Dormancy and Sprout Control in Root and Tuber Crops

Cheema, M. U. A

Thesis submitted in partial fulfilment for the degree of Doctor of Philosophy

Natural Resources Institute

University of Greenwich

UK

November, 2010

DECLARATION

I certify that this work has not been accepted in substance for any degree, and it is not concurrently being submitted for any degree other than that of Doctor of Philosophy (PhD) being studied at the University of Greenwich. I also declare that this work is the result of my own investigations except where otherwise identified by references and that I have not plagiarised another's work.

Student (Cheema, M. U. A) ……..... Date …….

Supervisors (Dr. Debbie Rees)……..... Date …….

(Prof. Andrew Westby) ……..... Date …….

ACKNOWLEDGEMENTS

Saying of Prophet Muhammad (PBUH), a person who is not thankful to his benefactors is not thankful to ALLAH

All and every kind of praises upon ALLAH who gave me the strength and who ever helped me in darkness and difficulties. I have a great honour to offer my heartiest gratitude to my worthy supervisor Dr. Debbie Rees and Prof. Andrew Westby for their guidance, useful suggestions, inspiring guidance and consistent encouragement without which this work would have never been materialized.

My sincere gratitude to Natural Resources Institute for financial assistance, without these contributions the study would not have been possible.

A special thanks to Dr. Mark Taylor at Scottish Crop Research Institute for giving invaluable advices and Dr. Richard Colgan who gave me technical guidance.

I wish to record my sincere appreciate to Dudley Farman for his assistance and support. Further thanks to all the staff of the Department of Enterprise, Trade and Food Management for their motivation and advice.

Cordial thanks to all my friends, family and especially to my grandfather, father and mother who gave me this opportunity, and who always motivated me to follow my dreams and complete what I have started.

ABSTRACT

Research objectives were to study dormancy/sprout control in potato and sweetpotato, and to identify novel sprout control strategies. Consistent with sprout stimulation by gibberellins (GAs) in potato, 10 mM GA_3 enhanced sprout length and number in sweetpotato roots and sprout growth was decreased by 20 ml/L piccolo (GA synthesis inhibitor).

Continuous application of 10 ppm ethylene or greater prevented sprouting in sweetpotato roots over 4 weeks storage at 25° C. Sprout growth was also inhibited by 1000 ppm aminoethoxyvinylglycine (AVG) (ethylene synthesis inhibitor) or 625 ppb 1-methylcyclopropene (1-MCP) (ethylene antagonist). Continuous ethylene treatment or single 1-MCP treatment could be a practical sprout control method for sweetpotatoes stored at >15° C. 5 ppm or greater ethylene increased root respiration rates, but this effect was reduced by 1-MCP or AVG. Sugar content in ethylene treated roots +/- 1-MCP or AVG were lower than untreated roots, with lowest levels in roots treated with ethylene alone.

Hormonal control of dormancy/sprout growth was studied in excised buds from potato tubers transformed to over-express a bacteria gene encoding 1-deoxy-D-xylulose 5 phosphate synthase (DXS), which exhibit arrested buds, Five weeks post-harvest over-expressing lines, DXS1 and DXS2, showed greater sprout growth compared to wild type when treated with 1 mM GA_3 or *t*ZR in 2009, whereas DXS1 showed less sprout growth 4 weeks post-harvest in 2007. There was no difference in DXS1 and DXS2 behaviour 4 months after harvest in 2007. The different behaviour of DXS1 over seasons and with time from harvest underlines how tuber state can change with maturity.

No consistent differences were found in chemical profile of peel among potato tubers of accessions with a range of dormancy characteristics. Although 1, 4-Dimethylnaphthalene

has been identified as a natural sprout suppressant, it could not be detected in any accession even though measurements were sensitive to below 100 ppb.

CONTENTS

FIGURES

TABLES

PLATES

ABBREVIATIONS

ABA	Abscisic acid
ACC	1-aminocyclopropane-1-carboxylic acid
ACO	ACC oxidase
ACS	ACC synthase
ANOVA	Analysis of variance
AVG	2-aminoethoxyvinylglycine
BSI	British Standard Institute
cDNA	Complementary deoxyribonucleic acid
CE	Control environment
CIPC	Isopropyl-N- (3- chlorophenyl) - carbamate
C_2H_4	Ethylene
DMN	1,4 Dimethylnaphthalene
DNA	Deoxyribonucleic acid
DPA	Diphenylamine
GA	Gibberellin
GC-MS	Gas chromatography linked with mass Spectrometry
HPLC	High pressure liquid chromatography
HPP	Hydrogen peroxide plus
IAA	Indole-3-acetic acid
ISO	International Organization for Standardization
KH_2PO_4	Potassium dihydrogen phosphate
KOH	Potassium hydroxide
MH	Maleic Hydrazide
NAA	1-Naphthaleneacetic acid
NaOCl	Sodium hypochlorite
NBD	2, 5-norbornadiene

PBZ	Paclobutrazole
pH	The negative log of hydrogen ion concentration
PLP	Pyridoxal phosphate enzymes
ppm	Parts per million
ppb	Parts per billion
RNA	Ribonucleic acid
RT-PCR	Real Time polymerase chain reaction
SPME	Solid phase micro-extraction
*t*ZR	*Trans* Zeatin riboside
1-MCP	1-Methylcyclopropene
%v/v	Percentage volume by volume

1 GENERAL INTRODUCTION

1.1 Dormancy control in root and tuber crops

Root and tuber crops are key staples in all regions of the world. Root and tuber crops provide the dietary base for 500 – 700 million people around the world. Of these, potato (*Solanum tuberosum*) is the most widely grown. With worldwide production at 300 million metric tons/year (FAOSTATS, 2007), potato is the world's fourth most important crop species. FAO statistics indicate that root and tuber crops are particularly important in the tropical countries of the world (Lancaster and Coursey, 1984). The main tropic tuber and root crops are cassava (*Manihot esculenta*), sweetpotato (*Ipomoea batatas*) and yam (*Dioscorea* spp). They play a vital role in the food security and economies of many countries, and their importance to poor people makes them key targets for making an impact on poverty world-wide.

Harvested root and tuber crops are living parts of the plant that continue to metabolise and respire after harvest. At harvest and for an indeterminate period after harvesting potato tubers are dormant and cannot sprout. In tubers destined for processing, maintenance of tuber dormancy is a critical aspect of successful potato storage. On the other hand, rapid termination of tuber dormancy is desirable for certain segments of the potato industry such as seed certification trials and same-season use of seed potatoes for southern markets. Sprouting is associated with quality loss e.g. increases in reducing sugar, increase in respiration, water loss and increase in glycoalkaloid content (Burton 1989; Suttle, 2004a). Given its economic importance, research into the control of dormancy is much more advanced in potato than the other root crops. However, the control of dormancy in other root and tuber crops is also of great developmental and economic importance.

Sweetpotato is a popular commodity in the United States, and is growing in popularity in Europe. Although root crops may be of different botanical origin, (e.g. tubers, rhizomes or roots) there are many common mechanisms involved in control of dormancy, and hence many lessons that can be learnt from findings on potato when studying other root crops. This project therefore involved trials on sweetpotato as well as potato. Like potato, storage potential of sweetpotato is limited through early sprouting under many conditions, specifically when stored under ambient temperatures as is the case in developing countries. Out of the ground and at ambient temperatures sweetpotato roots have shelf-lives that range from two to four weeks (Wenham, 1995). In some cases roots have been kept stored at ambient temperature in pits or clamps for longer periods (Tomlins *et al.,* 2007). In this situation sprouting is a very significant problem.

1.2 Project objectives

To increase understanding of dormancy and sprout control in potato and tropical root crops, specifically sweetpotato, and, where possible to identify novel appropriate strategies for controlling sprouting in both tropical root crops and potato tubers.

1.2.1 Specific objectives

- To investigate the role of growth hormones known to be important for potato dormancy/sprout control in the control of sprouting of sweetpotatoes using exogenous hormone treatment, hormone synthesis inhibitors and antagonists. Growth hormones to be considered are gibberellins and ethylene.

- To investigate the role of hormones in control of potato dormancy by examining the effect of growth hormones on tubers of transgenic potatoes (transformed to increase flux through the isoprenoid synthetic pathway).

- To look for chemicals within the surface tissues of potato which are associated with control of dormancy or sprout growth

2 REVIEW OF THE LITERATURE

2.1 Potato

Potato is the world's most widely grown root crop and the fourth largest food crop in terms of yield -- after maize, wheat and rice. The potato was introduced to Europe around 1600 and subsequently by European mariners to territories and ports throughout the world (CIP, 1998). Potato is most widely distributed crop in the world (Table 2-1), cultivated in about 150 countries, more than 100 of which are located in tropical and sub tropical zones (CIP, 2001 and CIP, 2002).

	2001	2003	2005	2007
Countries	million tonnes			
Developed	166.93	160.97	159.97	159.89
Developing	145.92	152.11	160.01	165.41
WORLD	312.85	313.08	319.98	325.30

Table 2-1 Potato production throughout the world- (Source FAOSTAT, 2007)

The potato has many uses to improve the quality of life. Throughout the world potatoes are used for human food as well as livestock feed. They are also processed into starch, flour, alcohol and distilled liquors.

Potato is a temperate crop which requires mild temperature during early growth and cool temperature during tuberization. 25˚ C is required at the time of germination, 20˚ C for vegetative growth and 15-18˚ C for tuberization and tuber development. Tuber growth is restricted below 10˚ C and above 30˚ C (Haverkort and Verhagen, 2008).

The main use of potato is for food. Over 66% of the world's production of potatoes in 2005 was for human consumption in different forms. The rest were used for animal feed, seed tubers or industrial purposes (FAO, 2007). Potatoes can be consumed in many different ways, either fresh or processed. Producing fresh potatoes throughout the year is unfeasible, hence long term storage is essential. The potato processing industry in many countries aims at maintaining an economic production of good quality food throughout the year which depends to a large extent on the quality of the potatoes on arrival at the factory. After harvest, however, there are many situations where the potatoes have to be stored for up to 8-9 months and during this period large changes in potato quality may occur, thus causing big losses.

In order to meet with these quality demands as much as possible, storage should be favorable to restrict the losses (Hessen, 1970; Suttle, 2007) and provide a uniform flow of tubers to fresh market and processing plants throughout the year. Storage should avoid excessive dehydration, rotting and sprouting. In the case of tubers destined for processing, storage should also decrease chances of high sugar accumulation which results in dark coloured fried products. The optimum storage temperature for potatoes to be processed in to chips or French fries is 7˚ C- 12˚ C to avoid sugar accumulation as low temperature storage is known to induce sugar accumulation in potato tubers. However potatoes stored at low temperatures such as 4˚ C can be used for processing after reconditioning. Reconditioning can be obtained by storing potatoes at 18˚ C - 22˚ C for 1-4 weeks depending on variety.

At the end of the dormancy period, potato tubers start to sprout. During storage, sprouting is one of main problems that can affect the quality of stored potatoes and cause major losses especially for higher temperature storage. Sprouting not only reduces the number of saleable potatoes but the increase in surface area due to sprout surfaces causes more transpiration (Afek and Warshavsky, 1998). Sprouting is considered to be one of the most

important physiological factors of post-harvest loss in the US (Suttle, 2004a). To preserve the quality of stored potatoes, sprouting has to be effectively controlled. For effective sprout control understanding of the dormancy/sprouting behaviour in tuber crops is of great importance. A range of sprout inhibitors are being used for longer storage but due to increasing awareness towards health hazards, there is a need to find alternative ways for good potato storage and for good quality potato supply.

Long term storage is required for potatoes destined for the processing industry and break of dormancy is required for seed tubers, as seed tubers can only be tested for various virus diseases once dormancy has been terminated.

Tubers have natural dormancy. Tubers are dormant when there is no visible growth due to endogenous and environmental factors (Hemberg, 1985; Burton, 1989). Potato tubers will start sprouting when environmental factors are favourable and endogenous factors allow them to sprout. Dormancy period differs from one cultivar to another.

Naturally occurring dormancy is regulated by endogenous bio-chemicals found within the tuber (reviewed by Hemberg, 1985). Many studies have been undertaken to investigate the endogenous regulators of tuber dormancy. However the progress to date is only a starting point and much needs to be learned about the complicated system involved in the tuber dormancy cycle. Alternative dormancy control processes can be used to develop the improved post-harvest storage technologies.

2.1.1 Taxonomy of Potatoes

The main potato species grown worldwide is *Solanum tuberosum* (a tetraploid with 48 chromosomes), belonging to the family Solanaceae. There are also some diploid (*Solanum stenotomum*) and triploid species (*Solanum Chaucha)* grown for food (Hijmans

and Spooner, 2001). The edible part of the potato plant is the tuber, which is an enlarged underground stem. Potato tubers come in different colours but most common are red and white. The eyes of tubers, where subsequent buds will form, are clearly visible.

2.1.2 Dormancy

Dormancy is a period in an organism's life cycle when growth, development, and (in animals) physical activity is temporarily suspended. This occurs in many plant species. It can be initiated by different factors including temperature variation; moisture stress, and day length (Hartmann *et al.*, 2002). Dormancy minimizes metabolic activity and therefore helps an organism to conserve energy. According to Hemberg (1985), dormancy in plants is the phase where there is no bud formation due to endogenous and exogenous conditions.

Dormancy in plants can be divided into three types; endodormancy is a condition caused by internal factors, paradormancy is a type of dormancy which is controlled by buds elsewhere *i.e.* plants maintain apical dominance and prevent axillary buds from growing through paradormancy, and ecodormancy is the inhibition of growth induced by environmental factors. For example, in summer with high temperature and moisture stress, plants will stop active elongation. As soon as environmental conditions become favourable, growth will start again (Crabbe and Barnola, 1996).

Alternatively, Hilhorst and Toorop (1997) defined two types of dormancy, known as innate dormancy and induced dormancy. In seeds, innate dormancy occurs during the development phase of seed and induced dormancy occurs when there are no favourable conditions. The same conditions can apply to bud dormancy. During the innate dormancy buds will not sprout even if there are favourable environmental conditions.

The physiological control of dormancy and sprouting in potato is very complex. Many studies have been conducted to look at the role of various plant hormones; including ethylene, auxins, abscisic acid, gibberellins and cytokinins (see section 2.3)

2.1.3 Mechanism of Dormancy

Plants have adapted to environmental conditions through evolutionary processes. One of the strategies developed to survive extreme conditions is dormancy. Dormancy, more specific endodormancy, is part of a plant's genetic design. The potato tuber has developed an endodormant phase to protect it against conditions in which the plant will otherwise not survive. Burton (1989) suggested that the duration of dormancy should be calculated from the time the tubers are initiated at which point the eyes are formed until sprouting commences.

A dormant bud is metabolically active even though no visible growth is observed (Burton, 1989), and DNA, RNA as well as proteins are synthesized by the resting organ (Suttle, 1996), but the process is much slower than in actively growing organs (Van der Schoot, 1996).

It is not clear how dormancy is initiated, although plant growth regulators clearly play an important role in the initiation and termination of dormancy. The first stage of dormancy takes place when a tuber is initiated. In this stage tubers act as sink organs and the buds are dormant.

In cell division four phases can be distinguished, namely the gap 1 (G1); synthesis (S); gap 2 (G2) and mitosis (M) phases (Figure 2-1). The G1 phase occurs between mitosis (M-phase) and DNA-synthesis (S-phase) and the G2 phase between DNA-synthesis and mitosis (Fairbanks and Anderson, 1999). A G0 phase also exists where non-cycling cells

are arrested. During the G-phases the cells prepare for the next phase of the cell cycle. Between the G1-S and G2-M phases are checkpoints of cyclin-dependent protein kinase (Cdk). Cdk must be bound to a protein, called cyclin, for the cycle to commence through the checkpoint to the next phase of cell division (Francis and Sorrell, 2001). Suttle (1996) stated that the nuclei of dormant buds are predominantly (77%) in the G1 phase, with only about 13% in the G2 phase.

Suttle (1996) mentions that especially one family of regulatory proteins, the P-34 kinases of which Cdc 2 kinase is the most important, is actively involved in cell division. These proteins are regulated by cyclins. In plants D-cyclins are necessary for G1 to S regulation. The D-cyclins react to external signals, and it has been found that cytokinins are able to induce D-cyclins to bind to Cdk proteins and initiate the G1 to S phase (Francis and Sorrell, 2001). Thus it is possible for cytokinins to stimulate non-cycling cells in the G0 phase to start division and act in the G1-S phase. Sucrose may be involved in the G1 to S transition by inducing CycD2, and the G1 to S phase may be blocked if an energy source is not available (Francis and Sorrel, 2001). Thus it is possible for cytokinins to stimulate non-cycling cells in the G0 phase to start division and act in the G1-S phase. Sucrose may be involved in the G1 to S transition by inducing CycD2, and the G1 to S phase may be blocked if an energy source is not available (Francis and Sorrel, 2001).

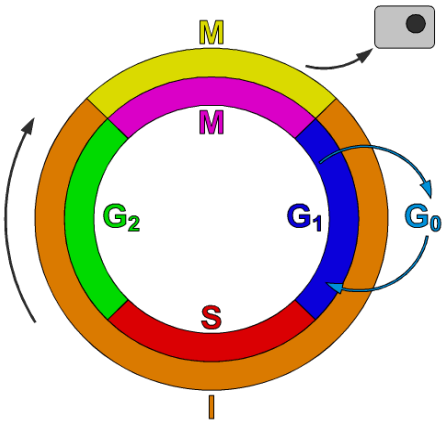


Figure 2-1 Cell Cycle showing different phases (http://en.wikipedia.org/wiki/S_phase)

Based on the available literature it is postulated that regulation of the cell cycle is key to regulation of dormancy, plant growth regulators clearly play an important part in regulating dormancy.

2.1.4 Physiological origin of sprouting

In potatoes, sprouts develop from identifiable buds (eyes); the buds at the apex of the tuber normally sprout first (Figure 2-2). These buds are called apical buds and are said to possess apical dominance, which is inhibition of the growth of lateral buds by the terminal bud of a plant shoot (Cline, 1997). When the apical buds are removed, or die, other buds are stimulated to sprout. When whole tubers are planted, generally the buds near the apex will develop.

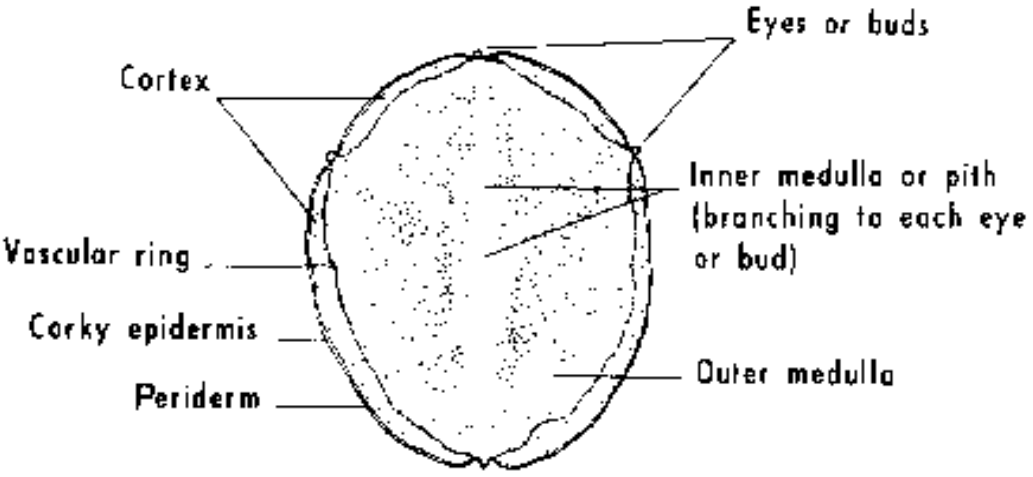


Figure 2-2 Cross section of a potato

2.1.5 Hormonal control of dormancy and sprouting

Control of dormancy is attributed to three groups of plant growth regulators namely abscisic acid (ABA), gibberellins (GA) and cytokinins (Arteca, 1996). The synthetic pathways of these plant regulators are shown in Figure 2-3.

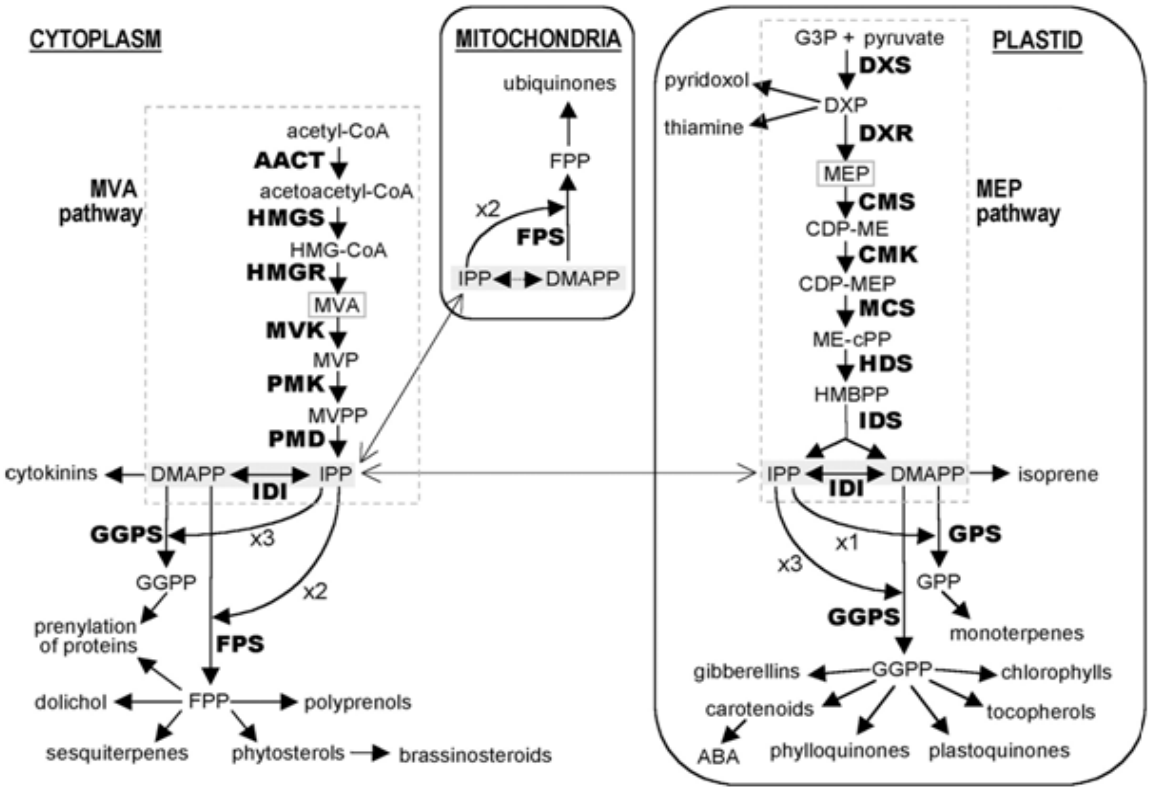


Figure 2-3 An overview of Isoprenoid biosynthesis pathways in the plant cell. HMG-CoA, Hydroxymethylglutaryl CoA;MVP, 5-phosphomevalonate; MVPP, 5 diphosphomevalonate; HBMPP, hydroxymethylbutenyl 4-diphosphate; FPP, farnesyl diphosphate; ABA, abscisic acid. Enzymes are indicated in bold: AACT, acetoacetyl CoA thiolase HMGS, HMG-CoA synthase HMGR, HMG-CoA reductase (EC MVK, MVA kinase); PMK, MVP kinase PMD, MVPP decarboxylase IDI, IPP isomerase (EC); GPS, GPP synthase; FPS, FPP synthase GGPS, GGPP synthase; DXS; DXR, DXP reductoisomerase CMS; CMK; MCS; HDS; IDS, IPP/DMAPP synthase (From Rodrguez-Concepcin and Boronat, 2002).

2.1.5.1 Abscisic acid (ABA)

Abscisic acid (ABA) is a naturally occurring growth inhibitor present in all organs of higher plants. ABA (a terpenoid) is produced in the chloroplasts and other plastids (Gardner *et al*., 1985; Arteca, 1996). ABA acts on various processes in plants, such as stomatal opening and closure, abscission, cold stress, and dormancy (Arteca, 1996). Its function is concentration dependent (Hartmann *et al.*, 2002) and transport of ABA takes place throughout the plant (Gardner *et al*., 1985).

ABA has been found to play a central role in dormancy regulation. Potato tubers have developed a dormant phase vital in surviving extreme cold winter conditions. ABA plays a pivotal role in the protection against cold stress (Arteca, 1996) and it has been proven that shorter days trigger the production of ABA (Gardner *et al.*, 1985). Various authors agree that the level of ABA is highest in freshly harvested tubers and that the level declines during storage (Coleman, 1987; Suttle and Hulstrand, 1994; Arteca, 1996; Suttle, 1996).

Leclerc *et al*. (1995) stated that small microtubers had a higher ABA content than field grown tubers and that the higher content was the reason for the longer dormant period. It has been suggested that it is only the initial level of ABA that is important in triggering dormancy (Hilhorst and Toorop, 1997; Biemelt *et al*., 2000), but Coleman (1987) and Suttle (2004a) are of the opinion that ABA is also important in maintaining dormancy.

According to Hill (1980) and Gardner *et al*. (1985) it is not necessarily the level of ABA, but the ratio of ABA to gibberellins that is the regulatory factor in maintaining dormancy. If the ratio is in favour of gibberellins, sprouting will commence and dormancy will be terminated (Hill, 1980). ABA is required to initiate dormancy, but there is not a definite level below which ABA must decrease for sprouting to commence (Claassens and Vreugdenhil, 2000).

Hemberg (1985) suggested that ABA is involved in the inhibition of DNA and RNA synthesis, while gibberellins (GA) are involved in the acceleration of DNA and RNA synthesis. So, it appears that ABA can maintain dormancy and GA can promote sprout growth.

ABA is produced to protect the tuber against cold damage and, through inhibition of DNA and RNA synthesis will arrest the cell in the G1 phase of the cell cycle until the GA: ABA ratio is in favour of gibberellins to promote cell division and sprouting. Most authors agree that exogenously applied ABA will inhibit sprouting (Hemberg, 1985; Burton, 1989; Suttle, 1996; Suttle, 2004a) but that it is concentration dependent (Suttle, 2004a).

As stated above, endogenous ABA is high in dormant tubers and declines during post-harvest storage. The effect of endogenous ABA on dormancy of potato tubers was observed by Suttle and Hultstrand (1994) using an in vitro microtuber system and the ABA biosynthesis inhibitor, fluridone. Treating developing microtubers with fluridone inhibited ABA accumulation by over 90% and resulted in premature sprouting. Addition of exogenous ABA to fluridone-treated microtubers restored ABA levels and prevented the premature sprouting. Application of fluridone to dormant microtubers also resulted in premature sprouting. These results showed that ABA is required for both tuber dormancy induction and maintenance.

Sorce *et al*. (1996) reported that ABA in eyes decreases as dormancy weakens and sprouting commences and application of exogenous ABA inhibits sprout growth.

Suttle and co-workers have conducted a number of studies to understand the hormonal control of tuber dormancy (reviewed in Suttle, 2004a). They concluded that both ABA and ethylene are required for the initiation of tuber dormancy, but only ABA is needed to maintain the dormant state.

Sophia *et al*. (2000) looked at the role of ABA in controlling the dormancy in potato tubers. The ABA was measured in six potato cultivars during storage. They found a continuous decrease in levels of ABA during storage, but there was no relation of this decline with rate of sprout growth indicating that the decrease in ABA might trigger dormancy break but did not influence rate of sprout elongation.

2.1.5.2 Gibberellins

Gibberellins (GAs) are growth promoters. There are several reports that exogenously applied gibberellins could break dormancy, the first of these was by Brian *et a*l. (1955). To date, over a hundred gibberellins have been isolated (Vivanco and Flores, 2000), but not all are active in plants. Gibberellins in the 13-hydroxylation group, especially GA_1 and GA_4 are the most active (Suttle, 1996; Vivanco and Flores, 2000; Suttle, 2004a; Suttle, 2004b). Increased activity is probably due to the lactone ring present in the structure of these gibberellins (Gardner *et al*., 1985). Gibberellins are synthesized through the mevalonic acid pathway (Figure 2-3). Rajala and Peltonen-Sainio (2000) as well as Arteca (1996) mentioned that all plants use the same pathway to produce gibberellins up to the GA_{12}-aldehyde phase from where different gibberellins are then synthesized. Gibberellins are mainly produced in the leaves but may also be synthesized in the roots and fruit (Gardner *et al.*, 1985; Vivanco and Flores, 2000).

Transport takes place mainly in the phloem of the plants and can be both up- and downwards (Kefeli, 1978). Gibberellins are generally considered to be responsible for cell elongation, rather than cell division (Kefeli, 1978; Vivanco and Flores, 2000; Francis and Sorrell, 2001), but may also play a role in stimulating cell division in meristematic areas (Kefeli, 1978; Roberts, 1988).

When gibberellins are applied to dormant tubers, dormancy can be broken according to Hemberg (1985); Coleman (1987); Burton (1989) and Fernie and Willmitzer (2001). Gibberellins may terminate dormancy by activating the synthesis of DNA and RNA (Bruinsma *et al.*, 1967; Clegg and Rappaport, 1970; Burton, 1989; Arteca, 1996) and by decreasing the duration of the cells in the G1 and S phases (Roberts, 1988). According to Francis and Sorrell (2001) gibberellins may affect the Cdc 2 kinase level at the G2-M checkpoint of the cell cycle and gibberellins may increase the rate at which cells are produced.

The large number of different gibberellins found makes it confusing to identify their various roles in potato tuber dormancy/sprouting. In many cases it appears that they can be transformed into other types. Thus Barendse (1975) suggested that GA_1, GA_4 and GA_7 are precursors of GA_3.

GA_1 is the most biologically active gibberellin (Jones *et al*., 1988). GA_{20} has been identified as the precursor of GA_1 (Jones *et al*., 1988). Xu *et al*. (1998) found that GA_1, GA_4, GA_9 and GA_{20} are present in potato plants and that GA_4 and GA_9 levels do not change during tuber development, but only the level of GA_1. These results concur with the suggestion of Jones *et al*. (1988) that GA_1 is biologically the most active gibberellin in tubers. Vreugdenhil and Sergeeva (1999) found the same range of gibberellins in the genotype *Solanum demissum*. Suttle (1996) mentioned that exogenously applied GA_{12} was metabolized in the shoot apices of potatoes to produce GA_1, GA_8, GA_{20}, GA_{29}, GA_{44}, GA_{51} and GA_{53}.

Carrera *et al*. (2000) showed that ectopic expression of GA_{20} oxidase in potato tubers led to increased GA and premature sprouting, whereas antisense inhibition of GA_{20} oxidase synthesis led to dwarfism, but there were no effects on tuber dormancy duration. This

supports the view that GAs can break dormancy but are not necessary for dormancy to end.

In order to confirm the role of GAs, Suttle (2004b) conducted a study to determine the effects of post-harvest storage duration on the endogenous content and bioactivities of selected GAs in relation to the dormancy status in Russet Burbank potatoes. The tubers used in these studies were completely dormant after 98 days of storage. Between 98 and 134 days of storage, dormancy began to end. Tuber dormancy weakened with further storage and tubers stored for 212 days or longer were completely non-dormant and exhibited vigorous sprout growth. Immediately after harvest, the endogenous contents of GA_{19}, GA_{20}, and GA_1 were relatively high (0.48-0.62 ng/g fresh weight, ppb). The content of these GAs declined between 33 and 93 days of storage. Internal levels of GA_{19}, GA_{20}, and GA_1 rose slightly between 93 and 135 days of storage reaching levels comparable to those found in highly dormant tubers immediately after harvest. Levels of GA_{19}, GA_{20}, and GA_1 continued to increase as sprout growth became more vigorous. Freshly harvested tubers were completely insensitive to exogenous gibberellins. As post-harvest storage continued, exogenous GAs (GA_1 and GA_{20}) promoted premature dormancy release. Sprout growth from non-dormant tubers was also promoted by exogenous GA in the following order of activity: $GA_1 = GA_{20} > GA_{19}$. Continuous exposure of developing tubers to inhibitors of GA biosynthesis did not extend tuber dormancy. This study (Suttle, 2004b) does not support a role for endogenous GA in potato tuber dormancy release but does suggest an involvement in the regulation of sprout growth.

Besides the synthesis of DNA and RNA, gibberellins are also believed to have an effect on the reducing sugar content (Hill, 1980; Mares, 1985). According to Hill (1980) the process of starch breakdown is gibberellin dependent. In seeds gibberellins are responsible for synthesis of enzymes such as amylase that is involved in the breakdown of starch into sugars.

Claassens and Vreugdenhil (2000) also mentioned that gibberellins in potatoes had a stimulating effect on the reducing sugar content, but it could not be proven whether gibberellins had an effect on starch breakdown. Coleman (1987) reported that gibberellins increased the synthesis of reducing sugars, but only after the storage tissues were no longer dormant.

Tuber wounding also has a stimulatory effect on the synthesis of gibberellins (Shih and Rappaport, 1970). Ewing *et al*. (1987) found that wounding had a stimulatory effect on the termination of dormancy, but could not establish what the mechanism was.

2.1.5.3 Cytokinins

Cytokinins are synthesized via the mevalonic acid pathway (Figure 2-3). All the cytokinins originate from isopentenyladenosine, a substrate found in the mevalonic acid pathway (Arteca, 1996; Vivanco and Flores, 2000). The cytokinins most prevalent in plants are those with an N6-side chain such as zeatin, isopentenyladenine and N6-benzyladenine (Vivanco and Flores, 2000; Mok and Mok, 2001).

The main effect of cytokinins is on cell division (Arteca, 1996; Francis and Sorrell, 2001; Hartmann *et al*., 2002; Vreugdenhil, 2004), but cytokinins also have an effect on cell enlargement (Arteca, 1996). Cytokinins act on the G1-S and the G2-M phases of the cell cycle (Roberts, 1988; Francis and Sorrell, 2001; Mok and Mok, 2001; Suttle, 2004a). In the G1-S transition, cytokinins function by inducing the CycD3 genes (Francis and Sorrell, 2001; Mok and Mok, 2001). Plants over-expressing CycD3 could maintain cell division without exogenously applied cytokinins. Cytokinins have also been found to be active in the G2-M transition of the cell cycle where induction of a histone-H-kinase, cdc2, takes place (Francis and Sorrell, 2001; Mok and Mok, 2001).

Hemberg (1985) mentioned that cytokinins applied exogenously can break dormancy of potato tubers, and the levels of endogenous cytokinins increase before the termination of dormancy. More than one form of cytokinin is found in potato tubers. Sattelmacher and Marschner (1978) as well as Van Staden and Dimalla (1977) found that zeatin riboside is the main component of cytokinins in potatoes, but Suttle and Banowetz (2000) stated that cis-zeatin and not cis-zeatin riboside, increase in tubers during dormancy and is responsible for the termination of dormancy. The authors also mentioned that isopentenyladenine and *trans*-zeatin levels increase in tubers during storage.

Suttle (1998b) found eight different forms of cytokinins present in potato tubers with isopentenyl adenine-9-glucoside the most abundant. The levels of the zeatin type cytokinins were comparable with that of the isopentenyl-type (IP) cytokinins. Isopentenyl adenine-9-glucoside is biologically inactive and serves as a precursor for zeatin-type cytokinins which are biologically active. The IP- type cytokinins must first be synthesized to zeatin-type cytokinins before dormancy can be terminated (Suttle, 1998b). Endogenous levels of cytokinins must increase before dormancy can be broken (Bana *et al.,* 1984; Suttle, 2004a).

Increase in cytokinin content coincides with a reduction of acid inhibitors like ABA (Claassens and Vreugdenhil, 2000). Tissue sensitivity to cytokinins is important in the regulation of dormancy (Turnbull and Hanke, 1985) and exogenously applied cytokinins were only effective at certain times in the dormancy period, mostly at the beginning and end of dormancy (Coleman, 1987).

Suttle (2001) found that cytokinins were unable to stimulate sprouting directly after harvest but that dormant tubers reacted to an injection of cytokinins at a dose dependent rate. Koda (1982) found that resting tubers, when wounded, exhibited a significant increase in cytokinins, seemingly zeatin glucoside, and that it could have an effect on

sprouting. Based on the available literature it is postulated that cytokinins are essential in the regulation of dormancy, probably acting in synergy with other hormones, especially gibberellins in terminating dormancy.

Tuber dormancy can be broken by addition of both natural and synthetic cytokinins (Hemberg, 1970). Potato tubers of the cultivar, Majestic, which has a long dormancy period were treated with water or with the cytokinins, kinetin or zeatin. The tubers treated with cytokinins broke dormancy after 2-3 days.

Potato lines transformed with a cytokinin biosynthesis gene show early sprouting (Ooms and Lenton, 1985). Transformed lines showed 100–200 fold higher concentrations of the biologically-active cytokinins, zeatin and zeatin riboside compared with untransformed potato lines.

Immunological techniques have confirmed that an increase in cytokinins is detected in tubers exiting dormancy (Turnbull and Hanke 1985; Suttle 2004a). Unfortunately the antibodies used in these studies recognized both active and inactive cytokinin metabolites.

Potato tuber buds normally remain dormant through the growing season until several weeks after harvest. Suttle (2004c, 2005) showed that synthetic cytokinins terminate dormancy. In another study, (Turnbull and Hanke, 1985) innate dormancy in the cultivar Majestic remained for 9 to 12 weeks in storage at 10° C, but was reduced to 3 to 4 weeks when the tubers were stored at 2° C and were treated with cytokinins. Applying cytokinins to tubers with innately dormant buds induced sprout growth within 2 days. The growth rate was comparable to that of buds whose innate dormancy had been lost naturally. Cytokinin treatment did not accelerate the rates of cell division and cell expansion in buds where innate dormancy had already broken naturally suggesting that cytokinins play a role in dormancy break but do not enhance sprout growth.

2.1.5.4 Auxins

Auxins are important growth regulators with many functions. The best known naturally occurring auxin is indole-3-acetic acid (IAA). IAA is mainly concerned with cell enlargements. In potatoes changes in endogenous levels of the auxin (IAA) are suggested to be more closely related to the regulation of subsequent sprout growth (Suttle, 2004a).

Suttle showed that endogenous auxin levels were low until after the end of dormancy and increased with sprout growth (Suttle 2004a). No evidence could be found to show that exogenous IAA terminates tuber dormancy (Suttle, 2004a). On the other hand the suggestion that IAA has a role in dormancy break is supported by a study conducted by Sorce *et al*. (2000). Free IAA increased towards the end of dormancy period and suggested that IAA break dormancy.

Auxins act over a limited concentration range and when applied in excess inhibit shoot growth (Jensen *et al.*, 1998). Suttle (2005) showed that natural and synthetic auxins inhibit sprout growth from non-dormant Russet Burbank tubers. It has been reported that auxin-induced shoot growth inhibition in certain species is mediated by both ethylene and ABA (Grossmann, 2000). In this situation, application of growth-inhibiting levels of auxin results in a stimulation of ethylene production which, in turn, results in shoot growth inhibition. However, others reports suggest that auxin-induced shoot growth inhibition is independent of ethylene synthesis and action (Valenzuela-Valenzuela *et al*., 2002). Suttle (2003) reported that the ethylene biosynthesis inhibitor aminoethoxyvinylglycine (AVG) reduced NAA-induced ethylene biosynthesis up to 80% but had no effect on sprout growth inhibition. He proposed that although auxin increased ethylene production in potato tubers ethylene had no effect in auxin-induced sprout growth inhibition. The mechanism-of-action of auxins as potato sprout inhibitors is not clear yet.

2.1.5.5 Ethylene

Ethylene (C_2H_4) is a unique plant hormone since it is the only hormone that is a simple gaseous hydrocarbon (Hopkins and Huner, 2004). Its effect has been known for centuries (treating plants with smoke or keeping different fruits and vegetables together promotes ripening and senescence). Yang and Hoffman (1984) describe it as the simplest olefin that exists in the gaseous state under normal physiological conditions and which regulates many aspects of plant growth, development and senescence.

There are various sources of ethylene in the atmosphere such as exhaust fumes from internal combustion engines or heaters, smoke, natural gas leaks and manufacturing plants (Blankenship, 2001). It is commercially available in ethylene releasing compounds such as Ethephon and is used to ripen fruit such as bananas, tomatoes, avocadoes, apples and pears, among others (Blankenship, 2001). Apart from these external sources, ethylene production occurs naturally in all plant organs such as roots, stems, leaves, buds, tubers, bulbs and flowers (Arshad and Frankenberger, 2002). Ethylene production can also be stimulated as a result of biotic and abiotic stresses. Ethylene is not only important in ripening, but also plays a vital role in other physiological activities of the plant including adventitious root formation, leaf and fruit abscission, flower induction, flower and leaf senescence, flower opening and tuber sprouting (Davies, 1987; Arshad and Frankenberger, 2002).

In the case of fruit, ethylene is present at low levels in the fruit during all stages of development, but it plays a more dominant regulatory role during the ripening phase of climacteric fruit (Kays, 1991). The diffusion of ethylene into and out of the plant tissues from both endogenous and exogenous sources can profoundly affect attributes of fruit quality such as colour, texture and flavour (Watkins, 2006). Two separate systems of ethylene production/action are present in the plant; system 1 and system 2. System 1 is

present throughout the development of climacteric and non-climacteric fruit, while system 2 is activated in climacteric fruit during ripening (Kays, 1991). In system 2, ethylene also stimulates its own synthesis, a process known as autocatalysis. Biale *et al*. (1954) defined autocatalysis as the capacity of the tissue to synthesize large quantities of ethylene in response to application of low concentrations of this gas. According to Serek *et al*. (2006) the presence of ethylene in the atmosphere or in the plant tissue causes a positive feedback, leading to a rise in the production of the hormone. Autocatalysis does not happen during system 1.

Ethylene is believed to be involved in the modulation of a number of potato tuber biochemical pathways and processes such as sprouting and sprout elongation. Ethylene have been implicated in dormancy regulation (Hemberg, 1985; Suttle, 1996) In general, ethylene or ethylene releasing compounds like Ethephon enhances release from dormancy and increases sprouting of potato tubers (Alam *et al*., 1994).

The involvement of endogenous ethylene in tuber endodormancy regulation is unclear. Rosa (1925) was the first to report an effect of ethylene on shortening the natural period of potato tuber dormancy. Subsequent studies by Denny (1926a, 1926b) failed to corroborate these findings. More recently, exogenous ethylene (or ethylene-releasing agents) has been reported to elicit seemingly contradictory responses. Depending on the concentration and duration of exposure, exogenous ethylene can either hasten or delay tuber sprouting. Relatively short-term (less than 3 days) exposure to ethylene results in the premature termination of tuber endodormancy (Kalt *et al*., 1999). Temporary treatment with exogenous ethylene has also been reported to stimulate the sprouting of partially dormant tubers (Alam *et al.*, 1994). In addition to its dormancy breaking effect, ethylene is also reported to break apical dominance leading to sprouting from lateral buds (Prange *et al.*, 1998; Kalt *et al.*, 1999; Wills *et al*., 2004; Prange *et al.*, 2005) which suggests ethylene can inhibit auxin synthesis/perception. On the other hand, where continuous exposure to

ethylene is maintained, inhibition of sprout elongation in tubers is observed (Rylski, 1974; Prange, 1998), and it is this effect that has been exploited most by the potato industry.

The range over which ethylene is effective for inhibiting sprout growth has been investigated by Daniels-Lake in a selection of North American potato varieties; Russett Burbank stored at 9°C was the most sensitive variety. It responded to ethylene between 0.4-400 ppm, and full suppression was achieved at concentrations greater than 4 ppm (Daniels-Lake *et al.*, 2005a). Studies over six seasons, found that Russett Burbank potatoes could be stored for up to 30 weeks at 9°C under 4 ppm of ethylene; ethylene delayed the onset of sprout appearance by 5-15 weeks, compared with air-stored samples and after 29 weeks storage ethylene treated potatoes had reduced sprout mass and sprout length compared with potatoes treated with the sprout suppressant CIPC although ethylene treatments increased the number of sprout initials (Daniels-Lake *et al.*, 2005a).

Although it is clear that continuous exposure to ethylene gas controls tuber sprout growth, for some potato cultivars it can also result in a darker fry colour associated with an increase in concentration of fructose and glucose. In addition to their effects on fry colour, fructose and glucose in tubers during the frying process can interact with free asparagine to form acrylamide via an N-glycoside intermediate as part of the Maillard reaction. Acrylamide results in discolouration and bitter off-notes in the fried product and more importantly, acrylamide is a potent neurotoxin and carcinogen (Mottram *et al.,* 2002).

Variation in sprout control by ethylene between varieties can be reduced by lowering the storage temperature below 6° C although this often stimulates low-temperature sweetening due to to the accumulation of reducing-sugars (fructose and glucose) (reviewed in Sowokinos, 2001). In an investigation to overcome processing quality difficulties associated with ethylene, Daniels-Lake *et al.* (2005a) investigated ethylene

concentrations in the range 0.4 to 400 ppm for effects on sprout control and fry colour. The authors concluded there were different dose dependent responses to ethylene for different aspects of sprouting and reducing sugar accumulation.

Variability in the degree of response to ethylene (4 ppm) is evident amongst cultivars (Daniels-Lake *et al*., 2008). For example cultivars Shepody and Asterix are more sensitive to ethylene than Russett Burbank with regards to fry colour, but less responsive than Russett Burbank in regard to sprout suppression. In contrast, Santana tubers are less responsive to ethylene in terms of fry colour and sprout inhibition than Russett Burbank. Previously, similar findings of cultivar variation in terms of respiration, fry colour and sprouting in response to ethylene have been reported (Rylski *et al.*, 1974; Pritchard and Adam, 1994; Prang *et al.*, 2005).

In summary, Continuous ethylene treatment of potato tubers is an effective sprout suppressor in commercial settings although it also resulted in undesirable accumulation of reducing sugars (Prange *et al*., 1998). Despite some of the limitations of ethylene as a sprout control agent such as effects on sugar levels and hence processing quality, and lack of consistent efficacy across all cultivars, ethylene has been used in potato stores in the UK on a commercial scale since 2001. There is no maximum limit for application levels of ethylene. It can be diffused and crops treated with it are widely considered as "residue free".

Currently, use of ethylene in the UK is controlled by the Pesticide Safety Directorate under a commodity approval. Ethylene is being supported through the EU pesticide approvals system (EU 91/414 fourth stage), and when successful this will supersede the current national controls.

Ethylene has been registered as a sprout suppressant for processing tubers in Canada, for use on the cultivar Russet Burbank since 2002. The approval requires a target

ethylene concentration of 4 ppm to be maintained between cycles of ventilation and throughout storage. Ethylene treatments in Canada are marketed under the *Eco Sprout Guard* brand name (Daniels-Lake and Prange, 2006).

2.1.5.5.1 Ethylene Biosynthesis

The ethylene biosynthesis pathway was elucidated by Adams and Yang (1979) and is often referred to as the Yang cycle (Figure 2.4) after Yang who discovered that 1 amino-cyclopropane-1-carboxylic acid (ACC) was the precursor for ethylene production. The last three reactions in the pathway have been the most studied and start with the conversion of methionine to S-adenosylmethionine (SAM) by the enzyme, methionine adenosyl transferase. SAM is converted to ACC by the enzyme ACC synthase (ACS). This is the rate-limiting step in ethylene biosynthesis. ACC is then converted to ethylene by ACC oxidase.

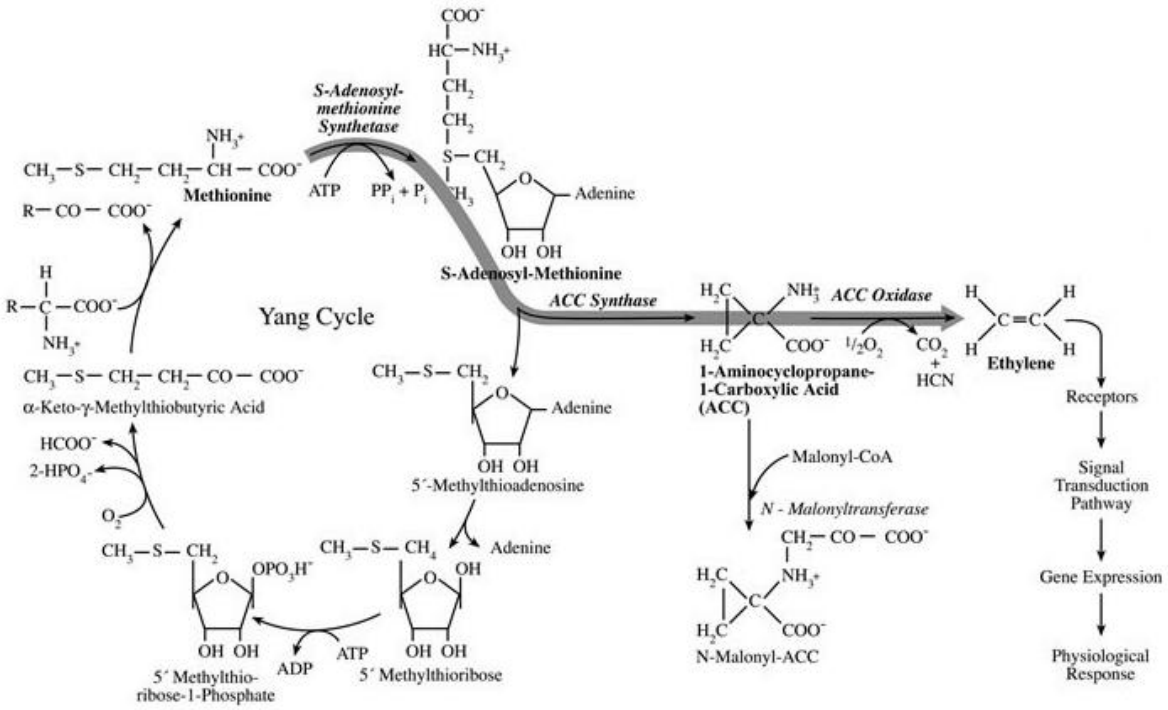


Figure 2-4 The ethylene synthesis pathway and Yang cycle. The initial precursor is the amino acid methionine and key regulatory enzymes in the pathway are ACC synthase and ACC oxidase (http://www.exonpress.com)

Dong *et al*. (1992) proposed that ACC was converted to ethylene by the following reaction:

1. $ACC + AH_2 + O_2 \xrightarrow[Fe_{2+},\ C0_2]{} ACC(O)\_ + O + H_2O$

2. $ACC(O) \qquad C_2H_2 + HCN + H_2O + CO_2$

2.1.5.5.2 Ethylene Perception

Plant cells constantly produce ethylene and in order to act as a regulator either responsiveness to ethylene must change or the amount of ethylene being produced must change. Both mechanisms are evident in plant tissues. During abscission the production rate of ethylene in the tissue does not change but an increase in auxin increases the sensitivity of cells to ethylene and initiates an abscission zone (Trewavas, 1983). The second type of response is typical of ripening fruits where an increase in the production rate of ethylene induces a rise in the respiration rate (climacteric). However, it is likely that there is also a change in the sensitivity of fruit to endogenous ethylene. This may be caused by the numbers of ethylene receptors present. Trewavas (1983) suggested the mechanism that regulates ethylene-dependent fruit ripening must be reliant upon the synthesis or activation of ethylene receptors within the plant. He concluded that after ethylene has bound to a receptor to form a complex; a signal must be transduced that leads to the synthesis of new ACC synthase and ACC oxidase and other ethylene inducible enzymes. (See Figure 2-5).

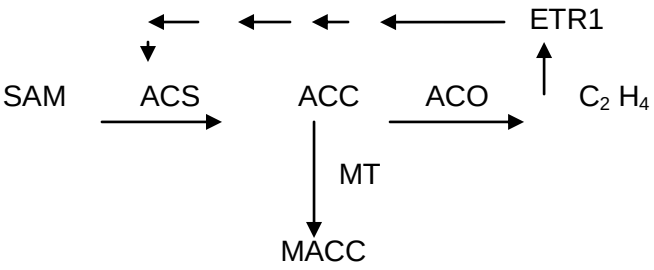


Figure 2-5 The final stages in the ethylene biosynthesis pathway showing the positive feedback loop

ACS- ACC synthase

ACO- ACC oxidase

MT- malonyl transferase

ETR1- ethylene receptor

⟶ ⟶ Positive feedback loop

2.1.5.5.3 Ethylene Binding

In order for exogenous ethylene to have any kind of effect, the plant must be sensitive to ethylene and it must have receptors to which this hormone can bind (Sisler and Yang, 1984). Receptors are membrane bound glyco-proteins that specifically and reversibly bind chemical signals, but unlike enzymes do not convert them chemically to a product. When the hormone is bound to the receptor, the latter is transformed to an activated state (Libbenga and Mennes, 1987). In a study done by Burg and Burg (1967), it was discovered that the ethylene receptor contains a metal ion, which was later discovered to be copper (Beyer, 1976), (Figure 2-6). Climacteric fruit become more sensitive to ethylene during ripening. Ethylene binding receptors were discovered in mung beans by Sisler (1979) and simultaneously in *Phaseolus vulgaris* by Bengochea *et al.* (1980). Binding sites are saturated at an ethylene concentration ranging between 10 - 100 µL.L-1 (ppm) (Sisler, 1991).

Ethylene binds to the membrane imbedded receptor which then activates certain signals, leading to the transcription of specific genes and the activation of enzymes, which in turn leads to a physiological response in the plant (Arshad and Frankenberger, 2002). The nature and structure of ethylene receptors has been studied in detail in arabidopsis, and the postulated structure is illustrated in Figure 2.6. In *Arabidopsis thaliana* 5 members of this receptor gene family have been identified, viz. ETR1, ETR2, ERS1, ERS2 and EIN4 (Srivastava, 2002; Serek *et al*., 2006).

Although it was originally assumed that ethylene sensitivity would increase with the number of binding sites, in fact many tissues that respond to ethylene have a low number of binding sites per cell and the greatest number of binding sites has been found in tissues in which no physiological function for the site has been observed (Silser, 1991). This is possible if the non-bound receptor is inhibiting the process under control, and the binding of ethylene cancels this inhibitory effect (Binder, 2008).

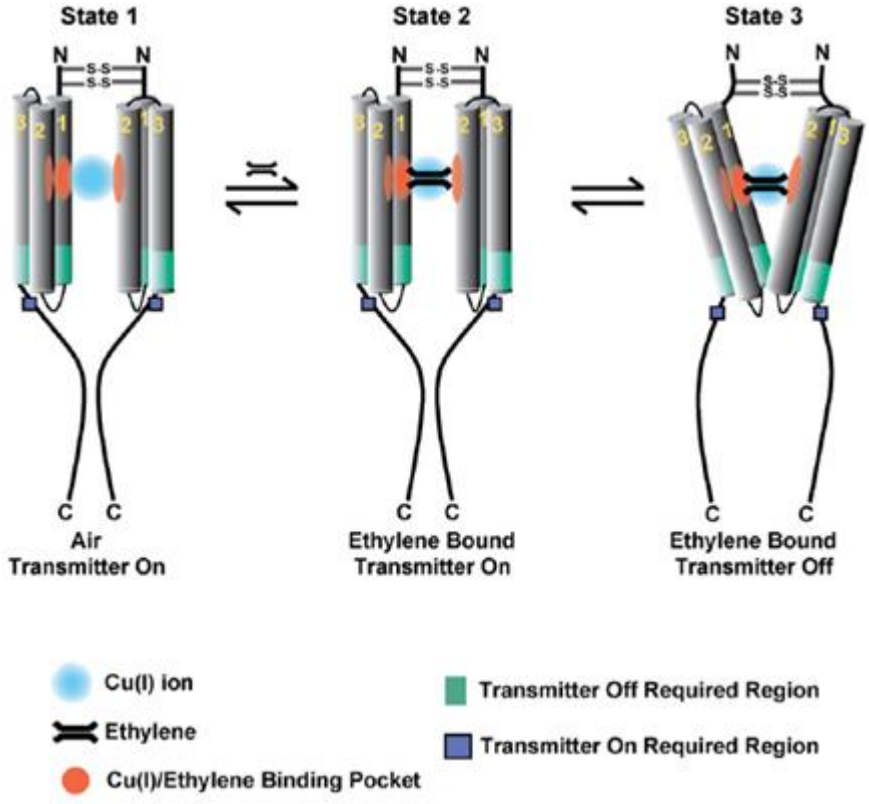


Figure 2-6 Structure of ethylene receptors (From Binder, 2008).

