Project title	The mechanisms behind onion bulb dormancy in relation to the potential for improved onion storage
Project number	CP 20
Project leader	Dr Phil White, HRI Wellesbourne
Report	Literature Review
Previous reports	None
Key workers	Gemma Chope, HRI, Wellesbourne, Warwick, CV35 9EF Tel. 01789 470382 Email: gemma.chope@hri.ac.uk Project supervisors are: Philip White and Laurence Trueman, HRI Wellesbourne, Leon Terry, Cranfield University.
Location of project	HRI Wellesbourne
Date project commenced	1 October 2003
Date completion due	30 September 2006
Key words	onion, allium, *Allium cepa*, bulb, storage, dormancy

Contents

1.0 Introduction

1.1 The genus *Allium*

The common onion (*Allium cepa*) belongs to the genus *Allium* (family *Alliaceae*), which consists of over 700 botanical species distributed throughout the temperate, warm temperate, boreal and tropical (mountainous areas only) zones of the Northern Hemisphere. Species within the genus are mostly perennial, bulbous plants. They exhibit a variety of phenologies (patterns of growth) consistent with the ecological diversification that has accompanied the evolution of *Alliums* (Fritsch and Friesen, 2002). For example, summer dormant and winter dormant species exist, adapted to dry summers and cold regions respectively (Brewster, 1994).

1.2 *Allium cepa*

Onions belong to the subgenus *Allium* and the section *Cepa*. The direct wild ancestor of onion is not known. Wild species in the section *Cepa* are petrophytes, which always grow in sites with a shallow soil layer such as rock crevices, stony slopes and river banks (Fritsch and Friesen, 2002). They have a long annual growth cycle (spring to winter) and take between three and ten years to reach flowering. The onion has been cultivated for around 5000 years and no longer exists as a wild species (Brewster, 1994). Onions have a biennial lifecycle, and are propagated by seeds, bulbs or sets. Selection for quicker growth probably took place during domestication. The storage organ of onions is a bulb, consisting of foliage leaf bases and swollen, bladeless inner sheathes. Onions flower between spring and early summer. The flowers are white with green stripes. Wide variation in the bulb characteristics, such as weight, shape, colour and flavour, exists between onion cultivars. A wide range of adaptations to photoperiod and temperature also exist, indicating intense selection.

1.3 Day length and bulb initiation

In temperate regions long days are required for bulbing to initiate. Day length, temperature and light spectral quality interact with the photoperiod (Brewster, 1990). Short day onions are somewhat misleadingly named, as although they form bulbs under short photoperiods at low latitudes, their behaviour is typical of other onions in that bulbing is faster as the photoperiod increases. Far red light, and to a lesser extent blue light, promotes bulbing, and red light inhibits it. The photoperiod required to induce bulbing can be reduced by a lower red to far-red ratio (Kahane *et al*., 1992). Temperature (including night temperature) is positively correlated with the rate of bulb development in an inductive photoperiod (Brewster, 1990).

1.4 Economic importance of the onion crop

Onion is the most economically important *Allium* crop, with an area of almost 9000 hectares harvested in the UK in 2002 (Table 1).

Table 1. The area of onions (harvested at a mature stage, not dehydrated) harvested, the yield and production from 1999 to 2002 in the UK and worldwide (FAO, 2003).

	Area Harvested (Ha)		Yield (Tonne/Ha)		Production (Tonne)	
Year	UK	World	UK	World	UK	World
2002	8950	2971750	32.7598	17.4693	293200	51914247
2001	8600	2895328	43.593	17.637	374900	51064783
2000	9100	2765515	43.1538	17.4729	392700	48321435
1999	9200	2731306	42.5435	171.503	391400	46842757

1.5 Dormancy and storage issues

Onion cultivars suitable for growing in the UK require long days for bulbing to occur, and so the summer crop must be stored over the winter. The aims of onion bulb storage are to maintain the quality of the crop and to meet consumer demands for extended availability of onions of a satisfactory quality. The principal biological factors in the deterioration of onion bulbs are respiration, resumption of growth and pathological breakdown. Class I onions are not permitted any signs of sprouting, but internal sprouting is allowed in class II provided that the sprout does not become visible within ten days of purchase. Bulbs with watery scale, and bacterial or fungal rots are deemed unsaleable.

The onion bulb is a naturally dormant organ adapted to maintain the viability of the plant during periods unsuitable for growth. In onion bulbs a dormant period where sprouting and rooting are not induced despite favourable conditions, is followed by a period where internal changes preparing the bulb for growth of shoots and roots occur. After dormancy as been broken, the bulb then proceeds towards flowering and seed production. The growth rate of the sprout inside the bulb is a major factor in determining the storage life of onions. If the biochemical changes controlling the dormancy period and sprout growth could be determined then it may be possible to extend the storage duration.

The time that onions can be stored for depends on many factors such as cultivar and pre and post harvest treatment. Long storing cultivars are available, but the new varieties of sweet onion grown for the fresh market generally do not store very well. Strategies for increasing the storage life of bulbs include low temperature (temperate countries) or high temperature (in the tropics) storage, controlled atmosphere storage where an increased proportion of carbon dioxide is maintained, and application of sprout inhibiting chemicals. Pre-harvest treatments also have a role to play.

2.0 Pre-harvest factors

2.1 Cultivar

Onion cultivars vary greatly in the properties that are important in storage life; dry matter content, bulb composition, number and toughness of outer skins and the depth of dormancy of mature bulbs. These factors are under both genetic and environmental control.

2.2 Nitrogen availability and irrigation

Sorensen and Grevsen (2001) investigated the effect of manipulation of the nitrogen supply and drought stress just prior to harvest on the storage life of cultivar Hyton. Exposure of plants to drought stress has been known to cause an increase in the concentration of growth inhibitory substances such as abscisic acid (ABA) and an increase in dry mass. The stress conditions were a deficit or surplus of 100kg N ha^{-1} (based on the content of mineral nitrogen in the top 25cm soil profile just prior to harvest), or lack of water for three weeks prior to harvest. Plants were harvested at 80% tops down and cured in the field for 2-3 weeks while protected from moisture. The bulbs were dried artificially at 25ºC for one week and then stored at 1ºC and 60-80% RH until April, May or June the following year. Plants grown at a nitrogen deficit matured later, with a later harvest date and reduced yield, whereas drought stressed planted matured earlier and yield was reduced. After storage, sprouting was recorded during a 40-day period at 15ºC to test shelf life. Shelf life in all treatments decreased with longer storage, and nitrogen deficiency slightly reduced shelf life compared to nitrogen excess or control conditions. The effect was greater in the first year of the trial, and in bulbs stored until May of both years, the effect was statistically significant. Dry matter concentration increased with increasing soil moisture deficit. Drought stress was the most effective treatment for reducing sprouting in the first year of the investigation. This was the year the water deficit in the top 25cm soil profile was greatest, 75% compared to 65% in the second year, and the dry mass of the drought stressed bulbs was greater than any of the other treatments (Sorensen and Grevsen, 2001). As bulbs were all stored until the same time despite different harvest dates, the reduced sprouting in nitrogen deficient bulbs could have been due to the later harvest date and therefore shorter storage time.

