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ROBERT SARPONG AMOAH

THE EFFECTS OF ETHYLENE ON SWEETPOTATO STORAGE

SCHOOL OF APPLIED SCIENCES

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Supervisor: Professor Leon Terry

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ABSTRACT

Sweetpotato (*Ipomoea batatas* (L.) Lam.) is an important food security crop in many parts of the developing world. Despite its economic value and growing contribution to nutrition and health in tropical and sub-tropical countries, sweetpotato has a short postharvest life. Inhibition of sprout growth to permit long-term storage and marketing of the roots is a major challenge in the sweetpotato industry. To date, no technically and economically satisfactory method is available to extend the tropical storage of sweetpotato. Previous studies have demonstrated the efficacy of both exogenous ethylene and the ethylene perception inhibitor 1-methylcyclopropene (1-MCP) in suppressing sprout growth in other root crops such as potato and onion, which display differential endodormancy. It has been proposed, through studies by others, that ethylene and 1-MCP may also enhance sweetpotato storage. In the present study, the physiological and biochemical effects of exogenously applied ethylene and 1-MCP were further investigated. In one set of experiments, 1-MCP (1.0 µl L^{-1}, 24 h) was applied to sweetpotato roots pre-storage, followed by storage in air at 15 or 20ºC. The 1-MCP treatment significantly reduced the incidence of root decay and marketable weight loss in a cultivar-dependent manner. No significant effects of 1-MCP on respiration rate, sprouting and the spatial concentrations of non-structural carbohydrates were found. In other experiments, roots were subjected to four concerted regimes of ethylene (10 µl L^{-1}, 25ºC) *viz.* (1) continuous ethylene supplementation throughout the storage; (2) truncated ethylene supplementation after dormancy break, followed by storage in air; (3) post-dormant ethylene supplementation after previous air storage and (4) continuous air storage. To facilitate these treatments, a laboratory-scale apparatus was designed and fabricated with the capacity to generate variable ethylene concentrations for parallel flushing of multi-storage chambers at flexible timings. Treatment effects on the physiological and biochemical variables in the roots were assessed during storage. The respiration rate of roots held continuously in exogenous ethylene was promoted 1.5-2.0-fold whilst concomitantly, sprout growth was retarded. Alongside, the levels of the monosaccharides glucose and fructose declined and are discussed in terms of their utilization for respiration and effect on solute potential.

Ethylene supplementation was also associated with higher incidence of root decay and water loss, culminating in increased loss of marketable weight. Sprouts grew vigorously

when the ethylene supplementation was truncated after dormancy break and the gaseous composition returned to air. On the other hand, applying exogenous ethylene immediately after dormancy beak effectively suppressed sprout growth to the same efficacy as the continuous treatment throughout storage. Post-dormancy ethylene supplementation also had greater benefits in terms of reducing weight loss and ethylene-induced rot symptoms. It is thus proposed that pre-storage treatment with 1-MCP, in combination with post-dormant ethylene supplementation may be the ideal application regime for sweetpotato storage. The mechanism by which exogenous ethylene inhibits sprout growth, in relation to the plant hormones abscisic acid (ABA) and zeatin riboside (ZR) were investigated. The growth inhibitor ABA was suppressed by exogenous ethylene suggesting that ethylene and ABA control dormancy via different pathways. The content of ZR was enhanced by exogenous ethylene but effective sprout growth was not promoted until the ethylene was removed. It thus suggests that exogenous ethylene may activate the biosynthesis of growth promoting ZR, but as long as ethylene is perceived, meristematic tissue sensitivity to ZR is impaired. Distinctive spatial effects of both ethylene and 1-MCP on the metabolism of phenolic compounds in sweetpotato were also observed and are discussed alongside their roles in dormancy break, stress injury restitution and defence against phytopathogens.

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NOTATIONS

α	alpha
^{o}C	degree celsius
β	beta
$	dollar
µl	microlitre
$µl\ L^{-1}$	microlitre per litre
µM	micromolar
%	percent
±	plus or minus
>	greater than
≥	equal to or greater than
≤	equal to or less than
1-MCP	1-methylcyclopropene
AA	effect of continuous treatment in continuous air
AAF	effect of continuous treatment in air on flesh tissue
AAS	effect of continuous treatment in air on skin tissue
ABA	abscisic acid
ABTS	2,2'-azinobis-(-3-ethylbenzothianzoline-6-sulfonic acid) diammonium salt
ACC	1-aminocyclopropane-1-carboxylic acid
ACO	S-adenosyl methionine oxidase
ACP	anaerobic compensation point
ACS	S-adenosyl methionine synthase

AE	effect of treatment transfer from air into ethylene
AEF	effect of treatment transfer from air into ethylene on flesh tissue
AES	effect of treatment transfer from air into ethylene on skin tissue
AMP	adenosine monophosphate
ANOVA	analysis of variance
ATP	adenosine triphosphate
AVG	aminoethoxyvinylglycine
AVRDC	Asian Vegetable Research Development Centre
BC	before Christ
BOC	British Oxygen Comapny
cc	cubic centimeter
C_2H_4	ethylene
CA	controlled atmosphere
Ca	calcium
ca.	circa (approximately)
CAC	The Codex Alimentarius Commission
ChAE	chlorogenic acid equivalent
CIP	The International Potato Center, Lima, Peru
CIPC	chlorpropham
Cl	chloride
CO_2	carbon dioxide
CoA	co-enzyme A
cm	centimeter
CRI	Crops Research Institute, Ghana

CSPI	Centre for Science in the Public Interest, United States of America
Cu	copper
cv.	cultivar
DAD	photodiode array detector
DFID	Department for International Development, United Kingdom
DM	dry matter
DNA	deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
DHZR	dihydro-zeatin riboside
DW	dry weight
EA	effect of treatment transfer from ethylene into air
EAF	effect of treatment transfer from ethylene into air on flesh tissue
EAS	effect of treatment transfer from ethylene into air on skin tissue
EE	effect of continuous treatment in ethylene
EEF	effect of treatment in continuous ethylene on flesh tissue
EES	effect of treatment in continuous ethylene on skin tissue
EAC	East African Community
EDIF	Export Development and Investment Fund, Ghana
EIN3	ethylene-insensitive3
ELSD	Evaporative Light-Scattering Detector
EPA	United States Environmental Protection Agency
et al.	and others
FAO	Food and Agriculture Organisation

FAOSTAT	Food and Agriculture Organisation Statistics
Fe	iron
FID	flame ionisation detector
FW	fresh weight
g	gramme
g^{-1}	per gramme
GA	gibberellin
GC	gas chromatography
GI	glycemic Index
Gy	gray
h	hour
h^{-1}	per hour
ha	hectare
HCl	hydrochloric acid
HortCRSP	Horticulture Collaborative Research Support Program
HP	horsepower
HPLC	high performance liquid chromatography
HPLC-MS	high performance liquid chromatography coupled with mass spectrometry
HWD	hot wire detector
IAA	indole acetic acid
IAR	Institute of Agricultural Research, Sierra Leone
I.D.	internal diameter
IITA	International Institute for Tropical Agriculture, Nigeria

IPA	isopentenyl adenine
ISHS	International Society of Horticultural Scientists
K	potassium
kg	kilogramme
kg^{-1}	per kilogramme
kGy	kilogray
kPa	kilo Pascal
L	litre
LS	lignification score
LSD	least significance difference
M	molar
MDG	Millennium Development Goals
MENA	methyl naphthalene-acetic acid
Mg	magnesium
mg	milligram
MH	maleic hydrazide
mL	millilitre
mm	millimeter
Mn	manganese
MOFA	Ministry of Food and Agriculture, Ghana
MS	mass spectrometer
MT	metric tonnes
n	number of replicates
Na	sodium

n.a.	not available
NASA	National Aeronautics and Space Administration
NCCov	North Carolina Covington
NCSU	North Carolina State University
ng	nanogram
nl	nanolitre
No.	number
NSP	non-starch polysaccharides
O_2	oxygen
O.D.	outside diameter
ORAC	oxygen radical absorbance capacity
P	phosphorus
PAL	phenylalanine ammonia lyase
PBS	phosphate-buffered saline
p	probability
pH	hydrogen ion concentration
PGR	plant growth regulator
PPO	polyphenol oxidase
PSD	Pesticides Safety Directorate, United Kingdom
QIEAC	Quality Infrastructure in East African Community
R^2	coefficient of determination
RH	relative humidity
RNA	ribonucleic acid
RTIMP	Root and Tuber Improvement and Marketing Programme, Ghana

ROS	reactive oxygen species
SAM	S-adenosyl methionine
SEM	standard error of the mean
SPA	sweetpotato anthocyanins
Spp	species
SPP	sweetpotato protein
STS	silver thiosulphate
TCA	tricarboxylic acid
TE	trolox equivalent
t/ha	tonnes per hectare
QIEAC	Quality Infrastructure in East African Community
QToF	quadruple time of flight
UK	United Kingdom
UNCTAD	United Nations Conference on Trade and Development
UPLC	Ultra Performance Liquid Chromatography
US	United States of America
USA	United States of America
USAID	United States Agency for International Development
USDA	United Stated Department of Agriculture
UV-VIS	ultraviolet-visible spectrum
v/v	ratio by volume
VEPEAG	Vegetables Producers Association of Ghana
viz	that is to say or namely
ZR	zeatin riboside

CHAPTER ONE: INTRODUCTION

1.1 Project Background

Sweetpotato (*Ipomoea batatas* (L.) Lam.) is a staple food for many small-holder farmers in Africa, Asia and Latin America. It has numerous agronomic advantages such as adaptability to diverse (including marginal) growing conditions, high yield and excellent health-promoting benefits (Islam *et al.*, 2003; Islam, 2006; CIP, 2006; Brinley *et al.,* 2008). As a result, sweetpotato has great potential to insure sustainable food supply and alleviate diseases in developing countries. In many regions in Africa, sensitization programmes are on-going to create awareness and promote the consumption of sweetpotato as a public health tool to improve nutrition and health. In Ghana, for example, there are systematic policies to include sweetpotato in the household food budget to enhance child and maternal nutrition (RTIMP, 2008). In particular, the yellow-orange-fleshed variety, which has been found to be rich in beta-carotene, is being promoted in most agricultural and nutritional programmes as a dietary therapy to combat vitamin A deficiency diseases that result in blindness and death of an estimated 250,000 – 500,000 African children each year (Low *et al.,* 1997, Van't Ooster, 1999). Awareness of the abundance of other beneficial food ingredients in sweetpotato has also enhanced its export to international horticultural markets (Thottappilly, 2009). Besides its value as food, sweetpotato has industrial uses such as for the manufacture of starch – an important economic activity in China.

Despite the many advantages, sweetpotato deteriorates readily in tropical and sub-tropical climates where it is mostly produced. Long-term storage of the edible sweetpotato root poses a major challenge to its food security role and marketing across the globe (Hall *et al.,* 1998; Rees *et al*., 2001; van Oirschot *et al*., 2003). Among the numerous storage problems (including weevil infestation and microbial decay), disorders such as pre-mature sprouting contribute greatly to the loss of marketing value. It is estimated that sweetpotato can sprout within two to three weeks under ambient storage conditions in the tropics (Rees *et al.,* 2003). In advanced countries, the cool chain is normally employed to store the roots using optimal storage conditions of 13.5^{o}C and a relative humidity of 90-

95%. This has been reported to extend the shelf-life up to a year (Picha, 1986b). Cold storage facilities, however, are not always accessible in developing countries due to economic and technical constraints. Other methods to suppress sprouting such as pre-harvest spraying with maleic hydrazide (MH) or postharvest treatment with chlorpropham (CIPC) are limited by health concerns associated with the chemical residues (Booth, 1974). The lack of suitable storage technologies among sweetpotato farmers in developing countries is a disincentive to large-scale investment into its production and limits its food security prospects. This has prompted the quest for viable storage alternatives that can be adapted by farmers in these regions.

This research explored the potential use of the phytohormone ethylene and the anti-ethylene gas 1-methylcyclopropene (1-MCP) to improve the storage of sweetpotato. Ethylene is produced naturally by all plant tissues. Synthetic ethylene is also commercially available as a plant growth regulator. Ethylene is known to be involved in the regulation of many physiological and biochemical processes in plant tissues that affect the quality of horticultural produce (Lin *et al.,* 2009). The effects of ethylene may be beneficial or deleterious depending on a matrix of factors such as the species, cultivar, type of tissue, growth stage and the ethylene application regime (Watkins, 2006). Consequently, many postharvest studies to improve the storage of specific crops are based on understanding the role of ethylene in mediating the physiological and biochemical processes within the produce. Ethylene-induced effects on the quality of climacteric fresh produce is now well established, and its management in the store is an important commercial tool for their shelf-life extension. Sweetpotato is classified as being non-climacteric in its postharvest behaviour. Currently, studies on the effects of ethylene on non-climacteric produce are mainly focused on understanding its role in mediating dormancy, sprout growth and senescence mechanisms in potatoes and onions. In this regard, studies done in the Plant Science Laboratory at Cranfield University have contributed an immense wealth of knowledge, which has helped to improve the storage of onion and potato. Findings relating to exogenous ethylene supplementation on potatoes and onions suggest that it is a useful tool to supress sprouting in non-climacteric produce without promoting adverse physiological disorders (Downes *et al*., 2010; Cools *et al*., 2011; Foukaraki *et al*., 2011). Given its convenience as a natural plant hormone, there is commercial interest in the use of ethylene for extending the postharvest life of onion and

potato. Downes *et al.* (2010) have proposed the possibility of integrating ethylene treatment with controlled atmospheres (CA) and low or high temperature for the storage of crops. The ethylene supplementation technology could also alleviate the storage of sweetpotato and other tropical produce (Cheema *et al*., 2010).

1-MCP is a gaseous compound that binds competitively and irreversibly to ethylene binding sites in plant tissues and prevents ethylene from exerting deleterious effects on produce. The suppression of ethylene regulated processes by 1-MCP has provided yet another tool for elucidating the role of this hormone in the development of higher plants (Huber, 2008). Since its discovery in the 1990s by Blankenship and Sisler (Blankenship, 2003), 1-MCP has been the centre of much research for a potential solution to the horticultural problems caused by ethylene. The synergistic effects between ethylene and 1-MCP on many crops have been exploited in commercial horticulture. For example, applying 1-MCP prior to continuous ethylene supplementation on potato cv. Russet Burbank has been reported to reduce fry colour caused by excessive sugar accumulation (Prange *et al*., 2005). In separate experiments by Downes *et al.* (2010) and Cools *et al.* (2011), it was observed that application of either ethylene or 1-MCP alone, or a treatment combination of ethylene with prior 1-MCP application for just 24 h before or after curing reduced sprout growth in the onion cv. Sherpa for up to four months.

The present study was based on the premise that previous findings relating to the use of ethylene and 1-MCP to improve the storage of potato and onion, may apply to sweetpotato as they share biological characteristics as being non-climacteric crops. Ethylene (10 µl L^{-1}) and 1-MCP (1 µl L^{-1}, 24 h) were applied to different consignments of sweetpotato during storage. Following the treatments, the physiological and biochemical changes were evaluated at different times during storage.

1.2 Aim and objectives

1.2.1 Aim

The broad aim of this thesis is to understand the physiological and biochemical changes in sweetpotato storage roots, as mediated by postharvest treatments with ethylene and 1-MCP.

1.2.2 Specific objectives

1. To investigate the effects of 1-MCP treatment on the dormancy, sprout growth, root weight, decay and the spatio-temporal concentration of sugars and phenolic compounds in sweetpotato roots during storage.

2. To design and fabricate a laboratory-scale ethylene supplementation system for storage trials.

3. To understand the effects of exogenous ethylene supplementation dormancy, sprout growth, root weight, decay and the spatio-temporal concentration of sugars and phenolic compounds in sweetpotato roots during storage.

4. To determine the optimal timing of ethylene supplementation that would extend the postharvest life of sweetpotato.

5. To understand the synergy between exogenous ethylene and endogenous hormones in mediating dormancy in sweetpotato roots.

1.3 Thesis structure

The report, herein, is divided into seven chapters. Chapter two is a review of the literature relating to the socio-economic importance of sweetpotato in tropical developing countries, the production agronomy, morphology and the physical and biochemical quality credentials of the tuberous roots which impact marketing and nutritional value. The pre-harvest and postharvest factors which affect sweetpotato deterioration in storage and the traditional storage practices are also reviewed. The final sections of Chapter Two review the characteristics of ethylene and 1-MCP, the current technologies involved in

the use of ethylene and 1-MCP for the storage of non-climacteric produce and the potential application for sweetpotato storage. The mediating role of ethylene in relation to other plant hormones is also highlighted.

Chapter three discusses the Materials and Methods used in the study. This includes a description of the sweetpotato roots acquired for the study, the instruments used for physiological and biochemical assessments, methods for sample preparation and the protocols for the quantification of non-structural carbohydrates and phenolic compounds. The statistical analytical procedures of data collected are also explained.

Chapter four presents the results for studies on the effects of 1-MCP on sweetpotato. The results are prefaced with a short introduction and the specific experimental design relating to the 1-MCP treatment. Three separate experiments were carried out using three sweetpotato consignments and therefore, the chapter is sub-divided into Experiment 1 (Section 4.5) Experiment 2 (Section 4.6) and Experiment 3 (Section 4.7). In the subsequent sections, the results for the three experiments are collectively presented and discussed.

Chapter Five involves studies on the effects of different timings of ethylene supplementation on sweetpotato. The chapter begins with a description of experimental design used to achieve ethylene concentration of 10 $\mu l\ L^{-1}$. The rest of the structure is similar to Chapter four as it also involved separate studies on three sweetpotato consignments and therefore divided into Experiment 4, 5 and 6 in different sections.

In Chapter Six, the effects of ethylene supplementation on the profile of endogenous hormones in sweetpotato are presented. In this chapter, selected samples from the sweetpotato consignment ('North Carolina Covington II') used in experiment 4 were analysed.

General discussions and conclusions of this project are presented in Chapter Seven. Chapter Seven is followed by a list of literature referenced in relation to the study. The results of ANOVA tables used for statistical inferences are presented in Appendices A, B, C for Chapters Four, Five and Six, respectively. Appendix D contains abstracts of oral presentations and posters at conferences attended.

In the course of the study, the following presentations were made at both local and international conferences:

- **Robert S. Amoah,** Sandra Landahl and Leon A. Terry. Understanding the biochemical and physiological changes in sweetpotato roots as mediated by ethylene and 1-methylcyclopropene (1-MCP) during storage. *Postgraduate Conference, Cranfield, UK, 26 September, 2013* (Oral presentation).
- **Robert S. Amoah** and Leon A. Terry. Effects of exogenous ethylene on the biochemistry and physiology of sweetpotato roots during storage. *SCI BioResources Young Researchers Conference, Reading, UK, 2 July 2013* (Oral presentation).
- **Robert S. Amoah,** Sandra Landahl and Leon A. Terry. A laboratory-scale design to generate variable ethylene concentrations for supplementation of crops during storage. *VI International Conference on Managing Quality in Chains, Cranfield, UK, 2-5 September, 2013* (Poster presentation).
- **Robert S. Amoah** and Leon A. Terry. Effects of ethylene on the biochemical and physiological changes in sweetpotato roots during storage. *XI International Controlled and Modified Atmosphere Research Conference, Trani, Italy, 3-7 June 2013* (Oral presentation).
- **Robert S. Amoah** and Leon A. Terry. Biochemical and physiological changes in stored sweetpotatoes as mediated by 1-methylcyclopropene (1-MCP). *ISHS Acta Horticulturae* 1012: VII *International Postharvest Symposium, Kuala Lumpur, Malaysia, 25-29 June 2012* (Oral presentation).

1.4 Declaration

All work in this study was carried out using facilities at the Plant Science Laboratory, Cranfield University, UK. With the exception of the sweetpotato consignment 'TIS-2', which was imported from Ghana, all sweetpotato samples were supplied by Barfoots of Botley, W. Sussex, UK, who also provided information regarding the production agronomy, temporary storage and transport. Information about 'TIS-2' was provided by the Agricultural Engineering Department of the University of Cape Coast, Ghana. Equipment to generate 10 µl L^{-1} for the ethylene supplementation experiments was

designed by the author. Extraction of phytohormones was done by the author under the supervision of Dr. Maria Del Carmen Almanar Gavidia, whilst the quantification of hormones using UPLC-QToF MS/MS was also done by the author under the supervision of Dr. José Juan Ordaz Ortiz. All other work, including physiological assessments and biochemical assays were done by the author.

CHAPTER TWO: LITERATURE REVIEW

2.1 Sweetpotato

Sweetpotato (*Ipomoea batatas (L) lam*) is a dicotyledonous plant, which belongs to the family Convolvulaceae. Plants in this family are characterised by having woody, herbaceous or often climbing stems. Convolvulaceae has about 1000 species grouped into 50 genera. *Ipomoea* is a large genus in the family and is composed of approximately 400 species. Most of the *Ipomoea* species are annual and perennial herbaceous vines. Sweetpotato (*Ipomoea batatas*) is one of the *Ipomoea* species. It is a vine-like, perennial herb, but cultivated as an annual (Lebot, 2009). Sweetpotato is the only species in the Convolvulaceae family that has economic value as a food (Woolfe, 1992). Although sweetpotato has similar physical appearance, postharvest handling and uses as potato, it is botanically different. Potato (*Solanum tuberosum*) belongs to the Solanaceae. Morphologically, the potato tuber is the tip of a stem whilst sweetpotato is an adventitious root (Thottappilly, 2009).

2.2 Economic importance, health and food security issues relating to sweetpotato

The global importance of sweetpotato is two-fold: its candidature as a food security crop and its health-promoting credentials (Terry, 2008). Because of its numerous agronomic advantages such as high yield, superior calorific value and adaptability to diverse climatic environments, sweetpotato has great potential for alleviating hunger, especially, for resource-poor households in developing countries. Many varieties of sweetpotato possess nutritionally beneficial phytochemicals (Brinley *et al.,* 2008; Islam, 2006). The roots also have a range of industrial and agricultural uses such as for livestock feed, the production of industrial starch, alcohol and bioplastics for manufacturing companies in China, Japan and the USA.

Besides the roots, other vegetative parts of sweetpotato, such as the leaves and vines, are eaten as valuable sources of vitamins and minerals (Kays, 1998; Scott and Maldonado, 1998; Hijmans and Huaccho, 2000; Thottappilly, 2009). New uses are generating more demand for the crop in Asia (Lebot, 2009) and other parts of the world.

2.2.1 Socio-economic importance of sweetpotato

Sweetpotato is cultivated as a staple crop by millions of small-holder farmers and low-income consumers in Africa, Asia, Latin America and other parts of the world (Scott and Maldonaldo, 1998). Over 98% of the global production of sweetpotato is consumed in developing countries (Scott and Maldonado, 1998; Thottappily, 2009). It is estimated that the annual per capita consumption of sweetpotato is 20 kg in Asia, 10 kg in Africa, 5 kg in Latin America, 7 kg in Japan and 2 kg in Canada (FAO, 2007). Sweetpotato is an important source of dietary calories in countries like the Solomon Islands (22.7%), Rwanda (17.6%), Papua New Guinea (14.2%), Uganda (12.8%) and Burundi (12.6%) (Woolfe, 1992). In the north-eastern and coastal savannah areas of Ghana, sweetpotato accounts for a significant share of the annual food production and consumption (Teye, 2010). Increasing sweetpotato production is therefore acknowledged as a means to achieve sustainable food security and alleviate poverty among the poorer segments of the rural population in these countries.

Compared to other economic crops, sweetpotato ranks seventh in terms of total global production volume, thirteenth from the viewpoint of the production (monetary) value of agricultural commodities and fifth on the list of developing countries' most valuable crops (Woolfe, 1992; Leobenstein and Thottappilly, 2009). The ranking differs in specific countries. In China, for example, sweetpotato ranks fourth as a staple food crop after rice, wheat and maize (Li *et al.,* 1992), whilst in Sierra Leone, it is the third staple crop after rice and cassava (IAR, 2009). Among the world's root crops, sweetpotato ranks second only to the potato in economic importance. Yet only in the last decade has the crop been the focus of intense, co-ordinated, global research to realize its potential as a source of food, feed, processed products and income (Scott and Maldonaldo, 1998).

2.2.2 Health benefits

Sweetpotato was ranked as the healthiest vegetable by nutritionists at the Centre for Science in the Public Interest, Washington DC (CSPI, 2013). Hill *et al.* (1992), Islam (2006), Pamplona-Roger (2006) and Ezekiel *et al*. (2013) have reported that sweetpotato has anti-carcinogenic properties and cardiovascular diseases-prevention effects. The

authors noted that, as a very good source of β-carotene, vitamin C and polyphenols, sweetpotato has high antioxidant capacity which helps to alleviate these conditions. Furthermore, they recommended the consumption of sweetpotato as a means to ameliorate conditions like colon cancer and asthma. Having a low glycaemic index (GI) rating, sweetpotato has been recommended for the glycaemic therapy of diabetes. An extract from the Japanese white-skinned sweetpotato cultivar, Caiapo, contains the active ingredient acidic glycoprotein, which has been shown to increase insulin sensitivity, and is used for the treatment of type-2 diabetes (Ludvik *et al.*, 2002).

A study shows that giving vitamin A supplements to children aged between six months and five years improves the immune system, reduces the rate of illness and child mortality by 24%, and can save more than 600,000 lives each year in low- and middle-income countries (Mayo-Wilson *et al.*, 2011; Thorne-Lyman and Fawzi, 2011). In many low-income countries, however, the largest contribution to vitamin A intake (up to 82%) comes from the pro-vitamin A carotenoids in plant-based foods (Van den Berg *et al.*, 2000). Thus, pro-vitamin A carotenoids from vegetable foods are very important in alleviating vitamin A deficiency diseases in these countries (Kidmose *et al.,* 2007). The yellow-orange fleshed varieties of sweetpotato have high β-carotene content and thus their consumption is being promoted to combat vitamin A deficiency diseases that results in blindness and death of an estimated 250,000 - 500,000 African children each year (Low *et al.,* 1997, Van't Ooster, 1999). In the United States of America (USA), sweetpotato consumption has increased slightly in recent years due mainly to national advertising campaigns on the nutritional aspects of the crop (Thottappilly, 2009). The National Aeronautics and Space Administration (NASA) has included sweetpotato as a food for research in Controlled Ecological Life Support Systems for long term space missions because of its nutrition, versatility and growth habits (Islam, 2007). According to Scott *et al.* (2000) sweetpotato is likely to increase its importance by 2020 in Asia, Africa and Latin America, where more than two billion people will depend on this crop for food, feed and income.

2.2.3 Origin, distribution and production trends

Sweetpotato is considered by most botanists as native to either Central or South America. The exact centre of origin and the routes and times for its dispersal to some of its present locations, are still unknown (Woolfe, 1992). Its cultivation has taken place since about 3000 BC (O'Brien, 1972). Sweetpotato spread easily to other parts of the world owing to its adaptability to varying (including marginal) environments. Currently, it is cultivated in more than 100 countries within the tropical and warm temperate regions as a staple and exportable food crop (Lebot, 2009).

The average global production in the last 10 years is about 122 million tonnes per annum (FAOSTAT, 2010). Approximately 85% of the total world production is grown in Asia, about 10% in Africa and only about 5% in all of the rest of the world. China dominates overwhelmingly with about 46% (*viz.* 3.9 million ha) of the total cultivated area (in 2009), and over 80% of the total global production (Hijmans *et al.,* 2000; Thottappilly, 2009; Lebot, 2009; FAOSTAT, 2010). Other important areas of production are Nigeria, the Lake Victoria region of East Africa, the Caribbean regions and many Oceania Island countries. Nigeria leads the production in Africa and is currently, second to China in the world (**Table 2.1**). The majority of what is produced in Africa, however, comes collectively from the southern and eastern parts of the continent. Japan and the United States of America (USA) are the only industrial nations that grow significant amounts of sweetpotatoes (CIP, 1987). Ghana is ranked 35th among the producer countries with an estimated production of 95,000 metric tonnes (on 68500 ha) (FAOSTAT, 2010). Taking into account the subsistent production which does not enter the formal marketing chain, the Crops Research Institute of Ghana, estimates the total figure at 200,000 metric tonnes (Asafu-Agyei, 2010).

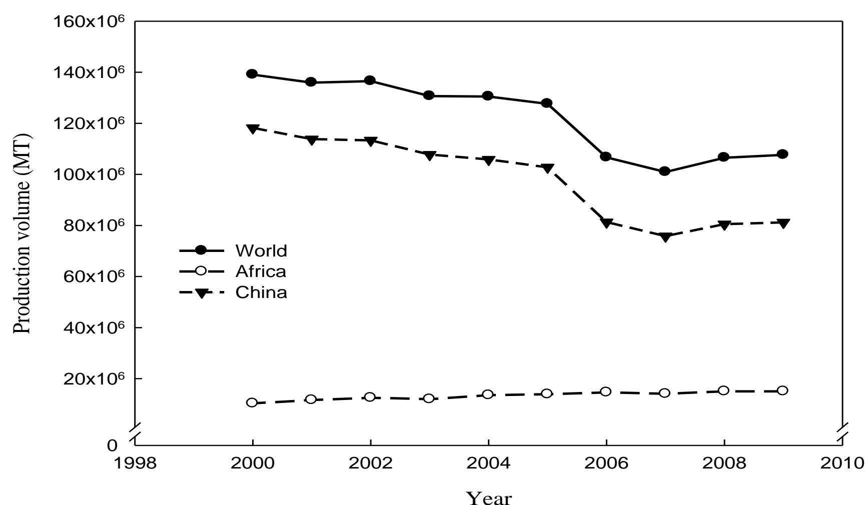


Figure 2.1 Sweetpotato production trend in China, Africa and the World (FAOSTAT, 2010)

The global trend of sweetpotato production has shown both increase and decline at various times, generally mirroring the trend in China (**Figure 2.1**). The global production has slightly declined in the last 10 years. Sweetpotato production in Africa alone, however, shows a steady upward trend where it is growing at about 5% per annum, especially in areas of increasing population and where cassava yields are being severely affected by the African Mosaic Virus (DFID, 2002).

Table 2.1 Sweetpotato production value (US $1000) and quantity (MT) by country in year 2011 (FAOSTAT, 2013)

Rank	Country	Production value (US$ 1000)	Production (MT)
1	China	2821877	75362000
2	Nigeria	249242	3300000
3	United Rep. of Tanzania	224567	3573302
4	Uganda	192898	2554000
5	Indonesia	147123	2192242
6	Viet Nam	92595	1362194
7	United States of America	84931	1223068
8	India	79047	1046600
9	Angola	78934	1045104
10	Burundi	72137	955103
11	Japan	765742	885900
56	Ghana	Not available	95000

2.2.4. Policy initiatives to boost sweetpotato production in Ghana

Following awareness of the food security prospects of sweetpotato, and as part of national efforts to achieve the Millennium Development Goals (MDGs), the Government of Ghana has recently intensified its campaign to feature it prominently in the household food budget (RTIMP, 2008). The crop is poised to play a major role as a public health tool for improved child and maternal nutrition, as well as a stand-by to overt national food crisis. Consequently, the Ministry of Food and Agriculture (MOFA) is sponsoring concerted studies to promote various aspects of sweetpotato's productivity, marketing and utilisation under the Root and Tuber Improvement and Marketing Programme (RTIMP, 2008). In support of this initiative, the Export Development and Investment Fund (EDIF) made available to Vegetable Producers Association of Ghana (VEPEAG), farm equipment worth GH¢136,000 (*ca.* $ 93,793) to enhance large-scale production of sweetpotatoes for local consumption and export (Afari-Mintah, 2007). A complementary USAID funded collaborative project (HortCRSP) between the Crops Research Institute of Ghana (CRI), the University of Ghana and Tuskegee University also aims to promote the production and consumption of the ß-carotene-rich orange-fleshed variety to curb malnutrition (HortCRSP, 2012). The project "Developing an efficient and cost effective postharvest technology for fresh sweetpotato roots" and related researches are also currently underway in Ghana to assist farmers to take advantage of the booming local and international trade in the horticultural market.

2.3 Life cycle and morphology of sweetpotato

2.3.1 The growth conditions and life cycle

Although it is naturally a perennial crop, sweetpotato is cultivated as an annual (Woolfe, 1992; Lebot, 2009). Sweetpotato cultivation is adaptable to a wide range of soils but it does better on well-drained, light and slightly acidic (pH range of 4.5 to 7.0), sandy-loam soils. The ideal temperature for growth is 24°C or higher. When temperatures fall below 10°C, growth is severely retarded. The crop is damaged by frost. Sweetpotato also requires relatively high light intensity as it occurs in summer but both flowering and root formation are promoted by short day length and therefore, temperate climates are not

ideal for growth. Sweetpotato may be vegetatively propagated from vines (consisting of long, thin stems and leaves), roots slips (sprouts) or tubers. The vines grow very rapidly and can cover the ground within a few weeks of planting (Woolfe, 1992).

The sweetpotato growth cycle may be divided into three phases: In phase 1 (from planting to 9.5 weeks), there is extensive growth of fibrous roots with moderate growth of the vines. In phase 2 (from 9.5 – 16 weeks), the vines grow extensively with tremendous increase in leaf area and initiation of storage root development. In the final phase, storage root bulking occurs with very little growth of the vines and fibrous roots. The duration of these phases vary with cultivars and environmental conditions (Edmond and Ammerman, 1971).

2.3.2 Storage root formation

The edible storage roots develop at the points where stem nodes are in contact with the soil, usually four to ten tuberous roots per plant. Root formation is initiated with the growth of white adventitious roots, which differentiate into four types: young roots, fibrous roots, pencil-form or tuberous roots, depending on the primary cambial activity and the amount of lignification of cells in the stele (Togari, 1950).

The underlying genetic mechanisms and the factors that promote storage root formation, the most important physiological process during sweetpotato growth are not well understood (Thottappilly, 2009). According to Wilson and Lowe (1973), root growth occurs by continuous meristematic activities of the vascular cambium in the stele and anomalous primary and secondary cambia in storage roots. Cambial strips unassociated with vascular tissues also develop within the secondary parenchyma, and contribute to the localized increase in girth. The storage root development is favoured by a combination of cool temperatures (22-24°C), high light intensity and adequate supply of potassium, which promote rapid activity in the cambium and little lignification of the roots. Low light intensity decreases both cambial activity and lignification and retards root development. Large amounts of soil nitrogen also decrease cambial activity but increase lignification, favouring the production of non-tuberous roots (Togari, 1950). Further

development of the tuberous roots depends on an increase in both the number and size of cells in the stele and on the development of starch granules in the cells. The number of tuberous roots is determined in the first 30 days after planting.

2.3.3 Maturity

Sweetpotato has the shortest growing cycle among the root crops in the tropics, a characteristic which makes it suitable as a famine crop. The growing period is normally between 3-7 months depending on the environment and cultivar. Within the tropics, most varieties are harvested as soon as the roots reach marketable size, often in 3-8 months after planting (Lebot, 2009). If harvested too early, yields are low. On the other hand, if the roots are left too long in the ground, they become increasingly prone to attack by the sweetpotato weevil, *Cylas* spp. and to various rots. The roots may also become fibrous or crack, with consequent poor quality. On the other hand, the storage roots may reach their regeneration phase and initiate the growth of new shoots (Woolfe, 1992; Thottappilly, 2009; Lebot, 2009).

2.3.4 Dormancy

Dormancy is the most important postharvest physiological stage as far as the storability and keeping quality of sweetpotato roots are concerned. How long it can be held in storage without intrinsic growth determines the postharvest life of the produce. Dormancy is exhibited by many plant species to counter what is often an unfavourable climate at the end of their growth cycle (Chokchaichamnankit *et al.,* 2009). This phenomenon is referred to as phenological escape. Potatoes, onions and yams are other examples of root crops that undergo dormancy. Different crops and cultivars have varying degrees of dormancy period, a major factor which accounts for the variations in their storage life (Ravi and Aked, 1996). Harvested sweetpotato roots may remain dormant for a very short duration and sprout quickly if there is favourable temperature and relative humidity. It has been argued that sweetpotato does not exhibit dormancy at all as the roots can be induced to sprout almost immediately after harvest when placed in favourable conditions (Cheema, 2010).

There is no universal consensus on the definition of dormancy more because the dormancy phenomenon is not fully understood. Burton (1989) defined a bud as being dormant when there is no visible growth. Hemberg (1985) attributed the inability of the bud to grow to inhibiting endogenous and exogenous factors. The dormancy regime is generally associated with reduced endogenous metabolic activity but the root retains the potential for future growth. These observations depict a cross-talk between endogenous variables and external environmental conditions in controlling dormancy. Various studies suggest three types of dormancy, namely, endodormancy, ecodormancy and paradormancy. Endodormancy is explained by Burton (1989) as primary, innate rest period and is dependent on the genetic attributes of the plant; therefore, dormancy will only be terminated after completing this phase of rest, regardless of the exogenous factors. On the other hand, ecodormancy is an extended rest period imposed by external factors such as temperature and humidity, and can be terminated by altering the conditions so as to make it more favourable for sprout growth (van der Schoot, 1996). Paradormancy is related to competitive bud growth in which growing buds suppress the others on the same organ. In sweetpotato for example, paradormancy occurs when proximal sprouts suppress sprouting of buds in the distal sections. With time, however, sprouts can also originate from the middle and distal parts of the tuber. The proximal dominance can be broken by slicing the tuber and allowing the individual slices to sprout (Onwueme and Charles, 1994).

There are disagreements between different authors regarding the physiological and biochemical mechanisms underlying dormancy. Explanations offered implicate changes in the sink activity, water status in the buds and hormonal balance. Champagnat (1989) and Petel *et al.* (1992) postulated an exchange of sink power between the bud initial and surrounding tissues, swapping their status during dormancy and dormancy break. During bulking and maturation of the root, it acts as a sink organ by accumulating starch while the buds remain dormant. At dormancy break, the storage root functions as a source organ by supplying growth nutrients to the growing buds. On the other hand, Faust *et al.* (1991) have shown that during dormancy, water in the buds is found in a more bound state while towards the release from dormancy, it becomes freer. They therefore suggested that the change in state of water controls the induction of dormancy and later, its release. Erez *et al.* (1997b) and Parmentier *et al.* (1998) objected the latter hypothesis with an indication

that the water status is rather correlated to their cold resistance. Plant hormones have also been implicated to control dormancy with the thought that a higher balance of growth retardants such as ethylene and abscisic acid induce dormancy whilst increasing biosynthesis of growth stimulators (e.g. cytokinnins and gibberellins) promote dormancy break (Crabbé, 1994), although the interactions and effects remain to be elucidated. It has also been postulated that the regulation of the cell cycle by plant hormones could be a key factor leading to the induction of dormancy and its release (Cheema, 2010).

The beginning of the dormancy phase is considered to be the point of physiological maturity of tubers, and is called the wilting point. In practice, however, harvesting time may not coincide with the onset of dormancy. The instance when sprouts become able to develop and start to grow is known as dormancy break (Pringles *et al.,* 2009). From the keeping quality point of view, the dormancy period is measured from the time of harvesting to the time of sprouting (Cho *et al*., 1983; Cutter, 1992).

2.3.5 Structure and morphology of sweetpotato roots

The mature sweetpotato root may be nearly cylindrical to spindle-shaped, weigh from 0.1 kg to over 1 kg, and extend from a few centimetres to over 30 cm in length. The tuberous root is attached to the parent plant by a rather long, stout stalk (proximal end), and often tapers to a thin root at the distal end (**Figure 2.2**). The root surface is covered by a thin layer of cork and may be smooth or irregularly ribbed. This is where many adventitious buds are located and where sprouting originates (Nonnecke, 1989). Root hairs on the surface tend to point towards the distal end. The proximal flesh is often tougher and darker in colour.

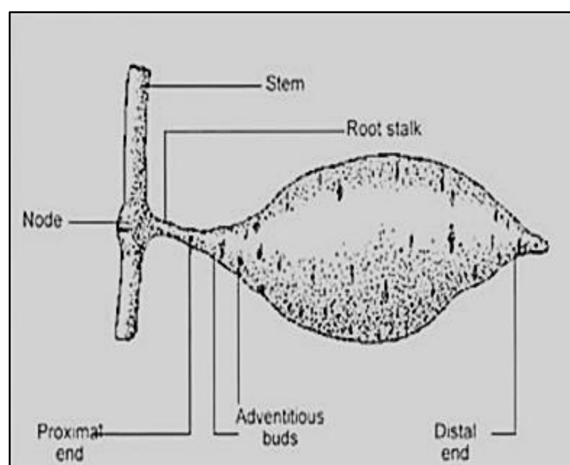


Figure 2.2 External features of sweetpotato root

The skin, as well as the root flesh, can contain significant amounts of carotenoid and anthocyanin pigments, which determine the colour of sweetpotato (Onwueme and Charles, 1994). The root skin colour ranges between red, purple, brown and white; and the flesh ranges from white through yellow, orange and purple depending on the relative abundance of these two pigments (Woolfe, 1992).

The internal structure of the root is complex and includes vascular tissues, parenchymatous storage cells, latex vessels, an epidermis and outer periderm (Lebot, 2009). The cortical parenchyma varies from very thin to very thick depending on variety. Lacticifers (latex ducts), which exudes a white, sticky latex when cut, are present throughout the root flesh. The cambium ring is where the latex vessels and central parenchyma are located (**Figure 2.3**). When the roots are cut, latex drops are produced and they darken very quickly due to oxidation.

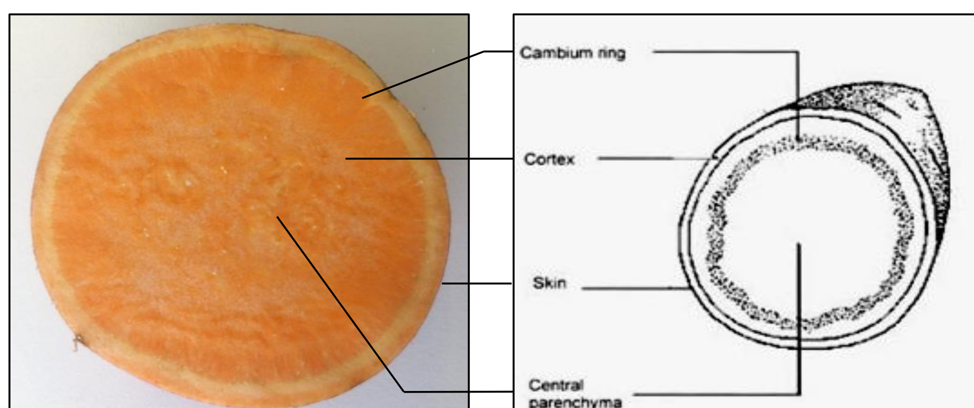


Figure 2.3 Transverse section through sweetpotato root

2.3.6 Major cultivars

Sweetpotato has broad genetic variability in terms of the number of cultivars. Many of them have been developed through breeding efforts, and through hybridization and mutations (Onwueme and Charles, 1994). They differ enormously in their physical and chemical characteristics, giving rise to different utility values and options. There are no unified names to describe the cultivars, as each is known by various local names in different regions. Differentiation between the cultivars is usually based on skin colour, flesh colour and the cooked texture. The skin colour may be yellow, orange, whitish, red, reddish purple, or tan. The flesh colour may be red, orange, salmon orange, yellowish orange, white or a combination of these (Nonnecke, 1989; Thottappilly, 2009). According to van Oirschot *et al*. (2003), sweetpotato cultivars are also distinguished, by their textural turnout after cooking, into three types:

1. Firm, dry, mealy fleshed
2. Soft, moist, gelatinous fleshed (called yams in the United States)
3. Very coarse fleshed (suitable only for animal feed or industrial uses).