2.1.5.5.4 Differential Expression of Ethylene Receptors

A lot of information about ethylene receptors is available in different plants. But there is lack of information about receptors in potato. Summary of available information is given below.

(a) Arabidopsis thaliana

The abundance of ethylene receptor mRNA transcripts has been studied in plant tissues at different stages of development. Hua *et al.* (1998) using RNA *in situ* hybridization found that the amount of RNA of the 5 receptors gene were generally low and ubiquitous. ERS1 was expressed ubiquitously in *Arabidopsis* and was found in: embryos, leaves and in cotyledons, hypocotyls and roots of etiolated seedlings. Strong expression was observed in young floral primordial, floral organ primordial and in the locules of anthers.

The patterns of expression of ETR1 in etiolated seedlings and leaves stem and flowers of *Arabidopsis* were similar to ERS1. However the abundance of ETR1 transcripts in etiolated seedlings and leaves was lower (Hua *et al*., 1998). ETR1 was also expressed strongly in the locules of anthers and developing carpels of flowers.

A similar pattern was found with ETR2 and expression was present in embryos, etiolated seedlings, leaves, stem and young floral primordial. In contrast to ETR1 and ESR1, ETR2 was not expressed strongly in the stamens, but was in developing carpels (Hua *et al.*, 1998)

(b) Muskmelon (*Curcumis melo*)

Northern blot analysis of CM-ETR1 and CM-ERS1 in melons fruits showed that the expression transcripts of these genes changed differently during fruit development (Sato-Nara K *et al.*, 1999). An increase in Cm-ERS1 was paralleled by an increase in fruit size

and thickening of pericarp and during this period the expression of CM-ETR1 remained low. The rise in expression of Cm-ETR1 occurred 64 days after pollination and this coincided with an increase in the rate of production of ethylene.

(c) Tomato (*Lycopersicon esculentum*)

Tomato has a family of putative ethylene receptors designated LeETR1 (Zhou *et al.*, 1996a; Lashbrook *et al.*, 1998), LeETR2 (Zhou *et al.*, 1996b) LeETR4 (Tiemen and Klee, 1999) and LeEYTR5 (Tieman and Klee, 1999). Abundance of transcript changes during ripening of fruit, with the expression of LeETR4 and NR RNA increasing during ripening of fruit (Lashbrook, 1998; Tieman and Klee, 1999) whereas LeETR1 and LeETR2 are expresses constitutively (Lashbrook, 1998)

(d) Mango (*Mangifera indica*)

A cDNA homologeus of ETR1 designated METR1 was isolated from mango (Martinez *et al.,* 2001). RNA blots demonstrated that the amount of mRNA of METR1 increased during fruit ripening, furthermore the abundance of mRNA of METR1 increased transiently during wounding of the tissue.

2.1.6 Aminoethoxyvinylglycine (AVG)

Aminoethoxyvinylglycine is a vinylglycine analog. Vinylglycine analogs are inhibitors of pyridoxal phosphate-linked enzymes (Yang and Hoffman, 1984). AVG is the most effective of the group and is most commonly used. It is an inhibitor of ACS (Yu *et al*., 1979). It is the active ingredient of a commercial product known as ReTain ® and has been used with success on products such as apples.

An early study on the action of AVG was carried out on apple by Adams and Yang (1979). They observed that AVG inhibited the conversion of methionine to ACC, but did not inhibit the conversion of methionine to SAM or the conversion of ACC to ethylene. These results indicated that AVG blocked the conversion of SAM to ACC catalysed by ACS.

Previous studies have been done on the pre - and postharvest application of AVG on various fruit types. In Red Delicious apple, Silverman *et al.* (2004) observed that AVG reduced ethylene production and starch degradation but had no significant effect on organic acids, colour and sugar. It also reduced ethylene and protein biosynthesis (Saltveit, 2005) and delayed fruit maturation in Cox's Orange Pippin apples (Johnson and Colgan, 2003). Romani (1983) observed that AVG delayed ripening of Bartlett pears. It also delayed ripening in muskmelon (Shellie, 1999). AVG reduced ethylene synthesis and fruit drop, and delayed fruit ripening in Kogetsu apples (Rath *et al*., 2006). Arctic Snow nectarines also exhibited delayed ripening, lower ethylene production and extended firmness after treatment with AVG (McGlasson *et al*., 2005). Torrigiani *et al*. (2004) reported that AVG delayed the softening, and reduced fruit drop in Stark Red Gold nectarines.

2.1.7 1-Methylcyclopropene (1-MCP)

1-MCP is a cyclopropene derivative used as a synthetic plant growth regulator. 1-MCP is an odourless, non toxic and gaseous product that binds irreversibly to the ethylene receptors and prevents ethylene action at very low concentrations (Serek *et al*., 2006; Watkins, 2006). 1-MCP has been registered and marketed by AgroFresh, a Rohm and Haas company under the commercial name of SmartFresh in 2002. In the US it has received FDA approval in the same year to treat apples and pears, and numerous other food and flower produce have been listed in applications for approval, including tomatoes.

Commercially, 1-MCP is available as Ethylbloc® and SmartFreshTM (Blankenship and Dole, 2003). Ethylbloc® is aimed for use on ornamental crops, while SmartFreshTM is aimed for use on edible crops. In the UK SmartFresh^{TM} is permitted for commercial use on apples, and for experimental use on pears and plums.

1-MCP competes with ethylene for its receptor sites. According to Sisler and Wood (1988) it binds permanently to the receptor site, whereas ethylene binds reversibly to the receptor. Ethylene is a pi-acceptor compound, i.e. it can accept electrons from metals into a vacant orbital. When ethylene binds to the metal in the receptor, electrons are withdrawn into the orbital of ethylene. This is followed by a rearrangement of the ligands, which surround the metal. One of the ligands moves away from the metal, while another moves towards it. Ethylene is then released and an active receptor complex is formed (Sisler and Serek, 1997, 1999). 1-MCP's mode of action is similar, but it is not lost from the complex and forms an inactive complex. In this way it blocks feedback regulation of autocatalytic ethylene production (Golding *et al*., 1998).

1-MCP has been used with success on flowers, potted plants, fruit and vegetables (Serek, 2006; Watkins, 2006). According to Blankenship (2001) the success of 1-MCP depends on a number of requirements such as the concentration, exposure time, treatment and maturity of the fruit. The efficacy of 1-MCP varies from product to product. In tomatoes 7 $nL.L^{-1}$(ppb) has been reported to be effective in delaying ripening (Wills and Ku, 2002), while 500 $nL.L^{-1}$ (ppb) was found to be effective in delaying ripening in unripe bananas (Harris *et al*., 2000).

1-MCP has also been found to be effective in delaying the effects of ethylene in vegetables. It was reported to suppress respiration in broccoli (Fan and Mattheis, 1999b) and cucumbers (Nillson, 2005). Pre-treatment with 1-MCP reduced ethylene-induced russet spotting of lettuce (Fan and Mattheis, 2000b) and yellowing of broccoli florets (Ku

and Wills, 1999; Gong and Mattheis, 2003). Jiang *et al*. (2002) reported that senescence in coriander leaves was significantly retarded after treatment of 1-MCP. Ethylene–induced degreening of cucumbers was also inhibited with 1-MCP (Nillson, 2005).

1-MCP is effective in delaying ripening of ethylene sensitive fruit. For example treatment, with 1-MCP delayed the softening in fruit such as apples (Tatsuki *et al*., 2007), apricots (Fan *et al*., 2000a), avocados (Feng *et al*., 2000; Hershkovitz *et al*., 2005), bananas (Jiang *et al*., 2004; Harris *et al.,* 2000; Zhang *et al.,* 2006), mangos (Jiang and Joyce, 2000), pears (Baritelle, 2001, Ekman *et al.,* 2004). Pre-treatment with 1-MCP before exposure to ethylene, resulted in a delay in the colour changes of avocados for 8 days (Feng *et al*., 2000; Woolfe et *al*., 2005). Fan *et al*. (2000a) also reported a delay in colour changes of apricots after treatment with 1-MCP. Banana fruit treated with 1-MCP displayed a delay in peel colour change. The ripened banana fruit did not turn bright yellow, but displayed an uneven dull yellow colour (Golding *et al*., 1998; Harris *et al*., 2000; Jiang *et al*., 2004). Treatment with 1-MCP was also found to be effective in delaying the degreening of non-climacteric fruit. When Indian lime fruit were treated with 200 or 500 $nL.L^{-1}$ (ppb) 1-MCP, yellowing was delayed for 21 days (Win *et al*., 2006). Degreening was effectively delayed by 1-MCP in Shamouti oranges (Porat *et al*., 1999) and pineapples (Selvarajah *et al*., 2001).

In addition to delaying fruit softening and degreening, 1-MCP was also found to suppress ethylene production and ripening for two weeks in Hass avocados (Feng *et al*., 2000; Woolfe *et al*., 2005). Ethylene production was suppressed in apple (Tatsuki *et al*., 2007), bananas (Golding *et al*., 1998; Zhang *et al.,* 2006) and Indian lime fruit (Jiang *et al*., 2004). Lou (2007) observed a delay in the peak of ethylene production in persimmon by about 10 days. 1-MCP even reduced the ethylene production in non-climacteric fruit such as strawberries (Bower *et al*., 2003). (Guillen, 2007) observed a delay in the ethylene peak of tomatoes treated with 1-MCP.

In certain fruit treated with 1-MCP the climacteric peak in respiration rate was delayed significantly. In avocados it was delayed for 6 days (Jeong *et al*., 2003) and by 8 days in plums (Dong *et al*., 2002), bananas (Golding *et al*., 1998) and apricots (Fan *et al*., 2000a). Papaya fruit that were treated with 1-MCP also displayed a delay in the respiration peak (Manenoi *et al*., 2007). 1-MCP extended the green life of guava fruit by 2- 4 days (Bosset *et al*., 2005).

1-MCP can also reduce the development of physiological disorders in fruit during storage. Internal browning, a symptom of chilling injury in banana, was effectively delayed or controlled by 1-MCP treatment (Jiang *et al*., 2004).

Treatment with 1-MCP has been used effectively in the ornamental industry. In potted *Schlumbergera truncata* it reduced ethylene production and bud abscission. In potted *Campanula carpatica* it suppressed ethylene production and extended flower life (Serek and Sisler, 2001). Cut carnations, petunia and delphinium flowers displayed a longer postharvest life when pre-treated with 1-MCP (Serek *et al*., 1995b; Ichimura *et al*., 2002).

2.1.7.1 1-MCP and Sprouting

Prang *et al*., (2005) observed the efficacy of 1-MCP as a preventative treatment for ethylene-induced fry colour darkening in potato (*Solanum tuberosum L*.) tubers, without reducing the effectiveness of ethylene as a tuber sprouting control agent. 1-MCP treatment can eliminate the adverse effect of ethylene on fry colour in potato tubers. Authors suggested that the effect of 1-MCP is dependent on the target tissue. The target tissue in potato is the tuber eye tissue where the sprouting is initiated. Tissues in the tuber eye are more metabolically active than the cortex, where sugars are metabolised. Therefore, possible turnover of ethylene binding sites could be rapid and thus, 1-MCP exhibits less effect on the tuber eye tissue.

Sprout suppression effect of 1-MCP in onions was reported by Chope *et al*. (2007). They found that sprout growth was lowered in 1-MCP treated onion bulbs. These results were supported by another study; where 1-MCP have sprout suppression effect in onion bulbs (Downes *et al.,* 2010). There are no reports that 1-MCP on its own inhibits sprouting in potatoes.

2.1.8 Summary from Suttle (2004a) of hormonal control

Suttle and co-workers have conducted many studies to understand the hormonal control of tuber dormancy. It is useful to consider an overview of their conclusions as to the respective roles of the different hormones and classes of hormones. Suttle (2004a) concluded that both ABA and ethylene are required for the initiation of tuber dormancy, but only ABA is needed to maintain the dormant state. Cytokinins are involved in dormancy break. Thus endogenous cytokinin levels are relatively low in highly dormant tubers and tubers are non-responsive to exogenous cytokinins. During dormancy tubers actively metabolise ABA and cytokinins to inactive products. As dormancy weakens, tuber ABA levels decline and tubers become increasingly sensitive to exogenous cytokinins. Sprout growth is accompanied by increases in both endogenous IAA and GA. Suttle did not consider the role of ethylene in dormancy break or subsequent sprout growth.

2.1.9 Investigation of dormancy and sprouting at the molecular level

Several research groups are looking at the molecular biology of dormancy. Quantitative trait loci (QTL) analysis indicates that dormancy is controlled by at least nine loci (Ewing 2004).

Major changes in gene expression during dormancy progression have been demonstrated (Bachem *et al*., 2000; Ronning *et al*., 2003) and transcripts and proteins unique to either dormant or growing meristems have been identified.

Identification of molecular markers defining the end of tuber dormancy prior to visible sprouting is of agronomic interest for potato growers and the potato processing industry. Faivre-Rampant *et al*. (2004) considered a large number of expressed sequence tags (ESTs) associated with dormancy break and identified one in particular as an auxin response factor gene (ARF6). They used *in situ* hybridisation to show that it was highly expressed in cells in the apical meristem while expression decreased on tuber initiation. No expression was found in dormancy.

Campbell *et al.* (1996) showed that dormant meristem cells of potato tubers were predominantly arrested in the G1/G0 phase of the cell cycle. In potato tubers, breakage of dormancy is associated with the reactivation of meristem function. Senning *et al*. (2010) reported in dormant meristems, cells are arrested in the G_1/G_0 phase of the cell cycle and re-entry into the G_1 phase followed by DNA replication during the S phase enables bud outgrowth. Up-regulation of genes involved in DNA replication might be one of the first events occurring after reactivation of potato tuber meristems. Deoxyuridine triphosphatase (dUTPase) is essential for DNA replication and was therefore tested as a potential marker for meristem reactivation in tuber buds. The corresponding cDNA clone was isolated from potato by PCR. By employing different potato cultivars, a positive correlation between dUTPase expression and onset of tuber sprouting could be confirmed. Moreover, gene expression analysis of tuber buds during storage time revealed an up-regulation of the dUTPase 1 week before visible sprouting occurred. Further analysis using an *in vitro* sprout assay supported the assumption that dUTPase is a good molecular marker to define the transition from dormant to active potato tuber meristems.

Sprouting was significantly accelerated in transgenic tubers cytosolically expressing an inorganic pyrophosphatase gene derived from *Escherichia coli*. The period of pre sprouting dormancy for transgenic tubers planted immediately after harvest is reduced by six to seven weeks when compared to wild-type tubers (Farre *et al.,* 2001)

2.1.10 Growth inhibitors found in potato volatile fraction

Some researchers have identified volatile compounds from potato tubers that are not defined as plant growth regulators, but appear to inhibit sprout growth. The natural levels of these inhibitory volatiles and their physiological role in potato dormancy/sprout control is discussed in more detail in Chapter 7.

Naphthalene and some of its alkyl-substituted compounds have been identified as natural volatiles produced by potatoes (Buttery *et al*., 1970; Meigh *et al*., 1973; Nursten and Sheen, 1974; Coleman *et al*., 1981; Oruna-Concha *et al*., 2001). Dimethylnaphthalene (DMN) isomers are a group of naphthalene-substituted compounds in which 2 methyl groups substituted two hydrogen atoms in the naphthalene ring. Ten isomers of dimethylnaphthalene have been identified (Alexander *et al*., 1983; Shinbo *et al*., 1998; Shinbo *et al*., 2000). Some of them, such as the 1,4- and 1,6- isomers, have shown potato sprout inhibiting effects (Meigh *et al*., 1973; Beveridge *et al.,* 1981a; Filmer and Rhodes, 1985). Endogenous growth inhibitors found in potato volatile fractions showing varying degrees of sprout-growth inhibitory activity includes:

- Monomethylnaphthalenes
- Dimethylnaphthalenes
- Trimethylnaphthalenes
- Benzothiazole
- 1, 4, 6-trimethylnaphthalene

- Diphenylamine
- Dibenzothiophene

1, 4- 1, 6-dimethylnaphthalene, 1, 4, 6-trimethylnaphthalene and diphenylamine have activities at least of the same order as that given by commercial suppressants such as CIPC (Meigh *et al.,* 1973).

2.1.10.1 Diphenylamine

Diphenylamine (DPA), $(C_6H_5)_2NH$, is a colourless solid, used as a pre- or postharvest scald inhibitor for apples. Its anti-scald activity is the result of its antioxidant properties, which protect the apple skin from the oxidation products of alpha-farnesene during storage.

Fractionation of volatile substances produced by potato tubers using GC was followed by assaying fractions for sprout growth inhibitory activity using a potato shoot-tip bioassay (Filmer and Rhodes 1985). A region of the chromatogram having high sprout-growth inhibitory activity was identified and subsequently further resolved by capillary column GC into several peaks, five of which gave well-defined mass spectra. Two of these compounds were identified as DPA and dibenzothiophene. DPA showed high growth inhibitory activity in the bioassay (at least as high as 1, 4-dimethyl naphthalene) and was shown to be an effective sprout suppressant for whole tubers. The compound was tested in small-scale storage trials using up to 0.5 tonne of potatoes to assess its potential as a sprout suppressant and inhibited sprout growth (Filmer and Rhodes, 1985).

A study was conducted to assess the efficacy of DPA for inhibition of sprouting during non refrigerated storage in a cool store 17- 30 degree C and 75 to 95% relative humidity. Double treatment of tubers with 1500 - 2500 mg DPA/Kg tuber weight for 10 – 115 days

significantly reduced sprouting of tubers until 80 days as compared to control after 2 weeks (Mehta, 2004).

Unfortunately the use of DPA on apples in the UK is no longer permitted, so that its use for sprout control of potato tubers is no longer feasible.

2.1.11 Commercial methods of sprout suppression

A main component of managing potato quality in storage is effective sprout inhibition. Sprouting causes increased weight loss, reduced tuber quality. During storage, physiological processes still continue in the potato tubers and these have consequences with respect to the quality of the tubers. Storage at low temperature can cause increased sugar contents in tubers. The processing industry requires low sugar content in potatoes. In order to meet with quality demands as much as possible, potatoes have to be stored in alternative conditions i.e. higher temperatures to avoid sugar accumulation. Sprouting during storage causes intensive respiration and increased evaporation. So in practice there will be need for alternative storage conditions to meet the quality demands and to reduce the health hazards caused by different chemicals (Afek *et al*., 2000)

A wide variety of methods have been suggested for the control of potato sprouting. These methods are described below.

2.1.11.1 Storing at low temperatures (2º to 4º C)

Low temperature storage (expensive and affects quality) can inhibit sprouting. However it results in an increase in reducing sugar levels (Es and Hartmans, 1987). Carey and Cronin (1990) indicated that the development of brown colour in fried potatoes is strongly affected by their reducing sugar content. The colour of fried potato products is an

important parameter in determining their level of acceptability by the consumers (O'Beirne *et al.*, 1985). Fried potatoes produce a brown colour due to the Maillard reaction between reducing sugars and amino acids (Habib and Brown, 1957). Low temperature is not recommended for processing potatoes and also in developing countries it is not a cost efficient way of storage.

Sprouting is only one of many factors to be considered in commercial storage conditions and a possibility of achieving control of sprouting is extremely limited in many countries. Increasing importance of processing industry as well as seed industry makes some other forms of sprout control than cold storage absolutely essential. So industry could not exist without satisfactory ways of suppressing sprout growth.

2.1.11.2 Use of chemical sprout suppressants

The use of chemical sprout inhibitors is the commercially preferred option for successful long-term storage of potato tubers, particularly the ones used for the processing industry. It is estimated that 52 % of ware potatoes stored in the UK from the 2004 harvest received chemical pesticide treatments (Anderson *et al*., 2006). The primary method for controlling sprouting of stored potatoes is by the application of the sprout-inhibiting chemical chlorpropham (Kleinkopf *et al*., 2003; Anderson *et al*., 2006).

Many chemical compounds are known to sprout inhibition as given below. Beveridge *et al*., 1979; Coleman and Coleman, 1986; Es and Hartmans, 1987; Hartmans *et al*., 1995; Prange *et al.*, 2005).

- Chlorpropham (CIPC)
- Maleic hydrazide

- Hydrogen peroxide
- Ethylene (See section 2.5.3)
- Jasmonates
- Ethanol
- Carvone
- Spearmint
- Peppermint
- Abscisic acid,
- Indole-acetic acid,
- Dimethylnaphthalene
- Diisopropylnaphthalene

Many chemical have been suggested as inhibitors but few of them are used commercially. The sprout inhibitors chlorpropham (CIPC) and maleic hydrazide (MH) have proved to be of particular value in potato storage industry. However, their application can be problematic due to health hazards (Lewis *et al.,* 1997).

2.1.11.3 CIPC

Its use as a potato sprout inhibitor was first reported by P. C. Marth in 1952 and later patented by the Pittsburgh Plate and Glass Co. It is the most effective post-harvest sprout inhibitor registered for use in potato storages globally for successful long-term storage of potatoes.

CIPC works by affecting plant cell division effectively preventing new growth and thereby suppressing sprout formation. However, cell division is also extremely important during the wound healing, or curing, period after potatoes are placed into storage.

Several problems have been encountered in the use of CIPC. It is known to inhibit the natural wound healing process. Wound healing requires the production of two to five new cell layers by cell division. If CIPC is applied before the wound healing process is complete, excessive losses due to tuber dehydration and disease can occur (Crafts and Audia, 1980). CIPC has been extensively used worldwide although regulation leading to lower residue tolerance levels have promoted re-evaluation of alternative sprout control methods in storage (Boyleston *et al*., 2001).

When CIPC is applied at high concentration to achieve effective sprout control, it will permanently impair the performance of seed tubers. It must never be used on seed potatoes to achieve a satisfactory number of plants per seed. Seed contamination and toxicology is of main concern and limiting the use of CIPC. Despite its successful use for potato storage, its availability may be become restricted (Lewis *et al*., 1997)

Due to the increasing safety and environmental concerns regarding this synthetic chemical, many countries have started to reassess the use of CIPC, and limits of the allowable levels of chlorpropham residues (MRL) in potatoes entering the market place have been set in many countries. A maximum residue limit of 10 mg/kg was approved in the EU countries and has been in place since 2007 (PRC, 2007). In the USA, the tolerance value is 30 mg/kg (Kleinkopf *et al*., 2003). The concerns regarding levels of CIPC residues and its toxicity have contributed to an interest in finding safer and more natural sprout inhibitors.

2.1.11.4 Maleic hydrazide (MH)

Maleic hydrazide is the general name for 6-hydroxy-3–(2H) pyridazenone. Its growth regulating properties were first described by Scheone and Hoffmann in 1949. Maleic hydrazide has been available for potato sprout control for many years (Crompton Royal

MH-30). MH is a systemic product and applied to actively growing vines in the field during the bulking period. It is subsequently translocated within the plant and inhibits cell division.

Application is usually about 4 weeks before harvest. Correct timing is critical as very early applications can cause injury to foliage, produce many small unmarketable tubers and hence reduce yield (Yada *et al.,* 1991). Delayed application can also reduce its efficacy (Yada *et al.,* 1991).

The efficacy of 0.3 % MH applied 3 weeks before cutting was observed in four cultivars. After harvest potatoes were stored in evaporative cool stores (15-29 ˚C) and 68-90% relative humidity. MH reduced the mean number of sprouted tubers by 27% up to 10 weeks (Kaul and Mehta 1994). Higher concentrations increased the effectiveness but also increased the undesirable effects. Tuber skin may become rutted and in certain cased growth cracks have been reported (Poapst and Genier, 1971).

2.1.11.5 Hydrogen peroxide (HPP)

Hydrogen peroxide can act as a sprout inhibitor and is part of a commercial product marketed as Hydrogen Peroxide Plus (Afek *et al*., 2000; Prange *et al*., 1997), although it has also been identified as a signal for dormancy break in other species such as grapevines (Perez and Lira, 2005). Thus a decrease in catalase activity, resulting in an increase in hydrogen peroxide is observed just prior to dormancy break in grapevines.

There are several hydrogen peroxide-based materials that are being evaluated for sprout suppression in storage (Afek *et al*., 2000). Some of these materials have suppressed sprouting by physically damaging the developing sprouts or buds before they can elongate. Repeated or continuous applications of many of these sprout suppressants are

necessary to achieve the long term sprout free condition in storage. In addition to that they have antimicrobial activity when applied to stored potatoes.

Hydrogen peroxide adversely affects the meristematic tissues that are formed after dormancy break in the tuber. HPP showed similar efficacy as compared to CIPC. Afek *et al.* (2000) observed that after six months of storage at 10ºC during which potatoes received 4 treatments with either CIPC or HPP, a 0 % sprouting rate was observed in case of CIPC and HPP and 84 % in control. In the samples that were taken after single treatment the percentages of sprouting after six months at 10 ˚C were 61, 58 and 87 % from HPP, CIPC and control respectively.

2.1.11.6 Carvone

A wide range of compounds and materials have been studied as potential sprout inhibitors. Several natural compounds were found to be effective sprout inhibitors, including several monoterpenes (e.g. carvone), spearmint and peppermint oils (D carvone), purified extracts from clove and substituted naphthalenes (Meigh *et al*., 1973; Beveridge *et al*., 1981a; Beveridge *et al.,* 1981b; Vaughn and Spencer, 1993; Kleinkopf *et al*., 2003). Many of these natural sprout inhibitors are commercially marketed in different countries.

S-(+)-Carvone is the main ingredient of oil available from caraway (50-70%) or dill seed (40-60%), now used as a commodity chemical in perfumes and food; its chemical formula is $C_{10}H_{14}O$, and it is a pale-yellowish or colourless liquid. This is slightly soluble in water and most soluble in alcohol. It boils at 231º C; it can be used in flavouring, liqueurs, perfumes, and soaps (www.en.wikipedia.org/wiki/Carvone).

S- Carvone the monoterpene commonly extracted from caraway oils is commercially marketed in Holland and Switzerland and several other countries (Ooesterhaven *et al*., 1995b). Talent is currently the only commercial sprout suppression formulation that uses carvone as its primary active ingredient.

The sprout inhibiting activity of this chemical rapidly weakens when treatments are discontinued, so making it suitable for seed tuber preservation (Lewis *et al*., 1997). Brown (2000) reported that plants grown from potato seed tubers treated with carvone produced more vigorous plants and higher yields than those treated with 1,4 DMN.

Treatment of potato tubers of the cultivar Kevin with the vapour of caraway essential oil at a dosage of 0.1 ml/Kg tubers applied regularly in four and six weeks periods successfully inhabited bud growth at 10˚ C. Weight loses by sprouting were 0.4 and 0.3 % whereas in control they were 7.5% (Cizkova *et al.,* 2000).

The effect of menthone ($C_{10}H_{18}O$) and neomenthol ($C_{10}H_{20}O$) vapours were compared to S (+) carvone for sprouts suppressants as well as for effect on soluble sugars levels, respiration, during high and low temperature storage. Menthone significantly inhibited sprouting without adversely affecting the percentage of glucose or sucrose contents. Menthone and neomenthole were 5 to 10 times more effective in suppression than S (+) carvone when applied together at 0.5 µl /L each (Coleman *et al*., 2001).

2.1.11.7 Spearmint and Peppermint

Spearmint (*Mentha spicata*) and peppermint (*M. piperita*) oils can be used as effective sprout suppressants to extend the storage period of potato tubers. In this case the active ingredient is D-carvone. One additional benefit in using these oils as sprout suppressants comes from the mint industry's interest in finding new markets for their crop. Different

application methods affect the efficacy of the product. A wick application of these oils gave better sprout control than thermal and cold aerosol (Frazier *et al*., 1998). This study suggested that both oils have potential for potato sprout suppression (Frazier *et al*., 1998).

2.1.11.8 Eugenol (BIOX A)

Eugenol ($C_{10}H_{12}O_2$) is an allyl chain-substituted guaiacol, i.e. 2-methoxy-4-(2-propenyl) phenol. It is a clear to pale yellow oily liquid extracted from certain essential oils especially from clove oil and cinnamon. It is slightly soluble in water and soluble in organic solvents. It has a pleasant, spicy, clove-like taste. Eugenol is used in perfumeries, flavourings, essential oils and in medicine (local antiseptic and analgesic)

A new product Biox A (eugenol) has been registered for sprout control in Idaho, Washington, California, Texas and Florida. This product has also received approval for use in the organic market (Kleinkopf and Frazier 2002).

Kleinkopf and Frazier (2002) conducted a comparative study of CIPC, Spearmint oil, Peppermint oil and Biox A. they observed that spearmint, peppermint oil and Biox A could be effectively used for potato sprout suppression. MCW-100 (HPP) was not successful at levels used in this study, but may be more effective at higher rates. In the case of these chemicals for long term storage multiple application are necessary. The cost of sprout suppression will vary with the number of applications. If the intended length of storage is longer than one month past dormancy break then cost will be significantly higher than with CIPC.

2.1.12 Other natural products as sprout suppressants

There are some natural products can be used to prevent sprouting as given below but they are not being used as commercial sprout suppressants.

Dormant corms of corn flag (gladiolus) were reported to have sprout inhibition effect. A leachate was prepared to treat non dormant tubers of potato. These were dipped in the leachate solution or distilled water (control) for 18 hrs. The percentage of sprouted tubers (1.68%) was significantly lower in leachate treatment as compared to the control (10.1 %).

Natural sprout inhibiters (Benzaldeyde, Salicylaldehyde and Thymol) were tested on potatoes stored at high temperature 24 ± 2 ˚C. The inhibiters were used for 8, 10 and 12 days at 500, 625 and 750 µl /L (Bolyston *et al*., 2001). Treatment with salicylaldehyde at 500 and 625 µl /L for 10 and 12 days significantly reduced sprouting compared to control up to 120 days without rotting (Mehta, 2004).

2.2 Sweetpotato

Sweetpotato (*Ipomoea batatas*) is the world's seventh most important staple crop, grown in over 100 countries of the world, covering an estimated total area of 9.2 million Ha, with an annual global production around 125 million tonnes (FAOSTAT, 2008). Almost 95% of the total production (Table 2-2) is in developing countries (CIP 1996). In terms of production Sweetpotato is the 3rd most important crop in roots and tuber crops after potato and cassava (FAOSTAT, 2008).

Region	Sweetpotato
Total World	129
Sub-Saharan Africa	11
South America	1
Asia	114
North and Central America	1.5
China	107
Brazil	0.5
Nigeria	2.5

Table 2-2 Production of sweet potato roots (million tonnes per annum) in selected regions and countries

Sweetpotato, a tuberous root vegetable, is a particularly popular food in southern and eastern Africa. Sweetpotatoes, native to Central America, are considered a staple in many countries and have been cultivated in Southern states since the 16th century. It gives better yield under tropical condition (Woolfe, 1992). In developing countries, sweetpotato is important because it is a food security crop for the poor (Hagenimana and Owori 1997, CIP Report 1996)

Nutritionally, sweetpotatoes are an excellent source of vitamin A (orange fleshed sweetpotato varieties) and a good source of potassium and vitamin C, B6, riboflavin, copper, pantothetic acid and folic acid.

2.2.1 Taxonomy of Sweetpotatoes

Sweetpotato (*Ipomoea batatas*) belongs to the Convolvulaceae family. It is hexaploid (6x = 90). Different theories of the evolution of *I. batatas* have been advanced by researchers, who consider it an autopolyploid derivative of *I. trifida (*a diploid, it is thought to be one of the likely progenitors of sweetpotato) and some studies support an allopolyploid origin of sweetpotato roots involving *I. trifida* and an unknown tetraploid parent (Woolfe, 1992).

2.2.2 Storage of sweetpotatoes

There appears to be no dormancy period in sweetpotato roots, so that harvested roots can generally be induced to sprout by being placed under appropriate conditions (20° C and above, and high humidity). There is no evidence of preformed eyes, as found in potato.

Storage techniques include physical (low temperature and controlled environment) and chemical treatments. Cold storage is definitely harmful for sweetpotato storage Sweetpotatoes are known to suffer from chilling damage at 12° C or below, while for potatoes, this type of damage occurs at 2° C or below. The extent of chilling damage usually depends on a time/temperature interaction. The most common symptoms are internal tissue breakdown, increased water loss, susceptibility to decay. When sweetpotatoes are chilled there is a marked increase in their susceptibility to infection by rot producing organisms. Chilling also reduces the seed value of the roots; in case of

severe chilling, sweetpotato roots cannot produce any sprouts when planted (Lyons, 1973).

Sweetpotato storage roots can be stored under controlled environments for several months. For example, in the USA, when roots are stored at temperatures of 13–15 ºC and high relative humidity, they can be kept for up to a year (Picha, 1986). During marketing under tropical conditions, where cold storage is too expensive, sweetpotato roots rarely kept for more than 2-3 weeks (Rees *et al.,* 2001; Tomlins *et al*., 2002) and can be subject to losses (Tomlins *et al*., 2007).

The use of temperature-controlled storage of sweetpotato is usually not economically feasible in tropical developing countries due to lack of resources. However, more than 80% of sweetpotato production is in developing countries (FAOSTAT, 2008).

Traditional storage technologies for sweetpotato roots have been reported in tropical countries such as Bangladesh (Jenkins, 1982), India (Prasad *et al*., 1981; Ray and Ravi, 2005), Tanzania (Tomlins *et al*., 2007) and Kenya (Karuri and Ojijo, 1994; Karuri and Hagenimana, 1995). The success of these storage technologies, however, has been variable (Ray and Ravi, 2005).

2.2.3 Sprout Control

Sprout production is an important economic consideration in commercial sweetpotato cropping. At planting time, vigorous and plentiful sprout production is required to minimise the cost of propagation material. However, sprout growth decreases the quality and value of roots for fresh market sales. Sprouting is generally controlled by manipulating the temperature and humidity under which the crop is stored. Sprout suppression would be useful for produce in transit as export to the northern hemisphere requires shipping

through equatorial conditions that promote sprouting. Control of sprout production in sweetpotatoes has been examined using a number of treatments. For sweetpotato roots, CIPC (Kushman, 1969), gamma irradiation (Bonsi and Loretan, 1988), naphthalene acetic acid (Paton and Scriven, 1989) all suppressed sprouting to some degree.

A mathematical model was used to observe the effects of hot water treatment on the chemical composition and sprouting of sweetpotato cv. Beniotome (Tanaka *et al*., 2001). Twenty weeks after the heat treatment, there were no significant changes in the level of starch; fructose; glucose; sucrose; maltose; and reducing and non reducing sugars between the pre- and post-treatment analysis. Sprouting was not observed in heat-treated samples after 20 weeks of storage compared with the 90% sprouting in the control (Tanaka *et al*., 2001).

A study to investigate the effect of sodium hypochlorite (NaOCl) concentrations and immersion times on sprout suppression in sweetpotato roots (*Ipomoea batatas*) was carried out. Treatments consisted of immersion for 20, 60 or 180 min in solutions containing 0, 0.33, 1.0, 3.0 or 9.0% NaOCl by volume, in all combinations. Evaluation after 102 days of storage showed that sprout numbers were significantly reduced by 3.0 and 9.0% NaOCl, but weight loss increased. No combination of factors gave comprehensive sprout inhibition while maintaining tuber quality at an acceptable level (Lewthwaite and Triggs, 1995).

Paton and Scriven (1989) showed the effect of naphthaleneacetic acid (NAA) on sweetpotatoes in terms of sprout inhibition at 25° C. During the study % sprouting and % weight loss were observed. NAA was applied by two methods either under reduced pressure as a 1 g litre^{-1} solution containing wetting agent or as a dust of 1, 10 or 100 mg /g of talc. NAA applied by both methods reduced sprouting by more than 50% up to 40 days, except the 1 mg NAA per gram of talc which reduced sprouting by 29%.

CIPC was most effect at relatively lower temperature than higher where multiple applications were required. It has been observed that CIPC inhibited sprouting when applied through fog and aerosol method. One aerosol application of 6.80 g of CIPC per 27 kg of potatoes significantly reduced sprouting at a storage temperature of 18˚ C or 26˚ C. but for 26˚ C two extra applications were required to maintain the level comparable to storage at 18˚ C with one application. Fog application also reduced sprouting by 30-60% in the top of the bin when applied at the rate of 1 pound per 1000 boxes of sweetpotatoes.

Gamma irradiation has been reported to inhibit sprouting of yams, potatoes and sweetpotatoes However, this technique has not yet been applied on a commercial scale in the tropics and is unlikely to be of practical value to farmers because of the cost of the high technology involved (FAO, 2002). Furthermore it is a treatment that is not widely acceptable to consumers or a permitted treatment for food commodities in some developed countries.

The following information outlines several similarities and differences between sweetpotatoes and potato (Table 2-3).

	Factor	Sweetpotato	Potato
1.	Scientific Name	*Ipomoea batatas*	*Solanum tuberosum*
2.	Plant family	*Convolvulaceae*	*Solanaceae*
3.	Plant group	Dicotyledon	Dicotyledon
4.	Chromosome number	2n=90 (hexaploid)	2n=48 (tetraploid)
5.	Origin	Tropical America (Peru, Ecuador)	Southern Peru
6.	Edible storage organ	Storage root	Stem Tuber
7.	Number/plant	4- 10	5-10
8.	Appearance	Smooth, with thin skin, no eyes or buds	Smooth, with thin skin, with eyes or buds
9.	Shape	Short, blocky, tapered ends	Short, oval, round,
10.	Dry matter	22 to 28%	18 to 25%
11.	Taste	Sweet	Starchy
12.	Beta carotene (Vit. A)	High (orange vars.)*	Low –medium
13.	Propagation	vegetative/vine cuttings	vegetative
14.	Storage	13 to 15^{o}C	4 to 7^{o}C
25.	Climatic requirements	Tropical and temperate	Temperate

Table 2-3 Differences and similarities between sweetpotatoes and potato

2.3 Conclusion

Successful storage of root and tuber crops depends on good control of sprouting. Worldwide most storage managers rely on synthetic sprout suppressants to control sprouting. Conversely, seed potato growers require safe, effective methods to prematurely terminate tuber dormancy. At harvest, potato tubers are dormant. Loss of tuber dormancy is accompanied by numerous biochemical changes, but many are detrimental to the nutritional and processing qualities of potatoes. Most work in this subject has been conducted on *Solanum tuberosum* owing to its worldwide importance. We have undertaken a detailed overview of the tuber sprout control utilising different techniques including physiological, biochemical and cell-biological techniques. It has

emerged that very little work has been carried out on sweetpotato storage. Growth hormones have been found to play a role in tuber dormancy regulation, but there is lack of knowledge about growth hormones in sprout control in sweetpotato roots. Giving it importance as a major root crop for food security for many tropical countries, we have established a series of experiments on potatoes and roots, using knowledge already available on potato to overcome the storage issue and to have a better understanding about dormancy and sprout control.

3 An examination of the role of gibberellins in sprout control in sweetpotato

3.1 Background

The control of potato dormancy and sprouting has been the subject of significant interest over many years. A number of different approaches have been adopted and data is available regarding, chemical sprout suppressants, hormonal, control of dormancy in potato tubers. The amount of information on sprout control in sweetpotato is much more limited, and no overall mechanism has been established.

Sprouting is an important factor limiting storage period of sweet potatoes and also it is economically important for seed roots. Investigations on sweetpotato root dormancy are of basic importance for both food products and seed roots.

Potato tubers and sweetpotato roots propagate vegetatively. The storage organ of the sweetpotato is a root; it can be used for vegetative propagation. This is in contrast to potatoes, which are tubers originated from the stem (Kays, 1985). In sweetpotatoes sprout buds are not visible prior to sprout growth, unlike the eyes of potato tubers. Most sprout suppressants, such as CIPC affect the meristematic cells of the sprouting loci as these loci are present in potato on the surface. But once sprouting has been started, the application of sprout suppressant may then inhibit further growth of the sprout initials. Sprout suppressants may cause food contamination with potentially toxic residues (Rastovski, 1987)

Plant hormones are involved in dormancy control and play an important role in maintenance or termination of dormancy in potatoes. Gibberellins (GA) stimulate sprouting (Clegg and Rappaport, 1970; Claassens and Vreugdenhil, 2000), but are often

not efficient on dormant tubers. It has been also reported that GA_3 decreases dormancy (Dogonadze *et al*., 2000). Research has focused mainly on the effect of gibberellins in potato tubers (See literature review 2.5.1), and no information exists on their application on sweetpotato roots.

Recent development of highly active growth retardants has enhanced the potential uses of chemical growth regulators. Among them, paclobutrazole (PBZ) is widely used. PBZ, a member of triazole plant regulator group, is a broad spectrum GA biosynthesis inhibitor and used widely in agriculture (Davis and Curry, 1991)

PBZ interferes with GA biosynthesis by inhibiting the oxidation of ent–kaurene to ent-kauronic acid through inactivating cytochrome P450-dependant oxygenases (Izumi *et al*., 1985). However the biosynthetic pathway from mevalonic acid to kaurene and kaurenic acid to GA_{12} aldehyde is not affected. The inhibitory effect of PBZ on GA synthesis is further supported by the fact that treated plants have lower GA concentrations (Steffens *et al.*, 1992) and some effects of PBZ could be reversed by GA application (Gilley and Fletcher, 1998)

Compared with other plant growth retardants, triazoles are potent and required in small quantities to inhibit growth (Davis *et al.*, 1988). The most noticeable effect of PBZ is internode compression resulting in compact short plants and reduced leaf area (Sebatian *et al*., 2002; Yeshitela *et al*., 2004).

PBZ extended the potato tuber dormany period during storage nearly 4 weeks as compare to control irrespective of the concentration used (Tekalign and Hammes, 2005).

Prohexadione calcium is another GA biosynthesis inhibitor. Prohexadione calcium effectively reduces the level of GA in the apple plant for three to four weeks after application. Prohexadione calcium does not persist in the plant or affect vegetative growth

the following season. Due to its short-term effect and lack of persistence, Prohexadione calcium is a flexible tool for vegetative growth management that can be applied at a variety of timings and used to develop user-specific growth management strategies (Evans *et al*., 1997).

In order to understand sprout control in sweetpotato roots, it is interesting to determine whether gibberellins have a similar role to their role in potato tubers. For that reason a series of trials was conducted to determine the effect of gibberellins and gibberellins synthesis inhibitors on sweetpotato sprouting.

Management of sprout growth knowing the effect of growth hormones in sweetpotatoes would be a valuable tool for areas where with relatively high ambient temperatures (20° C to 30° C) such as are normally experienced in tropical and subtropical lowlands.

3.2 Objectives

The objective of this study was to determine the role of GA in the control of sprouting in sweetpotato roots and to find hormone inhibitors that would minimise sprout production while maintaining root quality.

It was hypothesized that GA would be involved in promoting sprouting growth. Hence, there would be vigorous sprouting in the presence of gibberellins and there will be less growth in the presence of GA synthesis inhibitors.

3.3 Materials and Methods

The experiment was conducted in a controlled environment (CE) room at 25˚ C at the Natural Resources Institute, UK. Orange fleshed sweetpotato roots were imported from the USA through Greenvale. The experiment was carried out as a randomized complete block design with 10 treatments. Each treatment was replicated four times. A moderate humidity (80%) was maintained by putting roots in boxes and covering with plastic bags. Netting was also used as an insurance against insect infestation of facilities.

3.3.1 Optimum humidity for sprouting

A trial was conducted to examine the effect of relative humidity on sprouting in sweetpotato roots so that the appropriate humidities could be used in subsequent trials. The optimum humidity was found to be about 80-85 %. This was to get important insights into the physiological control of sprouting (Trial details and data are given in appendix 2)

Data on number/length of sprouts for each treatment were recorded weekly. Length of sprouts was measured by using a vernier caliper. The weight of each root was recorded at the start of the trial and at weekly intervals. The following treatments were used.

- 10^{-3}M GA_3
- 10^{-4} M GA_3
- 10^{-5} M GA_3
- 1 ml/L Piccolo® (Paclobutrazole)
- 5 ml/L Piccolo®
- 20 ml/L Piccolo®

- 2 g/L Regalis® (Prohexadione calcium)
- 6 g/L Regalis®
- 6 g/L Regalis® applied after 7 days of sprout initiation
- Control

3.3.2 GA_3 treatment

A 50 ml stock solution of 1 M GA_3 was made up in ethanol and used to make up 8 L of 10^{-3} M GA_3 by diluting 1000 fold with water. 1L of this was diluted 10 fold to give 10^{-4} M and the process repeated to give 10^{-5} M. Five drops of tween were added to each GA_3 treatment before dipping the roots to aid wetting. 20 roots were dipped for 2 hours in each GA_3 concentration (4 replicates of 5 roots). Roots were then blotted dry using paper towels, and left uncovered in the CE room for 1 hour to dry further.

3.3.3 Paclobutrazol (Piccolo) treatment

The commercial product Piccolo, which contains 4 g/L Paclobutrazol, was used at concentrations of 1, 5 and 20 ml per L. The treatment procedure using 8 L (volume of solution) was the same as described for GA_3.

3.3.4 Prohexadione Calcium (Regalis®) treatment

A commercial product Regalis® which is 10% w/w prohexadione-Calcium was used. BASF recommend usage at 6 g/L. For this experiment 2 g/L and 6 g/L was used at time 0, and another treatment was dipped in 6 g/L after 7 days (equivalent to initiation of sprouting for untreated controls). For convenience16 g and 48 g of Regalis® was each

made up in 1L water in advance. In order to get the best out of Regalis® a water conditioner X-Change was used for all Prohexadione Calcium treatments at a rate of 16 ml per 8L of water.

3.3.5 Statistical Analysis

All statistical analysis was carried out using GenStat 11th edition (VSN international Ltd UK). Least significant differences (L.S.D: *p*, 0.05) were calculated for separation of means. Analysis of variance (ANOVA) of repeated measurements was carried out to determine whether there were significant differences between treatments

3.4 Results and Discussion

The sprout growth (mm) and number of sprouts are shown in Tables 3-1, 3-2 and Figures 3-1 and 3-2. In order to determine the differences between treatments an analysis of repeated measurements was carried out (Table 3-3).

Pictures of representative roots are shown in Plate 3.1. The sweetpotato roots showed no delay in sprouting, consistent with the hypothesis that sweetpotato roots do not exhibit dormancy. Gibberellin (GA_3) treatments resulted in more vigorous sprout growth than other treatments. GA_3 was found to be more active for the stimulation of/and further growth of sprouts. Figure 3-1 shows that 10^{-3}M GA_3 treatment considerably increased sprout length over the period of 5 weeks of storage. In this trial, three concentrations of GA_3 were used to test the effect on sprout growth of sweetpotato roots but 10^{-3}M was more effective. Claassens and Vreugdenhil (2000) mentioned that GA_4 and GA_7 can be used for sprout stimulation and growth in potato tubers. They did however also mention that higher concentrations are needed in more dormant tubers to initiate sprouting. In this

study there was a concentration effect for GA_3 for both sprout growth and sprout number (Table 3-3). 10^{-3}M GA_3 significantly promoted sprout growth compared to the control, Piccolo treatments (except 20 ml/L) and Regalis treatments. 20 ml/L Piccolo showed significantly lower sprout growth than all other treatments including control. The Piccolo effect was concentration dependent. Regalis had no significant effect on sprout growth

In terms of number of sprouts, GA treatments also produced a higher number of sprouts per root as compared to other treatments and control (Figure 3-2). In 10^{-3}M GA_3 significantly higher numbers of sprouts were reported than all other treatments except 10^{-4}M GA_3, 10^{-5}M GA_3 and 5 ml /L Piccolo (Table 3-3). Higher concentrations of Piccolo 20 ml/L were found to be most effective in sprout inhibition. Piccolo (20 ml /L) considerably reduced the sprout growth compared to GA treated and all other treatments. Sprout length of roots treated with 20 ml /L piccolo was about 50% lower than the control (Figure 3-1). Number of sprouts remained significantly lower in 20 ml /L Piccolo treated roots (Figure 3-2). As for sprout growth there was no growth inhibiting effect of Regalis was reported as compared to control and GA treated roots

Table 3-3 shows the weight loss during the storage period. The weight loss increased steadily. No significant difference was observed for any treatments. (Figure 3-3, Table 3-4)

(A) (B)

(C) (D)

Plate 3-1 Effect of 10^{-3} M GA_3 (A), 20 ml/L Piccolo (B), Control (C) and 6 g/L Regalis (D) on sprout growth after 5 weeks of storage.

Table 3-1 Effect of gibberellins and gibberellin synthesis inhibitors on sprout growth (mm) of sweetpotatoes after 1, 2, 3, 4 and 5 weeks of storage. Each data point is the mean of four replicates each consisting of four roots.

Treatments	Average sprout growth per root (mm)				
	Weeks				
	1	2	3	4	5
10^{-3}M GA_3	13.1	25.2	38.1	72.5	128.3
10^{-4}M GA_3	11.7	23.9	34.6	56.0	101.2
10^{-5}M GA_3	7.7	20.2	31.2	55.9	98
1 ml/L Piccolo	8.0	19.9	41.4	60.9	94.6
5ml/LPiccolo	7.6	19.8	30.6	40.3	77.7
20ml/L Piccolo	3.9	9.8	15.2	26.4	41.4
2 g/L Regalis	5.4	13.7	25.8	45.6	77.2
6 g/L Regalis	5.0	15.9	37.8	51.9	94.5
6 g/L Regalis after 7 days of sprout initiation	6.1	21.2	41.1	56.9	83.4
Control	6.2	20.0	36.9	52.8	96.3
Treatment effect *p*	0.041	0.012	0.015	0.001	0.001
L.S.D (0.05)	5.5	10.8	18.8	16.1	30.1

Table 3-2 Effect of gibberellins and gibberellin synthesis inhibitors on no. of sprouts per root after 1, 2, 3, 4 and 5 weeks of storage. Each data point is the mean of four replicates each consisting of four roots.

Treatments	Average No. of sprouts per root				
	Weeks				
	1	2	3	4	5
10^{-3}M GA_3	3.50	4.06	5.18	6.43	7.47
10^{-4}M GA_3	2.94	3.75	4.31	4.88	6.25
10^{-5}M GA_3	2.94	3.69	4.38	4.62	6.68
1 ml/L Piccolo	2.62	2.19	4.81	5.31	5.56
5ml/LPiccolo	2.81	3.44	3.38	4.88	5.31
20ml/L Piccolo	1.56	3.00	2.36	3.56	4.06
2 g/L Regalis	2.06	3.12	3.31	4.19	5.06
6 g/L Regalis	2.00	2.38	3.94	4.88	5.75
6g/L Regalis after 7 days of sprout initiation	2.25	2.62	4.06	5.62	7.19
Control	2.06	3.02	3.93	4.75	5.25
Treatment effect *p*	0.532	0.251	0.018	0.41	0.016
L.S.D (0.05)	1.77	1.52	1.41	1.28	1.69

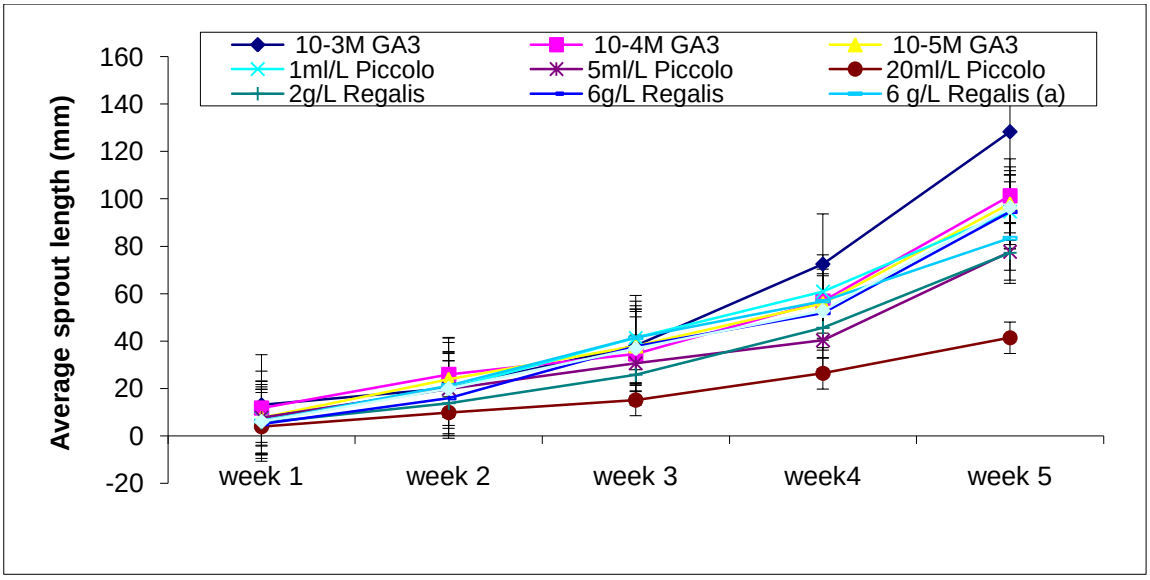


Figure 3-1 Effect of gibberellins and gibberellin synthesis inhibitors on average sprout length (mm) per root of sweetpotatoes. Each data point is the mean of four replicates each of which is four roots. Error bars indicate standard error of means

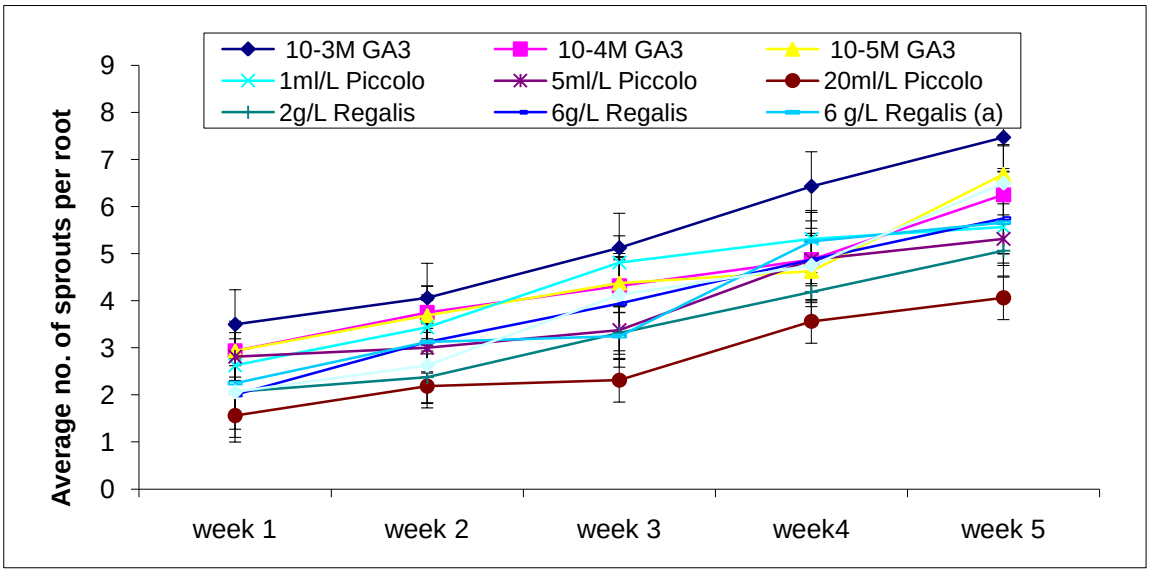


Figure 3-2 Effect of gibberellins and gibberellin synthesis inhibitors on no. of sprouts per root of sweetpotatoes. Each data point is the mean of four replicates each of with four roots. Error bars indicate standard error of means

Table 3-3 Overall Mean values of sprout growth (mm) and average no of sprouts per root with statistical analysis of repeated measurement

Treatments	Average sprout growth per root (mm)	Average no of sprouts per root
10^{-3}M GA_3	55.6	5.32
10^{-4}M GA_3	46.5	4.42
10^{-5}M GA_3	44.0	4.46
1 ml/L Piccolo	44.8	4.35
5 ml/LPiccolo	35.2	3.87
20 ml/L Piccolo	19.3	2.73
2 g/L Regalis	33.5	3.40
6 g/L Regalis	43.0	3.93
6 g/L Regalis after 7 days of sprout initiation	42.8	3.88
Control	42.4	4.01
Treatment effect *p*	0.001	0.009
L.S.D (0.05)	12.4	1.17

Table 3-4 Effect of gibberellins and gibberellin synthesis inhibitors on sweetpotato weight compared to initial weight (%) as influenced by different treatments. Mean values with results of statistical analysis of repeated measurements.