2.3 Temperature, humidity and carbon dioxide

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).

Wheeler *et al*. (1998) tested the theory that temperature and CO_2 concentration of the crop production environment has an effect on the postharvest sprouting rate of onions. Two Rjinsberger cultivars, Hysam and Sito, were used. The crops were grown in polythene tunnels with a temperature gradient of 2.5°C cooler to 2.5°C warmer than the external temperature. Treatments were elevated (532µmol mol^{-1}) and ambient (374µmol mol^{-1}) CO_2. Plants were harvested at 80% tops down and air-dried in the laboratory for two weeks before the tops and roots were removed. Visible sprouting at 11°C was assessed every 3-4 days. For each treatment a linear function described the relationship

between the number of bulbs sprouted and the duration of storage. Onset of sprouting was calculated by extrapolation, which may not have been the most accurate method as the rate of growth inside the bulb may be different to that outside the bulb. The time until onset of sprouting was not affected by growing season temperature, cultivar or CO_2 levels, but the sprouting rate increased with increasing temperature. At higher temperatures the time between transplantation and onset of bulbing, and the duration of bulbing was reduced. However, all crops were harvested at the same developmental stage therefore it is possible that an effect of temperature other than the effect on the rate of plant growth can modulate the post harvest storage behaviour of onion bulbs.

Rutherford and Whittle (1982) also observed that onions (cultivar Robusta) from growing seasons with lower mean sunshine hours and soil temperature could be stored for longer. In temperate climates, hot dry weather at the end of the growing season is needed to speed up leaf drying and to allow harvest of partially dry bulbs with no rain marks (Gubb and MacTavish, 2002).

2.4 Crop maturity at harvest.

The timing of harvesting impacts on both yield and storability. Common practice is to harvest bulbs at 50-90% tops down, sacrificing some yield for a greater number of intact skins. If bulbs are harvested too soon the water content in foliage leaves and the neck is too high and pathogens are encouraged. 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 (Gubb and MacTavish, 2002). Rutherford and Whittle (1982) found that early harvested bulbs dried and stored in the same manner as later harvested bulbs had lower carbohydrates levels, which were reduced further during sprouting, which occurred earlier. The consensus in Europe and the USA is that the optimum harvest time for storage onions is at 80-90% tops down.

2.5 Harvesting process

It is important that the physical damage to onion bulbs during harvesting is limited, especially with softer, sweeter onions, because wounding causes bulbs to sprout. Undercutting is usually done prior to lifting. Mechanised lifting is common practice, often done with modified potato lifters. When onions are stored in bulk, the tops are often removed to help 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).

2.6 Curing and drying

Onions for storage are cured and dried after harvest. 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 (Gubb and MacTavish, 2002).

3.0 Composition and biochemical changes in bulbs during storage

3.1 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 and is facilitated by evaporation and low-level respiration.

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 are therefore likely to have an effect on the properties of the skins (Hole *et al*. 2000). The ability of onions to withstand physical abuse during post harvest and post storage handling depends on the mechanical properties of the skins. Hole *et al.* (2000) found that humidification resulted in increased resistance of skins to breaking. Compliant skins are more 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.

The relative humidity of the storage environment is a compromise between keeping levels below that at which pathogens are encouraged and (<80%) 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 levels below 55%, therefore relative humidity in the storage environment should be maintained between 55-80%.

3.2 Respiration

Benkeblia *et al*. (2002) measured the respiration rate of Rouge Amposta onions (long storage type) harvested at the beginning of September and field dried for two weeks. Three sets of storage conditions were used; 4°C and 85% RH, 10°C and 80% RH, and ambient conditions of 20°C and 65% RH. The respiration rate generally increased during storage, rising from 0.12 to 0.19mmol kg^{-1} h^{-1} at 10°C and 0.17 to 0.30mmol kg^{-1} h^{-1} at 20°C after 24 weeks of storage. The respiration rate remained stable after eight weeks of storage at 4°C.

3.3 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 fresh weight (Rutherford and Whittle, 1982).

At 15-16˚C, fructose levels increase over the storage period (Salama *et al*. 1990; Pak *et al*. 1995) and fructan levels decrease (Suzuki and Cutcliffe, 1989; Ernst *et al*. 1998). Fructose concentration is higher in the outer leaves than the inner leaves (Darbyshire and Henry, 1978; Salama *et al*. 1990; Pak *et al*. 1995). A peak in soluble sugar content occurs between five and eight weeks after harvest (Salama *et al*. 1990; Benkeblia *et al*. 2002).

Pak *et al*. (1995) measured the glucose, fructose and sucrose contents were measured as well as fructans (calculated as total carbohydrate minus glucose, sucrose and fructose) in Rijnsberger Hysam onions. The bulbs were harvested at 80% tops down and cured for one week at 25-30°C. Storage was at 16°C, to be favourable for sprouting. Fructan content began to decrease two weeks before harvest. Carbohydrates may be converted before harvest to compensate for reduced photosynthetic ability due to the loss of green leaves. They also looked at enzymes involved in carbohydrate metabolism. The enzyme invertase was not found in the bulb throughout the experiment. Postharvest sucrose synthase activity in the shoot decreased very slightly, and increased in the bulb base, while activity in the inner and outer scales was consistently low. This suggests that the sucrose synthase pathway plays a more important role in sucrose degradation than invertase the pathway.

Salama *et al*. (1990) assessed changes in glucose, fructose and sucrose content over a storage period of twenty weeks in Sentinel onions grown on organic soil in New York State. Harvesting was by hand and bulbs were field cured for one week. The tops were removed and bulbs were cured in crates for two weeks at 25ºC and 60-70% RH. Six storage conditions were created using a combination of three temperatures, 0, 15, and 30ºC, and two relative humidities, 40 and 60%. Relative humidity had no effect on sugar levels, but temperature and storage duration did. Fructose was higher in bulbs kept at 0 and 15ºC and 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. Glucose was higher in the outer leaves than in the inner leaves, however Pak *et al*. (1995) and Darbyshire and Henry (1978) found no difference.