The commercial cultivars common in North American markets include the orange-fleshed type called Beauregard and the smaller copper-coloured, moist-fleshed cvs. Jewel and Garnet. Covington is a relatively new variety released from the Sweetpotato Breeding Program in the North Carolina State University (NCSU) (Truong *et al*., 2007). Other popular cultivars found in different parts of the world are the cvs. Centennial (bright copper orange skin, deep orange flesh), Porto Rico Bush (deep orange, sweet flesh), Tokatoka Gold (large, rounded smooth textured, popular in New Zealand) and Vardaman (golden yellow skin, deep red-orange flesh). The characteristics of some common Ghanaian cultivars are shown in **Table 2.2**.

Table 2.2 Some sweetpotato cultivars in Ghana; their characteristics and uses

Name of cultivar	Root skin colour	Flesh colour	Root yield (t/ha)	Dry matter (%)	Uses and products in Ghana and sub-region
Apomuden	Orange	Reddish	30	21.9	High beta-carotene; preferred by exporters; baby foods
Otoo	White	Light orange	23	32.2	Medium beta-carotene; boiled and deep-fried; export crop
Ogyefo	Pink	White	20	40.1	Boiled and fried as chips; good for starch extraction
Hi starch	Dark cream	Cream	18	40.0	High starch content (21%); mild sweetness; good for flour
Sauti	White	Yellow	19	40.2	Boiled and fried as chips; low sugar content
Faara	Pink	White	22	36.1	Excellent for fried chips and boiled as *ampesi*
Okumkom	Light pink	White	20	30.7	Early maturing; good for *ampesi*
Santom pona	Dark cream	Light yellow	17	34.4	Early maturing; tastes like yam; good for *ampesi*
Jukwa orange	Dark cream	Light orange	30	35.0	High dry matter; highly preferred for *ampesi* and chips

Source:Asafo-Agyei (2010)

2.4 Biochemical characteristics

The sweetpotato root possesses a variety of bio-active compounds which are utilized for the life processes in the plant (Woolfe, 1992). These compounds also contribute to the economic uses of the crop in a range of value-added products and important characteristics such as the calorific value, storability, processing suitability, organoleptic properties and nutritional value. The development of target compounds in certain cultivars for special end uses has the potential for transforming the crop's production, use, marketability, consumption and health benefits (Asafu-Agyei, 2010).

The biochemical characteristics of sweetpotato roots vary according to cultivar, climatic conditions, degree of maturity and the duration of storage after harvest (Palaniswami *et al.,* 2008). After harvest, several endogenous and environmental factors readily influence the compositional changes in sweetpotato and hence, the shelf-life, nutritional value and industrial utility of the roots. In the following sections, the biochemical compositions, as studied by different authors, are discussed. **Table 2.3** summarises the approximate composition in terms of the fresh weight.

Table 2.3 Chemical composition of fresh sweetpotato (Onwueme and Charles, 1994)

Component	% Composition in fresh weight
Moisture	50-81
Starch	8-29
Reducing sugars	0.5-2.5
Non-starch polysaccharides	0.5-7.5
Protein	0.95-2.40
Mineral matter	0.88-1.38
Vitamins:	mg/100g fresh weight
Carotene	1.0-1.2
Ascorbic acid	29-40

2.4.1 Dry matter content

The economic value of sweetpotato as a food or industrial raw material is the dry matter content and reflects the true biological yield (Teye *et al.*, 2011). Dry matter content in

excess of 35% is desired as a raw material in the industry (Mok *et al*., 1997). However, Rees *et al*. (2003) reported that cultivars with low dry matter content are associated with both longer shelf-life and wound-healing efficiency under low humidity conditions. Like other root crops, sweetpotato has high moisture content and low dry matter (Woolfe, 1992). The dry matter content of sweetpotato varies widely depending on cultivar, location, climate, soil type, cultivation practice and the incidence of pest and diseases (Bradbury and Holloway, 1988). The dry matter content varied from 13.6% to 35.1% in a number of sweetpotato lines grown in Taiwan (Anon., 1981) and from 22.9% to 48.2% in 18 cultivars grown in Brazil (Cereda *et al.,* 1982). The dry matter content determined for five varieties grown in Ghana ranged between 34.4 to 37.4 % (Aidoo, 2004) whilst for the eight major cultivars released by the Crops Research Institute (CRI) in Ghana, the dry matter contents ranged from 21 to about 40% (Asafu-Agyei, 2010). The constitutive bioactive compounds in the dry matter also vary (**Table 2.4),** and each component depends on one or more of the same factors which influence dry matter content (Woolfe, 1992).

Table 2.4 The approximate constituents of raw sweetpotato dry matter (Woolfe, 1992)

	Percent dry matter (% DM)	
Constituent	Average value	Ranges[a]
Starch	70	30-85
Total sugars	10	5-38
Total fibre (NSP + lignin)[b]	10	n.a.
Total protein	5	1.2-10.0
Lipids	1	1.0-2.5
Ash	3	0.6-4.5
Vitamins, organic acids and other components in low concentrations	<1	

DM, dry matter
[a]Published ranges
n.a. Not available
[b]Non-starch polysaccharides (NSP) and lignin calculated by difference.

2.4.2 Total Carbohydrates

Carbohydrates constitute the bulk (approximately 80-90%) of the dry matter of sweetpotato and consist of various proportions of starch and soluble sugars, with lesser amounts of pectins, hemicelluloses and cellulose (Woolfe, 1992). According to Duke (1983) the fresh root contains 25.6-31.0 g of total carbohydrates per 100 g. The carbohydrates play important functional roles in the biological systems of the living root and hence, influence the shelf-life and quality. For example, starch accumulates in the roots as an energy reserve whilst lignin, pectin, cellulose and hemicelluloses are important structural components of the cell walls. There are rapid and continual transformations of carbohydrates from one kind to another in physiologically active cells. Also carbohydrates are utilized in the formation of fats, proteins and other compounds. Some factors influencing the rate and direction of carbohydrate transformations are:

(a) **Temperature**: Low temperature storage generally favours starch-to-sugar transformation, resulting in a sweeter product.

(b) **Enzymes**: Most of the carbohydrate transformations occurring in plants are catalysed by enzymes. E.g. Starch-to-sugar hydrolysis is activated by starch amylase.

(c) **Hydrogen ion concentration** (pH): Enzymatic action is very sensitive to pH and therefore the pH of the medium has an effect on the rate and direction of the transformation.

The total carbohydrate content is influenced by such factors as cultivar, maturity, location and growth conditions (Hammet, 1974). It has been stated that the carbohydrate levels in crops have a direct correlation with the shelf-life (Chope *et al.*, 2012). Furthermore, the individual carbohydrates and their relative abundance influence the storage length and quality. These also vary not only with cultivars, environment and maturity of the root, but with storage conditions and cooking or processing, and have considerable influence on quality factors such as texture, including firmness, dryness, mouthfeel, and taste (Woolfe, 1992; Picha, 1987). Zhang *et al.*, (2002) observed a slight decrease in the carbohydrate content in sweetpotato during storage. The author also observed that a longer storage period of raw root prior to processing reduced product firmness and flour pasting viscosity but glucose and sucrose increased.

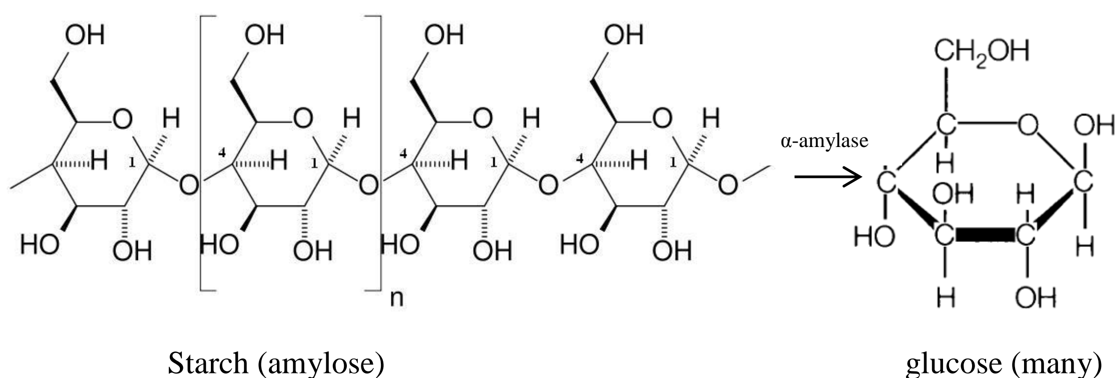
2.4.3 Non-structural carbohydrates

2.4.3.1 Starch

Starch is the commonest form of stored carbohydrates in plants. It is manufactured by the plant from glucose and on complete hydrolysis produces only glucose according to the equation:

$$(C_6 H_{10} O_5)x + xH_2O \longrightarrow x\, C_6 H_{12} O_6$$

Starch molecules are thus glucose polymers linked together by two types of glycosidic bonds: α-1,4 and α-1,6 which give rise to different structures for starch molecules. The unbranched chain containing several glucose units linked together by only α-1,4 glycosidic bonds is called the amylose (α-D-glucose subunits). Branched chains containing glucose subunits linked together by both α-1,4 and α-1,6 glycosidic bonds is called amylopectin. Starchy crops differ in the degree of polymerization (DP) of these polymers and the amylose:amylopectin ratio, which gives rise to different starch properties (Chen, 2003). Sweetpotato starch contains 8.5-38% amylose (Tian *et al.*, 1991). During the hydrolysis of starch, the enzyme α-amylase breaks down the glycosidic bonds to yield maltose or glucose (**Figure 2.4**).



Starch (amylose) glucose (many)

Figure 2.4 The hydrolysis of starch to glucose

Sweetpotato yields an important starch which is used commercially for sizing textiles and papers, for the manufacture of adhesives and in laundries. Extraction of starch from sweetpotato for such uses is a major economic enterprise in China, accounting for over 10% of the annual production of the crop (Wheatly and Bofu, 2000). In the food industry,

sweetpotato starch is used as an ingredient in noodles, bread, biscuits, cakes, juices, ice-cream and jam (Woolfe, 1992; Chen, 2003). High starch content is a desired attribute of sweetpotato as a food in the tropics (Mok *et al.*, 1997). In Ghana and other parts of the developing world, many staple foods derived from cereals and underground root and tubers are starch-based. The development of sweetpotato varieties with high starch content that can be integrated into many local food recipes is a major objective of the Crops Research Institute of Ghana (Asafu-Agyei, 2010).

Sweetpotato starch has great value in nutrition and health, as it has low glycaemic index (GI) rating (48 ± 6). This indicates slow digestion of the starch when eaten as food, causing only gradual rise in blood sugar levels (Foster-Powell *et al.*, 2002). This attribute makes sweetpotato a recommended diet for type-2 diabetic patients. By the same credential, the crop is also useful against obesity, as it produces feelings of satiety and relieves feelings of hunger for several hours (Pampola-Roger, 2006).

In the sweetpotato root, starch is the major carbohydrate of the dry matter. After harvest, the formation of starch from photosynthesis ceases so that the tissues of the root tuber depend on the internal reserve for their life processes. The amount of starch stored is therefore crucial for the longevity in storage. During storage, sweetpotato starch undergoes enzymatic transformation to glucose (**Figure 2.4**), especially if stored below 14°C, for onward caloric release during respiration of the living tissues (Wills *et al.,* 1989). During cooking, much of the starch is converted to maltose, which makes the cooked produce even more sugary to the taste (Ownueme and Charles, 2008). Exogenous ethylene induces the conversion of starch to sugars in potato. It also induced the hydrolysis of starch to sugars in apples (Thammawong and Arakawa, 2010).

There are notable genetic differences in the starch content among genotypes and varieties (Acedo *et al.,* 1996: Chen, 2003), which give rise to differences in their storability and utility for food or as industrial commodity. The starch content also varies with location, season, maturity and growth conditions, with an average of 18% FW but can range from 6.9 -30.7% FW (Tian *et al.,* 1991). Zhang *et al.* (2002) studied the carbohydrate contents of six sweetpotato genotypes and the changes during storage. The starch content was

found to range widely from 46.8% to 73.6% of the root dry weight (DW), with a mean value of 55.9% DW. The authors noticed only slight changes in the starch content in five of the six genotypes during 6-month storage. The sixth genotype (Hi-dry), which initially possessed the highest starch content (73.6%), rather showed more substantial decrease than the others. They also found that the dry matter and starch content of the fresh roots were positively correlated ($r = 0.92$, $p < 0.01$). The dry weight is correlated with the starch content (Li and Liao, 1983). A highly significant positive correlation ($r = 0.93$) was also found between the percentage starch and dry matter for Taiwanese cultivars (Woolfe, 1992) but Acedo *et al.* (1996) did not notice any consistent correlation between the starch and dry matter contents of five Filipino genotypes in a seed-board trial. A definite correlation between starch content and longevity in storage would be of interest to genetic breeders who may be eager to produce varieties with longer shelf-life. There is no published literature on the effect of ethylene on sweetpotato starch.

2.4.3.2 Sugars

Sugar is an important part of sweetpotato that makes the root sweet to taste but the composition, abundance and hence, the sweetness varies between cultivars, location, maturity and the storage regime such as temperature and duration (Kays and Horvat, 1984). Menezes *et al.* (1976) noted that roots harvested at 6 months after planting had the highest sugar concentration than those harvested earlier at 4 months and or later at 8 months. This indicates fluctuations in the sugar content during root development. Data presented by La Bonte and Picha (2000) also show fluctuations in fructose content in some cultivars during development. During storage at ambient temperatures, Acedo *et al.* (1996) noted an increase in sugar content. Storage at low temperatures increased the content of reducing sugars in sweetpotato as a result of increased invertase activity (Huang *et al.,* 1999).

The major types of sugars in sweetpotato are glucose, fructose and sucrose, their relative proportions varying among genotypes. Maltose exists in only low concentrations among some cultivars when raw. Cooked sweetpotatoes, however, have maltose resulting from starch hydrolysis. Sucrose is a disaccharide synthesised from the condensation of a molecule of α-glucose and a molecule of ß-fructose linked by a 1-2 glycosidic bond (**Figure 2.5**), which is facilitated by the enzyme sucrose synthase (Winter and Huber,

2000). Sucrose is the transport molecule of carbohydrate in plants to carry the energy to the cells where they are hydrolysed into glucose and fructose for energy release in the form of ATP. Sucrose is hydrolysed by the enzyme invertase to yield glucose and fructose. Glucose and fructose are structural isomers (glucose is an aldose and fructose is a ketose).

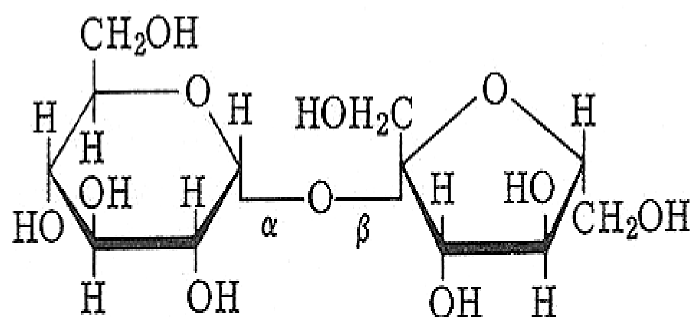


Figure 2.5 Structure of sucrose (glucose-left; fructose-right)

Various experiments indicate a wide range of sugar contents in different sweetpotato cultivars. In the southern Pacific, total sugars in sweetpotato roots ranges from 0.38-5.64% of the fresh weight (Bradbury and Holloway, 1988). Using high-performance liquid chromatography (HPLC) analysis, Picha (1985) recorded the total sugar content in the fresh weight of various American cultivars to range between 2.9-3.2 %. Total sugar content for conventional cultivars from Puerto Rico also ranged from 6.3-23.6 mg/100g dry weight (Martin and Deshpande, 1985) and from 5.6 to 38.9% (DW) in Louisiana samples (Truong *et al.,* 1986). Sucrose concentration exceeds other sugars in all raw cultivars. In some cultivars, glucose exceeds the concentration of fructose whilst in others, they occur in approximately equal amounts (Woolfe, 1992). Zhang *et al*. (2002) observed an increase in glucose and sucrose contents in the earlier stages of storage and then remained relatively constant with further storage period.

Literature suggests that exogenous ethylene increases the concentration of sugars in potato (Foukaraki *et al*., 2011), which results in darkening of the fry colour (Prange and Daniels-Lake, 2005) through Maillard reaction. Alongside, darkening of the fry colour could also be associated with the accumulation of acrylamide. Acrylamide discovered in foods such as fried potato, is thought to be a by-product of Maillard reaction produced as

a result of the reaction between asparagine and reducing sugars or carbonyl groups when heated at high temperatures for a long time (Mottram *et al*., 2002). Acrylamide has been reported to have neuro-toxic and carcinogenic effects. When potatoes were pre-treated with 1-MCP before ethylene the darkening effect was alleviated. In sweetpotato, Cheema *et al*. (2013) found an opposite effect as ethylene (10 µl L^{-1}, 25°C) caused a decline in the concentrations of glucose and fructose in the cvs. Bushbuck and Ibees after four weeks of storage. The authors also noted that 1-MCP treated roots maintained higher levels of the sugars than roots subjected to ethylene but both were below the air control check. On the other hand, Chegeh and Picha (1993) found that exposure of the cvs. Beauregard and Jewel to 0, 1, 10, 100 and 1000 ppm ethylene for 15 days at room temperature (21°C) resulted in increasing levels of sucrose and total sugar content with ethylene concentration but the monosaccharides fructose, glucose and maltose remained fairly stable.

High sugar content in sweetpotato is desirable in some food recipes such as a dessert (La Bonte and Picha, 2000) but it is a major drawback to its utility in many other food recipes, especially in the tropics (Asafu-Agyei, 2010). The Asian Vegetable Research Development Centre (AVRDC) reported in 1981 that a significant negative correlation exists between sugar and dry matter contents in sweetpotato lines with varying sugar concentrations. This indicates a possible development of a low-sugar, high dry matter variety (Woolfe, 1992). The development of non-sweet and high dry matter sweetpotatoes is a priority in the CRI's varietal breeding programme (Asafu-Agyei, 2010). Maintaining high starch content in the stored roots without significant degradation to exacerbate the sugary taste presents a major challenge during storage.

2.4.4 Structural carbohydrates

Organic compounds classed together as structural carbohydrates or non-starch polysaccharides include the pectic substances, cellulose and hemicelluloses. They are found in the middle lamella of plant cell walls. In nutrition, they are referred to as dietary fibres. They are not digestible by the human system but are important in diet to purge the digestive tract. There are suggestions that dietary fibres give protection against diverticulosis, cardiovascular diseases, colon cancer and diabetes (Woolfe, 1992). In the biological system of the living root, dietary fibres mainly play structural support function

by strengthening the cell walls. They therefore give distinctive textural properties like firmness, softness, coarseness, moistness or dryness to the sweetpotato roots. For example, ‘hardcore’, a disorder induced in raw roots by chilling temperatures and manifested in cooked roots as very hard areas has been correlated with the content of protopectin and other pectin fractions (Dianes *et al.,* 1976; Buescher *et al.,* 1976).

Cellulose has similar structure to starch but is formed from ß-1,4 glycosidic bonds. It occurs as a straight chain, highly fibrillar structure in the cell wall and is water insoluble. Cellulose, together with hemicelluloses form the structural framework of the cell wall. Pectic substances serve as binding or firming agents between the cells and therefore play key roles in the textural attributes of the sweetpotato root. Softening of plant tissue texture is attributed to insoluble protopectin, the native form of pectin, becoming soluble due to enzyme action. The breakdown or hydrolysis of pectic substances and solubility of hemicelluloses weakens the cell walls and the cohesive forces binding the cells together. The turnout is a soft textured root. The total fibre content and their stability against different storage regimes could therefore be decisive for the textural integrity of sweetpotato roots.

The total fibre content in sweetpotato has been variously determined. Total dietary fibre of raw sweetpotato samples from the Solomon Islands and Papua New Guinea ranged between 1.2- 2.62 % on fresh weight basis (Bradbury *et al*., 1984). The range depends primarily on varietal characteristics and location. van Oirschot *et al.* (2003) observed that storage did not affect the texture characteristics of five sweetpotato cultivars studied.

2.4.5 Total proteins

Plant proteins not only contribute to the nutritional value of food, but also play important biological functions in the living tissues. The main components of sweetpotato protein (SPP) are two major sporamins termed A and B, which together, account for more than 80% of the total proteins in the tuberous roots (Maeshima *et al.,* 1985; Guo and Mu, 2010). The total protein is referred to as crude protein. Every 100 g of the fresh root of sweetpotato is reported to contain 1.0-1.7 g of protein (Duke, 1983). Protein content of 0.95-2.4% FW has been quoted as average by Palaniswami *et al*. (2008). Woolfe (1992)

also stated that the total protein content in sweetpotato is on average 5% on a dry weight bases; it includes all nitrogenous compounds present. The protein content differs between cultivars and possibly, from year to year (Purcell *et al.,* 1976).

Fresh sweetpotato roots have been noted to possess higher concentration of soluble protein but this becomes low when the roots begin to sprout (Maeshima *et al.,* 1985) and then becomes almost absent after about one year storage (Li and Oba, 1985). Sporamin, the major storage protein in sweetpotato roots was highly expressed during the dormant period and lower expression was detected during the sprouting period (Chokchaichamnankit *et al.,* 2009). Evidence from the works of Purcell *et al.* (1976) and Li (1982) suggest that protein distribution in the root is not uniform, with higher concentration at the proximal end than the distal end. There might speculatively therefore be a possible correlation between protein spatial distribution and sprouting, as proximal sprouting is also noted to be higher than distal sprouting.

2.4.6 Polyphenols

Polyphenols, also called phenolic compounds, represent a group of complex organic substances which contain more than one phenol (carbolic acid) group. Phenols (C_6H_5OH) are aromatic (benzene) rings with six carbon atoms and a hydroxide group. Polyphenols are present in most plant tissues as secondary metabolites and play important functional and nutritional roles in the colour, flavour, taste, anti-oxidant activity, anti-fungal activity as well as enzymatic browning of food products (Goda *et al.,* 1996; Alonso *et al.,* 2003). Although phenolic compounds are generally thought to be correlated with health, issues about their bioavailability for utilization by the body tissues are uncertain (Terry, 2011; Cartea *et al*., 2011). Several variables such as intestinal absorption, metabolism of microflora and the nature of circulating metabolites, among others, are involved in promoting the bioavailability of food substances. The interactions between these variables in facilitating the absorption of specific food substances including polyphenols, however, are unknown (Terry, 2011). Studies in the last decades indicate that, in general, the absorption rate of dietary phenolics by the small intestine is very low (Crozier *et al*., 2009). Daily intake of foods rich in phenolics may therefore be necessary to derive the

full benefits of their health promoting potentials (Terry, 2011). In the biological system of plant tissues, polyphenols are known to provide protection against adverse environmental conditions (Duvivier *et al.,* 2010), and constitute the building blocks of lignin and dietary fibre (Nandutu *et al*., 2007).

2.4.6.1 Types and biosynthesis of phenolic compounds in sweetpotato

Several types of polyphenols have been identified in sweetpotato root tissues. Their characterisation in specific cultivars can reveal important information about their nutritional and health value as well as the functional roles they play in tissue metabolism. The phenolic compounds caffeic acid, *p*-coumaric acid, ferulic acid and sinapic acid belong to a class of aromatic acids (C_6-C_3 compounds) or phenylpropanoids called hydroxycinnamic acids or hydroxycinnamates. The hydroxycinnamic acids rarely occur in the free state and are often conjugated with glucose or quinic acid to form esters referred to as hydroxycinnamic acid esters (**Figure 2.6**). Hydroxycinnamic acid esters include chlorogenic acid and its derivatives (isochlorogenic acids). The phenylpropanoid pathway is used to synthesize the hydroxycinnamates, from which the hydroxycinnamic acid esters are also derived (**Figure 2.7).** Phenylpropanoid metabolism is stimulated by exogenous ethylene (Salveit, 1999); in response to wound stress and in defense of the tissues against pathogens (Labuza *et al*., 1992) by increasing phenylalanine ammonia lyase (PAL) activity. PAL activity has also been cited to increase in response to increasing cytokinins content (Gålis *et al*., 2002).

Walter and Purcell (1980) found that majority of the phenolics in sweetpotato were esters formed between quinic acid (1,3,4,5-tetrahydroxycyclohexanecarboxylic acid) and caffeic acid [3-(3,4-dihydroxyphenyl)-2-propanoic acid]. According to Truong *et al.* (2004) phenolics in sweetpotato include chlorogenic acid, isochlorogenic acids, caffeic acid and caffeylamide. Using high-performance liquid chromatography, coupled with mass spectrometry (HPLC-MS), Nandutu *et al*. (2007) isolated three isomers of mono-caffeoylquinic acid (CQA), three isomers of di-caffeoylquinic acid (di-CQA), one isomer of *p*-coumaroylquinic acid, one isomer of feruoylquinic acid and six isomers of caffeoylferuloyquinic acid from three Ugandan sweetpotato varieties *viz.* Nasport,

Tanzania and Spk004. Jung *et al.* (2011) also profiled the phenolic compounds in eight sweetpotato varieties using high-performance liquid chromatography (HPLC) and Folin-Ciocalteu (FC). The authors found three isomers of mono-caffeoylquinic acid, namely: 3-CQA (neo-chlorogenic acid); 4-CQA (cryptochlorogenic acid); 5-CQA (chlorogenic acid). They also found three isomers of di-caffeoylquinic acids: 3,5-diCQA, 3,4-diCQA, and 4,5-diCQA (i.e. isochlorogenic acid A, B and C, respectively).

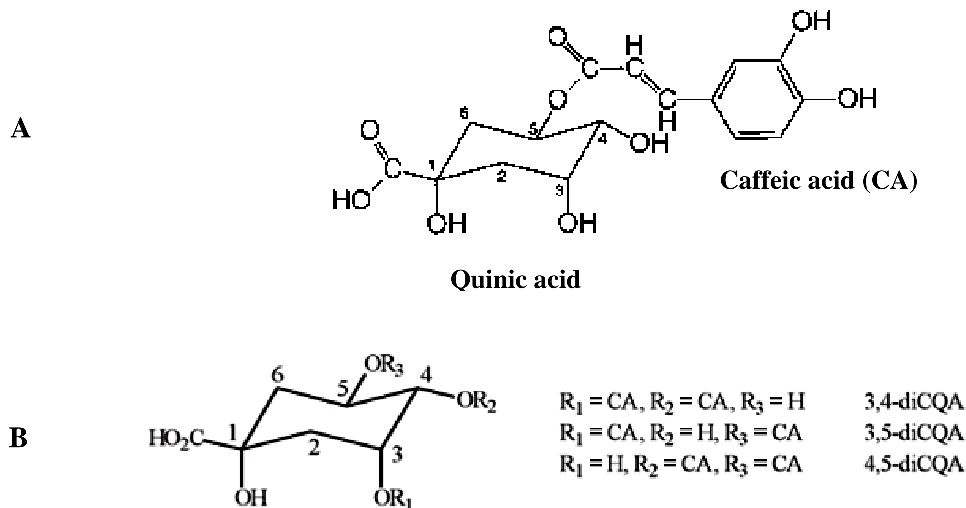


Figure 2.6 Chlorogenic acid and related compounds (A) structure of chlorogenic acid (B) di-esters of quinic with caffeic acid; (Modified from Clifford, 2003).

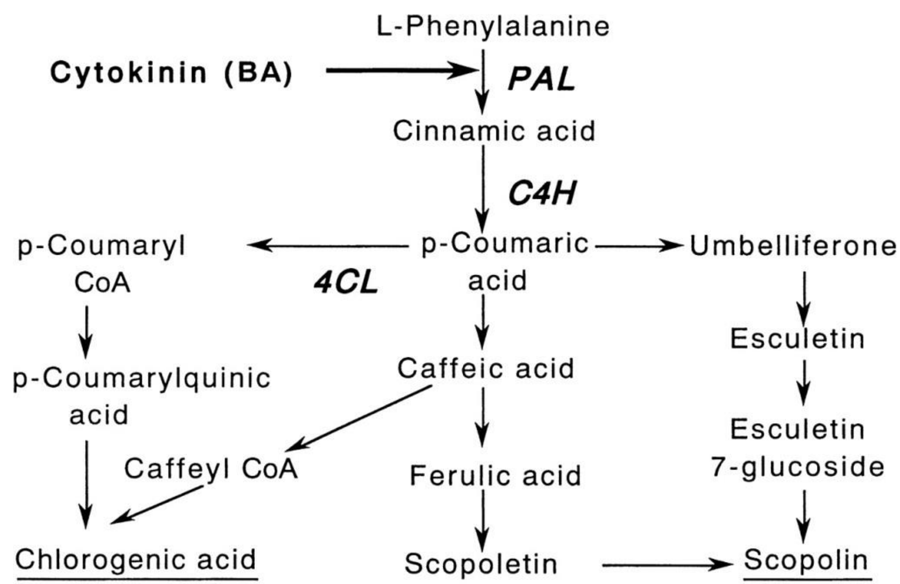


Figure 2.7 The phenylpropanoid pathway for the biosynthesis of phenolic compounds according to Harborne (1980). The thick arrow implies that cytokinin induces PAL gene expression.

2.4.6.2 Distribution of phenolic compounds in sweetpotato

Significant differences in phenolic contents among sweetpotato cultivars and between tissue zones were reported by Truong *et al.* (2004). Qualitative histochemical tests for spatial distribution of phenolics in sweetpotato indicate their presence in many tissues within the periderm, cortex and vascular bundles, with about 78% concentration in the outer 5-6 mm of tissue, which include the periderm and the secondary root tissue beneath the periderm and external to the cambium (Kim *et al.,* 1971; Walter and Schadel, 1981). These suggest that deep peeling of sweetpotato during processing will reduce browning but may presumably result in significant loss of nutritionally beneficial polyphenols. Jung *et al.* (2011) also found that the proximal ends of the roots contained higher concentrations of total phenolics than the middle and distal sections.

Varying concentrations of the phenolic compounds have been reported for different cultivars of sweetpotato. Truong *et al.* (2004) measured the total phenolic content in US commercial cultivars to range from 0.72 – 1.75 and 0.50 – 0.70 mg chlorogenic acid equivalent per g fresh weight (ChAE g^{-1} FW) for the outer root layers (0.5 cm including the skin and cortex) and inner tissue, respectively. The average phenolic contents in various sweetpotato cultivars have also been reported to range from 0.14 to 0.51 mg ChAE g^{-1} FW (Walter and McCollum, 1979). Teow *et al.* (2007) measured the total phenolics in different cultivars with distinctive colours. Purple-fleshed cultivars were noted to possess the highest total phenolic content (0.949 mg ChAE g^{-1} FW) whilst white-fleshed cultivars had the least content of phenolics (0.003 mg ChAE g^{-1} FW). Total phenolic content of 0.472 mg ChAE g^{-1} FW was obtained for an orange-fleshed variety (Teow *et al*., 2007). Comparatively, the phenolics content in strawberry and marionberry have been measured by Asami *et al.* (2003) to be 1.50 to 2.80 and 3.0 to 6.0 mg ChAE g^{-1} FW, respectively.

While there is a lot of literature about the phenolics concentrations in freshly harvested sweetpotato, there is comparatively limited information about the effect of storage time and ethylene treatment on the changes they undergo. In potato, and many crops, exogenous ethylene has been reported to boost phenolics biosynthesis.

2.4.6.3 Enzymatic browning

Polyphenols readily undergo several biochemical reactions with downstream effects on the sensory attributes and nutritional quality of food during storage or processing. Important among them is oxidation by the enzyme polyphenol oxidase (PPO) in the presence of free oxygen. This enzymatic oxidative reaction leads to the browning of plant tissues, which negatively affects their quality when cooked. Apples, potatoes and sweetpotatoes (**Figure 2.8**) are examples of crops that are affected by enzymatic browning. When the tissues are cut during processing or bruised during harvesting or handling, the vacuolar compartmentalized phenolic substances and the enzyme are brought together; and in the presence of oxygen, react to form quinones, which either polymerize with themselves or with amino acids and amino groups in proteins to form dark brown compounds called melanins (Woolfe, 1992). The degree of browning is significantly correlated with the phenolic content (Walter and Purcell, 1980). Processing may require a technique such as blanching, sulphiting, ascorbic acid or citric acid treatment to inhibit browning.

Figure 2.8 Enzymatic browning in sweetpotato cv. TIS-2

2.4.6.4 Polyphenols as natural food colorants

Despite the browning disadvantage, polyphenols have several beneficial uses as food ingredients (Ezekiel *et al*., 2013). The natural food colorants (anthocyanins) present in red-fleshed and purple sweetpotatoes can be used for developing functional foods (Oke and Workneh, 2013). Anthocyanins are a large group of water-soluble pigments that belong to a major class of polyphenols called flavonoids. Key anthocyanins found in high concentration in some sweetpotato cultivars are cyanidin and peonodin (Lebot, 2009). Interest in anthocyanin pigments in the consumer market has increased recently due to their potential health benefits and a range of colours they produce, with prospects as natural food colorants. They appear red, purple or blue according to pH and give corresponding coloration to fruits, vegetables, cereal grains and flowers. They are commonly used in acidic solutions as a red pigment in soft drinks, jams, confectionery and bakery products (Islam *et al*., 2003). Besides their functions as food colorants, they also possess physiological activities such as antioxidative, antimutagenic and antihypertensive potential and reduction of liver injury that improve the beneficial health properties of food (Yoshimoto, 2001). In the food industry, anthocyanins extracted from plant tissues are preferred by most consumers to synthetic pigments as natural food colorants (Mazza & Miniati, 1993). Such applications are widely used in Asia, South America, and Europe (Aoki *et al*., 2002; Jing and Guiti, 2005). The main drawback with plant sources of colorants, however, is their lower stability compared to synthetic colour equivalents. Oke and Workneh (2013) suggested that the relatively low cost of production of sweetpotatoes compared to other horticultural crops, is an incentive for the use of pigmented sweetpotatoes as sources of natural anthocyanins in the food industry. This suggestion was complemented by Ahmed *et al.* (2010) who stated that sweetpotato could be used as alternative food additive to enhance the colour with additional nutritional and health benefits. However, for the economy of pigment extraction, sweetpotatoes with high levels of anthocyanins are required.

Furuta *et al.* (1998) measured the anthocyanin content in purple-fleshed varieties of sweetpotato cultivated in Japan to be about 0.4 – 0.6 mg g^{-1} FW. Bridgers *et al.* (2010) on the other hand, measured the anthocyanins content of the purple-fleshed variety (origin not stated) to be 0.84-1.74 mg g^{-1} FW, which is comparable to some of the

commercial anthocyanin producing crops like blueberries, blackberries, cranberries and grapes. However, cooking effects may reduce the content in sweetpotatoes. Therefore the extractability and bioavailabity of pigments in sweetpotato needs to be ascertained. Some experiments have been conducted on the extraction and stability of sweetpotato anthocyanins (SPAs) (Kousuke *et al.,* 1990; Gongjian *et al.,* 2008). The stability of the SPAs when treated with different pH levels are the focus of these researches. In practice, however, sweetpotato may have to be transported from producer regions to industrialised countries for its utility, and the period lapse due to transportation protocols may be several weeks. Exposure to light and temperature, and possibly, time of storage and other conditions, can affect SPAs (Francis, 1989; Wrolstad, 2000). Efforts to prolong the shelf-life such as treatment with ethylene and 1-MCP may also affect the SPAs. Therefore the stability of SPAs as mediated by storage needs to be ascertained.

2.4.6.5 Antioxidant activity

The presence of several types of polyphenols, alongside β-carotene and vitamin C in sweetpotato, suggests that the crop could possibly be harnessed for beneficial use as an antioxidant against free radicals. Woolfe (1992) proposed the use of the liquid waste produced during the manufacture of starch or ethanol from sweetpotato as a source of antioxidant activity. The bioavailability of these phytonutrients for the body tissues, and therefore their implications for antioxidation activity, however, have not been confirmed.

Antioxidants are secondary metabolites characterised by having electron deficiencies due to their particular chemical structure, which makes them very reactive towards free radicals present in the body (Bridgers *et al*., 2010). Substances classified as free radicals or reactive oxygen species (ROS) are natural by-products of oxygen-involving metabolisms such as photorespiration and photosynthesis (He and Haeder, 2002; Apel and Hirt, 2004). Examples include superoxide, hydroxyl, peroxyl and alkoxy radicals. These radicals can be produced in excess by environmental stress factors like pollution, drought, temperature, excessive light intensities, and nutritional limitations. Their highly reactive potential is implicated to be responsible for some chronic diseases such as cancer and cardiovascular diseases; and is able to cause oxidative damage to proteins, DNA, and

lipids in humans (Jacobi and Burri, 1996). In the food industry, antioxidants are used to increase shelf-life by preventing deterioration resulting from oxidation reactions, especially lipid oxidation by free radicals. They accomplish this by being oxidised themselves, terminating the chain of oxidative reactions by the free radicals that tend to damage cells.

The antioxidant activity of substances are commonly expressed in relation to a reference scavenger called Trolox [(S)-(-)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid] against standard radicals such as DPPH, ABTS and ORAC. The antioxidant capacities [mg Trolox equivalent (TE) g^{-1} FW] of US sweetpotato cultivars evaluated against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical were measured in the outer layers and the inner layers to be 0.65-3.22 and 0.36-1.39, respectively. Against 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical, the outer and inner antioxidant activities were 0.23-0.62 and 0.37-0.50, respectively. Total phenolics were highly correlated with DPPH (r=0.94) and ABTS (r=0.98) antioxidant capacity (Truong *et al.,* 2004). Steed and Truong (2008) also measured the DPPH radical scavenging activities of a US purple-fleshed variety to be 47.0 – 87.4 µmol TE g^{-1}FW, whilst against the oxygen radical absorbance capacity (ORAC) it was measured to be 26.4 – 78.2 µmol TE g^{-1}FW. Padda and Picha (2008) also stated that the antioxidant activity of sweetpotato ranges from 1.3 to 4.6 mg g^{-1} DW. The anti-radical potential of sweetpotato, however, depends on the availability of the anti-oxidative substances during treatment and storage. Duvivier *et al.* (2010) noted a reduction in antioxidant activity in Taiwanese sweetpotato cv. Tainon 66 with increasing drying temperature. Degradation of β-carotene and vitamin C with heat treatment and storage has also been reported (Bechoff *et al.,* 2010; Bechoff *et al.,* 2011), which contributes to the reduction in the anti-oxidative potential. The antioxidant capacity of sweetpotatoes as mediated by various treatments and storage methods must therefore be evaluated.

2.4.7 Vitamins

The sweetpotato root is an important source of ascorbic acid, and contains moderate amounts of thiamine (B_1), riboflavin (B_2) and niacin as well as pyrodoxin and its

derivatives (B_6), pantothenic acid (B_5) and folic acid. Their chief importance, however, lies in their ability to produce variable and sometimes, large quantities of the carotenoids, which act as precursors of vitamin A (Kidmose *et al.,* 2007; Woolfe, 1992). Carotenoids largely account for the orange- and yellow-fleshed sweetpotato (Gross, 1991; Rodriguez-Amaya, 1997). HPLC analysis showed that the orange-fleshed varieties contained β-carotene as the principal carotenoid, along with other biologically active carotenoids, and would be valuable as one means in a multiple approach for the prevention of vitamin A deficiency (Lila, 2006). The vitamins present in sweetpotato per 100 g of roots are thiamine 0.09-0.14 g, riboflavin 0.05-0.10 g, vitamin C 16-22 mg, niacin 0.6-0.7 mg, and ascorbic acid 21-37 mg. These values vary from variety to variety and from location to location.

Most methods of preparing the tuber for consumption (boiling, baking, frying) result in a diminution of the content of the various vitamins. The content of ascorbic acid decreases as the tuber stays in storage (Woolfe, 1992; Onwueme and Charles, 1994). Changes in carotenoid pigments during curing and storage could also affect their quality in terms of colour and nutritional value. More recent research, however, generally confirms that carotenoids are relatively stable during storage although this may not be true for all cultivars and storage conditions. Many investigators have noticed an increase in total carotenoid content with storage when weight losses are not taken into account in the calculations. Others have noticed no significant change when weight losses are taken into account (Spiers *et al.,* 1945, 1953; Woolfe, 1992). The extent of change was found to depend on cultivar and storage temperature. Lu *et al.* (1986) did not find any apparent effect on the total carotenoids in sweetpotato with radiation doses of 2 kGy and 2 weeks of storage. The effects of many other storage treatments, such as ethylene and 1-MCP on the stability of carotenoids in sweetpotato, however, have not been reported.

2.4.8 Minerals

Like most foodstuffs, sweetpotato roots are sources of some minerals and trace elements. The predominant minerals in the sweetpotato tuber are potassium (K), sodium (Na), chloride (Cl), phosphorus (P), and calcium (Ca) (Onwueme and Charles, 1994). They are

a good source of P and though not having outstanding contents of iron (Fe) and (calcium (Ca), they can make modest contributions to the recommended daily intakes of these minerals in a quantity as little as 100 g, which also provide part of the daily allowance of magnesium (Mg), copper (Cu) and manganese (Mn) (Palaniswami *et al.,* 2008). 100 g of root is noted to contain 21-36 mg of Ca, 38-56 mg of P, 0.7-2.0 mg of Fe, 10-36 mg of Na, 210-304 mg of K, and 24 g of Mg. There is no information on changes in mineral constituents in sweetpotato roots during storage. If there are, they are likely to be insignificant since minerals are generally lost through physical removal or leaching into wash- or cooking water (Woolfe, 1992).

2.5 Sweetpotato quality characteristics

In recent years, due to trade globalisation, export of agricultural produce to international markets requires exporters to meet stringent quality and safety standards. Improving this ability to meet quality standards will facilitate greater international market access, reduce the impact of price competition, stimulate investment and mitigate risk, leading to increased exports (UNCTAD, 2007). Quality is defined for fresh produce as the totality of features and characteristics that bear on its ability to satisfy stated or implied needs. Quality attributes for fresh produce include the physical appearance as determined by the size, shape, colour, gloss and defects; textural feel, organoleptic or sensory properties, wholesomeness and nutritive value (UNCTAD, 2007).

Different countries or regional markets have developed their own specific quality standards for various produce, known as specifications. Member states of the East African Community (EAC) have drafted a standard aimed at fostering specification, grading and pricing in the international trade in sweetpotato under the Quality Infrastructure in East African Community (QIEAC) initiative (EAC, 2010). The Agriculture Marketing Service of the United States Department of Agriculture (USDA) (2005) has also developed a grading system for the marketing of sweetpotato. In all cases, subject to special provisions and tolerances allowed, good quality sweetpotato is defined as one that is smooth and firm with uniform shape, size and free from mechanical damage, defect and

disease. It should also have uniform peel colour and be typical of the variety. The produce is graded based on their degree of perfection as defined by tolerance limits.

The EAC grades sweetpotatoes into five classes as Extra Class, Class 1, Petite Class, Commercial Class and Class II, with specific tolerance limits for size, weight, appearance and defects. Sprouts in this quality grading system are classified as a defect; and is defined as when more than 10% of the roots in a lot have sprouts over 19 mm in length. The Agriculture Marketing Service of the United States Department of Agriculture (USDA) also has five similar grades for the marketing of sweetpotato namely; U.S Extra No. 1, U.S No. 1, US No. 1 petite, U.S Commercial and U.S No. 2. (Cantwell and Suslow, 2013) (**Table 2.5**).