Treatments	Weight (%) of initial weight
10^{-3}M GA_3	96.79
10^{-4}M GA_3	97.00
10^{-5}M GA_3	97.42
1 ml/L Piccolo	97.08
5ml/LPiccolo	96.92
20ml/L Piccolo	97.37
2 g/L Regalis	96.82
6 g /L Regalis	96.94
6g/L Regalis after 7 days of sprout initiation	96.99
Control	97.26
L.S.D treatments (0.05)	0.70
P value treatments	0.610

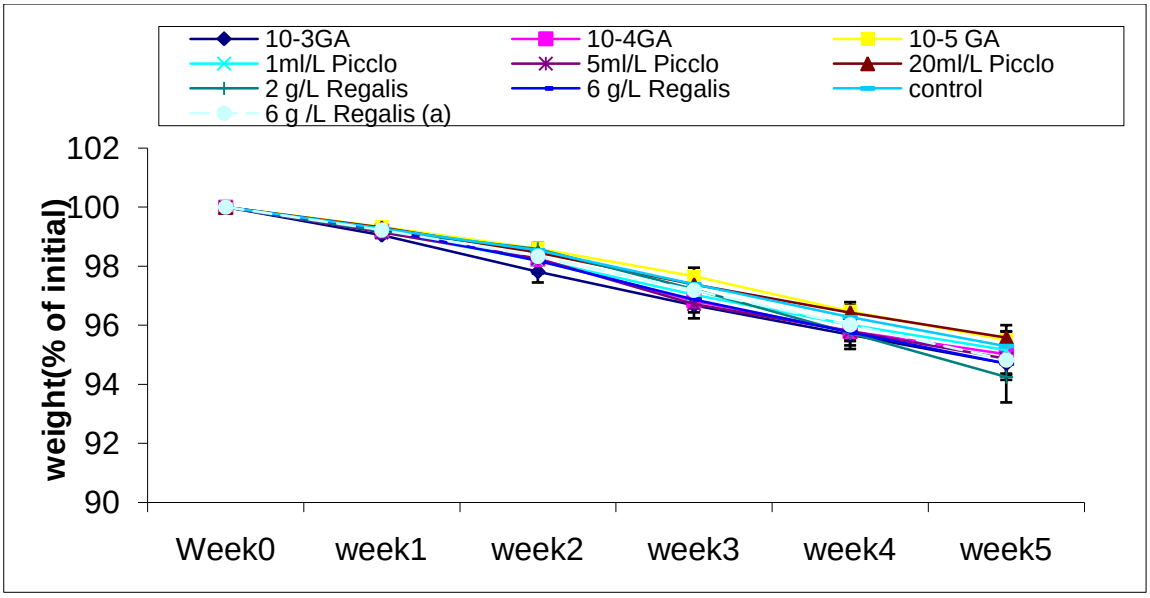


Figure 3-3 Effect of gibberellins and gibberellin synthesis inhibitors on sweetpotato initial weight (%) as influenced by different treatments. Each data point is the mean of four replicates each of with four roots. Error bars indicate standard errors.

3.5 Summary of main findings

In sweetpotato roots:

- Gibberellins (10^{-3}M GA_3) increases rate of sprout growth and number of sprouts.
- Piccolo (20 ml/L), an inhibitor of GA synthesis slows sprout growth and reduces number of sprouts.
- Regalis® shows no effect on sprout growth or number of sprouts.
- There is no indication of an effect on timing of sprout growth (dormancy). Roots for all treatments had sprouts after 1 week.

3.6 Conclusions

It has been observed in this study that sprouting of sweetpotato roots is controlled by the relative concentrations of growth promoters and inhibitors. Gibberellins (GA) are considered as growth promoters. Under the assumptions that GA would promote sprout growth, as it does in potatoes (Coleman *et al.,* 1987), accelerate growth was expected in GA treated sweetpotato roots. GA_3 treatments resulted in more sprout growth and a higher number of sprouts. It is in support with the results in potato where gibberellins are responsible for cell elongation associated with assimilate flow towards growing meristems (Low, 1975). In potatoes gibberellins are known to stimulate the synthesis of reducing sugars (Claassens and Vreugdenhil, 2000). It is suggested that gibberellins stimulate the synthesis of reducing sugars, and initiate the completion of the cell cycle and thus initiate growth. It has been observed that higher concentration of GA_3 had a greater stimulatory effect on sprout growth. In GA treated roots increase in growth rate can be associated to the increase in cells able to complete the cell cycle.

As it was assumed that GA synthesis inhibitors could inhibit sprout growth as in other crops. Paclobutrazole has been shown to extend dormancy in potato tubers (Tekalign and Hammes, 2005). Hence, in sweetpotato GA treated roots showed more sprout growth as compared to those treated with GA synthesis inhibitors. Piccolo had an inhibitory effect on sprout growth. This is possibly due to insufficient reducing sugars to start the cell growth as endogenous GA could be involved in stimulating synthesis of reducing sugars as well as DNA and RNA synthesis. In potatoes, Suttle (2004b) observed that endogenous GA content had a stronger effect on the sprout growth than on the termination of dormancy. Both Hemberg (1985) and Van Ittersum and Scholte (1993) observed that the endogenous GA concentration increased before dormancy was broken. The concentration increases even more once sprouting commenced (Suttle, 1996). Coleman (1987) reported that GA increased the synthesis of reducing sugars, but only after the

storage tissues were no longer dormant. Our study showed that sprout growth significantly decreased under the influence of 20 ml/L Piccolo. It clearly suggests that the effect of PBZ was functional in sweetpotatoes and the sprout growth was reduced as bioactive gibberellins synthesis could be blocked by Piccolo. Regalis, a GA synthesis inhibitor did not show a significant inhibitory effect. It was assumed that Regalis cannot be absorbed by the roots through skin directly. It has been used on foliage in apples where the epidermis contains many stomata, but apparently does not easily pass through the sweetpotato periderm. Regalis was used after 7 days of sprout initiation to see if the effect was greater where there were new fast growing tissues that might absorb the chemical more effectively. However, again there was no effect, possibly because the area of growing tissue was very small at the time of application.

The study suggests that higher concentrations of GA and GA synthesis inhibitors are involved in the sprout stimulation in the sweetpotato. Further studies on foliar application of growth hormones and hormones biosynthesis inhibitors before harvesting of roots could be of value. The suggestion is that growth hormone inhibitors or their analogues might provide safe sprout suppressants for sweetpotatoes. So alternative techniques such as plant growth regulators with low mammalian toxicity make their use advisable for sweetpotato roots.

Piccolo was not sufficiently effective to be used as a commercial sprout suppressant. Generally, ≤3 mm sprout length is considered to be good control during storage and piccolo did not maintain that level of control. The efficacy of Piccolo in sprout control is likely to be associated with its efficacy of GA inhibition. It would have been nice to know what levels of GA were present in the inhibitor treated roots.

4 Effect of ethylene on sprouting of sweetpotatoes in storage

4.1 Introduction

Ethylene is a plant hormone that has a wide range of physiological effects depending on the state of the plant tissues that it is acting on. Ethylene is a well known growth regulator but its role is unclear in many plant organs.

Elmer *et al*. (1932) observed that gases from ripe apples and pears had inhibiting effect on potato sprouts, and ripe fruits must be producing ethylene. There are varied reports of the effect of ethylene on potato sprouting. Continuous application of ethylene during storage has been shown to reduce sprout growth (Metlitski *et al*., 1982), although it has been concluded by Rylski *et al*. (1974) that ethylene decrease the dormant period but inhibit sprout growth, a conclusion consistent with an increase in respiration rate when ethylene was applied to dormant potato tubers (Micheal *et al*., 1990). Many studies showed ethylene as an inhibitor of sprouting in potatoes (Hughes *et al*., 1974). It has also been observed that sprout growth in onion was inhibited by exogenous ethylene by effecting leaf blade elongation (Bufler, 2009).

Despite some of the undesired effects of ethylene as a sprout control agent such as effects on sugar levels, ethylene has been used in potato stores in the UK on a commercial scale since 2001 Ethylene has also been registered as a sprout suppressant for processing tubers in Canada (Prange *et al*., 2005). There is no maximum application Level for ethylene, and crops treated with it are safe for health. For details see chapter 2, ethylene section 2.1.5.5.

As explained in pervious chapters, sweetpotatoes generally do not store well, except under ideal conditions (See section 2.2.2). In sweetpotatoes, sprouting is generally controlled by manipulating the temperature and humidity under which the crop is stored (Picha, 1986). Control of sprout production in sweetpotatoes has been examined using a number of treatments including, CIPC, gamma irradiation naphthalene acetic acid where all suppressed sprouting to some degree (For details see chapter 2, section 2.2.3). Sprout control in sweet potatoes is less studied as compared to potato tubers. New improved, cost effective technologies for sprout suppression in root crops would be useful for successful storage.

4.2 Objectives

The objective of the work described here was to determine whether ethylene would inhibit sprouting in sweetpotatoes as it does in potatoes. There are no published reports on effect of ethylene of sprouting during storage of sweetpotato roots.

4.3 Materials and Methods

White-fleshed sweetpotatoes cultivar bushbuck was obtained from a sweetpotato importer. The trial was conducted at the Natural Resources Institute, UK in August 2007 and 2008. The storage temperature was maintained at 25°C within incubators.

4.3.1 Trial to determine the effect of ethylene on sprout growth and number of sprouts (2007)

Ethylene was applied continuously throughout the entire storage trial period using a flow-through system with 3 L glass jars. Each treatment was applied to 4 jars, each of which contained three roots. Ethylene from a gas cylinder containing 5000 ppm ethylene in nitrogen (23.6 L cylinder, SIP analytical) and air from a compressor (1 L/min for each treatment) were mixed by using adjustable needle valves, and a flow meter to obtain the desired concentrations in the jars. Thus, the ethylene was used at 1, 4 and 20 ml/min flow rate, and the air was supplied at 1 L /min. The following treatments were applied.

4.3.1.1 Treatments in 2007

- Control (air)
- 5 ppm ethylene
- 20 ppm ethylene
- 100 ppm ethylene

The mixture of gases was supplied to the jars via nylon tubing through an airtight seal to the base of the jar where it was bubbled through water to maintain a high humidity (Plate 4-3). In order to avoid a buildup of ethylene in the laboratory, gas was removed from the top of each jar and directed through an exhaust tube to the outside of the building.

Ethylene concentrations were measured using a photovac gas chromatograph (Photovac model 10S50) with a photo ionization detector (Plate 4-1). The 10S50 is a self-contained air analyser. Samples were pumped in for 10 second and then run on column for 1

minute. High purity air was used as the carrier gas and internal temperature was maintained at 25 ˚C. A typical trace obtained is shown in plate 4-2.

Roots were assessed weekly for weight loss and for the presence of sprouting; a sprout was considered as any growth that was longer than 1 mm. length of sprouts was measured by using vernier caliper. The numbers of sprouts per root were noted at each observation.

Plate 4-1 Photovac gas chromatograph (10S50) with a photo ionization detector.

Plate 4-2 A typical trace obtained from the Photovac gas chromatograph (10S50)

Plate 4-3 Sweetpotato roots stored in incubator at 25º C fitted with air flow system (2007)

4.3.2 Trial to optimise ethylene levels for sprout control (2008)

This study was conducted in 2008. Ethylene was applied continuously throughout the entire storage trial period using a flow-through system with 5 L air tight plastic boxes. Each treatment was applied to 4 boxes, each of which contained three roots. Ethylene from a gas cylinder containing 2000 ppm ethylene in nitrogen (23.6 L cylinder, SIP analytical) and air from a compressor (1 L/min for each treatment) were mixed by using the same method as described in 4.3.1. The ethylene was used at 2.5, 5, 10 and 20 ml/min flow rate and the air was supplied at 1 L/min (Plate 4-3).

4.3.2.1 Treatments in 2008

The following treatments were applied.

- Control (air)

- 5 ppm ethylene
- 10 ppm ethylene
- 20 ppm ethylene
- 40 ppm ethylene

The mixture of gases was supplied to the sealed boxes via nylon tubing through an airtight seal to the far end of the box where it was bubbled through water to maintain a high humidity. Gas was removed from the other side of each box and directed through an exhaust tube to the exhaust system of the building.

Ethylene concentrations were measured using a photovac gas chromatograph with a photo ionisation detector (As described in 4.2.3).

Roots were assessed weekly for weight loss and for the presence of sprouting; a sprout was considered as any growth that was longer than 1mm and length was measured same as in 4.2.3. The numbers of sprouts per root were noted at each observation.

Respiration rates were determined by analysing the CO_2 content of air in the boxes by using GC chromatograph Model 93. The Model 93 was set up to measure carbon dioxide (CO_2), Oxygen (O_2) and nitrogen (N_2). GC was fitted with two packed columns. The machine was set up to use a thermal conductivity detector. Helium gas was used as carrier gas, Oven temperature was maintained at 90 ˚C while injector and detector temperature were maintained at 150 ˚C. 1 ml samples were injected into injection port by using gas tight syringe. Sample running time was 12 minutes. CO_2 was measured before sealing the jars and then 60 minutes after sealing the jars. Following equation was used for calculating respiration rate.

Respiration rate % Co_2 (mg)/h/kg= A /100 x (B -C) x1.96x1000/C

A= Difference in CO_2 before sealing and after sealing the boxes

B= Volume of Boxes in ml

C= Weight of sweetpotatoes in grams

The results were analysed using repeated measurement analysis of variance for all treatments and general analysis of variance for each time point.

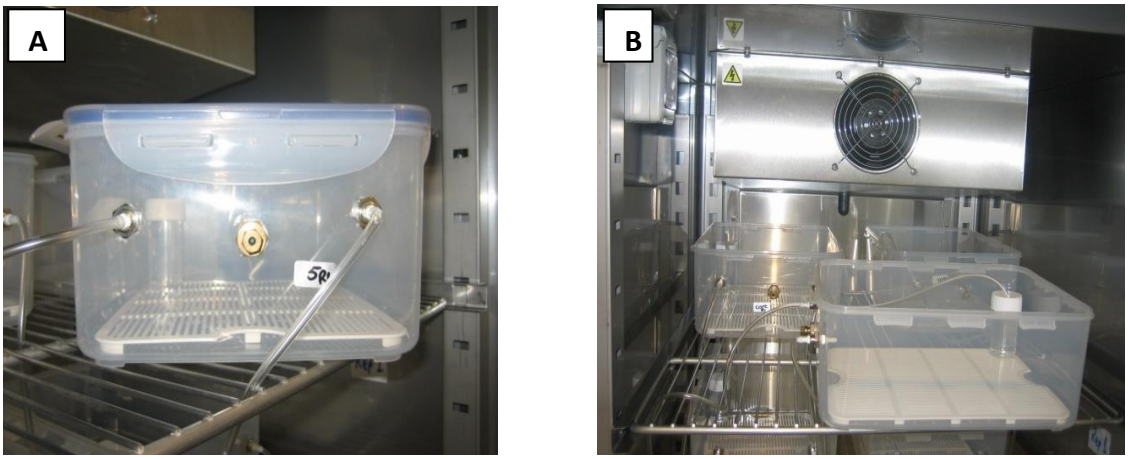


Plate 4-4 Plastic Boxes placed in incubator at 25˚ C fitted with air flow system (2008)

4.4 Results

4.4.1 Effect of ethylene on sprout growth, no of sprouts and weight loss (2007)

The time for sweetpotato roots to develop sprouts increased as the concentration of ethylene in the atmosphere increased (Figure 4-1). Sprouts were first observed on roots from the air control treatment, at 7 -8 days after the start of storage trial, but not until another 7 days later on roots treated with 10, 20 and 40 ppm ethylene in 2007. No sprouts

were observed on roots from 100 ppm ethylene treatment throughout the study in 2007. Air control roots had the most vigorous sprout growth. The number of sprouts was higher in the control than all other treatments.

Table 4-1 summarises the results for the 2007 trial. Ethylene treatments were significantly different from the control, although generally ethylene treatments were not significantly different from each other. Roots treated with 100 ppm were just significantly different from those treated with 5 ppm by the end of the storage period during 2007. The sprout length from the treatments 5 ppm and 20 ppm was likewise small compared to controls over the whole period. The length of longest sprout remained below 2 mm on roots treated with ethylene at all evaluation dates. The maximum sprout length on roots stored in air was longer than on roots from all other treatments, reaching 50 mm.

Figure 4-3 shows that as the ethylene concentration increases the rate of weight loss increases. Table 4.3 illustrated results of ANOVA of repeated measurements. However, no significant losses were found between ethylene treated roots and control. In that case increase in weight loss could be due to higher ethylene effects on respiration rate.

Plate 4-5 Sweetpotato roots after 28 days storage in air (control), 5ppm ethylene, 20ppm ethylene and 100ppm ethylene during 2007 (from left to right)

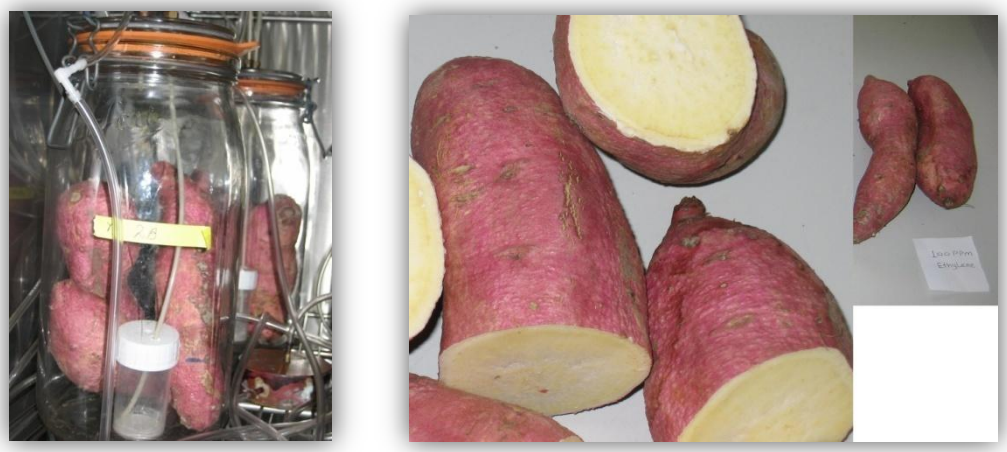


Plate 4-6 Sweetpotato roots after 28 days storage in 100 ppm ethylene (2007).

Table 4-1 Effect of ethylene on average sprout growth (mm) and number of sprouts per root of sweetpotatoes stored at 25º C after 1, 2, 3 and 4 weeks during 2007. Each data point is the mean of four replicates each consisting of three roots

Treatments	1st week		2nd week		3rd week		4th week	
	Sprout length (mm)	No. of sprouts	Sprout length (mm)	No. of sprouts	Sprout length (mm)	No. of sprouts	Sprout length (mm)	No. of sprouts
Control	1.75	1.50	2.81	3.08	6.73	5.17	15.59	6.33
5 ppm	0.00	0.00	1.00	1.33	2.33	1.58	2.85	1.75
20 ppm	0.00	0.00	0.25	0.25	0.45	0.42	1.04	0.67
100 ppm	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Treatment effect *P*	0.001	0.001	0.001	0.002	0.001	0.001	0.001	0.001
L.S.D (0.05)	0.76	0.46	0.759	1.403	2.30	1.297	4.49	1.64

Table 4-2 Mean values of sprout growth (mm) and average no of sprout with results of statistical analysis of repeated measurements in 2007

Average sprout growth (mm)				Average no of sprouts per root			
Control	5 ppm	20 ppm	100 ppm	Control	5 ppm	20 ppm	100 ppm
6.72	1.52	0.43	0.00	4.02	1.16	.33	0.00
L.S.D (0.05,Treatments)		1.92		L.S.D (0.05,Treatments)		1.21	
P value (Treatments)		0.001		*P* value (Treatments)		0.01	

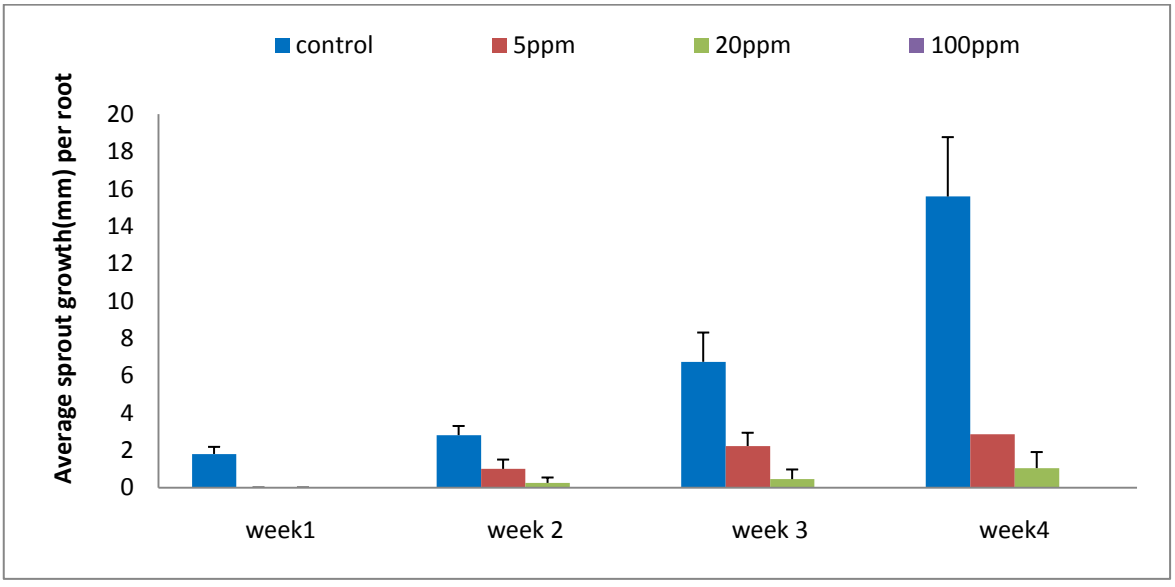


Figure 4-1 Effect of ethylene on average sprout growth (mm) of sweetpotato roots stored at 25ºC after 1, 2, 3 and 4 weeks in 2007. Each data point is the mean of four replicates each consisting of three roots. Error bars indicate standard errors of the means.

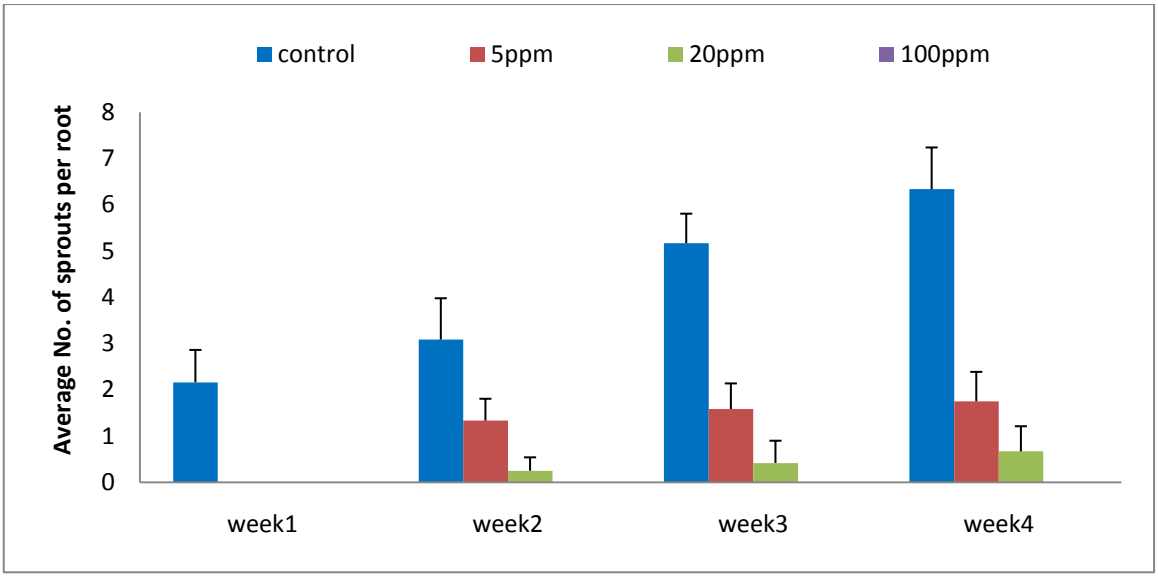


Figure 4-2 Effect of ethylene on average no. of sprouts per root after 1, 2, 3 and 4 weeks in 2007. Each data point is the mean of four replicates each consisting of three roots. . Error bars indicate standard errors of the means

Table 4-3 Mean values of % weight loss with results of statistical analysis of repeated measurements in 2007

Weight loss (% of initial)			
Control	5 ppm	20 ppm	100 ppm
97.84	97.62	97.27	96.88
L.S.D (0.05, Treatments)		1.27	
P value (Treatments)		0.396	

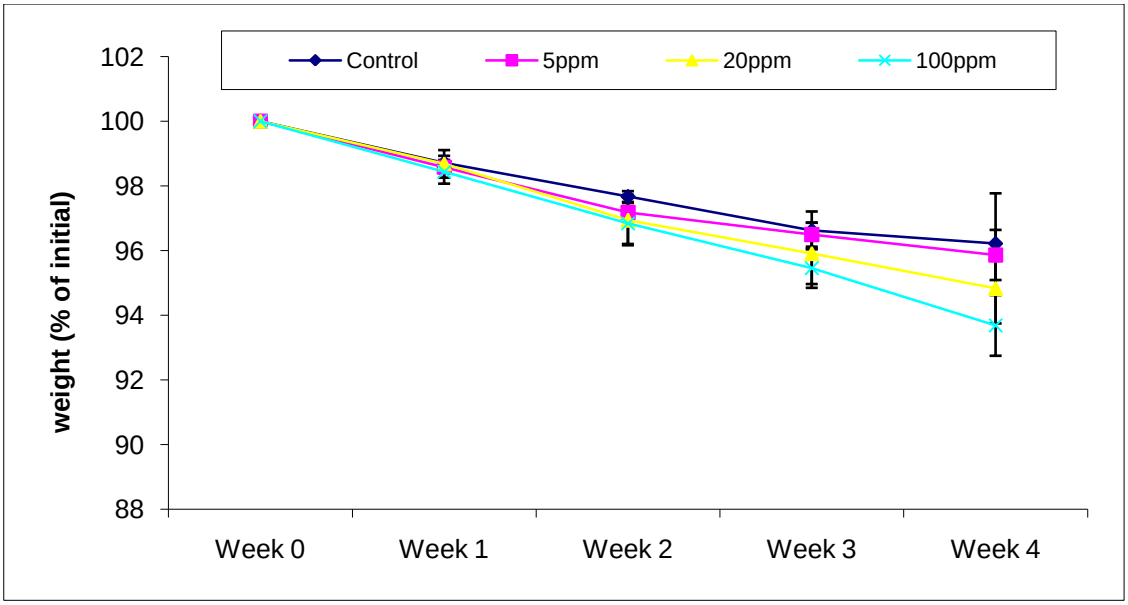


Figure 4-3 Effect of ethylene on % weight loss of sweetpotato roots after 1, 2, 3 and 4 weeks in 2007. Each data point is the mean of four replicates each consisting of three roots. Error bars indicate standard errors of the means.

4.4.2 Optimization of ethylene levels (2008)

Previously in the 2007 study it has been seen that ethylene can control sprouting in sweet potatoes therefore it was decided to optimize the ethylene levels in 2008 for future studies.

During 2008, a narrower range of ethylene levels was used to determine optimal ethylene levels for control of sprouting, and quality maintenance (Table 4-4 and Table 4-5). Ethylene at all concentrations, tested inhibited the sprout growth. There was no significant difference between different ethylene treatments throughout the trial. Sprout initiation in higher concentrations of ethylene was typically delayed longer than controls or lower concentration (5ppm). Sprout growth was inhibited in all ethylene concentrations. 10 ppm was considered most effective as in that treatment sprouts appeared 7 days later than in

5 ppm treatment. So it was assumed that 10 ppm would be more effective and economical than higher concentrations of ethylene. As for the 2008 trial, Figure 4-6 shows higher weight loss for roots stored at higher ethylene concentrations compared to controls. Higher weight loss in ethylene treated roots is associated with higher respiration rates. This is supported by Figure 4.7 which indicates that ethylene induces a higher respiration rate in roots. In 5 and 10 ppm, the respiration rate pattern was almost the same throughout the study except a higher respiration rate was noticed in 5 ppm treated roots after 4 weeks of storage. 20 and 40 ppm treatments showed higher respiration than 5, and 10 ppm treatments, in comparison with air where respiration rate was significantly lower than all ethylene treatments with no statistically difference in treated roots.

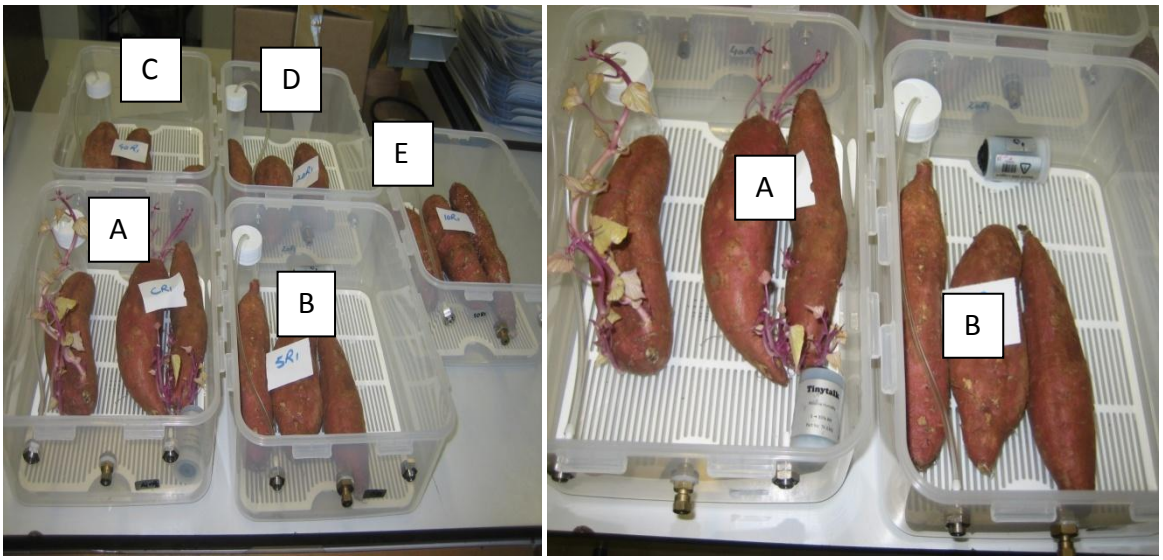


Plate 4-7 Sweetpotato roots after 28 days storage in air (control) A, 5ppm (B) ethylene, 10ppm (C), 20ppm (D) and 40ppm ethylene (E) (2008).

Table 4-4 Effect of ethylene on average sprout growth (mm) and number of sprouts per root of sweetpotatoes stored at 25º C during 1, 2, 3 and 4 weeks during 2008. Each data point is the mean of four replicates each consisting of three roots.

Treatments	1st week		2nd week		3rd week		4thweek	
	Sprout length (mm)	No. of sprouts	Sprout length (mm)	No. of sprouts	Sprout length (mm)	No. of sprouts	Sprout length (mm)	No. of sprouts
40 ppm	0	0	0	0	0.50	0.08	0.50	0.08
20 ppm	0	0	0	0	0.50	0.08	0.60	0.08
10 ppm	0	0	0	0	0.45	0.16	0.60	0.17
5 ppm	0.31	0.16	0.40	0.25	1.37	0.49	1.70	0.50
Control	3.47	2.24	14.04	3.17	17.74	4.91	32.80	7.66
Treatment effect *P*	.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
L.S.D	0.95	0.60	1.45	0.68	4.47	0.63	9.85	1.65

Table 4-5 means values of sprout growth (mm) and average no of sprout with results of statistical analysis of repeated measurements in 2008

Average sprout growth (mm)					Average no of sprouts per root				
Control	5ppm	10ppm	20ppm	40ppm	Control	5ppm	10ppm	20ppm	40ppm
17.1	1.01	0.25	0.26	0.25	4.50	0.354	0.08	0.04	0.04
L.S.D (0.05,Treatments)		3.84			L.S.D (0.05,Treatments)		0.85		
*P*value (Treatments)		0.001			*P*value (Treatments)		0.001		

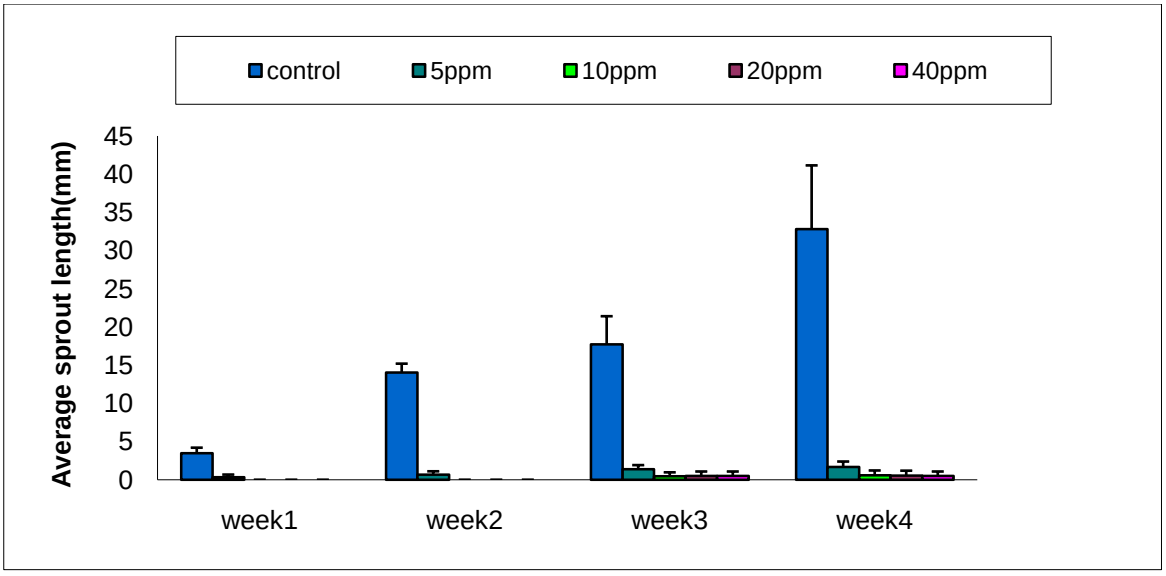


Figure 4-4 Effect of ethylene on average sprout growth (mm) of sweetpotato roots stored at 25ºC after 1, 2, 3 and 4 weeks in 2008. Each data point is the mean of four replicates each consisting of three roots. Error bars indicate standard errors.

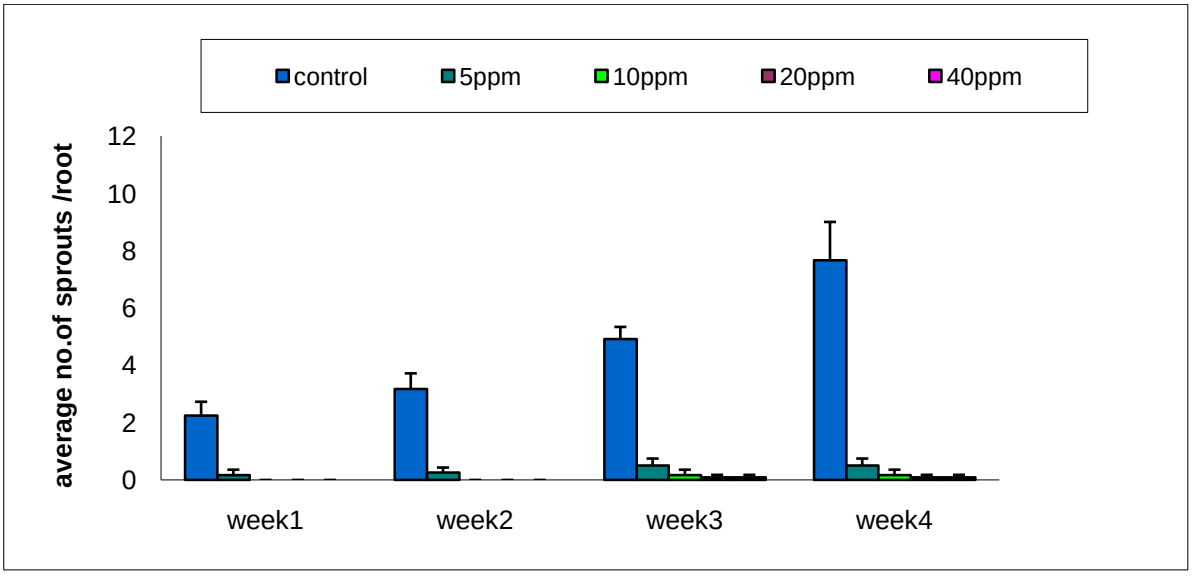


Figure 4-5 Effect of ethylene on average no. of sprouts per root after 1, 2, 3 and 4 weeks in 2008. Each data point is the mean of four replicates each consisting of three roots. Error bars indicate standard errors.

Table 4-6 Means values of % weight loss with results of statistical analysis of repeated measurements in 2008

Weight loss (% of initial)				
Control	5 ppm	10 ppm	20 ppm	40 ppm
96.52	96.06	95.54	95.51	95.08
L.S.D (0.05,Treatments)		1.34		
P value (0.05,Treatments)		0.229		

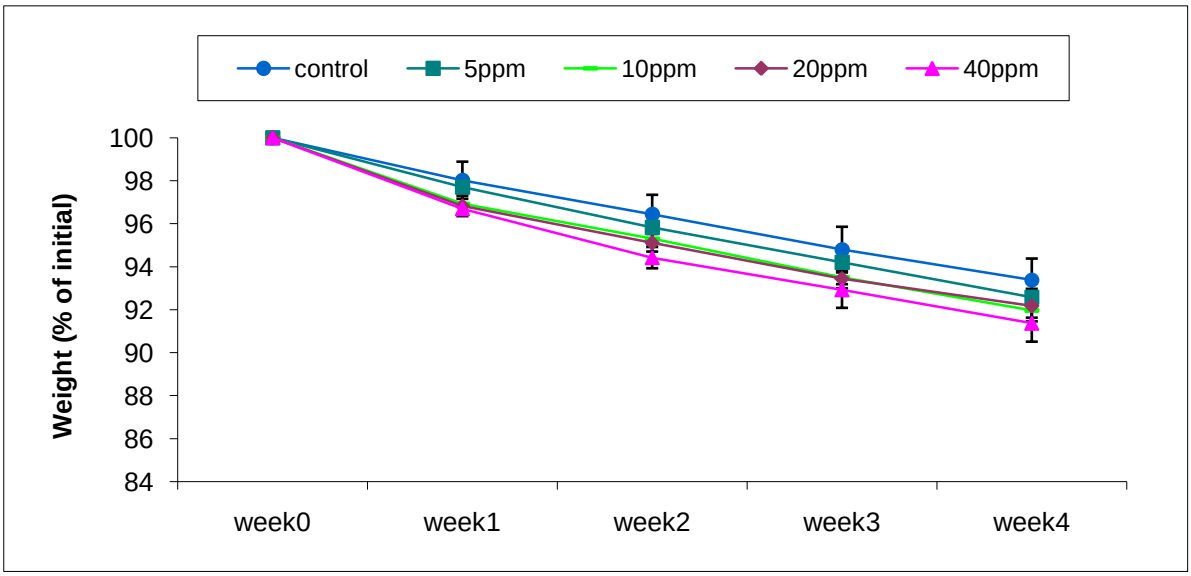


Figure 4-6 Effect of ethylene on % weight loss of sweetpotato roots after 1, 2, 3 and 4 weeks in 2008. Each data point is the mean of four replicates each consisting of three roots. Error bars indicate standard errors of the means.

Table 4-7 Means values of respiration rate CO_2 (mg)/h/kg with results of statistical analysis of repeated measurements in 2008.

Respiration rate CO_2 (mg)/h/kg				
Control	5 ppm	10 ppm	20 ppm	40 ppm
25.1	69.4	68.4	70.5	70.5
L.S.D (0.05, Treatments)		9.59		
P value (Treatments)		0.001		

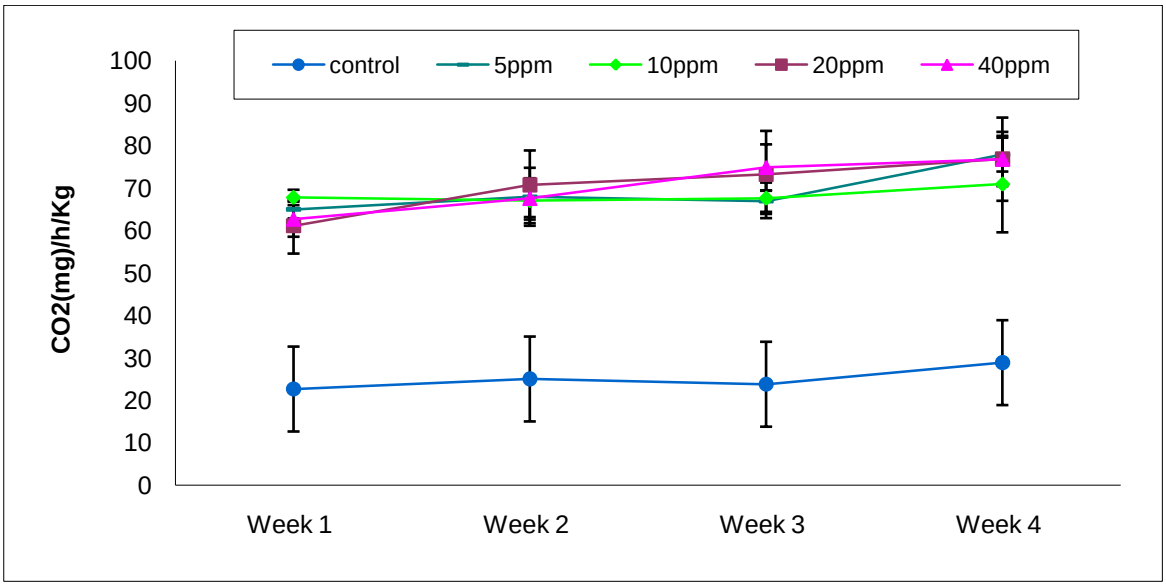


Figure 4-7 Effect of ethylene on respiration of sweetpotato roots after 1, 2, 3 and 4 weeks 1n 2008. Each data point is the mean of four replicates each consisting of three roots. Error bars indicate standard errors.

4.5 Summary of main findings

In sweetpotato roots:

- Continuous exposure to ethylene inhibits sprout number and growth rate in a concentration dependent manner, saturates in the range 10 – 40 ppm.

- Ethylene delays sprout growth.

- There are indications that ethylene increases slightly rate of fresh weight loss during storage.

- Ethylene increases root respiration rate three-fold. The effect saturates at 5 ppm, and there is no additional effect over the range 5 – 40 ppm

4.6 Discussion

The role of ethylene in potato tuber dormancy has been studied much more extensively than in sweetpotatoes. In potato tubers exogenous ethylene has been observed to either extend (Cvikrovai *et al.,* 1994) or terminate (Rylski *et al*., 1974) dormancy. It has been observed in potatoes that continuous ethylene inhibits sprout growth (Prange *et al*., 2005) and this is used commercially in potato industry.

Ethylene inhibition of sprout growth in sweetpotato has not been reported before.

Treatments with ethylene resulted in sprout growth inhibition and delays in sprout initiation at all concentrations tested. Time of appearance of sprouts was related to the concentration of ethylene used. All concentrations tested inhibited sprouting; 10ppm was considered most effective for further studies because it would be more cost effective than

higher concentrations and will be more effective than lower concentrations as there were more sprouts seen in 5 ppm treated roots in 2008.

Even though the differences were not statistically significant in terms of weight loss in both trials. Weight loss was greater in higher ethylene concentrations, possibly associated with higher respiration rates.

Respiration rate was not measured during the 2007 trial. During the 2008 trial, ethylene increased the respiration rate but this effect was saturated at the lowest ethylene level (5 ppm). The respiration remained low throughout the study in sprouting controls. Generally sprouting is associated with mobilization of sugars and an increase in respiration to provide energy for growth, but the opposite is seen. Given that sprouting would usually be expected to increase respiration, and the ethylene concentration effects are different, it can be concluded that the ethylene effect on sprouting and respiration are independent. This suggests that sprouting/ termination of dormancy is not associated with respiration in sweetpotato roots.

Increased respiration is usually associated with breakdown of starch into sugars. This is consistent with the observation that ethylene increases tuber respiration rate and accelerates the conversion of starch to sugars (Huelin and Barker, 1939; Haard, 1971; Reid and Pratt, 1972; Isherwood 1973; Day *et al.,* 1978; Schwobe and Parkin, 1990; Prange *et al.,* 1998).

This study suggests that ethylene increases the respiration rate but this effect is saturated at the lowest ethylene levels. There were dose dependent responses to ethylene for different aspects of sprouting in potato tubers and that “saturation” concentrations varied according to characteristic (Daniels-Lake, personal communication).

It is clear that sprout initiation was delayed by ethylene in this study. Sprout growth was also inhibited even when sprouts had appeared at low ethylene concentrations (5 ppm, 10 ppm). This is in support with the observation that in potato tubers, continuous ethylene exposure inhibits sprout growth (Furlong 1948; Rylski *et al*., 1974).

However data from this study suggest that ethylene is an inhibitor of sprout growth in sweetpotato roots. Further studies to investigate the effect of ethylene on quality and understand the mode of action in sweetpotato dormancy is necessary as it is increasingly apparent that ethylene plays a vital role in sweetpotato sprout control.

5 Effect of ethylene, an ethylene antagonist and an ethylene synthesis inhibitor on sprouting of sweetpotatoes in storage

5.1 Background

In the previous study (see chapter 4) it has been shown that continuous ethylene application (10 ppm) can control sprouting in sweetpotatoes effectively.

The working hypothesis in this thesis is that sweetpotato roots do not exhibit dormancy, and that ethylene is working by inhibiting sprout growth. In the case of potatoes, initially it was believed that continuous ethylene extended dormancy (Prange *et al.,* 1998), but more recent studies have indicated that ethylene inhibits sprout growth rather than extending dormancy (Prange *et al.,* 2005).

There are several ways in which ethylene synthesis and/or action can be inhibited, and these have all been used as tools to investigate how ethylene works

Silver nitrate to inhibit ethylene action

Silver nitrate ($AgNO_{3)}$ is capable of blocking the ethylene effect in plants. The ethylene receptor, ETR1, contains one ethylene-binding site per homodimer and binding is mediated by a single copper ion (Cu) present in the ethylene-binding site. The replacement of the copper co-factor by silver serves to lock the receptor (Zhao *et al*., 2002).

Another study suggested that $AgNO_3$ inhibits ethylene action by means of silver ions by reducing the receptor capacity to bind ethylene (Yang, 1985), which would result in higher

concentrations of ethylene in the tissues, thus inhibiting the earlier steps of its own pathway.

1-MCP to inhibit ethylene action

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

The effects of 1- MCP are very widespread. 1-MCP has been observed to reduce ethylene induced effects including senescence in a variety of potted flowering plants and cut flowers (Serek *et al*., 1995a, 1996; Sisler et *al,*. 1996a; Heyes and Johnson, 1998; Newman *et al*,. 1998).

Effects of 1-MCP on fruit and vegetables include inhibiting the ripening of apples (Fan and Mattheis, 1999a; Watkins *et al*., 2000; DeEll *et al.,* 2002), pears (Wild *et al*., 1999; Baritelle *et al*., 2001), stone fruit (Blankenship and Parker, 2001), bananas (Harris *et al*., 2000) and other tropical fruits (Ergum and Huber, 2001; Selvarajah *et al*., 2001), tomatoes (Canoles and Beaudry, 2001; Rohwer and Gladon, 2001), browning of broccoli (Ku and Wills, 1999) and the degreening of oranges (Porat *et al*., 1999). It also delays senescence of strawberries (Ku *et al*., 1999) which suggest that ethylene has effects on the regulation of ripening process in strawberries. 1-MCP is used commercially, especially during apple storage, in which case it can extend storage life as well as preventing some physiological disorders. For example, application of 1-MCP immediately after harvest greatly reduces superficial scald development in apples (Fan and Mattheis, 1999b; Calvo and Candan, 2001; Solomos e*t al*., 2001).

AVG as an ethylene synthesis inhibitor

The enzyme 1-aminocyclopropane-1-carboxylase (ACS) synthesises 1-aminocyclopropane-1-carboxylic acid (ACC), which, in turn, is converted to ethylene by ACC oxidase (ACO). CO_2, iron and ascorbic acid are key co factors for ACO. ACS is considered to be the rate-limiting enzyme in the biosynthesis of ethylene (Mathooko *et al*., 2001). Details can be seen in ethylene biosynthesis section in literature review (Chapter 2).

1- aminoethoxyvinylglycine (AVG) is a plant growth regulator -active ingredient in ReTain® that inhibits ACS (Boller *et al.*, 1979). AVG is known to be a general inhibitor of pyridoxal phosphate-linked enzymes. These enzymes use pyridoxal phosphate as a co factor (Yang and Hofmann, 1984). AVG inhibits by competing with binding of pyridoxal phosphate to the enzyme active site. AVG has been used to study the participation of ethylene synthesis in bud break in *in vitro*-grown shoots of *Hancornia speciosa* (a tropical fruit tree) (Pereira-Netto, 2001), Likewise, no increase in the multiplication rate was associated with AVG.

Like many other inhibitors, AVG may affect more metabolic pathways than those attributed to its mode of action (i.e., inhibition of ethylene biosynthesis).

It would have been useful to use $AgNO_3$ but in this study it was decided to use one ethylene antagonist (1-MCP) and one ethylene synthesis inhibitor (AVG) to learn more about the ethylene control of sprouting in sweetpotato.

5.2 Objectives

The objective of the work described in this chapter is to investigate the role of ethylene in control of sprouting in sweetpotato roots by observing the effect of, AVG (ethylene synthesis inhibitor) and 1-MCP (ethylene antagonist) on root sprouting in the presence and absence of exogenous ethylene. The effect of ethylene, 1-MCP and AVG on sugar accumulation was also investigated as this is important for root quality, as well as providing additional information on the control of sprouting.

5.3 Materials and Methods

White fleshed sweetpotatoes cultivar Bushbuck and Ibees were obtained from a sweetpotato importer in March and August 2009 respectively. The trials were conducted at the Natural Resources Institute UK.

The storage temperature was maintained at 25° C by using an incubator. The following treatments were applied.

- Air (Control)
- AVG + Air
- 1MCP + Air
- Ethylene (10ppm)
- AVG + Ethylene (10 ppm)
- 1MCP + Ethylene (10 ppm)

Ethylene (10 ppm) was applied continuously throughout the storage trial period using a flow-through system with 5.5 L plastic boxes. Each treatment was applied to 4 boxes, each of which contained three roots. 2000 ppm ethylene (5 ml/min) from a compressed gas cylinder (23.6 L cylinder 100 bar, SIP analytical) and air (1 L/min) from a compressor were mixed by using adjustable needle valves, and a flow meter to obtain the desired concentrations in the boxes (Plate 5-1).

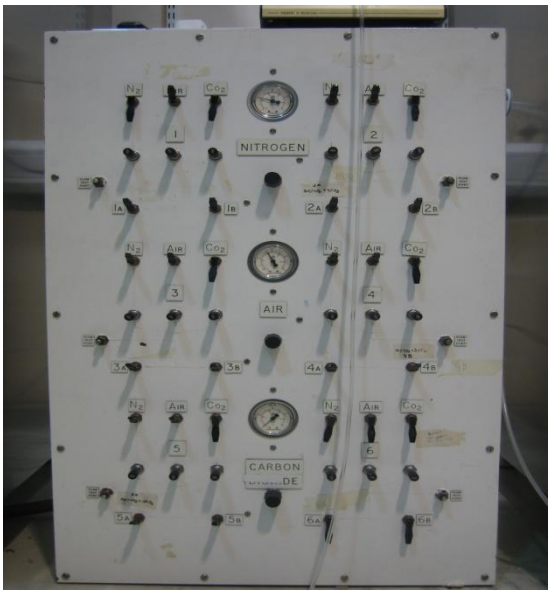


Plate 5-1 The valve assembly box to mix ethylene and air to obtain desired concentrations

The mixture of gases was supplied to the boxes via nylon tubing through an airtight seal to the bottom end of the box where it was bubbled through water to maintain a high humidity. Gas was exhausted to the outside of the building (Plate 5-2).

Plate 5-2 Boxes fitted with inlet and outlet and sampling port

For AVG treatment, roots were dipped for an hour in a 1000 ppm solution of AVG with no additional wetting. AVG, commercially known as Retain was provided by Valent Biosciences Corporation USA. After an hour sweetpotato roots were dried on paper towel before storing in boxes.

1-MCP commercially known as Smartfresh™ was obtained from Landseer Ltd. Roots were treated with 1-MCP (625ppb) for 24 hours at room temperature before they were stored with and without ethylene for 4 weeks. One tablet was put in a small plastic bottle fitted with small fans to facilitate smooth distribution of volatiles. Roots were kept in a 0.5 m^3 wooden box sealed with plastic sheet and a bottle contained Smartfresh™(Pink Tablet) ,activator tablet containing sodium bicarbonate (Blue Tablet) and activator solution containing citric acid was placed in the sealed area to treat the roots (Plate 5-3). The plastic bottle was fitted with a small fan to enhance the even distribution of 1-MCP during treatment.

Plate 5-3 SmartFresh™ treatment kit and box used to treat sweetpotato roots with 1-MCP

Ethylene concentrations were measured at the box outlets once a week using a Photovac gas chromatograph (GC-PID) with a photoionisation detector to ensure the ethylene was maintained at 10ppm throughout the entire experiment (see chapter 4).