Benkeblia *et al*. (2002) assessed the changes in soluble sugar and fructan levels occurring in Rouge Amposta onions. Onions were harvested at the beginning of September and field dried. Three sets of storage conditions were used; 4°C and 85% RH, 10°C and 80% RH, and ambient conditions of 20°C and 65% RH. The pattern of changes in total soluble sugars was similar for all storage conditions, but the peak total sugar level was less in the onions stored at 4°C. The concentrations of tetra, penta and hepta saccharides were greater at 10 and 20°C, probably because of the enzymatic hydrolysis of fructan polymers by depolymerases. At 10 and 20°C sprouts were visible after six and eight weeks respectively, and at 4°C sprouting was visible after 16 weeks. Conclusions were that the catabolism of carbohydrates was more dependent on the physiological stage than the temperature. Ernst *et al*. (1998) found no consistent differences in the fructan profile of different onion cultivars. Any differences found

were quantitative and they concluded that they were likely to be due to the stage in the life cycle of the plant.

Darbyshire and Henry (1978) studied the carbohydrate composition in recently harvested bulbs of the cultivar Cream Gold syn. Pukekohe Long Keeper. The bulbs were purchased and so curing and drying protocols are either unknown or not included. The results presented are based on a single bulb and due to the variation between bulbs their results may not be representative of the cultivar. The carbohydrate content of seven leaf bases from the inner to the outer of the bulb was determined. The percentage contribution of fructans of the total non-structural carbohydrates increased towards the centre of the bulb. Fructans with a DP of 3-9 were present in all leaf bases, and like total fructans became more concentrated towards the centre of the bulb. Increasing molecular weight was inversely correlated with concentration. Sucrose was lower in the outermost and innermost leaf bases than in the rest of the bulb.

Yamazaki *et al*. (2001) found little difference in the fructose, glucose, sucrose and fructans concentration in dormant and non-dormant cultivars of *Allium wakegi Araki*. During bulb development the concentrations of fructose and glucose decreased, while fructans and total carbohydrates increased, and no changes in sucrose concentrations were observed. After bulb development the percentage contribution of fructans to total carbohydrates exceeded 90%. When the bulbs were planted and began to sprout, concentrations of fructose and glucose increased and fructans and total carbohydrates decreased. Total carbohydrate content was similar in each cultivar, but before bulb development the contribution of fructans and fructose to total carbohydrates was 35% and 30% in the dormant cultivar and 70% and 10% in the non-dormant cultivar respectively.

3.3.1 Carbohydrate content and storage life

Suzuki and Cutcliffe (1989) found a significant but not large positive correlation between fructan content and percent marketable bulbs after two and four months storage in a three-year study with eight different onion cultivars. The bulbs were harvested in early October when nearly all tops were down, and dried in greenhouses in open boxes for five weeks. Bulbs were stored at 6-10°C and 40-60% RH, the conditions used by small producers and consumers. After two months the range of marketable bulbs per cultivar was 0 to 89%. No marketable bulbs were left after six months storage in any cultivar in any year. Sucrose occupied the largest proportion of WSC, followed by monosaccharides and fructans with a degree of polymerisation (DP) of 3 and 4. Fructans with a DP of 7 or more were also present.

Rutherford and Whittle (1982) found a correlation between higher fructose content at harvest and extended storage life. They measured the carbohydrate composition of Robusta onion bulbs over three growing seasons. Bulbs were harvested at full maturity in late September and immediately dried at 26°C and RH 70% for two weeks, then 10°C and 70% RH until the first week of November to complete the drying. Bulbs were stored at 4°C and 70% RH and sampled monthly until February when sprouts were visible. Total soluble sugars were constant from harvest throughout storage, but decreased when the onions began to sprout, although the amounts of glucose and fructose did not change during sprouting. Carbohydrate composition varied over the three seasons. The total sugar content of the bulbs did not change, although the

proportion of reducing and non-reducing sugars did. In the outer scales, fructans were hydrolysed into fructose and glucose.

This is in agreement with a lower proportion of bulbs sprouting per day in bulbs with higher levels of carbohydrate, as observed by Wheeler *et al*. (1998).

3.4 Organic acids

The major metabolic substances in the onion bulb are glucose, fructose, sucrose, malic acid and citric acid, with small amounts of succinic acid and fumaric acid. Pyruvic acid is also found in onions, but is discussed in the pungent flavours section. Citric acid increased in Sentinel onions (see carbohydrate section for harvesting practice) stored at 30ºC and was constant at 0 and 15ºC. The acid profile was different at 30ºC, but total acid levels were similar. Malic acid concentration was less at 0 and 15ºC than at 30ºC. These acid concentrations were only slighter greater at 15ºC than at 0ºC, so the higher levels were not due solely to respiration (Salama *et al*., 1990). Different citric to malic acid ratios at the higher temperature indicates an impact on metabolic processes, however it may be because heat causes the loss of a water molecule from malic acid, forming fumaric acid.

3.5 Flavour precursors and pungency

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 which contribute to perceived flavour. Ammonia and pyruvate are non-flavour products of this reaction. The composition and concentration of ACSOs determines the nature and intensity of flavour and odour. Different ASCOs are responsible for different components of flavour. Variations in flavour between cultivars and changes that may occur during storage are due to the differences and differential changes in the ASCOs present and alliinase activity. It is possible for pungency to increase, decrease or stay the same over time (Uddin and Mactavish, 2003). The amount of enzymatically produced pyruvate in onions is positively correlated with pungency (Havey, 1999).

Some of the ACSOs in onion are bound to glutamic acid as γ-glutamyl peptides and are not susceptible to the action of endogenous alliinase. In yellow sweet onions and Spartan Banner variety onions, γ-glutamyl transpeptidase in combination with exogenous C-S lyase enhanced pyruvate production in macerated onion. Addition of exogenous C-S lyase alone to macerated onion tissue also increased the pyruvate production slightly (Hanum, 1995). This implies that the reaction in onion tissue may not go to completion due to a sub optimal supply of endogenous C-S lyase.

Relative pungency is dependent on genetic and environmental factors. *Alliums* are efficient at sulphur uptake. This could be a survival mechanism: grazing animals may learn to avoid strong tasting plants. Selection for less enzymatically produced pyruvate results in a milder onion. The potential to reduce pungency by selection based on sugar content is a possibility that has not been fully explored (Havey, 1999).