Table 2.5 Size grading of US sweetpotato (USDA, 2005)

Grade	Length (in)		Diameter (in)		Weight (oz)
	Min	Max	Min	Max	Max
US Extra No.1	3	9	1¾	3¼	18
US No. 1 & US Commercial	3	9	1¾	3½	20
US No.1 Petit	3	7	1½	2¼	n.a.
US No.2	1½	n.a.	n.a.	n.a.	36

n.a. Not available

2.6 The pre-harvest factors affecting the shelf-life and quality of sweetpotato

2.6.1 Variety

Decisions to store fresh sweetpotato as a food security measure must consider the varietal responses to storage as evidence shows that, significant differential rates of sprouting, shrivelling, weight loss, weevil infestation and fungal decay exist between varieties when they are stored under the same conditions (Data, 1985; van Oirschot, 2000; Teye, 2010). Diamante and Data (1986) stated that, different varieties differ in their respiration rates, susceptibility to pathogens and dormancy; and this affects the effectiveness of some

storage methods. Mackay *et al.* (1989) supported this statement by confirming that, different varieties will respond differently to any storage method applied. van Oirschot (2000) found that physical characteristics such as periderm thickness and permeability, root size, root surface area/mass ratio and shape are different among cultivars. However, the author noted that these characteristics do not account for the variation in storability. It appears that genetic variations are a major factor in the storability of different sweetpotato cultivars. Differences in biochemical composition, alongside the morphological features, may combine to account for the varying storage behaviours of sweetpotato cultivars. The genetic basis for the differences in storability, however, has not been reported in available literature.

2.6.2 Cultural practices

Good cultural practices and sanitation such as timely weeding, pesticides and fertilizer application, irrigation and ridging have beneficial effects on the postharvest root quality of sweetpotato. Not only do good agricultural practices improve root yield in terms of saleable weight but also protect the roots against pests and diseases, and guarantee healthy roots that have greater capacity for better storage. For example, poor water supply leads to root cracks and abnormal root shapes and further predisposes the roots to weevil infestation. During tuberization, surface cracks normally appear on the soil and provide avenues for weevils to attack the roots. The eggs of sweetpotato weevils *Cylas puncticollis* (Bohe.) and *C. Brunneus* (Fabr.) laid in the vines find their way through soil cracks to the roots. The hatched larvae tunnel through the roots resulting in significant quality loss and yield reduction (Smit, 1997). On-farm yield losses due to sweetpotato weevils is reported to be up to 50% in India while experimental fields yield losses can range between 17-82% (Loebenstein and Thottappilly, 2009).

2.6.3 Maturity and harvesting

The postharvest system of sweetpotato begins with harvesting and it is a critical stage in the postharvest management process as any mistakes at harvest may be reflected and

magnified further down the supply chain. Important aspects to consider, in respect of harvesting, are the maturity and harvesting procedure. Based on the period of maturity, sweetpotato is classified into early maturing (3-4 months after planting), medium maturing (4-6 months after planting) and late maturing varieties (more than 6 months after planting) (Golokumah, 2007). Maturity is often indicated by the yellowing of the leaves, by which time, the roots would have reached marketable sizes. In the Southern states of the USA and other warm temperate regions where sweetpotato is produced, the roots must be harvested before frost sets in as they are sensitive to cold temperatures. In the warm tropical regions, due to lack of adequate storage capacity, in-ground storage is often practised by leaving the roots in the ground and harvested in piecemeal (depending on home consumption needs and market demand) (Smit, 1997). In these regions, harvesting is often spread over a period of 8-12 months to maintain supply of roots for the longest possible period Smit (1997). O'Hair (1991) stated that piecemeal harvesting presents problems in weevil-infested areas by providing a continuum for weevils. Leaving the roots in the soil for a long time also holds the land and does not make it available for the following cropping season, thus depriving the farmer of potential income. Smit (1997) remarked, however, that the practice of in-ground storage combined with piecemeal harvesting seems to contain a trade-off between the availability of stored food and weevil attack. In a study in Uganda, Smit (1997) did not find any significant difference between the undamaged yield in piecemeal harvesting and the corresponding yield at optimum harvest time of 6 - 7.5 months after planting. This, notwithstanding, availability of appropriate storage technologies would enhance productivity and income even more.

2.7 Postharvest factors affecting the shelf-life and quality of sweetpotato

2.7.1 Curing

Underground storage organs such as potato and sweetpotato roots have poorly developed cuticles and are thus subject to various degrees of damage during harvesting and handling, which potentially reduce their postharvest life and quality (Wills *et al.*, 1998; Cantwell and Suslow, 2013). Moisture loss and liability to parasitic and pathogenic attack of wounded roots is by far greater, in comparison to undamaged roots. Curing of sweetpotato

roots intended for storage is therefore an indispensable first step in a process that allows the industry to provide a year-round supply of high quality product to consumers (Kushman, 1975; Hall, 1993). It involves exposing the roots to a temperature of 29 – 30° C and relative humidity of 90-95 % for about 4-7 days (Wills *et al.,* 1998). During curing, wounds sustained during harvesting and handling become healed and coated with layers of suberized or lignified cells, which protect the root against water loss and pathogenic invasion (Walter *et al*, 1989; Snowdon, 1992; Afek *et al.,* 1998). In potato, there are two steps in the curing process. First is suberization – the production (biosynthesis) of suberin and its deposition in cell walls. Suberin is a healing substance containing wax and fatty acids which dry out and form a protective layer over the damaged area and thus prevents water loss and entry of micro-organisms. The second step is the formation of a cork cambium and the production of cork tissue in the bruised area. The cork cambium is a thin strip consisting of a few rows of narrow, thin-walled and roughly rectangular cells that originate from the outer layer of the collenchyma. It becomes meristematic and divides actively to give off new cells on both sides, forming the cork on the outside. The cork tissue seals the cut or bruised area and helps prevent the entrance of decay organisms.

In sweetpotato, the initial cell wall thickening is lignification (Rees *et al*., 2008). Wound healing in sweetpotato begins with desiccation of the surface cell layers, followed by lignification of the underlying cell layers and finally, the formation of a wound periderm by cell division (Artschager and Starret, 1931). Wound healing ability was found to be a major factor for the shelf-life of sweetpotato cultivars and lignin index was used to express the lignification potential of the cultivars. Under tropical marketing conditions, different cultivars were found to vary in their lignification indices. Generally, cultivars with high dry matter content were associated with low lignin index (van Oirschot, 2000). Curing of undamaged roots ‘sets’ or toughens the skin. It also enhances aroma by facilitating the synthesis of enzymes operative in flavour development during cooking. Uncured sweetpotatoes generally lack the visual appeal, shelf-life and culinary character of cured roots. Postharvest curing, however, has been reported to decrease the starch content while increasing the sugars and the sensation of moistness and sweetness (Picha, 1987; Wang *et al.,* 1998).

2.7.1.1 Techniques to ensure good skin quality

Good skin quality is essential to maintain the flesh moisture content and suppress diseases when stored for a long term. The quality and profitability of sweetpotato for the fresh market and processing are adversely affected by skin lesions. As sweetpotato skin is thin and soft (Wills *et al*., 1998), harvesting must be done with the greatest care to avoid surface wounds. To avoid this, the field is usually irrigated to soften the soil before harvesting but this can induce sprout growth (Golokumah, 2007). Another practice to toughen the skin is defoliation (removal of vines) 24 hours before harvesting. Golokumah (2007) reported that defoliation induces the production of lignin to fortify the skin against mechanical handling stresses. In potato, vine removal to enhance skin-setting has been practiced for over half a century. The idiom "skin-set" is used to indicate that the tuber has become resistant to skinning injury. Lulai (2008) explains the physiological mechanism of potato skin-setting as due to the maturation of the native periderm, leading to the outermost layer of cells, phellem, becoming tightly attached to the underlying cells and thereby becoming resistant to skinning injury. According to the author, vine killing initiates early senescence, which promotes tuber periderm maturation and associated skin-set development. Pre-harvest canopy removal was also demonstrated by La Bonte and Wright (1993) to reduce skinning damage in 'Beauregard' and 'Jewel' sweetpotato varieties combined.

2.7.2 Irradiation

The potential benefits of irradiation with gamma or electron beam in the postharvest handling of fruit and vegetables lie in both insect and disease disinfection, and in retarding aspects of produce development such as ripening and sprouting. Exposure of the produce to ionic radiation of too higher doses, however, may not only affect the invading organisms; it can also cause damage to the plant tissues and many aspects of produce metabolism in a way that may be detrimental. Due to the uncertainty about the deleterious effects of irradiation on food, national governments have been somewhat unwilling to approve it as a disinfection treatment for fresh fruit and vegetables, even though when used prudently, it can be effective.

Legal clearance for the inhibition of sprouting in potatoes, onions and garlic with irradiation has been given in some countries (Wilkinson and Gould, 1998) because toxicological tests on the required doses showed no detrimental effects on human consumption (Brewster, 1994). Sprouting in sweetpotato can be delayed by ionizing radiation (Matsuyama and Umenda, 1983). The exact dose to suppress sprouting without any adverse effect on the integrity of the roots and human health, however, has been variously reported. Lu *et al.* (1986) reported that treatment of the US cvs. Jewel and Georgia Jet with gamma radiation doses of 1.5 and 2.0 kGy decreased ascorbic acid and starch content. The radiation at those doses also caused softening of the texture of both cultivars yet the total carotenoids and colour of both cultivars were unaffected by the radiation treatment. However, sensory evaluation of the acceptability of baked sweetpotatoes showed consumer dissatisfaction at radiation doses $\geq$ 0.5 kGy. At a dose $\geq$ 0.4 kGy, Hallman (2001) noticed discoloration in the cooked flesh of the cv. Picadito, suggesting that this might be the minimum dose to cause objectionable loss to the commodity quality.

Commercial application of irradiation on sweetpotato so far, has been to combat pests. Because of the threat of potential spread of sweetpotato weevils from growing areas to consumer countries, regulatory bodies often require treatment of the roots to ensure that all weevil stages are dead. Ionisation irradiation of 165 Gy was approved as quarantine security by the California Department of Agriculture for the treatment of sweetpotato against damage caused by the weevil *Cylas formicarius elegantulus* infesting sweetpotatoes from Florida (Hallman, 2001). But even this was met with consumer objection. Approval by the USDA for Hawaiian farmers to disinfest the sweetpotato cv. Okinawan as an alternative to methyl bromide fumigation also raised public outcry. It appears that irradiation of sweetpotatoes as a measure to enhance its storage life will remain in the balance unless the related food safety issues are assured and attitudes change

2.7.3 Packaging and Storage

2.7.3.1 Importance of storage

Sweetpotato may be stored as dried chips or fresh roots, the former being more durable. Consumer preferences, however, encourage the production and marketing of fresh sweetpotato. The fresh roots are only seasonally available, with high off-season prices. Storage of fresh sweetpotato roots therefore offers the potential for farmers to earn higher income and guarantee year-round food security in regions where it is a staple crop (van Oirshcot *et al.,* 2003). The northern part of Ghana for example, has one growing season between May and September; therefore farmers have to wait for almost a year before the next harvest. This gives rise to gluts and low prices during the major harvest season, but scarcity, associated with higher prices in the lean season. In the USA, the prime season for fresh sweetpotatoes is from October to January. Cold storage in the USA, however, enables sweetpotato to be available during the rest of the year. On the other hand, the storage of sweetpotato roots in tropical and sub-tropical countries, presents a major challenge to farmers and retailers due to the unfavourable climate and lack of appropriate storage infrastructure. As a result, sweetpotato is hardly, if at all, stored in the marketing chain. In the tropics, sweetpotato is often stored in traditional structures with only modest success. Average shelf-life with various storage techniques in Ghana is just about two weeks (Golokumah, 2007) whilst van Oirschot *et al*. (2003) reported an average shelf-life of not longer than a week in the East African marketing chain. Both producers and consumers could benefit if storage enabled sweetpotato to be available for a longer period of time (Hall *et al*., 1998).

2.7.3.2 Storage systems

The storage structure is an important factor in determining the longevity of the roots. Many attempts to achieve long-term storage of sweetpotato with different storage structures in the tropics have been reported. They are generally classified as in-ground storage (delayed harvesting) combined with piecemeal harvesting (Smit, 1997), grass-lined pits (van Oirschot *et al*., 2003), open-floor heaping, clamp storage and storage in barns (van't Ooster, 1989). Many of these methods have evolved through indigenous

experience and their scientific basis and effects on the shelf-life, compositional changes and nutritional value have not been well evaluated. Furthermore, none of these methods is so far satisfactory, as they can hardly generate the ideal environmental conditions required for long-term storage. Four weeks or less appears to be the best that can be expected by any of these methods. Integrated methods of storage involving pre-treatment of the roots with chemicals or herbicidal repellents such as ash, *Lantana camara* and neem extracts to prevent weevil damage has been practiced in some areas in Ghana with modest results (Teye, 2010). In Trinidad, pre-harvest spraying with maleic hydrazide (MH) or treating the harvested tubers with methyl naphthalene-acetic acid (MENA) in acetone has been reported to inhibit sprouting and extend storage for 4-8 weeks (Kay, 1987). The use of chemicals, however, poses environmental and health risks.

2.8 Physiological activities that affect sweetpotato quality during storage

The tissues of the harvested roots are still living and therefore carry out a number of life functions. Although major activities such as the synthesis of dry matter will cease after harvest, a number of physiological processes take place in the produce. These activities may lead, in some cases, to deterioration in quality, while in other cases, it may be essential for the attainment of the desired quality. The main physiological activities are respiration, transpiration and the synthesis of organic substances.

2.8.1 Effect of respiration

Respiration of living tissues is the critical metabolic activity, as it is an energy release process for other metabolic functions. Starch is first degraded by the enzymes amylase and maltase to glucose. Sucrose can also be hydrolysed to fructose and glucose by the enzyme invertase. In the aerobic respiratory mechanism, glucose is firstly oxidized through glycolysis to the organic intermediate product pyruvate. Pyruvate is finally oxidized to carbon dioxide by the tricarboxylic acid pathway (TCA) in an oxygen requiring process, the enzymes of which are located in the mitochondria. The produced energy is stored as adenosine triphosphate (ATP) (Wills *et al.*, 1998; Zubay *et al.,* 1995). Respiration rates are measured in terms of the change in the concentration of carbon

dioxide (mL CO_2 kg^{-1} h^{-1}) or oxygen (mL O_2 kg^{-1} h^{-1}) in the store (De Baerdemaeker *et al*., 1999).

Several factors influence tissue respiration. Respiration increases with storage temperature and amount of respirable substrate (De Baerdemaeker *et al*., 1999). Also roots which are immature, starting to sprout or damaged have considerably higher respiration rates. Immature roots and sprouting buds are made up of actively dividing meristematic tissues with high rate of metabolism. Wound respiration of potato tuber has been shown to be dependent on the synthesis of RNA and proteins (Click and Hacket, 1963). It has been found in sweetpotato that, wound-induced respiration is linked to an increase in the content of the mitochondrial fraction in the tissue (Asahi *et al.,* 1965). The respiration also increases in response to infection by *Ceratocystis fimbriata* (Greksak *et al*., 1972).

Respiration results in a progressive depletion of the accumulated reserves of the dry matter as well as water, contributing to weight loss. Generally speaking, the rate of respiration is indicative of the rapidity with which compositional changes are taking place within the material (De Baerdemaeker *et al*., 1999). Products with relatively low overall rate of respiration are generally those which can be stored in the fresh condition without substantial loss of quality. Sweetpotato is classified as non-climacteric. For this class of produce, respiration decreases gradually after harvest, with consequent reduction in the metabolic activities until senescence and eventual death of the cells.

2.8.2 Effect of transpiration

Transpiration involves the transfer of moisture through the outer layers of the produce to the surface, from where it evaporates to the surroundings. Moisture loss affects the product quality, causing changes in appearance (e.g. wilting or shriveled surface), texture and flavour. It also reduces the mass of saleable product. Van Oirschot (2000) found that sweetpotatoes with high rates of water loss were more susceptible to rotting.

2.8.3 Synthesis of organic substances

Although the synthesis of dry matter ceases after harvest, the tissues remain capable of bringing about a wide range of metabolic transformations among the organic constituents. These include carotenoid pigmentation and synthesis of hormones and volatiles, which influence the quality and longevity in storage. The most important biochemical activities are starch-sugar inter-conversion during storage. At any given temperature, starch and sugar are in dynamic equilibrium, with some sugar degrading to CO_2 during respiration. Storage of potato and sweetpotato at ambient temperatures (20-25ºC) favours a net accumulation of starch. The critical temperature at which accumulation of sugar commences depends on the commodity. It is about 10ºC for potato and 15ºC for sweetpotato (Wills *et al*., 1998). The accumulation of sugar is undesirable in many starchy vegetables. Potato with high sugar content has a poor texture and a sweet taste when boiled; and when fried, excessive browning develops due to caramelisation and Maillard reactions between sugar and amino acid (Wills *et al*., 1998). The accumulation of sugar in potato stored at low temperatures can be largely reversed by raising the storage temperature to 10ºC or above (Wills *et al*., 1998).

2.9 Storage losses

Qualitative and quantitative losses arising from the postharvest storage of sweetpotato result from microorganisms, pests and physiological factors, or various combinations of these (Booth, 1974). Losses can also occur through mechanical damage sustained during harvesting and handling. Defects predispose the roots to microbial attack.

2.9.1 Losses due to microorganisms and pests

By far, postharvest diseases account for the greatest loss in storage, running to nearly hundred percent in extreme cases. Several microorganisms (mostly fungi) induce spoilage in stored sweetpotatoes. The most important postharvest diseases are Rhizopus soft rot, which is caused by *Rhizopus stolonifer* or *Rhizopus oryzae* and Java black rot, caused by *Botryodiplodia theobromae*. Also Fusarium root rot caused by *Fusarium solani,* bacterial

rot by *Erwinia chrysanthemi* and black rot caused by *Ceratocystis fimbriata* can be problematic (Kay, 1987; Clark, 1992). Microbial spoilage can be associated with a decrease in starch, total sugar, organic acid (ascorbic acid and oxalic acid) contents with concomitant increase in polyphenols and ethylene production (Ray and Ravi, 2005). The sweetpotato weevil (*Cylas* spp.) is the single most important storage pest in tropical regions for which no control measures are yet available.

2.9.2 Storage disorders

Disorders during storage may be physiological or through natural causes. Physiological disorders are abiotic in origin, caused not by diseases but by stresses related to excessive light, heat, cold, and moisture, or the mix of surrounding gases such as oxygen and carbon dioxide. Besides the direct effects on the roots, many postharvest disorders compromise the sweetpotatoes' natural defences, which in turn, increase susceptibility to infectious diseases. Thus hitherto healthy roots selected for storage can decay if the storage conditions are not well managed. These can result in weight loss, shrinkage, sprouting, chilling injury, pithiness and the development of hardcore. Changes in sensory properties and composition of the fresh produce may also be important during storage and such changes may be desirable or undesirable (van Oirschot *et al.,* 2002). In the USA the development of a soft and moist texture during storage is considered desirable (Hamman *et al.,* 1980). However, studies in Sierra Leone indicate that some sweetpotato cultivars may develop an undesirable taste during storage, which becomes apparent after frying (George and Kamara, 1988).

2.9.2.1 Physiological weight loss

Weight loss, particularly, through water loss is a major form of deterioration for sweetpotatoes in East Africa and promotes rotting (Rees *et al*., 1998). Physiological weight loss is ascribed mainly to respiratory water loss and conversion of dry matter into energy (dry matter loss) (De Baerdemaeker *et al*., 1999). Weight loss is often used as a measure of losses in postharvest studies of perishable commodities, not only because of

the actual economic loss due to the reduction in saleable weight, but also because it can reflect quality changes in the product (van Oirschot, 2000). There is evidence that the short shelf-life of sweetpotatoes is associated with accelerated weight loss (Rees *et al.,* 1998). Significant dry matter loss may result in pithiness with the formation of many small voids.

A wide range of weight loss in sweetpotato has been reported depending on the cultivar and storage system. van Oirschot (2000) evaluated the physiological weight loss for 39 East African cultivars. The author found a wide variation in the weight loss among the cultivars, which was linear with storage time. An average weekly weight loss of 5-15% was recorded. Respiration accounted for about 10% of physiological weight loss, the rest being due to water loss. Weight loss up to 35% or more was found to results in unmarketable roots. Amoah *et al.* (2010) compared the storage performance of two sweetpotato cultivars in a pit and an evaporative cooling barn. An average weight loss of 5% was recorded in the first two weeks of storage. The weight loss rose almost linearly to 32.5% after 12 weeks of storage. Weight losses ranging from 20% after 5 weeks (Jenkins, 1982), 40% after 1 month (Mbeza and Kwapata, 1995) and 27-40% after 20 days (George and Kamara, 1988) have been reported for pit storage in Bangladesh, mound-storage in Malawi and storage trials for various cultivars in Sierra Leone, respectively. The weight loss data presented in this section, however, are only physiological weight losses. Losses due to decay, which greatly affects the market profitability of sweetpotato business, were not accounted for. It would be interesting to also evaluate the net commercial loss resulting from the combination of the physiological and pathological losses as this constitutes the real loss to the farmer and determines the profitability of the sweetpotato business.

2.9.2.2 Sprouting

The root of sweetpotato sprouts readily after a short dormancy period of about two weeks during ambient storage in the tropics. The dormancy period can be so short that it has been suggested that sweetpotato does not exhibit dormancy (Cheema, 2010). To date, the underlying biochemical mechanism which triggers dormancy break has not been

exhaustively elucidated. It is thought that the dormancy and sprout growth mechanisms are regulated by endogenous hormones, which is discussed in Section 2.11. The sprouts originate from the vascular cambium region (Schlimme, 1966). In the beginning, nearly all the sprouts occur at the proximal end of the root (**Figure 2.9**), whilst the buds in the middle and distal sections of the root undergo paradormancy (Section 2.3.4). This suggests differential spatial metabolism, with higher abundance or otherwise, of a growth related factor in the proximal region compared to the other spatial sections. As the tuber ages in storage, however, sprouts can also originate from the other parts of the tuber (Onwueme and Charles, 1994).

Figure 2.9 Sweetpotato roots showing proximal dominance during sprouting

The inhibition of sprouting in sweetpotato is both necessary for its marketing in the developed world and to sustain food security in developing countries (Cheema *et al.,* 2010). USDA standards list sprouts over three-fourths of an inch (19 mm) long as defects (USDA, 2005). Sprouting occurs frequently during prolonged storage in conditions of high temperature and humidity. Wills *et al.* (1998) stated that during the rest period, dormancy is independent of temperature but once the rest period ends the rate of sprouting depends on temperature. At temperatures below 4°C, sprouting of potatoes rarely occurs. The onset of sprouting adversely affects the chemical characteristics and appearance of potatoes (Suttle, 2004b). Sprouting is always accompanied by weight loss and rapid respiration (Frazier *et al.,* 2004).

Chemical sprout suppressants such as isopropyl N-(3-chlorophenyl) carbamate (Chlorpropham or CIPC) and maleic hydrazide (MH) solution can be used but their use is limited by environmental, economic and managerial factors (Meena, 2012). Irradiation of sweetpotatoes is effective to suppress sprouting but the doses required are too high and may cause adverse biochemical and nutritional changes (Aljouni and Hamdi, 1988; Lu *et al.*, 1986). Low temperature storage is often the main tool used to control sprouting in sweetpotato. Cold storage, however, induces the conversion of starch to sugar, especially if stored below 12°C. Varietal storage trials in an evaporative cooling barn (24°C, 90% R.H.) by Amoah *et al.* (2010) recorded sprouting from the 4th week. By the 12th week, over 56% of the roots had sprouted.

2.9.2.3 Chilling injury

Sweetpotato is sensitive to chilling injury when stored below 12.5^{o}C or lower and can promote fungal decay, internal pulp browning, and root shrivelling (Cantwell and Suslow, 2013). Chilling injury is a serious concern in temperate zones during late-season harvests when the winter sets in. The extent of the chilling injury is a function of both the temperature and length of exposure.

2.10. Storage requirements to improve sweetpotato quality

An important and foremost principle for the successful storage of sweetpotato is that the roots themselves must be fit for storage (i.e. free from diseases or defects that can predispose them to storage disorders). It has been observed that even in the absence of temperature control, storage up to 4 months is possible where roots are carefully selected and stored in traditional pits or clamps (Hall and Devereau, 2000). This requires that the harvesting, curing and storage protocols, as described above, must be strictly adhered to. The roots must be packed in such a way as to minimize mechanical injury. Wooden crates, boxes or bins with ventilation slits are ideal for packaging and may be stacked over a few metres high depending on quantity. An important function of the storage structure is that it must be able to generate ideal conditions of temperature, relative humidity and atmospheric gas as varying environmental conditions can result in significant changes in

quality and nutritional composition (Dandago and Gungula, 2011). Furthermore, the structure must be able to prevent the entry of pests into the storehouse.

2.10.1 Temperature and relative humidity

Minimum temperature is important to slow down respiration rate and hence, reduce metabolic activities that lead to biochemical changes and the senescence of cells. Low temperature reduces the rate of sprouting, pest infestation and the development of rotting micro-organisms. A ventilation system is therefore required to remove the respiratory heat produced in the store. Sweetpotato, however, is sensitive to chilling injury at temperatures below 12°C. High relative humidity is also required to reduce moisture loss from the root skin. This is easier to attain since the roots give off respiratory moisture but may be further assisted with a humidifier. Cantwell and Suslow (2013) recommended the optimum relative humidity as > 95% for long-term storage and 70-90% for short-term handling for marketing. According to Woolfe (1992) and Picha (1986), under optimum temperature of 12-15°C and relative humidity of 85-90%, sweetpotato roots can store up to a year. Whilst these conditions are attainable in developed countries, farmers in tropical developing countries are restrained by lack of cold storage infrastructure and have to rely on traditional structures that can only create modest conditions for shelf-life extension (**Figure 2.10**).

Figure 2.10 Evaporative cooling barn for sweetpotato storage in Ghana

2.10.2 Controlled Atmosphere

The composition of the headspace gas can affect the shelf-life of stored produce. Controlled atmosphere (CA) storage refers to decreased oxygen and increased carbon dioxide concentrations in the headspace to subsequently reduce respiration rate. However, the levels need to be controlled carefully to prevent anaerobic respiration, as this can give off-flavours in response to high carbon dioxide concentration in the environment (Smittle, 1988; Yoo and Pike, 1996). The minimum oxygen level for each crop to prevent anaerobic respiration is called the anaerobic compensation point (ACP). Low oxygen levels also reduce ethylene production and hence, the physiological injury (Hewett, 1999).

Controlled atmosphere storage has been used to extend the shelf-life of many horticultural produce such as onions and potatoes. Storing onions in low oxygen (3 kPa) and high carbon dioxide levels of *ca.* 5 kPa have been noted to inhibit sprouting but some cultivars suffered side effects such as increased pungency. Potatoes have also been observed to suffer physiological injuries with high CO_2 and results in increased ethylene production. CO_2 levels of 20-30 kPa, however, do not affect the respiration and ethylene production of onions (Buescher *et al.,* 1979; Pal and Buescher, 1993). The influence of CA storage on the dormancy of some potato cultivars has also been studied (Thornton, 1933; Khanbari and Thompson, 1996), which indicate that different cultivars respond variously to different mix of gases. Therefore the effect of CA storage on each cultivar needs to be studied individually.

There is no commercial use of controlled atmospheres for sweetpotato storage (Cantwell and Suslow, 2013); neither has the economic benefits of CA storage been ascertained. Again the response of sweetpotato roots to different levels of O_2 and CO_2 has not been fully studied. This knowledge could be exploited to enhance sweetpotato shelf-life.

2.11 Plant Growth Regulators

Plant growth regulators (PGRs) or plant hormones control many plant functions and biosynthetic pathways, and have been ascribed a regulatory role in all aspects of plant

development from primordia formation and germination to senescence (Wills *et al.*, 1998). PGRs may be naturally occurring hormones in plant tissues or they may be synthetic compounds that mimic natural hormones. Synthetic hormone analogues are exogenously applied as pre-harvest sprays to foliage or as a liquid drench to the soil around a plant's base. Plant hormones (phytohormones) elicit a physiological response in a target cell by binding to a receptor protein in the plasma membrane.

Several types of endogenous plant hormones have been discovered. The major ones whose modulation of plant growth are remarkable and have therefore been widely studied are ethylene (C_2H_4), abscisic acid (ABA), gibberellins (GAs), cytokinnins, and auxins. Ethylene and ABA are generally known to be senescence promoters (or growth inhibition hormones) whilst auxins, gibberellins and cytokinins are classified as senescence inhibitors (or growth stimulatory hormones) (Kader, 1985, Suttle, 1998). Ethylene is the only plant hormone that exists as a gas and its higher diffusivity may explain its ubiquitous influence on plant life. Since phytohormones occur naturally, they might be expected to receive consumer acceptance as potential food additives. Despite their potency, however, few postharvest applications of PGRs have been implemented (Wills *et al.,* 1998).

2.11.1 Hormonal control of dormancy

The different conceptual views about the dormancy mechanism were discussed in Section 2.3.4. There have been extensive studies on the control of dormancy and sprouting in crops like potato and onion while literature to elucidate the dormancy mechanism in sweetpotato is limited (Ravi and Aked, 1996; Cheema, 2010). Much of the general knowledge gained about the process of dormancy control in other crops may be relevant to sweetpotato (Cheema, 2010). There is an indication from their general behaviour that dormancy in stored root crops may be influenced by phytohormones (Isenberg *et al.,* 1974). Evidence also suggests that, generally, more than one hormone is involved in dormancy; and the effects observed are due more to the hormonal balance than to the activity of any one hormone (Libermann, 1979). On the other hand, sensitivity to the growth substances may be the controlling factor rather than the hormonal level itself

(Trewavas, 1992). It is generally speculated that during dormancy, growth inhibition hormones suppress growth promoters so that the cells are in a quiescent state. A better understanding of the involvement of the individual phytohormones in the dormancy mechanism would assist the identification of potential targets for manipulation of the storage life. In the following sections, the biosynthesis, biochemistry, metabolism and regulatory mechanism of five major hormones associated with the dormancy and sprouting of dormant roots are discussed.

2.11.1.1 Ethylene biosynthesis, perception and response

From the context of postharvest technology, ethylene is the most studied among the plant hormones because it has dominant influence on the biochemistry and physiology of the latter stages of plant life, being found in higher concentrations in mature plant tissues. Ethylene is gaseous at room temperature and is produced by all tissues, the concentration increasing in response to biotic and abiotic stresses such as pathogen attack, wounding, temperature and chilling (Kader and Kasmire, 1984; Saltveit, 1999; Bleecke and Kende, 2000).

The synthesis of ethylene is through a pathway mediated by amino acid methionine and 1-aminocyclopropane-1-carboxylic acid (ACC) (Adams and Yang, 1979; Yang and Hoffman, 1984). Three key steps are involved in the mechanism of ethylene production (**Figure 2.11**). ATP and water are added to methionine, resulting in the formation of S-adenosyl-L-methionine (SAM). An enzyme 1-amino-cyclopropane-1-carboxylic acid synthase (ACC-synthase) facilitates the production of ACC from SAM. ACC is then oxidised to ethylene with the aid of an enzyme (ACC-oxidase). In addition to ACC, ACS produces 5'-methylthioadenosine (MTA), which is utilized for the synthesis of a new stock of methionine via a modified methionine cycle. This preserves the pool of methionine for continuous ethylene production (Alexander and Grierson, 2002). In the methionine-ACC pathway, ACC-synthase (ACS) and ACC oxidase (ACO) play pivotal roles in regulating the rate of ethylene synthesis, the former being the rate limiting step (Kende, 1993; Wills *et al.*, 1998). Many strategies to inhibit ethylene biosynthesis are therefore based on regulating ACC synthase (Bleecker and Kende, 2000). This can be

achieved by the application of aminoethoxyvinylglycine (AVG), which has been found to restrain the conversion of SAM to ACC (Yu *et al*., 1979, Wills *et al*., 1998). AVG is the active ingredient in ReTain, a commercial product used for the inhibition of ethylene synthesis (Cheema, 2010). Another strategy to inhibit the biosynthesis of ethylene is through controlled atmosphere (CA) storage by limiting the oxygen concentration in the headspace, thereby inhibiting ACO.

Ethylene perception by plants triggers a signal which is transduced through a transduction machinery to elicit specific physiological responses in target genes. Fruits, vegetables and flowers contain membrane bound, copper ion embedded receptors which serve as binding sites to absorb free atmospheric ethylene molecules. These binding sites can be blocked by 1-MCP (Blankenship, 2001). In the model plant Arabidopsis there is a family of five ethylene perception receptors, namely ethylene receptor 1 (ETR1), ethylene receptor 2 (ETR2), ethylene response sensor 1(ERS1), ethylene response sensor 2 (ERS2) and ethylene insensitive4 (EIN4). Their response to ethylene in Arabidopsis provides a framework for understanding how other plants also sense and respond to ethylene (Guo and Ecker, 2004). Prior to binding with ethylene, these receptors negatively regulate ethylene response pathway by upregulating CONSTITUTIVE TRIPLE RESPONSE 1(CTRI) activity which, in turn, downregulates the ethylene signal transduction along the pathway (Kieber *et al*., 1993). CTR1 is located downstream of receptors in the cytoplasm. Ethylene binds to the receptors via a copper co-factor, which results in the inactivation of receptor function and therefore stimulates ethylene responses. Ethylene is a π-acceptor compound and binds to the receptors by accepting an electron into its vacant orbitals, forming an inactive complex, which results in the release of ethylene (Sisler and Serek, 1997). This triggers signal transduction and a transcription response by ethylene controlled genes, leading to a physiological response in the plant. Downstream of CTRI are positive regulators of ethylene response, including ethylene insensitive2 (EIN2), ethylene insensitive3 (EIN3), ethylene insensitive5 (EIN5) and ethylene insensitive6 (EIN6). The functions of EIN3 is most understood and has been ascribed a primary transcription role by regulating the expression of ethylene response genes (Bleecker and Kende, 2000; Guo and Ecker, 2004).

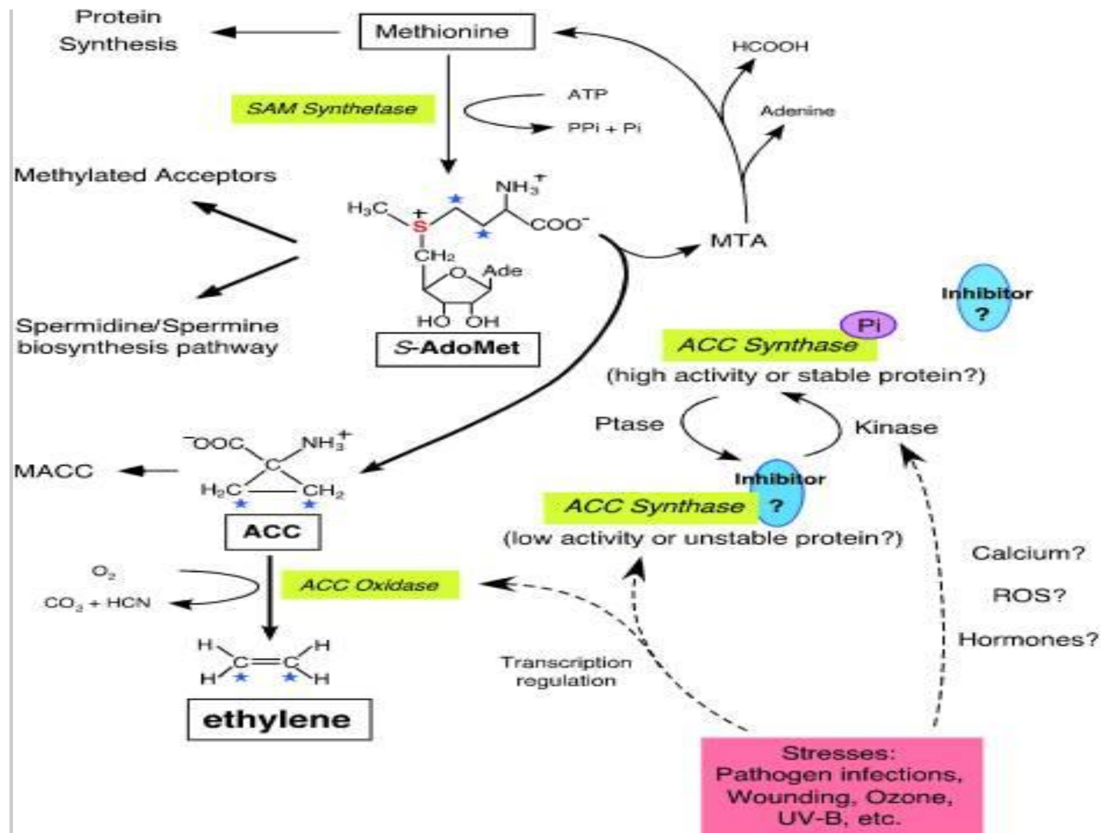


Figure 2.11 Biosynthetic pathway and regulation of ethylene (Wang *et al*., 2002)

2.11.1.2 Climacteric and non-climacteric crops

Crops have been classified based on their productivity and sensitivity to ethylene as climacteric or non-climacteric. Climacteric fruit and vegetables, may be defined as those which when harvested at physiological maturity, will ripen in response to high ethylene production. They show a respiratory peak within 3-10 days of harvest that correspond to a peak ethylene production (**Figure 2.12**), which initiates ripening on or off the tree (Hewett, 1999). Climacteric fruits can therefore be harvested before they are fully ripe and have much more flexibility in the rate at which they may be marketed. Ethylene production in climacteric fruits is autocatalytic, such that once stimulated, the ethylene production will continue even if the exogenous sources of ethylene are removed. The autocatalytic process enables them to produce large amounts of ethylene which induce ripening on or off the tree. Non-climacteric fruits do not exhibit autocatalytic ethylene production (Goldschmidt, 1977) and can only ripen on the tree as they do not show ethylene surge or respiratory peak once harvested. Rather the respiration rate and

ethylene production steadily decline with time. Both climacteric and non-climacteric fruits respond to exogenous ethylene or ethylene analogues with increased respiration and ethylene production (Atwell *et al*., 1999) but with non-climacteric fruits, it is only as long as the ethylene source is present (Hewett, 1999).

Two systems have been proposed to be operative in regulating ethylene production in climacteric and non-climacteric fruits. System 1 is functional during normal vegetative growth, is auto-inhibitory, and is responsible for producing basal ethylene levels in the tissues of both climacteric and non-climacteric fruit. System 2 operates during the ripening of climacteric fruit when ethylene production is autocatalytic (Alexander and Grierson, 2002). Non-climacteric fruit may be considered to be locked in the System-1 stage and only produce low levels of ethylene. System 2 comes into play during the ripening of climacteric fruit and during senescence and requires the induction of new isoforms of ACS and ACO.

The differentiation between climacteric and non-climacteric crops is now seen as two extremes of a continuum as many crops such as kiwi do progress through most of the ripening changes in the absence of any rise in ethylene and respiration (Atwell *et al*., 1999). Citrus fruits also exhibit climacteric-like behavior in the early stages of development and non-climacteric characteristics in the later stages of development (Katz *et al*., 2004). Although classically referred to ripening fruits, this differentiation has been extended to many dormant crops such as onion, potato and sweetpotato which exhibit characteristic non-climacteric behavior. Potato, sweetpotato and onion have low ethylene production. Ethylene production in onion and potato are < 0.1 µl kg^{-1} h^{-1} at 0-5°C and 0.1 µl kg^{-1} h^{-1} at 20°C, respectively (Knee *et al*., 1985). Ethylene production in sweetpotato is also measured to be < 0.1 µl kg^{-1} h^{-1} at 0°C (Cantwell and Suslow, 2013).

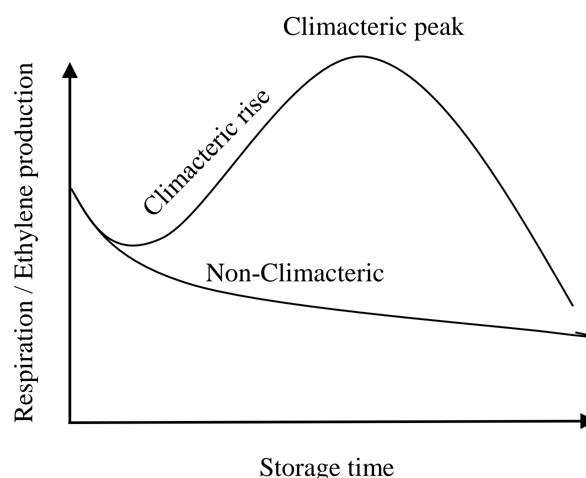


Figure 2.12 Climacteric and non-climacteric patterns of respiration in fruits and vegetables

Ethylene is physiologically active at extremely low concentrations, e.g. 0.1 µl L^{-1} and its effects may be desirable or undesirable (Kader and Kasmire, 1984). The known physiological and biochemical effects of ethylene on harvested produce include increased respiratory activity, increased activity of enzymes, increased permeability and loss of cell compartmentalization, and inhibition of auxin transport or metabolism (Pratt and Goeschl, 1969). Ethylene mediates many aspects of plant life cycle, including seed germination, root initiation, root hair development, flower development, leaf abscission, dormancy break, ripening and senescence (Lin *et al.,* 2009). Ethylene also provokes the conversion of starch to sugar and phenolic metabolism, which adversely affects the flavour and colour of cooked roots (Cantwell and Suslow, 2013).

2.11.1.3 Effects of ethylene on potato and onion

Studies indicate that the phenotypic response by crops to ethylene depends on the source, concentration, timing and duration of exposure in concert with the genotype and environmental conditions such as temperature, carbon dioxide, oxygen and the presence of ethylene binding inhibitors like 1-methylcyclopropene (1-MCP). Depending on the combination of these factors ethylene may elicit varied (sometimes contrasting) responses, with practical implications for the postharvest life and quality of produce. For example, exposure of some crops to low concentrations of endogenously produced

ethylene may promote dormancy break and sprout growth. In contrast, long-term exposure to high concentrations of exogenous ethylene also breaks dormancy but inhibits sprout growth.

The conventional sprout inhibitor chlorpropham (CIPC) used to supress sprouting in potato can leave pesticide residues with food safety concerns. Many authors have studied the application of exogenous ethylene as a proposed replacement. In experiments with short-term ethylene treatment (< 72 hr), Rylski *et al.* (1974) and Kader (1985) found that ethylene shortens the endodormancy period markedly. Prange *et al.* (1998), on the other hand, found that long-term (≥ 25 weeks) continuous exposure of potato tubers to ethylene (166 $\mu mol.m^{-3}$) resulted in sprouting being delayed by 5-15 weeks compared to untreated tubers. After sprouts appeared in the ethylene treatment, many failed to elongate, resulting in significantly larger numbers of small sprouts than large sprouts. In a later long-term experiment over 40 days, Kader (1985) also found that continuous ethylene treatment at 2 $\mu l\ L^{-1}$ inhibited sprouting in agreement with Prange *et al*. (1998). A similar response in potato has been observed by Daniels-Lake *et al.* (2005). Elmer (1936) earlier found that the dormancy of potato buds is preserved in the presence of external ethylene (emanating from apples), but once removed from the gas, the dormancy is broken and shoot growth is often more rapid than it might otherwise have been. On removal of ethylene after 40 days, Kader (1985) equally noted that sprouts appeared and grew at a rate similar to tubers in air. He concluded that ethylene can exert a dual effect on potato tubers by shortening duration of dormancy markedly, but inhibiting elongation of the sprouts. It has also been demonstrated in onion that while short or intermittent exogenous ethylene treatments induces dormancy break and leads to sprout growth, continuous exposure to ethylene inhibits sprout growth (Burton, 1952; Rylski *et al.,* 1974; Cools *et al*., 2011). These observations suggest that ethylene terminates dormancy at the biochemical and cellular level but appears to inhibit visible sprout cell differentiation and elongation. The differential responses between continuous and short-term ethylene treatment, however, remain to be fully explained.