Respiration rate was measured by using gas chromatography once a week. Carbon dioxide concentration was measured before sealing boxes and by taking a sample through the sampling port 60 minutes after sealing the boxes, and this was used to calculate the respiration rate (see chapter 4)

Roots were assessed once weekly for weight loss and for the presence of sprouting; a sprout was considered as any growth that was longer than 1mm. Sprout length was measured by using a vernier calliper. The numbers of sprouts per root were noted at each observation. At the end of the study (5 weeks) sprouts were detached from roots and roots were weighed for weight loss.

5.3.1 Sugar analysis

At the end of each experiment roots were sliced horizontally from the middle and then cut into small pieces (approximately $1cm^3$). Samples of each treatment were put into liquid nitrogen immediately after cutting, stored at -80° C and subsequently were freeze dried before being returned to storage at -80° C. In January 2010 freeze-dried samples were ground and extracted in water (1 g sample in 20 ml water) by shaking for one hour at room temperature (Plate 5-4). The extract was centrifuged for 5 minutes at 12000 rpm in 2ml tubes. The supernatant filtered through a 0.45 µm PTFE syringe filter. 20 µl samples were injected onto an HPLC column (Agilant Zorbax carbohydrate analysis column) maintained at 30°C using 75 % acetonitrile running at 1.5 ml/min as the mobile phase. Sugars were detected using a refractive index detector (Agilent 1200 refractive index detector). Data was analysed by using data system EZChrom 3.3 (Agilent). A typical trace/analysis of ethylene and air treated sample is shown in Plate 5-5.

The method for extracting sugars from freeze-dried sweetpotatoes using water has been compared with ethanol extraction and was found to give concentrations of sucrose, glucose and fructose that were very highly correlated in both methods (Rees *et al*., 1996).

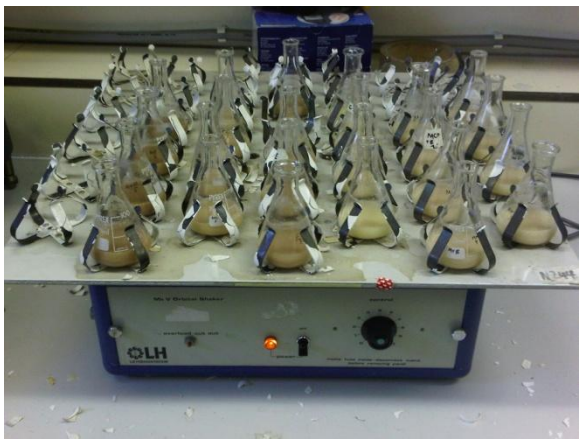


Plate 5-4 Samples during extraction on an Orbital Shaker

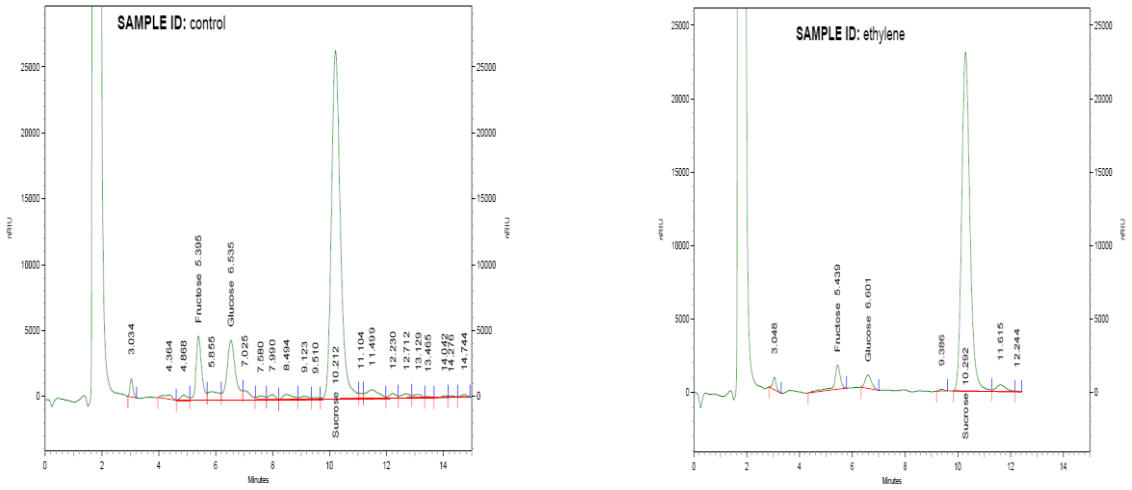


Plate 5-5 Sugar analysis, control (left) and ethylene (right)

5.4 Statistical analysis

The data were analysed using analysis of variance (ANOVA) for each parameter separately. All statistical analyses were performed using GenStat 11th edition for Windows Version 7.0.1.4. Least significant difference values (LSD; $P = 0.05$) were calculated.

5.5 Results

Sprouts were first observed on roots stored in air at approximately 5-7 days after the start of the storage trial, but no sprouts were observed on roots from all other treatments throughout the study except for AVG+Air when assessed in March 2009 (Plate 5-6).

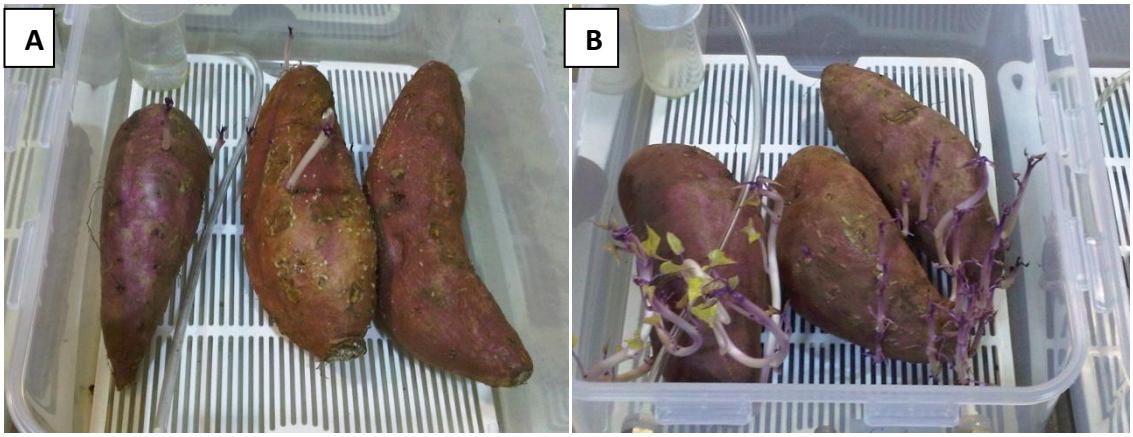


Plate 5-6 AVG+air treated roots (A), Control roots (B)

The results are summarized in Table 5-1 (results of statistical analysis with repeated measurements presented in Tables 5-2 and 5-3), and illustrated in Figures 5-1 and 5-2. All the treatments significantly inhibited sprout growth compared to the control. The sprout length from the treatment AVG+Air was likewise small as compared to controls over the whole period ($P \leq 0.001$). The overall average sprout length remained below 2 mm on roots treated with AVG+Air (Table 5.3). The only treatment other than air for which sprouting was observed was AVG+air. Although, sprouts were noticed on AVG+ Air treated roots, statistically fewer sprouts were reported than the air treated roots and did not increase during the 4 weeks of trial (Table 5-3).

These results were unexpected considering results in potatoes, because as exogenous ethylene inhibits sprout initiation in sweetpotato roots (Chapter 4), it was thought that inhibition of endogenous ethylene production by AVG might increase the sprout growth. Conversely, it was found that AVG did not appear to increase the sprout growth.

In potatoes 1-MCP although an ethylene antagonist does not counteract the ethylene inhibition of sprouting. The same was observed here. However 1-MCP on its own also completely inhibited sprout initiation, which was not expected.

Figure 5-3 and Table 5-4 shows weight loss data. Although the effects were only just statistically significant, weight loss was greatest in the sprouting controls than the other treatments. This could be due to water loss through sprouts. Weight loss was less in 1-MCP+air compared to other treatments after four weeks of storage, although not statistically significant (Figure 5-3).

Figure 5-4 and Table 5-5 shows the respiration rata data. Ethylene treated roots were found to have higher respiration rates compared to air treated roots (Figure 5.4). Neither AVG nor 1-MCP changed this effect. Although AVG would not be expected to affect exogenous ethylene effects, it is interesting that this phenomenon is not sensitive to 1-MCP. The lowest respiration was recorded in 1-MCP treated roots but there was no difference from air treated roots and AVG treat roots.

Table 5.6 illustrates the sugar concentration data. Ethylene on its own has reduced monosaccharide (glucose and fructose) concentrations in roots significantly compared to all other treatments. The difference in sucrose concentration between treatments was just significant. The highest sucrose levels were reported in roots treated with ethylene alone. AVG and 1–MCP with or without ethylene showed higher levels of fructose and glucose than for ethylene treatment alone. Significantly higher fructose and glucose contents were reported in the air control.

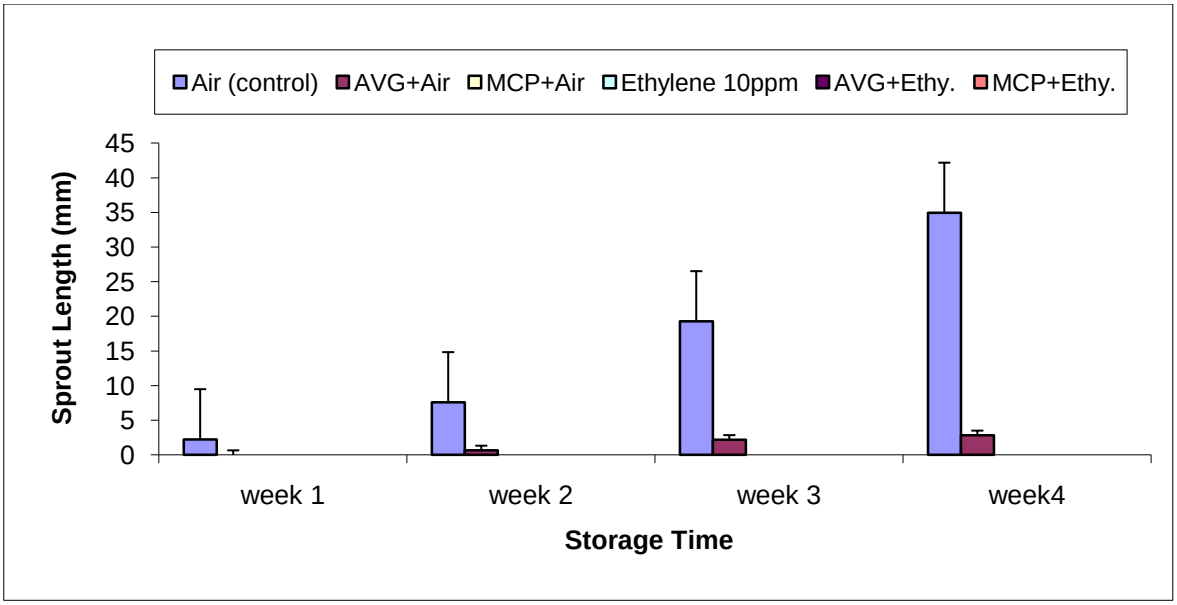


Figure 5-1 Effect of ethylene, AVG and 1-MCP on sprout inhibition of sweet potato roots (Bushbuck) after 1, 2, 3 and 4 weeks. Each data point is the mean of four replicates each consisting of three roots. Error bars are S.E.M

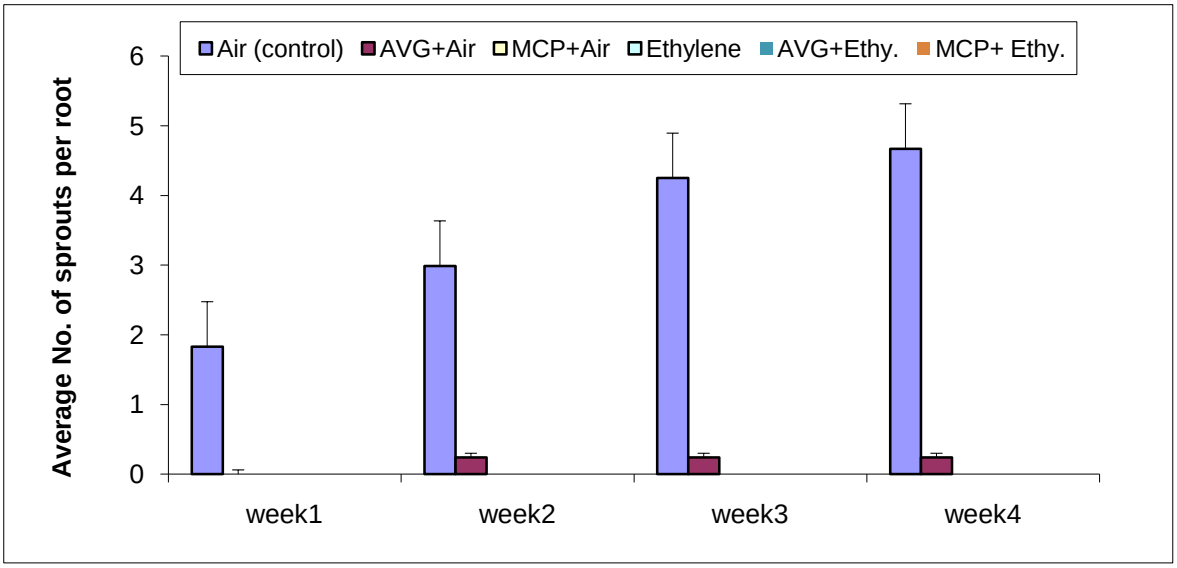


Figure 5-2 Effect of ethylene, AVG and 1-MCP on sprout inhibition of sweet potato roots (Bushbuck) after 1, 2, 3 and 4 weeks. Each data point is the mean of four replicates each consisting of three roots. Error bars are S.E.M

Table 5-1 Effect of ethylene, AVG, and 1-MCP on average sprout growth (mm) and no. of sprouts per root of sweetpotatoes (Bushbuck) stored at 25˚ C after 1, 2, 3 and 4 weeks. Each data point is the mean of four replicates each consisting of three roots.

Treatment	After 1 weeks		After 2 weeks		After 3 weeks		After 4 weeks	
	Average growth (mm)	No. of sprouts/ root	Average growth (mm)	No. of sprouts/ root	Average growth (mm)	No. of sprouts/ root	Average growth (mm)	No. of sprouts/ root
Air (control)	2.25	2.00	7.59	2.91	19.28	4.25	35.69	4.66
AVG+Air	0.00	0.00	0.67	0.25	2.20	0.25	2.85	0.25
MCP+Air	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ethylene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AVG+Ethy.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MCP+Ethy.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Treatment effect *p*	0.001	.001	0.001	0.001	0.001	0.001	0.001	0.001
L.S.D(0.05)	0.33	0.29	0.69	0.36	2.13	0.48	3.15	0.48

Table 5-2 Overall mean values of sprout growth (mm) in cultivar Bushbuck with results of statistical analyses of repeated measurements

Average Growth (mm)					
Air (control)	AVG+air	MCP+air	Ethylene	AVG+Ethy.	MCP+Ethy.
16.20	1.43	0.00	0.00	0.00	0.00
L.S.D (0.05)			1.33		
Treatment effect *p*			0.001		

Table 5-3 Overall mean values of average no. of sprout sin cultivar Bushbuck with results of statistical analyses of repeated measurements

Average number of sprouts					
Air (control)	AVG+air	MCP+air	Ethylene	AVG+Ethy.	MCP+Ethy.
3.45	0.19	0.00	0.00	0.00	0.00
L.S.D (0.05)			0.25		
Treatment effect *p*			0.001		

Table 5-4 Overall mean values of % weight loss in cultivar Bushbuck with results of statistical analyses of repeated measurements

Weight compared to initial (%)					
Air (control)	AVG+air	MCP+air	Ethylene	AVG+Ethy.	MCP+Ethy.
95.062	95.94	96.59	96.17	96.38	96.28
L.S.D (0.05)			0.92		
Treatment effect *p*			0.040		

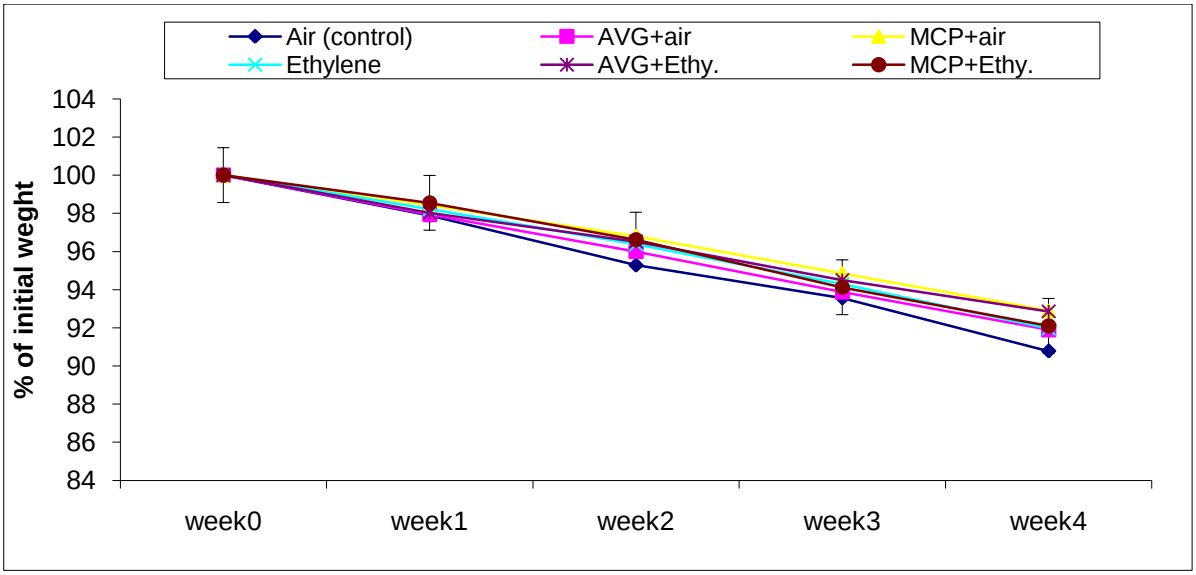


Figure 5-3 Effect of ethylene, AVG and 1-MCP on % weight loss of sweet potato roots (Bushbuck) after 1, 2, 3 and 4 weeks. Each data point is the mean of four replicates each consisting of three roots. Error bars are S.E.M

Table 5-5 Overall mean values of respiration rate in cultivar Bushbuck with results of statistical analyses of repeated measurements

CO2 (mg)/h/kg					
Air (control)	AVG+air	MCP+air	Ethylene	AVG+Ethy.	MCP+Ethy.
53.25	48.21	46.83	64.47	65.46	61.32
L.S.D (0.05)			6.86		
Treatment effect *p*			0.001		

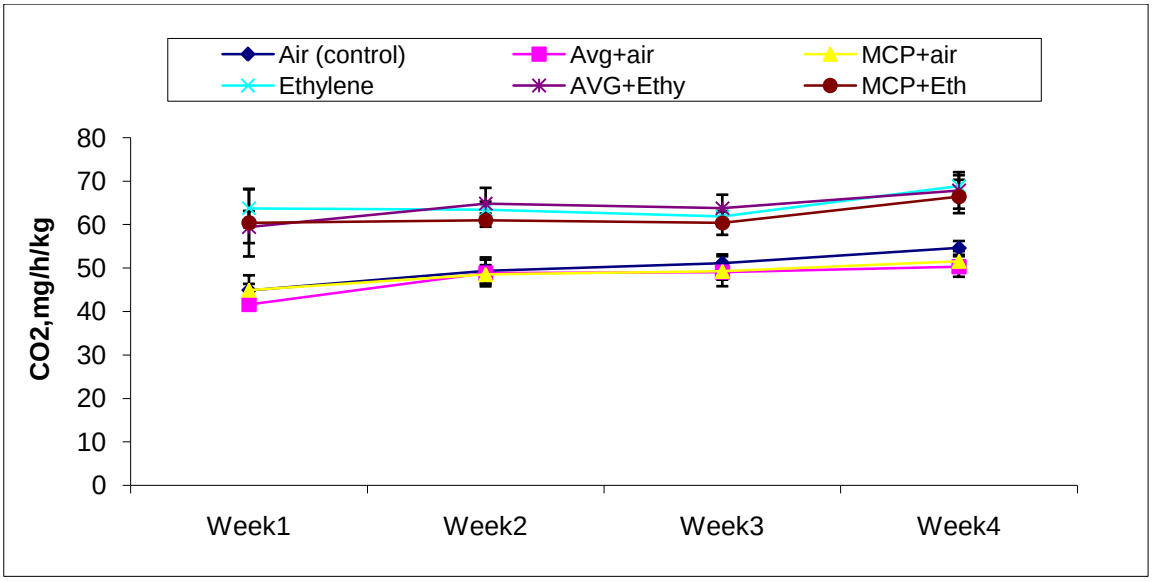


Figure 5-4 Effect of ethylene, AVG and 1-MCP on respiration rate of sweet potato roots (Bushbuck) after 1, 2, 3 and 4 weeks. Each data point is the mean of four replicates each consisting of three roots. Error bars are S.E.M

Table 5-6 Effect of ethylene, AVG, and 1MCP on fructose, glucose and sucrose contents of sweetpotatoes (Bushbuck) stored at 25 C after 1, 2, 3 and 4 weeks. Each data point is the mean of four replicates.

Treatments	Fructose(mg/g)	Glucose (mg/g)	Sucrose (mg/g)
Air (control)	15.83	26.39	136.6
AVG+air	10.95	15.52	136.8
MCP+air	12.03	17.20	133.4
Ethylene	7.32	6.87	147.8
AVG+Ethy.	12.29	13.03	138.1
MCP+Ethy.	12.17	14.12	133.0
L.S.D (0.05)	1.37	3.83	11.84
P value	0.001	0.001	0.014

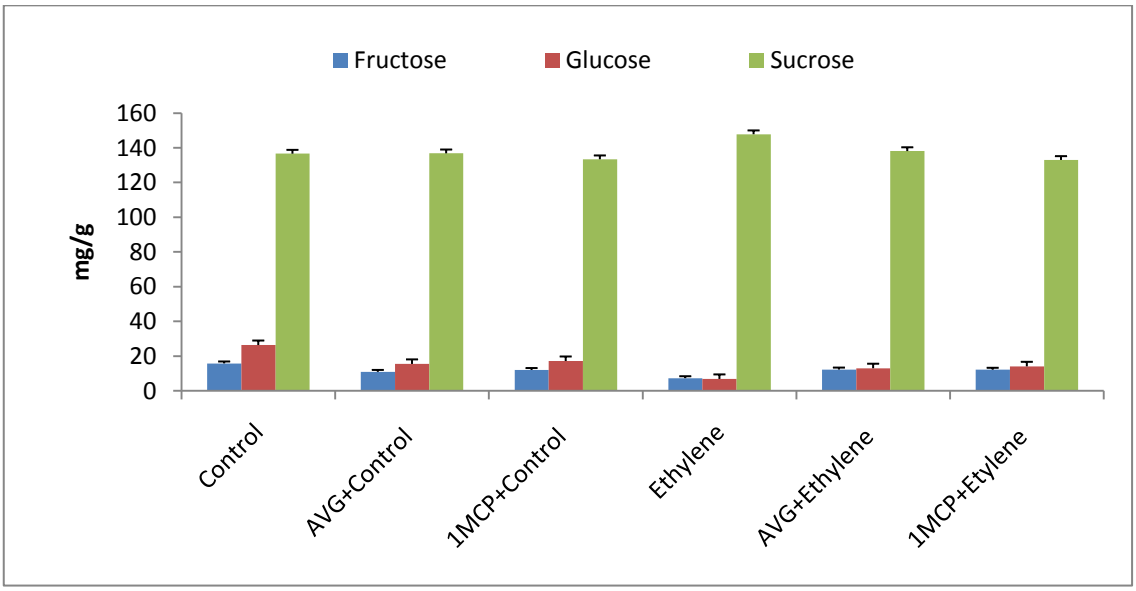


Figure 5-5 Sugar contents in sweetpotato roots (Bushbuck) treated with ethylene, 1 MCP, AVG and Control in March 2009. Each data point is the mean of four replicates. Error bars are S.E.M

The experiment was repeated to reconfirm the results in August 2009. Sprouting was not completely inhibited in 1-MCP and ethylene treated roots as for the first experiment but results of the repeated study in August 2009 were in line with first study. Thus sprouting was much greater in the controls than any other treatment. Although, a different variety was used in the repeated study it behaved similarly to that used in the first study (Figure 5-6 and Figure 5-7)

Weight loss data agrees with first experiment, greatest weight loss was noted for air treated roots and least weight loss was observed for 1-MCP treated roots (Table 5-10 and Figure 5-8)

Table 5-11 and Figure 5-9 shows respiration data. In this case respiration showed more distinction between treatments. Ethylene increased respiration rate. Clear reduction of this effect was observed with 1-MCP and AVG. 1-MCP alone showed the lowest respiration rate significantly different from ethylene treated roots.

Sugar concentration data is presented in Table 5-12. Lowest fructose and glucose were detected in ethylene treated roots. The overall mean concentration of fructose and glucose sugars was approximately 2-fold lower in ethylene-treated roots than the roots treated with air (control). Highest glucose and fructose were reported in air treated roots. (Figure 5-10). Results were in line with first experiment.

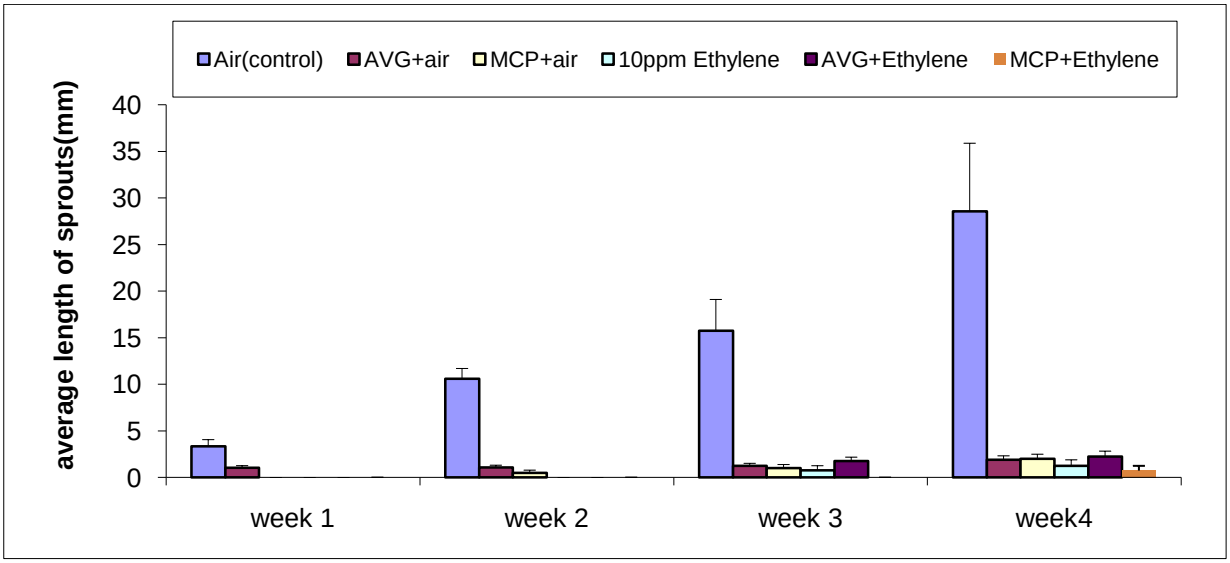


Figure 5-6 Effect of ethylene, AVG and 1-MCP on inhibition of sprouting in sweetpotato roots (Ibees) after 1, 2, 3 and 4 weeks. Each data point is the mean of four replicates each consisting of three roots. Error bars are S.E.M.

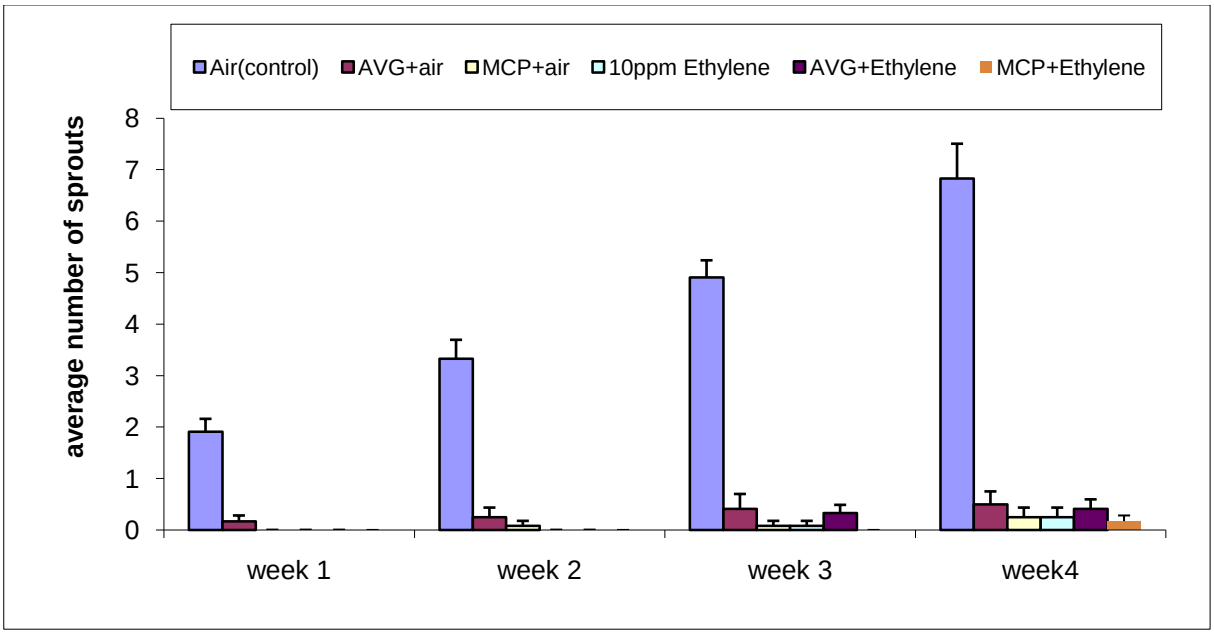


Figure 5-7 Effect of ethylene, AVG and 1-MCP on inhibition of sprouting in sweetpotato roots (Ibees) after 1, 2, 3 and 4 weeks. Each data point is the mean of four replicates each consisting of three roots. Error bars are S.E.M.

Table 5-7 Effect of ethylene, AVG, and 1-MCP on average sprout growth (mm) and no. of sprouts per root of sweetpotatoes stored at 25˚ C after 1, 2, 3 and 4 weeks. Each data point is the mean of four replicates each consisting of three roots.

Treatment	After 1 weeks		After 2 weeks		After 3 weeks		After 4 weeks	
	Average growth (mm)	No. of sprouts/ root	Average growth (mm)	No. of sprouts/ root	Average growth (mm)	No. of sprouts/ root	Average growth (mm)	No. of sprouts/ root
Air	3.34	1.91	10.58	3.33	15.75	4.91	28.55	6.83
AVG+Air	1.05	0.17	1.07	0.25	1.24	0.42	1.88	0.50
MCP+Air	0.00	0.00	0.50	0.08	1.00	0.08	2.00	0.25
Ethylene	0.00	0.00	0.00	0.00	0.75	0.08	0.00	0.25
AVG+Ethy.	0.00	0.00	0.00	0.00	1.75	0.33	2.25	0.42
MCP+Ethy.	0.00	0.00	0.00	0.00	0.00	0.00	0.75	0.17
Treatment effect *p*	0.001	.001	0.001	0.001	0.001	0.001	0.001	0.001
L.S.D (0.05)	1.08	0.31	1.30	0.54	3.36	0.49	6.01	0.43

Table 5-8 Overall mean values of sprout growth (mm) in cultivar Ibees with results of statistical analyses of repeated measurements.

Sprout growth (mm)					
Air (control)	AVG+Air	MCP+Air	Ethylene	AVG+Ethy.	MCP+Ethy.
15.55	1.31	0.69	0.50	0.56	0.19
L.S.D (0.05)			3.04		
Treatment effect *p*			0.001		

Table 5-9 Overall mean values of Average no of sprouts per root in cultivar Ibees with results of statistical analyses of repeated measurements.

Average no of sprouts per root					
Air (control)	AVG+air	MCP+air	Ethylene	AVG+Ethy.	MCP+Ethy.
3.97	0.33	0.10	0.08	0.18	0.04
L.S.D (0.05)			0.43		
Treatment effect *p*			0.001		

Table 5-10 Overall mean values of weight loss in cultivar Ibees with results of statistical analyses of repeated measurements.

Weight loss					
Air (control)	AVG+air	MCP+air	Ethylene	AVG+Ethy.	MCP+Ethy.
95.59	96.60	96.64	95.80	96.40	96.50
L.S.D (0.05)			0.81		
Treatment effect *p*			0.033		

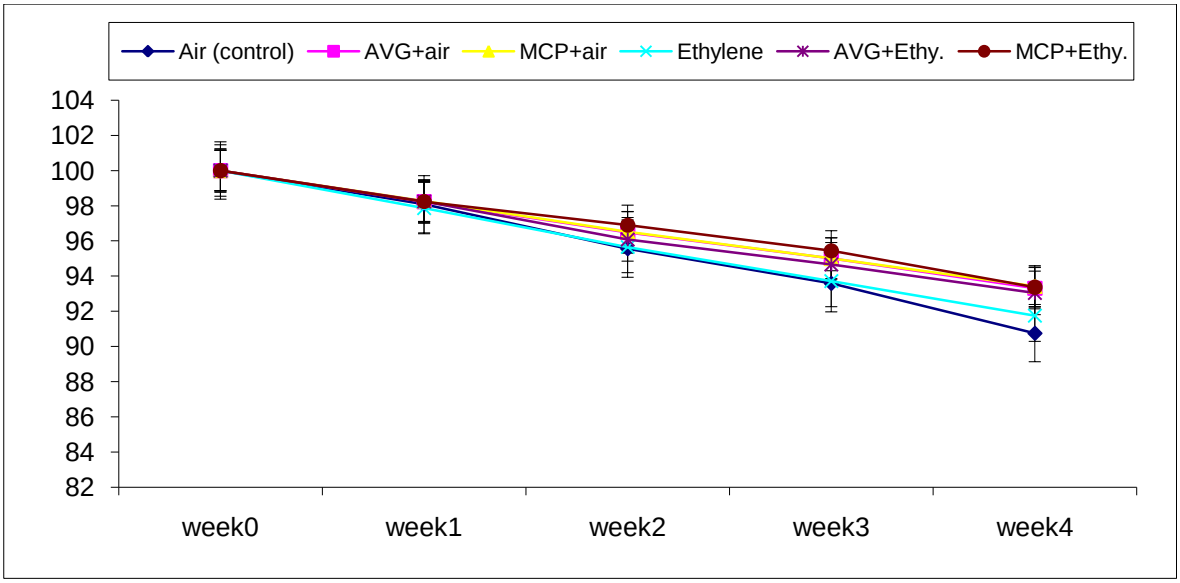


Figure 5-8 Effect of ethylene, AVG and 1-MCP on % weight loss of sweetpotato roots (Ibees) after 1, 2, 3 and 4 weeks. Each data point is the mean of four replicates each consisting of three roots. Errors bars are S.E.M

Table 5-11 Overall mean values of respiration rate in cultivar Ibees with results of statistical analyses of repeated measurements.

CO_2 (mg)/h/kg					
Air (control)	AVG+air	MCP+air	Ethylene	AVG+Ethy.	MCP+Ethy.
30.43	31.70	26.72	48.06	43.61	39.15
L.S.D (0.05)			8.49		
Treatment effect *p*			0.001		

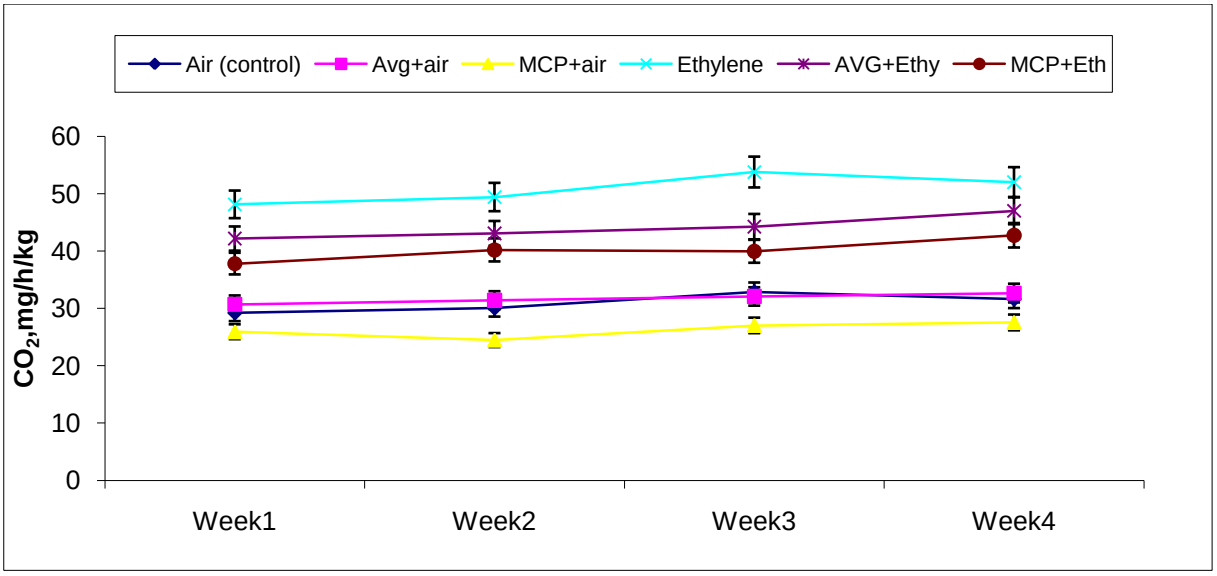


Figure 5-9 Effect of ethylene, AVG and 1-MCP on respiration rate of sweetpotato roots (Ibees) after 1, 2, 3 and 4 weeks. Each data point is the mean of four replicates each consisting of three roots. Errors bars are S.E.M

Table 5-12 Effect of ethylene, AVG, and 1MCP on fructose, glucose and sucrose contents of sweetpotatoes (Ibees) stored at 25 C after 1, 2, 3 and 4 weeks. Each data point is the mean of four replicates.

Treatments	Fructose(mg/g)	Glucose (mg/g)	Sucrose (mg/g)
Air (control)	56.7	105.4	165.7
AVG+air	46.2	69.2	151.5
MCP+air	51.9	78.9	170
Ethylene	24.5	41.1	157.9
AVG+Ethy.	40.5	73.7	163.8
MCP+Ethy.	46.2	76.5	166
L.S.D (0.05)	7.52	9.96	15.28
P value	0.001	0.001	0.018

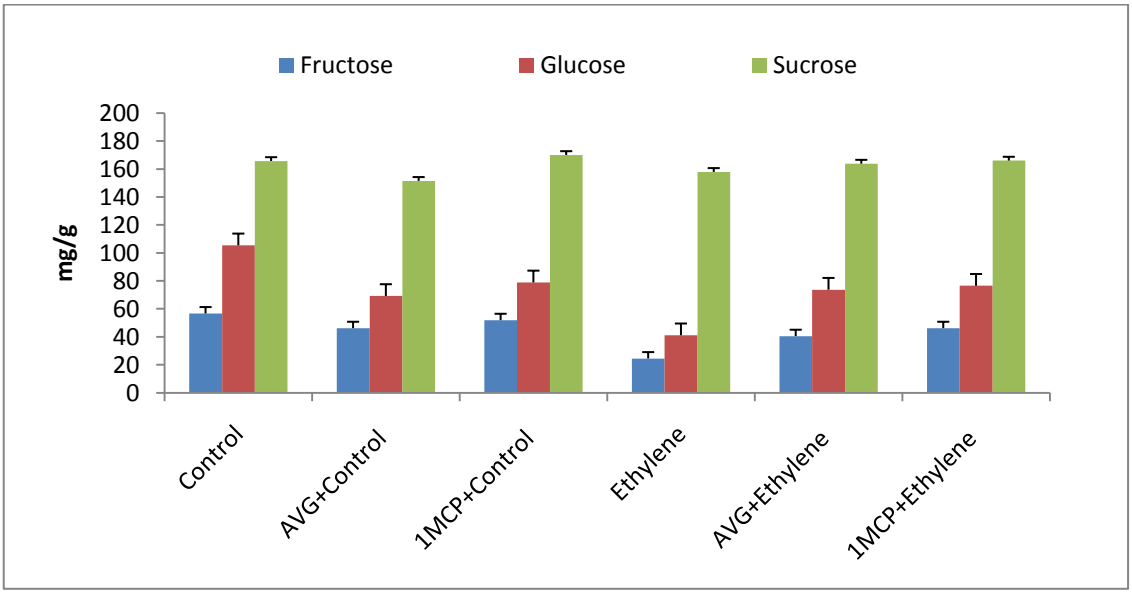


Figure 5-10 Sugar contents in sweetpotato roots (Ibees) treated with ethylene, 1 MCP, AVG and Control in August 2009. Each data point is the mean of four replicates. Error bars are S.E.M.

5.6 Summary of main findings

In sweetpotato roots:

- As observed in Chapter 4 continuous exposure to ethylene inhibits sprout growth and number.
- Treatment with 1-MCP, ethylene antagonist, inhibits sprout growth and number in the presence and absence of ethylene.
- Treatment with AVG, an ethylene synthesis inhibitor, inhibits sprout growth and number in the presence and absence of ethylene.
- Root respiration is increased by ethylene. This effect is reduced by 1-MCP and by AVG.
- Ethylene leads to a decrease in concentrations of fructose and glucose.

5.7 Discussion

Dormancy and sprout growth

It has been known that ethylene has effects on potato tuber dormancy and sprouting (reviewed in Rylski *et al*., 1974, Prange *et al*., 1998). The effect of exogenously applied ethylene on potato sprouting depends on the duration of exposure (Timm *et al.,* 1986). Ethylene has dormancy breaking effect in potato tubers (Prange *et al.*, 2005). On the

other hand, where continuous exposure to ethylene is maintained, inhibition of sprout elongation in tubers is observed (Rylski, 1974; Prange, 1998).

In this study the involvement of ethylene in sweetpotato sprouting was studied not only from direct exposure to the ethylene, but by looking at the effects of AVG and 1- MCP.

In this chapter it has been shown that all treatments (ethylene +/- 1-MCP, +/- AVG), inhibited sprouting compared to air treated roots.

The results of the ethylene effect on sprout growth are consistent with those in potatoes.

1-MCP on its own also inhibited sprout growth. As 1-MCP blocks ethylene receptors, therefore blocking ethylene effects, this suggests ethylene is necessary for sprout initiation. Results are in line with sprout suppressant effect of 1-MCP in onions cultivar Sherpa (Downes *et al.,* 2010)

The application of AVG did not increase the elongation of sprouts and number of sprouts, but inhibited sprouting; also consistent with the hypothesis that endogenous ethylene is necessary for sprout initiation. Prange *et al,* (2005) found an increased number of sprouts and initials in the presence of ethylene. AVG is known to reduce ethylene production. The results of this study suggest that the rate of ethylene inhibition was sufficient to have an effect of sprouting. In potatoes, Stow and Senner (unpublished data) found that AVG did not increase the elongation of sprouts. This suggests that endogenous ethylene synthesis was inhibited and tubers remained dormant for longer time, hence less sprout elongation was observed. Application time of AVG seems to be important. If the dormancy is already broken then the AVG effect on sprouting could be different from when applied after dormancy is broken.

There was no sprout growth when 1-MCP and AVG were applied with continuous ethylene (10ppm) presumably due to the dominant sprout inhibitory effect of continuous ethylene exposure. Measurement of endogenous ethylene concentrations would be useful to understand the effect of different levels of endogenous ethylene production and to investigate how much ethylene production was reduced by AVG.

The inhibition of sprouting by 1-MCP and AVG is consistent with a hypothesis that ethylene is needed for sprout initiation in sweetpotatoes, in other words there is a two step process. Firstly, a certain amount of ethylene is required for sprout initiation and then continuous exposure to exogenous ethylene could lead to sprout growth inhibition.

Although 1-MCP will extend dormancy in potatoes it does not completely inhibit sprouting (R. Colgan Personal communication), and in this way potatoes behave differently from sweetpotatoes.

Respiration and sugar concentrations

In potatoes, ethylene is used to control sprouting, but has a limitation particularly at low temperature when it can increase sugar accumulation in tubers (a problem for processing). However, it has been observed where potatoes are treated with 1-MCP (625 ppb) followed by ethylene treatment, 1-MCP does not counteract the ethylene inhibition of sprout growth but can reduce sugar accumulation (Prange, *et al*., 2005). However, there is variability between varieties in response to 1-MCP (Daniels-Lake, 2008). According to Blankenship (2001), success of 1-MCP depends on number of factors such as the concentration, exposure time and maturity of the tissues. There is already evidence that the concentration of 1-MCP needed to be effective varies considerably between species. In the case of fruit, in tomatoes, 7ppb is effective for delaying ripening (Wills and Ku, 2002), while 500ppb was found to be effective in delaying ripening in unripe bananas (Harris, *et al*., 2000). In potatoes, there is possibility that 1-MCP concentration (625 ppb)

was not sufficiently blocking the ethylene receptors to affect sprouting. If different concentrations were used, perhaps we would have seen 1-MCP sprout inhibitory effect of potatoes tubers.

In the study reported here ethylene alone increased respiration rate and reduced monosaccharide sugar accumulation in sweetpotato roots. This suggests that some process other than sprout growth which requires energy was stimulated by ethylene in sweetpotato, hence increased respiration and use of monosaccharide. That process was partially inhibited by 1-MCP and AVG, whereas the 1-MCP effect is easily explained by its inhibition of ethylene action, AVG cannot just be acting by inhibiting ethylene synthesis because exogenous ethylene was added. However, AVG inhibits pyridoxal phosphate enzymes (PLP), PLP acts as a co-enzyme in all transamination of amino acids (Toney, 2005). AVG inhibits protein synthesis in tomato (Saltveit, 2005), so the ability of AVG to reduce protein synthesis may be a factor to inhibit respiration rate. Respiration data distinctly showed that inhibitory effect of 1-MCP and AVG (Figure 5-9). Alternatively, ethylene overcomes the 1-MCP and AVG effect on respiration. Comparatively low respiration rate was reported in 1-MCP+air and AVG+air treated roots. The slight increase in respiration in control as compare to 1-MCP alone treated roots was probably a result of respiration by sprouts after 2 weeks. Low respiration rate effect of 1-MCP in sweetpotato roots is in line with the effect of 1-MCP on respiration in onions –Sherpa, (Downes, *et al.,* 2010)

Highest concentrations of fructose and glucose were reported in control roots. Highest sugar concentration suggested mobilization of starch. When a root sprouts, it will often become sweeter as starch is converted to sugar to provide energy and to nourish the growing sprouts. In ethylene-treated roots the lowest fructose and glucose concentrations were reported. However differences in sugar levels leads to the idea that sugars in sweetpotato are probably mainly in the vacuole, and are therefore available to be used by

respiration to drive processes. This suggest that the monosacharides might be used primarily as substrates for fueling respiration and to produce carbon skeletons that can be then used in other continuous reactions of ethylene treated roots.

Higher fructose and glucose levels were observed in 1-MCP and AVG treated roots than ethylene treated roots on its own. This may be because the 1-MCP and AVG treatments were counteracting the effect of ethylene and up regulating the mechanisms related to sugar accumulation and inhibiting the metabolism of monosaccharides. This suggests that AVG and 1-MCP result in different biochemical responses to ethylene treatment. Two different varieties of sweetpotatoes were used. This study has shown that response to ethylene, AVG and 1-MCP is not limited to one variety. No rotting or other disorders were reported in any treatment.

It can be concluded that for sweetpotato in developing countries markets, ethylene can be used to suppress growth of sprouts at room temperature. Although the results from this study suggest that 1-MCP and ethylene reduce sprout growth and may play a role in endogenous ethylene production, break of dormancy or initiation of sprout growth in sweetpotato roots. To further understand the mechanism of sprout suppression using ethylene, 1-MCP and AVG, molecular techniques are required to determine differences in gene expression in response to treatments.

6 Investigating the role of gibberellins and cytokinins in the control of dormancy and sprout growth in potatoes *(Solanum tuberosum* L) using potato accessions transformed for the isoprenoid synthetic pathway.

6.1 Introduction

Several classes of plant hormones have been implicated in the control of dormancy and sprouting in potato. These include gibberellins and cytokinins, but the precise mechanisms of control have not yet been fully elucidated (see literature review section 2.1.5). According to Suttle *et al*. (2004a) cytokinins are important for dormancy break and gibberellins for promoting subsequent sprout growth, however, gibberellins have also been found to break dormancy.

Isoprenoid biosynthetic pathways provide a wide range of metabolites that are essential for both plant development and storage organ food quality. Over 22,000 different isoprenoids have been identified in plant species, forming a structurally and functionally diverse group of metabolites. The isoprenoid-derived phytohormones, gibberellins, cytokinins and abscisic acid are involved in plant defense, aroma and flavour. Carotenoids which are important micronutrients in plant-derived food are also isoprenoids.

At the Scottish Crop Research Institute (SCRI), as part of a programme to increase carotenoid levels, potato tubers were transformed to over express a bacterial gene encoding 1–deoxy–D-xylulose 5 phosphate synthase (DXS), a key enzyme of isoprenoid biosynthesis. Some of the transgenic lines (DXS1 and DXS2) produced tubers with elongated shape and also showed an early tuber sprouting phenotype, in which the eyes sprouted, but the sprouts were arrested at any early stage until the normal time for

dormancy break (Morris *et al*., 2006). In order to account for the sprouting phenotype, the concentration of growth regulators were measured in transgenic and control tubers. The major difference observed was an increase in the cytokinin, *trans*-zeatin riboside (*t*ZR) measured at harvest. In addition, in some *DXS* expressing lines, tuber carotenoid content increased compared to controls. The significant difference in gibberellin content was a decrease in the level of inactive GA_{29} in DXS2 tubers compared with wild type, but the level of $GA_{1,}$ which is known to play a role in potato sprouting, was not observed to change in *dxs* expressing lines compared with controls (Morris *et al.,* 2006).

Both cytokinins and gibberellins stimulate sprouting by activating certain phases of the cell cycle. For example, Hill (1980) observed that cytokinins alter the growth of axillary buds and that applying gibberellins will accelerate bud growth.

In the present study the effects of adding gibberellins or cytokinins to wild type and transgenic lines were observed in order to elucidate the role of these hormones in the control of dormancy break and sprout growth. The transgenic tubers must have broken dormancy for sprout growth to be initiated, but as the bud growth was arrested, they presumably re-entered some form of “meta- dormant state”. It was hypothesized that owing to a higher level of endogenous cytokinins this “meta-dormant state” would be terminated more easily by using cytokinins and gibberellins in transgenic lines, compared to the wildtype. The effect of cytokinins and gibberellins was also evaluated with regard to the time-dependent manner in which the dormant buds reacted to applications of these hormones. A system of excised buds was used. An eye and a cylinder of tissue around it was cut out of the tuber, and placed in a Petri-dish partially submerged in medium. This system allows easy treatment with plant hormones.

6.2 Materials and Methods

Potatoes were grown in a glasshouse at SCRI. The day-time temperature was maintained at 20° C and nocturnal temperature at 15° C. Tubers were harvested in April 2007 at which time they were in deep rest. Tubers were grown again to repeat the experiment during 2009 and were harvested during August 2009. The tubers were washed and stored in the dark at 3-4° C before starting the experiment. They were used four and twelve weeks after harvest in 2007 and five weeks after harvest during 2009. It was observed that tubers during 2007 were bigger and had more eyes per tuber. In 2009, tubers were smaller and had fewer eyes per tuber.

The following potato lines were used.

- DXS1 – *DXS* expressing
- DXS2 – *DXS* expressing
- DXS19 – *DXS* non-expressing
- DXS25 – *DXS* non-expressing
- Empty vector (EV)
- Wild type (WT)

6.2.1 Experiment 1: Testing sprout growth behaviour of excised buds

In this experiment two tubers from each of the six lines were selected. Each individual bud (eye) was excised within a cylindrical plug of tissue using a cork borer (internal diameter 0.5 cm) and cut to a length of 5-6 mm. The cork borer was kept sterile throughout the procedure by flaming with industrial methylated spirit. Eyes on each tuber were divided into three groups depending on the distance from the apical bud. Each group was

analysed as a separate block. The eyes were washed for 15 minutes in a sterile Sprout Release Assay (SRA) buffer (20 mM MES, 300 mM Mannitol and 5 mM Ascorbic acid, pH 6.5) the assay buffer was replaced and the 15 minute washing process repeated two more times. The eyes were then drained and transferred to sterile petri dishes containing filter paper soaked in water. Petri dishes were sealed to avoid contamination. Thereafter all dishes were placed in the dark at 22° C for 10 days and assessed for sprout growth.

Plate 6-1 Eyes excised from a tuber of DXS 1 are dipped in sterile Sprout Release Assay (SRA) buffer

6.2.2 Experiment 2: The effect of hormones on sprout growth of excised buds at four, five and twelve weeks after harvest

This series of experiments (Experiments 2a, 2b and 2c) was conducted during the years 2007 and 2009. In these experiments the same six potato lines (DXS1, DXS2, DXS19, DXS25, EV and WT) were used. Potato discs, each containing an eye, were excised with a potato cork borer as described for experiment 1. The eyes excised from each line were divided into three groups (blocks). (Details of how blocking was set up are described

individually for each experiment below) Each group of discs was stored in separate glass beakers filled with SRA buffer until the excision process was completed. This was a longer process than for experiment 1, so that the discs were dipped for 2-3 hours until all the eyes were excised from each cultivar. They were then drained and washed 2 more times for 15 minutes each in SRA. Then they were transferred to sterile Petri dishes marked to divide them into three segments for the three blocks. Samples were subjected to the different treatments. Solutions of hormones were made up in SRA. For the treatments, 8 ml of each solution was poured into 6 different Petri dishes (one Petri dish per line), so that the tissue cylinders were immersed to about half-way up. Each Petri-dish was divided into three segments for the three replicates. (Note separate Petri-dishes were not used for each replicate, as this would have required too much expensive growth hormone.). After one hour, eyes in each group were placed in sterile Petri dishes containing water soaked sterile filter papers and sealed Petri dishes were kept in the dark at 20-22^{o} C.

6.2.2.1 Experiment 2a: The effect of hormones on sprout growth of excised buds at four weeks after harvest (2007)

During this study the eyes excised from each line were divided into three groups (blocks) depending on the distance from apical bud. After washing, the eyes from each group were divided randomly into 11 samples (6-8 eyes per group). One sample from each block (6-8 eyes /sample) was frozen immediately in liquid nitrogen and stored at – 70^{o} C for later analysis. (Thus three replicate samples were frozen for each line). The remaining 10 samples of each block were subjected to the following five treatments (2 samples per treatment): 1 hour in gibberellic acid; GA_1 (1 mM) or GA_3 (1 mM), the cytokinin, *trans*–zeatin riboside (*t*ZR) (1 mM), sucrose (100 mM) and SRA buffer as a control. GA1 was obtained from OlChemIm Ltd Czech Republic and all other chemicals were obtained from

Sigma chemicals. Sealed Petri dishes were kept in a dark room and sprout growth was observed on a daily basis. After 3 days, half of the samples were frozen in liquid nitrogen in cryogenic vials, before transferral to a -70º C freezer for later analysis. After 10 days when good sprout growth was observed for some treatments, the sprouts were separated from plugs with sterile sharp blades and then plugs and sprouts were frozen separately in liquid nitrogen and stored at -70º C until further analysis.