Hamilton *et al*. (1998) grew Texas Grano 1015Y onions on clay and loam soil in a major onion producing area. The clay soil had a higher sulphur content than loam. Two treatments were applied to each soil type: no additional sulphur fertiliser or 22.4kg of sulphur per hectare. Over most of course of crop growth the sulphur content of the fields was stable, but increased during the month before harvest. Irrigation was terminated around this time and so the increase may have been a result of the crystallisation of sulphur salts in the soil. The sulphur treatment applied was not sufficient to effect a significant change in the sulphur content of the soil. Pyruvic acid concentration generally decreased during bulb maturation in the field. This was likely to be due to the dilution effect during the rapid growth period of the bulb. In this investigation pyruvic acid levels showed no response to sulphur nutrition. This was probably because the sulphur treatments applied were too low.

3.6 Plant Growth Regulators

Over winter storage in the UK a gradual change in the relative composition of plant growth regulators occurs as the levels of inhibitors drop and the levels of growth promoters rise. Gibberellins (GAs) have a first peak in December, followed by peaks of cytokinins and auxins. A second gibberellin peak is accompanied by sprouting in March. 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). Therefore before an external sprout is visible, there are important internal changes occurring in the apparently dormant onion. ABA levels are high at the onset of dormancy, reaching a maximum approximately one month after harvest. The levels then decrease until rising again during sprouting. The roles and mode of action of plant growth regulators are unknown, but is probably a complex phenomenon involving the combined action of several endogenous hormones (Gubb and MacTavish, 2002).

Abdel-Rahman and Isengard (1974) investigated the role of an auxin - indole acetic acid (IAA), gibberellic acid (GA), kinetin (K), abscisic acid (ABA), maleic hydrazide (MH) and ethephon (2-chloroethylphosphoric acid, CEPA) in the dormancy and regrowth of onion bulbs (c.v. Elba Globe). The treatments used were 100ppm (in 1ml) of GA, IAA, ABA, or MH and combinations of either MH or ABA with IAA, GA and/or K, with the addition of the following treatments in the second year: 100ppm ethephon and combinations of ethephon, GA, IAA and/or K. Treatments were applied to fully mature bulbs via injection. Three weeks after treatment the plants were killed by spraying with onion-top killer (neodecanoic acid) and plants were harvested three days later. The bulbs were stored at 3˚C for 4-6 weeks and planted in moist vermiculite in a growth chamber at 26˚C and 85% RH in the dark. Treatment effects were assessed in terms of effect on plant growth, bulb dimensions, days between injection and leaf senescence, and dormancy breaking of harvested bulbs denoted by rooting and sprouting observations. Injection of ABA alone had the greatest effect on the length of the dormant period – 74 days, compared to 43 days for control bulbs. By the end of the trial period, only 75% of the bulbs treated with ABA had sprouted compared to 100% for all other treatments, including the control. The sprout length was also shorter. This experiment is limited in measurements were only taken at the end of the storage period, by which time most bulbs had external sprouts regardless of treatment. Therefore the effects of treatment on the rate of sprouting could not be assessed. Combinations of plant growth regulators were not able to reverse the inhibitory effect of MH. However,

treatment with a combination of MH, IAA, GA and K resulted in 25% sprouting, compared to 0% when treated with MH alone. Treatment with IAA, GA, K or their combinations delayed the onset of leaf senescence, while treatment with ABA reduced the time to senescence. ABA reduced sprouting, but effects were lessened when combined with other plant growth regulators especially K, and K + IAA + GA. They observed that onion bulbs produce ethylene at much greater concentrations at the end of dormancy than at the beginning. Ethephon alone had little effect on the dormant period but when applied in combination with ABA it reduced the effect of ABA on dormant period.

When Thomas (1969) investigated the role of plant growth regulators in onions, he placed his bulbs in storage after two months in trays in the field after harvest. He assumed that after two months the bulbs would be ready to sprout under favourable conditions. A long storing (Rjinsburger) and a short storing cultivar (Lancastrian) were used. Storage was in bins at an average temperature of 5˚C from mid November to April. Samples were taken at fortnightly intervals. Visible sprouting was recorded, but when non-sprouted bulbs were cut open at the end of the storage period well developed internal sprouts were found. This would have reduced the saleability of the onions. The sprouting behaviour of each cultivar was different, but in both cultivars auxin activity was detected at the time of sprouting and persisted as sprouting continued. GA was detected in the early period of storage but decreased until none could be detected just prior to sprout emergence. The levels of inhibitors in bulbs with internal signs of sprouting were low when compared with the levels in non-sprouting or fully sprouted bulbs, and more inhibitor was present in the long storing cultivar at the beginning of the storage period than in the short storing cultivar. Auxin appeared to inhibit sprouting especially during the later stage of the storage period. This was probably due to reducing elongation of shoot cells and reducing growth rate of the shoot inside the bulb. Gibberellin treatments resulted in long fine roots, suggesting a role in cell elongation, while auxin treatments resulted in thicker and shorter roots, suggesting a role in cell division. GA content was more likely to be a result of sprouting rather than a cause, as levels were low in non-sprouted and internally sprouted bulbs. Non-sprouted bulbs were used for hormone assays, but true non sprouted bulbs would have become increasingly difficult to identify towards the end of storage period. Therefore the later stages of the experiment were more likely to pick up changes caused by sprouting rather than those causing sprouting.

3.6.1 Abscisic acid

ABA is a phytohormone that is naturally occurring in plants. The initial stages of ABA biosynthesis occur in chloroplasts and other plastids. The amount of ABA in the plant is a balance between synthesis and degradation. Development, environmental conditions such as drought stress, and other growth regulators affect these processes. ABA is synthesised from a carotenoid precurser, which is cleaved inside the plastid to form xanthonin. Xanthonin is then converted to ABA via abscisic alcohol, in the cytoplasm (Cutler and Krochko, 1999). ABA has many physiological effects, many to do with response to water and cold stress, including bulb and seed dormancy, germination inhibition, stomatal closure and inhibition of cell elongation.

3.6.1.1 Abscisic acid, bulbing and dormancy

Matsubara and Kimura (1991) found endogenous ABA in all onion bulb tissues (leaf, growing tip, bulb and leaf sheath), but the actual amount varied according to age and tissue type. After harvest storage temperature affected ABA concentrations. At low temperature storage ABA rapidly decreased after one month, whereas at high temperature storage a rapid decrease occurred a month later. *In vitro* studies did not support the theory that ABA is the primary cause of bulbing, as addition of ABA (0.1-5 mgl^{-1}) did not induce bulbing, although the number of leaves was decreased.

