissue using 100% methanol at a ratio of 1ml methanol to 1g of tissue. The sample was lyophilised and then rehydrated with sterile distilled water.
[P] = The ABA extract was acidified to pH 3 by adding an equal volume of citrate phosphate buffer. A reversed phase SepPak C18 Plus cartridge (Waters Ltd., Hertfordshire) was primed with a strong solvent (5 ml 100% methanol) followed by a weak solvent (5 ml 5% acetic acid) under gravity. The column was not allowed to dry before 2 ml of the extract was loaded. After loading the extract, 5 ml 30% methanol was passed through the column. The ABA was then eluted from the column with 5 ml 60% methanol. The eluate was lyophilised and rehydrated with sterile distilled water.