6.2.2.2. Experiment 2b: The effect of hormones on sprout growth of excised buds at five weeks after harvest (2009)

The above mentioned study was repeated during the year 2009 five weeks after harvest. Tubers in each line were divided randomly into three groups (replicates), so that in this case blocking was in terms of tubers. After washing, the eyes from each group were divided randomly into 5 samples (6-8 eyes per sample). One of these samples (6-8 eyes /sample) was frozen immediately in liquid nitrogen and stored at – 80º C for later analysis. (Thus three replicate samples were frozen for each line). The remaining 4 samples of each replicate were subjected to the following four treatments: 1 hour in gibberellic acid; GA_3 (1mM), the cytokinin, *trans*–zeatin riboside (*t*ZR) (1mM), a combination of GA_3 and *t*ZR and SRA buffer as a control. All chemicals were obtained from Sigma chemicals. Sealed Petri dishes were kept in the incubator at 20-22° C. Sprout growth was observed on a daily basis.

6.2.2.3 Experiment 2c: The effect of hormones on sprout growth of excised buds at twelve weeks after harvest (2007)

This experiment was conducted to re-check the effect of growth hormones on excised tuber eyes for tubers at 4 months after harvest during 2007. Due to shortage of material,

in this experiment only two tubers from each of the six lines were selected. Each individual bud (eye) was cut out as described for experiment 1. The eyes were dipped in sterile SRA for about 20 minutes until all the eyes were excised from a cultivar, then drained and washed 2 times more for 15 minutes, drained and transferred to sterile Petri dishes. After washing, the eyes from each cultivar were divided randomly into two samples (6-8 eyes per group). The two samples of each cultivar were subjected to the following treatments: treatment up to 1 hour in gibberellic acid; GA_3 (1 mM) and SRA buffer as a control. For the treatments 15 ml of each solution was poured into 6 different Petri dishes (one Petri dish per line), so that the tissue cylinders were immersed. (A larger volume of solution was necessary for this experiment compared to experiment 2 due to the smaller number of discs). The sealed Petri dishes were kept in the dark room at 20-22° C. Sprout growth was observed once a week. After a week when most of the eyes were grown, the sprouts were separated from plugs with sterile sharp blades and then plugs and sprouts were frozen in liquid nitrogen and stored at -80º C until further analysis.

6.2.3 Experiment 3: Effect of hormones on sprout growth of whole tubers at 4 months after harvest (2007).

This study was conducted to determine the effect of growth hormones on the whole potato tubers and to see how this related to the behaviour of excised buds. Four tubers for each line, four months after harvest, were used to assess the effect (Two for each treatment). The method of hormone treatment was essentially as described in (Suttle, 2004). Using a 16 gauge needle, a cavity (3-5 mm deep) was made immediately below each bud of each tuber. 5 µl of GA_3 1µg/1µl (highest concentration used in Suttle 2004) was used for injection under each bud (2 tubers/treatment). For the control 5 µl SRA buffer was injected below the buds. The tubers were stored in the dark at 22º C. Sprout growth was measured after one week and then again after four weeks.

6.2.4 Experiment 4: Comparison of lines in terms of Gene Expression using Real time PCR.

6.2.4.1 Sample preparation

During experiment 2b, eyes with sprouts if they had grown, were separated from the tissues cylinders (excised buds) of each sample, placed in 2ml eppendorf tubes, frozen in liquid nitrogen and stored at -80º C. Individual samples were removed from the -80ºC freezer then frozen eyes/sprouts were ground in eppendorf tubes by adding steel beads and placing for 1-2 minutes in a bead mill (Qiagen Tissue Lyser).Total frozen tissue was ground to a fine powder and then added to 450 µl RLT buffer (RNeasy Lysis Buffer)

6.2.4.2 RNA extraction

RNA was extracted from each sample following the plant extraction method from RNeasy using a RNeasy Plant Mini Kit by QIAGEN.

The lysate was transferred to a QIAshredder spin column placed in a 2 ml collection tube and spun for 2 minutes at full speed. The supernatant was carefully transferred to a new micro centrifuge tube for further use. 0.5 volume of ethanol (96-100%) was added to the cleared lysate. Then samples (650 µl) were placed in RNeasy spin column placed in 2ml collection tube and centrifuged for 15 s at 10,000 rpm. Flow through was discarded to reuse the collection tube. Buffer RW1 (700 µl) was added to RNeasy spin Column and centrifuged at 10,000 rpm for 15s to wash the spin column membrane. Buffer RPE (500 µl) was added to the RNeasy column and centrifuged for 2 minutes at 10,000 rpm to make sure that no ethanol was carried over. Finally the RNeasy spin column was placed in a new 1.5 ml collection tube and 30 µl RNase-free water was added to spin column

membrane. It was centrifuged at 10,000 rpm for 1 minute to elute the RNA. Samples were DNase treated using DNAse I (Qiagen) following the Qiagen-on-column produce.

6.2.4.3 Estimation of RNA concentration

Total RNA was quantified by measuring the absorbance at 260 and 280 nm by using a Bio Photometer. Absorbance ratios at 260:280 give information on the purity of the nucleic acid. A ratio of 1.7 to 2.0 indicates RNA is of good quality and free of protein contamination.

6.2.4.4 Real Time PCR analysis

A RT-PCR assay was applied to analyse *dxs* gene expression in 6 potato tuber lines. Total RNA were reverse transcribed in 20 µl reaction samples. 10 µl RNA samples were added to master mix (see appendix 4). cDNA synthesis was allowed to occur at 37° C for 60 minutes using a thermal cycler. After 1 hour, the reaction was inactivated by heating at 70°C for 5 minutes. cDNA was then diluted by using RNase free water. 4 µl of cDNA was mixed in 16 µl of PCR mix (the recipe is given in appendix 3). Primers used for DXS gene are given in appendix 3. Real-time PCR was carried out using SYBR Green PCR Master Mix and samples were amplified by using the prism sequence detector. SYBR Green dye was used in samples. SYBR Green is a nucleic acid stain with many uses including double stranded DNA (dsDNA) quantification in real time PCR and gel electrophoresis. Upon binding to double stranded DNA, its fluorescence intensity becomes 1,000 times that of its unbound state. The following thermal cycle conditions were used.

1. 95˚ C for 2 minutes. During this initialization step the template DNA and primers fully dissociates. This step occurs only once in the reaction.

2. 95˚C for 15 seconds. This denaturation step dissociates the DNA targets produced during previous cycles by disrupting the hydrogen bonds between complementary bases. This exposes the bases and allows the primers to bind to them in the next step.

3. 56˚C for 15 seconds. During this annealing step, the primers bind to their complementary sequences in the target DNA.

4. 68˚C for 20 seconds. This is the extension step when the DNA polymerase extends the DNA strand starting from the primers, assembling from the 5’ to 3’ end of the new DNA strand by adding the complementary dNTP to the elongating strand.

5. Repeat steps 2-4 for 40 cycles. After each cycle, the number of DNA strands is theoretically doubled if 100% efficient.

6. Final Elongation. This step was performed at the higher temperature for 20 minutes to ensure any remaining single-stranded DNA was fully extended.

Real time PCR results were expressed as CT (cycle threshold) values. This value corresponds to the cycle at which the fluorescence of the SYBR Green dye reaches above the threshold or background fluorescence value. In quantifying gene expression, Relative expression levels were calculated and primers validated by using the CT method.

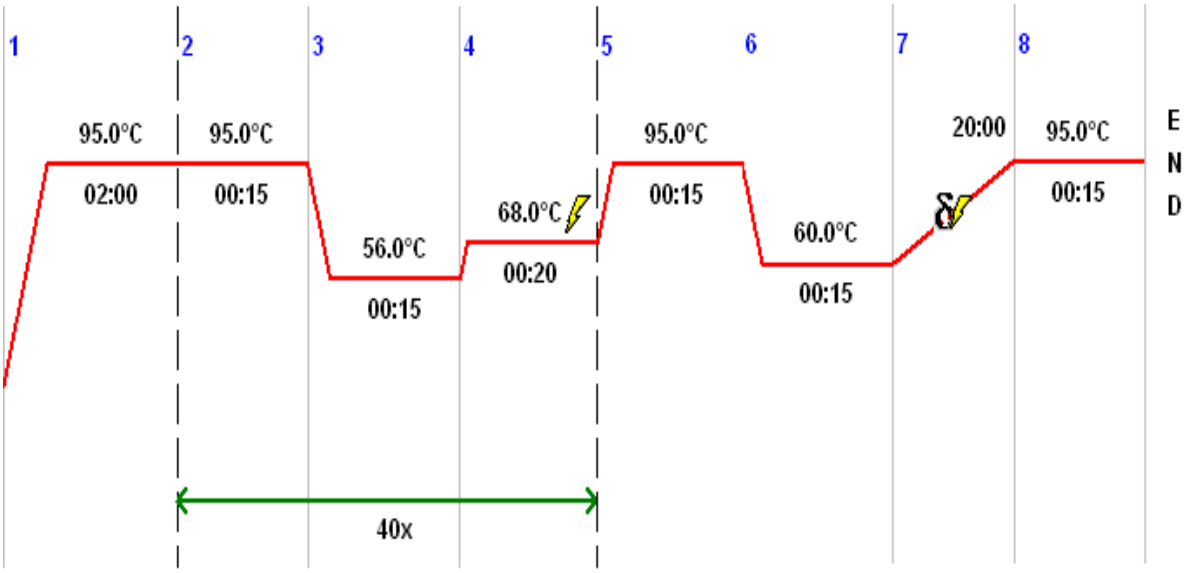


Plate 6-2 PCR conditions used for RT-PCR analysis

6.3 Statistical analysis

ANOVAs were carried out using GenStat (11th edition) statistical package. Repeat measurement analysis of variance was performed to see the difference in lines. Graphs were plotted with a standard error of means (SEM) value at each point unless otherwise stated.

6.4 Results

At harvest and at the start of the experiment tubers of DXS1 and DXS2 had buds as observed previously (Morris *et al*., 2006). These buds did not develop further after harvest. No buds were present on the tubers of other lines.

In 2007, it was observed that the line DXS1 had more eyes per tuber (9-10 eyes per tuber), than DXS2 (7-8 eyes per tuber), and that other lines had about 5-6 eyes per tuber.

DXS 1 and 2 were elongated compared to the other lines, and this was most pronounced for DXS 1. During the repeat study in 2009, it was observed that all of the tubers were smaller than tubers used during the first study in 2007. In 2009 DXS1 and 2 did not differ in shape from the other lines, but had the same arrested bud phenotype as in 2007.

During the trial a few of the buds on DXS1 and DXS2 developed discolouration. (15 out of 300 for DXS1 and 2 out of 220 for DXS2). In the case of DXS2, discolouration did not appear to prevent further sprout growth. No signs of rotting were observed in any tissue disc throughout the whole experiment.

Plate 6-3 DXS 1 tuber (left) and DXS 2 tuber (left) 2007

6.4.1 Experiment 1: Testing sprout growth behaviour of excised buds.

This experiment was conducted to check that the removal of apical dominance by the excision of buds did not induce sprout growth, in which case this experimental system would not be useful to study hormonal effects, as all lines would exhibit sprout growth. Sprout growth was assessed visually during the 10 days of storage; there was no further sprout growth in DXS1 and DXS2 and no sprout initiation in any of the other four cultivars.

6.4.2 Experiment 2: a and b. The effect of hormones on sprout growth of excised buds at four, five and twelve weeks after harvest.

In this experiment tubers were assessed four weeks after harvest in 2007 and five weeks after harvest in 2009. Sprout growth at four, six and ten days after incubation in SRA buffer (control) or treatment with GA_1, GA_3, *t*ZR, a combination of GA_3 and *t*ZR or sucrose was expressed in terms of mean sprout length (Figures 6.1-6.6) and percentage of eyes with sprout growth (i.e. % sprouts showing growth in DXS1 and DXS2, and % eyes sprouting for the other lines) (Figures 6-1-6.6).

None of the lines exhibited any sprout growth after incubation with SRA buffer alone (data not shown). However in almost all other cases, 3-4 days after growth hormone treatment excised eyes that had been treated with either gibberellins or cytokinins had begun to exit dormancy. One clear, but confusing result was that whereas sprout growth was evident in all lines except DXS1 during 2007, in contrast all lines including DXS1 showed sprouting during the repeat study in 2009. DXS2 exhibited more growth over the period of 10 days than the other lines in 2007, whereas in 2009, DXS1 response was similar to that of DXS2.

In the case of GA_1 (2007) (Figure 6.1) DXS2 exhibited the most vigorous growth rate with mean sprout length significantly higher than all the other lines after 4, 6 and 10 days. The % sprouts growing was greater for DXS2 than the other lines and was statistically higher than DXS25 and EV. There was no significant difference between DXS19, DXS25, EV and WT.

In the case of GA_3 although DXS2 initially exhibited the fastest sprout growth rate, the difference was less marked than for GA_1, It was not significantly different from the EV accession which was more vigorous by ten days. There was no significant effect on

average sprout rate in 2007. However, in the later study DXS1 and DXS2 both exhibited significantly more rapid growth rate as compared to all other lines. DXS1 and DXS2 showed higher sprout growth as compared to other lines. WT was less responsive to GA_3 during both years. WT showed significantly less average sprout growth than others except DXS19 in 2009.

*t*ZR induced sprout initiation/growth in all lines except DXS1 in 2007, but in 2009 sprout growth in DXS1 was parallel to DXS2. The rate of sprout growth tended to be lower than induced by GAs during both trials. During 2007, DXS2 showed the most vigorous growth, followed by DXS19 which was significantly different from other lines. In terms of average growth WT showed less growth as compared to DXS2 and DXS19 and was not significantly different from DXS25 and EV. In 2009 DXS1 and DXS2 showed significantly higher sprout rate and average sprout growth than other lines. WT remained with lowest sprout growth in 2009 but with no difference to other lines except DXS1 and DXS2 which were significantly higher.

The combination of GA_3 and *t*ZR was only tested in 2009 and was the most effective treatment for which lines showed higher growth rate and sprout growth. Statistically higher average sprout growth was observed in DXS1 and DXS2 than other lines with no difference in between DXS1 and DXS2. Sprout rate were also significantly higher in DXS1 and DXS2 but there were no significant differences between DXS19, DXS25, EV and WT.

Sucrose had no effect on any line in 2007, but was not included in the 2009 trial.

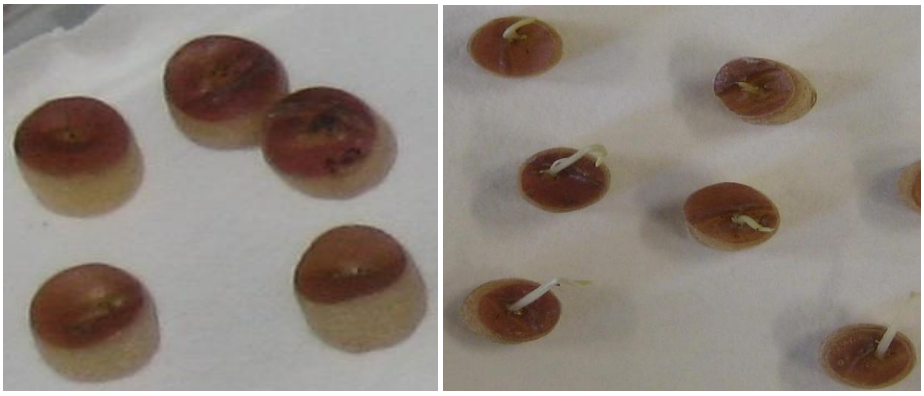


Plate 6-4 Excised buds from transgenic line DXS1 (left) and DXS2 (right) 10 days after treatment with GA_3 in 2007.

Plate 6-5 Excised buds from transgenic line DXS1 (left), DXS2 (right) 10 days after treatment with GA_3 in 2009.

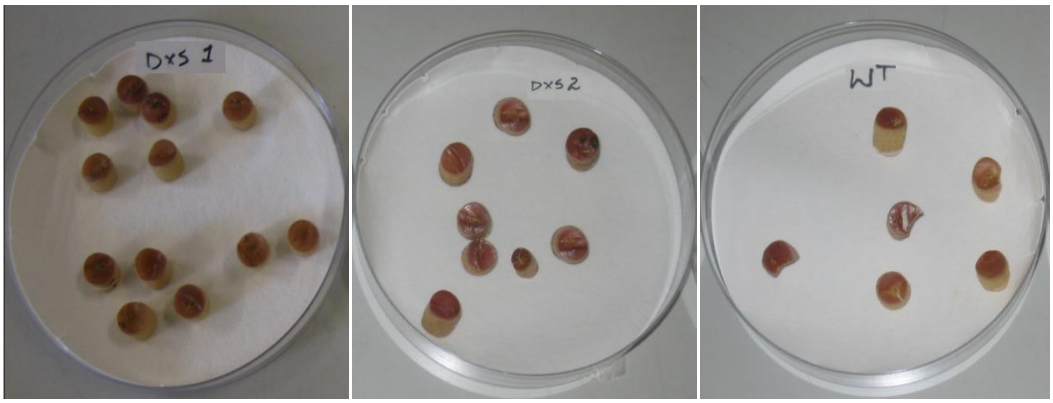
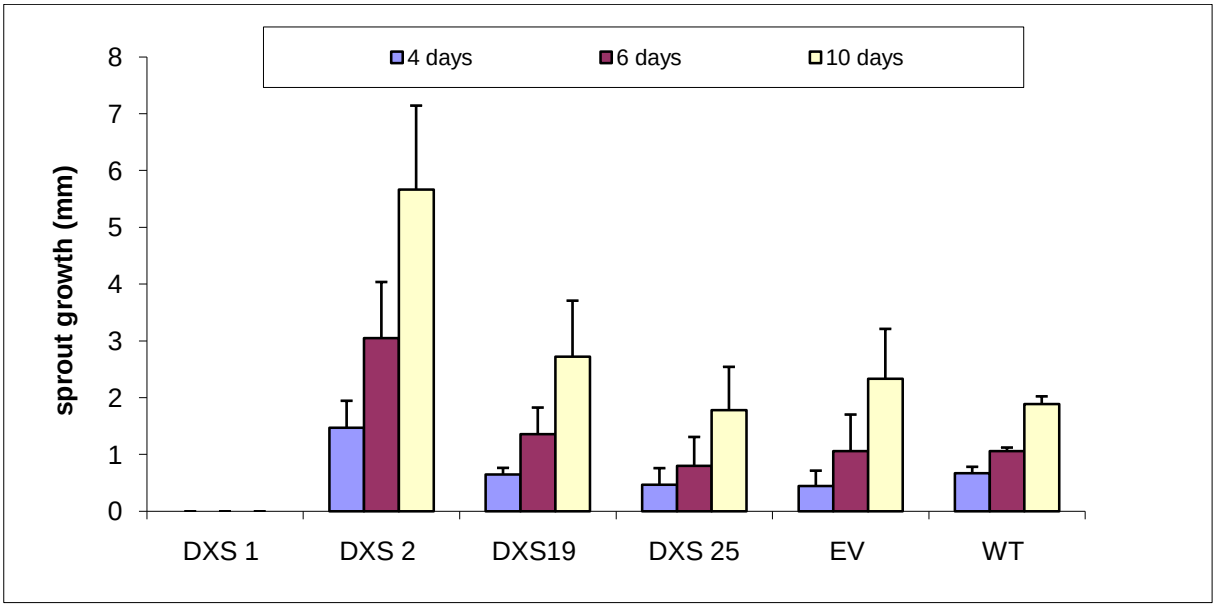
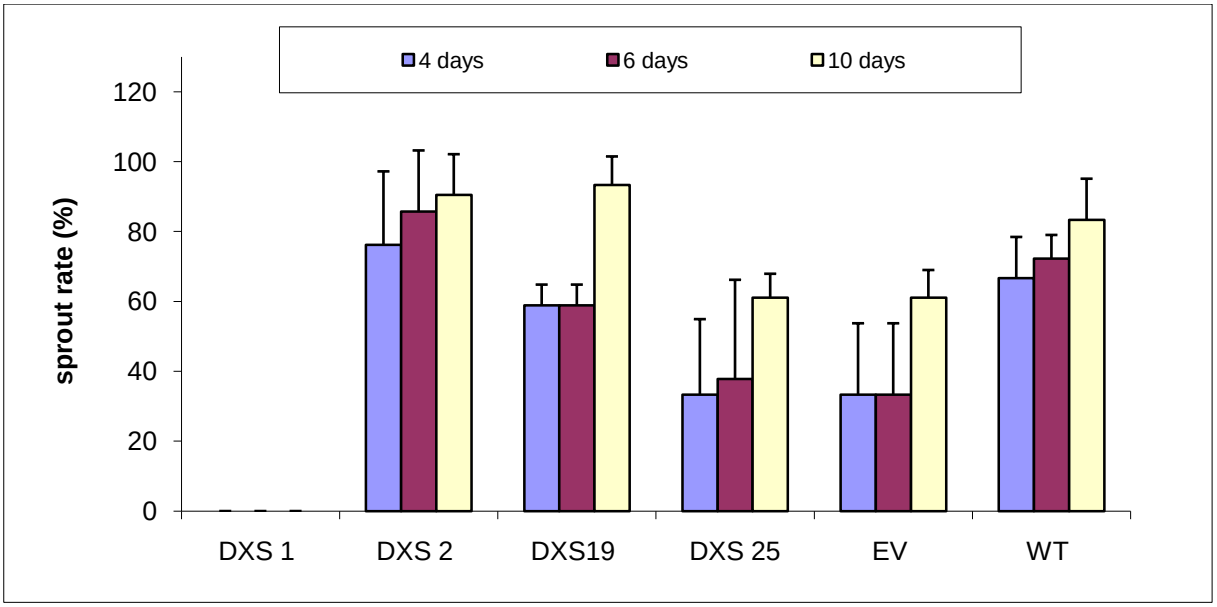


Plate 6-6 Excised buds from transgenic line DXS1 (left), DXS2 (centre) and wildtype (left), 10 days after treatment with SRA buffer in 2009.



(A)

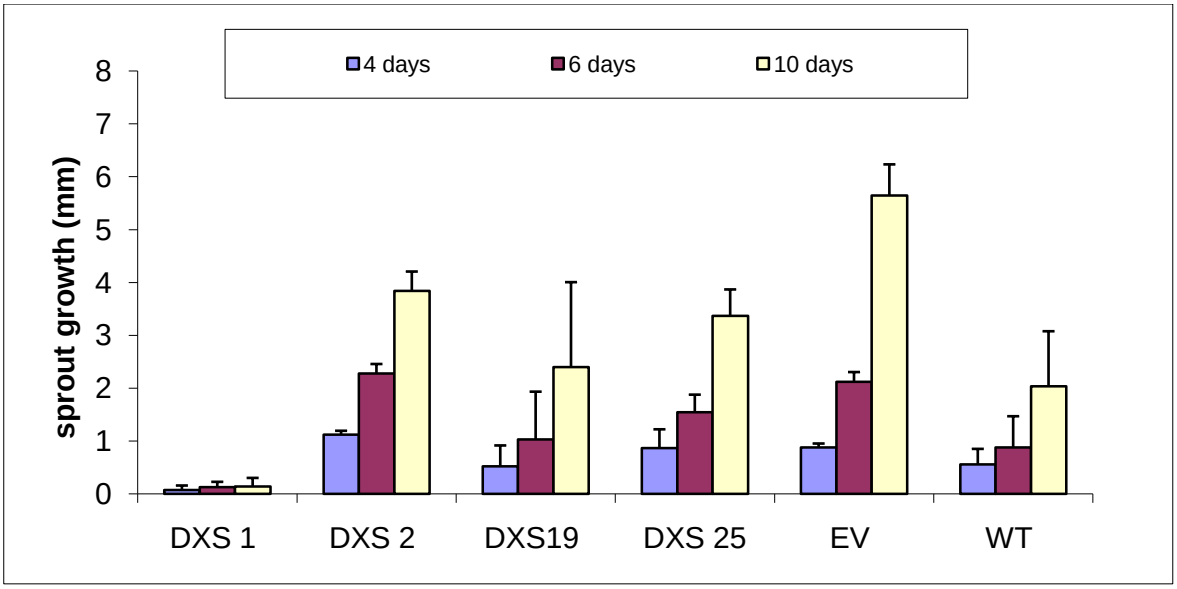


(B)

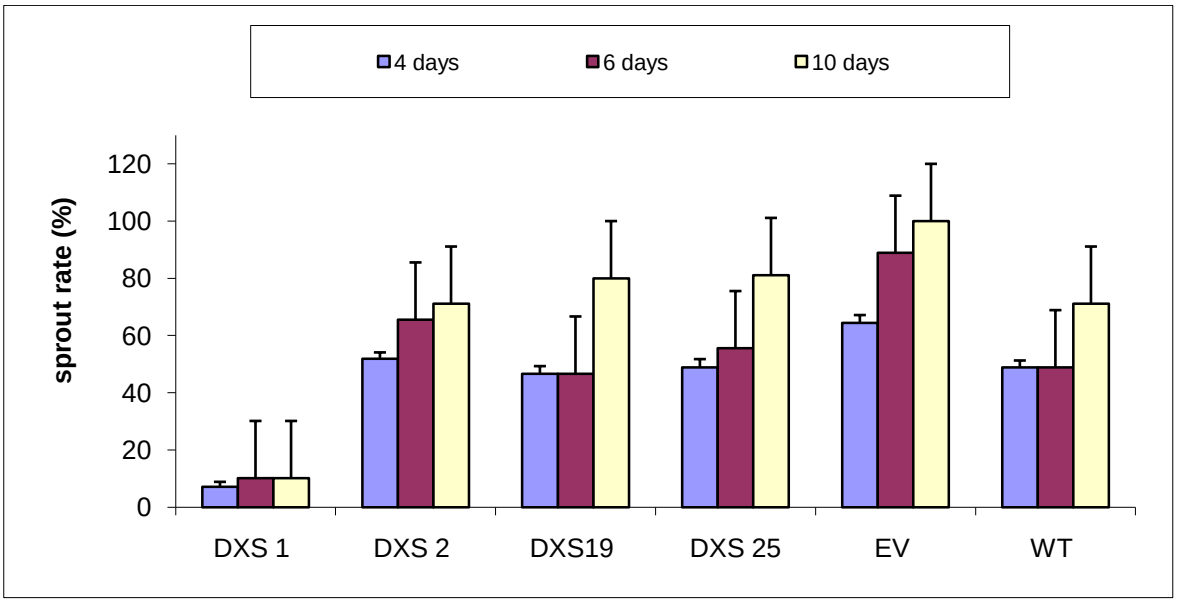
Figure 6-1 Average sprout growth (mm), A and sprout rate (%), B after 4, 6 and 10 days for eyes excised from potato tubers of 6 lines after treatment with GA_1 in 2007. Each data point is the mean of three replicates each consisting of 6-10 excised eyes. Error bars indicate SEMs of the replicates.

Table 6-1 Mean values of sprout growth (mm) and % sprouting after GA_1 treatment with results of statistical analysis of repeated measurements in 2007

Average sprout growth (mm)						Sprout rate (%)					
DXS1	DXS2	DXS19	DXS25	EV	WT	DXS1	DXS2	DXS19	DXS25	EV	WT
0	3.39	1.57	1.03	1.27	1.25	0	84.1	64.4	44.1	42.6	75.9
L.S. D(0.05) (Lines)		1.58				L.S. D (0.05) (Lines)		39.63			
P value (Lines)		0.016				*P* value (Lines)		0.009			



(A)

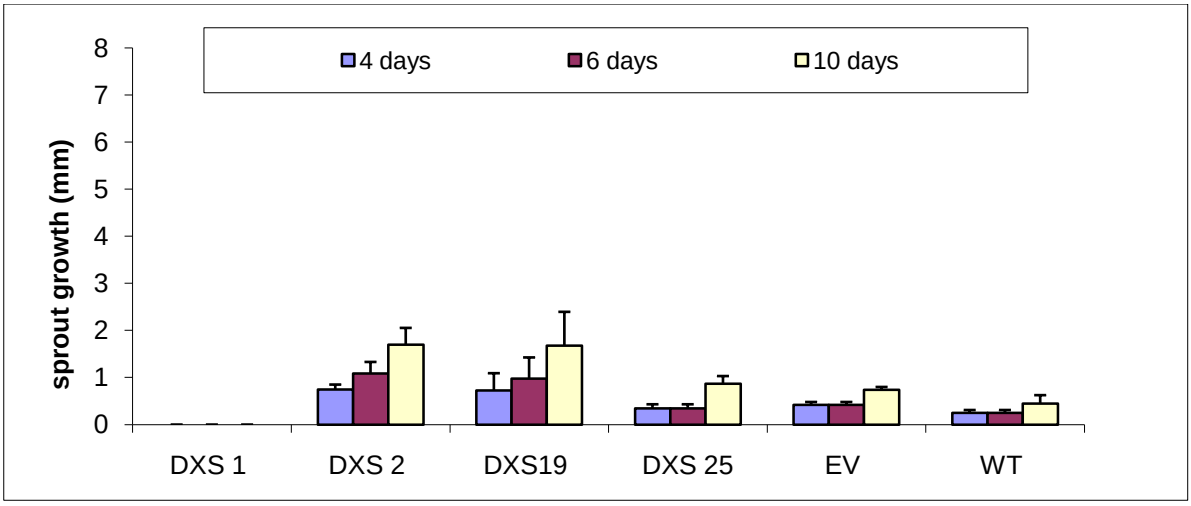


(B)

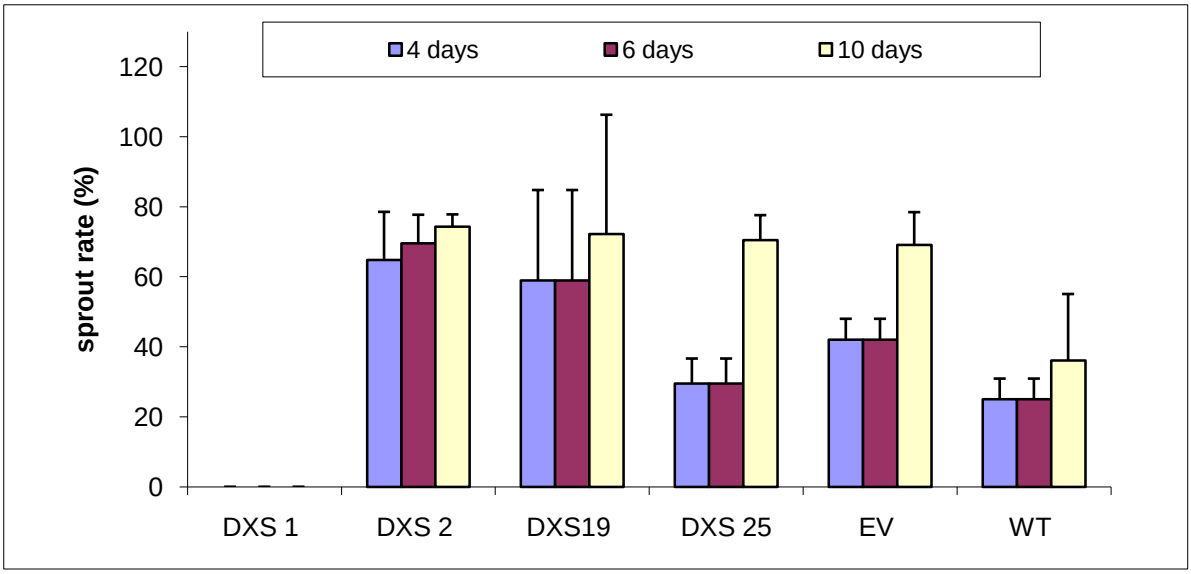
Figure 6-2 Average sprout growth (mm), A and sprout rate (%), B, after 4, 6 and 10 days for eyes excised from potato tubers of 6 lines after treatment with GA_3 in 2007. Each data point is the mean of three replicates each consisting of 6-10 excised eyes. Error bars indicate SEMs of the replicates.

Table 6-2 Mean values of sprout growth (mm) and % sprouting after treatment of GA_3 with results of statistical analysis of repeated measurements in 2007.

Average sprout growth (mm)						Sprout rate (%)					
DXS1	DXS2	DXS19	DXS25	EV	WT	DXS1	DXS2	DXS19	DXS25	EV	WT
0.12	2.41	1.31	2.07	2.87	1.15	9.2	62.8	57.8	61.9	84.4	56.3
L.S. D (0.05) (Lines)		1.27				L.S. D (0.05) (Lines)		33.65			
P value (Lines)		.008				*P* value (Lines)		.011			



(A)

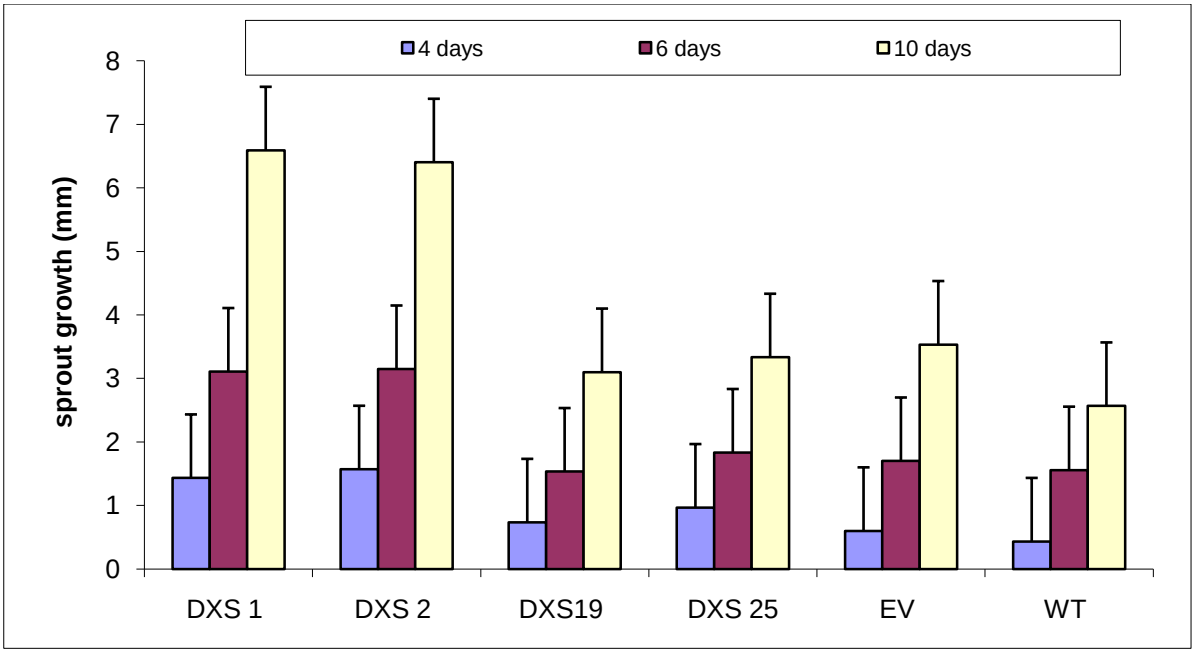


(B)

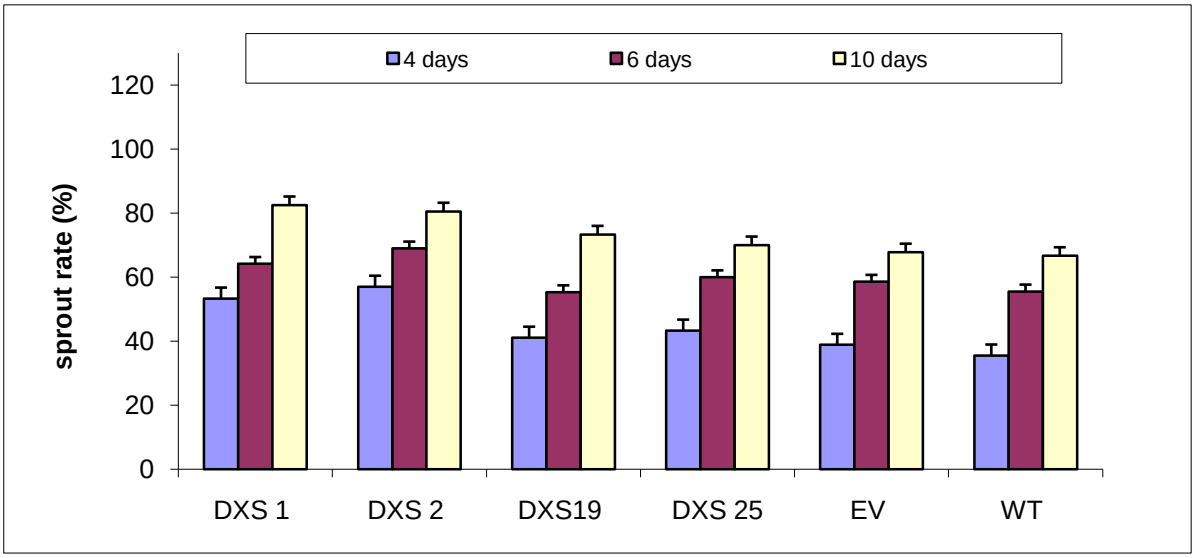
Figure 6-3 Average sprout growth (mm),A, and sprout rate (%),B after 4, 6 and 10 days for eyes excised from potato tubers of 6 lines after treatment with *t*ZR in 2007. Each data point is the mean of three replicates each consisting of 6-10 excised eyes. Error bars indicate SEMs of the replicates.

Table 6-3 Mean values of sprout growth (mm) and % sprouting after *t*ZR treatment with results of statistical analysis of repeated measurements in 2007

Average sprout growth (mm)						Sprout rate (%)					
DXS1	DXS2	DXS19	DXS25	EV	WT	DXS1	DXS2	DXS19	DXS25	EV	WT
0	1.17	1.12	.51	.52	.31	0	69.5	63.3	43.2	51.1	28.7
L.S. D (0.05) (Lines)		0.51				L.S. D (0.05) (Lines)		29.78			
P value (Lines)		0.003				*P* value (Lines)		0.004			



(A)

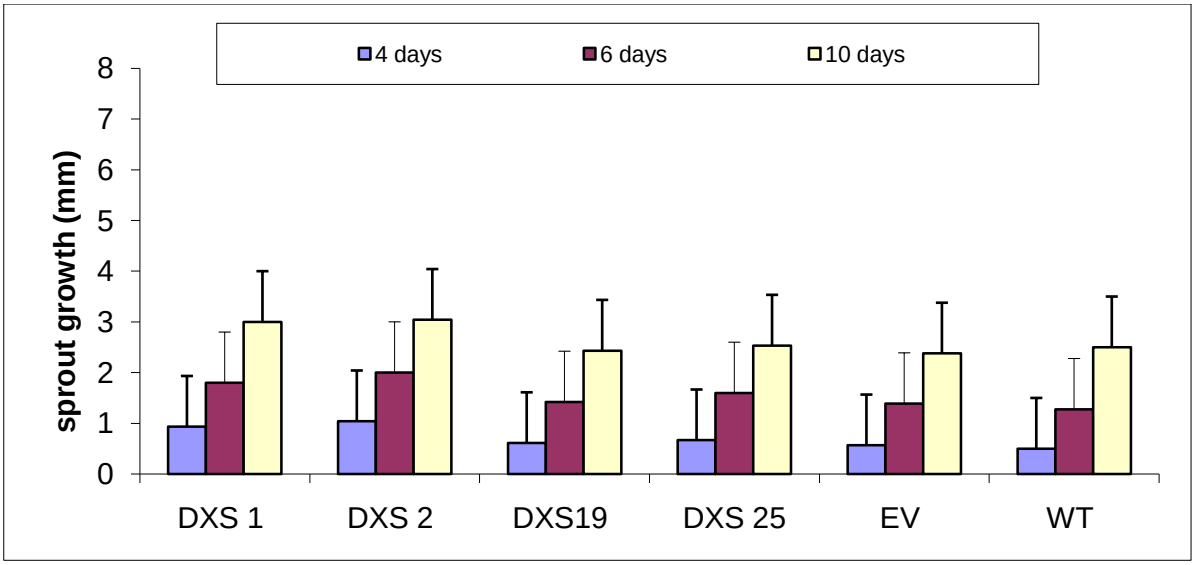


(B)

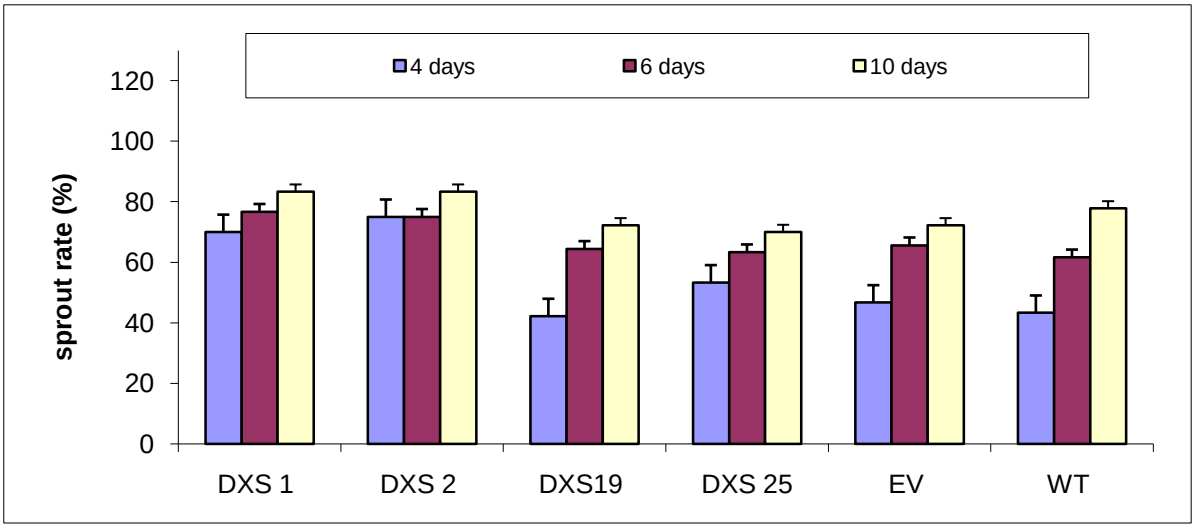
Figure 6-4 Average sprout growth (mm),A and sprout rate (%),B, after 4, 6 and 10 days for eyes excised from potato tubers of 6 lines after treatment with GA_3 in 2009. Each data point is the mean of three replicates each consisting of 5-10 excised eyes. Error bars indicate SEMs of the replicates.

Table 6-4 Mean values of sprout growth (mm) and % sprouting after GA_3 treatment with results of statistical analysis of repeated measurements in 2009

Average sprout growth (mm)						Sprout rate (%)					
DXS1	DXS2	DXS19	DXS25	EV	WT	DXS1	DXS2	DXS19	DXS25	EV	WT
3.71	3.71	1.78	2.04	1.94	1.51	66.6	68.8	55.9	57.7	55.1	52.5
L.S. D (0.05) (Lines)		0.40				L.S. D (0.05) (Lines)		8.06			
P value (Lines)		0.001				*P* value (Lines)		0.005			



(A)

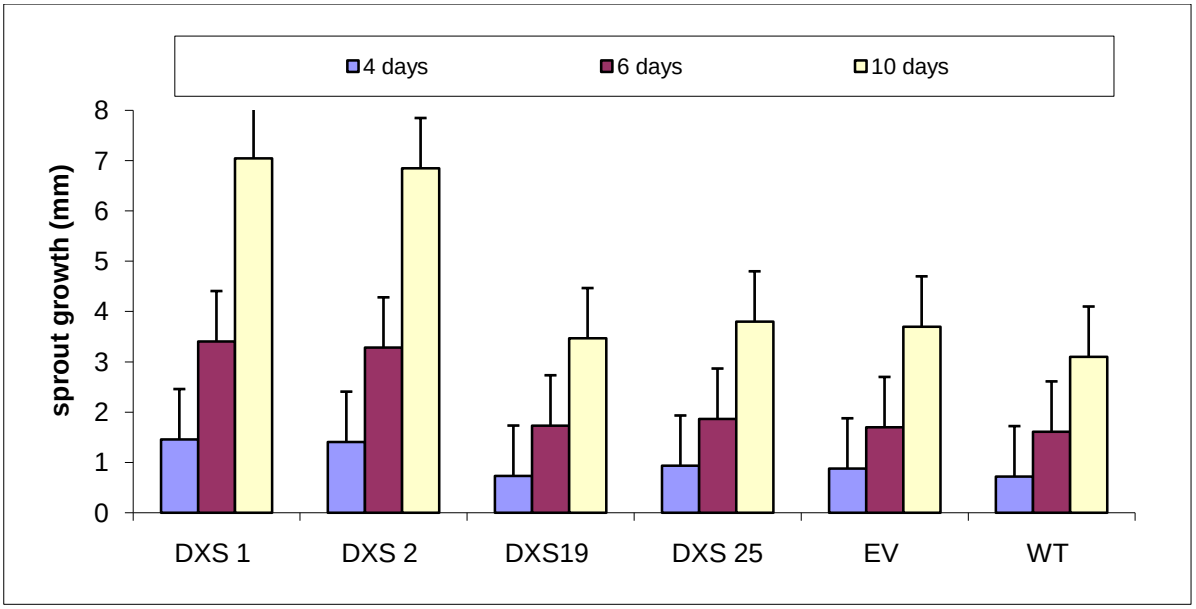


(B)

Figure 6-5 Average sprout growth (mm),A and sprout rate (%),B, after 4, 6 and 10 days for eyes excised from potato tubers of 6 lines after treatment with *t*ZR in 2009. Each data point is the mean of three replicates each consisting of 5-10 excised eyes. Error bars indicate SEMs of the replicates.

Table 6-5 Mean values of sprout growth (mm) and % sprouting after *t*ZR treatment with results of statistical analysis of repeated measurements in 2009

Average sprout growth (mm)						Sprout rate (%)					
DXS1	DXS2	DXS19	DXS25	EV	WT	DXS1	DXS2	DXS19	DXS25	EV	WT
1.91	2.04	1.48	1.60	1.44	1.38	76.7	77.8	59.6	62.2	61.5	60.7
L.S. D (0.05) (Lines)		0.29				L.S. D (0.05) (Lines)		11.59			
P value (Lines)		0.003				*P* value (Lines)		0.013			



(A)

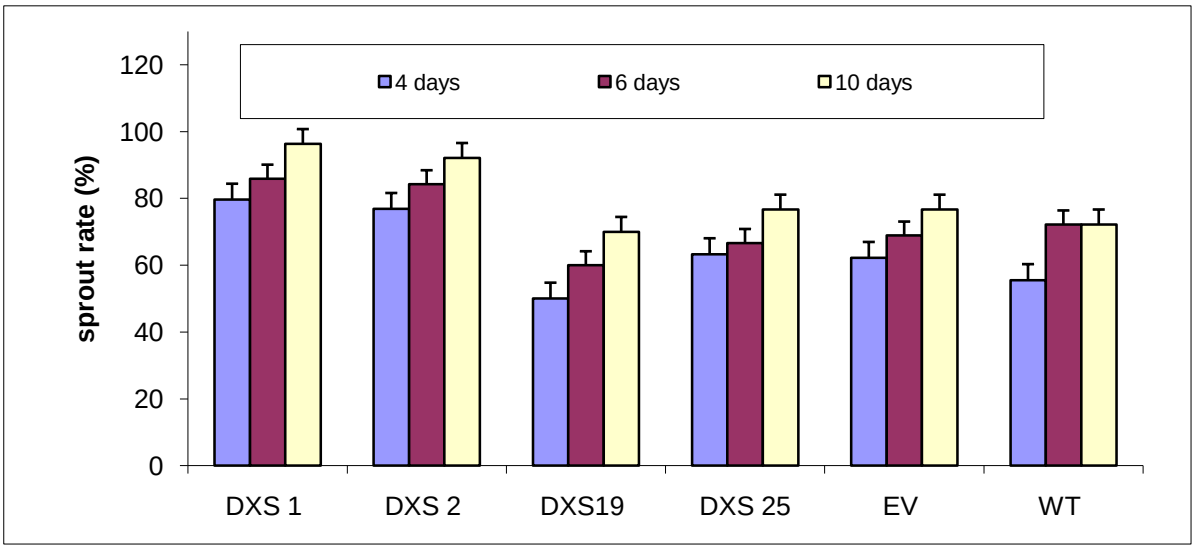


Figure 6-6 Average sprout growth (mm) and sprout rate (%) after 4, 6 and 10 days for eyes excised from potato tubers of 6 lines after treatment with GA_3 + *t*ZR in 2009. Each data point is the mean of three replicates each consisting of 5-10 excised eyes. Error bars indicate SEMs of the replicates.

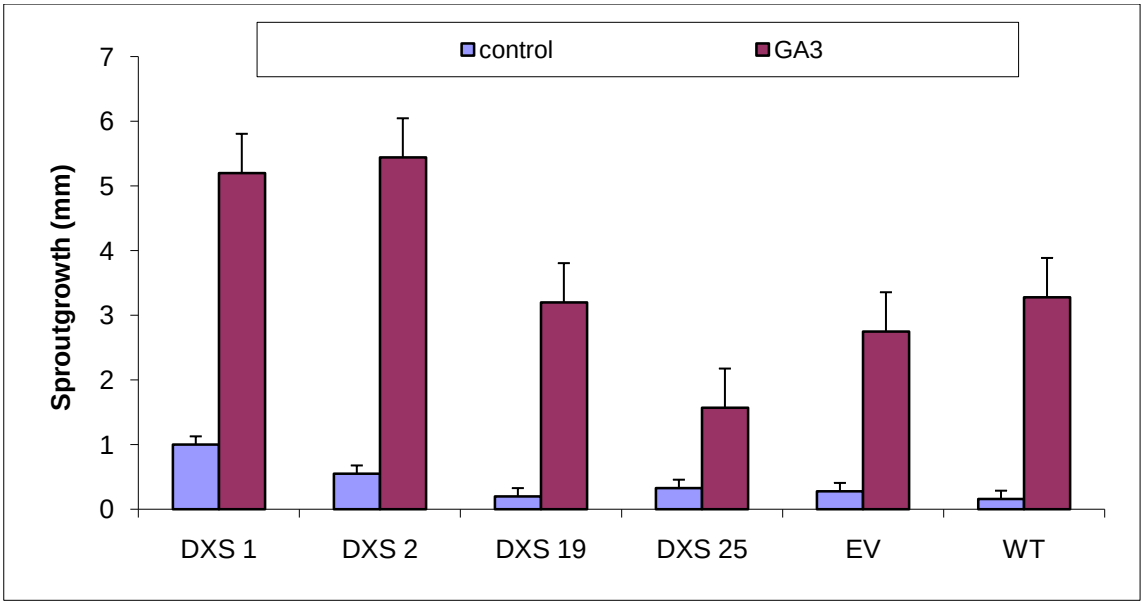
Table 6-6 Mean values of sprout growth (mm) and % sprouting after GA_3+ *t*ZR treatment with results of statistical analysis of repeated measurements in 2009

Average sprout growth (mm)						Sprout rate (%)					
DXS1	DXS2	DXS19	DXS25	EV	WT	DXS1	DXS2	DXS19	DXS25	EV	WT
3.96	3.83	1.97	2.20	2.09	1.80	87.28	84.41	60.0	68.8	69.2	66.6
L.S. D (0.05) (Lines)		0.50				L.S. D (0.05) (Lines)		11.66			
P value (Lines)		0.001				*P* value (Lines)		0.002			

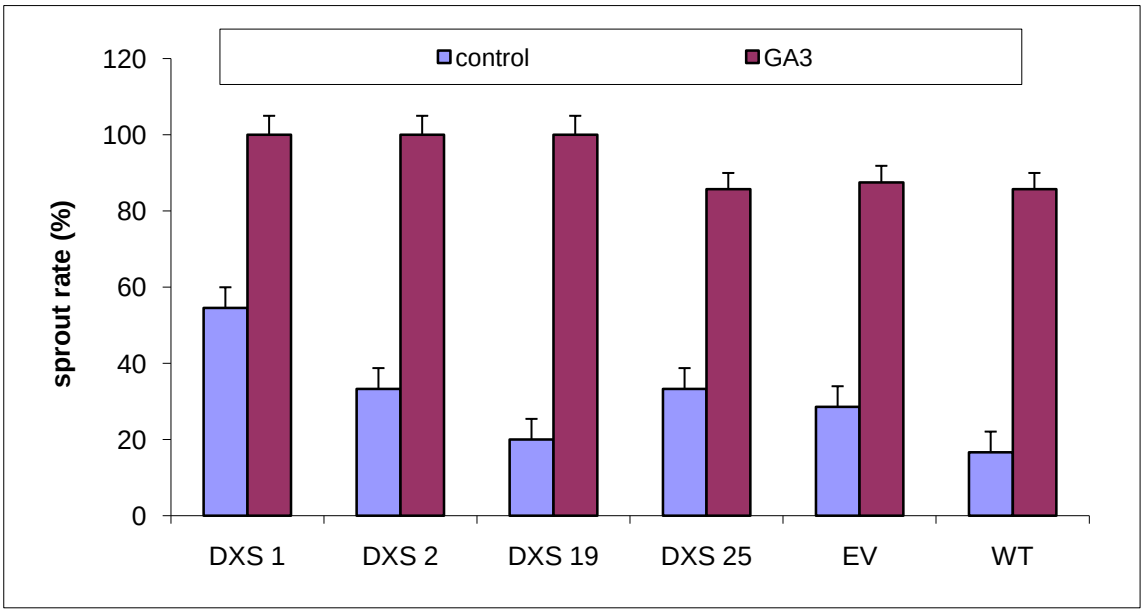
6.4.3 Experiment 2c. The effect of hormones on sprout growth of excised buds at four months after harvest. In 2007

This experiment was conducted to check the surprise result that DXS1 did not respond to gibberellins or trans-zeatin riboside in Experiment 2a. As in experiment 2a,b in this experiment, average sprout growth (mm) (Figure 6.7) and % sprout growth (i.e. % sprouts showing growth in DXS1 and DXS2, and % eyes sprouting for the other lines) (Figure 6.8) were assessed. Due to shortage of material, only GA_3 was tested, and a full statistical analysis was not possible. Three to four days after growth hormone treatment excised eyes had begun to exit dormancy, and sprout growth was evident in all lines. Contrary to its behaviour at four weeks, DXS1 showed vigorous growth and DXS1 and DXS2 had more growth over the period of 6 days compared to all others.

In terms of % sprout rate DXS1, DXS2 and DXS19 showed higher rates (up to 100%) than other three lines



(A)



(B)

Figure 6-7 Effect of 1 mM GA_3 on sprout growth (mm), A, and control on sprout rate (%), B, of excised eyes from 6 potato lines (4 months after harvest) after 7 days. Each data point is the mean of 6-10 excised eyes. Error bars indicate SEMs of the replicates.