In the quest to elucidate ethylene behaviour, Foukaraki *et al.* (2011) compared the effects of continuous and post-dormant ethylene supplementation on potato. The authors found that ethylene supplementation after dormancy break was as effective in inhibiting sprout

growth as continuous ethylene from the beginning of storage. This suggests that ethylene elicits different responses in tissues depending on the dormancy phase and the physiological needs of the crop. In other words, exposure of dormant tissues to exogenous ethylene may terminate endodormancy but the sprouts become paradormant. In the paradormancy phase, the ethylene may inhibit activities that lead to cell division and differentiation. Libermann (1979) suggested that ethylene may not act in isolation but can be associated with interactions with absisic acids, gibberellins, cytokinins and auxins. Thus the level of antagonisms and synergies between ethylene and related hormones may inhibit or promote growth.

2.11.1.4 Practical applications of ethylene

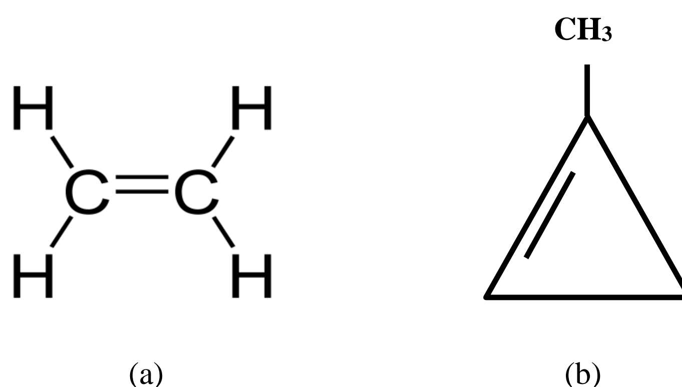
Trials now confirm that ethylene is a viable commercial alternative to chemical sprout suppressants such as chlorphropham (CIPC) and maleic hydrazide (MH) for sprout suppression in potato for markets where fry colour is unimportant (Prange *et al.,* 1998). In Canada, ethylene has been registered under the trade name “Eco Sprout Guard”, for use as a potato sprout suppressant since 2002 (Daniels-Lake *et al.,* 2005). The use of ethylene for the commercial storage of potatoes was introduced in the UK by Greenvale-AP in 2001 (British Potato Council, 2006). In 2003, the sprout inhibition effect of continuous ethylene treatment was approved for commercial sprout suppression in onions and potatoes in the UK. A target safety limit of 10 µl L^{-1} was defined by the Pesticides Safety Directorate (PSD). This was revised upwards to 50 µl L^{-1} for seed potato in 2006.

Currently in the UK, two companies, Biofresh and Restrain, currently supply ethylene generating systems to suppress sprout elongation in onion and potato in stores. The Biofresh system is a fixed installation which introduces pure ethylene from pressurized cylinders and releases the gas into the ventilation system of the storeroom. The Restrain system is a portable installation that generates ethylene catalytically from ethanol-based fuel. BioFresh and Restrain also differ in their management of ethylene, with BioFresh recommending that ethylene supplementation be applied early in storage (before dormancy break) while Restrain recommends ethylene supplementation at the time of dormancy break (British Potato Council, 2006).

Trials with continuous ethylene supplementation on potato were found to darken the colour when fried; suggesting that ethylene also promotes the conversion of starch to sugars in potato. The fry colour intensity was observed to depend on the ethylene concentration (Daniels-Lake *et al.,* 2005). The researchers found that ethylene dose of 0.4 µl L^{-1} on potato cv. Russet Burbank stored at 9°C over 25 weeks resulted in lighter fry colour but with poorer sprout inhibition effect than doses of 4 µl L^{-1} or higher. They thus suggested that a trade-off dose has to be determined between potato fry colour and sprout inhibition. The different ethylene treatments did not significantly affect tuber disease incidence or dry matter content in comparison with the controls (CIPC treated and untreated tubers) but the controls incurred about 50% greater loss of tuber mass.

2.11.1.5 The use of 1-MCP and other methods to inhibit detrimental effects of ethylene

1-methylcyclopropene (1-MCP) is a derivative of cyclopropene used as a synthetic plant growth regulator. 1-MCP (chemical formula: C_4H_6) binds competitively and irreversibly to ethylene receptors in plants such that it antagonises the latter (Sisler, 2006; Blankenship and Dole, 2003).



(a) (b)

Figure 2.13 Molecular structures of (a) Ethylene and (b) 1-methylcyclopropene

Methods to enhance the shelf-life of climacteric produce were originally based on purging ethylene from storage compartments (Bleecker and Kende, 2000) or by inhibiting its production with anti-ethylene compounds (Watkins, 2006) such as silver thiosulfate

(STS) and aminoethoxyvinylglycine (AVG). Since its discovery in the 1990s by Blankenship and Sisler (Blankenship, 2003), 1-methylcyclopropene (1-MCP) has been the centre of many researches for a potential solution to many horticultural problems caused by ethylene. Published literature confirms the efficacy of 1-MCP in inhibiting ethylene action on climacteric produce. Given its stronger binding effect to ethylene receptors (10 times higher affinity for ethylene receptors than that of ethylene) (Blankenship and Dole, 2003), 1-MCP has been exploited in commercial horticulture to restrain endogenously produced ethylene from exerting adverse influences on climacteric produce.

Industrial 1-MCP product is formulated as a stable powder in which it is complexed with γ-cyclodextrin so that it is easily released as a gas when the powder is dissolved in water (Watkins, 2006; Nanthachai *et al*., 2007). Effective concentrations range from 2.5 nl L^{-1} to 1 µl L^{-1} and are most commonly applied at 20-25°C but lower temperatures can be used in some commodities. Treatment durations of 12-24 h are sufficient to achieve a full response (Blankenship and Dole, 2003). Toxicological tests showed that 1-MCP is non-toxic, leaves negligible residue on crops and is active at very low concentrations (EPA., 2002). Therefore in 1999, the US Environmental Protection Agency (EPA) approved its use on ornamentals and marketed as EthylBloc® by Floralife, Inc. (Walterboro, SC). Its commercialization on edible crops was undertaken by AgroFresh, Inc., a subsidiary of Rohm and Haas (Springhouse, PA), under the trade name SmartFresh™ (Watkins, 2006). 1-MCP is registered for use in several countries on apples, cut flowers and many other crops.

Interest in the study of 1-MCP lies both in its use as a tool to elucidate ethylene action and to maintain produce quality (Watkins, 2006). There have subsequently been trials on 1-MCP effects on non-climacteric produce such as potato and onion, which is dominated by work done by Prange *et al*. (2005), Pruski *et al*. (2006), Chope *et al*. (2007a), Downes *et al*. (2010), Cools *et al*. (2011), Foukaraki *et al*. (2012) and Chope *et al*. (2012). Observations from these studies showed that the application of 1-MCP to potatoes prior to ethylene treatment greatly reduces the darkening of fry colour, whilst maintaining adequate sprout control, though for some cultivars, it may be necessary to periodically re-apply the 1-MCP to retain good fry colour throughout the storage period (Prange and

Daniels-Lake, 2005). Chope (2006) also treated the onion cv. SuppaSweet1 (SS1) with 1-MCP (1µl L^{-1}) and stored at temperatures 4, 12 and 20ºC. Sprout growth was reduced in the bulbs, and approximately 2-fold greater concentrations of sucrose, glucose and fructose were maintained in 1-MCP-treated bulbs stored at 12ºC compared to the untreated bulbs. Furthermore, Downes *et al*. (2010) found that for the onion cvs. Sherpa and Wellington, pre-treatment with 1-MCP (1µl L^{-1}, 24 h) before or after curing, followed by continuous ethylene supplementation inhibited sprout growth for up to four months in storage.

Studies on the effect of 1-MCP on sweetpotato is limited; and sweetpotato is absent from the list of 1-MCP-tested crops by Watkins (2006). Thus the ideal application regime such as the concentration and temperature are unknown. 1-MCP concentration interacts with temperature such that low concentrations applied over a long period may be as effective as high concentrations (Blankenship and Dole, 2003). In a more recent study, Cheema *et al*. (2013) found that 1-MCP (625 nl L^{-1}, 25ºC), suppressed sprouting in sweetpotato cvs. Bushbuck and Ibees for, at least, 4 weeks. How 1-MCP, though an ethylene binding inhibitor, elicited the same effect as ethylene was found to be intriguing. Cools *et al*. (2011) found in a transcriptional analysis of onion bulbs during storage that both exogenous ethylene and 1-MCP down-regulated a transcript with similarity to an ethylene receptor. Also, they both down-regulated the ethylene transcriptional regulator, ethylene-insensitive3 (EIN3) and suggested that, this may explain why both compounds suppressed sprouts.

Other techniques to suppress sprouting in onions and potato include cold temperature storage and the use of potassium permanganate-based ethylene scavengers. At low temperatures, endogenous ethylene production and respiration rates are low and therefore, catabolic activities leading to senescence are also reduced. In the presence of potassium permanganate, the produced ethylene is adsorbed so that it is purged from the environment. Activated charcoal is reported to have similar effect (Fridborg and Eriksson, 1975). In respect of the use of ethylene scavengers, Terry *et al*. (2007a) noted that palladium-based scavengers were even more efficient. These reports have set the stage for similar study on the effect of ethylene and 1-MCP on sweetpotato.

2.11.1.6 Effects of ethylene on sweetpotato

Like potato and onion, sweetpotato is regarded as non-climacteric with low ethylene production (Cantwell and Suslow, 2013). It may therefore be expected to respond to ethylene in a similar way to potato and onion. Cheema *et al.* (2010) have studied the effect of exogenously applied ethylene on the sweetpotato cv. Bushbuck during storage. Ethylene was applied continuously for 4 weeks in storage jars at the concentrations 5 µl L^{-1}, 20 µl L^{-1} and 100 µl L^{-1} and maintained at 25°C in an incubator. The control comprised a jar of sweetpotato roots flushed continuously with air. The authors found that the time for the roots to develop sprouts increased with the ethylene concentration in the atmosphere, with no sprouts found on the 100 µl L^{-1} treated roots throughout the study. They also found that higher ethylene concentrations resulted in yet higher root respiration and weight loss although the differences were not significant. Based on their earlier assessment, they recommended 20 µl L^{-1} ethylene treatment as effective concentration to control sweetpotato sprouting. This dose is, however, higher than the safety limit of 10 µl L^{-1} approved for table stock potato by PSD, and its acceptability for the treatment of sweetpotato against sprouting is likely to be queried. In another experiment, ethylene concentration of 10 µl L^{-1} was found to be effective against sweetpotato sprouting (Cheema *et al*., 2013). To date, however, issues regarding the optimum timing of ethylene supplementation and associated effects on the physiological, biochemical and nutritional quality attributes of sweetpotato have not been investigated. Earlier, Buescher *et al.* (1975) found that ethylene applied continuously to fresh sweetpotato roots resulted in reduced firmness after cooking, but had adverse effects on flavour and colour. Exposure of sweetpotato to ethylene has also been reported to result in the alleviation of chilling injury symptoms, such as the severity of hardcore, on some varieties. Further research is needed to ascertain the effects of various combinations of ethylene treatment on the physiological and biochemical attributes of sweetpotato.

2.11.2 Abscisic acid

Abscisic acid (ABA) plays a major role in several steps of plant growth and development, such as stomatal closure, embryo and seed dormancy, seed germination and the adaptation

to environmental stresses. It has been established that ABA is present in various plant roots and root extracts (Tietz, 1971; Rivier *et al*., 1977). ABA is classified alongside ethylene as a growth retardant and is therefore implicated to have a role in the sprout growth inhibition complex. ABA interaction with ethylene in regulating endodomancy is, to date, not known (Suttle, 1998). According to Suttle (2004a), while both ethylene and ABA are needed to initiate dormancy, only ABA is required to maintain the dormant state. Several reports indicate changes in endogenous ABA levels between the dormancy period and the initiation of sprouting in potato tubers, which suggest its involvement in these physiological processes. They all point to dormancy break as being associated with reduced ABA concentration. ABA concentration has also been shown to be dependent on storage temperature. Coleman and King (1984) noted in stored potato that ABA concentration was highest at a low temperature of 2°C, with the lowest concentration being found at 20° C. When different tuber cultivars were transferred from the storage environment of 10°C to 20°C, the duration of dormancy positively correlated with initial ABA concentration at 10°C. No threshold ABA concentration for sprouting was determined.

A study by Chope (2006) on ABA in three onion cvs. Renate, Ailsa Craig and SS1, stored in a controlled atmosphere (CA-3% CO_2, 5% O_2; 2°C) showed a decline in the concentrations at the same rate from the inception of storage, with sprouting occurring at minimum ABA concentration of *ca.* 50-120 ng g^{-1} DW. Applying ABA analogue (PBI-365) and exogenous ABA as pre-harvest sprays or as postharvest bulb soaks on six onion cultivars did not increase endogenous bulb ABA. On storage, ABA concentration decreased until sprouting at minimum level (75-150 ng g^{-1} DW). The author also noted that ABA concentration was highest at the lowest of the three storage temperatures 4, 12 and 20°C. After sprouting, ABA concentration increased.

It has also been reported that exogenous ABA, applied in-vitro at concentrations of 0.01, 0.1, 1.0 and 10 mg L^{-1} to nodal segments of sweetpotato cv. Jewel inhibited axillary bud growth and root development (Jarret and Gawel, 1991). There is the possibility therefore that ABA is involved in the dormancy of sweetpotato. The changes in ABA levels in sweetpotato in relation to the dormancy mechanism, however, have not been reported.

2.11.3 Gibberellins and cytokinins

Gibberellins are growth promoters associated with cell division and elongation. More than a hundred members of gibberellins have been isolated (Vivanco and Flores, 2000). No plant appears to possess all of the gibberellins; nor are the various gibberellins equally active in plants. They have all been assigned 'gibberellin numbers' (GA_x) and are usually referred to by these rather than by conventional chemical nomenclature. Gibberellins are involved in a wide range of developmental responses including stimulation of cell elongation (particularly of stems and grass leaves), breaking of seed dormancy, and speeding up germination. GA_1 is the most active gibberellin in the promotion of cell elongation. They may also play a role in stimulating cell division in meristematic areas (Kefeli, 1978; Roberts, 1988).

Gibberellins are noted to induce dormancy break in potatoes (Hemberg, 1985; Coleman, 1987) and appear to have a key role in stimulating subsequent sprout growth Gibberellins promote the growth of sprouts by stimulating the synthesis of DNA and RNA (Suttle, 2004a). Rossouw (2008) treated tuber segments (cut at the stolon end and placed on moist cotton wool, or left intact and dry) of the Greenhouse potato cvs. Caren and Up-to-date and others from private institutions, namely Cult. A, Cult. B and Hermes with a gibberellin concentration of 0.1 g L^{-1}. Sprouts were stimulated within 9 days whereas untreated tubers (control) sprouted much later. The researcher also noted that the concentration of gibberellins affected the time of dormancy break, number of sprouts and sprout growth rate. Higher gibberellins concentration (0.1 g L^{-1}) produced more and longer sprouts than lower concentration (0.005 g L^{-1}). Cheema *et al.* (2010) also stated that for sweetpotatoes, levels of GAs (GA_1, GA_{19} and GA_{20}) increased as sprout growth became more vigorous.

Cytokinins are growth hormones associated with cell division and differentiation (Mok and Mok, 2001; Hartmann *et al.*, 2002); and therefore functions as a growth promoter. Cytokinins appear to play a role in the development of tubers by accelerating cell division and expansion. The presence of cytokinins in root extracts and in root exudates supports the view that roots are a site for cytokinin biosynthesis (Letham and Palni, 1983). The major cytokinin in sweetpotato root is zeatin riboside (ZR); and the endogenous level in the root is maximum just after tuberization. Kefeli (1978) suggested that cytokinins

function better when applied in combination with other growth hormones. Kucera *et al.* (2005) added that cytokinins interact with other growth regulators in dormancy and germination regulation. Its combined effect with gibberellins on ware potato is discussed below.

Suttle (2004a) noted that tissue sensitivity to gibberellins is time-dependent. Immediately after harvest, potato tubers were found to be completely insensitive to GAs but over time, GAs (GA_1 and GA_{20}) supported premature dormancy release. On the other hand, Coleman (1987) stated that cytokinin sensitivity is prevalent at the beginning and end of dormancy and tubers will be less sensitive to cytokinin application between these two periods. A treatment combination of cytokinins and gibberellins resulted in earlier termination of dormancy than when either cytokinin or gibberellin was applied alone (Rossouw, 2008). Combining these observations, it may be deduced that, the synergy between cytokinins and gibberelins possibly results in the former initiating sprouting through meristematic tissue division whilst the latter effect sprout growth.

2.11.3.1 Effects of gibberellins on sweetpotato

Drawing lessons from its effects on potato, Cheema *et al.* (2010) also studied how GA and GA synthesis inhibitors affect sweetpotato sprout growth. Orange-fleshed sweetpotato roots were dipped for 2 h at 25° C in various concentrations of GA_3 and GA inhibitors, paclobutrazol and prohexadione calcium, derived from the commercial products Piccolo and Regalis, respectively. The higher gibberellins concentration (10^{-3}M GA_3) resulted in increased sprout length and number than concentrations of 10^{-4} M GA_3 and 10^{-5} M GA_3, respectively. Whereas the result for the GA synthesis inhibitor, Regalis, was inconsistent, the Piccolo treatment reduced the sprout length as well as the number of sprouts per root, the effect increasing with higher concentration of the inhibitor. This observation indicates a similar effect of GAs on both potato and sweetpotato and may suggest similar hormonal interaction in the dormant roots.

2.11.4. Auxins

Auxin is also a growth promoter and its interaction with GA and cytokinins in effecting dormancy break may be significant. According to Isenberg *et al.* (1974), dormancy in onions correlates with low levels of auxin, gibberellins and cytokinin. Thomas (1969) postulated that auxin may be more involved in the actual dormancy break in onions since an increase in auxin levels was observed in the early stages of sprouting. The role of auxin in dormancy break is also evidenced by the fact that the sprout growth inhibitor MH has been shown to have anti-auxin properties (Äberg, 1953). In studies by Abdel-Rahman and Isenberg (1974) onion bulbs treated with MH had low auxin content, alongside low levels of gibberellin and cytokinin. It has been established that the transport of auxin is inhibited by ethylene. Thus the presence of exogenous ethylene may restrain growth promoting hormones and consequently, inhibit sprout growth. The influence of ethylene on dormancy may therefore be through its interaction and modulation of the growth hormones rather than a direct involvement in tissue metabolism. This could be either by regulating their contents or tissue sensitivity to the respective hormones.

2.12 Conclusions

Sweetpotato is valued as an important food security crop in especially tropical developing countries. Its importance is likely to increase in the next decades owing to findings relating to numerous health-promoting phytochemicals, which have created opportunities for diversified uses of the crop. The food security potential and other numerous benefits are, however, discounted by the high postharvest losses. Research has improved agronomic practices and yield. However, there have not been corresponding studies to improve the postharvest storage of sweetpotato. To date, farmers in the tropics continue to rely on traditional storage methods, which have not yielded any economically beneficial improvements in the shelf-life.

The plant growth regulator ethylene is known to regulate many physiological and biochemical activities that affect the postharvest quality of crops. Currently, several postharvest studies to improve storage are based on understanding how ethylene mediates metabolic activities in specific crops. The ethylene supplementation technology is

particularly attractive because it is a natural plant hormone. Studies have led to ethylene being commercially applied to inhibit sprouting in related non-climacteric crops such as potato and onion in some countries like the UK and Canada. Expectations are that ethylene may likewise suppress sprouting in sweetpotato. Published works have shown the possibility of applying ethylene to inhibit sweetpotato sprouting but there can also be deleterious side effects, especially as it has been shown to accumulate sugars in potato. The application of 1-MCP prior to exogenous ethylene has been found to alleviate sugars accumulation without compromising the ethylene's sprout suppression effect. Compared to potato and onion, there is paucity of knowledge regarding how ethylene mediates dormancy in sweetpotato and its interaction with endogenous hormones. The effects of ethylene on the constitutive metabolites such as non-structural carbohydrates and phenolic compounds are also largely unknown. In the subsequent chapters, the effects of ethylene and the anti-ethylene gas 1-methylcyclopropene on the physiological and biochemical changes in sweetpotato are presented.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Introduction

Common instrumentations and methods were used in the various experiments in this study. Therefore in this chapter, the plant materials, experimental procedures and analytical protocols generally used for evaluating the target biochemical and physiological variables are discussed. Some experimental designs and procedures were specific to individual experiments and will be discussed in the appropriate sections. This chapter also discusses the statistical procedure used for data analysis. **Figure 3.1** is an overview of the various experiments performed in the study.

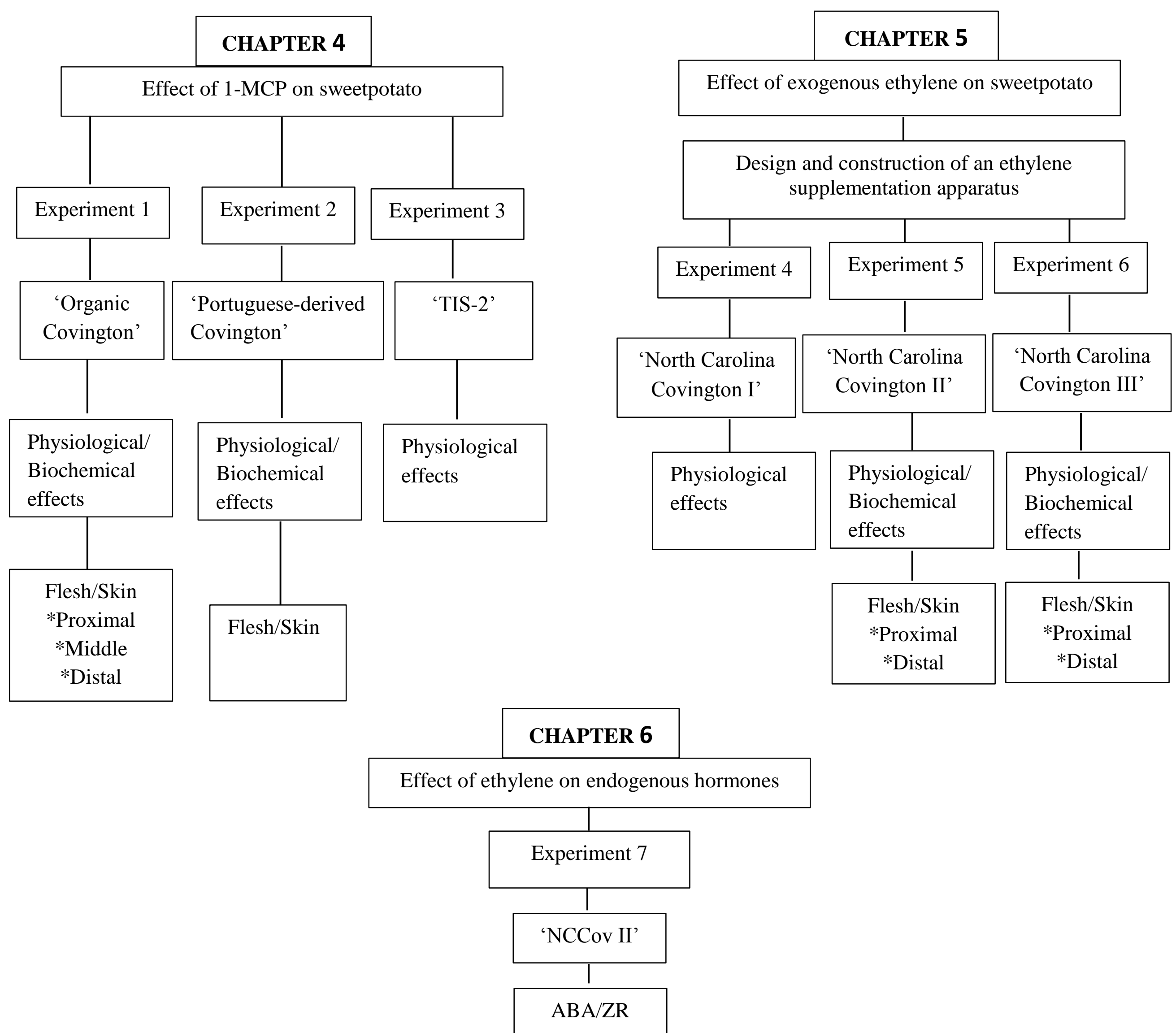


Figure 3.1 Overview of experiments in the study

3.2 The plant materials

In all, six sweetpotato consignments were analysed in 7 experiments (**Figure 3.1**). The 1-MCP experiments were repeated with two consignments of the cv. Covington ('Organic Covington' and 'Portuguese-derived Covington') and one consignment of the cv. TIS-2 (**Figure 3.2**). The ethylene experiments were also repeated with three separate consignments of the cv. North Carolina Covington, designated as 'NCCov I', 'NCCov II' and 'NCCov III,' respectively (**Figure 3.3**). The agronomic history, previous storage conditions and number of replicates, however, differed from one consignment to another based on the supply, over which the author had no control. The target physiological and biochemical analysis carried out on each consignment also differed and shall be explained in detail in the respective sections for each experiment.

All the Covington consignments were supplied by Barfoots of Botley Ltd. (W. Sussex, UK) – a fresh produce processing and distribution company to UK supermarkets. Covington is a newly released cultivar from the Sweetpotato Breeding Programme, North Carolina State University (Truong *et al*., 2007). It is an orange-fleshed cultivar, credited with high ß-carotene content and widely sold in the USA and on the international market. The cv. TIS-2 was supplied from Ghana by the University of Cape Coast. 'TIS-2' is a thick skinned, white-fleshed and high yielding cultivar grown and marketed in the Cape Coast municipality of Ghana. Details on the agronomy and storage conditions of all the consignments are described in the respepctive sections.

Figure 3.2 Sweetpotato consignments used for 1-MCP experiments: (A) 'Organic Covington', (B) 'Portuguese-derived Covington' and (C) 'TIS-2'

D E F

Figure 3.3 Sweetpotato consignments used for ethylene experiments: (D) 'North Carolina Covington I' (E) 'North Carolina Covington II' and (F) 'North Carolina Covington III'

'NCCov II' and 'NCCov III' were part of the same harvest but supplied at different times, NCCov II being supplied 10 days after harvest whilst 'NCCov III' was supplied five months later after pre- storage at 14°C at Barfoots. In experiment 7, involving the effects of ethylene on the endogenous hormones in sweetpotato, selected samples from 'NCCov II' were investigated. Another trial involving the use of a 'Senegalese-derived Covington' was also partially performed as the experiment was accidentally compromised mid-way by hydrocarbon pollution from a Hydrovane compressor in the laboratory.

3.3 Experimental precautions

In all cases before the individual experiments, the roots were sorted, and only wholesome ones were used. Wholesome roots were grouped according to the number of treatment boxes, ensuring fairly uniform sizes across the groups to reduce variability in the experimental units (**Figure 3.4 A**). The groups were randomly selected into labelled treatment boxes. Handling was carefully done to avoid bruises which could pre-dispose the roots to micro-organisms. The treatment boxes were also initially disinfected with iso-propanol and dried. Within the storage room, the treatment boxes were randomly placed on shelves (**Figure 3.4 B**). Storage of the roots was done in the dark except when the rooms were temporarily opened for inspection or sampling. The storage boxes were

treated as the experimental units and the roots within as pseudo-replicates. Each treatment box contained two subsets of roots. One subset of roots was designated as non-destructive sub-samples and was used for physiological assessments. A second subset was kept in the same box and selected at random for biochemical assays.

A B

Figure 3.4 **A.** Sorted sweetpotato roots for the trials **B**. Randomised placement of treatment boxes on shelves in the storage room

3.4 Design of a laboratory-scale ethylene supplementation apparatus

The system to generate 10 µl L^{-1} ethylene for parallel supplementation of different storage boxes was designed and fabricated by the author. The ethylene treatment was accomplished with a custom-built flow-through system. The root samples were stored in water-sealed, 81.2 L polypropylene boxes, covered with transparent lids and flushed with the aforementioned concentrations of air/ethylene mix, respectively. Certified ethylene in nitrogen (BOC, Surrey, UK) of stock concentration 5000 µl L^{-1}, compressed into a cylinder (50 L capacity, 200 bar) was diluted (*ca.* 500 parts of air to 1 part of ethylene) to 10 µl L^{-1} with air from a 100 L capacity Hydrovane Compressor (HVO2, Flitwick, UK) at approximate flow rates of 1.0 L min^{-1} and 2.0 mL min^{-1} (air/ethylene), respectively.

This was achieved by connecting two calibrated mass flow control valves (Sierra, The Netherlands: model 840-L-2-OV1-SV1-E-V1-S1 and 840-L-2-OV1-SV1-D-V1-S1 for air and ethylene, respectively) in line with the air and ethylene streams to regulate their flow and blend them at a T-junction downstream **(Figure 3.5).** The calibrated maximum flow rates of the air and ethylene valves were 13.5 and 0.03 L min^{-1}, respectively. A third air flow control valve (model 840-L-2-OV1-SV1-E-V1-S1) was connected in a parallel air stream for direct flushing of the control boxes. All the valves were connected to a multi-channel Mass Flow Controller (MFC-4, Sable systems, NV, US) to regulate their actual flow rates. The desired ethylene concentration K (µL L^{-1}) was achieved with the derived model:

$$K = \{(x\%\ of\ 0.03)/(\ y\%\ of\ 13.5)\} * 5000$$

Where $x\%$ is the % of the full-scale setting of the ethylene flow control valve

$y\%$ is the % of the full-scale setting of the air flow control valve

With the optimum choice of x and y values, different concentrations and flow rates could be achieved. The pure air (for the control treatments) and ethylene/air mix (10 µl L^{-1}) were applied to the crop storage boxes via gas distribution manifolds (HNL Engineering Ltd., Stockton-On-Tees, UK) and flexible nylon tubing. The manifolds further assist gas mixing by creating turbulence in their expanded hollow tubes. The gases were bubbled through bowls of water placed in the boxes to maintain a relative humidity of *ca*. 70-95%. Tropical climatic conditions were maintained by keeping the room temperature constant at 25ºC. The humidity and temperature in the storage boxes were regularly monitored using Gemini data loggers (Tiny-tag Ultra 2, 0-95% RH, -25ºC to 85ºC Temperature, Part No. TGU-4500). Outlet tubing was also connected to the opposite ends of the boxes (front) to provide a continuous flow-through system and maintain the CO_2 concentration in the boxes below 0.5% (Reid and Pratt, 1972). CO_2 levels in the boxes were checked by injecting headspace gas samples into a gas chromatograph (GC model 8340, DP800 integrator, Carlos Erba Instruments, Herts, UK) fitted with a Hot Wire Detector (HWD), analytical column porapak (2 m length, 6 mm O.D./4 mm I.D.) and calibrated with 10% CO_2 standard (BOC, Surrey, UK). The exhaust gas was removed from the outlet tubing of each box and directed to the outside of the building through the room ventilation system.

Periodically, the headspace gas in each box was analysed as previously described by Terry *et al.* (2007). A 60 mL plastic syringe was used to withdraw and inject samples (*ca.* 10 mL per injection) into a gas chromatograph (GC model 8340, DP800 integrator, Carlos Erba Instruments, Herts, UK) fitted with flame ionisation detector (FID, 250°C) and analytical column (Porapak, 2 mm length, 6 mm O.D./4 mm I.D.) (**Figure 4.1**) to ascertain a concentration of *ca.* 10 µl L^{-1} in the ethylene-flushed boxes and *ca.* 0 µl L^{-1} in the air-flushed boxes. The calibration standard was 10.6 µl L^{-1} isobutylene. The Mass Flow Controller was adjusted correspondingly until the appropriate concentrations in the respective boxes were achieved.

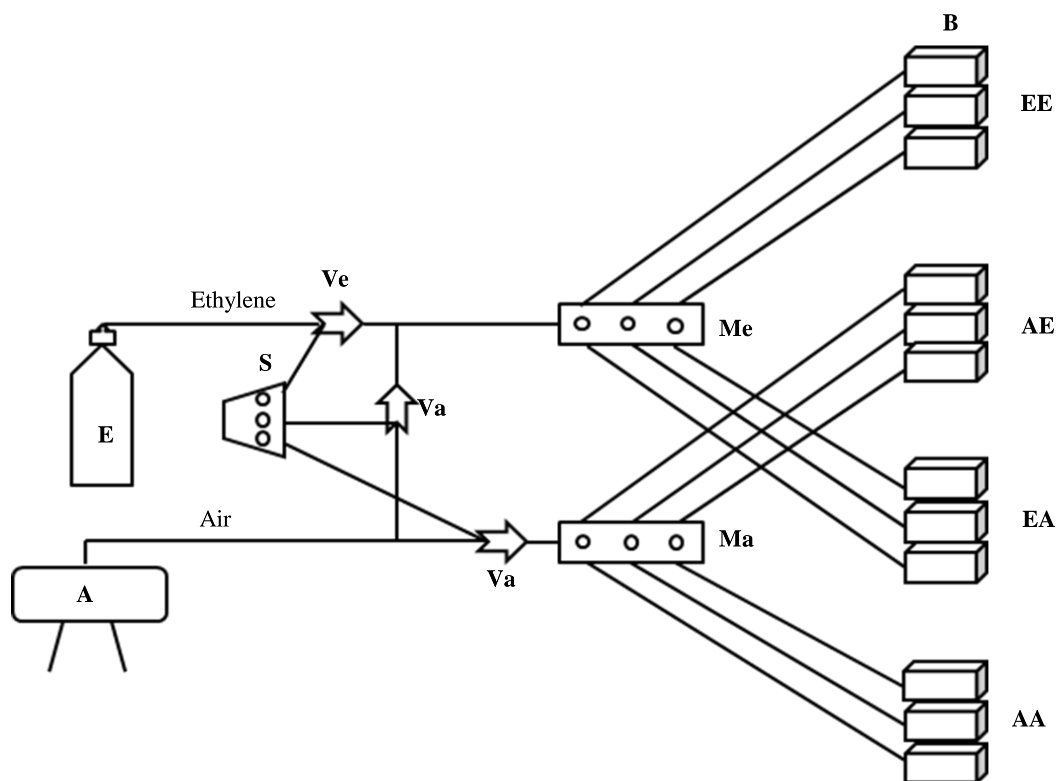


Figure 3.5 Schematic diagram of the ethylene supplementation setup

A. Hydrovane air compressor **E.** Ethylene cylinder **Ve.** Ethylene flow control valve **Va.** Air flow control valve **S.** MFC-4 Sable gas mixer **Me.** Ethylene distribution manifold **Ma.** Air distribution manifold **B.** Crop storage boxes **EE**. Continuous ethylene-flushed boxes **EA**. Ethylene, then air-flushed boxes **AE**. Air, then ethylene-flushed boxes.

3.5 Non-destructive assessments

3.5.1 Weight loss

Each subset of non-destructive roots was numbered and weighed at the beginning of storage and at the period outturns. Weight loss was determined as the difference between their initial weights and the weights at the successive outturns. Roots showing extensive rotting and unmarketable were removed from the stock and the mean weight of the remaining roots was calculated (Rees *et al.,* 2003). In the 1-MCP experiments (Experiments 1, 2, and 3), the total marketable weight loss (physiological plus decay) for each batch of non-destructive roots was calculated (percentage of initial fresh weight). This was done to give an indication of the actual commercial loss due to the treatments. In the ethylene experiments (Experiments 4, 5 and 6), the physiological weight loss (respiratory losses) for the individual roots was determined (percentage of initial fresh weight).

3.5.2 Incidence of diseases

Root rot sheets were prepared according to treatments and replications. At the respective outturns, the diseased roots (showing 10% decayed surface) in each batch of non-destructive roots were removed, counted and added to the number of previously discarded roots from the batch to give an indication of cumulative decay. The mean number of decayed roots per treatment was then calculated.

3.5.3 Sprouting

Sprouting of sweetpotato roots is a major variable used to assess the physiological quality of marketed produce. A sprout is considered as a defect; and a lot is downgraded as having sprouted if 10% of the roots have sprouts > 19 mm (EAC, 2010). At the evaluation outturns in the experiments, the number of sprouted roots, the mean number of sprouted buds per root, and the maximum sprout length (mm) per root in the non-destructive sub-samples were recorded. For the purpose of assessing the biological activity in the roots, any bud growth up to 1 mm or more was counted as a sprout. Colour photographs were

taken at the periodic outturns to serve as references. Sprout length was measured with the aid of a calliper and a linear scale (ruler). The percentage sprouted roots was calculated as:

Percentage sprouted roots = (Number of sprouted roots/Total number of roots) x 100 %

3.5.4 Respiration

The respiration of roots selected for biochemical assays were measured using a Sable Respirometry System (Model 1.3.8 Pro, Sable Systems International, NV, USA) (**Figure 3.6**) as described by Collings *et al.* (2012). The Sable Respirometry System is a real-time automated method for dynamic measurement of in-situ respiration rates of fresh produce to avoid modified atmosphere-induced stress. The individual roots were taken from the treatment boxes and immediately incubated in 3 L, air-tight glass jars, connected in series to a carbon dioxide detector (CA-10, Firmware version 1.05), an oxygen detector (FC-10, Firmware version 3.0) and a water vapour pressure detector (RH-300) of the Sable System equipment to measure CO_2 (%), O_2 (%), and RH% respectively, in the effluent gas. Simultaneously, flow rate and barometric pressure were recorded which were used in software calculations. The jars were continuously flushed with filtered air (*ca*. 600 ml min^{-1}) using an 80 HP pump (HIBLOW, Techno Takatsuki Co. Ltd., Philippines). The dynamic flow-through mode was important to avoid any stress-induced changes as the same roots were used for downstream biochemical assays. An auto-sampling program was created with an MUX flow multiplexer to sample the effluent air from the jars in sequence for 5 min each, allowing 2 min equilibrating time between the jars. Each jar was sampled a total of three times, giving three readings of O_2 and CO_2 per root and the average calculated. The respiration rates were analysed with ExpeData Release 1.3.8, Version: PRO software and expressed in mL CO_2 $kg^{-1}h^{-1}$.

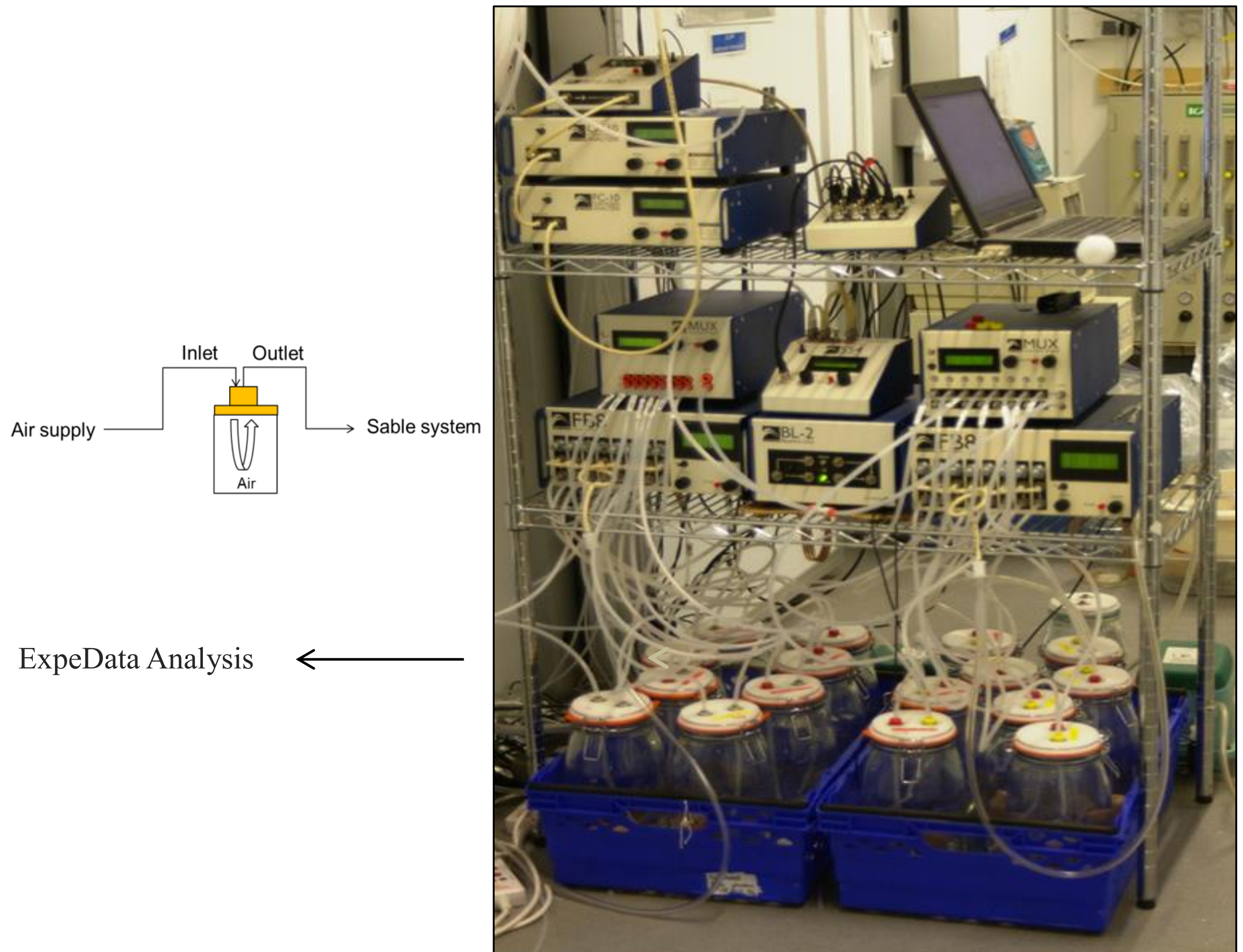


Figure 3.6 Measuring the respiration of sweetpotato roots with Sable System respirometer

3.6 Biochemical assays (destructive assessments)

Immediately after measuring the respiration, roots were further assessed for the effects of the treatments on the non-structural carbohydrates and phenolic compounds in the spatial regions *viz*. the proximal (stem end), middle, and distal (root end) segments for both skin and flesh tissues (**Figure 3.7**).

3.6.1 Sample preparation for biochemical assays

The sample preparation steps are summarized in **Figure 3.7**. The roots were washed under running tap water and dried with a soft paper towel. Each root was then cut into the

proximal, middle and distal segments (each segment was approximately a third of the root length). The segments were manually peeled with a sharp knife to obtain two types of tissue: skin (*ca*. 1.0-1.5 mm from the outer surface) and flesh. The skin and flesh tissues were snap-frozen in liquid nitrogen, each divided into two and stored at -40° C and -80° C respectively, until further analysis. Approximately 10 g of the frozen tissue (-40° C) were weighed (fresh weight, FW) and freeze-dried (Scan Vac, Västerås, Sweden) in the dark for 7 days. After lyophilisation, the samples were re-weighed to obtain the dry weight (DW). The dry matter content (mg g^{-1} FW) was subsequently calculated. The samples were then ground to powder and stored at -40° C in readiness for non-structural carbohydrates, phenolics and antioxidant capacity assays.