Yamazaki *et al*. (2002) studied the role of ABA and gibberellins in the regulation of *Allium wakegi* (a hybrid between Japanese bunching onion, *A. fistulosum*, and shallot, *A.cepa* L. Aggregatum group) bulb dormancy in Japan. Endogenous gibberellins were identified in *A. wakegi*, and the concentrations of ABA and GAs in the bulbs were measured in relation to bulb development and dormancy. The plants were harvested at full maturity in mid May and dried in a plastic film house for two weeks, with roots and aerial parts removed. Over the summer the bulbs were placed in plastic netted bags and hung under a canopy under natural conditions. Bulbs were planted in moist vermiculite in early September. Basal leaf sheath samples were collected from February to September. Bulb development was followed by measuring the ratio of the maximum diameter of the basal leaf sheath to the minimum diameter of the neck. Bulb dormancy was defined as the number of days from planting to sprouting with roots and aerial parts removed. The concentration of free ABA in the basal leaf sheath after harvest was positively correlated with the number of days to sprouting. Conjugated ABA changed only slightly during the sampling period and is therefore unlikely to play a major role. All gibberellins showed a temporal increase shortly before bulb swelling and rapidly decreased after and remained at a low level throughout the storage of bulbs. A peak in gibberellins occurred after dormancy had been induced. Gibberellins are probably related to elongation of the shoot rather than to dormancy. A one-month time lag was observed between the peak ABA concentration and the peak number of days to sprouting.

Yamazaki *at al*. (1999a) examined the relationship between ABA content and the state of bulb dormancy in *Allium wakegi* bulbs exposed to bulb inducing and non bulb-inducing conditions in Japan. The effect of the application of ABA and fluridone (fluridone inhibits ABA synthesis) on bulb development and dormancy was examined. *Allium wakegi* forms dormant bulbs under long day conditions. ABA treatment was applied two months after planting (November); some plants were then subjected to long days (LD)(14h photoperiod) while others kept under natural short days. Bulbs were excised from plants exposed to LD for different time periods and soaked in 0, 50, 500µM aqueous (+)-ABA. Fluridone (5ml of 0, 5, 25, 125µM aqueous solution – 1% acetone water) was applied to plants in pots, which were then placed in a controlled environment of 25˚C in the day and 20˚C at night under LD. Development of bulbs and bulb dormancy (number of days to sprouting on moist vermiculite) were monitored. Plants kept under natural short days did not form bulbs, and endogenous levels of ABA were low throughout – a maximum level of 5ng g^{-1} FW compared to a peak level of 20ng g^{-1} FW for plants subjected to 60 days under LD (in the middle of the deeply dormant period). Under LD, bulbs became dormant at the 30^{th} day of LD, were deeply dormant for 60 days and then were gradually released from dormancy. No dormancy

was observed after 155 days. At the end of the treatment endogenous ABA concentration in the treated bulbs was higher than in the control bulbs, even though 5µM and 50µM had little or no effect. However bulbs were used for endogenous ABA quantification after being washed in water and so inefficient removal of the exogenous ABA treatment may have been the cause of elevated ABA levels. Treatment with 500µM ABA increased the days to sprouting, having the greatest effect in bulbs subjected to 60 days LD. Fluridone at 25µM accelerated sprouting. These results suggest that ABA is important in the dormancy of *Allium wakegi*, but is not involved in bulb formation as bulb scales were formed in all plants, and the leaf sheaf ratio was similar regardless of fluridone application.

3.6.1.2 Sensitivity to abscisic acid

Yamazaki *et al*. (1999b) aimed to determine the ABA content and sensitivity to ABA of the bulbs in dormant (Kiharabansei No. 1) and nondormant cultivars (Ginoza) of *Allium wakegi*. Endogenous levels of ABA were measured 0, 3, 6, and 9 days after planting in moist vermiculite two weeks after harvest, when Kiharabansei was dormant. Bulbs of both cultivars (with outer scales removed) were soaked in 0, 10, 100, or 1000µM (+)-ABA for 24 hours. This was repeated with the nondormant cultivar. Bulb development and dormancy were recorded. This experiment was repeated when the bulbs of the dormant cultivar were partially released from dormancy. The number of days to sprouting of the dormant cultivar decreased with increasing delay in planting, indicating that there is a definite dormant period. A time lag of one month was again discovered. Exogenous ABA inhibited sprouting in the dormant cultivar at a level of 1000µM, but did not have an effect on the nondormant cultivar. ABA content on the bulbs increased during bulb development and reached a maximum level shortly after harvest. The level then decreased throughout the storage period. ABA concentrations were similar and followed a similar pattern in the nondormant cultivar. Peak concentrations were at the same time but slightly higher in the nondormant cultivar (33ng g^{-1} FW) than the dormant cultivar (26ng g^{-1} FW). These results suggest that sensitivity to ABA could be an important factor in onion dormancy.

As in onions, ABA levels in potatoes decrease during storage. Suttle and Hulstrand (1994) found that application of fluridone to potatoes decreased endogenous ABA concentration. Conjugated ABA was low, as in *A. wakegi*. Exogenous (+)-ABA only had an effect on internal ABA levels in the tubers after nine weeks. Failure of exogenous (+)-ABA to affect endogenous ABA was attributed to limited uptake period, suboptimal treatment concentration and oxidative catabolism of ABA. Untreated microtubers remained dormant throughout the nine-week investigation period, but those treated with fluridone progressively lost dormancy - 96% had sprouted by the end of nine weeks. Application of exogenous (+)-ABA to tubers treated with fluridone resulted in a dose dependent increase in endogenous ABA, and an increase in sprouting percentage. ABA is only active as a growth inhibitor when applied to dormant tubers. The authors interpret the findings of this investigation to suggest that ABA restores dormancy rather than acts as a growth inhibitor, however it could be that sensitivity to ABA is greater during the dormant period.

In stored onion bulbs the major nucleolar organising regions (NORs) that form the visible nucleoli in the leaf base outer epidermis (equatorial region) become activated when cells are exposed to ambient conditions in all regions of the bulb leaf base except

in the apex. Activated nucleoli increase in size and number and change morphologies from round and oval to elongated oval and dumbbell shapes. Minor NORs become active and form visible nucleoli between 6 and 24h of exposure to ambient conditions, reduced to 3h in the presence of ethylene (Karagiannis and Pappelis, 1994).

Karagiannis and Pappelis (1994) aimed to find out whether plant growth regulators were involved in the maintenance of the karyoskeleton essential for normal cellular activities. The following null hypotheses were tested in sweet, yellow Spanish onions:

1. No selective ribosomal cistron regulation can be demonstrated in quiescent epidermal cells from basal, equatorial and near-apical (2-1cm from dead cells at the apex) tissue harvested from the third outer most turgid leaf base and treated with solutions of ABA, GA_3, IAA and kinetin (K).
2. No major or minor NORs will be activated in apical (1-0cm from dead cells at the apex) epidermal cells in an advanced state of senescence when treated with the four plant growth regulators (PGRs).