6.4.4 Experiment 3: Effect of hormones on sprout growth of whole tubers at 3 months after harvest (2007).

In experiment 3 average sprout growth (mm) and number of sprouts per tuber was determined visually during storage; after 6 days there was no further sprout growth in all of the six lines due to GA_3. But after 28 days GA_3 treated tubers were different from control (Fig. 6-8 and 6-19). In GA_3 treated tubers there were a number of eyes with multiple sprouts, whereas in the controls there was only one thicker, shorter sprout (Plate 6-7)

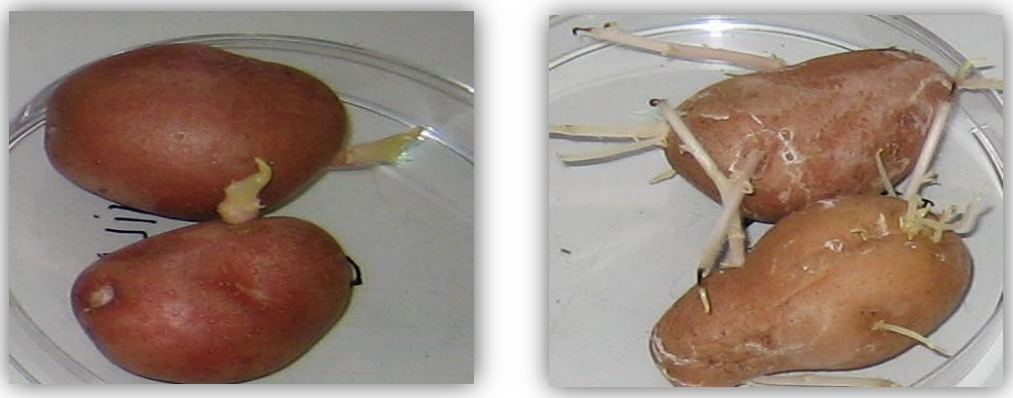


Plate 6-7 Difference of sprout growth in GA_3 treated tubers and control

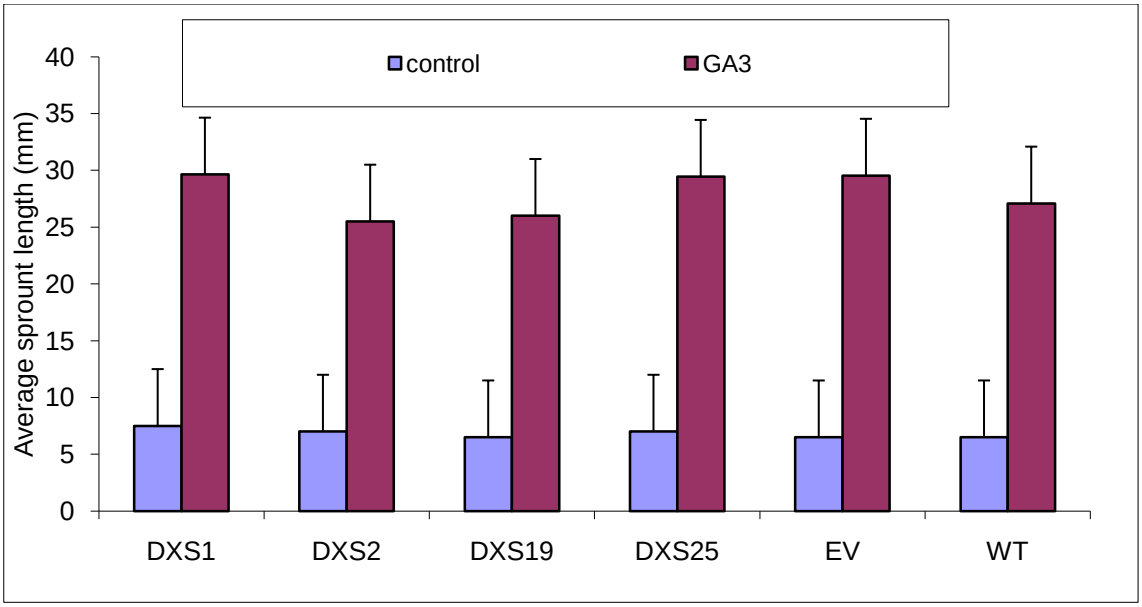


Figure 6-8 Effect of 1µg/1µl GA_3 and control on average sprouts growth (mm) from 6 potato lines (4 months after harvest) after 28 days. Each data point is the mean of sprouts of two tubers.

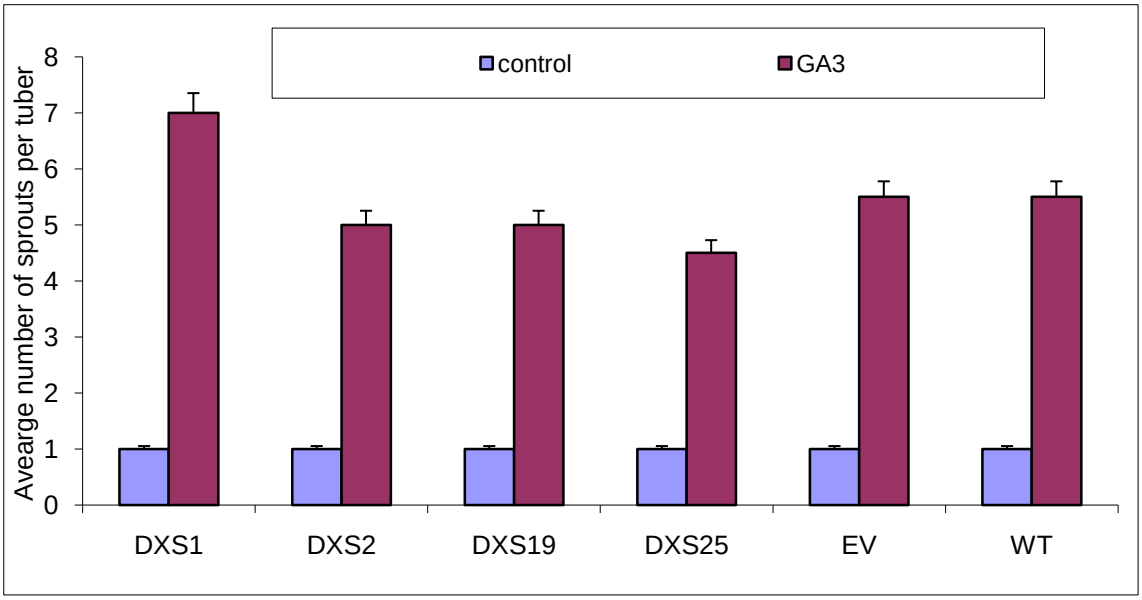


Figure 6-9 Effect of 1µg/1µl GA_3 and control on total average no. of sprouts per tuber from 6 potato lines (4 months after harvest) after 28. Each data point is the mean of sprouts of two tubers.

6.4.5 Experiment 4 Comparison of lines in terms of Gene Expression using Real time PCR.

In order to understand the behaviour of the different lines real time PCR was undertaken to check the level of expression of the DXS gene in each line. The results of real-time assays for *dxs* gene expression are shown in Figure 6-10. RNA purity was considered good as 260:280 ratio measured by spectrophotometer was 1.90 or above in all samples. Tissue samples were taken in 2007 but were found to be of poor quality and so could not be analysed. However this study was conducted in 2009 in order to gain insight into the mechanism and effect of *dxs* gene over-expression on tuber dormancy /sprouting. The housekeeping gene Ubiquitin (*UBQ*) showed similar level of expression in all lines indicating that samples were of a similar cDNA concentrations. As expected DXS1 and DXS2 exhibited *DXS* expression. There was no significant difference between these lines. As expected WT, EV and the non-expressing line DXS19 exhibited no expression. Surprisingly, DXS25 also exhibited expression at a level as high as DXS1 and DXS2

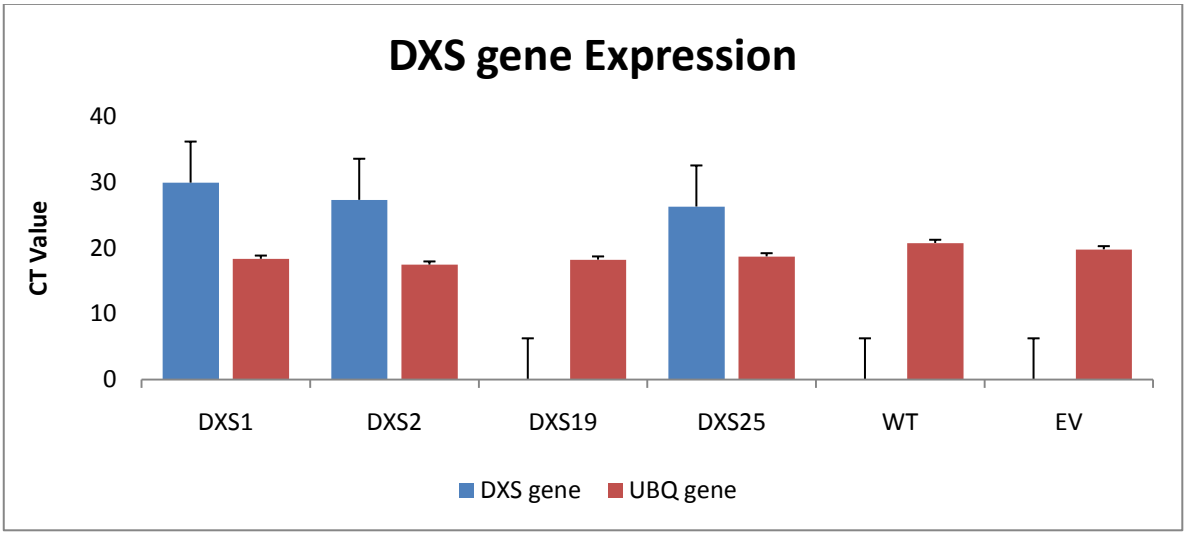


Figure 6-10 *DXS* and *UBQ* gene expression in 6 lines.

6.5 Summary of main findings

- Removal of apical dominance by excision of potato eyes from tubers is not sufficient to terminate dormancy.

- Dormancy of potato discs used in these experiments could be broken by addition of either gibberellins or the cytokinin *t*ZR.

- DXS1 and DXS2 tubers must have exited dormancy at the point that eye sprouting occurred. However, they seem to have re-entered a "meta-dormant" state. In 2007 DXS1 seems to have been in a deeper dormant state than the other lines that could not be broken by either gibberellins or *t*ZR. In the other cases exit from the "meta-dormant" state could be triggered by either gibberellins or *t*ZR in the same way that dormancy could be broken in the other lines.

- Addition of GA3 induces multiple buds in whole tubers, but not in excised buds.

6.6 Discussion

In this study we started from the assumption that in both DXS1 and DXS2 sprouts could be induced to grow more easily than in other lines because they had broken dormancy due to elevated cytokinin levels at harvest. Surprisingly although both lines had clearly developed buds before harvest, in 2007 DXS1 and DXS2 behaved very differently from each other, and buds on DXS1 could not be induced to grow by addition of gibberellins or *t*ZR until 4 months after harvest. However in a repeat study in 2009, DXS1 tubers behaved similarly to DXS2 tubers.

It was observed that the phenotype of DXS1 was more "extreme" than DXS2. Thus it tended to be more elongated and had more buds than DXS2. One possible difference in

DXS1 could be that it has higher levels of endogenous cytokinins after 4 weeks of harvest, and that associated with this is a lower sensitivity to exogenous growth hormones. There are examples in the literature of cases where the presence of high levels of a hormone is associated with low sensitivity. There are also cases where the response to a hormone may differ depending on its concentration. Thus in potatoes it has been reported that low levels of ethylene promote sprouting and high level of ethylene inhibits sprouting (Rylski *et al*., 1974). However real-time PCR results indicated no difference in the level of *dxs* expression in the two lines

In this study the cytokinin *t*ZR or a combination of gibberellins and *t*ZR terminated dormancy as soon as 4 days after treatment. Cytokinins are important hormones in the termination of tuber dormancy. Gibberellins have more effect on sprout growth and also contribute to termination of dormancy. It was observed during the 2007 study that DXS1 was not responsive to concentration applied after 4 weeks of harvest, in contrast same concentration of growth hormones were effective after 5 weeks of harvest during 2009 and 12 weeks after harvest during 2007. There is another possibility that concentration we used were not effective for DXS1 after 4 weeks of harvest. Tuber age and tuber size could also be factor responsible for treatment effectiveness. Sprout growth in DXS1 showed time dependent manner and could also be dose dependent.

We found that DXS over-expressed lines were more responsive towards growth hormones as compare to wild type. This study underlines and importance of physiological stage for hormone responses. The response of tissues can change with physiological stage. The result for DXS 25 suggests that gene expression of DXS might change with physiological stage.

7 An investigation of the volatile profile of tubers from potato lines that differ in their dormancy characteristics

7.1 Introduction

Volatile plant compounds perform a wide range of functions (Dudareva *et al*., 2004). Many floral volatiles have anti-microbial or anti-herbivore activity (Hammer *et al*., 2003), and so act to protect valuable reproductive parts of plants from enemies. Both floral and vegetative parts of many plant species produce volatiles with distinctive smells, i.e. can be detected by the human nose. The discovery of the gaseous hormone ethylene 70 years ago brought the knowledge that at least some of the compounds released may have physiological significance without any distinctive smell to humans (Knudsen *et al*., 1993).

Potato tubers have been found to produce a range of volatiles that play different physiological roles. A range of aromatic hydrocarbon volatiles have been isolated from potato skin and some of these compounds are believed to suppress sprouting (Nursten and Sheen, 1974; Meigh *et al*., 1973; Coleman *et al*., 1981;. Methyl – substituted naphthalenes have been identified as natural volatiles produced by potatoes (Coleman *et al*., 1981), and some of these are associated with sprout suppression. Among these, 1, 4-dimethylnaphthalene and 1, 6-dimethylnaphthalene showed sprout suppression activity comparable to the commercial sprout suppression chemical chlorpropham (Meigh *et al*., 1973; Filmer and Rhodes, 1985).

This chapter reports on a study of the volatile profile that was conducted on a range of accessions of potato tubers obtained from the Scottish Crop Research Institute (SCRI), selected specifically for a wide range in dormancy period. It was hypothesised that dormant accessions would have higher 1, 4-DMN concentrations and that they would

have a volatile profile distinct from the less dormant lines which might include other chemicals with sprout suppressant properties

7.2 Objectives

The objectives of this study were;

- To determine whether the natural levels of 1, 4-DMN present in dormant and non-dormant accessions of potatoes are consistent with its hypothesized physiological role in sprout control.

- To look for differences in volatile profile between dormant and non-dormant potato accessions in order to identify other naturally occurring sprout suppressants.

7.3 Materials and Methods

This experiment was conducted in 2006. 18 accessions of tetraploid *S. tuberosum* with a wide range in dormancy period (nine defined as “dormant” and nine defined as “non-dormant”) were obtained from the Scottish Crop Research Institute (SCRI) in April 2006. Some tubers were used for immediate analysis and others were used for further propagation. Tubers used immediately were stored at 4˚ C for 4 months before they were sent to NRI for the study, where they were kept at 4˚ C before analysis.

These 18 accessions with two other commercial varieties included as controls, Santee and Desiree, were grown at Hadlow College Kent U.K. Standard agronomic practices were utilized. Tubers were harvested in September 2006. At harvest, the tubers were washed with tap water then placed for one week at 16˚ C in the dark at 80% relative

humidity to promote wound healing (curing). Then they were stored in an incubator at 3-4˚ C for further study. The accessions supplied by SCRI, U.K. were as follows. They were defined as “Dormant” or “Non-dormant” through observation of dormancy period made at SCRI.

- NTB-14 Dormant
- NTB-16 Dormant
- NTB-25 Dormant
- NTB-26 Dormant
- NTB-41 Dormant
- NTB-42 Dormant
- NTB-59 Dormant
- NTB-96 Dormant
- NTB-112 Dormant
- NTB- 57 Non dormant
- NTB-85 Non dormant
- NTB-128 Non dormant
- NTB-199 Non dormant
- NTB-224 Non dormant
- NTB-273 Non dormant
- NTB-275 Non-dormant
- NTB-340 Non-dormant
- NTB-427 Non dormant

Sprouting observation was carried out at NRI to select most dormant and non-dormant accessions. 2 tubers from each accession were stored at room temperature for 4 weeks. Sprout length was measured on a weekly basis.

Four methods were tested to collect volatiles near the surface of the tubers that might be involved in control of dormancy. Methods 1, 2 and 3 were tried on tubers supplied directly from SCRI, while Method 4 was tried on tubers grown by Hadlow College.

Samples were run using GC-MS (GC - Agilent 6890). The column was an Agilent DB5; 30 m long x 0.25 µm x 250 µm. GC temperature was maintained at 50° C for 2 minutes and then programmed to rise to 240° C at 6° C/min. Helium gas was used as carrier gas. Samples were analysed by using a mass spectrometer detector (Agilent 5973MSD)

7.3.1 Volatile Collection Method 1

On 16 April six accessions of potato tubers (3 dormant and 3 non-dormant) were taken out from storage in an incubator at 3-4º C and then placed separately at ambient temperature for 4-5 hours. One tuber of each of these accessions was placed separately in 500 ml sealed glass jars, with sampling ports fitted with resealing silicon seals. The glass jars were kept at ambient temperature overnight. The accessions used for the experiment are given below.

- NTB-14 dormant
- NTB-41 dormant
- NTB-96 dormant
- NTB-57 non-dormant
- NTB-224 non-dormant
- NTB-275 non-dormant

A solid phase micro-extraction fibre was inserted through a seal in each glass jar for 30 minutes to absorb the volatiles and then the fibres were transferred into the injection port of GC-MS, with the help of the syringe-like handling device, where desorption of the analyte took place and analysis was carried out on the GC-MS for separation and quantification of the analytes (Plate 7-1)

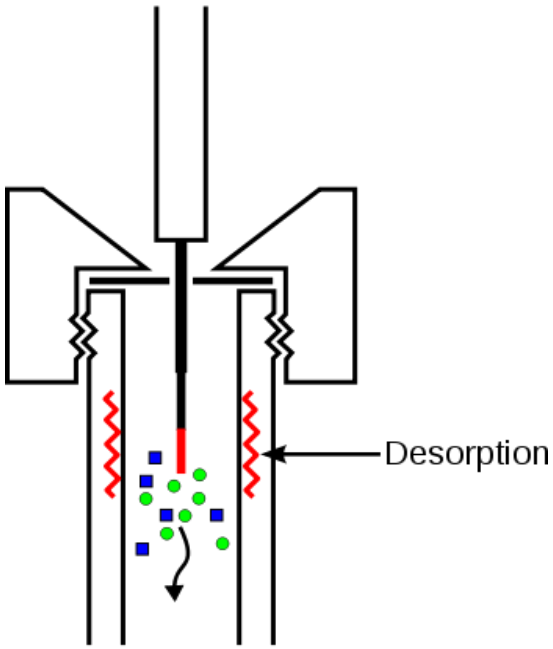


Plate 7-1 Solid phase micro-extraction (SPME) showing fiber with syringe-like holding device

7.3.2 Volatile Collection Method 2

A solid phase micro-extraction (SPME) fiber was rubbed directly over the uncut surface of a potato tuber, NTB-14 (dormant) and NTB-57 (non dormant) for about 1 minute and then GC-MS was used for analysis.

7.3.3 Volatile Collection Method 3

One tuber of NTB-14 (dormant), and one of NTB-57 (non dormant) were peeled to a thickness of about 1-2 mm, using a potato peeler. The peel and remaining tuber was placed in two separate beakers and sealed for 2-3 hours using aluminium foil. A solid phase micro-extraction fiber was then inserted into each beaker for 30 minutes before analysis.

7.3.4 Volatile Collection Method 4 (peel extraction)

In this study freshly grown tubers were used, on the basis that they would be more deeply in dormancy and might be producing more volatiles than more mature tubers. In November 2006, tubers from 18 accessions of potato (9 dormant and 9 non-dormant) were removed from storage (4º C) allowing 4 weeks of storage and then placed separately at ambient temperature for 4-5 hours. Then each tuber was peeled to a thickness of about 2-3 mm, using a potato peeler. Peel from each tuber was wrapped in aluminium foil and dropped into liquid nitrogen. Frozen samples were transferred into pots and stored in a -80° C freezer prior to analysis.

For analysis, the samples were freeze dried (48 hours). Dried samples were ground in pestle and mortar and divided into three parts. These were extracted (.50g) in glass bottles in 5 ml 1:1 diethylether:hexane (vol:vol) or 5 ml ethyl acetate +/- dimethylnaphthalene (10 ppm), samples were placed overnight in a fridge after being shaken for 15 minutes using hands. Then a sample of supernatant was taken. Final samples were analysed by GC-MS. More peaks were detected in extracts with ethyl acetate and it was decided to use this extraction method for further samples.

7.4 Results

7.4.1 Assessment of volatiles profiles using volatile collection (Method 1, 2 and 3)

There was no reliable peak difference between samples. No clear differences were seen in dormant (Figure 7-1) and non dormant tubers (Figure 7-2). Although peaks were observed at the retention time attributed to 1, 4-DMN levels were so low that no conclusion could be made as to whether 1, 4 DMN was present. These volatile collection methods do not allow quantification of volatile content so that no prediction from the scientific literature could be make as to whether 1, 4-DMN or any other volatile would be observed. It was observed that sprouting was initiated in all dormant tubers by the last week of April, approximately 5 months after harvest.

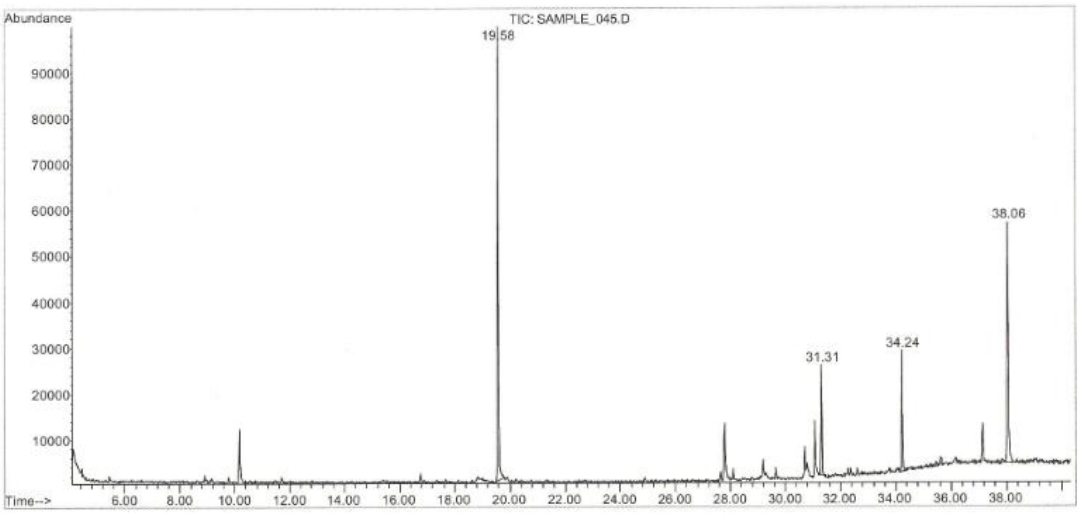


Figure 7-1 A GC trace obtained from a SPME collection of volatiles from NTB- 25 (dormant tuber)

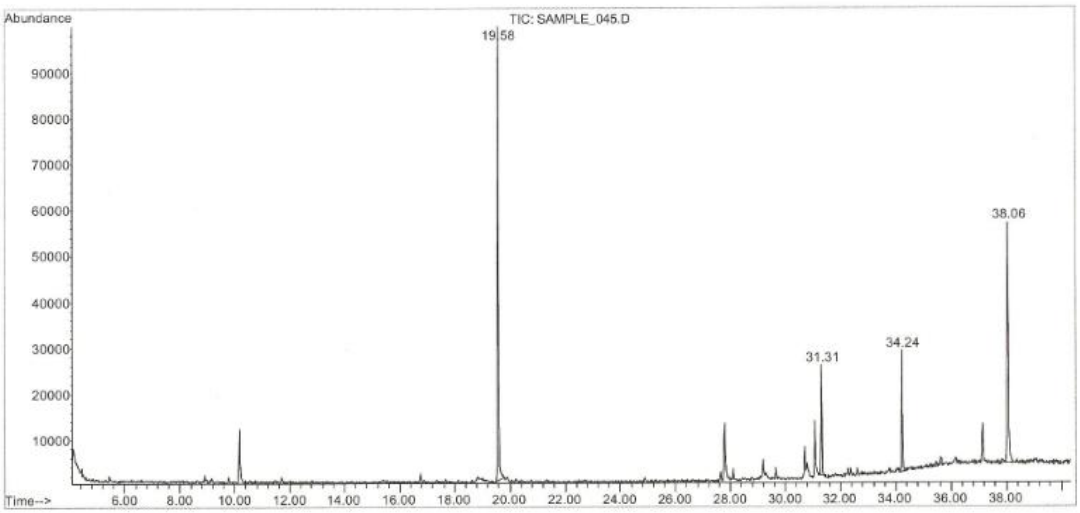


Figure 7-2 A GC trace obtained from a SPME collection of volatiles from NTB- 273 (non dormant tuber) with SPME

7.4.2 Assessment of volatile profiles obtained by peel extraction (Method 4)

On the basis of SCRI findings, it was assumed that dormant and non dormant tubers behave differently in terms of sprouting. Figure 7-3 illustrates the differences in sprout rate of the 14 accessions (seven dormant and seven non dormant). Originally, we were supplied with 18 accessions, but during further propagation we were able to obtain production from 15 accessions. So we selected seven dormant and seven non dormant accessions for comparing sprout growth. Results were in support with SCRI findings as dormant accessions remained dormant for longer, and accessions defined as non-dormant showed considerably higher sprout growth. On the basis of these findings, four dormant and four non dormant accessions were selected for further studies (peel extraction). Among chosen accessions most dormant were , NTB 16, NTB 25, NTB 26, NTB 59 and most non dormant accessions were and NTB 273, NTB 275, NTB 340 and NTB 427.

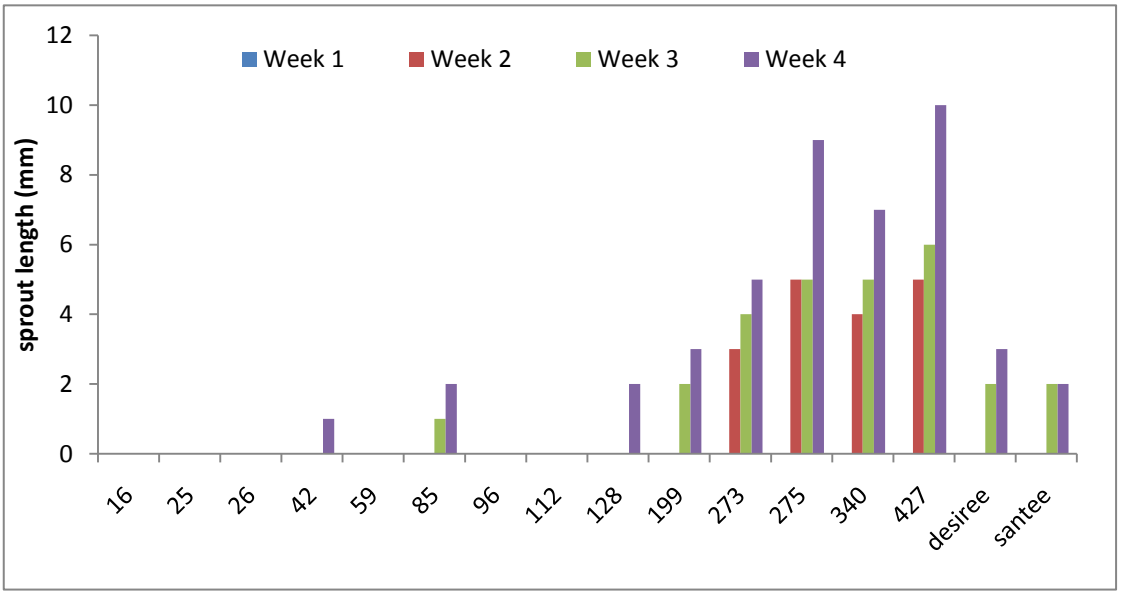


Figure 7-3 Comparison of average sprout growth of apical bubs when they were stored at room temperature 25˚C for 4 weeks. Each data point is the mean of 2 tubers.

There were no obvious consistent differences in GC traces obtained from dormant tubers compared to non dormant tubers. Figure 7-2 shows typical GC traces obtained from peel extracts of NTB-16, 25, 26 and 59 (dormant accessions) which include the standard dimethylnaphthalene and Figure 7-3 shows the same for non dormant accessions (NTB-273, 275, 340 and 427). No peaks were observed near to the retention time of 1, 4-DMN retention time in traces where the standard was not used (data not shown). Mass spectrometry indicated that peaks observed were mainly straight chain hydrocarbons. These are likely to be membrane lipids, and are unlikely to have a sprout suppressant function, as they are unlikely to inhibit meristem activity and signaling compounds usually have more complex ring structures.

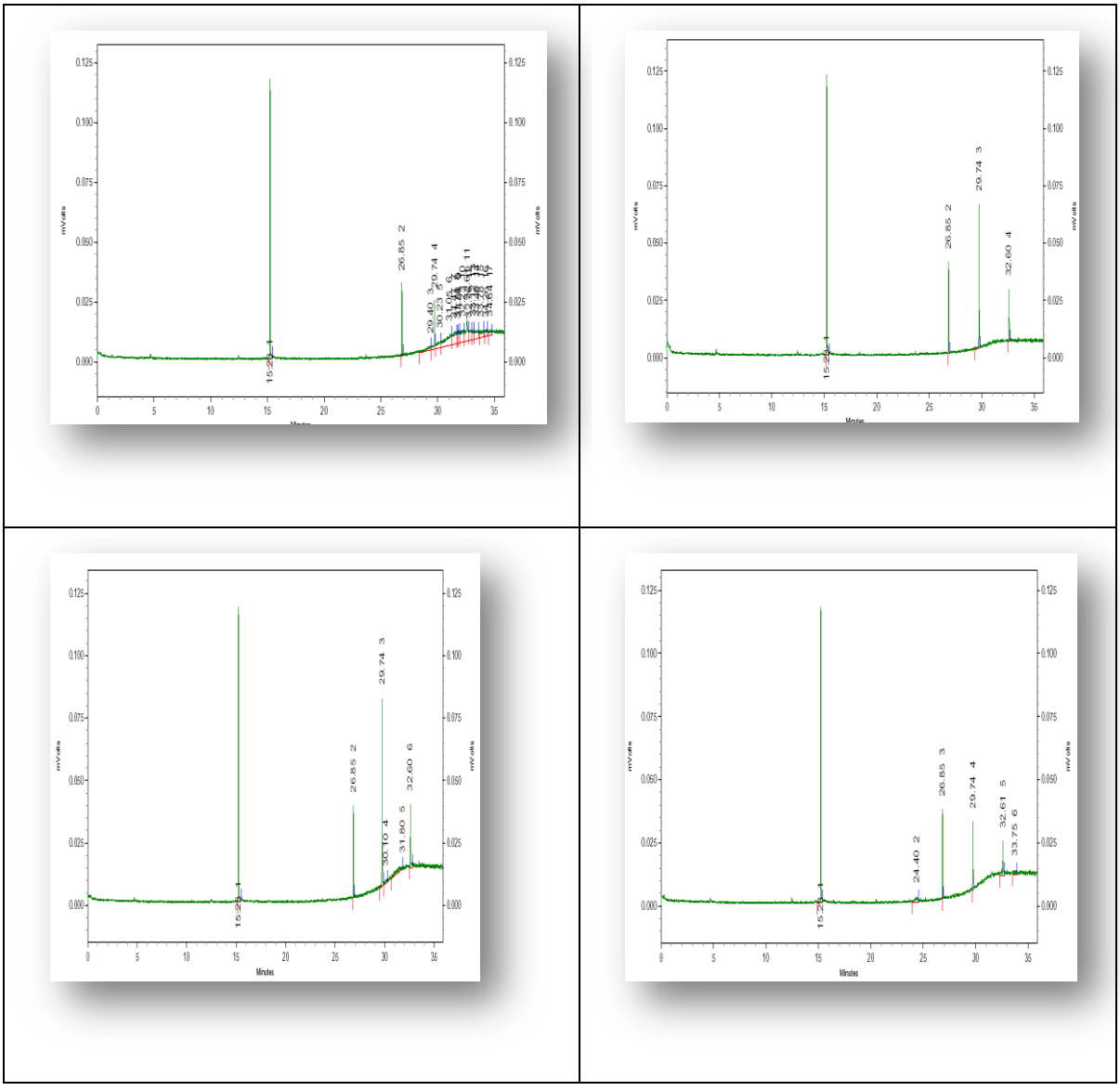


Figure 7-4 A range of peaks in NTB-16, 25, 26 and 59 (Dormant tuber peel extraction in diethyl ether) with standard 1, 4 DMN at rate of 10ppm/fresh weight.

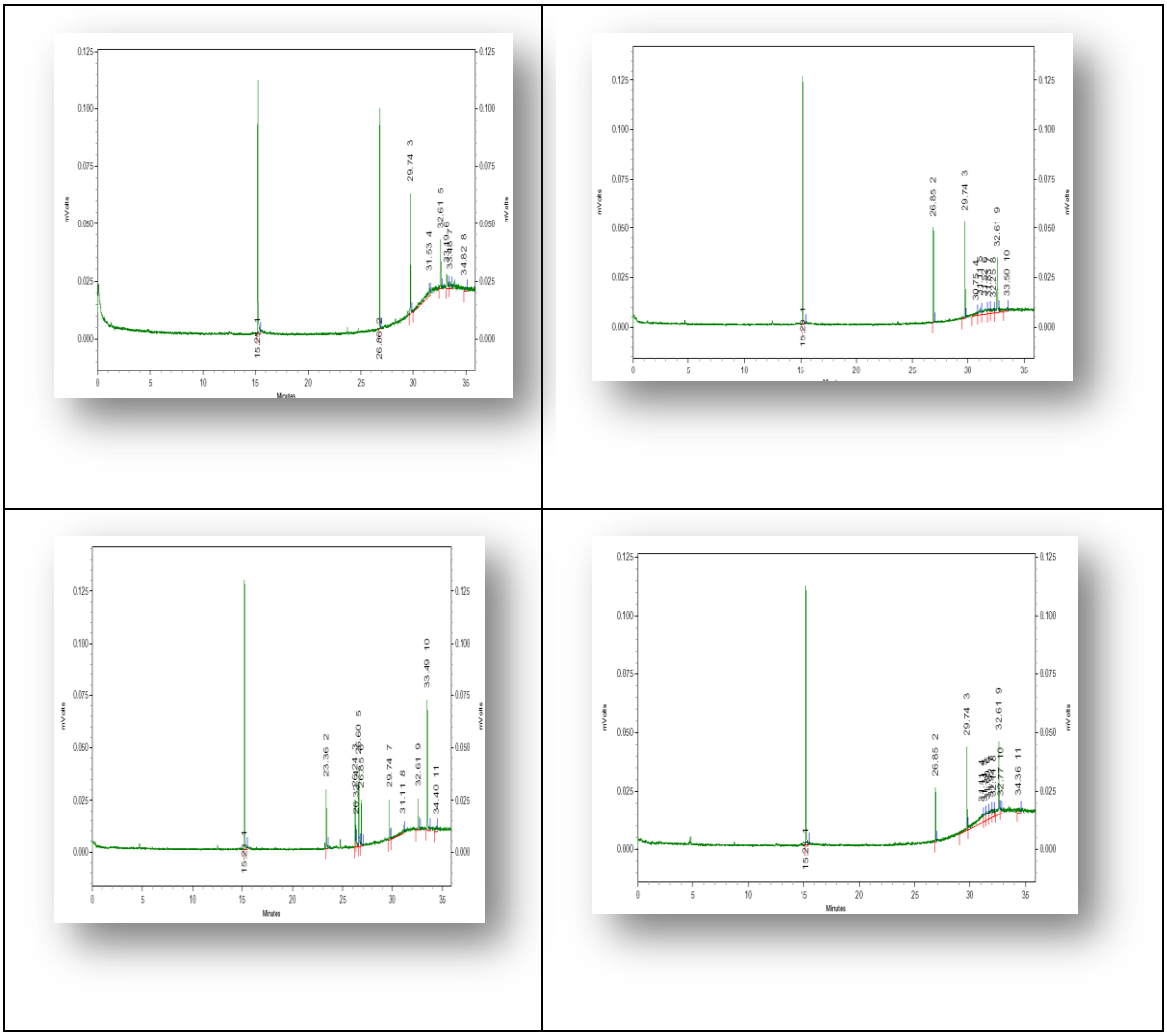


Figure 7-5 Typical GC traces obtained from diethyl ether extracts of tuber peel of dormant potato accessions, NTB- 273, 275, 340 and 427. A standard, 1, 4- DMN is included at 10ppm/fresh weight.

With the equipment being used there was no method available for profiling of the volatile range. Therefore in order to check for any differences in volatile profile between dormant and non-dormant accessions, a manual method was used. The total peak area over 5 second periods of retention time was calculated. This is illustrated in Figure 7-6. No consistent differences can be observed between dormant and non-dormant accessions.

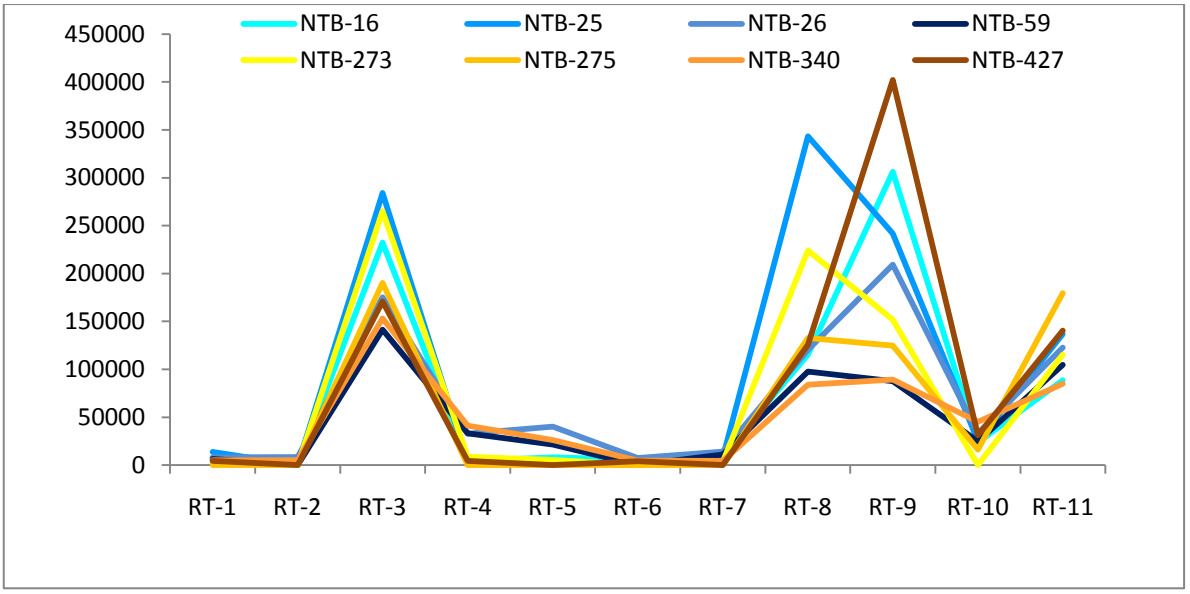


Figure 7-6 Comparison of different peaks in dormant and non dormant accessions. Each data point is mean of three replicates

7.5 Summary of main findings

- Potato accessions previously defined as dormant and non-dormant differed in sprouting characteristics as predicted.
- 1, 4-DMN the natural sprout suppressant could not be detected from any of the accessions, even though the system was sensitive to below 100ppb.
- No difference in volatile profile from peel extracts could be detected

7.6 Discussion

It is believed that dormancy is controlled by endogenous and exogenous factors. As early as 1960s, scientists identified several naturally occurring volatile bio chemicals that evolve from stored potatoes and began evaluating their ability to suppress sprouting. 1, 4-DMN was identified as one of the most potent natural sprout suppressants (Meigh *et al*., 1973).

The objective of this work was to collect the volatiles produced by potato tubers that might be involved in dormancy control and search for the natural 1, 4-DMN. It was hoped to collect a sufficient amount of natural 1, 4-DMN for qualitative and quantitative measurements. A series of experiments were conducted for this purpose. In the first experiment, the volatiles from one potato tuber in a jar were collected over 24 hours at ambient temperatures. A few small peaks were eluted around the retention time of 1, 4-DMN. However, it was not possible to confirm the identity of these peaks, due to the small size of these peaks and the relatively high noise in the baseline. It was decided to repeat the experiment with freshly produced tubers as the age of tubers could have contributed to the loss of some of the volatiles, therefore reducing the collected amount of potato volatiles in samples. With SPME, the amount of extraction is very small compared to the sample volume. As a result, exhaustive removal of analytes to the extracting phase could not occur.

8 Conclusion and Future Work

Root and tuber crops are key staples in all regions of the world. Of these, potato (*Solanum tuberosum*) is the most widely grown. With worldwide production at 300 million metric tons/year it is the world's fourth most important crop species. However, yams (*Dioscorea* spp), sweetpotato (*Ipomeae batatas)* and cassava (*Manihot esculenta*) are also vital for food security in many tropical regions of the world.

The control of dormancy in root and tuber crops is of great developmental and economic importance. In potato tubers destined for processing, maintenance of tuber dormancy is a critical aspect of successful potato storage. Reversibly, rapid termination of tuber dormancy is desirable for certain segments of the potato industry such as seed certification trials and same-season use of seed potatoes for southern markets. Sprouting in potato tubers is associated with quality loss e.g. increases in reducing sugar, increase in respiration, water loss and increase in glycoalkaloid content (Burton, 1989; Suttle 2004a).

Sweetpotato (*Ipomoea batatas*) is a major tropical root crop grown in Sub-Saharan Africa and it plays a vital role in the food security and economies of many countries. The importance of this crop to poor people makes it a key target for making an impact on poverty world-wide. Storage potential of sweetpotato is limited through early sprouting under many conditions. This has highlighted the need for increasing the understanding of the mechanisms of sweetpotato root dormancy and sprouting in order to generate new ideas for storage potential in order to extend storage life.

8.1 Present state of knowledge on dormancy control in potatoes

The use of low temperature storage to extend shelf life and prolong dormancy for ware potatoes is widespread. However, the temperatures used for storage must be kept above a certain level to prevent low temperature conversion of starch to sugar and consequent poor processing quality. Further, in some situations (e.g. African highlands, and some parts of Central America) controlled temperature storage is not feasible. A number of sprout suppressants have been identified for use with potato. Of these, CIPC (isopropyl-N-chlorphynyl carbamade) is the most effective in current use for ware potatoes, but permitted levels are being reduced due to human health concerns. A recent Environmental Protection Agency mandate, from the requirements of Food Quality Act (FQPA) of 1996, resulted in a reduction in allowable CIPC residue on fresh potatoes in the United States from 50 ppm to 30 ppm (Kleinkopf *et al.,* 2003). For storage of seed potatoes, where sprout inhibition must be reversible, the most commonly used commercial sprout suppressants are dimethylnaphthalene and carvone (a natural product that can be isolated from caraway seeds) (USEPA, 1995 and Brown *et al*., 2000).

A number of studies have been conducted to understand the hormonal control of tuber dormancy (see details in chapter 2) (reviewed in Suttle, 2004a). They concluded that both Abscisic acid (ABA) and ethylene are required for the initiation of tuber dormancy, but only ABA is needed to maintain the dormant state. Cytokinins are involved in dormancy break. Thus endogenous cytokinins levels are relatively low in highly dormant tubers and tubers are non-responsive to exogenous cytokinins. During dormancy tubers actively metabolise ABA and cytokinins to inactive products. As dormancy weakens, tuber ABA levels decline and tubers become increasingly sensitive to exogenous cytokinins. Gibberellins are also required for sprout growth promotion. Recently ethylene has been introduced as a method to control potato sprouting. It has been registered for

commercial use in the UK since 2003 (Prange *et al*., 2005). Suttle was also looking at the possibility of using synthetic forms of plant hormones for sprout control (Suttle, 2005).

8.2 Sprout control in sweetpotato

Given its economic importance, research into the control of dormancy is much more advanced in potato than the other root crops. While research on the improvement of storage of sweetpotato has not received attention. Improving dormancy /sprouting behaviour is essential to achieve improved postharvest storage of sweetpotatoes and these developments will contribute to reducing poverty and enhancing food security and income growth.

Although root crops may be of different botanical origin, (tubers or roots), there are many common mechanisms involved in control of dormancy/sprouting. Sweetpotato is a subterranean storage organ which accumulates starch. Although the harvested part of sweetpotato is also often referred to as a tuber, it is in fact a lateral root in which starch has accumulated. Potato tubers have defined places to sprout (eyes) but sweetpotato roots don't have any defined areas to sprout. Although potato and sweetpotato are not related, and the storage organs are of different botanical origin (tuber *vs.* root), it is known that many of the sprout suppressants used for potato, including CIPC, maleic hydrazide and methyl esters of alpha naphthalene acetic acid are also effective on sweetpotato (Paton and Scriven 1989). The effectiveness of carvone is not widely reported. Interestingly hot water treatment has been tested and found to be fairly effective for extending dormancy in sweetpotato (Tanaka *et al*., 2001)

Before comparisons between potato and sweetpotatoes can be made it is important to consider the similarities and differences between the two fresh produce types that may have implications on interpretation. Potatoes and sweet potatoes both form underground storage organs (tuber and root), and are important crop species comprising various cultivars displaying a range of potential storage lives. Storage life can be influenced by both pre- and postharvest factors, and the role of hormones in control of dormancy and sprouting is considered to be of importance. However, potatoes have a many preformed growing points (eyes) and sweetpotatoes have no growing point (eye). Taking into account these similarities and differences between the two crops will allow considered conclusions to be drawn from studies concerning dormancy control.

8.3 Project Conclusions

A brief summary of the conclusions of the project is given below.

An examination of the role of gibberellins in sprout control in sweetpotato

In sweetpotato roots:

- Gibberellins (10^{-3} M GA_3) increase rate of sprout growth and number of sprouts.
- Piccolo (20 ml/L), an inhibitor of GA synthesis slows sprout growth and reduces number of sprouts.
- Regalis shows no effect on sprout growth or number of sprouts.
- There is no indication of an effect of Gibberellins on timing of sprout growth (dormancy). Roots after all treatments had sprouts after 1 week

- Stimulation of sprout growth by GA is consistent with behaviour of potato tubers

To investigate the involvement of ethylene in the sprout control by storing roots in continuous presence of ethylene

In sweetpotato roots:

- Continuous exposure to ethylene inhibits sprout number and growth rate in a concentration dependent manner. The effect saturates at 10 - 40 ppm.

- Ethylene delays sprout growth.

- There are indications that ethylene increases rate of fresh weight loss during storage.

- Ethylene increases root respiration rate three-fold. There is no ethylene concentration effect over the range 5 – 40 ppm

Effect of ethylene, an ethylene antagonist and an ethylene synthesis inhibitor on sprouting of sweetpotato in storage

In sweetpotato roots:

- As observed in Chapter 4 continuous exposure to ethylene inhibits sprout growth and number.

- Treatment with 1-MCP, an ethylene antagonist, inhibits sprout growth and number in the presence and absence of ethylene.

- Treatment with AVG, an ethylene synthesis inhibitor, inhibits sprout growth and number in the presence and absence of ethylene.

- Root respiration is increased by ethylene. This effect is reduced by 1-MCP and by AVG.

- Ethylene leads to a decrease in concentrations of fructose and glucose.

Investigating the role of gibberellins and cytokinins in the control of dormancy and sprout growth in potatoes *(Solanum tuberosum* L) using potato accessions transformed for the isoprenoid synthetic pathway

In potato tubers

- Removal of apical dominance by excision of potato eyes from tubers is not sufficient to terminate dormancy.

- Dormancy of potato discs used in these experiments could be broken by addition of either gibberellins or the cytokinin *t*Zr.

- DXS1 and DXS2 tubers must have exited dormancy at the point that eye sprouting occurred. However, they seem to have re-entered a "meta-dormant" state. In 2007 DXS1 seems to have been in a deeper dormant state than the other lines that could not be broken by either gibberellins or *t*ZR. In the other cases exit from the "meta-dormant" state could be triggered by either gibberellins or *t*ZR in the same way that dormancy could be broken in the other lines.

- Addition of GA3 induces multiple buds in whole tubers, but not in excised buds.

An investigation of the volatile profile of tubers from potato lines that differ in their dormancy characteristics

In potato tubers

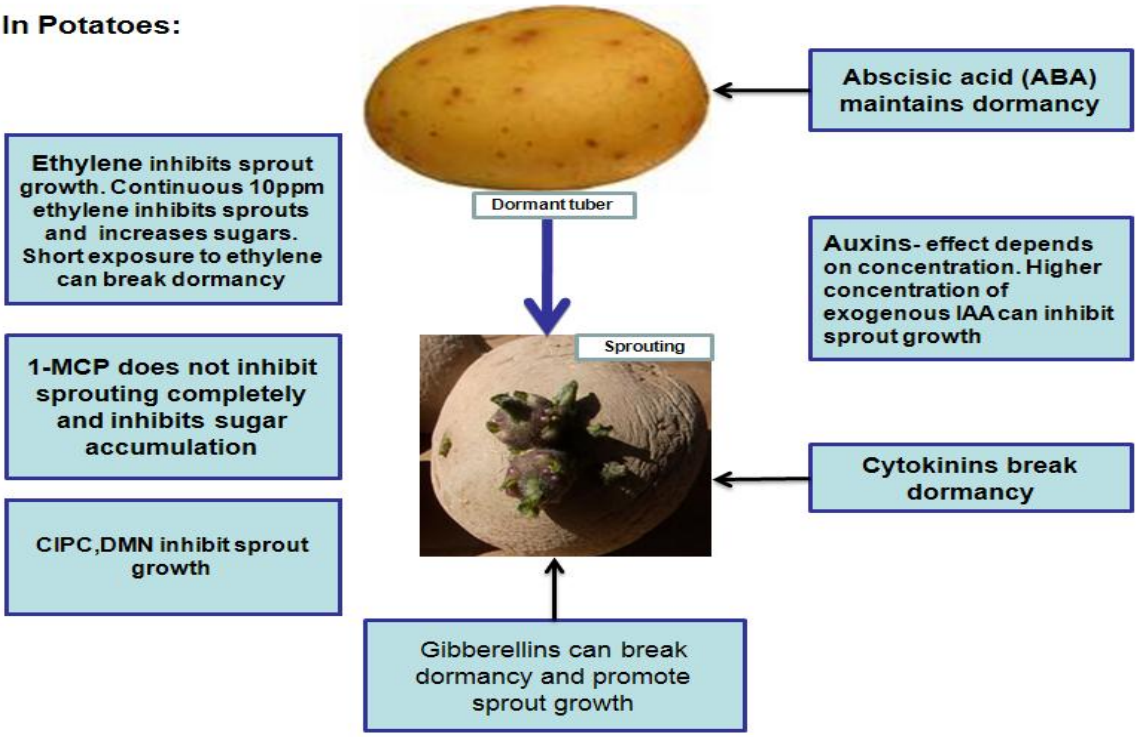
- Potato accessions previously defined as dormant and non-dormant differed in sprouting characteristics as predicted.

- 1, 4-DMN the natural sprout suppressant could not be detected from any of the accessions, even though the system was sensitive to <100 ppb.

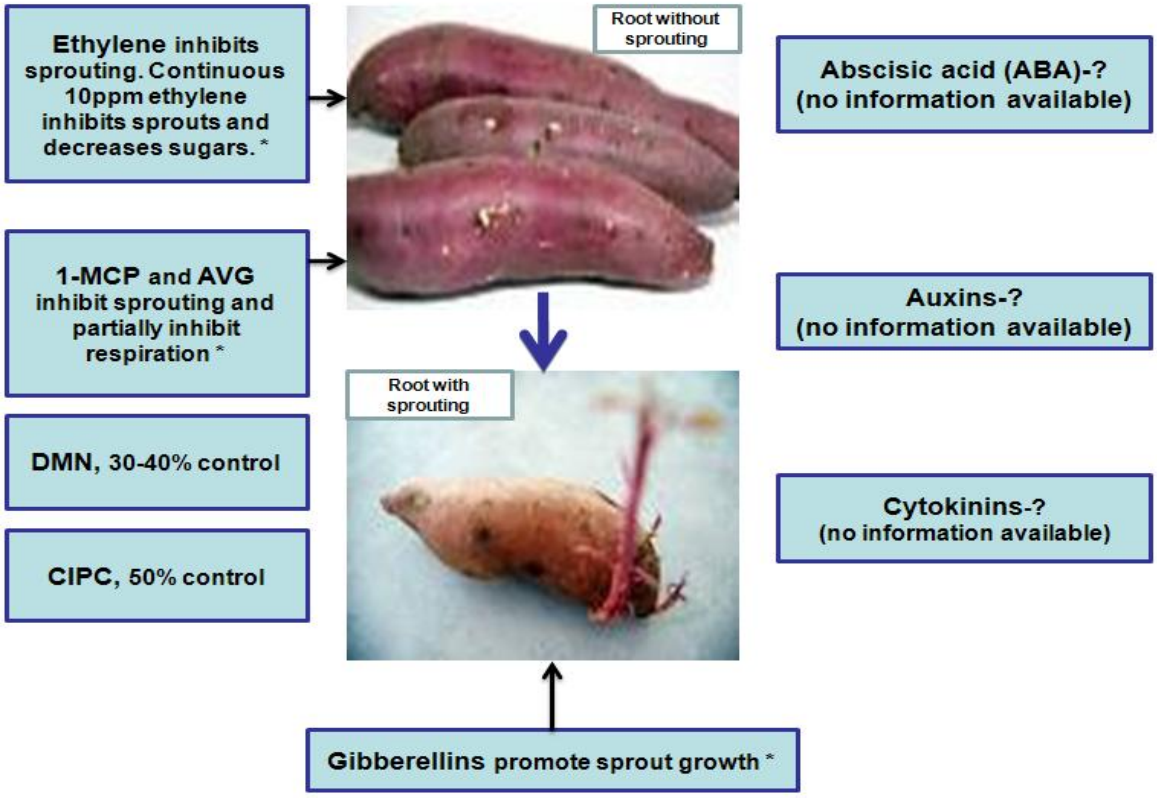
- No difference in volatile profile from peel extracts could be detected.

A comparison of the the control of sprouting in potatoes and sweetpotatoes

In Potatoes:



In Sweetpotatoes:



* Results are presented first time in this thesis or associated publications.

Gibberellins stimulate sprouting in sweetpotato, consistent with effects on potato

It is likely that dormancy and sprout suppression are under the control of a combination of factors, and in sweetpotatoes the role of growth hormones, specifically gibberellins in these processes was previously unreported. Gibberellins (GA) have been demonstrated to play a functional role in promoting sprout growth in potatoes (Suttle, 2004a). It was postulated that GAs are important in controlling dormancy and sprouting in sweetpotato roots. To test this hypothesis, novel work to investigate the role of GAs and GAs synthesis inhibitors was undertaken. A treatment of GA_3 was shown to promote sprout growth in stored roots and roots treated with gibberellins synthesis inhibitor (Piccolo) was shown to reduce sprout growth (Chapter 3).

The effect of gibberellins on sweetpotato is consistent with the effects on potato suggesting that control of sprout growth is similar in the two species. This work has been published in Cheema *et al*. (2008). Further investigation of the mechanism by which this occurred would be valuable.