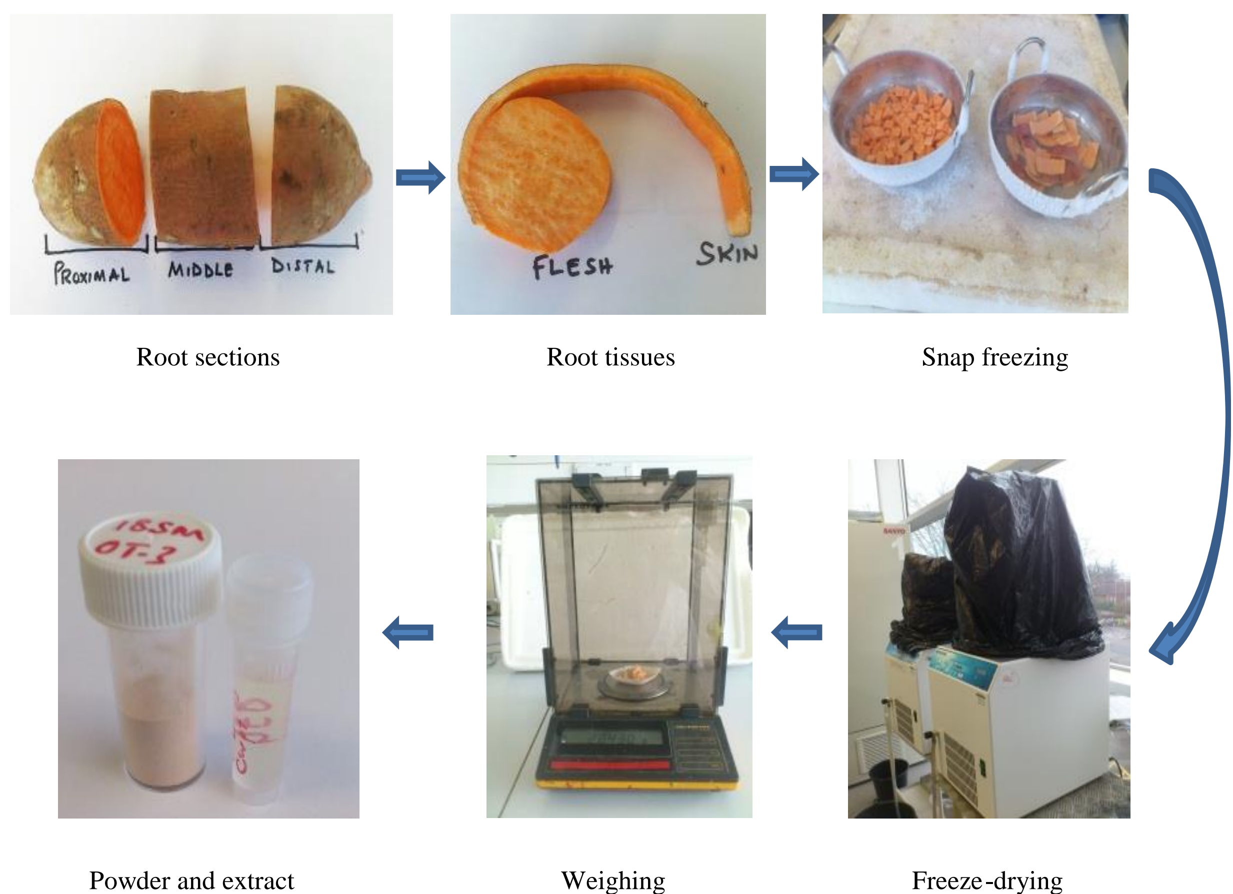


Figure 3.7 Sample preparation steps

3.6.2 Extraction and quantification of non-structural carbohydrates

The soluble sugars were extracted according to the protocol developed by Chope *et al.* (2007a) with slight modifications. The powdered samples (150 mg) were weighed into 7 mL polystyrene Bijou vials (Sterilin, Staffs, UK) and 3 mL of HPLC grade extraction solvent [62.5:37.5 methanol:water (v/v)] added. The mixture was incubated in a shaking water bath (Clifton, UK) at 55° C for 15 min. During the incubation period the vials were vortexed for 20s every 5 min (Vortex Genie 2, Scientific Industries, NY, USA) to prevent layering. The extracts were left to cool at room temperature and the supernatant filtered through 0.2 µm Millipore syringe driven filters (Cronus, PTFE 0.2 µm) (Jaytee Biosciences, Kent, UK) and stored at -20° C pending further analysis. Before analysis, the samples were diluted (dilution factor 10) with HPLC grade water.

Identification and quantification of the sugars were done using Agilent 1260 Infinity HPLC system coupled to Agilent 1260 Infinity Evaporative Light-Scattering Detector (ELSD) (Agilent Technologies Inc., Germany) (**Figure 3.8**). The stationary phase column was a Prevail Carbohydrate Es 5µ, GRACE, USA (250 mm x 4.6 mm; Part No. 35101; Serial No. N908718) fitted with a security guard cartridge. The mobile phase comprised of HPLC grade water (solvent A) and 100% acetonitrile (solvent B). A gradient program (A: 0-15 min, 20-50%; 15-20 min, 50-20%) was used to facilitate a linear increase/decrease of the amount of water in acetonitrile, and 5 min post run equilibration of the column at 20% of A. The HPLC operating conditions were facilitated with a Chemsoft software program to generate a pump flow rate of 1.0 mL min^{-1}, 30°C column temperature and 20 µL injection volumes of the calibration standards and the samples. The concentrations of the identified sugars were calculated by comparison with the standard peak areas using GenStat calibration models generated for the standard curves. Authentic calibration standards of fructose, glucose, sucrose and maltose were obtained from Sigma-Aldrich Co., (UK) and prepared to concentrations ranging from 0.1 – 2.5 mg mL^{-1} for sucrose (sucrose is disparately more abundant in sweetpotato than the other sugars and required separate calibration curve created with different standard concentrations) and 0.05 – 1.25 mg mL^{-1} (fructose, glucose and maltose). A typical chromatogram for the elution of the sugars is shown in **Figure 3.9**.

Figure 3.8 HPLC-ELSD system used to quantify sugars

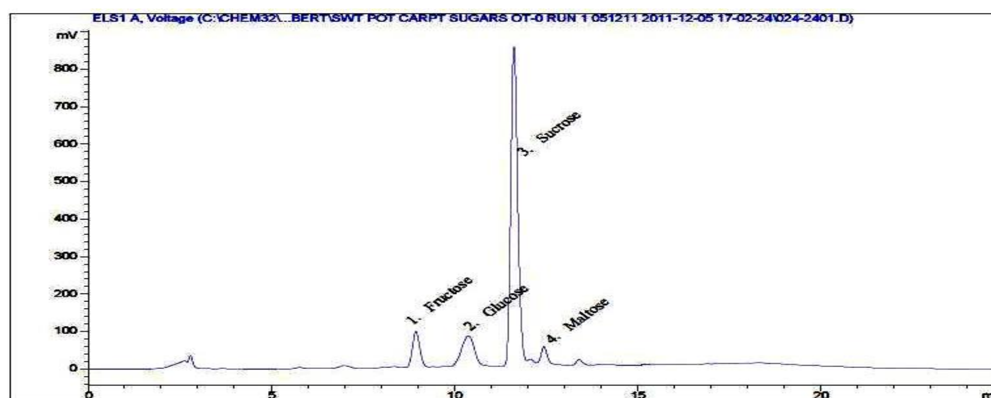


Figure 3.9 Typical gradient HPLC chromatogram showing the elution order of non-structural carbohydrates in sweetpotato

3.6.3 Extraction and quantitation of phenolic compounds

3.6.3.1 Extraction of phenolic compounds in sweetpotato

The samples were initially prepared as described in Section 3.5.1. The phenolic compounds were extracted from 100 mg of the freezed-dried powder with 1.5 mL of acidified aqueous methanol (methanol:water:HCl; 70:29.5:0.5 v/v). The mixture was

incubated in a shaking water bath at 35° C for 1.5 h. The cooled samples were filtered as described for sugars in Section 3.5.2 and stored at -20° C until further analysis.

3.6.3.2 Method development for the quantitation of phenolic compounds using HPLC

Before the main assays, a method adopted from Giné Bordonaba and Terry, (2008), was developed for the quantitation of phenolic compounds in sweetpotato. Evaluation of different methods for the analysis of sweetpotato phenolics has shown that the reversed-phase high performance liquid chromatography (HPLC) method gives the most accurate, specific results (Walter and Purcell, 1979). The HPLC method is able to separate the caffeoylquinic acid esters and enable the quantitation of the individual. Even in many methods that attempt to quantify the individual phenolic compounds, the concentrations are usually quantitated in terms of the more commercially available standard, chlorogenic acid (Walter and Purcell, 1979; Royal Society of Chemistry, 2003; Truong *et al*., 2007; Teow *et al*., 2007). The need to closely examine the bioactivity of each phenolic compound, however, highlights the importance of separating and quantifying them independently. The HPLC method, however, has been found to be expensive and lengthy. In particular, the gradient elution procedures which give accurate results typically require more than 100 min for a single injection (Islam *et al*., 2002; Truong *et al*., 2007). In more improved methods the total analysis time for individual phenolic acid quantification in sweetpotato roots ranged from 20-45 minutes (Walter and Schadel, 1981; Hayase and Kato, 1984; Son *et al*., 1991; Yoshimoto *et al*., 2004). In the current study, a gradient method was developed, which distinctly separated the phenolic peaks with remarkably short elution period of less than 15 min (**Figure 3.11**) followed by column equilibation.

The phenolic compounds were analysed using Agilent 1200 HPLC system, which comprised of a binary pump (Agilent, Berks- UK) and Agilent 1200 DA G1315B/G1365B photodiode array with multiple wavelength detectors (DAD) (**Figure 3.10**). The eluents were detected and quantified at UV-VIS wavelength of 280 nm against a reference detector set at a wavelength of 600 nm. The calibration standards consisted of a mix of chlorogenic, caffeic, *p*-coumaric and ferulic acids (Sigma-Aldrich Co., UK); and

iso-chlorogenic acids A, B and C (Oskar Tropisch, Germany). Initial trials showed that the skin tissues of sweetpotato had about 6-fold higher concentration of phenolic compounds than the flesh tissues. It was therefore necessary to generate two different linear calibration curves for separate quantitation of the skin and flesh samples. A highly sensitive balance (Sartorius MC 5, AG. Göttingen, Germany) was used to weigh the phenolic standards into acidified aqueous methanol [70:29.5:0.5 methanol:water:HCl (v/v)] to concentrations up to 0.05 mg mL^{-1} (calibrated against the skin samples) and (up to 0.02 mg mL^{-1} calibrated against the flesh samples). The calibration curves were made with at least five data points for each standard.

The mobile phase solvents comprised of HPLC grade water stabilized with 0.5% formic acid (line A) and 100% acetonitrile (line B). A gradient program was developed with Chemsoft software to facilitate a linear increase/decrease of the amount of water in acetonitrile (A: 0-13 min, 95-72%; 13-18 mins, 72-30%; 18-20 mins, 30-95%) and 5 min post run equilibrating period. The stationary phase column was an Agilent Zorbax eclipse XDB-C18 column (4.6 mm x 150 mm, 5µm particle size) fitted with a C18 Opti guard column. The operating conditions were facilitated with the Chemsoft software program to set the pump flow at 1 mL min^{-1}, autosampler injection volume of 5 µL and column temperature at 25°C. The data was presented in Agilent ChemStation Rev. B.02.01 software. **Figure 3.11** is a typical chromatogram showing the elution order of the phenolic compounds. The identification of the compounds was based on their HPLC retention times and the profiles of the UV-VIS spectra in comparison with those of the pure standards. These were further confirmed by fraction collection of individual eluting compounds and injecting them into LC-MS for structural analysis and comparing their m/z ratios to that of standard compounds. The phenolic compounds were found to be derivatives of caffeoylquinic acid with identical spectral profiles (**Figure 3.12**).

The concentrations of phenolic compounds in Experiment 1 ('Organic Covington') were quantified on the basis of the equivalent UV absorption by a series of chlorogenic acid standards (Walter and Purcell, 1979). This was done by comparing the sample peak areas of the HPLC chromatograms to that of the chlorogenic acid standards and expressing their quantities as milligrams of chlorogenic acid equivalent per 100 gram of fresh weight of the sample (mg ChAE /100g FW) (Truong *et al.*, 2007). In the subsequent experiments,

however, the individual phenolic compounds were calibrated with their respective pure standards so the quantitated values were expressed in absolute terms as mg/100 g FW.

Figure 3.10 HPLC-DAD system used to quantify the phenolic compounds

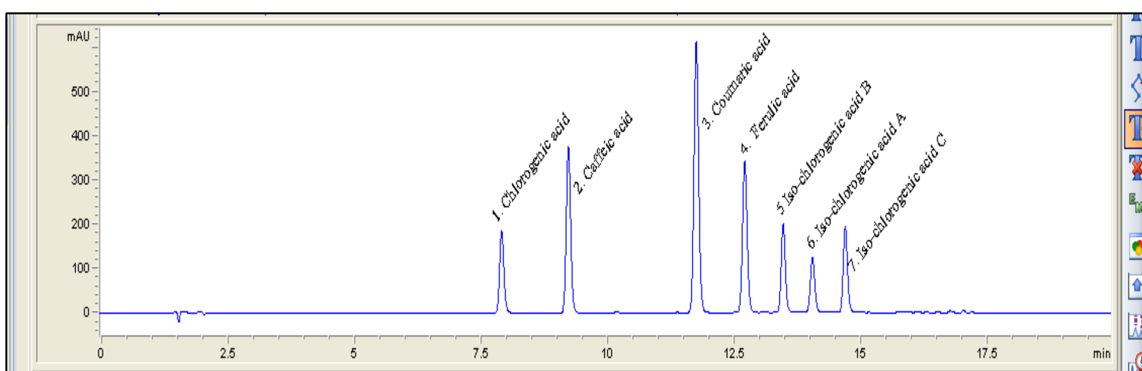


Figure 3.11 A typical HPLC gradient chromatogram showing the elution order of the phenolic standards in sweetpotato

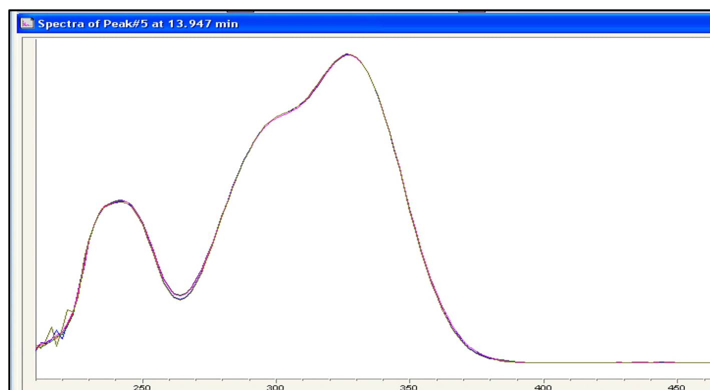


Figure 3.12 Typical spectral profile of a phenolic compound

3.6.4 Antioxidant capacity assays using DPPH radical scavenging

The antioxidant activity of substances are commonly measured in relation to a reference scavenger called Trolox [(S)-(-)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid] against a standard radical such as 2,2-diphenyl-1-picrylhydrazyl (DPPH). The DPPH method for measuring antioxidant capacity is based on monitoring its absorbance at a characteristic wavelength, using a UV-Vis spectrophotometer. The DPPH radical forms a deep purple colour in solution with a strong absorbance at 515 nm. As the DPPH is reduced by the antioxidant, it loses its absorbance and the colour intensity reduces, eventually becoming yellowish (Brand-Williams *et al*., 1995).

In the current study, the antioxidant capacity was measured for phenolic compounds in the samples extracted in Section 3.5.3. The frozen extracts were vortexed and 200 µL was diluted with 800 µL of 0.2 M phosphate-buffered saline (PBS at pH 7.4) in a small vial. Methanolic DPPH radical was prepared to a concentration of 2.5 mg per 100 mL by weighing 5 mg DPPH into 200 mL methanol (HPLC grade). Aliquots of 3.9 mL of the methanolic DPPH were transferred into 1-cm path length, 4 mL cuvettes and 100 µL (0.1 mL) of the diluted samples added and mixed up using a 1 mL pipette. The mixture was kept in a dark at ambient temperature for 60 min. A blank was prepared with 3.9 mL methanolic DPPH added to 100 µL PBS (pH 7.4) without the sample and also stored in the dark for 60 min.

A trolox calibration curve was generated by weighing 25 mg of trolox into a 100 mL volumetric flask and dissolving with 5 mL methanol. This was made up to volume with PBS buffer (pH 7.4). Aliquots of 0.5 mL, 1.0 mL, 2.0 mL, 4.0 mL, 6.0 mL and 8.0 mL of the diluted trolox was taken into 10 mL volumetric flasks and made to volume with PBS (pH 7.4) to give trolox standard concentrations of 50, 100, 200, 400, 600 and 800 μM, the initial concentration being 1000 μM.

After 60 minutes incubation in the dark, the absorbance of the samples, blank and standard concentrations were measured with a Camspec M501 Single Beam Scanning UV/Visible Spectrophotometer (Camspec Ltd., UK) with the wave-length scan set at 515 nm. The experiment was performed in triplicate and the mean values calculated. The percentage inhibition of the standard trolox concentrations and the samples were calculated according to Padmanabhan and Jangle (2012).

$$\% \text{ Inhibition} = 100 (A_o - A)/A_o$$

Where A_o = Absorbance of the blank sample

A = Absorbance of the test extract or the standard with known concentration

The percentage inhibition was plotted against trolox concentrations for the standards and used to calculate the antioxidant capacity of the samples in μMol TE g^{-1}DW or μMol TE g^{-1}FW.

3.6.5 Extraction and quantification of phytohormones

In Experiment 7, the endogenous phytohormones in selected tissues of 'NCCov II', previously treated with either continuous ethylene or continuous air were assayed. The previous experiments showed dominance of biological activities such as sprouting and accumulation of phenolic compounds in the proximal segments, so only the proximal tissues (flesh and skin) were examined. In addition, only samples (n=168) in selected outturns *viz*. baseline, OT-2, OT-3, OT-4, and OT-5 were examined.

3.6.5.1 Method for the extraction and quantification of phytohormones in sweetpotato using UPLC/QTOF/MS

A method for the extraction and quantification of endogenous phytohormones was developed after repeated trials to profile the endogenous hormones present and determine the sample weights which will give the highest recovery. Abscisic acid and zeatin riboside were the main phytohormones identified in 'NCCov II' and the methods for extraction and quantification is described as follows.

Freeze-dried powdered samples (*ca*. 150 mg) were weighed into 15 mL polypropylene tubes and phytohormones were extracted by adding 4950 µL of acidified methanol in water (methanol:water:HCl = 75:20:5, v/v) and spiked with 50 µL of internal standard mix containing d_4-ABA and d_3-DHZR at 400 ng mL^{-1}. A blank solution was prepared with 5000 µL of the extraction solvent containing no sample. A double blank was also prepared with 4950 µL of the extraction solvent spiked with 50 µL of the same internal standards but without any sample. The mixture were cold extracted overnight (12 h) at -20°C. They were then vortexed (20 s) and centrifuged (Heraeus Labfuge 400R Centrifuge, Thermo Scientific, Fischer, UK) for 15 min at 4500 rpm, 4°C. After centrifugation the supernatant was transferred into 15 mL Agilent purification cartridges and shaken to remove lipids and plant pigments. The residue was re-extracted with 2000 µL of the extraction solvent for 30 min and added to the first extract in the Agilent cartridges. The cartridges were centrifuged (4500 rpm, 15 min, 4°C) and the supernatant was filtered into 15 mL tubes using 0.2 µm syringe filters (Jaytee Biosciences Ltd., Kent, UK). The samples were firstly concentrated for 1 h at 22°C in a vacuum evaporator (miVac, Quattro Concentrator, Genevac Ltd., England) to evaporate the methanol and then freeze-dried (Scan Vac, Västerås freeze-dryer, Sweden) overnight.

The dried powder was reconstituted with 1 mL formic acid (1.0 M) and vortexed. Oasis MCX cartridges (6cc/150 mg, 60 µm) (Waters Corporation, Massachusetts, U.S.A.) were used to purify the phytohormones in the reconstituted samples. The cartridges were first conditioned with 5 mL methanol (100%) and then equilibrated with 5 mL formic acid (1.0 M). The reconstituted samples were then loaded into the cartridges, which retained the phytohormones whilst the eluted fraction was disposed. Methanol (2 mL) was then added to elute S-ABA into 15 mL tubes, followed by addition of 2 mL of 0.35 M NH_4OH

in 60% methanol to elute the cytokinins. The eluted solutions were combined and dried in a vacuum evaporator, followed by freeze-drying as previously described. After drying, the powder was reconstituted with 400 µL of the mobile phase A (0.1% formic acid in HPLC grade water), vortexed to homogenise and transferred into eppendorff vials (selected samples were reconstituted with 350 µL of the mobile phase A plus 50 µL of d_6-ABA at 400 ng mL^{-1} to determine recovery rate). They were then centrifuged for 5 min at 13000 rpm. The supernatant was filtered through 0.2 µm syringe filters into brown Agilent vials and stored at -40ºC pending analysis.

Aliquots (5 µL) of the samples were injected into Agilent 1290 Infinity UPLC/QToF/MS system comprising an Infinity 1290 UPLC, a thermostatted column compartment (TCC) operated at 30ºC, a binary pump system (Agilent, Berks – UK) and a 6540 UHD accurate mass Quadruple Time of Flight (UPLC/QToF/MS) with electrospray ionization source (ESI). The stationary phase column was ZORBAX Rapid resolution, HD Eclipse Plus, C18 (2.1 mm x 50 mm, 1.8 µm, Agilent, USA) while the mobile phase was a binary system comprising 0.1% formic acid in deionized water (A) and 0.05% formic acid in LC-MS grade acetonitrile (B). The elution gradient was as follows: 0 min, 4%B, 7.0 min; 26%B; 10 min, 40%B; 10.6 min, 100%B and held for 1 min. Finally, the column was re-equilibrated for 1.35 min to initial conditions. The source conditions for the MS were set as follows: gas temperature (150ºC), sheath gas temperature (400ºC), gas flow (11 $Lmin^{-1}$) and fragmentor voltage (165V). Identification of the individual compounds was done through comparison of retention times and mass spectra with that of reference compounds. Quantification was based on internal standards calibration curves ranging between 10 ng mL^{-1} to 3000 ng mL^{-1}.

3.7 Statistical Analysis

The data was first subjected to Shipro-Wilk normality test and plotted for residuals to verify assumptions for the Analysis of Variance (ANOVA). The ANOVA tables were generated using GenStat for Windows, Version 14 (VSN International Ltd., Herts., UK) and used to identify statistically significant trends. The means between treatments were separated with the Least Significant Difference (LSD) and declared to be significant at

95% confidence level (i.e. significance level *P*= 0.05). Data for weight loss, decay and sprouting were analysed as repeated measurements. General ANOVA was used to analyse the respiration rate, dry weight, sugars and phenolics. The ANOVA tables show the main effects of the treatments, tissues, root sections, time of storage and the interactions between these factors. Baseline values of the biochemical variables were evaluated at the start of each experiment. These were compared to the means of the corresponding factors after treatment. In experiments five and six which involved nested factorial swap treatments, the data was analysed with the GenStat structure: Tissue*Section* Baseline/(P1*T1/(T2*P2)) and has been elaborated in **Table GS** (Appendix B).

Where P1 = Treatments before swap P2 = Treatments after swap

T1= Outturns before swap T2 = Outturns after swap

CHAPTER FOUR: EFFECTS OF 1-METHYLCYCLOPROPENE (1-MCP) ON THE PHYSIOLOGY AND BIOCHEMISTRY OF SWEETPOTATO ROOTS DURING STORAGE

4.1 Introduction

Sweetpotato has a short shelf-life of about 2-3 weeks (Rees *et al*., 2001). Its long-term preservation largely depends on how storage conditions can be managed to enable it to stay dormant without adverse quality changes such as sprouting, decay, loss of marketable weight and nutritional losses. Many of these detrimental changes can be accelerated through endogenously produced ethylene by the roots or through inadvertent exposure to short-term, low levels of exogenous sources of ethylene (Saltveit, 1999). Endogenous ethylene production can be induced by abiotic stresses such temperature, oxygen, and carbon dioxide levels (Kader, 2002). In storage practice, the ethylene binding inhibitor, 1-methylcyclopropene (1-MCP), is often applied to restrain the deleterious effects caused by ethylene in crop tissues. There are indications from studies on the application of 1-MCP that it is capable of inhibiting sprout growth in produce such as onion cvs. SS1 (Chope *et al*., 2007a) and Sherpa (Downes *et al*., 2010; Cools *et al*., 2011). Foukaraki *et al.* (2011) also found that, alongside the inhibition of sprout growth in potato cv. Marfona, 1-MCP reduced sugar accumulation caused by ethylene supplementation and could thus help curb the browning of tissue during frying.

Sweetpotato shares characteristics with onion and potato as exhibiting non-climacteric behaviour and undergoes dormancy. As a result, findings relating to potato and onion may be expected to apply to sweetpotato; and indeed, the potential use of 1-MCP on sweetpotato was reported by Cheema *et al*. (2013) who found that a concentration of 625 nl L^{-1} applied for 24 h inhibited sprouting in sweetpotato cvs. Bushbuck and Ibees by at least four weeks. The work reported herein, further elucidates the broader spectrum of 1-MCP effects on sweetpotato storage roots in relation to changes in both the physiological quality attributes and the spatio-temporal flux of key metabolites that affect growth and development such as non-structural carbohydrates and individual phenolic compounds.

4.2 Aim and objectives of the study

4.2.1 Aim

To investigate the effects of 1-MCP treatment on the physiology and biochemistry of sweetpotato roots during storage.

4.2.2 Specific objectives

To determine the effects of the application of 1-MCP (1.0 µl L^{-1}/24 h) on the respiration rate, carbohydrate and phenolics metabolisms, dormancy period, initiation and progression of sprout growth, marketable weight and decay of sweetpotato roots during storage.

4.3 Hypothesis

The experiments were carried out with the following hypotheses:

4.3.1 Null Hypothesis

HO: Treatment of sweetpotato storage roots with 1-MCP has no significant effects on the biochemical and physiological characteristics.

4.3.2 Alternate Hypothesis

HA: Treatment of sweetpotato storage roots with 1-MCP has significant effects on the biochemical and physiological characteristics.

4.4 Experimental design

To reject above hypothesis or otherwise, three experiments were conducted as completely randomised designs using three different consignments of sweetpotato (*viz.* 'Organic Covington', 'Portuguese-derived Covington' and 'TIS-2') described in Section 3.2. One consignment was used per experiment. In each experiment, the consignment was divided into two lots. Each lot was further divided into three replicate batches and placed in water-

sealed 264 L polypropylene boxes (**Figure 4.1**). One lot (in three treatment replicates) was treated with 1-MCP (1.0 µl L^{-1}) for 24 h followed by storage in air. The other lot (also in triplicates) was stored continuously in air to serve as control check. Roots in each box were sub-divided into two sub-samples; designated as destructive sub-samples (used for biochemical assays) and non-destructive sub-samples (used for physiological assessments). The non-destructive roots were separated by placing them in a small basket within the treatment box and assessed periodically for weight loss, decay and sprout growth. At periodic outturns, roots from the destructive sub-samples were also randomly selected and assessed for respiration, followed immediately by biochemical assays (non-structural carbohydrates, phenolics and antioxidant capacity). The respiration and biochemical assays of baseline samples as received (Day 0) were assessed before the 1-MCP treatment.

4.4.1 1-MCP treatment

1-MCP was applied as described by Chope *et al*. (2007a) with slight modifications. To release a concentration of 1.0 µl L^{-1} of 1-MCP gas in the treatment boxes, 1.47 g of 1-MCP powder (Smart-fresh, 0.14% Rohm and Haas PA, Lot 005737873) was weighed into three 50 mL conical flasks and sealed with Nescofilm (Bando Chemical Ind. Ltd., Kobe, Japan) (**Figure 4.1**). To each flask, 5 mL of warm water (*ca.* 50°C) was injected through the Nescofilm using a needle and syringe, shaken to mix and quickly placed in the storage box for 24 h. In each treatment box, an 8x8 cm electric fan (Nidec beta SL, Nidec, Japan) was installed to circulate the released gas. Periodically, the headspace gas in each box was analysed by taking samples with repeated withdrawal-displacements using a 20 mL plastic syringe and injecting into a gas chromatograph (GC model 8340, DP800 integrator, Carlos Erba Instruments, Herts, UK) fitted with flame ionisation detector (EL 980 FID, 250°C) and a stainless steel analytical column Chromosorb PAW (2 m length, 6 mm O.D./4 mm I.D.) (**Figure 4.1**). The GC was calibrated with 0.97 µl L^{-1} isobutylene (Certified Standard from British Oxygen Company - BOC, Surrey, UK). After 24 h, the boxes were opened and the roots were transferred into air storage on trays in a temperature controlled room. Simultaneously as the 1-MCP treatment, control roots were kept in boxes under identical conditions without 1-MCP treatment. Each box was

treated as an experimental unit with the individual roots as pseudo-replicates (Pat Bellamy, Personal Communication).

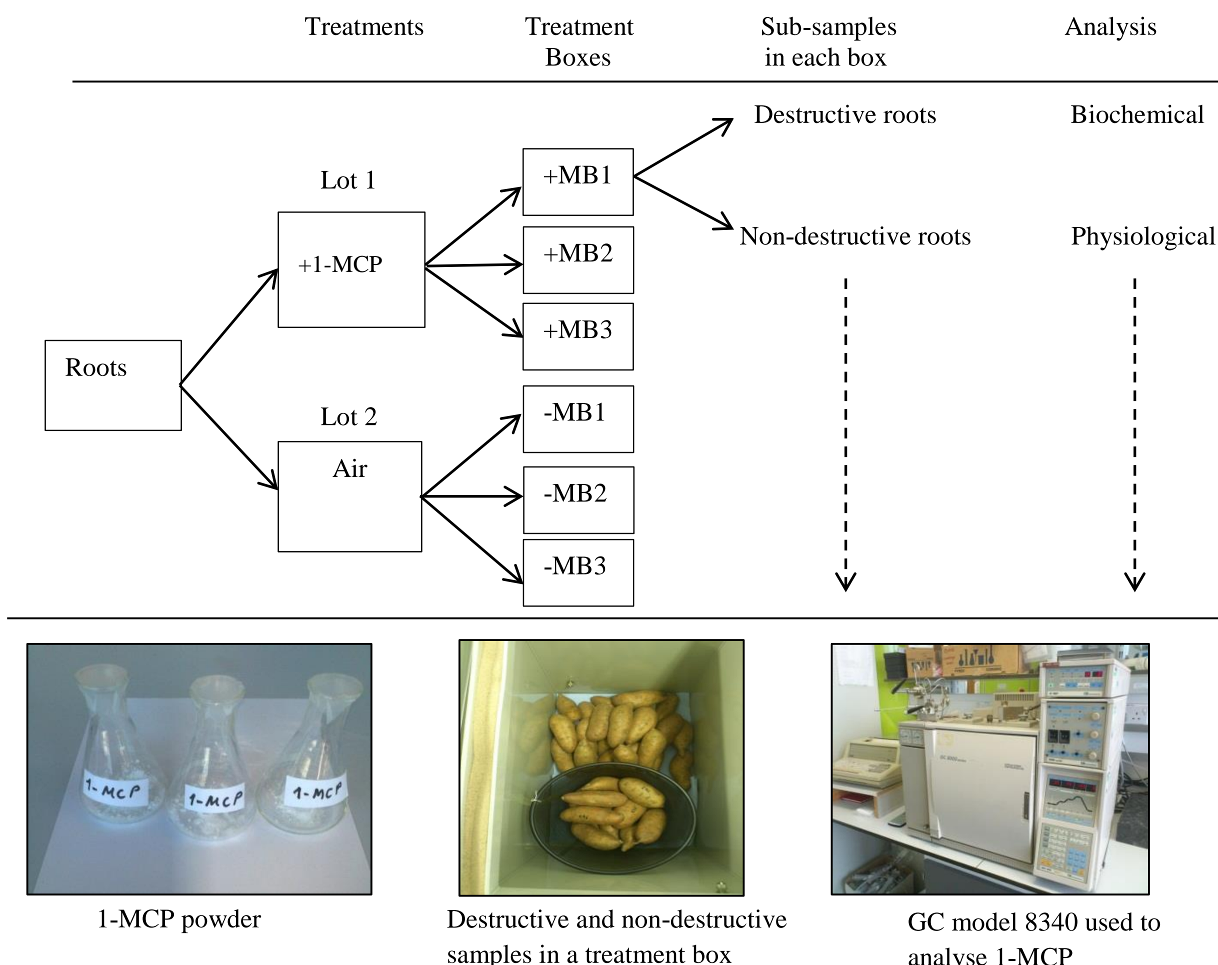


Figure 4.1 Schematic layout for 1-MCP treatment

4.4.1.1 Treatment effectiveness

The concentration of 1-MCP was measured periodically in the treatment boxes as described in Section 4.4.1 above. The treatment achieved a mean 1-MCP concentration of 1.02 µl L^{-1} in the boxes (**Figure 4.2**).

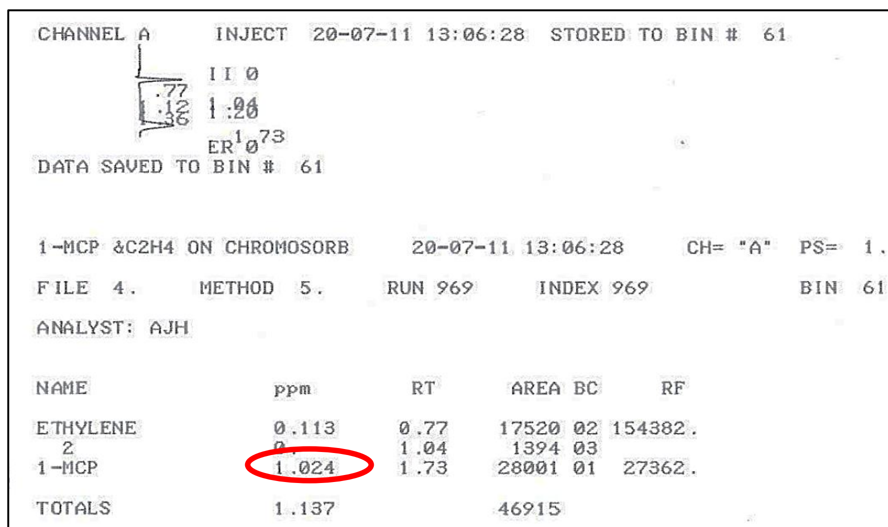


Figure 4.2 Typical chromatogram for 1-MCP treated box as measured with the GC

4.5. Experiment One: Effect of 1-methylcyclopropene (1-MCP) on the physiology and biochemistry of 'Organic Covington'

4.5.1 Sourcing and conditioning of the 'Organic Covington'

In the first trial (2011), the sweetpotato consignment 'Organic Covington' (**Figure 3.1 A**) was sourced from Barfoots of Botley Ltd., UK. The crop was originally cultivated using conventional agronomic practices in Barnes Farms, North Carolina, USA, and harvested in November 2010. Pre-storage treatment involved curing (30°C, 95% relative humidity, 7 days) before being shipped to Barfoots where it was stored for *ca.* 7 months at 14°C as a commercial stock for distribution to retail markets in the UK (Justin Creasy, Personal communication). Samples were collected from the commercial storage and sent to Cranfield University for the trial in July, 2011. Thus it is estimated that the samples were *ca.* 8 months old upon delivery. After sorting, a total of 150 wholesome roots were obtained and apportioned according to the treatments and replicates as described in the experimental details below.

4.5.2 Experimental details specific to the ‘Organic Covington’ trial with 1-MCP treatment

The experiment was generally carried out as described under the relevant sections (Sections 3.5 to 3.6) in Chapter 3. The schematic layout for the experiment is also as presented in **Figure 4.1**. The storage temperature was 15ºC (Some control root checks were also stored at 25ºC to monitor the effect of temperature on sprouting). There were 10 roots per non-destructive sub-sample and 15 roots per destructive sub-sample in each storage box. The non-destructive sub-samples were evaluated at 2-week intervals over 12 weeks (84 days) while the destructive root samples for biochemical assays were assessed every four weeks, except the respiration which was measured later on day 70, 84, 98 and 112 (**Table 4.1**). Root respiration was measured with the Sable Respirometry System as described in Section 3.5.4. At each outturn for the biochemical assays, 3 roots were randomly sampled from each box (n = 9 per treatment). The roots were then prepared for biochemical analysis as described in Section 3.6. The spatial distribution of the non-structural carbohydrates and phenolic compounds in both the flesh and skin tissues were separately assessed in the proximal, middle and distal roots sections. The results were analysed with the statistical procedure described in Section 3.7.

Table 4.1 Evaluation schedules for physiological and biochemical assessments of ‘Organic Covington’

Evaluation day after storage	Physiological	Biochemical
0	√	√
14	√	
28	√	√
42	√	
56	√	√
70	√	*
84	√	√*
98		*
112		*

* Respiration measurements

4.6 Experiment Two: Effects of 1-methylcyclopropene (1-MCP) on the physiology and biochemistry of 'Portuguese-derived Covington'

4.6.1 Sourcing and conditioning of the 'Portuguese-derived Covington'

For the second trial, a fresher consignment, 'Portuguese-derived Covington' (**Figure 3.1 B**), grown in an experimental field in Portugal was supplied by Barfoots of Botley Ltd. The crop was harvested in October 2011, followed by postharvest curing at 30^oC, 95% relative humidity for 7 days. After curing, it was transported to Barfoots for temporary storage at 14^oC for 2 weeks (Justin Creasy, Personal communication). Samples were collected from the storage and sent to Cranfield University in October 2011, *ca*. 3-4 weeks after harvest. A total of 300 wholesome roots were obtained after sorting. They were allocated to the treatments as described in the experimental details below.

4.6.2 Experimental details for the 'Portuguese-derived Covington' trial with 1-MCP treatment

The experiment was designed as described under Section 4.4 using the same experimental layout in **Figure 4.1**. The non-destructive sub-samples in each box consisted of 20 roots whilst the destructive sub-samples in the boxes were made up of 30 roots each. The experimental storage temperature was 15^oC (As in Experiment 1, some control root checks were also stored at 25^oC to monitor the effect of temperature on sprouting). The non-destructive roots were evaluated at periodic intervals over a total of 112 days whilst the destructive roots were also analysed over 130 days at irregular periods (**Table 4.2**). At each outturn, 4 roots were randomly selected from each box (n=12 per treatment) for respiration measurements and subsequent biochemical assays. In this trial, biochemical assays (non-structural carbohydrates and phenolics) were done for only the flesh and skin tissues. This was accomplished by pooling samples from the proximal, middle and distal sections of each root. The results were analysed with the statistical procedure described in Section 3.7

Table 4.2 Evaluation schedules for physiological and biochemical assessments of ‘Portuguese-derived Covington’

Evaluation day after storage	Physiological	Biochemical
0	√	√
3		√
12	√	
28	√	
32		√
29	√	
43	√	
53	√	
55		√
67	√	
98	√	
101		√
112	√	
130		√

4.7 Experiment Three: Effect of 1-methylcyclopropene (1-MCP) on the physiology and biochemistry of ‘TIS-2’

4.7.1 Sourcing and conditioning of the ‘TIS-2’

The sweetpotato cultivar TIS-2 was supplied by the University of Cape Coast, Ghana. The crop was planted in August 2011 at the School of Agriculture Farms and harvested in December 2011. Postharvest treatments involved curing at 28°C/90% relative humidity for 7 days and then transported by air within 2 days to the UK. The consignment arrived at Cranfield University in January 2012, approximately, 2 weeks after harvest. In all, 300 wholesome roots were supplied and allocated to the treatment boxes as described below.

4.7.2 Experimental details specific to the 'TIS-2' trial with 1-MCP treatment

An identical layout as described in **Figure 4.1** was used for the 'TIS-2' experiment. Each treatment box contained 50 roots: 12 roots in the non-destructive sub-samples and 38 in each destructive sub-sample. The rate of decay in 'TIS-2' was very high so the whole experiment was carried out in a relatively short period of 32 days. Sampling for assessments were irregularly spread over the storage period (**Table 4.3**). Four (4) replicate roots were randomly picked from the destructive sub-samples in each treatment box (n=12 per treatment) for respiration and dry weight assessments. Biochemical assays involving non-structural carbohydrates and phenolic compounds could not be done in this experiment. The experimental storage temperature for this trial was 20ºC.

Table 4.3 Evaluation schedules for physiological and biochemical assessments of 'TIS-2'

Evaluation day after storage	Physiological	Biochemical
0	√	√
1	√	
15	√	
16	√	√
24	√	
25	√	√
31	√	
32		√

4.8 Results

4.8.1 Effect of 1-MCP on sprouting

No incidence of root sprouts was noticed in any of the three 1-MCP experiments over the entire evaluation periods, irrespective of treatment and storage temperature (15 or 20ºC). Control root checks of 'Organic' and 'Portuguese-derive Covington' stored at 25ºC, however, sprouted after approximately 4 weeks.

4.8.2 Effect of 1-MCP on the incidence of diseases

There was cultivar-dependent effect of 1-MCP on root decay. The 1-MCP treatment significantly reduced the incidence of decay in the 'Organic Covington' (**Figure 4.3**), than the control (*p*-value < 0.05). In the 'Portuguese-derived Covington', significant reduction in the rate of decay by 1-MCP was recorded only after about 100 days of storage but not before. The interactive treatment effect for the 'Portuguese-derived Covington', however, was not statistically significant (*p*-value > 0.05). In the cv. TIS-2, 1-MCP treatment did not have any significant effect on the decay. Diseases in the 'Organic Covington' were first noticed after *ca.* 3 weeks of storage whilst for the much fresher 'Portuguese-derived Covington', the earliest incidence of decay occurred after *ca.* 7 weeks of storage. At the end of the storage period (84 days) the control roots in the 'Organic Covington' recorded about 2-fold greater root decay than the 1-MCP treated roots (47% : 23%). The 'Portuguese-derived Covington' was the most resistant cultivar to diseases; recording 20% decay in the control compared to 5% in the 1-MCP treated roots after 130 days of storage at 15ºC. The thick skinned cv. TIS-2 stored at 20ºC was highly vulnerable to root decay. The 'TIS-2' roots remained disease-free for only the first two weeks, after which soft and black rots initiated from the distal ends (**Figure 4.4**) and progressed quickly towards the opposite ends. After just 31 days of storage 75 and 58% of the 1-MCP treated roots and control roots, respectively, had decayed. However, the overall treatment effect over time was not statistically significant between the 1-MCP treatment and the control (*p*-value > 0.05).

Two distinct types of decay were observed; tip (proximal) rot and end (distal) rot. Both types of rot initiated from the respective root ends and progressed through the middle to the opposite end. Tip rot mostly affected the Covington roots whilst end rot predominantly affected 'TIS-2'.