Their data led them to reject the first null hypothesis and to accept the second. A $0.5cm^2$ section of outer epidermis was removed from each region. Nucleoli were classified according to shape and number per nuclei. The following treatments were applied: 10^{-5} M ABA, 10^{-5} M GA_3, 10^{-5} M IAA, 10^{-9} M K and water (control). Samples were taken after 3 and 6 hours of incubation in the dark at 23±1ºC. At T=0 90% of nucleoli were small and round, and 10% were small and oval. Apical and equatorial tissues were not statistically different from this, but less major nucleoli were activated in the basal tissue. In the controls at T=3h, about 25% of the major nucleoli had enlarged to become elongated oval or dumbbell shape. ABA treatment inhibited nucleolar activation in basal, equatorial and near apical tissue compared to the controls. No minor nucleoli were visible and no major nucleoli were activated in apical tissue. GA_3 stimulated major NOR activation in basal (T=6) and equatorial and near apical (T=3) regions, as did K. IAA activated major NORs in basal tissue and but inhibited those in equatorial and near apical regions. This supports a role for ABA in inhibition of cell elongation, and an inductive role for auxins, and a variation in the sensitivity of different tissues.

4.0 Dormancy and dormancy breaking

4.1 Changes in dormancy over time

Carter *et al*. (1999) hypothesized that control of cell division (in the shoot apex) may be a key regulatory process in bulb dormancy and sprouting. To test this they studied the expression of the cell cycle regulated gene histone 2A in the cultivar Robusta, and within a group of cultivars with varying storage lives. Bulbs were harvested at maturity and allowed to dry, then stored in cooled ambient conditions. The first sign of sprouting was taken as visible emergence of the leaves from the neck of the bulb. Samples were taken in the field and throughout storage (mid October to August. Onions (cv. Robusta) grown under 2 weeks short days (8h) and 32 long days (18h) formed definite bulbs, whereas control bulbs subjected only to short days did not. At the end of the growing period histone 2A levels were barely detectable in the leaf tissue, and levels in the bulb was about 2.5 times greater than in the root. Levels followed the same pattern, but were less in the root and bulbs of the short day control plants, and greater in the leaves of the long day plants. Photoperiod did not have a great effect on the expression. In the stored bulbs the final measurement coincided with 74% sprout emergence, the

remainder of the bulbs decayed prior to sprout emergence. Sprouts did not appear until after 140 days of storage (early April). 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. Staining with TTC revealed a changing pattern of respiration activity during storage. 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 increased staining of the root tips indicated an increase in metabolic rate. 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 same tests were carried out on other cultivars selected as very good or very poor storers. A peak in histone 2A appeared in all cultivars during storage, but at the same time of year as Robusta (March to April) and not coinciding with the developmental stage of the bulbs. Long storing non-sprouted bulbs were planted to confirm viability. This experiment confirms that the histone 2A peak is not related to sprouting time. Histone 2A is an important component of chromatin and expression pattern is linked to cell division activity. The genetic basis of control of sprout leaf elongation is not known, but will play a large part in the determination of storage capacity.

4.1.1 Root dormancy

Ernst *et al*. (1999) tested the hypothesis that absence of starch in the primary thickening meristem (PTM) is indicative of onion bulb dormancy by investigating the effects of cultivar and temperature on the temporal pattern of starch occurrence in the PTM. The PTM is located near to the apical meristem and is responsible for stem thickening and root initiation. Experiments were carried out over three seasons in three cultivars; Southport White Globe F1 (very early sprouting), Golden Bear F1 (early sprouting), Super Bear F1 (late sprouting) and Stuttgarter Riesen (late sprouting). Bulbs were harvested at 80% tops down, dried in open sheds and stored at 16-20˚C. An overlapping mean was used to compensate for bulb-to-bulb variation. Leaf ratio steeply increased 40-60 days after harvest except in Super Bear in which the steep increase occurred at 90 days. When the bulbs were dried for three weeks the steep increase in starch in the PTM preceded (early cultivars) or accompanied (late cultivars) the steep increase in sprout growth as determined by leaf ratio measurements. Only the early sprouting cultivar Golden Bear was used in the temperature experiment. At 15˚C sprouting occurred after 7 weeks and at 1˚C and 30˚C it occurred after 10 weeks. At 15˚C the increase in starch was almost immediate and again preceded sprouting. At 1˚C the increase was delayed and slow, but still preceded sprouting. At 30˚C starch dropped to non-detectable concentrations and stayed low during delayed sprouting, this effect was reversed when bulbs were moved to 15˚C. They also looked at the effect of cytokinins on sprouting in bulbs used immediately after harvesting. Bulbs were kept at 30˚C for 6 weeks and then injected with benzyladenine (a cytokinin) (BA) or solvent (control), then left at 30˚C for a further week. In bulbs injected with BA there was an increase in leaf ratio compared to the control. No starch was found in the PTM in either treatment. They concluded that a low concentration of starch is an indicator of root dormancy and not sprout dormancy based on the following:

1. Increase of starch does not consistently interact with sprout growth.
2. Storage at high and low temperatures inhibited sprouting but acted differently on starch. High temperatures may have imposed root dormancy and low temperatures slowed root growth.
3. Cytokinin induced sprouting at high temperature but starch was not detected.

Sprout dormancy may be determined by root dormancy. If the PTM is inactive no roots can be formed and so cytokinins are not supplied for sprouting.

It has been shown that bulbs with roots sprout earlier in dry storage than those with the roots removed. Therefore the root system may provide substances that promote sprout growth or elongation. Miedema and Kamminga (1994) grew and stored bulbs with varying dormancy characteristics. Cultivar differences in derooted bulbs were more pronounced than in rooted bulbs. It was suggested that cytokinins exert their action by stimulating cell division in the sprout meristem or by increasing the sink activity of the sprout. Wounding of the growth plate promoted sprout growth and may have done so by facilitating gas exchange and promoting respiration.

4.2 Temperature and dormancy breaking

Temperature has a profound effect on the dormancy period and storage life of onion bulbs. In general sprouting is inhibited by low and high temperature, and encouraged at intermediate temperatures. In developed temperate countries, onions are kept in large, specialised stores. Ventilation is forced, usually around 5ºC, but can be as low as -1ºC. This is the most important storage strategy in the UK. Moisture loss is increased at temperature ranges 0-10ºC and 27ºC+, and decreased at 11-27ºC. Increased sprouting and root growth is observed at 5-20ºC. In warmer climates high temperature storage is a practical option. High temperature storage conditions are a compromise between rotting loss and sprouting loss. At temperatures above 25ºC sprouting is inhibited, but moisture loss is high. High temperature storage conditions are generally 25-32ºC, and 60–75% RH (Brice *et al*. 1995). The high temperature inhibition of sprouting is likely to be related to the dormancy observed in hot seasons in wild *Alliums* (Gubb and MacTavish, 2002).