Ethylene inhibits sprouting in potatoes and sweetpotatoes, and stimulates respiration

Continuous exposure to ethylene (4 µl/L, ppm) has been shown to be an effective method of sprout control in potatoes (Prange *et al*., 2005). Increasing the concentration of ethylene to 40 µl l/l increased inhibition of sprout growth, but not enough to justify the additional costs of applying this chemical in a commercial environment (Daniels-Lake *et al*., 2005). This is in line with results reported in Chapter 4 where sweetpotato roots exposed to 10ppm ethylene for 4 weeks stored at 25°C showed sprout inhibition compared with untreated controls. This is the first report of ethylene control of sprouting in sweetpotato and is reported in Cheema *et al.* (2008). Increased respiration rates were

reported when sweetpotato roots were exposed to higher concentrations of ethylene (No rotting was observed in any of the treatments.) Higher respiration rates associated with ethylene are seen in many commodities, for example strawberries (Lannetta *et al*., 2006) and sugarbeet roots (Fugate *et al*., 2010).

In potatoes there is an increase in sugars associated with ethylene treatment, but the observation for sweetpotatoes was that sugars decreased – even though in both cases respiration increased. One explanation for this is that in potatoes with low sugar reserves, starch is mobilized, whereas sweetpotatoes have sugars stored in vacuoles which can be used.

Study suggested that ethylene play a vital role in sweetpotato roots sprout control and if understood in more detail there would be the potential to manipulate these parameters in order extend to storage life of sweetpotato roots. Constant supply of ethylene will be key during storage of sweetpotato roots. Perhaps storage efficiency can be boosted in developing countries by storing ethylene producing crops together with sweetpotato roots.

The effects of 1-MCP treatment on potato and sweetpotato

Unlike sweetpotatoes, exposure of stored potatoes to ethylene has the undesired side-effect of darkening the fry colour upon processing. This is caused by an accumulation of fructose and glucose in tubers stored in the presence of ethylene. Fry colour darkening can be prevented by application of 1-MCP (0.9 µl /L) prior to ethylene exposure and at subsequent monthly or bimonthly intervals (Daniels-Lake *et al*., 2005a). Thus, 1-MCP reduces the rate of ethylene-induced sugar accumulation in potato tubers, despite the fact that it does not block the ethylene inhibitory effect on sprout growth. Although ethylene can be used as a method of sprout control for potatoes, it reduces the true dormant period (defined by the number of days from planting to shoot emergence) in comparison with control tubers stored in air, (Pruski *et al*., 2006). In potatoes, 1-MCP does not completely

inhibit sprouting. Possibly it could be that potatoes have preformed eyes and are more metabolically active than growing sites of sweetpotatoes. There could be rapid production of ethylene binding sites in potato eyes. Taken together, this suggests that ethylene breaks dormancy in potatoes, but subsequently suppresses sprout elongation. To investigate the effect of 1-MCP on sweetpotato roots a study was conducted (chapter 5). Interestingly 1-MCP strongly inhibited sprout growth in sweetpotato roots. The simplest explanation of the inhibitory effect of 1-MCP on sprouting is that ethylene is necessary for initiation of sprouting. This suggests a two stage process as in potato tubers; break of dormancy, sprout growth. 1-MCP partially inhibited the ethylene stimulation of respiration and resulted in a corresponding level of sugars half way between that in ethylene treated and control roots. Lower respiration rate was reported in 1-MCP treated roots.

An alternative approach for the 1-MCP effect could be put forward in which blocking of the negative feedback of ethylene towards its own synthesis leads to an increase in endogenous ethylene. Ethylene production by whole grapefruit was increased four days after treatment with 1-MCP (0.05 µl/L/kg/h) compared with untreated controls (0.005 µl/L/kg/h) (Mullins *et al*., 2000). However, the increase in ethylene production was observed soon after 1-MCP treatment and may therefore have been a transient effect. The authors concluded that as ethylene biosynthesis is under negative feedback control, the increase in ethylene concentration was caused by 1-MCP binding to ethylene binding proteins, thus blocking the negative feedback effect of ethylene on its own biosynthesis and leading to uncontrolled ethylene production. If 1-MCP also increases ethylene production in roots, this could explain the sprout inhibiting effect of 1-MCP. This study suggests the use of an inhibitor of ethylene perception to extend storage of roots. It would be a logical extension to this work to investigate the effects of 1-MCP on a range of cultivars and to monitor sugar contents concentration throughout storage.

Response to AVG of sweetpotato roots

AVG inhibits sprouting. This fits with the hypothesis that sprouting is a two step process, and that ethylene is needed for dormancy break (sprout initiation). AVG partially inhibits ethylene stimulated respiration. One explanation is that AVG affects more metabolic pathways than those attributed to its mode of action in the inhibition of ethylene biosynthesis. AVG inhibits pyridoxal phosphate enzymes (PLP), (Toney, 2005). PLP acts in all transamination of amino acids. Thus AVG inhibits protein biosynthesis in tomato (Saltveit, 2004) and starch degradation in apples (Silverman *et al.*, 2004).

Strength of dormancy and break of dormancy in potato tubers

It has been observed that GAs can break dormancy (Willmitzer, 2001) but are more active in sprout initiation (Suttle, 2004b). According to Suttle and Banowetz (2000) cytokinins are responsible for termination of dormancy but have no effect on further sprout growth. In the preset thesis a system of excised buds was used (chapter 6). Contrary to the view that GAs are not involved in dormancy break, GAs appeared to break dormancy and also to promote sprout growth in excised buds of all lines (except DXS1 in 2007). The excised buds appeared to break dormancy more easily than whole tubers. GA promoted sprouting within 2-3 days, where for whole tubers sprout growth was delayed for 28 days. Treatments with cytokinin and gibberellins resulted in significantly higher growth rates than control treatments. The increase in growth rate can be ascribed to the increase in cells able to complete the cell cycle. An increase in cell number would lead to an increase in growth rate (Doonan, 1996). In 2007, DXS1 showed no response to growth regulator after 4 week of harvesting. But after 12 week of harvest and in 2009 after 4 week of harvest, buds were responsive to treatments. DXS1 and DXS2 were more responsive to GA and *t*ZR. This suggests that initially DXS1 was in a deeper state of dormancy than the other lines. The fact that DXS1 and DXS2 had buds indicates that at some point, presumably when on the plant in the soil, the eyes must have broken dormancy, and then

returned to a dormant state. The concept of potato eyes going in and out of dormancy in this way is quite novel, and provides an exciting system to study dormancy. There could be the possibility that the response to exogenous hormones changes with endogenous levels of hormones. Thus in 2007 DXS1 was not responsive to exogenous levels we used. With increased DXS expression it is expected that cytokinin levels are higher. DXS1 behaved the same as DXS2 in 2009 even after 4 weeks of harvest. It was noted that tubers used in 2007 were much bigger than tubers that were used in 2009. There could be possibility that other agronomic factors contribute toward endogenous levels of growth hormones.

Does 1, 4-DMN act as a sprout suppressant *in vivo* and are there other identifiable sprout suppressants

In chapter 7 a study was conducted to look at the chemical composition of a range of potato lines that differed in dormancy characteristics. Observations confirmed the classification of the lines, suggesting that the dormancy characteristics were stable over seasons. Given the postulated role of 1, 4-DMN as an intrinsic sprout suppressant, it was assumed that more 1, 4 DMN will be present in dormant tubers than non dormant tubers. Although a signalling compound such as gibberellins can have an effect at very low levels (0.5 ppb) a compound with a direct inhibitory effect on the meristem (as postulated for 1, 4-DMN) would have to be present at higher concentrations. Likewise the presence of CIPC in potato tubers suggests that 1-DMN suppress sprout growth instead of extending dormancy. However the mode of action of 1, 4 DMN is different from CIPC and not clear yet (Campbell *et al.,* 2010). In this study no reliable peak was detected in either dormant or non dormant tubers. Results are in support of investigations conducted by Walker *et al.* (2004) where they found very low levels of 1, 4 DMN and assumed that it could be due to environmental or soil contamination. Having contamination is not impossible in potatoes. A detailed study is needed where potatoes can be grown with extra care to avoid contamination. There is also need to develop a system where bigger quantity can be used

to collect volatiles and then samples can be run on GC where more concentrated samples can be injected to check the natural production of 1, 4 DMN in tubers.

It had been hoped that intrinsic sprout suppressants could be identified in the peel. No obvious differences in peel composition were observed between dormant and non-dormant accessions.

Sweetpotatoes and dormancy

Sweetpotato roots can be stored for months at low temperature (13-15˚ C). However, sprouting could be induced in all sweetpotato roots tested in this study at 25 C˚, suggesting that sweetpotato roots have “ecodormancy” and can be influenced by external factors. This is also supported by the observation that planted roots subjected to water deficit do not sprout and can be stored for longer (water stress), but they start growing as water is given to roots (Richard Gibson personal communication). 1-MCP data also provides strong evidence of a two stage activation of sprouting i.e.; dormancy with an ethylene requirement for the initial activation.

No eyes were visible on roots. When sweetpotatoes sprout, the bud seems to form below a uniform periderm. Non- uniform sprouting was observed over whole root in series of experiments. Distribution pattern of sprouts was cultivar dependent.

Sweetpotatoes appear to respond to most sprout suppressants (i.e. CIPC) used for potato. These often act on preformed meristems. This suggests that sweetpotato roots have growing meristems below the surface from where sprouting begins.

8.4 Future work

It is clear that suggestions in this study were based on the available knowledge in potatoes. Significant progress has been made in establishing the sprout inhibiting effect of ethylene for storage extension in sweetpotato roots. However it was not possible to test or confirm all the aspects of dormancy/sprouting during this project, and there is clearly a need for further work.

Unlike potatoes, sweetpotato roots have no obvious eyes. It has been suggested that they do not have a preformed meristem, so that sprouting sites are not predetermined but are created when sprouting is stimulated. Microscopy of roots would also be useful to understand the sprouting behavior in sweetpotato roots. Effects of CIPC, which attacks growing meristems, could be used to test the hypothesis that sweetpotato roots have a preformed active mersitem below the surface. Sweetpotatoes do not sprout when stored at 15ºC, CIPC can be applied at this temperature and then roots moved to 25˚ C to allow them to sprout. If sweetpotato roots have a growing meristem under the skin then there will be no growth as CIPC acts directly on growing tissues, however if the meristem forms at the point of sprout stimulation CIPC would not be effective..

Ethylene has clearly been identified as playing an important role in sweetpotato root development (Chapters 4 and 5). Therefore, the mechanisms controlling ethylene biosynthesis within the roots during the different physiological stages in stored roots should also be investigated in more detail.

Measurements of endogenous level of growth hormones including ethylene, estimation of 1-aminocyclopropane-1-carboxylate synthase activity (ACS) and more accurate assessment of effects on sugar content at different time periods of storage would be of value in order to obtain better understanding of mechanism underlying dormancy and

sprouting processes. To elucidate the role of ethylene the effect of multiple applications of 1-MCP should also be checked.

Different concentration of growth regulators should be used in sweetpotato roots to see the effect on sprouting. In the long-term it would be useful to identify molecular markers of sprout suppression and dormancy in combination with physical and biochemical traits, as this will further enhance the understanding of the genetic mechanisms underlying these physiological processes.

Unlike sweetpotato, in the case of yams (*Dioscorea* spp*.)*, standard potato sprout suppressants are not found to be effective, such as CIPC which acts directly on the growing meristems. Yams are said to have no preformed meristem (Wickham *et al*., 1984) and behave in contrary manner to potato and sweetpotato; i.e., GA inhibits sprouting (Okagami and Nagao, 1971; Okagami and Tanno, 1993). It will be of value to compare the sprouting behaviour of sweetpotato roots and yams to understand the dormancy/ sprouting.

References

Adams, D.O. and Yang, S.F. (1979). Ethylene biosynthesis: identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. *Proceedings of the National Academy of Sciences, USA* **76**, 170-174.

Afek, U. and Warshavsky, S. (1998). Problems in storage of potatoes in Israel. In: Potatoes in Hot Climates, (D. Levy, ed.), Israel Agresearch, *Journal of the Agriculture Research Organisation, Agricultural Research Organization (ARO), Volcani Center, Israel* **9**, 97-114.

Afek, U., Orenstein, J. and Nuriel, E. (2000). Using HPP (Hydrogen Peroxide Plus) to inhibit potato sprouting during storage. *American Journal of Potato Research* **77**, 63-65.

Alam, S.M.M., Murr, D.P. and Kristof, L. (1994). The effect of ethylene and of inhibitors of protein and nucleic acid syntheses on dormancy break and subsequent sprout growth. *Potato Research* **37**, 25-33.

Alexander, R., Kagi, R. I. and Sheppard, P. N. (1983). Relative abundance of dimethylnaphthalene isomers in crude oils. *Journal of Chromatography* **267**, 367-372.

Anderson, H. M., Garthwaite, D. G. and Thomas, M. R. (2006). Potato stores in Great Britain 2004. York: *Central Science Laboratory*. Report number: 205. (Online) Available from:http://www.csl.gov.uk/aboutCsl/scienceGroupsAndTeams/fsg/pesticides/documents/potstores2004.pdf (Accessed 10 July 2007).

Arshad, M. and Frankenberger, W.T. (jr). (2002). Ethylene: Agricultural Sources and Applications, Kluwer Academic / Plenum Publishers, New York.Hopkins, W.G. and Huner, N.P.A. 2004. Introduction to Plant Physiology, 3rd ed. Wiley and Sons, USA.

Arteca, R.N. (1996). Plant growth substances: *Principles and Applications*, Chapman and Hall, New York.

Bachem, C., Van der Hoeven, R., Lucker, J., Oomen, R., Casarini, E., Jacobsen, E., and Visser, R. (2000). Functional genomics analysis of potato tuber life-cycle. *Potato Research* **43**, 297 – 312.

Baker, A., Brown, P.H. and Blake, M.R. (2000). Use of S-Carvone and 1, 4-Dimethylnaphthalene as Sprouting Inhibitors during Potato Storage. *Global Research and Development* (Eds. SM Paul Khurana *et al.*).

Bana, A., Bieliska-Czarnecka, M. and Klocek, J. (1984). Activity of endogenous cytokinins in potato tubers during dormancy and sprouting. *Bulletin of the Polish Academy of Sciences Chemistry* **32**, 37-41.

Barendse, G.W.M. (1975). Biosynthesis, metabolism, transport and distribution of gibberellins. In: H.N. Krishnamoorthy (ed.). *Gibberellins and plant growth*. 65-85. John Wiley and Sons, Inc, New York.

Baritelle, A.L., Hyde, G.M., Fellman, J.K. and Varith, J. (2001). Using 1-MCP to inhibit the influence of ripening on impact properties of pear and apple tissues. *Postharvest Biology and Technology* **23**, 153-160.

Bengochea, T., Dodds, J.H., Evans, D.E., Jerie, P.H., Niepel, B., Shaari, A.R. and Hall, M.A. (1980). Studies on ethylene binding by cell-free preparations from Cotyledons of *Phaseolus vulgaris* L. *Planta* **148**, 397-406.

Beveridge, J.L. (1979). Volatile potato sprout suppressant chemicals. *Ph.D. Thesis*, Glasgow University of Glasgow.

Beveridge, J.L., Dalziel, J. and Duncan, H.J. (1981a). The assessment of some volatile organic compounds as sprout suppressants for ware and seed potatoes. *Potato Research* **24**, 61-76.

Beveridge, J.L., Dalzeil, J. and Duncan, H.J, (1981b). Dimethylnaphthalene as a sprout suppressant for seed and ware potatoes. *Potato Research* **24**, 77-88.

Beyer, E.M. (1976). A potent inhibitor of ethylene action. *Plant Physiology* **58**, 268 -271.

Beyer, E. M. Jr. (1979). Effect of silver ion, carbon dioxide and oxygen on ethylene action and metabolism. *Plant Physiology* **63**, 169- 173.

Biale, J. B., Young, R. E. and Olmstead, A. J. (1954). Fruit Respiration and Ethylene Production. *Plant Physiology* **29**, 168 – 174.

Biemelt, S., Hajirezaei, M., Hentschel, E. and Sonnewald, U. (2000). Comparative analysis of abscisic acid content and starch degradation during storage of tubers harvested from different potato varieties. *Potato Research* **43**, 371-382.

Binder, M.B. (2008). The ethylene receptors: Complex perception for a simple gas. *Plant Science* **175**, 8-17.

Blankenship, S. M. (2001). Ethylene effects and benefits of 1-MCP. *Perishables Handling Quarterly*. Issue No. 108.

Blankenship, S.M. and Parker, M.L. (2001). Influence of 1-methylcyclopropene on Shelf Life of Peach and Nectarine Cultivars. *HortScience* **36**, 423-430

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

Boller, T., Herner, R.C. and Kende, H. (1979). Assay for and enzymatic formation of an ethylene precursor, 1-aminocyclopropane-1-carboxylic acid. *Planta* **145**, 293–303.

Bonsi, C. K. and Loretan, P. A. (1988). Sprout inhibition of gamma irradiated sweet potatoes. *HortScience* **23**, 827-830.

Bosset, E., Jacomino, A.P., Pinheiro, A.L. and Kluge, R.A. (2005). Delay of ripening of 'Pedro Sato' guava with 1-methylcyclopropene. *Postharvest Biology and Technology* **35**, 303-308.

Bower, J.H., Biasi W.V. and Mitcham, E.J. (2003). Effects of ethylene and 1-MCP on the quality and storage life of strawberries. *Postharvest Biology and Technology* **28**, 417- 423.

Boyleston, T.D., Powers. J.R., Weller, K.M. and Yanj, J. (2001). Comparison of sensory differences of stores Russet Burbank potato treated with CIPC and alternative sprout inhibitors. *American Journal of Potato Research* **78**, 99-107.

Brown, P, Blake, M, Baker, A, (2000). Carvone – A sprout regulator for seed potatoes? Australian Potato Research, Development and Technology Transfer Conference Proceedings, *Tasmanian Institute of Agricultural Research*, 1-3.

Brian, P.W., Hemming, H.G., and Radley, M. (1955). A physiological comparison of gibberellic acid with some auxins*. Plant Physiology* **8**, 899-912.

Bruinsma, J., Sinnema, A. Bakker, D. and Swart, J. (1967). The use of gibberellic acid (GA) and N- Dimethylaminosuccinamic acid (B9) in the testing of seed potatoes for virus infection. *European Potato Journal* **10**, 136-152.

Buchanan, B.B., Gruissem, W. and Jones, R. L. (2000). Biochemistry and molecular biology of plants. Third edition. American society of plant physiologists, Rockville, Maryland.

Bufler, G. (2009). Exogenous ethylene inhibits sprout growth in onion bulbs. *Annals of Botany* **103**, 23-28.

Burg, S.P. and Burg, E.A. (1967). Molecular requirements for the biological activity of ethylene. *Plant Physiology* **42**, 144-152.

Burton, W.G. (1989). *The Potato*. 3rd edition. Longman scientific and Technicaal. Essex, 470-504.

Buttery, R.G, Seifert, R.M, and Ling, L.C. (1970). Characterization of some volatile potato compounds. *Journal of Agriculture and Food Chemistry* **18**, 538-539.

Calvo, G. and Candan, A.P. (2001). Control de escaldadura Superficial mediante laaplicación de 1-metilciclopropene (1-MCP) en manzanas cv. Granny Smith. *Horticultura Argentina* **20**, 76-86.

Campbell, M A., Suttle, J.C. and Sell, T.W. (1996). Changes in cell cycle status and expression of p34cdc2 kinase during potato tuber meristem dormancy. *Plant Physiology* **98**, 743-752.

Campbell, M.A., Gleichnser, A., Alsbury, R., Horvath, D.P. and Suttle, J.C. (2010). The sprout inhibitors chlorpropham and 1, 4-dimethylnaphthalene elicit different transcriptional profiles and do not suppress growth through a prolongation of the dormant state. *Plant Molecular Biology* **73**,181-189.

Canoles, M. and Beaudry, R. (2001). Effects of single and continuous application of 1-methylcyclopropene on ripening of tomato. *HortScience* **36**, 35-37.

Carey, A.T., and D.A. Cronin, D.A. (1990). A comparative study of cold-sweetening in two potato cultivars. *Irish Journal of Food Science and Technology* **14**, 95-107

Carrera, E., Garcia-Martinez, J.L. and Prat, S. (2000). Changes in GA20-oxidase gene expression strongly affect stem length, tuber induction and tuber yield of potato plants. *Plant Journal* **22**, 247-256.

Cheema, M.U.A., Rees, D., Westby, A and Taylor, M. (2010) Hormonal Control of Sprouting of sweetpotatoes in Storage. *Acta Horticulturae* **858,** 173-178

Chope, G. A., Terry, L. A. and White, P. J. (2007). The effect of 1-methylcyclopropene (1-MCP) on the physical and biochemical characteristics ofonion cv. SS1 bulbs during storage. *Postharvest Biology and Technology* **44**, 131-140.

CIP (1996). CIP sweetpotato facts. A compendium of key figures and analysis for 33 important sweetpotato-producing countries. *International Potato centre.*

CIP (1998). *International Potato Center*, Annual Report 1998.

CIP (2001). *International Potato Center*, Annual Report 2001.

CIP (2002). *International Potato Center*, Annual Report 2002.

Cizkova, H J., Vacek, M.V., Sevcik,R. and Kratka, J. (2000). Caraway essential oil as potential inhibitor of potato sprouting, *Rostlinna Vyroba* **46**, 501–507.

Claassens, M.M.J. and Vreugdenhil, D. (2000). Is dormancy breaking of potato tubers the reverse of tuber initiation? *Potato Research* **43**, 347-369.

Clegg, M.D. and Rappaport, L. (1970). Regulation of bud rest in tubers of potato, Solanum tuberosumL. VI. Biochemical changes induced in excised potato buds by gibberellic acid. *Plant Physiology* **45**, 8-13.

Cline M.G. (1997). Concepts and terminology of apical dominanc*e. American Journal of Botany* **84,** 1064-1069.

Coleman, E.C., Ho, C.T, and Chang, S.S. (1981). Isolation and identification of volatile compounds from baked potatoes. *Journal of Agriculture and Food Chemistry* **29**, 42-48.

Coleman, W.K. and Coleman, S.E. (1986). The effects of Bromoethane and Ethanol on potato (Solanum tuberosum) tuber sprouting and subsequent yield responses. *American Potato Journal* **63**, 373-377.

Coleman, W.K. (1987). Dormancy release in potato tubers: a review. *American Potato Journal* **64**, 57-68.

Coleman, W.K., Lonergan, G. and Silk, P. (2001). Potato sprouts growth suppression by menthone and neomenthol, volatile oil components of Minthostachys, Satureja, and Mentha species. *American Journal of Potato Research* **78**, 345-354.

Crabbe, J. and Barnola, P. (1996). A new conceptual approach to bud dormancy in woody plants. In: G.A. Lang (ed.). Plant dormancy: Physiology, biochemistry and molecular biology. 83-107. *CAB International*, Oxon.

Crafts, C. C and Audia. W. V. (1980). Some effects of isopropyl N-(3-chlorophenyl) carbamate on respiration water uptake, and ion leakage of potato tissue *American Potato Journal* **36**, 386-393.

Cvikrovai, M., Sukhova, L.S., Eder, J. and Korableva, N.P. (1994). Possible involvement of abscisic acid, ethylene and phenolic acids in potato tuber dormancy. *Plant Physioogy andl Biocehmistry* **32**, 685-691.

Daniels-Lake, B. J., Prange, R. K., Nowak, J., Asiedu, S. K. and Walsh, J. R. (2005a). Sprout development and processing quality changes in potato tubers stored under ethylene: 1. Effects of ethylene concentration. *American Journal of Potato Research* **82**, 389-397.

Daniels-Lake, B.J. and R. Prange, (2006). Maintaining good fry colour when using ethylene sprout inhibitor (abstract). PAA/Solanaceae Genomics meets Biodiversity conference July 23-27, Madison, Wisconsin, USA.

Daniels-Lake, B.J., Prange, R.J. Bishop, S.D. and Hiltz, K. (2008). 1-Methylcyclopropene counteracts fry color darkening attributable to carbon dioxide and ethylene interaction. *Hortscience* **43**, 2112-2114.

Davies, P.J. (1987). *The Plant Hormones*: Their nature, occurrence and functions. In: Davies, P. J. (ed). Plant hormones and their role in plant growth and development. pp. 1-11. Martinus Nijhoff Publishers. Dordrecht.

Davis, T.D., Steffens, G.L. and Sankhala, N. (1988). Triazole plant growth regulators. *Horticultural Reviews* **10**, 63-105.

Davis, T.D. and Curry, E.A. (1991). Chemical regulation of vegetative growth. *Critical Reviews in Plant Sciences* **10**, 151-188.

Day, D.A., Arron, G.P., Christoffersen, R.E. and GG Laties, G.G. (1978). Effect of ethylene and carbon dioxide on potato metabolism. *Plant Physiology* **62**, 820-825.

DeEll, J.R., Murr, D.P., Porteous, M.D. and Rupasinghe, H.P.V. (2002). Influence of temperature and duration of 1-methylcyclopropene (1-MCP) treatment on apple quality. *Postharvest Biology and Technology* **24**, 349-353.

Denny, F.E. (1926a). Hastening the sprouting of dormant potato tubers. *American Journal of Botany* **13**, 118-125.

Denny, F.E. (1926b). Second report on use of chemicals for hastening the sprouting of dormant potato tubers *American Journal of Botany* **13**, 386-396.

Dogonadze, M.Z., Korableva, N.P., Platonova, T.A., and Shaposhnikov. G.L. (2000). Effects of gibberellin and auxin on the synthesis of abscisic acid and ethylene in buds of dormant and sprouting potato tubers. *Applied Biochemistry and Microbiology* **36**, 507-509.

Dong, J.G., Fernandez-Maculet, J.C. and Yang, S.F. (1992). Purification and characterisation of 1-aminocyclopropane-1-carboxylate oxidase from apple fruit. *Proceedings of National Academy of Sciences* **89**, 9789- 9793.

Dong, L., Lurie, S. and Zhou H.W. (2002). Effect of 1-methylcyclopropene on ripening of 'Canino' apricots and 'Royal Zee' plums. *Postharvest Biology and Technology* **24**, 135-45.

Doonan, J. (1996). Plant growth: roots in the cell cycle. *Current Biology* **6**, 788-789.

Downes, K., Chope, G.A. and Terry, L.A. (2010). Postharvest application of ethylene and 1-methylcyclopropene either before or after curing affects onion (*Allium cepa* L.) bulb quality during long term cold storage. *Postharvest Biology and Technology* **55**, 36-44.

Dudareva, N., E. Pichersky and J. Gershenzon. (2004). Biochemistry of plant volatiles. *Plant Physiology* **135**, 1893-1902.

Ekman J.H., Clayton M. Biasi, W.V., and Mitcham E.J. (2004). Interactions between 1-MCP concentration, treatment interval and storage time for 'Bartlett' pears. *Postharvest Biology and Technology* **31**, 127-36.

Elmer, O .H. (1932). Growth inhibition of potato sprouts by the volatile products of apples. *Science* **75**, 19-22.

Ergun, M. and Huber. D.J. (2004). Suppression of ethylene perception extends shelf-life and quality of 'Sunrise solo' papaya fruit at both pre-ripe and ripe stages of development. *European Journal of Horticultural Science* **69**, 184-192.

Es, A.V. and Hartmans, K.J. (1987). Starch and sugar during tuberization, storage and sprouting. In: *Storage of Potatoes: Post-harvest Behavior, Store Design, Storage Practice, Handling*, 79-113. A Rastovski, A van Es (eds.), Pudoc, Wageningen, The Netherlands.

Evans, R. R., Evans, J.R. and Rademacher, W. (1997). Prohexadione calcium for suppression of vegetative growth in eastern apples. *Acta Horticulturae* **451**, 663-666.

Ewing, L.L., Mcmurry, S.E. and Ewing, E.E. (1987). Cutting as a method of breaking dormancy in microtubers produced in vitro. *American Potato Journal* **64**, 329-332.

Ewing, E.E., Simko, I., Omer, E .A. and Davies, P. J. (2004). Polygene Mapping as a Tool to Study the Physiology of Potato Tuberization and Dormancy. *American Journal of Potato Research* **81**, 281-289.

Faivre-Rampant, O., Gilroy, E.M., Hrubikova, K., Hein, I., Millam, S., Loake, G.J., Birch, P., Taylor, M., and Lacomme, C. (2004). Potato virus X-induced gene silencing in leaves and tubers of potato. *Plant Physiology* **134**, 1308-1316.

Fan, X. and Mattheis, J.P. (1999a). Methyl jasmonate promotes apple fruit degreening independently of ethylene action. *HortScience* **34**, 310-312.

Fan, X. and Mattheis, J.P. (1999b) Impact of 1-methylcyclopropene and methyl jasmonate on apple volatile production. *Journal of Agricultural and Food Chemistry* **47**, 2847-2853.

Fan, X., Argenta, L. and Mattheis, J.P. (2000a). Inhibition of ethylene action by 1-methylcyclopropene prolongs storage life of apricots. *Postharvest Biology and Technology* **20**, 135-142.

Fan, X. and Mattheis, J.P. (2000b). Reduction of ethylene-induced physiological disorders of carrots and iceberg lettuce by 1-methylcyclopropene. *HortScience* **35**, 1312-1314.

Fairbanks, D. J. and Andersen, R. W. (1999). Genetics: *The Continuity of Life*. Brooks/Cole Publishing Company, Pacific Grove, California.

FAO (2002). Internet web site of the Food and Agriculture Organization of the United Nations. At http://apps.fao.org/

FAO (2007). International year of the potato 2008. (Online) Available from: http:// www.potato2008.org/en/index.html (Accessed 16 th March 2008).

FAOSTAT (2007). Internet web site of the Food and Agriculture Organization of the United Nations. At http://apps.fao.org/

FAOSTAT (2008). Internet web site of the Food and Agriculture Organization of the United Nations. At http://apps.fao.org/

Farre, E.M. and Trethewey, R.N. (2001). Acceleration of potato sprouting by the expression of a bacterial pyrophosphatase. *Nature Biotechnology* **19**, 268–272.

Feng, X., Apelbaum, A., Sisler, E.C. and Goren, R. (2000). Control of ethylene responses in avocado fruit with 1-methylcyclopropene. *Postharvest Biology and Technology* **20**, 143- 150.

Fernie, A.R. and Willmitzer, L. (2001). Molecular and biochemical triggers of potato tuber development. *Journal of Plant Physiology* **127**, 1459-1465.

Filmer, A.A.E, and Rhodes, M.J.C. (1985). Investigation of sprout-growth-inhibitory compounds in the volatile faction of potato tubers. *Potato Research* **28**, 361-377.

Francis, D. and Sorrell, D.A. (2001). The interface between the cell cycle and plant growth regulators: a mini review. *Plant Growth Regulations* **33**, 1-12.

Frazier, M.J., Kleinkopf, G.E and Brandt, T.L. (1998). Effects of spearmint and peppermint oil used as alternative sprout and disease suppressants. *American Journal of Potato Research* **75**, 276. (abst).

Frazier, M.J., Kleinkopf, G.E and Brandt, T.L. (2000). Spearmint oil and peppermint oil used as alternative sprout suppressants. *American Journal of Potato Research* **77**, 399. (abst).

Fugate, K.K., Suttle, J.C. and Campbell, L.G. (2010). Ethylene production and ethylene effects on respiration rate of postharvest sugarbeet roots. *Postharvest Biology and Technology* **56,** 71-76.

Furlong, C.R. (1948). Summer potato storage in clamp and cool storage. *Agriculture* (London) **55**, 81-85.

Gardner, F.P., Pearce, R.B. and Mitchell, R.L. (1985). *Physiology of crop plants*, Iowa State University Press, Ames, Iowa.

Gilley, A. and Fletcher, R.A. (1998) Gibberellin antagonizes paclobutra- zol-induced stress protection in wheat seedlings. *Journal of Plant Physiology* **153**, 200-207.

Golding, J.B., Shearer, D., Wyllie. S.G. and McGlasson, W.B. (1998). Application of 1-MCP and propylene to identify ethylene-dependent ripening processes in mature banana fruit. *Postharvest Biology and Technology* **14**, 87-98.

Gong, Y. and Mattheis, J.P. (2003). Effect of ethylene and 1-methylcyclopropene on chlorophyll catabolism of broccoli florets. *Plant Growth Regulation* **40**, 33-38.

Grossmann, K. (2000). Mode of action of auxin herbicides: a new ending to a long drawn out story. *Trends in Plant Science* **5**, 506-508.

Guillen, F., Castillo, S., Zapata, P.J., Mart´inez-Romero, D., Serrano, M. and Valero, D. (2007). Efficacy of 1-MCP treatment in tomato fruit. Duration and concentration of 1-MCP treatment to gain an effective delay of postharvest ripening. *Postharvest Biology and Technology* **43**, 23-27.

Haard, N.F. (1971). Differential response of cold-stored potato tubers to ethylene. *American Potato Journal* **48**, 183-186.

Habib, A.T., Brown, H. D. (1957). Role of reducing sugars and amino acids in the browning of potato chips. *Food Technology* **11**, 85-89.

Hagenimana, V. and Owori, C. (1997). Feasibility, acceptability, and production costs of sweetpotato-based products in Uganda, 276-281. CIP Program Report 1995-96, CIP, Lima, Peru.

Hammer, K.A., Carson, C.F. and Riley, T.V. (2003). Antifungal activity of the components of *Melaleuca alternifolia* (tea tree) oil. *Journal of Apllied Microbilogy* **95**, 853–860.

Harris, D.R., Seberry, J.A., Wills, R.B.H. and Spohr, L.J. (2000). Effect of fruit maturity on efficacy of 1-methylcyclopropene to delay the ripening of bananas. *Postharvest Biology and Technology* **20**, 303-308.

Hartsman, K.J., Diepenhorst, P., Bakker, W. and Gorris, L.G.M. (1995). The use of carvone in agriculture: sprout suppression of potatoes and antifungal activity against potato tuber and other plant diseases. *Industrial Crops and Products* **4**, 3-13.

Hartmann, H.T., Kester, D.E., Davies, F.T. and Geneve, R.L. (2002). *Plant Propagation*: Principles and Practices, 7th edition. Prentice Hall, New Jersey.

Haverkort, A. J. and Verhagen. A. (2008). Climate Change and Its Repercussions for the Potato Supply Chain. *Potato Research* **51**, 223-237.

Hemberg, T. (1970). The action of some cytokinins on the rest-period and the content of acid growth-inhibiting substances in potato. *Plant Physiology* **23**, 850-858.

Hemberg, T. (1985). Potato rest. In: P.H. Li (ed.). *Potato Physiology*. 353-379. Academic Press Inc, London.

Hershkovitz, V., Saguy, S.I. and Pesis, E. (2005). Postharvest application of 1-MCP to improve the quality of various avocado cultivars. *Postharvest Biology and Technology* **37**, 252-264.

Hessen, J.C. (1970). Storage and handling of potatoes for the chip industry. European Regioanl 7th Convention .*Potato Chip Institute international*.

Heyes, J. A. and Johnson, J. W. (1998). 1-Methylcyclopropene extends *Cymbidium* orchid vaselife and prevents damaged pollinia from accelerating senescence. *New Zealand Journal of Crop and Horticultural Science* **26**, 319-324.

Hijmans, R.J. and Spooner D.M. (2001). Geographic distribution of wild potato species. *American Journal of Botany* **88**, 2101-2112.

Hilhorst, H.W.M. and Toorop, P.E. (1997). Review on dormancy, germinability, and germination in crop and weed seeds. *Advances in Agronomy* **61**, 111-155.

Hill, T.A. (1980). *Endogenous plant growth substances*, 2nd edition. The Camelot Press Ltd., Southampton.

Hopkins, W.G. and Huner, N.P.A. (2004). *Introduction to Plant Physiology*. 3rd edition. Wiley and Sons, USA.

Hua, J., Sakai, H., Nourizadeh, S., Chen, Q.G., Bleecker, A.B., Ecker, J.R and Meyerowitz E.M. (1998). EIN4 and ERS2 are members of the putative ethylene receptor gene family. *Plant Cell* **10**, 1321-1332.

Huelin F.E, and Barker, J. (1939). The effect of ethylene on the respiration and carbohydrate metabolism of potatoes. *New Phytologist* **38**, 85-104.

Hughes, D.L., Takahashi, B., Timm, H. and Yamaguchi, M. (1974). Influence of ethylene on sprout development of seed potato. *American Journal of Potato Research* **50**, 439-444.

Ichimura, K., Shimizu, H., Hiraya, T. and Hisamatsu, T. (2002). Effect of 1methylcyclopropene (1-MCP) on the vase life of cut carnation, Delphinium and sweet pea flowers. *Bulletin of the National Institute of Floricultural Science* **2**, 1-8.

Isherwood, F.A. (1973). Sugar-starch interconversion in *Solanum tuberosum*. *Phytochemistry* **12**, 2579-2591.

Izumi, K., Kamiya, Y., Sakurai, A., Oshio, H. and Takahashi, N. (1985). Studies of sites of action of a new plant growth retardant(E)-1-4 chlorophenyl-4,4dimethyl-2-(1,2,4-triazol-l-yl)-1-penten-3-ol (S33O7) and comparative effects of its stereoisomersin a cell-free system from *Cucurbita maxima. Plant Cell Physiology **26***, 821-827.

Jakubowicz, M. (2002). Structure, catalytic activity and evolutionary relationships of 1-aminocyclypropane-1-carboxylate synthase, the key enzyme of ethylene synthesis in higher plants. *Acta Biochimica Polonica* **49**, 757- 774.

Jan W.D., Thornton, M.K. and Shafii, B. (2010). Sprout Suppressing Residue Levels of 1, 4 dimethylnaphthalene (1,4DMN) in Potato Cultivars. American Journal of Potato Research **87**, 434-445

Jeong, J., Huber, D.J. and Sargent, S.A. (2003). Delay of avocado (*Persea americana*) fruit ripening by 1-methylcyclopropene and wax treatments. *Postharvest Biology and Technology* **28**, 247-257.

Jenkins, P.D. (1982). Losses in sweet potatoes (*Ipomea batatas*) stored under traditional conditions in Bangladesh. *Tropical Science* **24**, 17-28.

Jensen, P., Hangarter, R. and Estelle, M. (1998). Auxin transport is required for hypocotyl elongation in light-grown but not dark-grown Arabidopsis. *Plant Physiology* **116**, 455-462.

Jiang, Y., and Joyce, D.C. (2000). Effects of 1-methylcyclopropene alone and in combination with polyethylene bags on the postharvest life of mango fruit. *Annals of Applied Biology* **137**, 321-327.

Jiang. W., Sheng, Q., Zhou, X.J., Zhang, M.J.and Liu, X.J. (2002). Regulation of detached coriander leaf senescence by 1-methylcyclopropene and ethylene. Postharvest Biology and Technology **26**, 339-345.

Jiang, Y., Joyce, D.C., Jiang, Wand Lu, W. (2004). Effects of chilling temperatures on ethylene binding by banana fruit. *Plant Growth Regulation* **43**, 109-115.

Johnson, D.S. and Colgan, R.J. (2003). Low ethylene controlled atmosphere induces adverse effects on the quality of 'Cox's Orange Pippin' apples treated with aminoethoxyvinylglycine during fruit development. *Postharvest Biology and Technology* **27**, 59-68.

Jones, M.G., Horgan, R. and Hall, M.A. (1988). Endogenous Gibberellins in the potato, Solanum tuberosum. *Phytochemistry* **27**, 7-10.

Kader, A. A. (1983). Postharvest quality maintenance of fruits and vegetables in developing countries. In: Lieberman, M. (ed). *Post-harvest physiology and crop preservation*. pp. 455 – 470. Plenum Press. London.

Kalt, W., Prange, R.K. and Daniels-Lake, B.J. (1999). Alternatives compounds for the maintenance of processing quality of stored potatoes*. Journal of Food Processing and Preservation* **23**, 71-81.

Karuri, E.G. and Ojijo, N.K. (1994). Storage studies on sweet potato roots: experiences with KSP20 cultivar. *Acta Horticulturae* **368,** 441-452.

Karuri, G.E. and Hagenimana, V. (1995) Use of ambient conditions and sawdust in storage of sweetpotato (*Ipomoea batatas* L.) roots in Kenya. *Zimbabwe Journal of Agricultural Research* **33,** 1-9.

Kaul, H.N. and Mehta, A. (1994). Wound healing in potato tuber tissue: cultivar variation. *Journal of the Indian Potato Association* **21**, 247-250.

Kays S.J. (1985). Chapter 4. The physiology of yield in sweet potato p 79-133, In: *Sweet potato products: A natural resource for the tropics*. Bouwkamp JC ed. CRC Press Inc, Boca Raton, Florida. ISBN 08493 5428 5.

Kays, S.J. (1991). Postharvest Physiology of Perishable Plant Products. Van Nostrand Reinhold, New York.

Kefeli, V.I. (1978). Natural plant growth inhibitors and phytohormones, Dr W. Junk b.v. Publishers, Boston.

Kende, H. (1989). Enzymes of ethylene biosynthesis. *Plant Physiology*, **91**, 1-4.

Kende, H. (1993). Ethylene biosynthesis. *Plant Physiology* **44**, 283-307.

Kende, H and Boller, T. (1981). Wound ethylene and 1-aminocyclopropane-1-carboxylate synthase in ripening tomato fruit, *Planta* **151**, 476-481.

Kleinkopf, G.E. and Frazier, M.J. (2002). Alternative sprout suppressants for stored potatoes. University of Idaho, College of Agricultural and Life Sciences. Proceedings: *Winter Commodity Schools* **34**, 183-187.

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

Knee, M. (1984). 1-aminocyclopropane-l-carboxylic acid levels in ripening apple fruits. *Journal of Experimental Botany* **35**, 1794-1799.

Knee, M. (1985). Evaluating the practical significance of ethylene in fruit storage. In: Roberts, J.A. and Tucker, G.A. Editors, *Ethylene and Plant Development* Butterworths Press, London, 297–315.

Knee, M. (1985c). Metabolism of 1-aminocyclopropane-l-carboxylic acid during apple fruit development. *Journal of Experimental Botany* **35**, 670-678.

Knowles, N.R., Knowles, L.O, and Haines, M.M. (2005). 1, 4-Dimethylnaphthalene Treatment of Seed Potatoes Affects Tuber Size Distribution. *American Journal of Potato Research* **82**, 170-190.

Knudsen, J.T., Tollsten, L. and Bergstrom, G. (1993). Floral scents – a checklist of vol**a**tile compounds isolated by head-space techniques. *Phytochemistry* **33**, 253–280.

Koda, Y. (1982). Effects of storage temperature and wounding on cytokinin levels in potato tubers. *Plant Cell Physiology* **23**, 851-857.

Ku, V.V.V. and Wills, R.B.H. (1999). Effect of 1-methylcyclopropene on the storage life of broccoli. *Postharvest Biology and Technology* **17**, 127-132**.**

Ku, V.V.V., Wills, R.B.H. and Ben-Yehosua, S. (1999). 1-Methylcyclopropene can differentially affect the postharvest life of strawberries exposed to ethylene. *HortScience* **34**, 119-120.

Kushman, L.J. and Wrights, F.S. (1969). Sweet potato storage. USDA Agriculture Handbook no. 358.

Lancaster, P.A and Coursey, D.G. (1984). Traditional postharvest technology of perishable tropical staples. *FAO Agricultural Services Bulletin* **59**, FAO, Rome.

Lannetta, P. P. M., Laarhoven, L. J., Medina-Escobar, N., James, E. K., Mcmanus, M. T.,Davies, H. V. and Harren, F. J. M. (2006). Ethylene and carbon dioxide production by developing strawberries show a correlative pattern that is indicativeof ripening climacteric fruit. *Physiologia Plantarum* **127**, 247-259.

Lashbrook, C.C., Tieman, D.M. and Klee H.J. (1998). Differential regulation of the tomato ETR gene family throughout plant development. *Plant Journal* **15**, 243-252.

Leclerc, Y., Donnelly, D.J., Coleman, W.K. and King, R.R. (1995). Microtuber dormancy in three potato cultivars. *American Potato Journal* **72**, 215-223.

Lentza-Rizos, C. and Balokas, A. (2001). Residue levels of chlorpropham in individual tubers and composite samples of postharvest-treated potatoes. *Journal of Agricultural and Food Chemistry* **49**, 710-714.

Lewis, M.D., Kleinkopf, G.E. and Shetty, K.K. (1997). Dimethylnaphthalene and diisopropyle-naphthalene for potato sprout control in storage:1 Application and efficacy. *American Potato Journal* **74**, 183-197.

Lewthwaite, S.L. and Trigs, C.M. (1995). Sprout suppression insweetpotato roots following immersion in sodium hypochlorite solutions. *New Zealand Journal of Crop and Horticultural Science* **23**, 283-287.

Libbenga, K.R. and Mennes, A.M. (1987). Hormone binding and its role in hormone action. In: Davies. *Plant hormones and their role in plant growth and development*, pp. 194-22. Martinus Nijhoff Publishers. Dordrecht.

Lopez- Gomez, R., Campbell, A., Dong, J.G., Yang, S.F. and Gómez – Lim, M.A. (1997). Ethylene biosynthesis in banana fruit: isolation of a genomic clone to ACC oxidase and expression studies. *Plant Science* **123**, 123-131.

Low, V.H.K. (1975). Role of gibberellins in root and shoot growth. In: H.N. Krishnamoorthy (ed.). Gibberellins and Plant Growth. 101-112. John Wiley and Sons, Inc, New York.

Luo, Z. (2007). Effect of 1-methylcyclopropene on ripening of postharvest persimmon (Diospyros kaki L.) fruit. *Food Science and Technology* **40,** 285-291.

Lyons, J.M. (1973). Chilling injury in plants. *Annual Review of Plant Physiology* **24**, 445-466.

Malik, J.N. (1995). Chapter 1. The potato crop- An introduction. In: Potatoes in Pakistan, Word Mate Pakistan. ISBN: 969-8301-00-3.

Manenoi, A., Bayogan, E.R.V., Thumdee, S., andPaull, R.E. (2007). Utility of 1-methylcyclopropene as a papaya postharvest treatment. *Postharvest Biology and Technology* **44**, 55–62.

Mares, D.J. (1985). Carbohydrate metabolism in developing potato tubers. In: P. H. Li (ed.). *Potato Physiology*. 280-318. Academic Press Inc, London.

Martinez, P.G., Lopez Gómez R. and Gómez-Lim, L.A. (2001). Identification of an ETR1-homologue from mango fruit expressing during fruit ripening and wounding. *Journal of Plant Physiology*. **158**, 101-108.

Mathooko, F.M., Tsunashima, Y., Owino, W.Z.O., Y. Kubo, Y. And Inaba, A. (2001). Regulation of genes encoding ethylene biosynthesis enzymes in peach (*Prunus persica* L.) fruit by carbon dioxide and 1-methylcyclopropene, *Postharvest Biology and Technology* **21**, 265–281.

McGlasson, W.B., Rath, A.C. and Legendre, L. (2005). Preharvest application of aminoethoxyvinylglycine (AVG) modifies harvest maturity and cool storage life of "Arctic Snow' nectarines. *Postharvest Biology and Technology* **36**, 93 -102.

Mehta, A. (2004). Sprout suppression in stored potatoes by natural growth inhibitor – diphenylamine. *Journal of Food Science and Technology* **41**, 539-541.

Meigh, D.F., Filmer, A.E. and Self, R. (1973). Growth-inhibitory volatile aromatic compounds produced by Solanum tuberosum tubers. *Phytochemistry* **12**, 987-993.

Metlitskii, LV, Korableva, NP, Sukova, LS, Pershutin, AN, Litver, N.N, (1982). Use of Hydrel to prevent tuber germination during storage with concurrent reduction of disease-induced losses. *Applied Biochemistry and Microbiology* **18**, 96-103.

Michael, S., Reid. S. and Pratt, H.K. (1990). Effects of Ethylene on Potato Tuber Respiration. *Plant Physiology* **49**, 252-255.

Mok, D.W.S. and Mok, M.C. (2001). Cytokinin metabolism and action. *Annual Review of Plant Molecular Biology* **52**, 89-118.

Morris, W.L., Ducreux, L.J.M., Hedden, P., Millam, S. and Taylor, M.A. (2006). Overexpression of a bacterial 1-deoxy-D- xylulose 5- phosphate synthase gene in potato tubers perturbs the isoprenoid metabolic network: implications for the control of the tuber life cycle. *Journal of Experimental Botany* **57**, 3007-3018.

Mottram, D.S., Wedzicha, B.L. and Dodson, A.T. (2002) Acrylamide is formed in the Maillard reaction. *Nature* **419**, 448-449.

Mullins, E. D., McCollum, T. G. and McDonald, R. E. (2000). Consequences on ethylene metabolism of inactivating the ethylene receptor sites in diseased nonclimacteric fruit. *Postharvest Biology and Technology* **19**, 155-164.

Newman, J.P., Dodge, L.L. and Reid, M.S. (1998). Evaluation of ethylene inhibitors for postharvest treatment of *Gypsophila paniculata* L, *Horticulture Technology* **8**, 58-63.

Nillson, T. (2005). Effects of ethylene and 1-MCP on ripening and senescence of European seedless cucumbers. *HortScience* **36**, 113-125.

Nursten, H. E. and Sheen, M. R. (1974). Volatile flavor components of cooked potato. *Journal of the Science of Food and Agriculture* **25**, 643-663.

NZFSA. (2006). Proposals to Amend the New Zealand (Maximum Residue Limits of Agricultural Compounds. *Food Standards* 2006. (online) Available from. http://www.nzfsa.govt.nz/consultation/mrl/page-15.htm (Accessed on 15th March 2006)

O'Beirne, D., Walshe. P and Egan, S. (1985). Searching for quality in cooked and processed potatoes. *Farm Food Research* **16**, 71–73.

O'Hagan, N. (1991). Substituted naphthalenes and their role as potato sprout supressants. Ph.D. Thesis, Glasgow, University of Glasgow.

Okagami, N. and Nagao, M. (1971). Gibberellin-induced dormancy in bulbils of *Dioscorea. Planta* **101**, 91-94.

Okagami, N and Tanno, N. (1993). Gibberellic acid-induced prolongation of the dormancy in tubers or rhizomes of several species of East Asian *Dioscorea*. *Plant Growth Regulation* **12**, 119-123.

Ooms, G. and Lenton, J.R. (1985). T-DNA genes to study plant development: precocius tuberisation and enhanced cytokinins in a tumefaciens transofrmed potato. *Plant Molecular Biology* **5**, 205-212.

Oosterhaven, K., Hartmans, K.J., Scheffer, J.C and van der Plas, LHW. (1995b). Inhibitory effect of S-carvone on wound healing of potato tuber tissue. *Physiologia Plant* **93**, 225-232.

Oruna-Concha, M. J., Duckham, S. C. and Ames, J. H. (2001). Comparison of volatile compounds isolated from the skin and flesh of four potato cultivars after baking. *Journal of Agricultural and Food Chemistry* **49**, 2414-2421.

Oteef, M.D.Y. (2008). Analysis of the potato sprouts inhibitor. *PhD Thesis,* Glasgow University U.K

Paton, J.E. and Scriven, F.M. (1989). Use of NAA to inhibit sprouting in seetpotatoes (*ipomea batatas*). *Journal of the science of food and agriculture* **48**, 421-427.

Pereira-Netto, A.B. (2001). Effect of inhibitors of ethylene biosynthesis and signal transduction pathway on the multiplication of in vitro-grown Hancornia speciosa, *Plant Cell Tissue Organ Culture* **66**, 1–7.

Perez, F.J. and Lira, W. (2005. Possible role of catalase in post-dormancy bud break in grapevines. *Journal of Plant Physiology* **162**, 301-308.

Petersen, M.A., Poll, L. and Larsen, L.M. (1998). Comparison of volatiles in raw and boiled potatoes using a mild extraction technique combined with GC odour profiling and GC–MS. *Food Chemistry* **61**, 461–466.

Picha, D.H. (1986). Weight loss in sweet potatoes during curing and storage: contribution of transpiration and respiration. *Journal of the American Society for Horticultural Science* **11**, 889-892.

Poapst, P. A. and Genier. C. (197l). Storage disorder in Kennebec potatoes, caused by high concentrations of maleic hydrazide. *Canadian Journal of Plant Science* 50, 591-593.

Porat, R., Weiss, B., Cohen, L., Daus, A., Goren, R. and Droby, S. (1999). Effects of ethylene and 1-methylcyclopropene on the postharvest qualities of 'Shamouti' oranges. *Postharvest Biology and Technology* **15**, 155-163.

Prange R., Kalt, W., Daniels-Lake, B., Liew, C., Walsh, J., Dean, P, Coffin, R., and Page, R. (1997) Alternatives to currently used potato sprout suppressants. *Conference Proceedings, Postharvest News and Information* **8**, 37-41.

Prange R.K., Kalt, W., Daniels-Lake, B.J., Liew, C.L., and Page, R.T. (1998). Using ethylene as a sprout control agent in stored Russet Burbank *potatoes. Journal of American Society for Horticultural Sciences* **123**, 463-469.

Prange, R. K., Daniels-Lake, B. J., Jeong, J-C. and Binns, M. (2005). Effects of ethyleneand 1-methylcyclopropene on potato tuber sprout control and fry colour. *American Journal of Potato Research* **82**, 123-128.

Prasad, S.M., Srinivasan G. and Shanta P. (1981). Post harvest loss in sweet potato in relation to common method of harvest and storage. *Journal of Root Crops* **7,** 69-73.

PRC. (2007). Annual Report of the Pestiside Residual Committee. (Online) available from:http://www.pesticides.gov.uk/prc.asp?id=1937andlink=%2Fuploadedfiles%2FWeb%5FAssets%2FPRC%2FPRC%5FAnnual%5FReport%5F2006%2Epdf (Accessed, 9th August, 2008).

Pritchard, M.K. and Adam, L. (1994). Relationships between fry color and sugar concentration in stored Russet Burbank and Shepody potatoes. *American Potato Journal* **71**, 59-68.

Pruski, K., Prange, R. K., Daniels-Lake, B. J., Nowak, J., Astatkie, T. and Ronis, D. H. (2006). Growth-room and field studies with seed tubers treated with ethylene and 1-methylcyclopropene (1-MCP) during storage. *American Journal of Potato Research* **83**, 149-160.

Rajala, A., and Peltonen-Sainio, P. (2000). Manipulating yield potential in cereals using plant growth regulators. In: A.S. Basra (ed.). *Plant growth regulators in agriculture and horticulture: Their role and commercial uses*. 27-49. Food Products Press, Inc, New York.

Rando, R. (1974). Chemistry and Enzymology of k.sub.cat Inhibitors. *Science* **185**, 320-324.

Rakitin, Y. V. (1973). Action of maleic hydrarizide as an inhibitor ofpotato sprouting. Gidrazid Maleinovoi Kisloty Regul. *Rosta Rast.Chem, Abs*. **80**, 340.

Rastovski, A. (1987). Storage losses. *In*: Rastovski, A. and A. van Es (Eds), *Storage of Potatoes: Post-harvest Behavior, Store Design, Storage Practice, Handling*. Pudoc, Wageningen, The Netherlands, 177-180.

Rath, A.C., Kang, I., Park, C., Yoo, W. and Byun, J. (2006). Foliar application of aminoethoxyvinylglycine (AVG) delays fruit ripening and reduces pre-harvest fruit drop

and ethylene production of bagged ‘‘Kogetsu’’ apples. *Plant Growth Regulation* **50**, 91-100.

Ray R.C. and Ravi V. (2005). Post Harvest Spoilage of Sweetpotato in Tropics and Control Measures. *Critical Reviews in Food Science and Nutrition* **45**, 623 - 644.

Rees, D., Bancroft, R., Matters, D and Pollard, A. (1996). Physiological basis for storage life extension in fresh Sweetpotato. *NRI Final Technical Report.*

Rees, D., Kapinga, R., Mtund, A.K., Chilosa, D., Rwiza, E., Kalima, M., Kiozya, H and Munshi, R. (2001). Damage reduces both market value and shelf-life of sweetpotato: a case study of urban markets in Tanzania. *Tropical Science* **41**, 1–9.