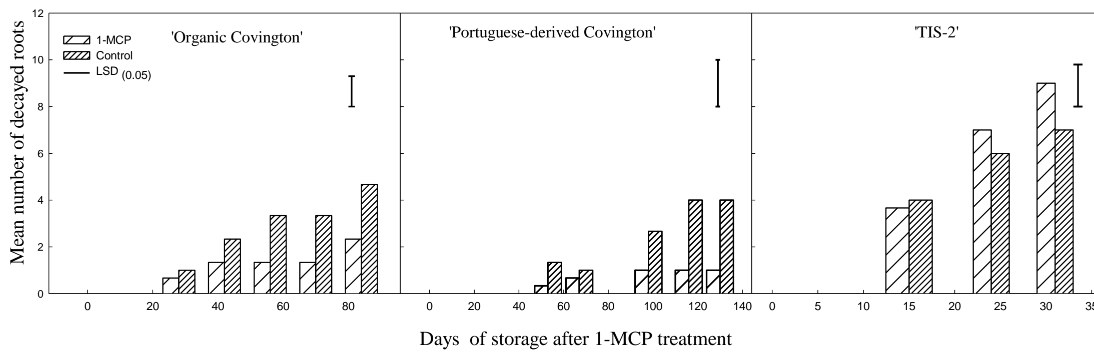
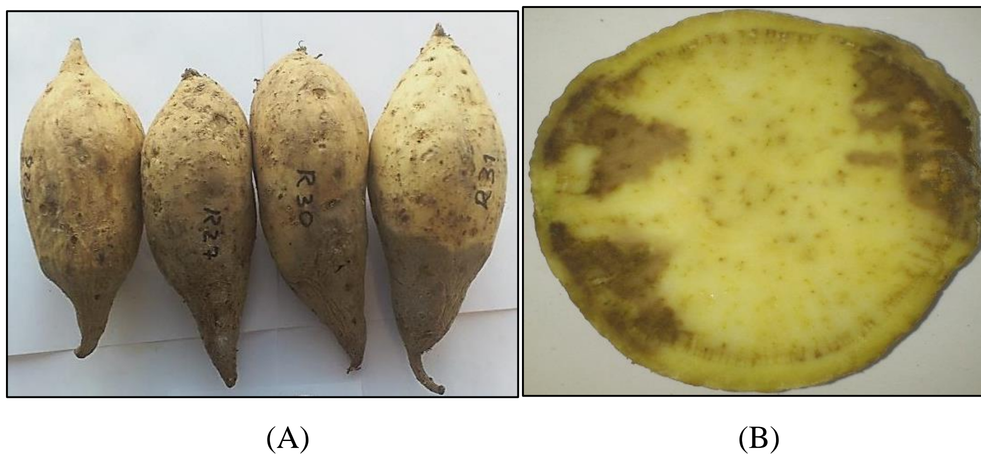


Figure 4.3 Effect of 1-MCP on decay during the storage of ‘Organic Covington’ (15°C), ‘Portuguese-derived Covington’ (15°C) and ‘TIS-2’ (20°C). The ‘Organic Covington’, ‘Portuguese-derived Covington’ and ‘TIS-2’ were treated with 1-MCP 32, 4 and 2 weeks after harvest, respectively. Each data point is the mean of three treatment boxes containing 10 roots per box for ‘Organic Covington’, 20 roots per box for ‘Portuguese-derived Covington’ and 12 roots per box for ‘TIS-2’, respectively. L.S.D $_{(0.05)}$ bars are shown. The legend applies to all the graphs. The ANOVA tables are presented in Appendix A: Tables A1, A2 and A3 for ‘Organic Covington’, ‘Portuguese-derived Covington’ and ‘TIS-2’, respectively.



(A) (B)

Figure 4.4 (A) End rot and (B) internal decay in ‘TIS-2’

4.8.3 Effect of 1-MCP on marketable weight loss

Sweetpotato is often marketed on weight basis. In the 1-MCP experiments, the commercial weight loss resulting from decay was evaluated. The control roots of the two

Covington consignments (‘Organic’ and ‘Portuguese-derived Covington’) lost significant weight (as a result of the removal of decayed roots) compared to the 1-MCP treated roots (p-value < 0.05). In the ‘TIS-2’, however, no significant difference was found between the 1-MCP- treated and the control roots on the marketable weight loss (**Figure 4.5**).

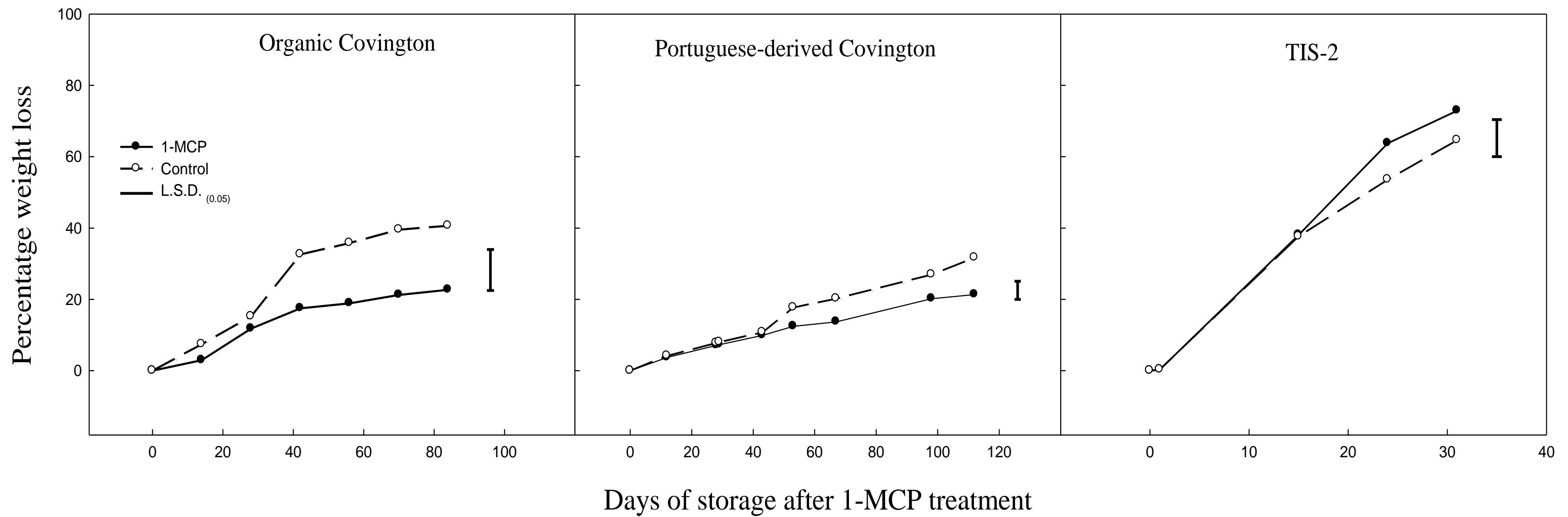


Figure 4.5 Effect of 1-MCP on weight loss during the storage of 'Organic Covington' (15ºC), 'Portuguese-derived Covington' (15ºC) and 'TIS-2' (20ºC). The 'Organic Covington', 'Portuguese-derived Covington' and 'TIS-2' were treated with 1-MCP 32, 4 and 2 weeks after harvest, respectively. Each data point is the mean of three treatment boxes containing 10 roots per box for 'Organic Covington', 20 roots per box for 'Portuguese-derived Covington' and 12 roots per box for 'TIS-2', respectively. The bars indicate L.S.D $_{(0.05)}$. The legend applies to all the graphs. The ANOVA tables are presented in Appendix A: Tables A4, A5 and A6 for 'Organic Covington', 'Portuguese-derived Covington' and 'TIS-2', respectively.

4.8.4 Effect of 1-MCP on respiration rate

Respiration rate was measured as the volume of CO_2 (mL CO_2 kg^{-1} h^{-1}) evolved by the root samples. In all the experiments (Experiments 1, 2 and 3) the 1-MCP treatment did not have any significant effect on the respiration of the roots. The respiration of 'Organic Covington', as measured from 70 days after storage, was fairly constant with mean values 7.7 and 6.2 mL CO_2 kg^{-1} h^{-1} for the 1-MCP treated and control roots, respectively. The initial respiration of the relatively fresher consignment 'Portuguese-derived Covington' was higher (*ca.* 16 mL CO_2 kg^{-1} h^{-1}) and dropped significantly during storage along a curve characteristic of non-climacteric crops to *ca*. 6.6 mL CO_2 kg^{-1} h^{-1}. The mean respiration values of the 1-MCP-treated and control roots of the 'Portuguese-derived Covington' were 10.8 and 11.0 mL CO_2 kg^{-1} h^{-1}, respectively. For the 'TIS-2', after an initial transient drop from *ca*. 20.0 mL CO_2 kg^{-1} h^{-1}, the respiration of roots treated with 1-MCP rose above the control respiration rate, reaching a peak level (25.8 mL CO_2 kg^{-1} h^{-1}) after 16 days of storage and declined during the latter stages of storage along a typical non-climacteric curve (**Figure 4.6).** The respiration of 'TIS-2' control roots also declined consistently in a non-climacteric pattern from *ca*. 20.0 to 13.6 mL CO_2 kg^{-1} h^{-1}. The differences in the respiration between the treatments, however, was not significant.

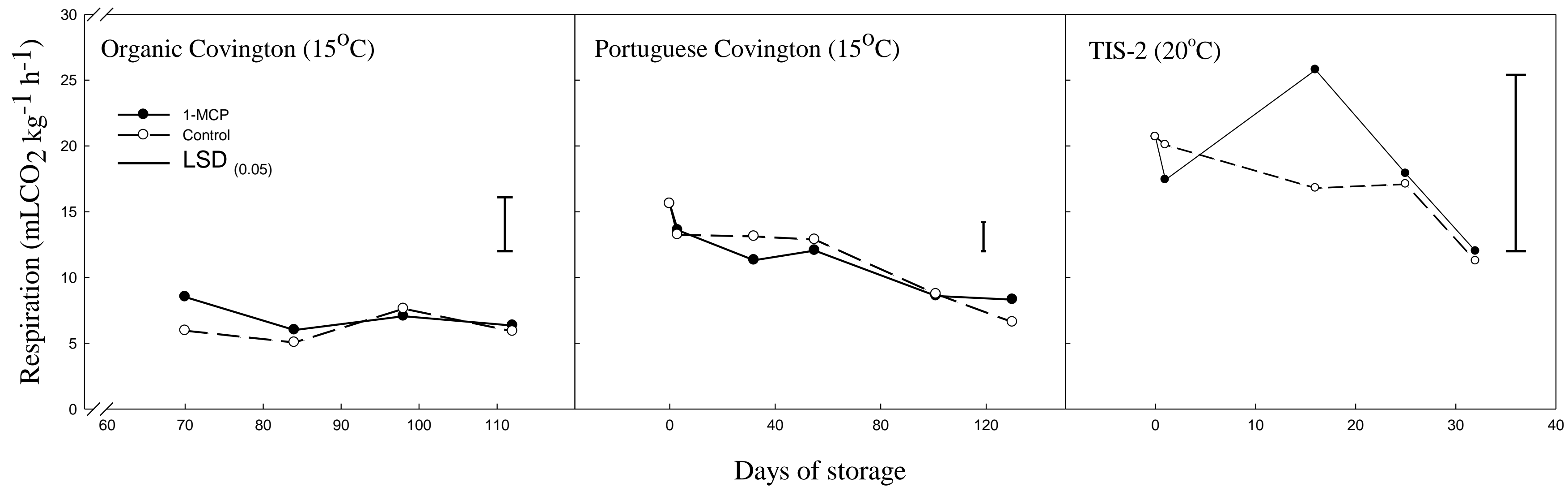


Figure 4.6 Effect of 1-MCP on the respiration rate of 'Organic Covington' (15°C), 'Portuguese-derived Covington' (15°C) and 'TIS-2' (20°C) during storage. The 'Organic Covington', 'Portuguese-derived Covington' and 'TIS-2' were treated with 1-MCP 32, 4 and 2 weeks after harvest, respectively. Each data point is the mean of three treatment boxes consisting of 3 roots per box for 'Organic Covington' (n=9), 4 roots per box for 'Portuguese-derived Covington' (n=12) and 4 roots per box for 'TIS-2' (n=12), respectively. L.S.D $_{(0.05)}$ bars are shown. The legend applies to all the graphs. The ANOVA tables are presented in Appendix A: Tables A7, A8 and A9 for 'Organic Covington', 'Portuguese-derived Covington' and 'TIS-2', respectively.

4.8.5 Effect of 1-MCP on dry weight

The spatial variations of the dry weight as a proportion of the fresh weight (% FW) were evaluated in the three experiments (**Figures 4.7 - 4.9**). The 2 weeks old consignment 'TIS-2' had the highest dry matter proportion of the fresh weight (baseline means 31.8 and 39.2 % FW for the skin and flesh, respectively), followed by the 'Portuguese-derived Covington' (baseline means 26.0 and 25.1 % FW for the skin and flesh, respectively). The *ca.* 8-month old consignment, 'Organic Covington', had the least dry matter content with baseline means of 22.4 and 19.4 % FW for the skin and flesh tissues, respectively.

The treatment effect on the dry weight was dependent upon the consignment. For the 'Organic Covington', the dry weight of the 1-MCP-treated roots (mean 21.1% FW) was significantly higher than that of the control roots (mean 20.6%) (p-value < 0.05). Also the skin tissues (mean 21.5 % FW) had significantly higher dry weight compared to the flesh tissues (mean 19.4 % FW). The differences between mean dry weight for the proximal (21.0 % FW), middle (20.7 % FW) and distal (20.9 % FW) sections, however, were not significant (**Figure 4.7**). There was an initial transient increase in the dry weight (% FW) of the skin in all the root sections up to, at least 28 days; followed by a decreasing trend. The initial increase in the 1-MCP treated proximal skin tissues, however, reached further to *ca.* 23 % FW in 56 days and remained fairly stable at that level. The dry weight (% FW) in the flesh tissues within the root sections was fairly constant.

In contrast to the 'Organic Covington', the dry weight (% FW) in the control roots of the 'Portuguese-derived Covington' was significantly higher than the 1-MCP treated roots (**Figure 4.8**). The mean values were 24.2 and 25.0 % FW for the 1-MCP treated and the control roots, respectively. Consistent with the 'Organic Covington', however, the skin tissues had significantly higher proportion of dry weight (mean value 26.2 % FW) than that of the flesh tissues (mean value 23.0 %FW). The proportion of dry weight in the flesh tissues of the 'Portuguese-derived Covington' decreased with time but that in the skin was fairly stable.

In 'TIS-2', no significant difference was found in the dry weight (% FW) between the 1-MCP-treated and the control roots (mean values 37.4 and 38.8% FW, respectively). However, contrary to the observations made for the 'Organic' and the 'Portuguese-

derived Covington', the dry weight (% FW) in the flesh tissue of 'TIS-2' was significantly higher than in the skin (p-value < 0.001) (mean values 40.1 and 35 % FW for the flesh and the skin, respectively). Also, against the backdrop that there was increasing decay with time, the dry weight (as a proportion of the fresh weight) tended to increase in both tissues (**Figure 4.9**).

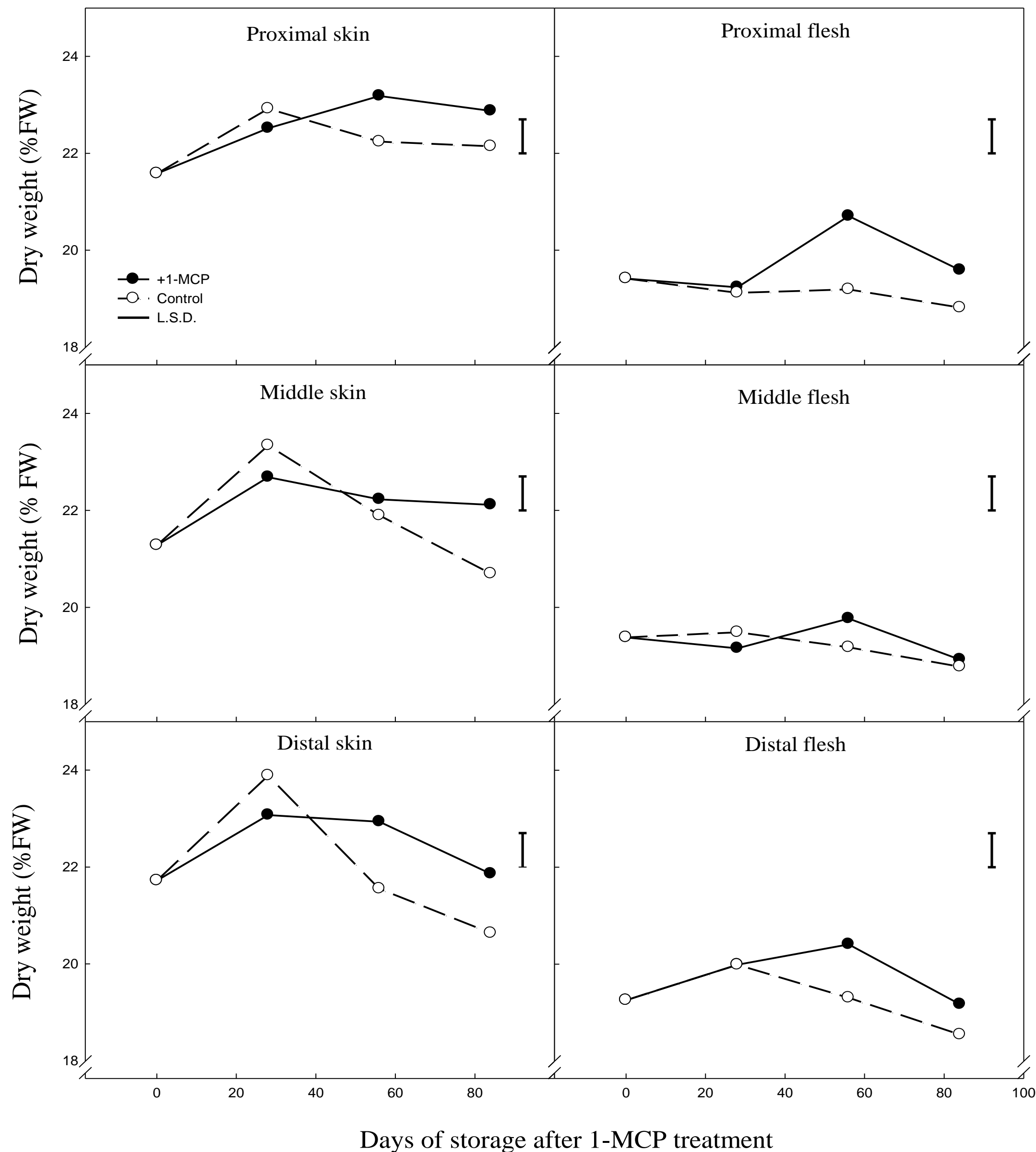


Figure 4.7 Effect of 1-MCP on the spatial variation of dry weight (% FW) of 'Organic Covington'. The roots were treated 32 weeks after harvest. Each data point is the mean of three treatment boxes consisting of 3 roots (pseudo-replicates) per box (n=9). L.S.D. $_{(0.05)}$ bars are shown. The legend applies to all the graphs. The ANOVA table is presented in Appendix A: Table. A10.

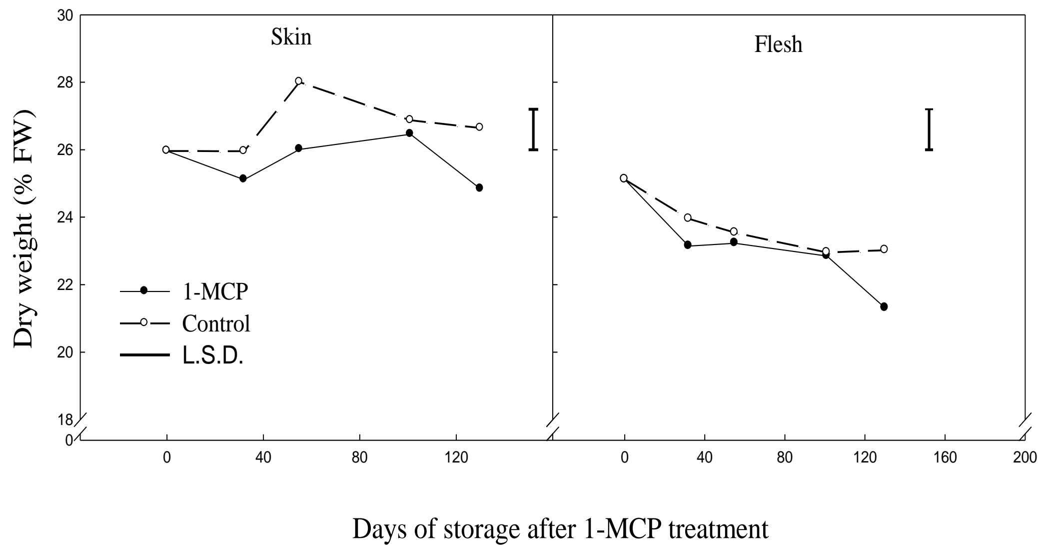


Figure 4.8 Effect of 1-MCP on the spatial variation of dry weight (% FW) of 'Portuguese-derived Covington'. The roots were treated 4 weeks after harvest. Each data point is the mean of three treatment boxes consisting of 4 roots (pseudo-replicates) per box (n=12). L.S.D. $_{0.05}$ bars = 1.2 are shown. The legend applies to all the graphs. The ANOVA table is presented in Appendix A: Table. A11.

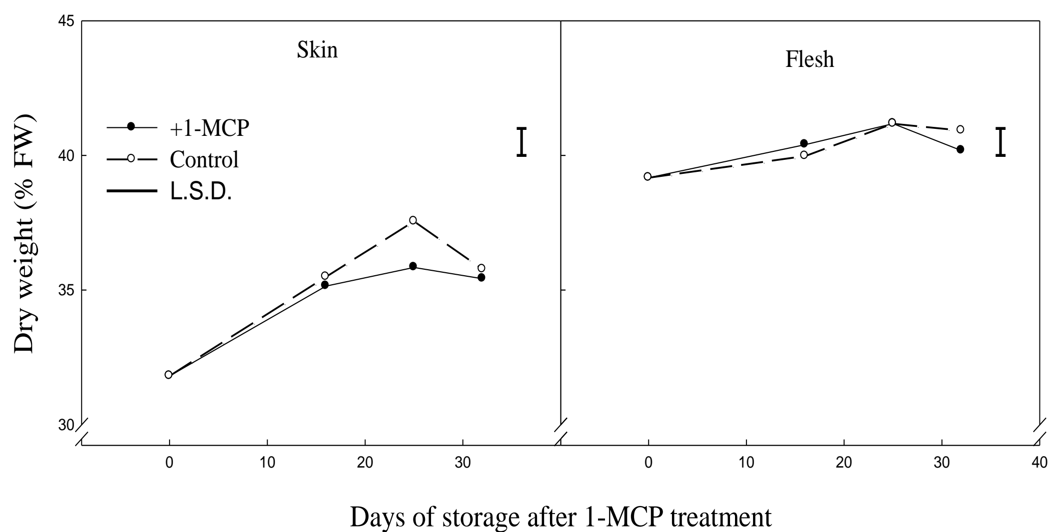


Figure 4.9 Effect of 1-MCP on the spatial variation of dry weight (% FW) of 'TIS-2'. The roots were treated 2 weeks after harvest. Each data point is the mean of three treatment boxes consisting of 4 roots (pseudo-replicates) per box (n=12). L.S.D$_{0.05)}$ bars = 1.0 are shown. The legend applies to all the graphs. The ANOVA table is presented in Appendix A: Table. A12.

4.8.6 Effect of 1-MCP on the phenolic compounds in 'Organic Covington'

4.8.6.1 Effect of 1-MCP on total phenolics in 'Organic Covington'

The total phenolics content in the skin tissues of 'Organic Covington' was *ca.* 11-fold greater compared to the flesh tissues. 1-MCP treatment significantly reduced the total phenolics concentration in the flesh tissues (mean values 18.5 and 22.4 mg ChAE/100 g FW for the 1-MCP-treated and the control roots, respectively). There was, however, no treatment effect in the skin tissues (p-value > 0.05). Significantly higher concentration of the total phenolics was also recorded in the proximal section of the roots (mean value in the flesh: 25.9 mg ChAE /100 g FW) than in both the middle and distal sections. The middle sections of the roots had the lowest content of phenolic compounds (mean value in the flesh: 15.9 mg ChAE/100 g FW). The 1-MCP treatment significantly accentuated the proximal dominance of phenolic compounds **(Figure 4.10).**

There was a high linear correlation (R^2 = 0.95) between the total phenolics and the antioxidant capacity as measured in the baseline roots (Results in Appendix D).

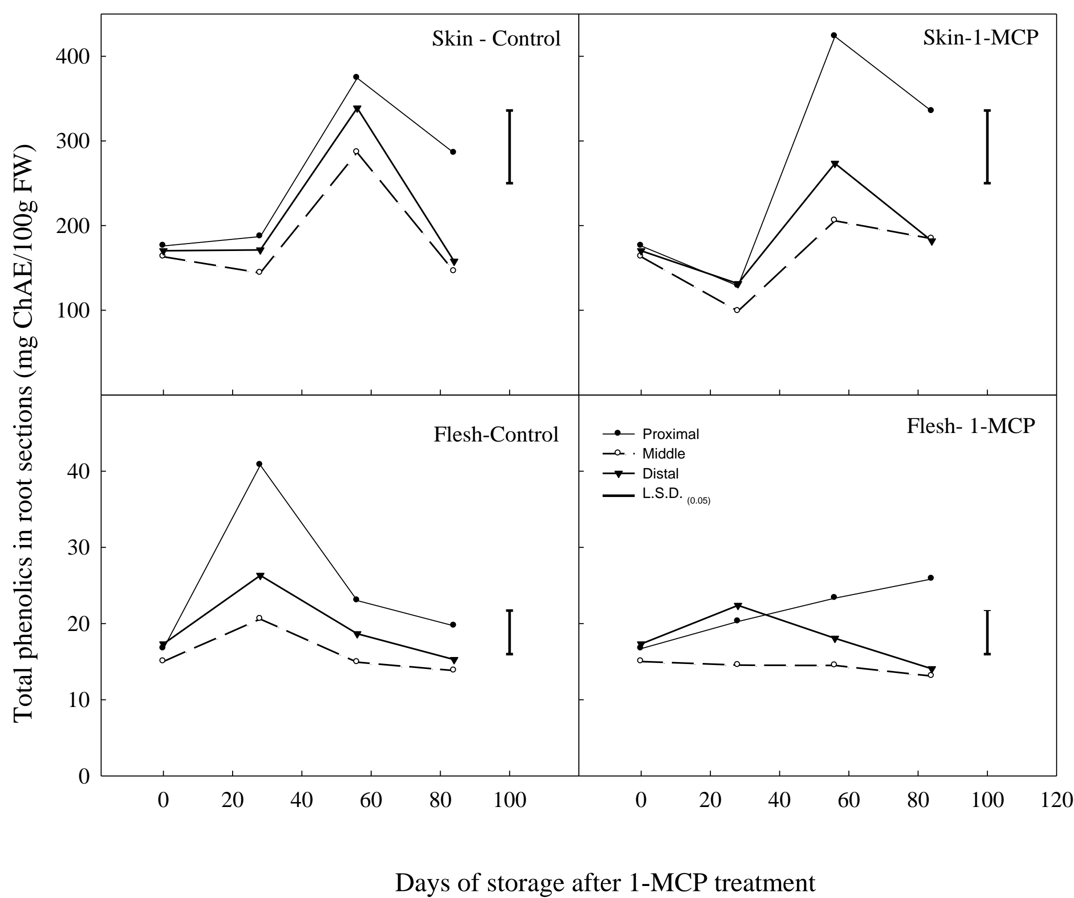


Figure 4.10 Effect of 1-MCP on the spatial concentrations (mg ChAE/100g FW) of total phenolics in 'Organic Covington'. Each data point is the mean of three treatment boxes consisting of 3 roots per box (n=9). L.S.D. $_{0.05}$ bars are shown. The legend applies to all the graphs. The ANOVA table is presented in Appendix A: Tables A13 and A14.

4.8.6.2 Spatial concentrations of individual phenolic compounds in 'Organic Covington'

There was non-homogenous spatial distribution of the individual phenolic compounds in the roots such that, iso-chlorogenic acid A (3,5-di-*O*-caffeoylquinic acid) was the most abundant polyphenolic in the skin tissues of 'Organic Covington' whilst chlorogenic acid (5-*O*-caffeoylquinic acid) dominated in the flesh tissues across all the root sections (**Figure 4.11**) Also, all the individual phenolic compounds were significantly more abundant in the skin than in the flesh tissues. Coumaric and ferulic acids were found in trace quantities below quantification levels. The detailed distribution and changes in the individual phenolic compounds over storage time are presented in **Figures 4.12 – 4.16**.

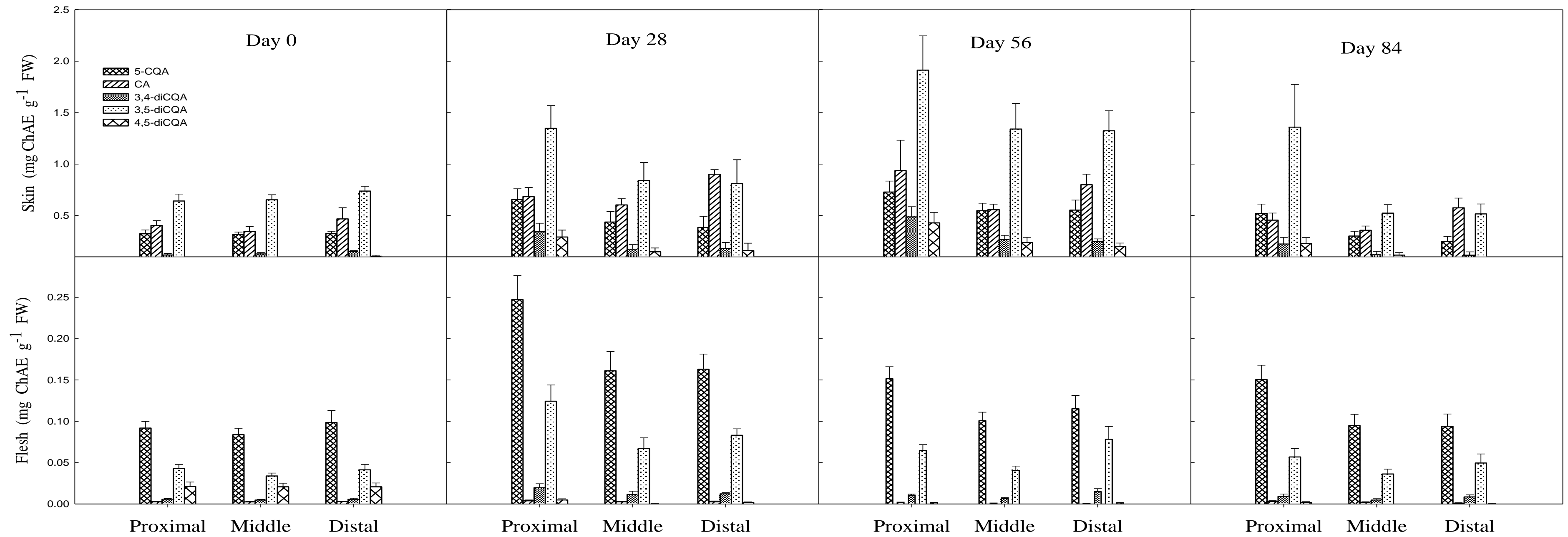


Figure 4.11 The spatial concentrations (mg ChAE g^{-1} FW) of the major phenolic compounds in 'Organic Covington'. Each data point is the mean of three treatment boxes consisting of 3 roots per box (n=9). Standard errors of the means are shown. The legend applies to all the graphs.

4.8.6.3 Effect of 1-MCP on individual phenolic compounds in 'Organic Covington'

All the individual phenolic compounds were significantly more abundant in the skin compared to the flesh tissues (**Table 4.4**). Chlorogenic acid and its isomers (Isochlorogenic acid A, B and C) were significantly more abundant in the proximal sections of the roots. Caffeic acid concentrations in the proximal and distal sections, however, were not significantly different. 1-MCP treatment reduced the contents of all the individual phenolic compounds, which affected the spatial regions differentially. The contents of chlorogenic acid and its isomers in the proximal skin tissues were not affected by the 1-MCP treatment, leaving a significantly higher balance of the phenolics in the proximal sections. In contrast to chlorogenic acid and its isomers, the 1-MCP treatment significantly reduced the caffeic acid concentration in the skin but not in the flesh. In general 1-MCP significantly reduced the phenolic compounds in the middle and distal sections of the roots whilst comparatively, the contents in the proximal tissues were less affected (**Figures 4.12-4.16**).

Table 4.4 Baseline concentrations of phenolic compounds in the tissues of 'Organic Covington'

	Concentration (mgChAE/100g FW)	
Phenolic Compound	Flesh	Skin
Chlorogenic acid	9.1	32.1
Isochlorogenic acid A	3.9	67.7
Isochlorogenic acid B	0.5	13.1
Isochlorogenic acid C	2.1	9.3
Caffeic acid	0.3	40.5

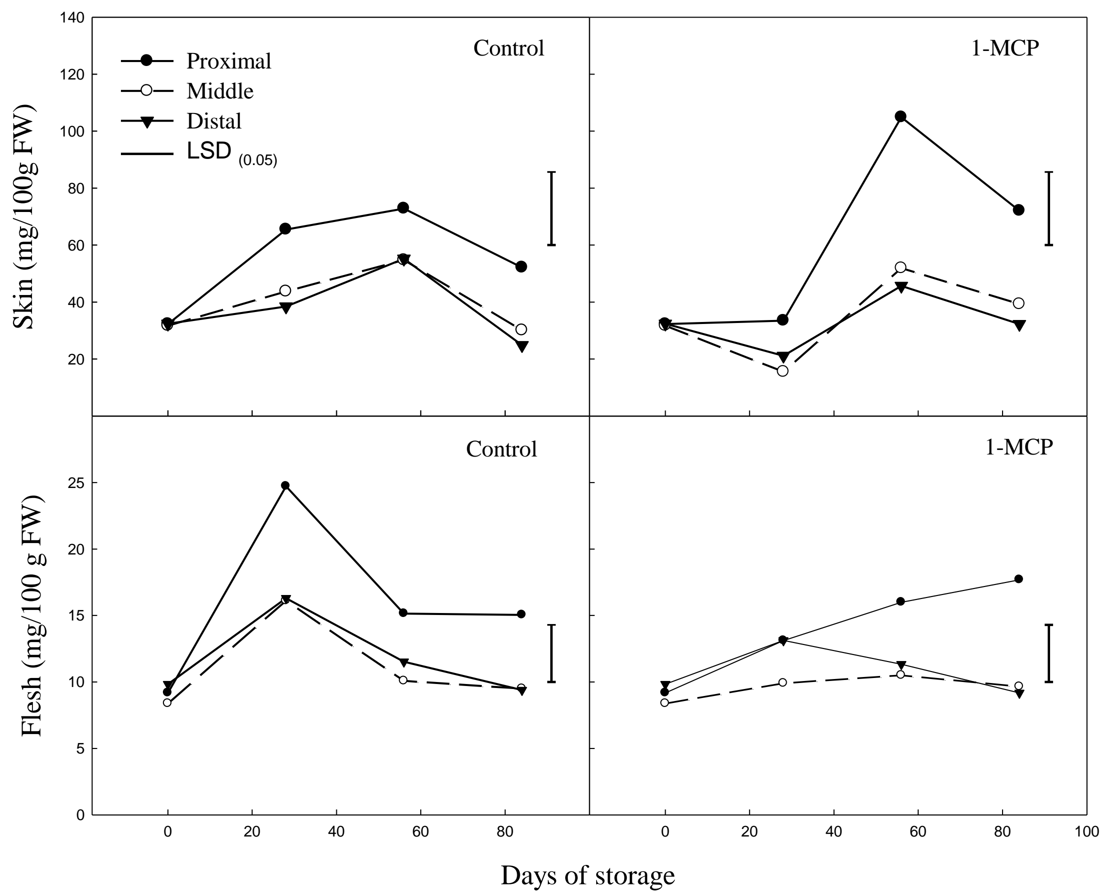


Figure 4.12 Effects of 1-MCP on the spatial concentrations (mg ChAE/100 g FW) of chlorogenic acid in 'Organic Covington'. Each data point is the mean of three treatment boxes consisting of 3 roots per box (n=9). LSD $_{(0.05)}$ bars are shown. The legend applies to all the graphs. The ANOVA table is presented in Appendix A: Tables A15 and A16.

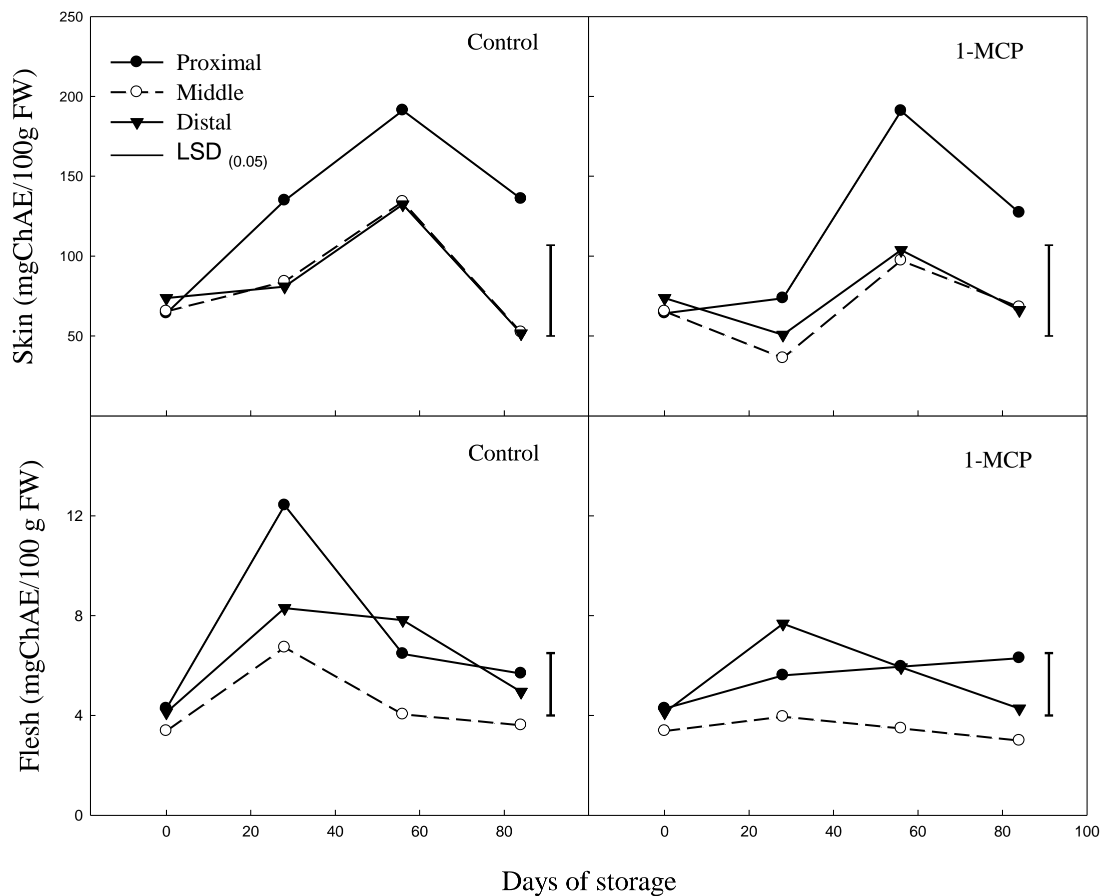


Figure 4.13 Effects of 1-MCP on the spatial concentrations (mg ChAE/100 g FW) of iso-chlorogenic acid A (3-5 di-CQA) in 'Organic Covington'. Each data point is the mean of three treatment boxes consisting of 3 roots per box (n=9). LSD $_{(0.05)}$ bars are shown. The legend applies to all the graphs. The ANOVA table is presented in Appendix A: Tables A17 and A18.

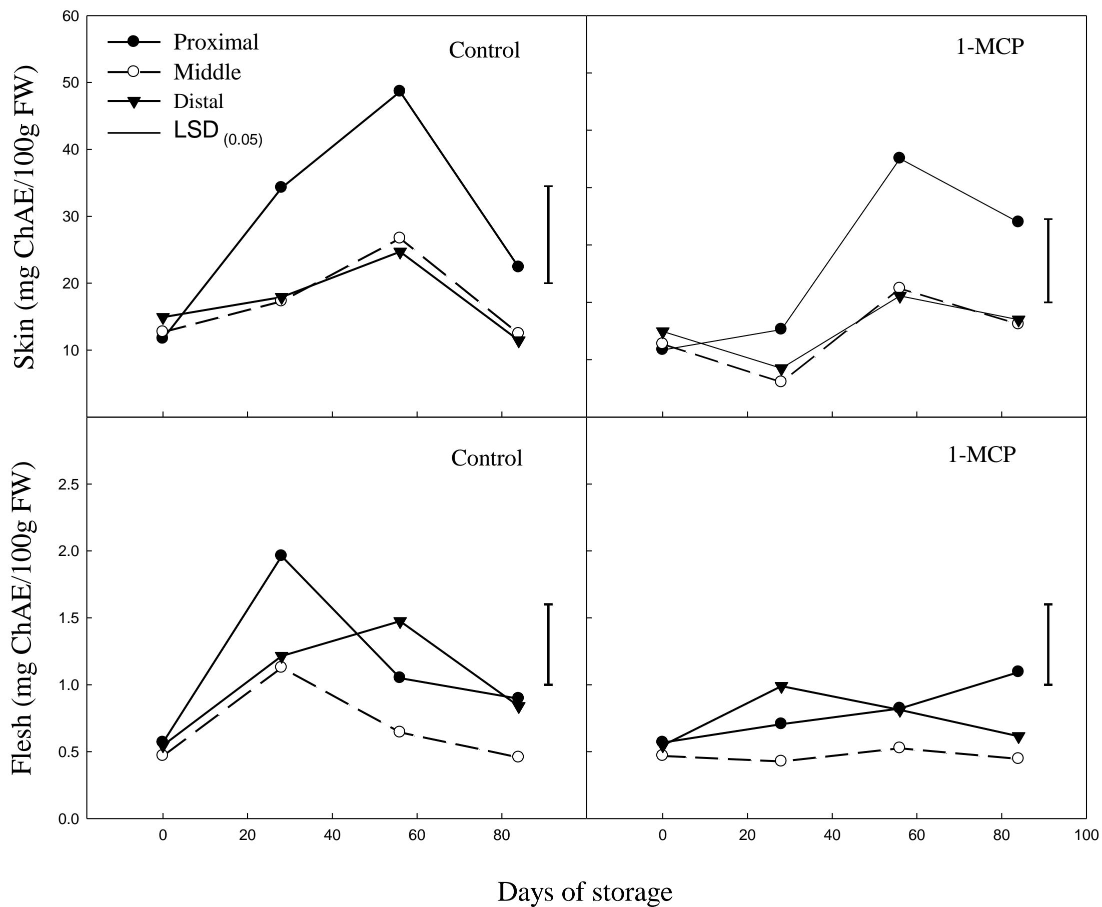


Figure 4.14 Effects of 1-MCP on the spatial concentrations (mg ChAE/100 g FW) of iso-chlorogenic acid B (3-4 di-CQA) in 'Organic Covington'. Each data point is the mean of three treatment boxes consisting of 3 roots per box (n=9). LSD $_{(0.05)}$ bars are shown. The legend applies to all the graphs. The ANOVA table is presented in Appendix A: Tables A19 and A20.