Brice *et al*. (1995) looked at two three-month storage seasons (April to June) in the Yemen. The red cultivar Baftain was field dried for three days. The bulbs were stored in bins under various ventilation regimes which aimed to maintain conditions of 26-30ºC and 60- 75% RH. Control bulbs were placed adjacent to the bins. The main effect of ventilation was to reduce fluctuations in temperature and humidity in comparison with the prevailing ambient conditions. There was little difference between the control bulbs and the ventilated bulbs after 19 weeks of storage, but after 31 weeks ventilated bulbs suffered less rotting and weight loss, and the majority of bulbs were marketable. Rotting in the control bulbs was thought to be due to localised heating. Rejected bulbs were five times less in the ventilated bulbs due to reduced rates of external sprouts, bacterial infection and dehydration.

Miedema (1994a) selected ten onion cultivars based on large differences in time to sprouting. Bulbs were grown from seed and harvested at 50% tops down. Drying conditions were 25ºC for 10-20 days and bulbs were stored at the same temperature until use. Experiments were carried out at a range of temperatures: 5, 10, 15, 20, 25, 30 and 35ºC, with relative humidity ranging from 90-95% at 5ºC to 30-40% at 35ºC. Visible

sprouting was recorded. The time to 50% sprouting and rooting was calculated by interpolation. Short-term temperature treatments of 15-35ºC for three weeks were applied immediately after harvesting and defoliation. Time to sprouting and rooting after planting in moist vermiculite at 15ºC was recorded. Each cultivar differed in the response to temperature. Optimum temperatures for sprouting ranged from 10 to 25ºC. Some had a sharp optimum and others had a broader range. 10-20ºC was the optimum temperature range for sprouting in dry storage of most cultivars. Sprouting was inhibited at 30ºC. Decay of unsprouted bulbs was more prevalent at 30ºC. In the experiment on sprouting of different cultivars at 10ºC, all bulbs opened at the end of the 315 day storage period contained internal sprouts. Post harvest treatment 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. The post harvest treatments indicate that exposure of the bulbs to high temperatures during curing and drying may reduce the level of dormancy and therefore reduce storage time. Inter and intra cultivar differences were observed in the traits measured.

4.3 Controlled atmosphere

Uddin and MacTavish (2003) reported that after nine weeks of controlled atmosphere storage pyruvate concentration decreased, whereas in ambient atmosphere storage the pyruvate concentration increased. The decrease in pyruvate concentration was accentuated at the greater carbon dioxide concentration. Conditions of temperature and humidity, 0.5±0.5ºC and ±3% RH, for each treatment (21±0.2% O_2 and 0.1±0.1% CO_2, 2±0.2% O_2 and 2±0.2%, and CO_2 2±0.2% O_2 and 8±0.2% CO_2). The difference was statistically significant but may not have been detectable by humans as it was less than 1µmol and the range of human detection is between 1 and 7µmol. ASCO 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.

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 (Gubb and MacTavish, 2003).

4.4 Sprout inhibitors

Potatoes, like onions require sprout inhibition combined with proper storage management for long-term storage. Kleinkopf *et al*. (2003) reviewed the current status of sprout inhibition in storage. The major sprout inhibitor used on potatoes is chloropropham (CIPC). CIPC stops sprouting by inhibition of cell division, more specifically it interrupts spindle formation during active mitosis. CIPC is applied as an aerosol in storage. As with many chemicals the residue limits are being reviewed and have been lowered. The storage conditions and use of CIPC treatments depend on the end use of the commodity i.e. fresh market, dehydration and freezing, and chips. Maximum application rates apply. A consistent residue throughout the potato pile is therefore desirable.

Sprout inhibition failure in storage can be due to many reasons.

1. Product failure or incorrect application rate.
2. Temperature differences in the potato pile due to bad ventilation systems.
3. Disease hotspots or excess dirt restricting the airflow.
4. Field stressed potatoes may respond less well to the inhibitor than non stressed ones.
5. Fluctuating humidity and temperature can accelerate physiological ageing of potatoes, which then respond differently.
6. Late season application i.e. after dormancy break.

Different cultivars respond differently to the treatment most often due to differing length of dormancy period.

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

Marcano *et al*. (2003) carried out a study to determine the mitotoxic and clastogenic effects of maleic hydrazide as well as the ultrastructural alterations induced at root tips of *A. cepa* at different exposure times. In normal cells the ultrastructure of the nucleolus has mixed granular and fibrillar components. In those treated with maleic hydrazide, the granular components were centrally located and were surrounded by the fibrillar components, an arrangement known as nucleolar segregation. 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. The mitotic index allows estimation of the frequency of cell division. MH caused a dose dependent reduction in the mitotic index compared to the control, which remained steady. The effect of concentration was 1.93 times higher than the effect of the time of exposure. Clastogenic effects were also observed. Maleic hydrazide has no effect on sugar and organic acid composition of the bulbs (Salama *et al*. 1990).

4.4.2 Ethylene

There are conflicting reports on the effect of ethylene during storage. If endogenous ethylene does stimulate sprouting then research into ethylene blockers such as methyl cyclopropene (MCP) may be worthwhile.

Benkeblia and Selslet-Attou (1999) looked at the effect of ethephon (1ml of100ppm injected into centre of the bulb), and, ethephon and cold treatment (cooled for 3 weeks at 9ºC / 70% RH, then 18ºC for 24h before treatment) on Rouge Amposta onions in Algeria. The bulbs were harvested in August and dried in the field for two weeks. Storage conditions were in 12kg trays in the dark at 18ºC and 70% RH for six months. There was little variation in ethylene production. Sprouting was earlier in the bulbs cooled and treated with ethephon (50% at 2 months, 100% at 4 months) than in the

control and ethephon treated bulbs (50% at 3 months, 100% 6 months). They concluded that ethephon does not have a significant effect on the break of dormancy and sprouting of onion bulbs.

Ethylene has not got a clearly defined action in potatoes, but commercial scale trials indicate that continuous ethylene exposure may allow for effective long-term sprout control (Kleinkopf *et al*. 2003).