Reid, M.S. and Pratt, H.K. (1972). Effects of ethylene on potato tuber respiration. *Plant Physiology* **49**, 252-255.

Roberts, L.W. (1988). Hormonal aspects of vascular differentiation. In L.W. Roberts, P.B. Gahan and R. Aloni (eds). Vascular differentiation and plant growth regulators, 22-38.

Rodrguez-Concepcin. M., Boronat, A. (2002). Elucidation of the Methylerythritol Phosphate Pathway for Isoprenoid Biosynthesis in Bacteria and Plastids. A Metabolic Milestone Achieved through Genomics. *Plant Physiology* **130**, 1079-1089.

Rohwer, C.L. and Gladon, R.J. (2001). 1-Methylcyclopropene Delays Ripening of Pink and Light Red Tomatoes. *HortScience* **36**, 100-105.

Romani, R., Labavitch, J., Yamashita, T., Hess, B. and Rae, H. (1983). Preharvest AVG treatment of ‘Bartlett’ pear fruits: effects on ripening, color change, and volatiles. *Journal of American Society of Horticultural Science* **108**, 1046-1049.

Ronning, C.M., Stegalkiua, S.S., Ascenzi, R.A., Bougri, O., Hart, A.L., Utterbach, T.R., Vanaken, S.E., Riedmuller, S.B., White, J.A., Cho, J., Pertea, G.M., Lee, Y., Karamycheva, S., Sultana, R., Tsai, J., Quakenbush, J., Griffiths, H.M., Retrepo, S., Smart, C.D., Fry, W.E., Van der Hoeven, R., Tanksley, S., Zhang, P., Jin, H., Yamamoto, M.L., Baker, B.J., and Buell, C.R. (2003) Comparative analyses of potato expressed sequence tag libraries. *Plant Physiology* **131**, 419-429.

Rosa, J.T. (1925). Shortening the rest period of potatoes with ethylene gas. Potato Association of America, *Potato News Bulletin* **2**, 363-365.

Rylski, I., Rappaport, L., and Pratt, H.K. (1974). Dual effects of ethylene on potato dormancy and sprout growth. *Plant Physiology* **53**, 658-662.

Salisbury, F. B. and Ross, C. W. (1991). *Plant Physiology*, 4th edition. Wadsworth publishing company, Belmont, California.

Saltveit, M.E. (2005). Aminoethoxyvinylglycine (AVG) reduces ethylene and protein biosynthesis in excised discs of mature-green tomato pericarp tissue. *Postharvest Biology and Technology* **35**,183-190.

Sattelmacher, B. and Marschner, H. (1978). Cytokinin activity in stolons and tubers of Solanum tuberosum during the period of tuberization. *Plant Physiology* **44**, 69-72.

Sato-Nara, K., Yuhashi, K.I., Higashi, K., Hosoya, K., Kubota, M. and Ezura, H. (1999). Stage- and tissue-specific expression of ethylene receptor homolog genes during fruit development in muskmelon. *Plant Physiology* **120**, 321-330.

Schoene, D.L. and Hoffmann, O.L. (1949). Maleic hydrazide, a unique growth regulant. *Science* **109**, 588.

Schwobe, M.A. and Parkin, K.L. (1990). Effect of low temperature and modified atmosphere storage on sugar accumulation in potatoes (*Solanum tuberosum*). *Journal of Food Processing and Preservation* **14**, 241-252.

Sebastian, B., G. Alberto, A.C. Emilio, A.F. and A.F. Juan. (2002). Growth, development and colour response of potted *Dianthus caryophyllus* cv. Monriaan to paclobutrazol treatment. *Scientia Horticulturae* **1767**, 1-7.

Selvarajah, S., Bauchot, A.D. and John, P.J. (2001). Internal browning in cold-stored pineapples is suppressed by a postharvest application of 1-methylcyclopropene. *Postharvest Biology and Technology* **23**, 167-170.

Senning, M., Uwe, S. and Sonnewald, S. (2010). Deoxyuridine triphosphatase expression defines the transition from dormant to sprouting potato tuber buds. *Molecular Breeding* **10**, 1007-1032.

Serek, M., Tamari, G., Sisler, E. C. and Borochov, A. (1995a). Inhibition of ethylene-induced cellular senescence symptoms by 1-methylcyclopropene, a new inhibitor of ethylene action. *Physiologia Plantarum* **94**, 229-232.

Serek, M., Sisler, E. C. and Reid, M.S. (1995b) Effects of 1-MCP on the vase life and ethylene response of cut flowers. *Plant Growth Regulation* **16**, 93-97.

Serek, M., Sisler, E.C. and Reid, M.S. (1996). Ethylene and the post-harvest performance of miniature roses. *Acta Horticulturae* **424**, 145–149.

Serek, M. and Sisler, E.C. (2001). Efficacy of inhibitors of ethylene binding in improvement of the postharvest characteristics of potted flowering plants. Postharvest Biology and Technology **23**, 161-166.

Serek, M., Woltering E.J., Sisler, E.C. Frello, S. and Sriskandarajah, S. (2006). Controlling ethylene responses in flowers at the receptor level. *Biotechnology Advances* **24**, 368-381.

Shellie, K.C. (1999). Muskmelon (*Cucumis melo* L.) fruit ripening and postharvest quality after a preharvest spray of aminoethoxyvinylglycine. *Postharvest Biology and Technology* **17**, 55-62.

Shih, C.Y. and Rappaport, L. (1970). Regulation of bud rest in tubers of potato, Solanum tuberosumL.VII. Effect of abscisic acid and gibberellic acids on nucleic acid synthesis in excised buds. *Plant physiolology* **45**, 33-36.

Shinbo, T., Sudo, Y., Shimabukuro, Y., Kanamori, T., Masuoka, T., Iwatsubo, T., Yamasaki, A., Ogasawara, K. and Mizoguchi, K. (1998). Preparation of a cyclophane-bonded stationary phase and its application to separation of naphthalene derivatives. *Journal of Chromatography* **803**, 95-101.

Shinbo, T., Shimabukuro, Y., Kanamori, T., Iwatsubo, T., Nagawa, Y. and Hiratani, K. (2000). Separation of aromatic isomers on cyclophane-bonded stationary phases. *Journal of Chromatography* **877**, 61-69.

Silverman, F.P., Petracek, P.D., Noll M.R and Warrior, P. (2004). Aminoethoxyvinylglycine effects on late-season apple fruit maturation. *Plant Growth Regulation* **43**, 153-161.

Sisler, E.C. (1979). Measurement of ethylene binding in plant tissue. *Plant Physiology* **64**, 538-542.

Sisler, E. C., and Yang, S. F. (1984). Ethylene, the gaseous plant hormone. *BioScience* **34**, 234 -238.

Sisler, E.C. and Wood, C. (1988). Competition of unsaturated compounds with ethylene for binding and action in plants. Plant Growth Regulation **7**, 181-191.

Sisler, E.C. (1991). Ethylene-binding components in plants. In: Mattoo AK, Suttle J.C, editors. The Plant Hormone Ethylene. Boca Raton, FL: CRC Press; 81-99.

Sisler, E.C., Dupille, E and Serek. M. (1996a). Effect of 1-methylcyclopropene and methylenecyclopropane on ethylene binding and ethylene action on cut carnations. *Plant Growth Regulation* **18,** 79-86.

Sisler E.C. and Serek, M. (1997). Inhibitors of ethylene responses in plants at the receptor level: Recent advances. *Plant Physiology* **100**, 577- 582.

Sisler, E. and Serek, M. (1999). Compounds controlling the ethylene receptor. *Botanical Bulletin of Academia Sinica* **40**, 1-7.

Solomos, T., Trivedi, P. and .Mattoo, A. (2001). Effect of MCP on Apple Fruit Ripeningand Scald Development. *HortScience* **36**, 200-206.

Sophia. B., Hajirezaei. M., Hentschel. E. and Sonnewald. U. (2000). Comparative analysis of abscisic acid content and starch degradation during storage of tubers harvested from different potato varieties. *Potato Research* **43**, 371-382.

Sorce, C., Paiggesi, A., Ceccarelli, N., and Lornzi, R. (1996). Role and metabolism of abscisic acid in potato tuber dormancy and sprouting. *Journal of Plant Physiology* **149**, 548 -552.

Sorce, C., Lorenzi, R. and Ranalle, P. (1997). The effects of (S)-(+)-carvone treatments on seed potato tuber dormancy and sprouting. *Potato Research* **4,** 155-161.

Sorce, C., Lorenzi, R., Ceccarelli, N. and Ranalli, P. (2000). Changes in free and conjugated IAA during dormancy and sprouting of potato tubers. *Australian Journal of Plant Physiology* **27**, 371-377.

Sowokinos, J.R. (2001). Biochemical and molecular control of cold-induced sweetening in potatoes. *American Journal of Potato Research* **78**, 221-236.

Srivastava, M.L. (2002). Plant growth and Development: Hormones and environment. Academic Press, USA.

Steffens, G.L., Lin,J.T., Stafford,A.E., MetzgerJ.D. and Hazebroek, J.P. (1992). Gibberellin content of immature apple seeds from paclobutrazol-treated trees over three seasons *Journal of Plant Growth Regulation* **11**,165-170.

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

Suttle, J.C. (1996). Dormancy in tuberous organs: problems and perspectives. In: G.A. Lang (ed.). Plant dormancy: Physiology, biochemistry and molecular biology. 133-143. CAB International, Oxon.

Suttle J.C. (1998b). Postharvest changes in endogenous cytokinins and cytokinin efficacy in potato tubers in relation to bud endodormancy. *Physiologia Plantarum* **103**, 59-69.

Suttle, J.C. and Banowetz, G.M. (2000). Changes in cis-zeatin and cis-zeatin riboside levels and biological activity during potato tuber dormancy. *Physiologia Plantarum* **109**, 68-74.

Suttle, J.C. (2001). Dormancy-related changes in cytokinin efficacy and metabolism in potato tubers during postharvest storage. Plant Growth Regulation **35**, 199-206.

Suttle, J.C. (2003). Auxin-induced sprout growth inhibition: role of endogenous ethylene. *American Journal of Potato Research* **80**, 303-309.

Suttle, J.C. (2004a). Physiological regulation of potato tuber dormancy. *American Journal of Potato Research* **81**, 253-262.

Suttle, J.C. (2004b). Involvement of endogenous gibberellins in potato tuber dormancy and early sprout growth: a critical evaluation. *Journal of Plant Physiology* **161**, 157-164.

Suttle, J.C. (2004c). Breaking tuber dormancy with synthetic cytokinins. *American Journal of Potato Research* **82**, 92.

Suttle, J.C. (2005). Hormone analoges as novel sprout – control agents.*Valley Potato Grower Magazine* **70**, 20-21.

Suttle, J. C. (2007). Dormancy and sprouting. In: Vreugdenhil, D. and Bradshaw, J. (Eds.) Potato biology and biotechnology.Amsterdam: Elsevier Science B.V.

Tanaka, S., Urasa, R.L., Tanaka, F., Morita, k. and Sugawara, T. (2001). Modelling of sweetpotato hot water treatment for sprout control. *Journal of the Japanese Society of Agriculture Machinery* **63,** 62-67*.*

Tatsuki, M., Endo, A. and Ohkawa, H. (2007). Influence of time from harvest to 1-MCP treatment on apple fruit quality and expression of genes for ethylene biosynthesis enzymes and ethylene receptors. *Postharvest Biology and Technology* **43**, 28-35.

Tieman, D.M., and Klee, H.J. (1999). Differential expression of two novel members of the tomato ethylene-receptor family. *Physiologia Plantarum* **120**,165–172.

Tekalign, T. and Hammes, P.S. (2004). Growth responses of potato (Solanum tuberosum) grown in hot tropical lowland to applied paclobutrazol: 2. Tuber attributes. *New Zealand Journal of Crop and Horticultural Science* **33**, 43-51.

Tomlins, K. I., Ndunguru, G. T., Rwiza, E. and Westby, A. (2002). Influence of pre-harvest curing and mechanical injury on the quality and shelf life of sweet potato (*Ipomoea batatas* (L) Lam) in East Africa. *Journal of Horticultural Science and Biotechnology* **77**, 399-403.

Tomlins, K.I., Ndunguru, G.T., Kimenya, F. T., Ngendello,N., Rwiza,E., R Amour, Q van Oirschot and A Westby, A. (2007). On Farm evaluation of methods for storing fresh sweet potato roots in East Africa. *Tropical Science* **47**, 197-210.

Toney, M. D. (2005). Reaction specificity in pyridoxal enzymes. *Archives of Biochemistry and Biophysics* **433**, 279-287.

Torrigiani, P., Bregoli, A.M., Ziosi, V., Scaramagli, S., Ciriaci, T., Rasori, A., Biondi, S. and Costa, G. (2004). Pre-harvest polyamine and aminoethoxyvinylglycine (AVG) applications modulate fruit ripening in Stark Red Gold nectarines (Prunus persica L. Batsch). *Postharvest Biology and Technology* **33**, 293- 308.

Trewavas, A.J. (1983). Is plant development regulated by changes in the concentration of growth substances or by changes in the sensitivity to growth substances? *Trends in Biochemical Science* **8**, 354-357.

Turnbull, C. G. N and Hanke, D. E. (1985). The control of bud dormancy in potato tubers evidence for the primary role of cytokinins and a seasonal pattern of changing sensitivity to cytokoinins. *Planta* **165**, 359-365.

UC-SAREP (2002). 1, 4-Dimethylnaphthalene for use as a post-harvest adjuvant. USDA NationalOrganicProgram.\(Online) Available from: http://www.ams.usda.gov/nop/NationalList/TAPReviews/14Dimethylnaphthalene.pdf \(Accessed June 2006).

USEPA (1995). 1, 4-Dimethylnaphthalene; exemption from the requirement of a tolerance. Washington: U.S. Government Printing Office. \[Online] Available from: http://frwebgate.access.gpo.gov/cgi-bin/getpage.cgi?dbname=1995_registerandposition=allandpage=7457 (Accessed 2nd April 2006).

USEPA (1997). Raw and processed food schedule for pesticide tolerance reassessment. Washington: U.S. Government Printing Office. \[Online] Available from: http:// frwebgate .access.gpo.gov/cgi-bin/getpage.cgi?position=allandpage=42020anddbname=1997_register (Accessed 2nd April 2006).

Van Altvorst, A. C. and Bovy, A. G. (1995). The role of ethylene in the senescence of carnation flowers, a review. *Plant Growth Regulation* **16**, 43- 53.

Van Der Schoot, C. (1996). Dormancy and symplasmic networking at the shoot apical meristem. In: G.A. Lang (ed.). Plant dormancy: Physiology, biochemistry and molecular biology. 59-79. *CAB International*, Oxon.

Van Ittersum, M.K. and Scholte, K. (1993). Shortening dormancy of seed potatoes by a haulm application of gibberellic acid and storage temperatures regimes. *American Potato Journal* **70**, 7-19.

Valenzuela-Valenzuela, J.M., Lownds, N.K and Sterling, T.M. (2002). Ethylene is not involved in clopyralid action in yellow starthistle (*Centaurea solstitialis* L.). Pesticide Biochemistry and Physiology **72**, 142-152.

Van Staden, J. and Dimalla, G.G. (1977). The distribution of cytokinins in tuberizing potatoes. *Annals of Botany* **41**, 741-746.

Vaughn, S.F. and Spencer, G.F. (1993). Naturally-occurring aromatic compounds inhibit potato tuber sprouting. *American Potato Journal* **70**, 527-533

Ververidis, P. and John, P. (1991). Complete recovery *invitro* of ethylene forming enzyme activity. Phytochemistry **30**, 725-727.

Vivanco, J.M. and Flores, H.E. (2000). Control of root formation by plant growth regulators. In: A.S. Basra (ed.). Plant growth regulators in agriculture and horticulture: Their role and commercial uses. 1-16. Food Products Press, Inc, New York.

Vreugdenhil, D. and Sergeeva, L.I. (1999). Gibberellins and tuberization in potato. *Potato Research* **42**, 471-481.

Vreugdenhil, D. (2004). Comparing potato tuberization and sprouting: Opposite phenomen. *American Journal of Potato Research* **81**, 275-280.

Walker, R., Macleod, I., Gracie, A., Davies, N., Brown, P. and Hingston, T. (2004) Evaluating a product for enhancing dormancy and storage qualities of potatoes.Devonport: Serve-Ag Research. Report number: PT01038.

Wang, H. and Woodson, W. R. (1989). Reversible inhibition of ethylene action and interruption of petal senescence in carnation flowers by Norbornadiene. *Plant Physiology* **89**, 434- 438.

Watkins, C. B. (2006). The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. *Biotechnology Advances* **24**, 389-409.

Watkins, C.B., Nock, J.F. and Whitaker, B.D. (2000). Responses of early, mid and late season apple cultivars to postharvest application of 1-methylcyclopropene (1-MCP) under air and controlled atmosphere storage conditions. *Postharvest Biology and Technology* **19**, 17-32.

Watkins, C. B. and Miller, W. B. (2005). A summary of physiological processes or disorders in fruits, vegetables and ornamental products that are delayed or decreased, increased, or unaffected by application of 1-methylcyclopropene (1-MCP). (online). Department of Horticulture, Cornell University, Ithaca, NewYork. Available at: http://www.hort.cornell.edu/department/faculty/watkins/ethylene/index.htm. (Accessed: 6 August 2009).

Wenham, J.E. (1995). Post-harvest deterioration of cassava: a biotechnology perspective. Plant Production and Protection Paper FAO No 130. FAO Plant Production and Protection, Rome Italy pp.99.

Whitehead, C. S. (1994). Ethylene sensitivity and flower senescence. In: Molecular and cellular aspects of plant reproduction. Edited by Scott, R. J. and Stead, A. D. Cambridge University Press, Cambridge.

Wickham, L.D., Passam D.H.C. and Wilson L.A. (1984). Dormancy responses to post-harvest application of growth regulators in Dioscorea species. 2. Dormancy responses in ware tubers of D. alata and D. esculenta. *Journal of Agricultural Science* **102**, 433–436.

Wild, H.P.J.de, Woltering, E.J., and Peppelenbos, H.W. (1999). Carbon dioxide and 1-MCPinhibit ethylene production and respiration of pear fruit by different mechanisms. *Journal of Experimental Botany* **50**, 837-844.

Wills, R., McGlasson, B., Graham, D., Joyce, D. and Terry Lee, T. (1998). *Postharvest*: an Introduction to the Physiology and Handling of Fruit, Vegetables and Ornamentals. 4th edition. CAB International. UK.

Wills, R.B.H. and Ku, V.V.V. (2002). Use of 1-MCP to extend the time to ripen of green tomatoes and post-harvest life of ripe tomatoes. *Post-harvest Biology and Technology* **26**, 85-90.

Wills, R.B.H., Warton, M.A. and Kim, J.K (2004). Effect of low levels of ethylene on sprouting of potatoes in storage. *Hortscience* **39**, 136-137.

Win, T.O., Srilaong, V., Heyes, J., Kyu, K.L. and Kanlayanarat, S. (2006). Effects of different concentrations of 1-MCP on the yellowing of West Indian lime (Citrus aurantifolia, Swingle) fruit. *Postharvest Biology and Technology* **42**, 23-30.

Woolfe, J.A. (1992). *Sweet Potato. An untapped food resource*. Cambridge University Press, ISBN 0 521 4029.

Woolfe, A.B., Requejo, T. C., Cox, K.A., Jackman, R.C., Gunson, A., Arpaia, M.L. and White, A. (2005). 1-MCP reduces physiological storage disorders of “Hass” avocados. *Postharvest Biology and Technology* **35**, 43-60.

www.biologie.uni-hamburg.de/b-online/e31/31.htm (Accessed 14th September 2007).

www.en.wikipedia.org/wiki/Carvone (Accessed 21st July 2010).

www.exonpress.com (Accessed 17th May 2008).

www.igrox.co.uk (Accessed 17th May 2008).

Xu, X., Van Lammeren, A.A.M., Vermeer, E. and Vreugdenhil, D. (1998). The role of Gibberellin, Abscisic acid, and Sucrose in the regulation of potato tuber formation in vitro. *Plant Physiology* **117**, 575-584.

Yada, R.Y., Coffin, R.H., Keenan, M.K., Fitts, M., Dufault, C and and Tai, G.C. (1991). The effect of maleic hydrazide (potassium salt) on potato yield, sugar content andchip color of Kennebec and Norchip cultivars. *American Potato Journal*, **68**, 705-709.

Yang, S.F. and Hoffman, N.E. (1984). Ethylene biosynthesis and its regulation in higher plants. *Annual Review of Plant Physiology* **35**, 155–189.

Yang, S.F. (1985). Biosynthesis and action of ethylene. *HortScience* **20**, 41-45.

Yeshitela, T., Robbertse, P.J and Stassen, P.J.C. (2004). Paclobutrazolsuppressed vegetative growth and improved yield as well as, fruitquality of Tommy Atkins mango (*Mangifera indica*) in Ethiopia. *New Zealand Journal of Crop and Horticulture Science* **32**, 281-293.

Yu, Y.B., Adams, D.O. and, Yang, S.F. (1979). Regulation of auxin-induced ethylene production in mungbean hypocotyls: role of I-aminocyclopropane-l-carboxylic acid. *Plant Physiology* **64**, 1074-1077.

Yu, Y.B., Adams, D.O. and Yang, S.F. (1979). 1-Aminocyclopropane carboxylate synthase, a key enzyme in ethylene biosynthesis. *Archives of Biochemistry and Biophysics* **198**, 280- 86.

Zhang, M.J., Yue-Ming, J., Wei-Bo, J. and Xue-Jun, L. (2006). Regulation of ethylene synthesis of harvested banana fruit by 1-Methylcyclopropene. *Food Technology and. Biotechnology* **44**, 111-115.

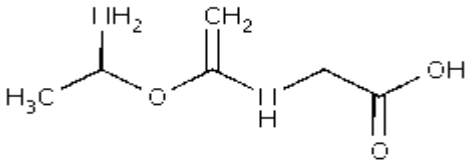
Zhao, X.C., Qu, X., Mathews, D.E. and Schaller, G.E. (2002). Effect of ethylene-pathway mutations upon expressionof the ethylene receptor ETR1 from *Arabidopsis*. *Plant Physiology* **130**, 1983-1991.

Zhou D., Kalaitzis, P., Mattoo, A.K. and Tucker. M.L. (1996a). The mRNA for an ETR1 homologue in tomato is constitutively expressed in vegetative and reproductive tissues. *Plant Molecular Biology* **30**, 1331-1338.

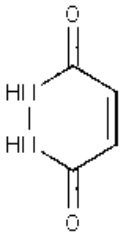
Zhou D., Mattoo, A.K. and Tucker, M.L. (1996b). Molecular cloning of a tomato cDNA (accession No.U4279) encodingan ethylene receptor. *Plant physiology* **110**, 1435-1440.

Appendix 1

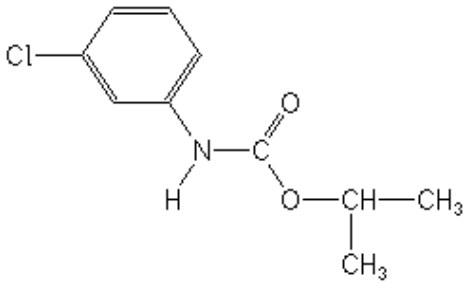
Chemical structures of different compounds.



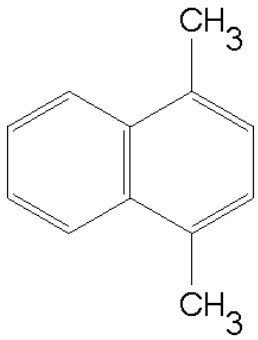
Amino ethoxyvinyleglycine (AVG)



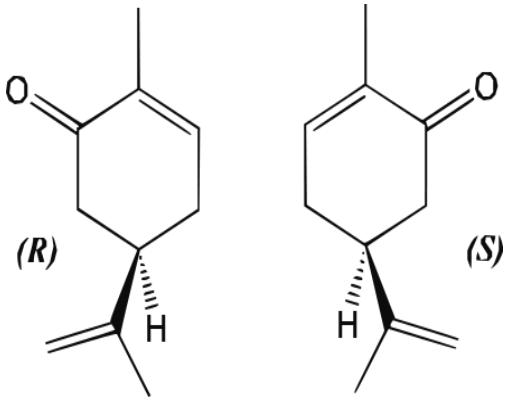
Maleic Hydrazide



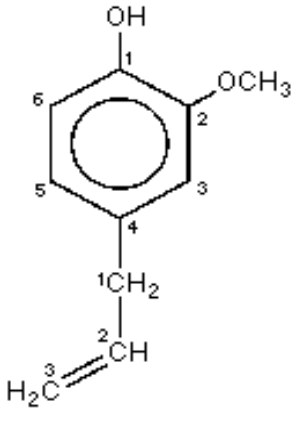
Chlorophenyl Isopropyl Carbamate (CIPC)



1, 4 Dimethylnaphthalene (DMN)



Carvone



Eugenol

1-Methylcyclopropene (1-MCP)

Appendix 2

The effect of varying relative humidity on sprouting in sweetpotato (*Ipomoea batatas, L.*)

Introduction

This study examines the effect of relative humidity on sprouting in sweetpotato. This will provide important insights into the physiological control of sprouting. A set of experiments to investigate methods of sprout control in sweetpotato was planned. It was therefore particularly important to know how critical humidity was for rate of sprouting, so that subsequent experiments could be planned with appropriate conditions.

Material and Methods

The experiment was carried out as a split plot design with three treatments as given below.

- 60% RH and 25 ºC
- 80% RH and 25 ºC
- 90% RH and 25 ºC

Each of the three treatments was replicated four times.

White fleshed sweetpotato roots were obtained from a local supermarket. Sweetpotato roots were placed in two different humidity controlled incubators at 25ºC with humidity controls set at 60% relative humidity (RH) and 80% RH respectively. 90% RH was obtained by putting the roots in semi sealed plastic bags in the incubator set at 80% RH.

Sprout numbers, length, root weight and respiration rates were recorded after 7, 14 and 28 days on each root. A sprout was considered as any growth that was longer than 1mm. Respiration rates were assessed by sealing each root in a glass gar and measuring CO_2 build up after two hours with a CO_2 meter.

Humidity was measured by using tiny talk-Gemini data loggers placed at the top and bottom of each incubator

Results

The number of sprouts per root is shown in below Figure 1. Sprouting was not eliminated by any of the levels of relative humidity tested. During the first two weeks sprout emergence was slower at 60% RH as compared to 80% and 90% RH. However, by four weeks of storage time the number of sprouts per root was the same for all humidities. Figure 2 shows effect of humidity on sprout length per root. In case of 60% and 80% humidity sprout length was the same over the storage period. After 3 weeks of stoarge there was a significant humidity effect on sprout length ($p<0.05$). LSDs indicated that 60 and 80 % RH resulted in significantly shorter sprouts than 90%RH (Table 1).

Figure 3 show the respiration rates. Respiration rate increased steadily with both relative humidity and storage time. However there were no significant differences in respiration rates in all treatments. There were lower weight losses at 90% RH as compare to 60% and 80% (Figure 4). Higher relative humidity is usually assumed to be optimum to keep the roots quality for a longer period. In this study 90%RH showed less weight loss as compare to others with no significant effect on sprout rate over the whole period of storage.

Table 1 Effect of relative humidity on mean no. of sprouts per root and average sprout length (mm) for sweetpotato roots stored at 25º C for 21 days at a range of relative humidities. Each data point is the mean of four replicate samples each of which consisted of three roots

Treatments	Average no of sprouts per root	Average sprout length (mm)
60 % RH	10.08	4.50
80 % RH	9.08	4.17
90 % RH	9.91	5.12
Treatment effect *p*	1.94	0.042
L.S.D (0.05)	3.59	0.72

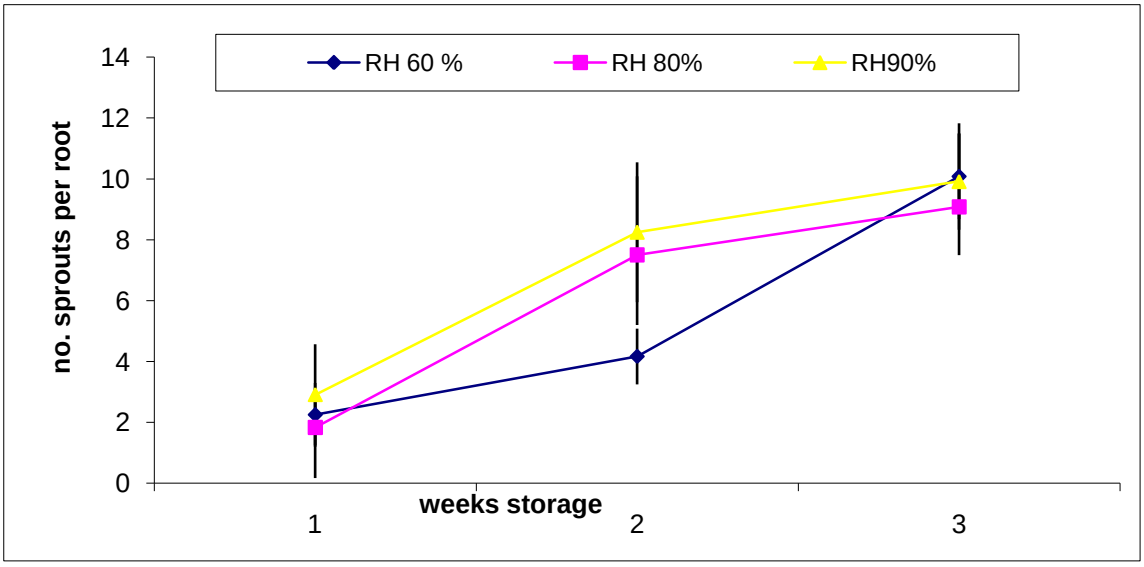


Figure 1 Effect of relative humidity on mean no. of sprouts per sweetpotato root stored at 25º C at a range of relative humidities. Each data point is the mean of four replicate samples each of which consisted of three roots.

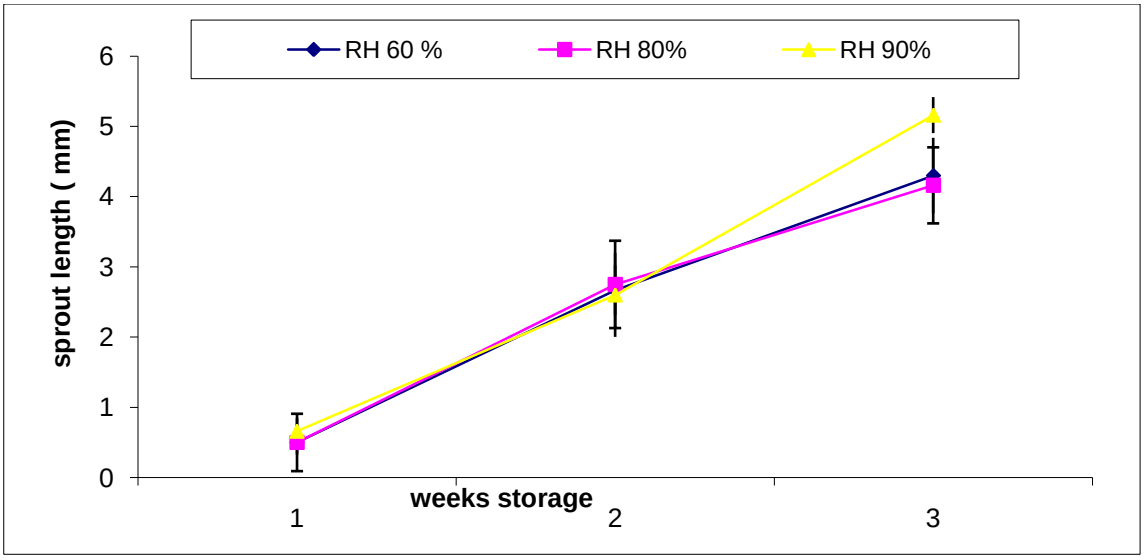


Figure 2 Effect of relative humidity on average sprouts length stored at 25º C at a range of relative humidities. Each data point is the mean of four replicate samples each of which consisted of three roots.

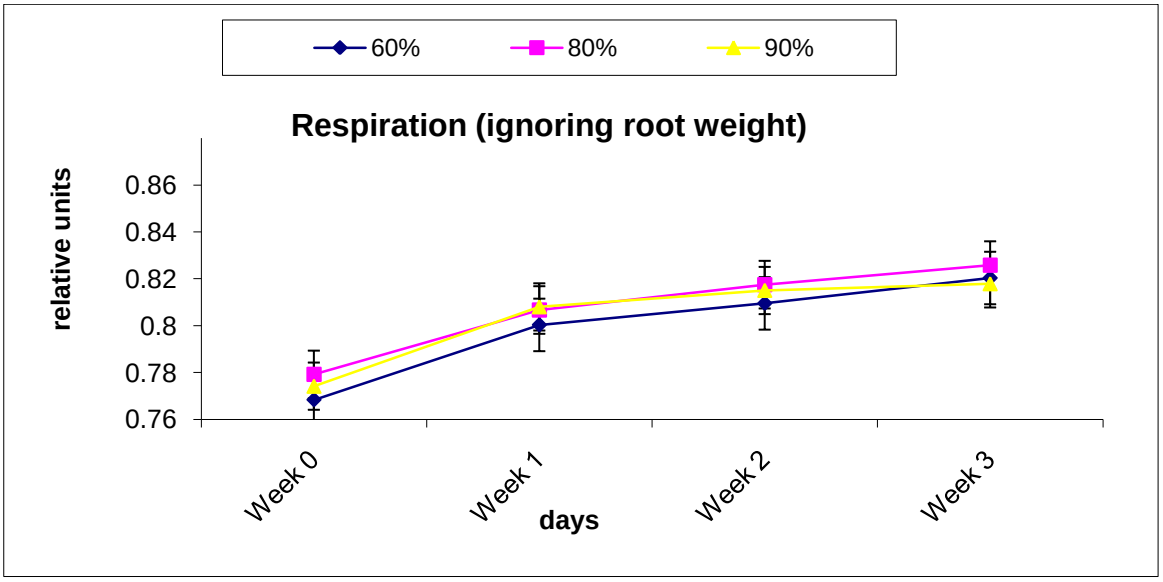


Figure 3 Effect of relative humidity on respiration rate (%) per root stored at 25º C at a range of relative humidities. Each point is the mean of four replicate samples each of which consisted of three roots.

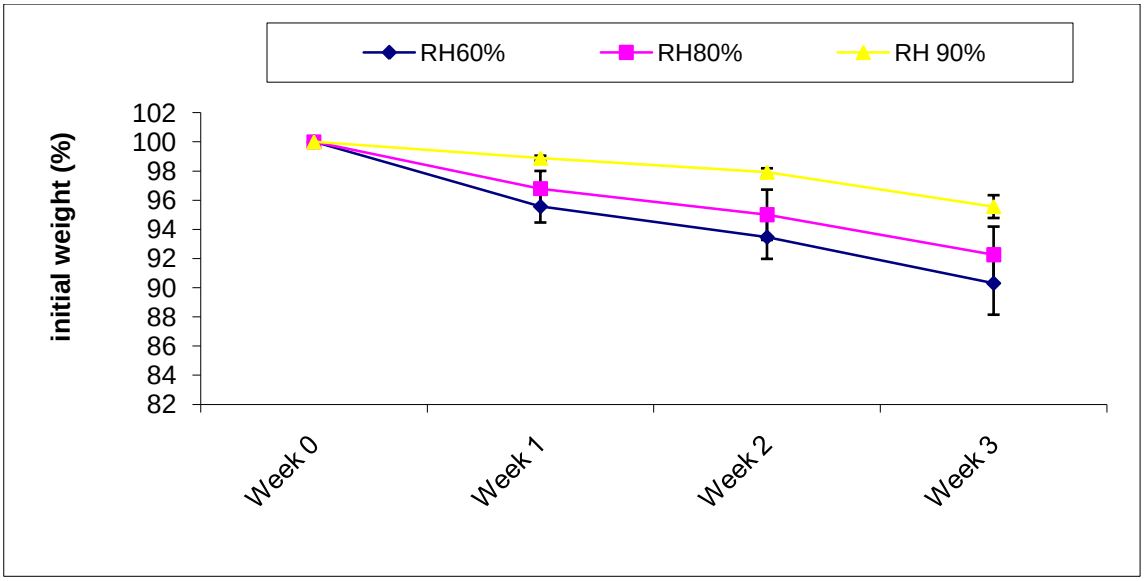


Figure 4 Effect of relative humidity on % initial weight per root stored at 25º C at a range of relative humidities. Each point is the mean of four replicate samples each of which consisted of three roots.

At lower relative humidity roots dry quickly as compare to higher humidity. Generally the higher relative humidity leads to a lower the respiration rate, but in this study no considerable differences were reported in terms of respiration rate. For later experiments on root storage, a higher relative humidity of 90% was selected to maintain the root quality while studying sprouting/dormancy behavior.

Appendix 3

Preparation of 2X reverse transcription master mix

The kit components were allowed to thaw on ice.

Referring to below table below, we calculated the volume of components required for number of reactions.

Component	Volume / reaction µl
10X RT buffer	2.0
25X dNTP mix	0.8
10X RT primers	2.0
RT inhibitor	1.0
Reverse transcriptase	1.0
Nuclease free H_2O	3.2
Total per reaction	10

Preparation for RT-PCR Product

The following components were mixed in PCR tubes for the PCR reaction. The PCR reaction was performed with 30 cycles of denaturation. 15 sec at 95˚ C, annealing, 15 sec at 56˚ C and extension 20 sec at 68˚ C.

Component	Volume / reaction µl
PCR Master Mix	8.0
Forward Primer	1.35
Reverse Primer	1.35
cDNATempelate	4.0
Syber Green	1.0
water	4.3
Total	20.0

The following DXS primers were.

DXS forward primer: GGCATCAGCGTACCAAAGTT

DXS reverse primer: CTATAACGATGGCCCGTCAG

Appendix 4

Analysis of Variance

Chapter 3

Analysis of variance (Table3-3)

Sprout growth

Variate: Week_1, Week_2, Week_3, Week_4, Week_5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replications stratum	3	673.1	224.4	0.53	
replications.Subject stratum					
treatments	3	11201.6	3733.9	8.90	0.001
Residual	9	3775.2	419.5	4.10	
replications.Subject.Time stratum					
d.f. correction factor 0.4019					
Time	4	67583.5	16895.9	165.19	<.001
Time.treatments	12	7504.3	625.4	6.11	0.002
Residual	48	4909.5	102.3		
Total	79	95647.1			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Analysis of variance (Table 3-3)

Number of Sprouts

Variate: Week_1, Week_2, Week_3, Week_4, Week_5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replications stratum	3	7.1750	2.3917	0.90	
replications.Subject stratum					
treatments	4	83.7463	20.9366	7.91	0.009
Residual	12	31.7437	2.6453	6.60	
replications.Subject.Time stratum					
d.f. correction factor 0.3879					
Time	4	126.1962	31.5491	78.71	<.001
Time.treatments	16	12.1287	0.7580	1.89	0.123
Residual	60	24.0500	0.4008		

Total	99	285.0400

(d.f. are multiplied by the correction factors before calculating F probabilities)

Analysis of variance (Table3-4)

Weight loss

Variate: Week0, Week_1, Week_2, Week_3, Week_4, Week_5, Week_6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	3	4.1075	1.3692	0.83	
replication.Subject stratum					
treatment	9	12.0105	1.3345	0.81	0.610
Residual	27	44.4166	1.6451	8.30	
replication.Subject.Time stratum					
d.f. correction factor 0.3137					
Time	6	1223.3373	203.8895	1028.21	<.001
Time.treatment	54	10.4680	0.1939	0.98	0.495
Residual	180	35.6931	0.1983		
Total	279	1330.0328			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Chapter 4

Analysis of variance (Table 4-2)

Sprout growth

Variate: Week_1, Week_2, Week_3, Week_4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	3	17.637	5.879	1.02	
replication.Subject stratum					
treatment	3	461.415	153.805	26.58	<.001
Residual	9	52.081	5.787	3.20	
replication.Subject.Time stratum					
d.f. correction factor 0.4076					
Time	3	186.666	62.222	34.46	<.001
Time.treatment	9	309.875	34.431	19.07	<.001
Residual	36	65.002	1.806		
Total	63	1092.676			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Analysis of variance (Table 4-2)

Number of sprouts

Variate: Week_1, Week_2, Week_3, Week_4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	3	5.0885	1.6962	0.73	
replication.Subject stratum					
treatment	3	160.3108	53.4369	23.10	<.001
Residual	9	20.8212	2.3135	11.61	
replication.Subject.Time stratum					
d.f. correction factor 0.4839					
Time	3	30.0330	10.0110	50.24	<.001
Time.treatment	9	34.0990	3.7888	19.01	<.001
Residual	36	7.1736	0.1993		
Total	63	257.5260			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Analysis of variance (Table 4-3)

Weight loss

Variate: Week_0, Week_1, Week_2, Week_3, Week_4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	3	11.6091	3.8697	1.21	
replication.Subject stratum					
treatment	3	10.6347	3.5449	1.11	0.396
Residual	9	28.8099	3.2011	4.53	
replication.Subject.Time stratum					
d.f. correction factor 0.3490					
Time	4	239.0823	59.7706	84.67	<.001
Time.treatment	12	10.3097	0.8591	1.22	0.341
Residual	48	33.8838	0.7059		
Total	79	334.3294			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Analysis of variance (Table 4-5)

Sprout length

Variate: Week_2, Week_3, Week_4, Week_5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	3	81.042	27.014	1.08	
replication.Subject stratum					
treatment	4	3521.322	880.331	35.29	<.001
Residual	12	299.337	24.945	2.72	
replication.Subject.Time stratum					
d.f. correction factor 0.3674					
Time	3	436.388	145.463	15.89	<.001
Time.treatment	12	1341.806	111.817	12.21	<.001
Residual	45	411.988	9.155		
Total	79	6091.883			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Analysis of variance (Table 4-5)

Number of sprouts

Variate: Week_2, Week_3, Week_4, Week_5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	3	1.2931	0.4310	0.35	
rep.Subject stratum					
ethylene_levels	4	245.5056	61.3764	50.39	<.001
Residual	12	14.6167	1.2181	5.73	
rep.Subject.Time stratum					
d.f. correction factor 0.5159					
Time	3	17.5931	5.8644	27.60	<.001
Time.ethylene_levels	12	51.0944	4.2579	20.04	<.001
Residual	45	9.5625	0.2125		
Total	79	339.6653			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Analysis of variance (Table 4-6)

Weight loss

Variate: Week_1, Week_2, Week_3, Week_4, Week_5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	3	16.5186	5.5062	1.44	
replication.Subject stratum					
treatment	4	25.0092	6.2523	1.64	0.229
Residual	12	45.8368	3.8197	8.82	
replication.Subject.Time stratum					
d.f. correction factor 0.4544					
Time	4	724.3359	181.0840	418.25	<.001
Time.treatment	16	7.3323	0.4583	1.06	0.417
Residual	60	25.9773	0.4330		
Total	99	845.0100			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Analysis of variance (Table 4-7)

Respiration rate

Variate: Week_2, Week_3, Week_4, Week_5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	3	577.41	192.47	1.24	
replication.Subject stratum					
treatment	4	25498.03	6374.51	41.14	<.001
Residual	12	1859.47	154.96	1.67	
replication.Subject.Time stratum					
d.f. correction factor 0.6651					
Time	3	1120.22	373.41	4.01	0.029
Time.treatment	12	462.18	38.52	0.41	0.903
Residual	45	4187.69	93.06		
Total	79	33704.99			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Chapter 5

Analysis of variance (Table 5-2)

Sprout growth

Variate: Week_1, Week_2, Week_3, Week_4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	3	7.598	2.533	0.81	
replication.Subject stratum					
treatment	5	3404.445	680.889	216.74	<.001
Residual	15	47.123	3.142	2.91	
replication.Subject.Time stratum					
d.f. correction factor 0.4817					
Time	3	517.470	172.490	159.61	<.001
Time.treatment	15	2135.407	142.360	131.73	<.001
Residual	54	58.358	1.081		
Total	95	6170.401			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Analysis of variance (Table 5-3)

Number of of sprouts

Variate: Week_1, Week_2, Week_3, Week_4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	3	0.63310	0.21103	1.81	
replication.Subject stratum					
treatment	5	156.47801	31.29560	268.43	<.001
Residual	15	1.74884	0.11659	3.09	
replication.Subject.Time stratum					
d.f. correction factor 0.7381					
Time	3	3.52199	1.17400	31.16	<.001
Time.treatment	15	14.69329	0.97955	26.00	<.001
Residual	54	2.03472	0.03768		
Total	95	179.10995			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Analysis of variance (Table 5-4)

Weight loss

Variate: Week_0, Week1, Week2, Week3, Week4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	3	3.3106	1.1035	0.58	
replication.Subject stratum					
treatment	5	29.3410	5.8682	3.10	0.040
Residual	15	28.3760	1.8917	4.85	
replication.Subject.Time stratum					
d.f. correction factor 0.5157					
Time	4	998.6153	249.6538	640.30	<.001
Time.treatment	20	20.7428	1.0371	2.66	0.014
Residual	72	28.0728	0.3899		
Total	119	1108.4585			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Analysis of variance (Table 5-5)

Respiration rate

Variate: Week_1, Week_2, Week_3, Week_4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	3	188.29	62.76	0.76	
replication.Subject stratum					
treatment	5	5432.81	1086.56	13.09	<.001
Residual	15	1245.01	83.00	2.07	
replication.Subject.Time stratum					
d.f. correction factor 0.8109					
Time	3	886.24	295.41	7.38	<.001
Time.treatment	15	799.85	53.32	1.33	0.235
Residual	54	2162.14	40.04		
Total	95	10714.35			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Analysis of variance (Table 5-10)

Weight loss

Variate: Week_0, Week_1, Week_2, Week_3, Week_4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	3	3.2986	1.0995	0.75	
replication.Subject stratum					
treatment	5	24.1857	4.8371	3.29	0.033
Residual	15	22.0301	1.4687	5.51	
replication.Subject.Time stratum					
d.f. correction factor 0.6778					
Time	4	810.3229	202.5807	760.61	<.001
Time.treatment	20	17.6037	0.8802	3.30	0.001
Residual	72	19.1765	0.2663		
Total	119	896.6175			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Analysis of variance (table 5-8)

Sprout growth

Variate: Week_1, Week_2, Week_3, Week_4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	3	60.821	20.274	1.24	
replication.Subject stratum					
treatment	5	2972.011	594.402	36.34	<.001
Residual	15	245.328	16.355	2.32	
replication.Subject.Time stratum					
d.f. correction factor 0.3973					
Time	3	392.630	130.877	18.58	<.001
Time.treatment	15	1292.890	86.193	12.24	<.001
Residual	54	380.331	7.043		
Total	95	5344.010			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Analysis of variance (Table 5-9)

Number of sprout

Variate: Week_1, Week_2, Week_3, Week_4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	3	0.59606	0.19869	0.61	
replication.Subject stratum					
treatment	5	196.35301	39.27060	120.63	<.001
Residual	15	4.88310	0.32554	3.86	
replication.Subject.Time stratum					
d.f. correction factor 0.7449					
Time	3	14.69792	4.89931	58.16	<.001
Time.treatment	15	38.05903	2.53727	30.12	<.001
Residual	54	4.54861	0.08423		
Total	95	259.13773			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Analysis of variance (Table 5-11)

Respiration rate

Variate: Week_1, Week_2, Week_3, Week_4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	3	267.914	89.305	0.70	
replication.Subject stratum					
treatment	5	6334.046	1266.809	9.98	<.001
Residual	15	1903.876	126.925	31.77	
replication.Subject.Time stratum					
d.f. correction factor 0.6257					
Time	3	58.313	19.438	4.87	0.015
Time.treatment	15	45.145	3.010	0.75	0.663
Residual	54	215.717	3.995		
Total	95	8825.010			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Chapter 6

Analysis of variance (Table 6-1)

Sprout growth

Variate: days4, days10, days_6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	2.3083	1.1541	0.51	
Rep.Subject stratum					
Lines	5	55.2780	11.0556	4.85	0.016
Residual	10	22.8051	2.2805	7.15	
Rep.Subject.Time stratum					
d.f. correction factor 0.5455					
Time	2	29.3869	14.6934	46.06	<.001
Time.Lines	10	14.8040	1.4804	4.64	0.011
Residual	24	7.6557	0.3190		
Total	53	132.2379			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Analysis of variance (Table 6-1)

Sprout rate (%)

Variate: days 4, days_6, days10

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		2948.8	1474.4	1.04	
Rep.Subject stratum						
Lines	5		41508.4	8301.7	5.83	0.009
Residual	10		14234.7	1423.5	8.17	
Rep.Subject.Time stratum						
d.f. correction factor 0.9349						
Time	2		4850.1	2425.1	13.92	<.001
Time.Lines	10		2854.1	285.4	1.64	0.165
Residual	23	(1)	4008.1	174.3		
Total	52	(1)	67668.7			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Analysis of variance (Table 6-2)

Sprout growth

Variate: days4, days_6, days10

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	2.7236	1.3618	0.92	
Rep.Subject stratum					
Lines	5	44.5827	8.9165	6.02	0.008
Residual	10	14.8050	1.4805	5.11	
Rep.Subject.Time stratum					
d.f. correction factor 0.6750					
Time	2	46.8693	23.4347	80.83	<.001
Time.Lines	10	19.5081	1.9508	6.73	<.001
Residual	24	6.9578	0.2899		
Total	53	135.4465			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Analysis of variance (Table 6-2)

Sprout rate (%)

Variate: days4, days_6, days10

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	4171.2	2085.6	2.03	
Rep.Subject stratum					
Lines	5	27764.5	5552.9	5.41	0.011
Residual	10	10263.9	1026.4	6.79	
Rep.Subject.Time stratum					
d.f. correction factor 0.6606					
Time	2	5509.6	2754.8	18.22	<.001
Time.Lines	10	2029.8	203.0	1.34	0.295
Residual	24	3628.2	151.2		
Total	53	53367.2			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Analysis of variance (Table 6-3)

Sprout growth

Variate: days4, days_6, days10

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.51110	0.75555	3.10	
Rep.Subject stratum					
Lines	5	9.51819	1.90364	7.82	0.003
Residual	10	2.43548	0.24355	9.15	
Rep.Subject.Time stratum					
d.f. correction factor 0.6502					
Time	2	2.41944	1.20972	45.46	<.001
Time.Lines	10	1.27439	0.12744	4.79	0.005
Residual	24	0.63860	0.02661		
Total	53	17.79719			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Analysis of variance (Table 6-3)

Sprout rate (%)

Variate: days4, days_6, days10

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	4480.90	2240.45	2.79	
Rep.Subject stratum					
Lines	5	29107.59	5821.52	7.24	0.004
Residual	10	8040.58	804.06	13.05	
Rep.Subject.Time stratum					
d.f. correction factor 0.5330					
Time	2	3306.97	1653.49	26.83	<.001
Time.Lines	10	2241.49	224.15	3.64	0.027
Residual	24	1478.84	61.62		
Total	53	48656.38			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Analysis of variance (Table 6-4)

Sprout growth

Variate: days4, days_6, days10

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.7161	0.8581	5.78	
Rep.Subject stratum					
Lines	5	44.2734	8.8547	59.69	<.001
Residual	10	1.4836	0.1484	0.90	
Rep.Subject.Time stratum					
d.f. correction factor 0.5757					
Time	2	100.3802	50.1901	303.03	<.001
Time.Lines	10	14.7770	1.4777	8.92	<.001
Residual	24	3.9751	0.1656		
Total	53	166.6052			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Analysis of variance (Table 6-4)

Sprout rate (%)

Variate: days4, days_6, days10

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	43.89	21.95	0.37	
Rep.Subject stratum					
Lines	5	1988.96	397.79	6.74	0.005
Residual	10	589.79	58.98	1.34	
Rep.Subject.Time stratum					
d.f. correction factor 0.9762					
Time	2	7370.32	3685.16	83.50	<.001
Time.Lines	10	244.50	24.45	0.55	0.831
Residual	24	1059.24	44.13		
Total	53	11296.69			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Analysis of variance (Table 6-5)

Sprout growth

Variate: days4, days_6, days10

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.67273	0.33636	4.40	
Rep.Subject stratum					
Lines	5	3.13496	0.62699	8.20	0.003
Residual	10	0.76470	0.07647	1.83	
Rep.Subject.Time stratum					
d.f. correction factor 0.6543					
Time	2	33.62296	16.81148	401.90	<.001
Time.Lines	10	0.22553	0.02255	0.54	0.783
Residual	24	1.00391	0.04183		
Total	53	39.42479			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Analysis of variance (Table 6-5)

Sprout rate (%)

Variate: days4, days_6, days10

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	509.12	254.56	2.09	
Rep.Subject stratum					
Lines	5	3190.10	638.02	5.24	0.013
Residual	10	1217.48	121.75	2.21	
Rep.Subject.Time stratum					
d.f. correction factor 0.9853					
Time	2	4160.13	2080.06	37.78	<.001
Time.Lines	10	955.81	95.58	1.74	0.131
Residual	24	1321.21	55.05		
Total	53	11353.84			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Analysis of variance (Table 6-6)

Sprout growth

Variate: days4, days_6, days10

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.2448	0.1224	0.54	
Rep.Subject stratum					
Lines	5	43.3085	8.6617	38.17	<.001
Residual	10	2.2694	0.2269	1.26	
Rep.Subject.Time stratum					
d.f. correction factor 0.5699					
Time	2	122.8196	61.4098	339.85	<.001
Time.Lines	10	16.4543	1.6454	9.11	<.001
Residual	24	4.3368	0.1807		
Total	53	189.4334			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Analysis of variance (Table 6-6)

Sprout rate (%)

Variate: days4, days_6, days10

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	315.70	157.85	1.28	
Rep.Subject stratum					
Lines	5	5164.30	1032.86	8.38	0.002
Residual	10	1233.25	123.33	2.40	
Rep.Subject.Time stratum					
d.f. correction factor 0.9016					
Time	2	2324.17	1162.09	22.60	<.001
Time.Lines	10	208.89	20.89	0.41	0.918
Residual	24	1234.23	51.43		
Total	53	10480.55			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Appendix 5

Publications and presentations

Cheema, M.U.A., Rees, D., Westby, A and Taylor, M. (2010) Hormonal Control of Sprouting of sweetpotatoes in Storage. *Acta Horticulturae* **858** 173-178.

Cheema, M.U.A, Rees, D., Taylor, M. and Westby, A. (2007). Control of sprouting in root and tuber crops. Poster presented at the International Symposium on Prospects of Horticultural Industry in Pakistan Faisalabad, Pakistan 28th to 30th March.

Bishop, C., Rees, D., Cheema, M.U.A., Harper, G. and Stroud, G. (2011) Chapter 16. Potatoes in: Rees, D., Orchard, J.E. and Farrell, G. (eds) (2011). *Crop Post-Harvest: Science and Technology* Volume 3: Perishables Blackwell Science Ltd, UK. In press

Rees, D., Westby, A., Tomlins, K., van Oirschot, Q.E.A., Cheema, M.U.A. Cornelius, E. and Amjad, M (2011) Chapter 18. Tropical Root Crops. in: Rees, D., Orchard, J.E. and Farrell, G. (eds) (2011). *Crop Post-Harvest: Science and Technology* Volume 3: Perishables Blackwell Science Ltd, UK. In press