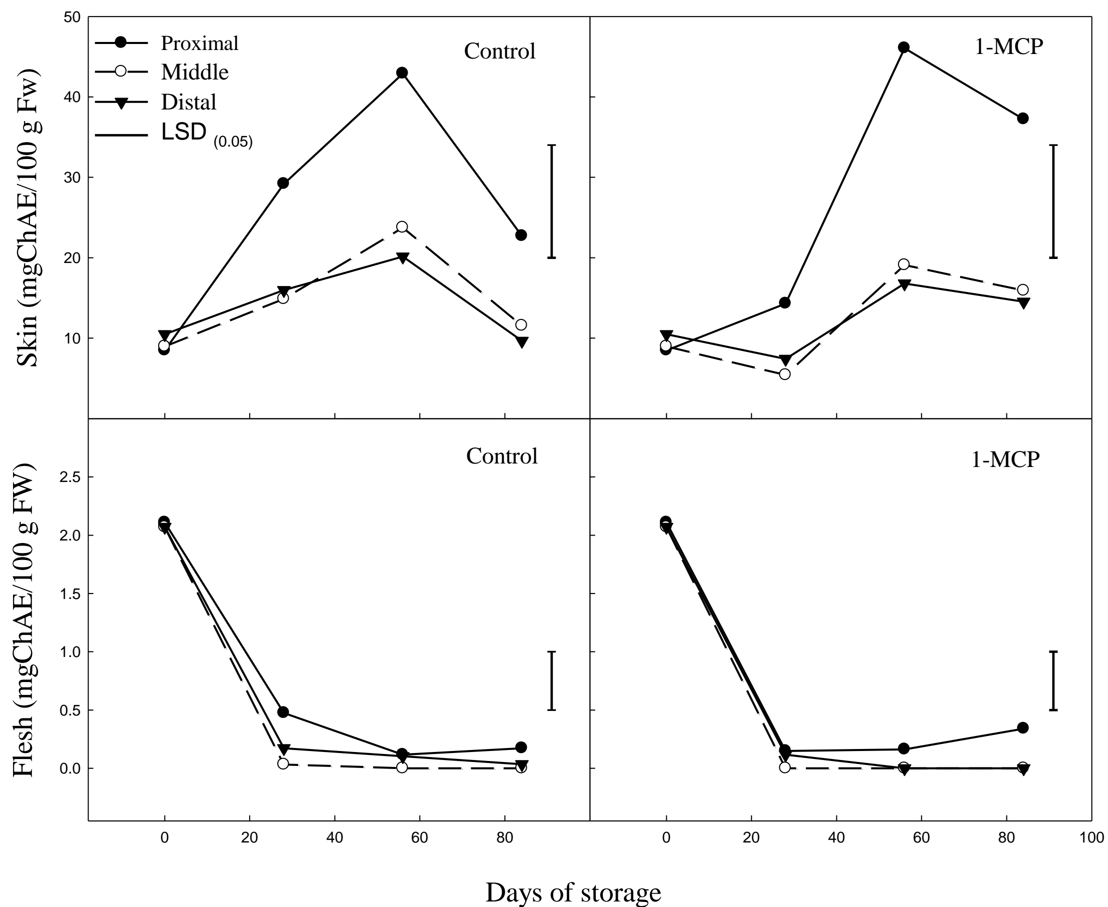


Figure 4.15 Effects of 1-MCP on the spatial concentrations (mg ChAE/100 g FW) of isochlorogenic acid C (4-5 di-CQA) in 'Organic Covington'. Each data point is the mean of three treatment boxes consisting of 3 roots per box (n=9). LSD $_{(0.05)}$ bars are shown. The legend applies to all the graphs. The ANOVA table is presented in Appendix A: Tables A21 and A22.

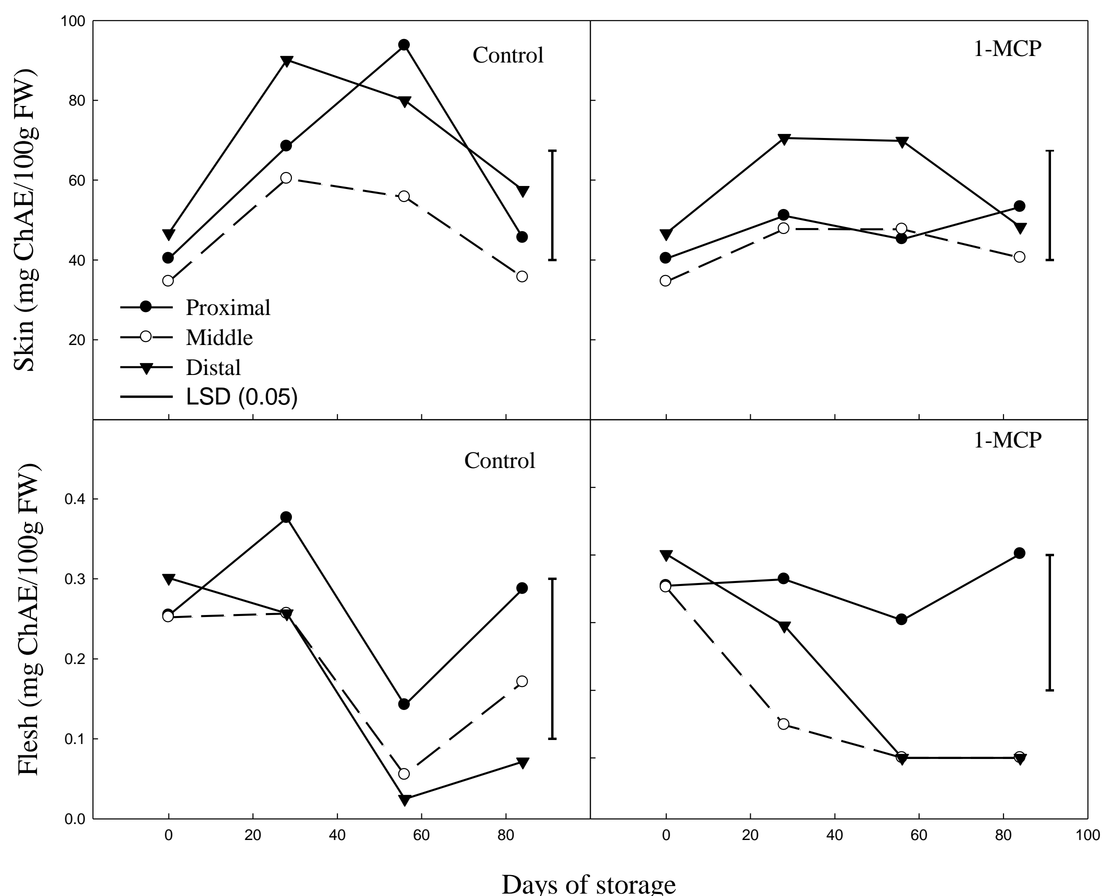


Figure 4.16 Effects of 1-MCP on the spatial concentration (mg ChAE/100 g FW) of caffeic acid in 'Organic Covington'. Each data point is the mean of three treatment boxes consisting of 3 roots per box (n=9). LSD $_{(0.05)}$ bars are shown. The legend applies to all the graphs. The ANOVA table is presented in Appendix A: Tables A23 and A24.

4.8.7 Effect of 1-MCP on the phenolic compounds in 'Portuguese-derived Covington' (Experiment 2)

In Experiment 2, the spatial sections (proximal, middle and distal) were pooled for the skin and flesh tissues. The major phenolic compounds identified in the tissues of the Portuguese-derived covington were also chlorogenic acid, caffeic acid, iso-chlorogenic acid B, iso-chologenic acid A and iso-chlorogenic acid C, which eluted in that order. In this experiment, all the individual phenolic compounds in the samples were calibrated with their respective pure standards so the quantitated values were expressed in absolute

terms as mg/100 g FW. As with the case of the 'Organic Covington', it was also found in the 'Portuguese-derived Covington' that chlorogenic acid dominated in the flesh tissues whilst iso-chlorgenic acid A was the most abundant phenolic compound in the skin (**Table 4.5**). Caffeic acid was found in the skin but was not present in the flesh tissues throughout storage. Iso-chlorogenic acids B and C were also virtually absent at the beginning of storage but their concentrations increased with time. The total phenolics concentration was *ca.* 15 fold higher in the skin compared to the flesh tissues. The concentrations of all the phenolic compounds within the skin tissues increased during the initial period (*ca.* 55 days) of storage but tended to decline for chlorogenic acid and Isochlorogenic acid A afterwards. Within the flesh tissues, the content of all the individual phenolic compounds did not vary with time. There was no significant effect of 1-MCP on the concentration of the individual phenolic compounds except caffeic acid whose concentration in the skin tissues of the control roots was just statistically higher than the 1-MCP-treated roots. (**Figure 4.17**).

Table 4.5 Baseline concentrations of phenolic compounds in the tissues of 'Portuguese-derived Covington'

	Concentration mg / 100g FW	
Phenolic Compound	Flesh	Skin
Chlorogenic acid	9.8	101.5
Isochlorogenic acid A	5.4	112.4
Isochlorogenic acid B	0.1	6.2
Isochlorogenic acid C	0	4.4
Caffeic acid	0	7.4
	15.3	231.9

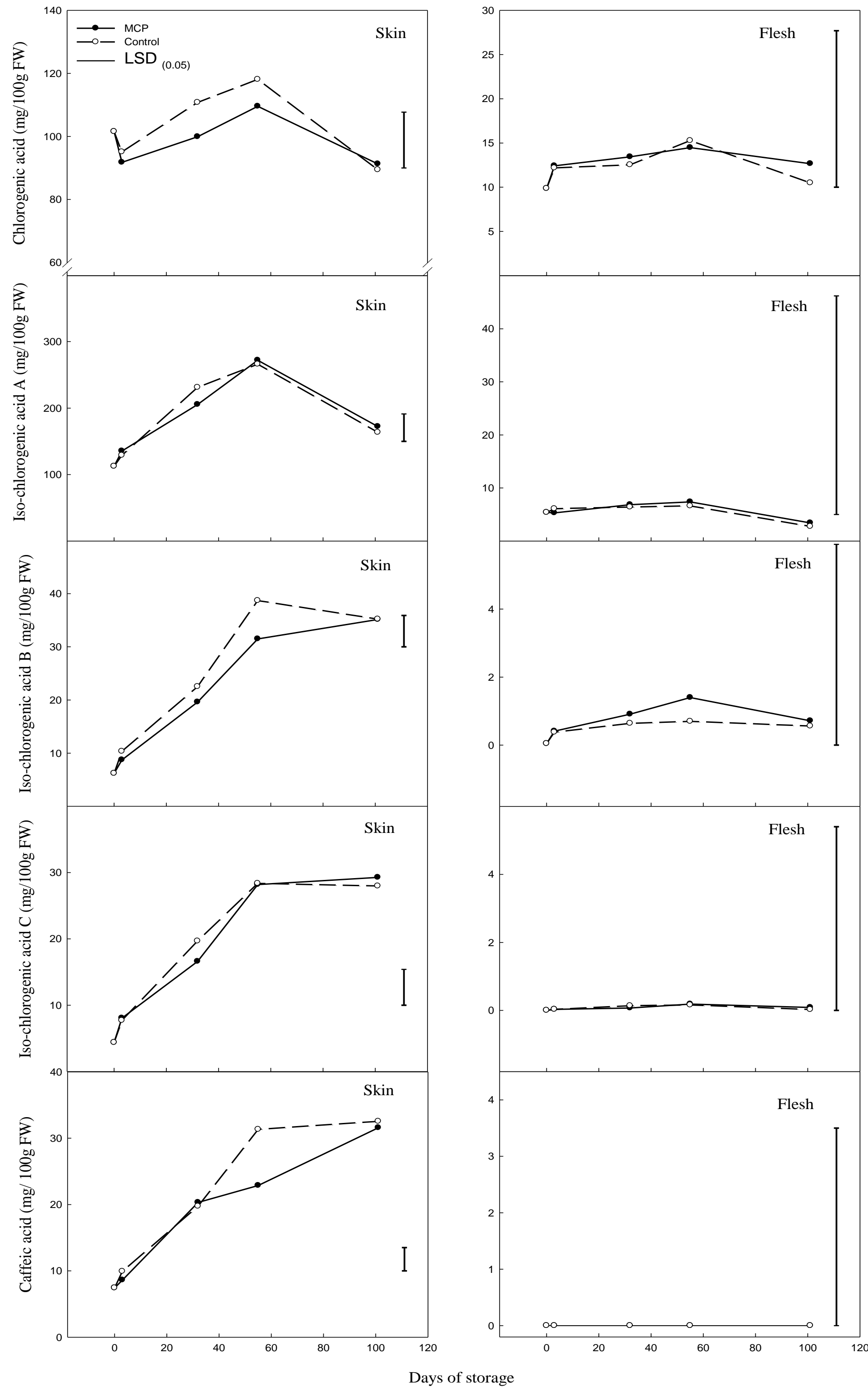


Figure 4.17 Effects of 1-MCP on the spatial concentration (mg /100 g FW) of phenolic compounds in 'Portuguese-derived Covington'. Each data point is the mean of three treatment boxes consisting of 4 roots per box (n=12). LSD $_{(0.05)}$ bars are shown. The legend applies to all the graphs. The ANOVA table is presented in Appendix A: Tables A25 - A29.

4.8.8 Effects of 1-MCP on sugars

The non-structural carbohydrates found in the two consignments of the sweetpotato cv. Covington are shown in **Figures 4.18- 4.20**. The average concentration of the sugars (mg g^{-1}FW) occurred in the order: sucrose > glucose > fructose > maltose. Maltose was detected in 'Organic Covington' only.

4.8.8.1 Sugars in 'Organic Covington' (Experiment 1)

In 'Organic Covington', fructose, glucose and sucrose had significantly higher concentrations in the flesh than the skin tissues (p-value < 0.05) (**Figure 4.18**). Maltose had approximately the same concentration in the flesh as the skin. The mean baseline (no treatment) concentrations in the flesh tissues were 2.4, 5.5, 8.3 and 46.9 mg g^{-1} FW (representing 0.2, 0.6, 0.8 and 4.7% FW) for maltose, fructose, glucose and sucrose, respectively. In the skin tissues, the relative concentrations were 2.8, 3.1, 3.2 and 38.1 mg g^{-1} FW (representing 0.3, 0.3, 0.3 and 3.8% FW) for maltose, fructose, glucose and sucrose, respectively. Fructose was significantly more concentrated in the distal than the proximal sections. Maltose on the other hand, was more abundant in the proximal than the other root sections. The distribution of glucose and sucrose between the sections was not significant. With storage time, the levels of fructose, glucose and maltose significantly declined in both tissues but sucrose and maltose remained fairly stable. No significant effect of 1-MCP treatment was found in the concentrations of the individual sugars within the tissues of 'Organic Covington'. The concentrations of the individual sugars across the root segments (proximal, middle, and distal) were also not significantly different between the treatments except for glucose, where concentrations in the proximal control tissues dropped after 30 days of storage (**Figure 4.19**).

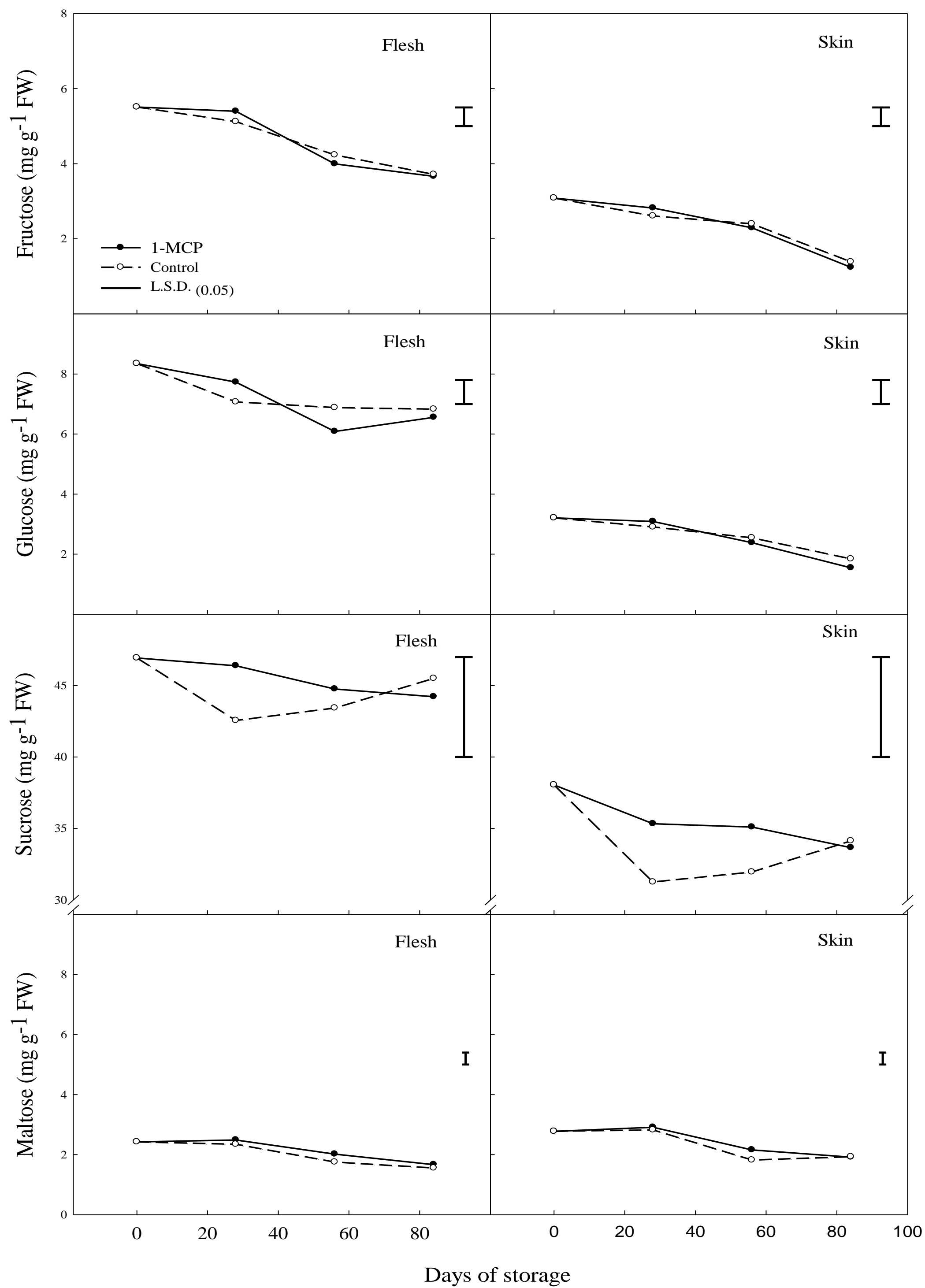


Figure 4.18 Effects of 1-MCP on non-structural carbohydrates in the tissues of 'Organic Covington' during storage at 15°C. L.S.D. $_{(0.05)}$ bars are shown. Each data point is the mean of three treatment boxes consisting of 3 roots per box (n = 9). The legend applies to all the graphs. The ANOVA table is presented in Appendix A: Tables A30 - A33.

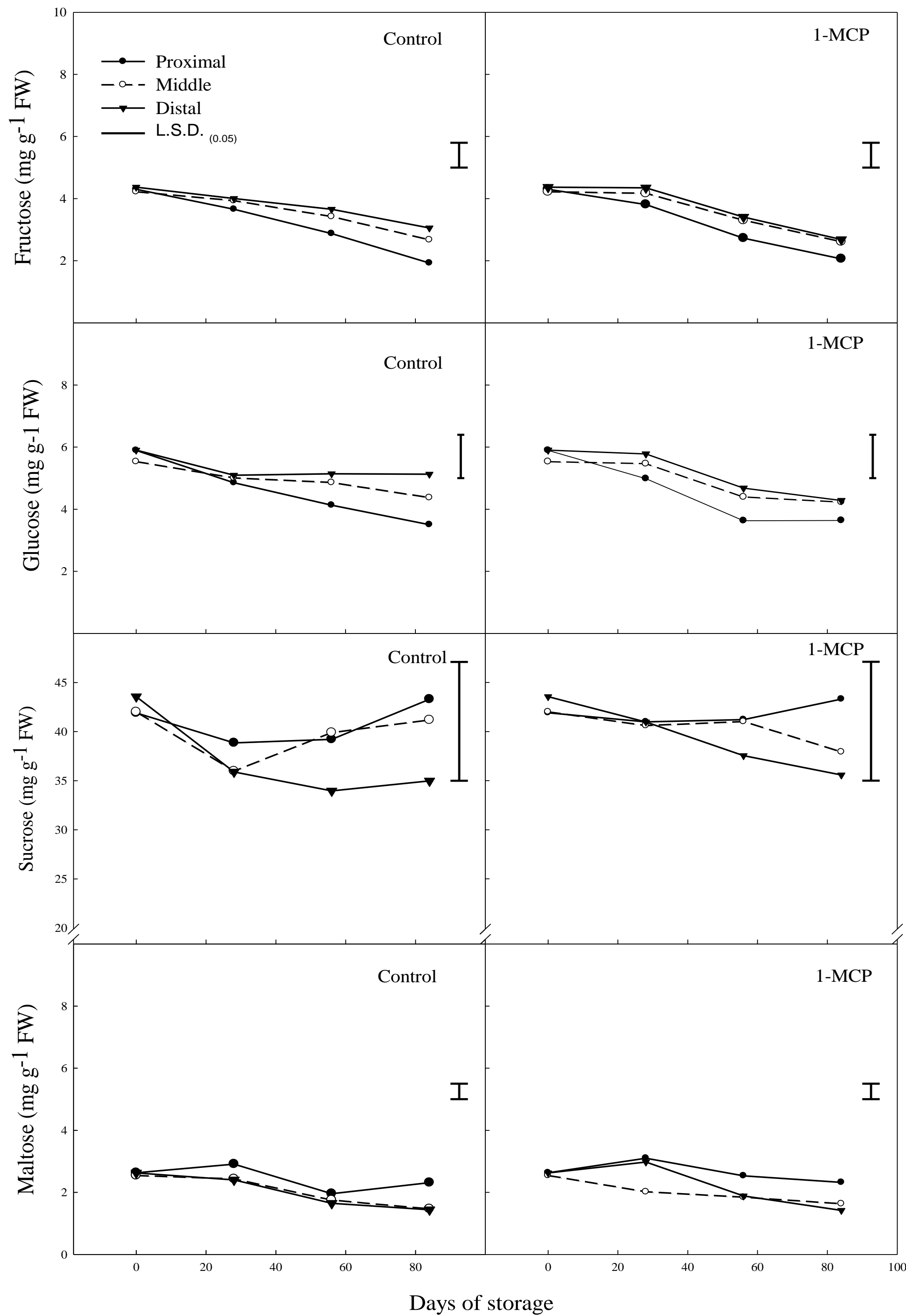


Figure 4.19 Effect of 1-MCP on non-structural carbohydrates in the sections of 'Organic Covington' during storage at 15°C. Each data point is the mean of three treatment boxes consisting of 3 roots per box (n=9). Standard errors of the means are shown. The legend applies to all the graphs. The ANOVA table is presented in Appendix A: Tables A34 - A37.

4.8.8.2 Sugars in 'Portuguese-derived Covington' (Experiment 2)

Maltose was not found in the 'Portuguese-derived Covington'. Fructose, glucose and sucrose were significantly more abundant in the flesh than in the skin tissues at all evaluation times (**Figure 4.20**). The mean baseline (without treatment) concentrations in the flesh were 13.7, 15.4 and 40.7 mg g^{-1} FW for fructose, glucose and sucrose, respectively. In terms of percentages of the fresh weight, their contents were 1.4%, 1.5% and 4.1% for fructose, glucose and sucrose, respectively. In the skin tissues, the relative baseline concentrations for fructose, glucose and sucrose were 6.9, 8.1 and 41.9 mg g^{-1} FW, representing 0.7%, 0.8% and 4.2% FW, respectively. As in the case of 'Organic Covington', the levels of fructose and glucose in the tissues significantly declined with storage time. Sucrose concentration, however, was fairly stable throughout the storage. In either tissue of 'Portuguese-derived Covington', there were no significant differences in the concentration of the individual sugars between the treatments.

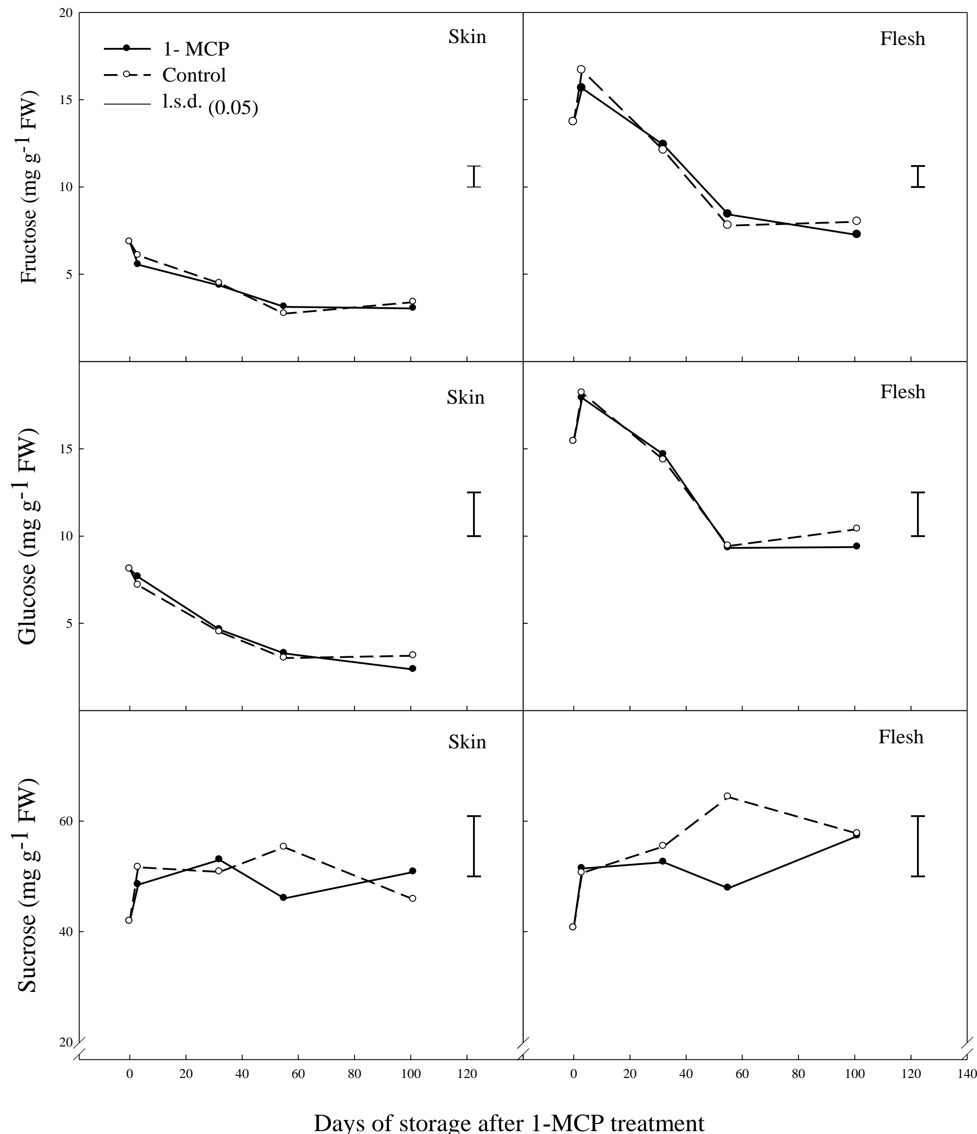


Figure 4.20 Effects of 1-MCP on non-structural carbohydrates in the tissues of Portuguese-derived Covington during storage at 15°C. Each data point is the mean of three treatment boxes consisting of 4 roots per box (n=12). Standard errors of the means are shown. The legend applies to all the graphs. The ANOVA table is presented in Appendix A: Tables A38 - A40.

4.9 Discussion

The effects of 1-MCP on many aspects of sweetpotato storage such as the respiration rate, weight, incidence of diseases and sprout growth have been reported (van Oirschot 2000; Truong *et al.,* 2007; Teow *et al.,* 2007; Cheema *et al.*, 2013). There is, however, no literature on how 1-MCP mediates the spatial flux of non-structural carbohydrates and

phenolic compounds in sweetpotato. In the present study, 1-MCP was applied at a concentration of 1.0 µl L^{-1}, 24 h at 15°C (‘Organic Covington’ and ‘Portuguese-derived Covington’) and 20°C (‘TIS-2’). The effects on the incidence of diseases, weight, respiration rate, sprouting, and the spatial distribution of individual non-structural carbohydrates and phenolic compounds were investigated.

4.9.1 Effect of 1-MCP on diseases

This current study lasted longer than that by Cheema *et al*. (2013) and enabled the long term effects of 1-MCP treatment on diseases to be evaluated. Disease symptoms were recorded after approximately 3, 7 and 2 weeks in ‘Organic Covington’, ‘Portuguese-derived Covington’ and ‘TIS-2’, respectively. The incidence of diseases in ‘Organic Covington’ was significantly reduced thoughout storage by the 1-MCP treatment compared to the air control. However, for ‘Portuguese-derived Covington’, there was a significant reduction in the incidence of diseases by 1-MCP treatment after *ca*. 100 days of storage but not earlier. In ‘TIS-2’ on the other hand, 1-MCP was entirely ineffective in curbing decay showing that 1-MCP effect is cultivar dependent. In an unpublished report by Villordon Arthur (2012, Personal communication) the application of 1-MCP (1.0 µl L^{-1}, 24 h) during the curing of sweetpotato cvs. Beauregard and Evangeline achieved 10% lower incidence of rot symptoms in the early periods of storage (up to 133 days) compared to the control checks. However, the effectiveness against decay diminished during subsequent storage. In comparison, Cheema *et al*. (2013) found no rots when four weeks old sweetpotato cvs. Bushbuck and Ibees were treated with 1-MCP (625 nl L^{-1}, 24 h) and stored at 25°C for a period of four weeks. These conflicting results suggest that variables such as cultivar, age, 1-MCP concentration, storage temperature and duration may all be important in determining 1-MCP effect on sweetpotatoes. It has been stated that for every commodity, 1-MCP effectiveness is dependent on variables such as the concentration, temperature and duration of exposure (Blankenship and Dole, 2003). Nanthachai *et al*. (2007) also reported that different produce absorb 1-MCP at differential rates, which can vary as much as 30-fold between commodities. Factors such as fresh weight, water content, soluble dry matter and peel thickness were found by the authors to affect 1-MCP absorption rate. The cv. TIS-2 used in the current study, for example, had thick skin

which could constitute a barrier for gas diffusion, and hence reduce the influx of 1-MCP. These varied results shows that it is important to carry out precise and detailed studies on 1-MCP application methods for specific crops.

The efficacy of 1-MCP in alleviating rot symptoms in the ‘Organic Covington’ may be related to its antagonistic role to ethylene. It has been reported that microbial decay can be promoted by ethylene in some fruit and vegetables depending on the crop-pathogen system (Palou *et al*., 2003). Ethylene softens some fruit and vegetables, alters the texture by causing loss of cell compartmentalization and increases permeability (Pratt and Goeschl, 1969; Saltveit, 1999). This can subsequently pre-dispose the tissues to microbial attack. In a positive feedback relationship, the microbial attack induces copious amounts of ethylene which escalates decay symptoms (Hyodo *et al*., 2003). Arancibia *et al*. (2013) also reported that when sweetpotato cv. Beauregard was treated with ethephon (an analogue of ethylene) pre-harvest, there was a higher incidence of tip (proximal) rot compared to the controls and that, higher rate of ethephon resulted in yet higher tip rot incidence. This suggests that ethylene-related decay affects root tips (proximal ends). In the present study tip rot mostly affected the Covington roots whilst ‘TIS-2’ was predominantly affected by end rot. It appeared that 1-MCP was more effective against ethylene-induced proximal tip rot by blocking ethylene perception by the proximal receptors.

A B

Figure 4.21 Tip rot (A) in ‘Organic Covington’ and end rot (B) in ‘TIS-2’

4.9.2 Effect of 1-MCP on weight loss

Weight loss in sweetpotato has been associated with physiological disorders and reduced quality (Rees *et al.,* 1998; van Oirschot, 2000), and it is used as a measure of postharvest loss of marketable produce. The precise economic losses have not been quantified but it is estimated to range between 35-95% in developing countries (Rees *et al*., 2003). Rees *et al*. (2003) also suggested that such high figures require confirmation. Data presented by van Oirschot (2000) showed that between 5-15% of total weight loss in sweetpotato stored in East African climate was due to respiration, the rest being attributed to microbial decay. It is evident therefore that decay is the most important factor of economic losses in the marketing of sweetpotato. In the present study, the effect of 1-MCP treatment on the total market losses, emanating from decay was evaluated. It was found that 1-MCP treatment significantly reduced the marketable weight loss in a cultivar dependent manner, being more effective for the two Covington consignments but not 'TIS-2'. The results here were apparently a consequential effect of the removal of decayed roots. This suggests that 1-MCP, through its suppression of diseases, may be effective in reducing economic losses in the marketability of some sweetpotato cultivars. The effect of 1-MCP on physiological weight loss alone was not evaluated in the current study. As there was no incidence of diseases in the study by Cheema *et al*. (2013), it may be concluded that the weight loss they measured was attributed to physiological losses (respiratory water and dry matter losses). The authors found that weight loss was greatest in air storage in comparison with 1-MCP and other treatments. It is thus possible that 1-MCP may reduce both physiological and pathological weight losses.

4.9.3 Effect of 1-MCP on respiration

Ethylene is known to boost tissue respiration and therefore, the blocking of ethylene perception by 1-MCP is expected to produce the opposite effect. In line with this expectation, 1-MCP is reported to suppress respiration in potato (Foukaraki *et al*., 2011). Also Cheema *et al.* (2013) reported that 1-MCP reduced the respiration of sweetpotato cv. Bushbuck stored at 25°C for 4 weeks. In the current study, however, 1-MCP did not show any significant effects on the respiration rates of all the sweetpotato consignments

tested. For the 'Organic Covington', this result may be expected as the roots were already too old (*ca*. 32 months) and its non-climacteric respiration is expected to have declined to basal levels after the long term storage. 'TIS-2', though much fresher, was thick skinned and the 1-MCP gas diffusion into the tissues was probably limited. The results for the 'Portuguese-derived Covington', however, was not expected but could possibly be attributed to the low storage temperature (15ºC) at which respiration rate and therefore, metabolic activity is usually low and may thus not elicit any marked differences between the treatments.

4.9.4 Effect of 1-MCP on sprouting

No incidence of sprouting was observed in this study probably for the same reason of low temperature storage and low respiration rate. Indeed, low temperature is used as effective postharvest tool against sprouting in sweetpotato. Other investigators (Picha, 1986; Wolfe, 1992) have found that when stored at low temperatures sweetpotato can keep up to a year without significant quality loss. Chope (2006) found that 1-MCP inhibited sprout growth in onions stored at 4 or 12ºC but not at 20ºC, suggesting temperature dependence of 1-MCP effectiveness against sprouting. It is also possible that the low temperatures of 4 or 12ºC were adequate to inhibit sprouting in onion. However, when sweetpotatoes were stored at a higher temperature (25ºC), Cheema *et al*. (2013) reported that sprout growth in sweetpotatoes was inhibited by 1-MCP. It has been stated that variability exists among crops in their response to 1-MCP (Daniels-Lake *et al*., 2008). Blankenship (2001) also stated that variables such as the concentration, exposure time and maturity of the tissues affect the efficacy of 1-MCP. The conflicting results based on different crops and storage temperatures supports the view that the effect of 1-MCP is both crop and temperature dependent.

4.9.5 Effect of 1-MCP on dry matter

The dry matter content (% FW) of sweetpotato is highly variable between cultivars. Literature confirms that the dry matter ranges from as low as 13% FW to 48% FW. The

differences are attributed to variables such as the genetic characteristics of the cultivar, age, location, climate, soil type, cultivation practice and the incidence of pest and diseases (Bradbury and Holloway, 1988). The wide variability in the dry matter proportion was evident in the current study, where it ranged from *ca.* 19% FW in 'Organic Covington' to as high as *ca.* 39% FW in 'TIS-2'. The range of values obtained in the present study is therefore in agreement with reported dry matter concentrations in sweetpotato. In particular, the Ghanaian cv. TIS-2, which had high dry matter content concurs with what was found by Aidoo (2004).

1-MCP treatment increased the dry matter (% FW) content of 'Organic Covington' whilst for the 'Portuguese-derived Covington', the dry matter proportion was reduced by 1-MCP. On the other hand, no significant effect of 1-MCP on the dry matter content of 'TIS-2' was observed. The dry matter (% FW) of the skin of 'TIS-2', however, increased significantly with time, an observation which is not consistent with the fact that respiration utilizes dry matter reserves. The dry matter proportion, however, is affected not only by respiratory dry matter loss but also, the rate of water loss. Excessive dehydration compared to dry matter conversion in respiration could lead to a net increase in the dry matter (% FW). Despite the thick skin, 'TIS-2' showed severe symptoms of water loss as the roots wilted and this could increase dry matter (% FW). The high moisture loss through the skin is also consistent with the observation that the skin of 'TIS-2' contained more moisture that the flesh tissues.

The economic value of sweetpotato as a food or industrial raw material is the dry matter content; and the dry matter reflects the true biological yield (Teye *et al.*, 2011). Cultivars with high dry weight content in excess of 35% are desired as industrial raw materials (Mok *et al.*, 1997) and are preferred for food in the tropics (Asafu-Agyei, 2010). There is a positive correlation between root dry matter content and starch (Li and Liao, 1983; Woolfe, 1992; Zhang *et al.*, 2001) and tends to be negatively correlated with sugar levels (AVRDC, 1981; Rees *et al.*, 2008). Rees *et al.* (2003) reported that low dry matter content is associated with both wound-healing efficiency and longer shelf-life. Thus the wide variations in the dry matter among sweetpotato cultivars provide the opportunity to select the appropriate cultivar for long term storage or utilization for specific purposes. The current study has established that the dry weight (% FW) is differentially affected by

1-MCP in a cultivar-dependent manner emphasizing the need to evaluate 1-MCP effect of specific crops intended for storage.

4.9.6 Effect of 1-MCP on phenolic compounds

The phenolic compounds in sweetpotato have been measured by different authors as discussed in Section 2.4.6.1. The content and spatial distribution of the individual compounds obtained for the 'Organic' and 'Portuguese derived Covington' in the current study are within the range of values found by other authors in a variety of sweetpotato cultivars (Walter and McCollum, 1979; Truong *et al*., 2004; Teow *et al*., 2007; Truong *et al*., 2007 and Jung *et al.*, 2011). Truong *et al*. (2004) found that the total phenolic contents in the skin and flesh of a 2-week old 'North Carolina Covington' stored at 13-15°C were 150.1 and 57.1 mg ChAE/100g FW, respectively. The value in the skin is comparable to what was found in the current study for 'Organic Covington' (162 mg ChAE /100g FW) though the flesh value obtained was much lower (15.9 mg ChAE /100g FW). The flesh contents of chlorogenic acid found in the present study are, however, comparable to that reported by Truong *et al.* (2007). In some cases, however, for example caffeic acid, the values in the current study were much higher compared to the reported values by Truong et al. (2007). The difference could possibly be due to the growing and storage conditions, among other factors. Estimates of phenolic content in sweetpotato reported by many authors are baseline values, often measured immediately after harvest and sometimes do not reflect the changes during storage. The study reported herein shows that temporal effect on the content of phenolic compounds can be significant and must be taken into account when estimating the concentration of phenolics over a specific storage period. Furthermore, the current study recorded variability of phenolics concentration across the root sections, which highlights the importance of sampling across the entire root length. For example, significantly higher concentrations were found in the proximal than the middle and distal tissues. This finding concurs with what was reported by Jung *et al*. (2011). The lowest concentration was found in the middle sections of the roots where samples are normally taken from. This demonstrates that the concentration can be underestimated if the samples taken are not representative of the entire tissues.

In many crops, 1-MCP has been reported to suppress phenolics biosynthesis (Salveit, 2004; MacLean, *et al*., 2006) in opposition to ethylene effect. MacLean *et al.* (2006) reported that 1-MCP inhibited an increase in chlorogenic acid in pears and the peel tissues of cv. Red Delicious apples during storage. It is not yet known if 1-MCP mediates the distribution of phenolics in sweetpotato and many other crops. Such knowledge could be valuable in unravelling the mechanism of ethylene action in crops. The present study, investigated the spatial effects of 1-MCP in 6 regions; *viz*. the skin and flesh tissues of the proximal, middle and distal sections. To the best of the knowledge of the author, this is the first report regarding 1-MCP effect on the spatial concentrations of phenolic compounds in sweetpotato. A consistent observation was made in Experiment 1 that, although 1-MCP generally reduced phenolics in the consignment 'Organic Covington', the contents in the middle and distal sections of the roots were most suppressed, leaving a significantly higher balance of phenolic compounds in the proximal tissues. To link this result with the incidence of diseases, it was also noted that microbial attack mostly initiated from either the root tip or the root end and therefore, the end-to-end concentration of phenolic compounds may be important in providing resistance against diseases. It may be speculated that the dominance of phenolics in the proximal tissues, as enhanced by 1-MCP, provides a context to explain its mechanistic suppression of ethylene-induced rot symptoms and thus warrants further investigation.

The analysis of the spatial distribution of the individual phenolics in both 'Organic' and 'Portuguese-derived Covington' also identified for the first time that iso-chlorogenic acid A (3,5-di-*O*-caffeoylquinic acid) and chlorogenic acid (5-*O*-caffeoylquinic acid) were the dominant phenolic compounds in the skin and flesh tissues, respectively. Furthermore, these major compounds were highly concentrated in the proximal sections of the roots. They are therefore likely to be related to diseases control as suggested by Friedman (1997). Further research is required to ascertain if this distribution of phenolics in the tissues is unique to the sweetpotato cv. Covington or the pattern is similar in other cultivars.

4.9.7 Effect of 1-MCP on non-structural carbohydrates

There is a wide variability of sugar content among different cultivars of sweetpotato and the value in each case, depends on variables such as the cultivar, origin, age after harvest and storage conditions. Also there are changes in the content of individual sugars during development and storage (La Bonte and Picha, 2000). The content of sugars obtained in the present study are well within the range of values found by Picha (1986), La Bonte and Picha (2000) and other authors. It was noted in Experiments 1 and 2, however, that sucrose was fairly stable but maltose, fructose and glucose declined with time. Wills *et al*. (1998) stated that at ambient temperatures (*ca*. 20-25^oC), starch-sugar balance in sweetpotato is heavily biased towards the accumulation of starch, whilst below 15^oC, there is accumulation of sugars. In line with this expectation, low temperature (<15^oC) storage increased the content of reducing sugars in sweetpotato (Huang *et al.,* 1999). However, Acedo *et al.* (1996) also found an increase in sugar content during storage at ambient temperatures, suggesting that there can still be increased starch hydrolysis at ambient temperatures. In the current study where 'Organic' and 'Portuguese-derived Covington' were stored at exactly 15^oC, the content of the monosaccharides declined. This suggests limited starch hydrolysis at this critical temperature. Also, the relatively constant values for sucrose suggests that invertase activity at 15^oC was low such that there was also little sucrose hydrolysis to monosacharides.