4.4.3 Other chemicals

Benkeblia and Varoquaux (2003) looked into the possibility of using nitrous oxide as a method of extending the storage period of onions. Nitrous oxide is similar to carbon dioxide in terms of relative stability and high solubility in water, and is permitted for food use. It 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. The influence of N_2O applied at different concentrations for different time periods on sprouting and rotting of onion bulbs, as well as on respiratory stress and soluble sugars was assessed. The French cultivar Rouge Amposta was organically grown, harvested in September and field dried for two weeks. The onions were kept at 18ºC before treatment. Experiments were carried out over two seasons. Three concentrations of N_2O were used; 50kPa, 80kPa and 100kPa (made up to 100kPa with nitrogen), 100kPa nitrogen and controls which were placed in boxes with open lids. Treatment periods were 5, 10 and 15 days. Following treatment, storage conditions were in a ventilated room at 18±0.1ºC, and 65±1%RH. Measurements were taken every 15 days. Respiration rates in the control bulbs did not change. The respiration rate was the lowest in bulbs treated for 5 days for all concentrations, and was approximately half that of the control. 10 and 15 days treatment caused an increase to approximately 20% less that of the control. 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. Five days treatment decreased rots, whereas ten and fifteen days of treatment increased rots. Accumulation of malic acid could be due to its metabolic function during the glycolytic pathway where it is the result of transformation of succinate.

Alternative inhibitors are being investigated for use in potatoes. Monoterpene extracted from caraway oil is marketed under the name Talent. Essential oils extracted from plants have the added benefit of antimicrobial properties. These include spearmint and peppermint oil, however they have high volatility and so repeated or continuous application is necessary. Biox-A or eugenol is an extract of clove and has been approved for use on organic potatoes. It is thought to function by causing physical or chemical damage to developing buds. Substituted napthalenes such as 1, 4-Sight (1, 4-dimethyl napthalene) and Amplify (2, 6-diisopropyl napthalene) are thought to act by hormonal action. Hydrogen peroxide probably adversely affects the meristematic tissue formed after natural dormancy is lost (Kleinkopf *et al*. 2003).

4.4.4 Irradiation

Benkeblia *et al*. (2002) applied ionising radiation using a ^{60}Co source at a dose of 0.15 kGy at 20°C 17 and 19 days after harvest to Rouge Amposta onions. Three sets of storage conditions were used: 4°C and 85% RH, 10°C and 80% RH, and ambient condition of 20°C and 65% RH. The respiration rate in ionised bulbs generally decreased. The decrease is likely to be caused by degeneration of meristematic cells and the death of the sprout caused by the radiation, which slows down the complete respiratory pathway including glycolysis. After 24 weeks the percentage of sprouted bulbs ranged from 3% (irradiated) to 7% (control) with no significant difference. Radiation treatment was most effective at preventing sprouting at 20°C (5% after 24 weeks, compared to 75% control).

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

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

5.0 Conclusions

These trials are difficult to compare and contrast because of differences in the harvesting methods and curing, drying and storage processes. Treatments are applied in different concentrations and the measurement criteria are not always the same. Many procedures have an effect on the storage life of the bulb. Also different cultivars have different properties and so as what applies for one may not be generally applicable, therefore care should be taken when proposing a blanket model to cover all cultivars. The literature is limited in this respect. Shelf life can also be affected by these treatments, however most of the research in this review does not take this as a consideration.

Controlled atmosphere stores and refrigerated stores are expensive. If bulb dormancy was better understood than the need for these measures could be reduced, and a cost-effective solution developed, whether markers to help breeding for longer dormancy or pre or postharvest treatments that inhibit sprouting.

Further elucidation on the role of ethylene and the relative importance of degradation and sensitivity to ABA on dormancy and the rate of sprout growth is required. This could be achieved with the use of 1-MCP in the case of ethylene (Blankenship and Dole, 2003) and with the use of ABA analogues that are more resistant to degradation (Abrams *et al*., 1997) and analysing the expression of genes involved in abscisic acid degradation. This should be carried out in long, medium and short storing cultivars and the results compared. The effect of the use of alternative inhibitors, such as peppermint and spearmint oils, that have been used to inhibit sprouting in potatoes would also be beneficial.

References:

Abdel-Rahman, M. and Isengard, F. M. R. (1974). The role of exogenous plant 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, 89-97.

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

Benkenblia, N. and Varoquaux, P. (2003). Effect of nitrous oxide (N_2O) 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., 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, 196-175.

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

Brewster, J. L. (1994). **Onions and other vegetable Alliums**. CAB International, Wallingford.

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

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 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, 461-470.

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

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.

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

Food and Agriculture Organisation of the United Nations (FAO). (2003). **FAOSTAT Agriculture data**. [WWW]. http://apps.fao.org/page/collections?subset=agriculture. Accessed 13th January 2004.

Fritsch, R. M. and Friesen, N. (2002). Evolution, domestication and taxonomy. In: Rabinowitch, H. D. and Currah, L. (Eds.) (2002). **Allium crop science: recent advances**. CABI Publishing, Cambridge.

Gubb, I. R. and MacTavish, H. S. (2002). Onion pre- and postharvest considerations. In: Rabinowitch, H. D. and Currah, L. (Eds.) (2002). **Allium crop science: recent advances**. CABI Publishing, Cambridge.

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 Horticultuae**, 74, 249-256.

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

Havey, M. J. (1999). Advances in new alliums. 374-378. In: Janick, J. (Ed.), **Perspectives on new crops and new uses**. ASHS Press, Alexandria, VA.

Hole, C. C., Drew, R. L. K. and Gray, D. (2000). Humidity and mechanical properties on onion skins. **Postharvest biology and biotechnology**, 19, 229-237.

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

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**, 176, 145-155.

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

Marcano, L., Carruyo, I., Del Campo, A. and Montiel, X. (2003). Cytotoxicity and mode of action of maleic hydrazide in root tips of *Allium cepa* L. **Environmental**

Research, Article in press, corrected proof. Available online at doi:10.1016/S0013-9351(03)00121-X

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

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

Pak, C., van der Plaas, L. H.W. and Douwe de Boer, A. (1995). Importance of dormancy and sink strength in sprouting of onions (*Allium cepa*) during storage. **Physiologia Plantarum**, 94, 277-283.

Rutherford, P. P. 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.

Suttle, J. C. and Hulstrand, J. F. (1994). Role of endogenous abscisic acid in potato microtuber dormancy. **Plant Physiology**, 105, 891-896.

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

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.

Thomas, T. H. (1969). Role of growth substances in regulation of onion bulb dormancy. **Journal of Experimental Botany**, 20, (62), 124-137.

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

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, 223-229.

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

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

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 and Biotechnology**, 73, (6), 750-754.

APPENDIX

Poster displayed at the
Carrot and Onion conference
26th and 27th November, 2003