1-MCP did not impose any significant effects on the sugars in any of the 'Covington' roots when stored at 15^oC. This lack of effect may be explained by Nanthachai *et al*. (2007) who found that, no correlation exists between soluble dry matter, including sugars, and 1-MCP absorption. This suggests that when applied on its own, 1-MCP may not elicit any effect on soluble sugars. Data presented by Cheema *et al*. (2013) on sweetpotato cvs. Ibees and Bushbuck stored at 25^oC, however, showed a decline in the monosaccharides by 1-MCP (625 nl L^{-1}). 1-MCP has also been reported to reduce sugar accumulation in potatoes (Foukaraki *et al.* 2011; Prange *et al.* (2005). Possibly, 1-MCP action in reducing sugars is through its blocking of ethylene perception and not by direct involvement in the metabolism of sugars.

4.10 Conclusions

This study was premised on the hypothesis that 1-MCP treatment has no effect on the physiology and biochemistry of sweetpotato during storage. In many respects, the study has corroborated earlier works by other investigators on sweetpotato, especially with regards to findings on decay. As a step to further gain understanding of the bioactivity in sweetpotato roots in relation to the decay phenomenon, it was noted in the current study that 1-MCP influences the spatial distribution of phenolic compounds in the root sections leaving a significantly higher balance of the phenolics in the proximal sections. This may explain the mechanistic effect of 1-MCP against decay as phenolic compounds are known to play a role in curbing the incidence of diseases. Previous studies by other authors associate proximal rot with ethylene. Thus as an ethylene antagonist, 1-MCP may be effective against the ethylene-induced proximal or tip rot. Secondly, isochlorogenic acid A and chlorogenic acid were found to be the most abundant phenolic compounds in the skin and flesh tissues of sweetpotato cv. Covington, respectively. These two compounds were also found in high abundance in the proximal sections. As suggested by Friedman (1997), they may have a role in diseases control, which needs to be investigated. Also it was found that 1-MCP did not affect the content of sugars in sweetpotato cvs. Organic and Portuguese-derived Covington and may therefore not alter the nutritional status when applied to improve storage. It was also noted in this study that storage of sweetpotato at 15°C was effective in inhibiting sprouting suggesting that, the application of 1-MCP as a means to forestall sprouting may only be beneficial and better evaluated if the roots are to be stored in higher temperature environments such as the tropics.

CHAPTER FIVE: EFFECT OF DIFFERENT TIMINGS OF EXOGENOUS ETHYLENE SUPPLEMENTATION ON THE PHYSIOLOGY AND BIOCHEMISTRY OF SWEETPOTATO ROOTS DURING STORAGE

5.1 Introduction

The phytohormone ethylene is well-known to be involved in the regulation of many physiological and biochemical processes in plant tissues that affect the quality of horticultural produce (Lin *et al.,* 2009). Consequently, many postharvest studies to improve the storage of certain crops are based on understanding the role of ethylene in mediating these processes. Current understanding of the biological activity of ethylene suggests that it may elicit variable (sometimes contrasting) responses in plant tissues depending on a matrix of factors, including the type of tissue, growth stage, concentration and timing of exposure to the tissue. These distinctive effects of ethylene merit more precise studies into its influence on various aspects of plant metabolism (Huelin and Barker, 1939). Ethylene effects on the quality of climacteric fresh produce is now well established because of its great agronomic importance (Bleecker and Kende, 2000). Currently, studies on the effects of ethylene on non-climacteric produce are mainly focused on understanding its role in mediating dormancy, sprout growth and senescence mechanisms in potatoes and onions. Findings relating to the continuous ethylene supplementation on potatoes and onions suggest that it could be an efficient tool to supress sprouting in non-climacteric produce without promoting adverse physiological disorders (Prange *et al.,* 1998; Daniels-Lake *et al*., 2005; Downes *et al*., 2010; Cools *et al*., 2011; Foukaraki *et al*., 2011). Based on these findings, and given its convenience as a natural plant hormone, there has been commercial interest in the use of ethylene for extending the storage life of sweetpotato (Cheema *et al*., 2013). However, the optimal timing of ethylene supplementation and the detailed effects on the physiological and biochemical profiles in sweetpotato is uncertain.

Currently in the UK, two ethylene supplementation options have been proposed by two companies which supply equipment for the generation of ethylene. The company, BioFresh, recommends that ethylene should be applied prior to dormancy break while the

other company, Restrain, suggests ethylene supplementation at the time of dormancy break (British Potato Council, 2006). In the present study, sweetpotato roots were subjected to four concerted regimes of ethylene (10 µl L^{-1}) treatment *viz*. (1) continuous ethylene supplementation throughout the storage; (2) truncated ethylene supplementation after dormancy break, followed by storage in air; (3) post-dormant ethylene supplementation after previous air storage and (4) continuous air storage as a control check. The effects of the treatments on the physiological quality attributes as well as the spatial profiles of the non-structural sugars and individual phenolic compounds in the roots during the storage were assessed.

5.2 Aim and objectives of the study

5.2.1 Aim

The aim of this study was to determine the effects of timing of exogenous ethylene supplementation on sweetpotato.

5.2.2 Specific objectives

1. To design a laboratory-scale ethylene supplementation apparatus for parallel flushing of multi-chamber storage boxes.
2. To determine how different timings of exogenous ethylene supplementation affect the respiration rate, carbohydrate and phenolics profiles, the dormancy phase, initiation and progression of sprout growth, weight and decay in sweetpotato roots during storage.

5.3 Hypothesis

5.3.1 Null Hypothesis

It was hypothesised that:

HO: Different timings of ethylene supplementation impose no significant changes in the biochemical and physiological quality characteristics of sweetpotato.

5.3.2 Alternate Hypothesis

HA: Different timings of ethylene supplementation impose significant changes on the biochemical and physiological quality characteristics of sweetpotato.

5.4 Experimental design

The hypothesis was tested by investigating three consignments of sweetpotato cv. North Carolina Covington in three separate experiments. The consignments were designated as 'NCCov I', 'NCCov II' and 'NCCov III', respectively. Experiment 4, involving studies on 'NCCov I' was conducted as a completely randomised design. The roots were divided into two lots and stored in either continuous ethylene (10 µl L^{-1}) or continuous air throughout the storage. Each lot was sub-divided into three and placed in treatment boxes. Experiments 5 and 6 involving 'NCCov II' and 'NCCov III' were nested factorial designs as described by Foukaraki (2012) with slight modifications. Each consignment was divided and stored under four different regimes of exogenous ethylene supplementation *viz.* 1) continuous ethylene at 10 µl L^{-1} throughout the storage (EE); 2) initial storage in 10 µl L^{-1} ethylene, followed by transfer into air storage after dormancy break (EA); 3) initial air storage, followed by storage in 10 µl L^{-1} ethylene after dormancy break (AE); and 4) control, made up of storage in air throughout (AA). Dormancy break was defined as the time point when approximately 10% of the roots in the control treatment had sprouts ≥ 1mm. The treatments were carried out in triplicate boxes (experimental units), containing the pseudo-replicate roots. The experimental layout is summarised in **Figure 5.1.**

For 'NCCov I', the swap treatments were not carried out (continuous ethylene, EE or continuous air, AA only) and assessments were carried out for only root weight loss, sprout growth, decay, respiration rate and dry weight. Swap treatments were done for 'NCCov II' and 'NCCov III' and the biochemical assays (respiration, dry weight, sugars and phenolics) were carried out in addition to the physiological profiling. Each box of 'NCCov II' and 'NCCov III' contained two sub-samples of sweetpotato roots – non-destructive and destructive sub-samples. The non-destructive sub-samples were numbered individually for the physiological profiling whilst the destructive sub-samples

were randomly sampled for spatial analysis of sugars and phenolic compounds at each out-turn.

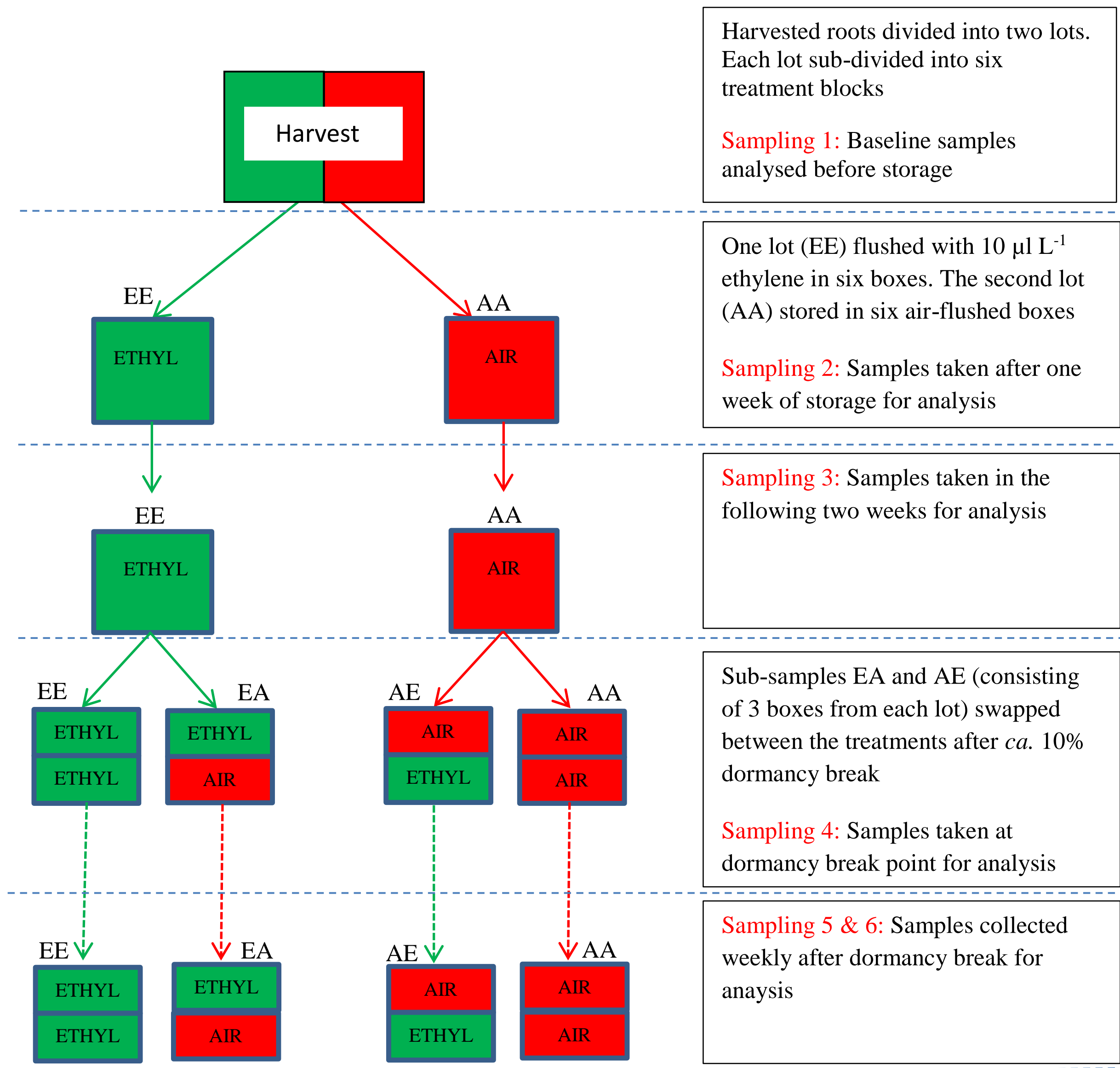


Figure 5.1 Design layout of ethylene experiments

5.4.1 Design of a laboratory-scale ethylene supplementation apparatus

A laboratory-scale apparatus for parallel flushing of the storage boxes with ethylene was designed as described in Section 3.4.

5.5 Experiment Four: Effects of exogenous ethylene supplementation on the physiology and biochemistry of 'NCCov I' (Jim Summelin Covington)

5.5.1 Sourcing and conditioning of the plant material

In Experiment 4, the sweetpotato consignment 'NCCov I' was sourced through Barfoots of Botley, UK. It was originally grown by FARMPAK in Jim Summelin Farms, North Carolina, USA, and harvested in October 2011, followed by postharvest curing (30°C, 90% RH, 7 days), packaging and shipment to Barfoots of Botley Ltd. At Barfoots, it was stored at 14° C as a commercial stock. Samples were taken from the commercial storage and delivered to Cranfield University in July 2012 for the experiment. Thus the NCCov I was *ca.* 8 months old on delivery for the experiment.

5.5.2 Specific details relating to NCCov I in Experiment 4

The experimental design and layout are as summarized in **Figures 5.1 – 5.2**. The storage temperature was 25°C. The analytes in the experiment were measured with the methods described under the relevant sections (Sections 3.3 to 3.5) in Chapter 3. Each storage box contained 15 non-destructive roots and numbered individually for repeated measurements of weight, sprout growth, and decay. Sprout growth was measured in three terms: (1) percentage of roots that sprouted; (2) mean number of sprouted eyes per root; and (3) maximum sprout length per root. The 15 non-destructive roots (pseudo-reps) were evaluated at irregular intervals over 4 weeks (48 days). There were also 30 destructive roots selected periodically at the rate of four roots per treatment box (n = 12 per treatment) for respiration and dry weight analysis (**Table 5.1**).

Table 5.1 Evaluation schedules for physiological and biochemical assessments of 'NCCov I'

No. of days	Physiological	Biochemical
0	√	√
13	√	
14		√
21	√	
28	√	√
29	√	
35	√	√
42		√
48	√	

5.6 Experiments Five and Six: Effect of different timings of ethylene supplementation on the physiology and biochemistry of 'NCCov II' and 'NCCov III' (Anderson East Farm Covington)

The Covington consignments 'NCCov II' and 'NCCov III' were part of the same harvest but supplied at two different times for the analysis. The crop was grown in a commercial field (Anderson East Farm) by FARMPAK, USA, for supply to Barfoots of Botley Ltd., for onward distribution to UK supermarkets. It was harvested in October 2012. Special arrangements were made for two distinct supplies at different times for the experiments. 'NCCov II' was delivered 10 days after harvest to Cranfield University via Barfoots. 'NCCov III' was held in storage at Barfoots (14°C) pending delivery to Cranfield University in February 2013. Thus there was *ca.* 5 months storage interval between the time of analysis of 'NCCov II' and 'NCCov III'.

5.6.1 Specific details relating to 'NCCov II' (Experiment 5)

The experimental design is as shown in **Figures 5.1 – 5.2**. The storage temperature was 25°C. The analytes in the experiment were also measured with the methods described under the relevant sections (Sections 3.3 to 3.5) in Chapter 3. Each treatment box contained 7 non-destructive roots numbered individually for repeated measurements of weight, sprout growth, and decay (n = 21 per treatment and outturn for physiological

assessment). There were also 15 destructive roots per treatment box, three of which were selected per outturn (n=9) for the respiration and downstream biochemical assays. Measurement of sprout growth was as described in Section 5.5.2. The sampling schedules are summarised in Table 5.2.

Table 5.2 Evaluation schedules for physiological and biochemical assessments of 'NCCov II'

No. of days	Physiological	Biochemical
0	√	√
7		√
8	√	
14		√
15	√	
22	√	
23		√
30	√	
38	√	
48	√	√
49		√

5.6.2 Specific details relating to 'NCCov III' (Experiment 6)

The experimental design was as summarized in **Figures 5.1 - 5.2**. The storage temperature was also 25°C and the analytes in the experiment were measured with the methods described under the relevant sections (Sections 3.3 to 3.5) in Chapter 3. Each treatment box contained 10 non-destructive roots numbered individually for repeated measurements of weight, sprout growth, and decay (n= 30). There were also 25 destructive roots in each treatment box, three of which were randomly selected per outturn (n=9) for respiration and biochemical assays. Sprout growth was as described in Section 5.5.2. The sampling schedules are summarised in **Table 5.3**.

Table 5.3 Evaluation schedules for physiological and biochemical assessments of 'NCCov III'

No. of days	Physiological	Biochemical
0	√	√
8	√	
9		√
14	√	√
21	√	√
28	√	
32		√

5.7 Results for the gas flow parameter settings and treatment effectiveness achieved with the ethylene-dosing apparatus

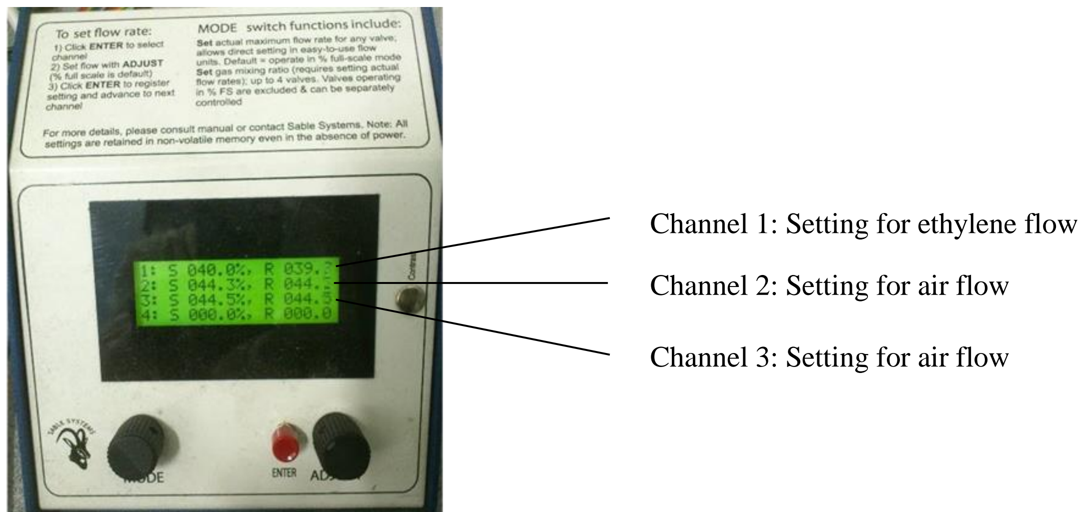
The ethylene supplementation apparatus permitted the concentration (K) and the gas flow rate to be varied using the model:

$$K = \{(x\%\ of\ 0.03)/(y\%\ of\ 13.5)\} * 5000$$

Where $x\%$ is the percentage of the calibrated full-scale (FS) setting (0.03 L/min) of the ethylene flow control valve.

$y\%$ is the percentage of the calibrated full-scale (FS) setting (13.5 L/min) of the air flow control valve.

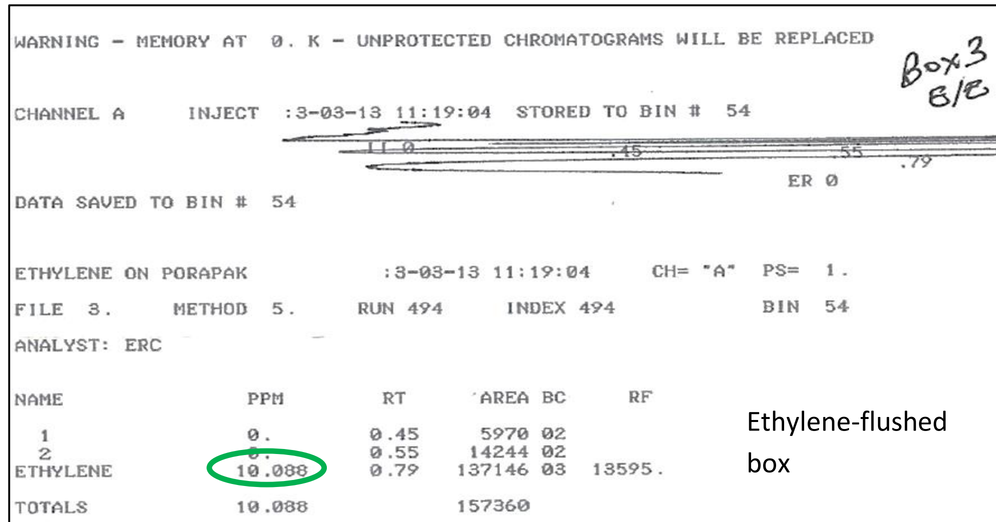
An excel program was developed to facilitate the variation of the ethylene concentration and the corresponding gas flow rates as shown in **Figure 5.2** below:



Flow channel	Valve	Max flow (FS)	% of FS	Flow (L/min)			
1	Ethylene	0.03	40.0%	0.012	Flow ratio: Channel 1/Channel 2	0.00200652	
2	Air	13.5	44.3%	5.9805	K = [(Flow ratio)*5000]	10.0	ppm
3	Air	13.5	44.50%	6.0075	Total flow rate:	5.9925	L/min

Figure 5.2 Excel program for gas concentration and flow rates in storage boxes. Gas flow through channels 1 and 2 are mixed to achieve the desired ethylene concentration (K) according to the flow settings, whilst air flow rate through channel 3 is set for flushing the control boxes.

The ethylene concentration in the treatment boxes was measured with the GC as described in Section 3.4. Typical chromatograms of the ethylene- and air-flushed boxes are shown in **Figure 5.3**. A mean ethylene concentration of 10.1 µl L^{-1} was obtained for the exhaust gas of the ethylene-flushed boxes whilst 0.2 µl L^{-1} was recorded for the air-treated boxes (**Figure 5.4**). The 0.2 µl L^{-1} recorded in the control boxes was as a result of endogenous ethylene production by the roots themselves. When opened, it took an average of 2-3 h for the concentration in each box to build up to 10 µl L^{-1}. The mean relative humidity and temperature in the boxes, as measured with the Tiny Tag data logger, were 60-95% and 24.8 °C, respectively **(Figure 5.5**). The CO_2 concentrations in the headspace were *ca*. 0.1 % (**Figure 5.6**).



WARNING – MEMORY AT 0. K – UNPROTECTED CHROMATOGRAMS WILL BE REPLACED

Box 3 E/E

CHANNEL A INJECT :3-03-13 11:19:04 STORED TO BIN # 54

.45 .55 .79

ER 0

DATA SAVED TO BIN # 54

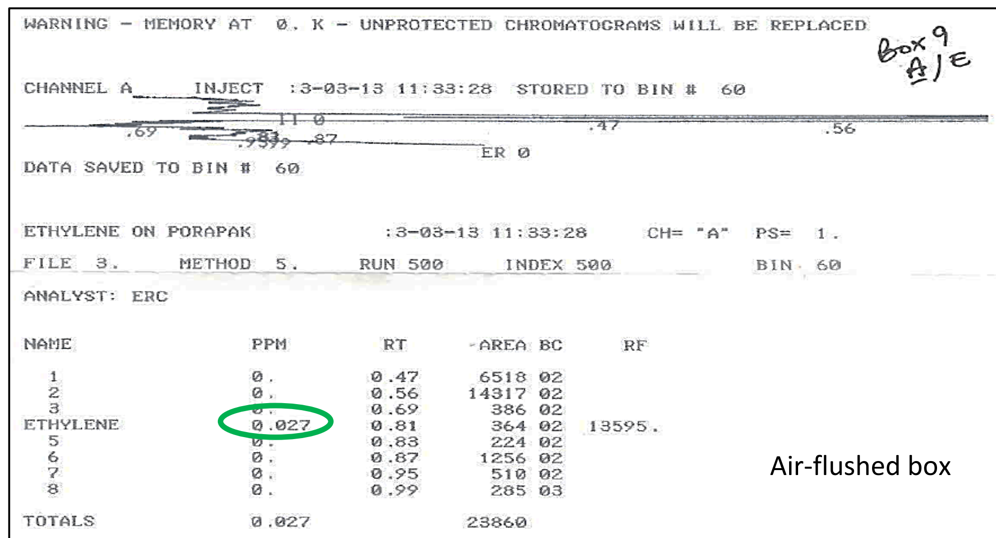
ETHYLENE ON PORAPAK :3-03-13 11:19:04 CH= "A" PS= 1.

FILE 3. METHOD 5. RUN 494 INDEX 494 BIN 54

ANALYST: ERC

NAME	PPM	RT	AREA	BC	RF
1	0.	0.45	5970	02	
2	0.	0.55	14244	02	
ETHYLENE	10.088	0.79	137146	03	13595.
TOTALS	10.088		157360		

Ethylene-flushed box



WARNING – MEMORY AT 0. K – UNPROTECTED CHROMATOGRAMS WILL BE REPLACED

Box 9 A/E

CHANNEL A INJECT :3-03-13 11:33:28 STORED TO BIN # 60

.69 .47 .56 .87

ER 0

DATA SAVED TO BIN # 60

ETHYLENE ON PORAPAK :3-03-13 11:33:28 CH= "A" PS= 1.

FILE 3. METHOD 5. RUN 500 INDEX 500 BIN 60

ANALYST: ERC

NAME	PPM	RT	AREA	BC	RF
1	0.	0.47	6518	02	
2	0.	0.56	14317	02	
3	0.	0.69	386	02	
ETHYLENE	0.027	0.81	364	02	13595.
5	0.	0.83	224	02	
6	0.	0.87	1256	02	
7	0.	0.95	510	02	
8	0.	0.99	285	03	
TOTALS	0.027		23860		

Air-flushed box

Figure 5.3 Typical GC chromatograms for ethylene- and air-flushed boxes

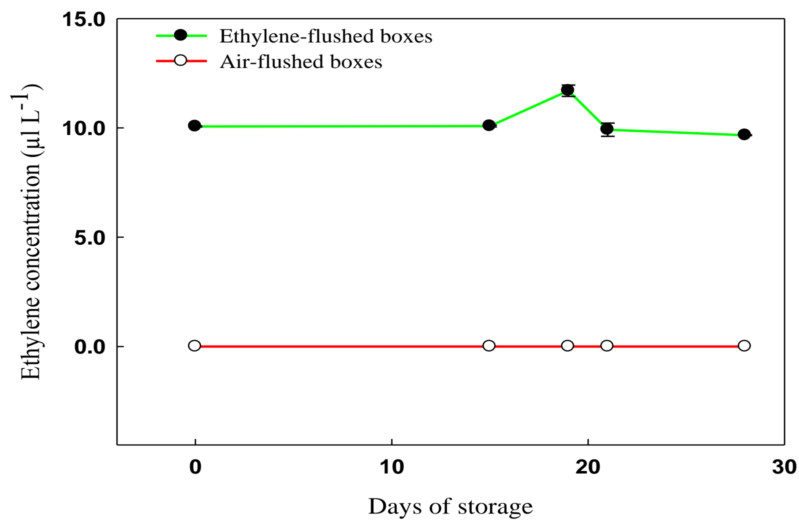


Figure 5.4 Typical variation of ethylene concentration in a storage box. Each data point is the mean for six replicate boxes. The bars shown are standard errors of the means.

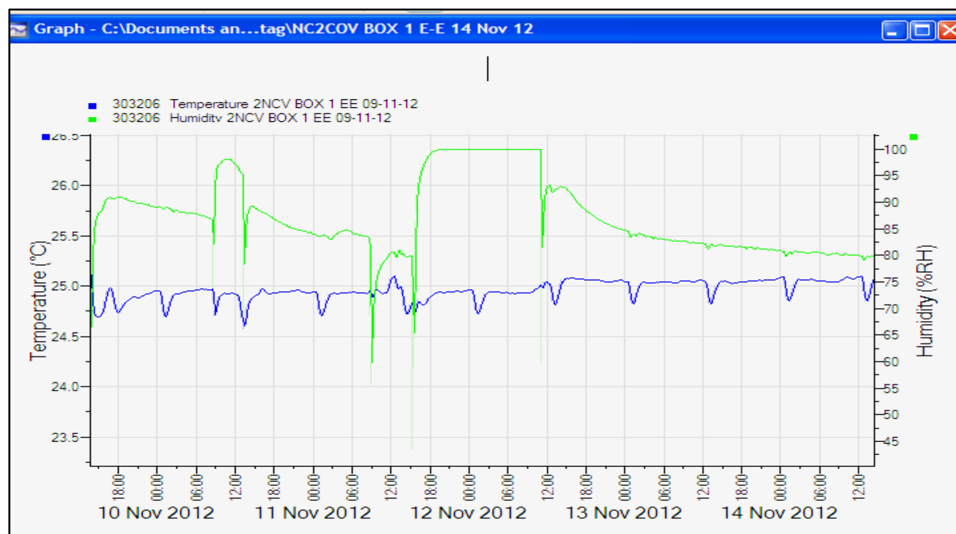


Figure 5.5 Typical temperature and relative humidity variations in the storage boxes as measured over 5 continuous days with a Tiny Tag data logger.

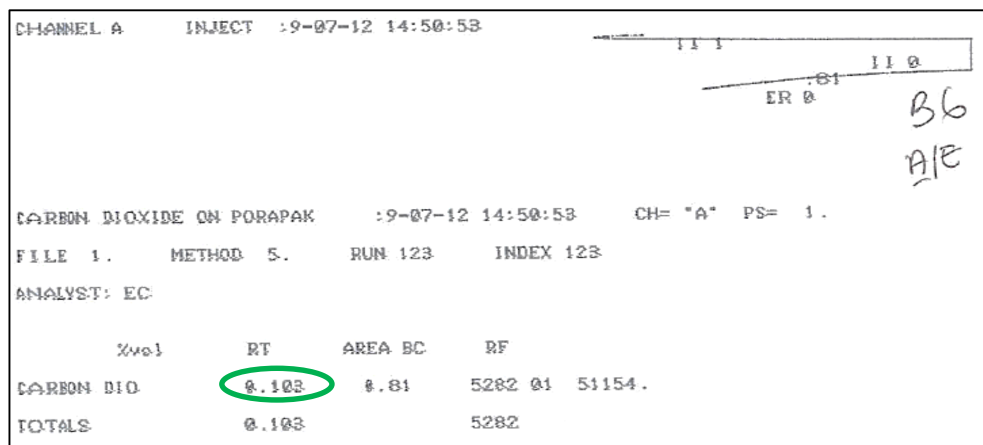


Figure 5.6 Typical GC chromatogram for CO_2 concentration in a storage box

5.8 Results for Experiments Four, Five and Six

5.8.1 Physiological variables

5.8.1.1 Effect of ethylene supplementation on respiration rate

In all the Covington consignments studied in Experiments 4, 5 and 6, the respiration rate of roots stored in continuous ethylene were significantly elevated by *ca.* 1.5 - 2.0-fold compared to those stored in air (p-value < 0.01). The ethylene-treated roots showed a characteristic respiratory response to ethylene with an initial transient, climacteric-like increase to a peak value (*ca.* from 13.8 to 27.0 mL CO_2 kg^{-1} h^{-1} for 'NCCov I'; from 16.8 to 26.5 mL CO_2 kg^{-1} h^{-1} for 'NCCov II'; and 16.8 to 36.2 mL CO_2 kg^{-1} h^{-1} for 'NCCov III'). The duration of the "climacteric-like rise" depended on the age of the crop, being relatively short (*ca.* 7 days) for the fresher consignment 'NCCov II' whilst for the previously stored consignments 'NCCov I' and 'NCCov III', it lasted between 20 and 30 days, respectively. The peaks were followed by typical non-climacteric decline (**Figure 5.7**).

In the air storage, the respiration of the older roots, 'NCCov I' and 'NCCov III', remained fairly stable (mean values 15.8 and 19.4 mL CO_2 kg^{-1} h^{-1}, respectively) whilst that of the fresher roots, 'NCCov II', dropped from the baseline respiration of 16.8 to 6.4 mL CO_2 kg^{-1} h^{-1} along a characteristic non-climacteric curve. There was no noticeable change in

the respiratory pattern in 'NCCov I' and 'NCCov II' at dormancy break. In the 'NCCov III', however, dormancy break was coincident with accentuated increase in the respiration rate of the ethylene treated roots to a peak value. When the ethylene supplementation in 'NCCov II' was truncated after dormancy break, and sub-samples of the roots were immediately transferred into air, there was a significant drop in the respiration rate to the equivalent level observed in continuous air. Conversely, the respiration rate of roots transferred from air into ethylene increased reciprocally, reaching a similar level as for roots held under continuous ethylene within *ca*. 28 days. An identical, but transient effect was observed in 'NCCov III'. Thus, the immediate response of the roots to the transition from ethylene into air, or vice-versa, was the swapping of their respiration status.

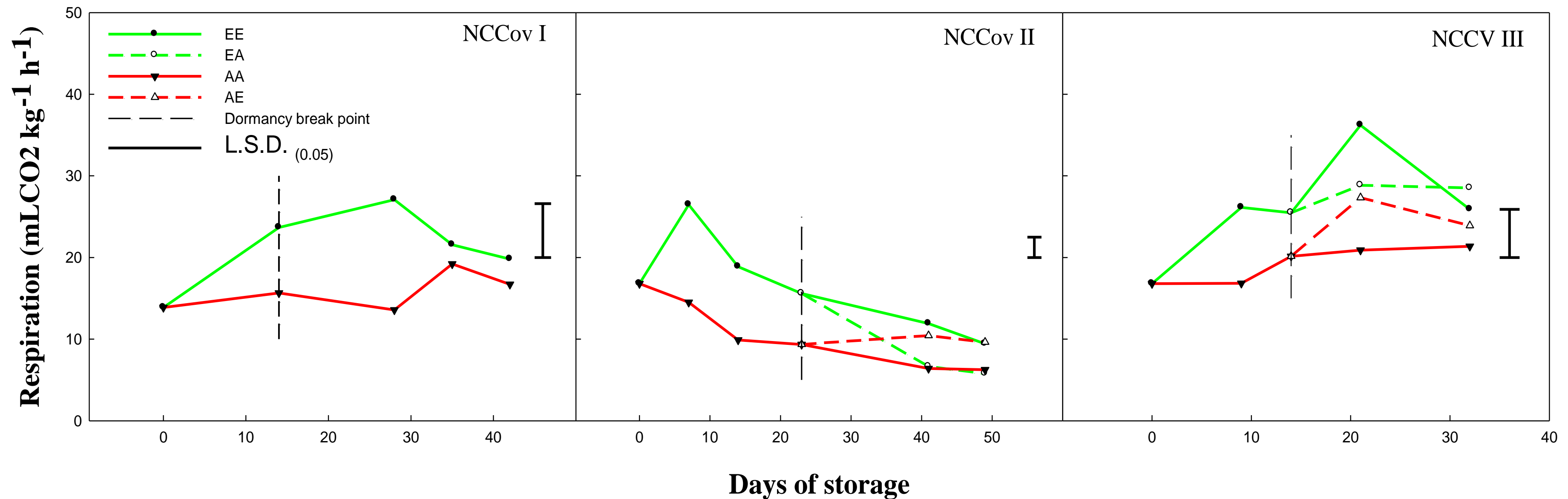


Figure 5.7 The respiration curves for the 'Covington' consignments 'NCCov I', 'NCCov II' and 'NCCov III' in continuous ethylene (EE); ethylene, followed by air (EA); continuous air (AA); or air, followed by ethylene (AE). Each data point for 'NCCov I' is the mean of three boxes consisting of 4 roots per box (n=12). Each data point for 'NCCov II' and 'NCCov III' before dormancy break is the mean of six boxes consisting of 3 roots per box (n=18). Each data point for 'NCCov II' and 'NCCov III' after dormancy break is the mean of three boxes consisting of 3 roots per box (n=9). L.S.D. $_{(0.05)}$ bars are shown. The legend applies to all the graphs. Analysis of Variance Tables (ANOVA) are in Appendix B, Tables B1-B3.

5.8.1.2 Effect of ethylene supplementation on weight loss

Results for the physiological weight loss (measured as perecentage of initial stored weight) in the roots as affected by the respective treatments is shown in **Figure 5.8**. In all the 'Covington' consignments studied, roots stored in continuous ethylene incurred significantly higher weight loss than those stored in air (p-value < 0.01). There was a significant benefit in terms of weight when 'NCCov II' and 'NCCov III' roots were treated with ethylene after dormancy break compared to those roots treated continuously with ethylene from the beginning of storage. The older consignments 'NCCov I' and 'NCCov III' lost weight at a faster rate than the freshly harvested consignment 'NCCov II'.

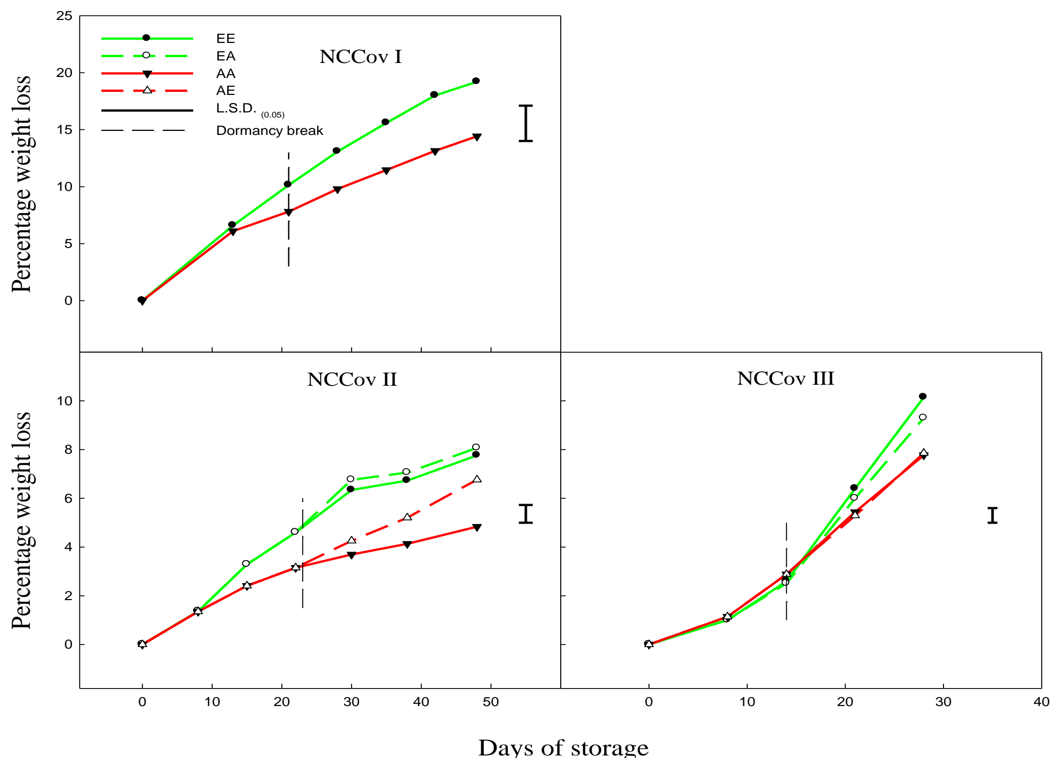


Figure 5.8 Percentage weight loss of the 'Covington' consignments 'NCCov I', 'NCCov II and 'NCCov III' in continuous ethylene (EE); ethylene, followed by air (EA); continuous air (AA); or air, followed by ethylene (AE). Each data point of 'NCCov I' is the mean of three treatment boxes containing 15 roots per box (n=45). Each data point of 'NCCov II' and 'NCCOv III' before dormancy break is the mean of six treatment boxes containing 7 roots per box for 'NCCov II' (n=42) and 10 for 'NCCov III' (n=60). Each data point of 'NCCov II' and 'NCCOv III' after dormancy break is the mean of three treatment boxes containing 7 roots per box for 'NCCov II' (n=21) and 10 for 'NCCov III' (n=30). L.S.D. $_{(0.05)}$ bars are shown. The legend applies to all graphs. ANOVA tables are in Appendix B: Tables B4-B6.

5.8.1.3 Effect of ethylene supplementation on dry weight

The dry weight as a proportion of the fresh weight (% FW) depends on the relative rate at which roots lose moisture or dry matter through respiration and transpiration. The dry weight (% FW) increases if the tissues lose more water than they lose dry matter and vice-versa. The mean dry weight of 'NCCov I' (baseline values of the skin and flesh: 19.0 and 18.1 % FW, respectively) fluctuated throughout storage within the range 18.3 -19.2 % FW without any consistently significant temporal effect (Mean in air and ethylene storage: 18.7 and 18.9 % FW, respectively) (**Figure 5.9**). Ethylene supplementation and the resulting elevation in root respiration and sprout inhibition did not significantly affect the proportion of the dry matter to the fresh weight.

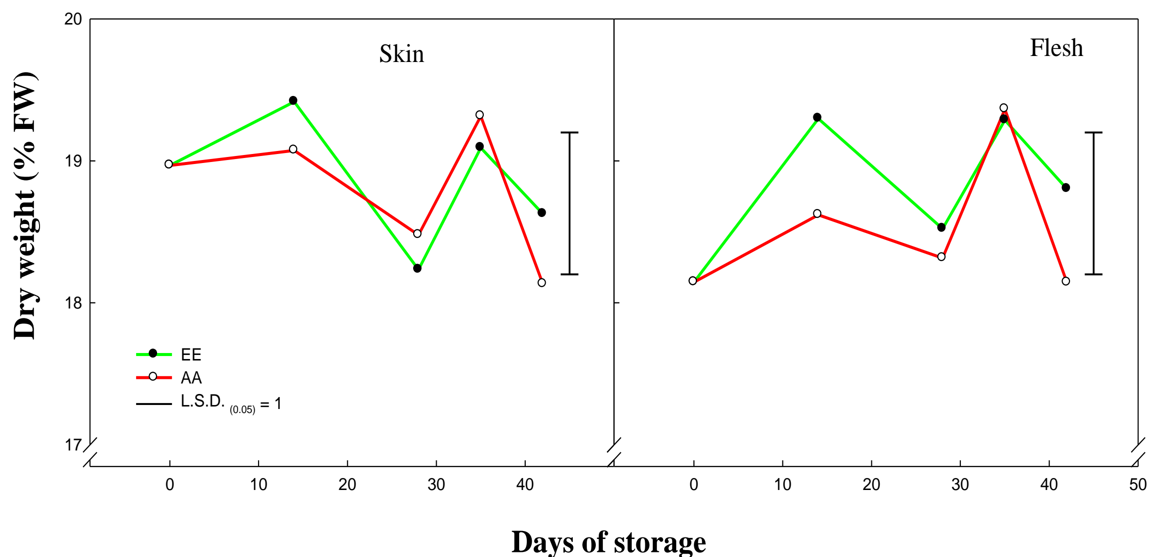


Figure 5.9 Dry weight (% FW) of the Covington consignment 'NCCov I' in ethylene or air. Each data point is the mean of three treatment boxes consisting of 4 roots per box (n=12). L.S.D. bars are shown. The ANOVA table is in Appendix B7.

For the freshly harvested consignment 'NCCov II', however, continuous ethylene supplementation resulted in a significant drop in the dry weight proportion of the fresh weight compared to both the storage in air and the post-dormant swap from air into ethylene (**Figure 5.10**). Dormancy break was associated with a further drop in the dry

weight proportion of the ethylene treated roots. The dry weight proportion of the skin (mean value: 22.6 % FW) was much higher than that of the flesh tissues (mean value: 22.1 % FW). Also a significantly higher proportion of the dry weight was found in the proximal (mean 23.4% FW) than the distal (mean 21.3% FW) section.

No significant difference in the dry weight (% FW) was found between the ethylene-treated and control roots of 'NCCov III' (**Figure 5.11**). Likewise, the dry weight was not significantly different between the continuous ethylene and post-dormant transfer of roots from air into ethylene. The dry weight proportion in the control skin tissues followed a somewhat parabolic curve, falling to a minimum in about a week after dormancy break and rising thereafter. The corresponding proximal skin dry weight proportion (% FW) curve for the ethylene-treated roots was a W-shape, with dormancy break being coincident with the transient increase in the middle. The swap treatments did not significantly change the dry weight proportions in the tissues. As opposed to 'NCCov II', the dry weight proportion (% FW) in the distal tissues of 'NCCov III' was significantly higher than the proximal tissues.

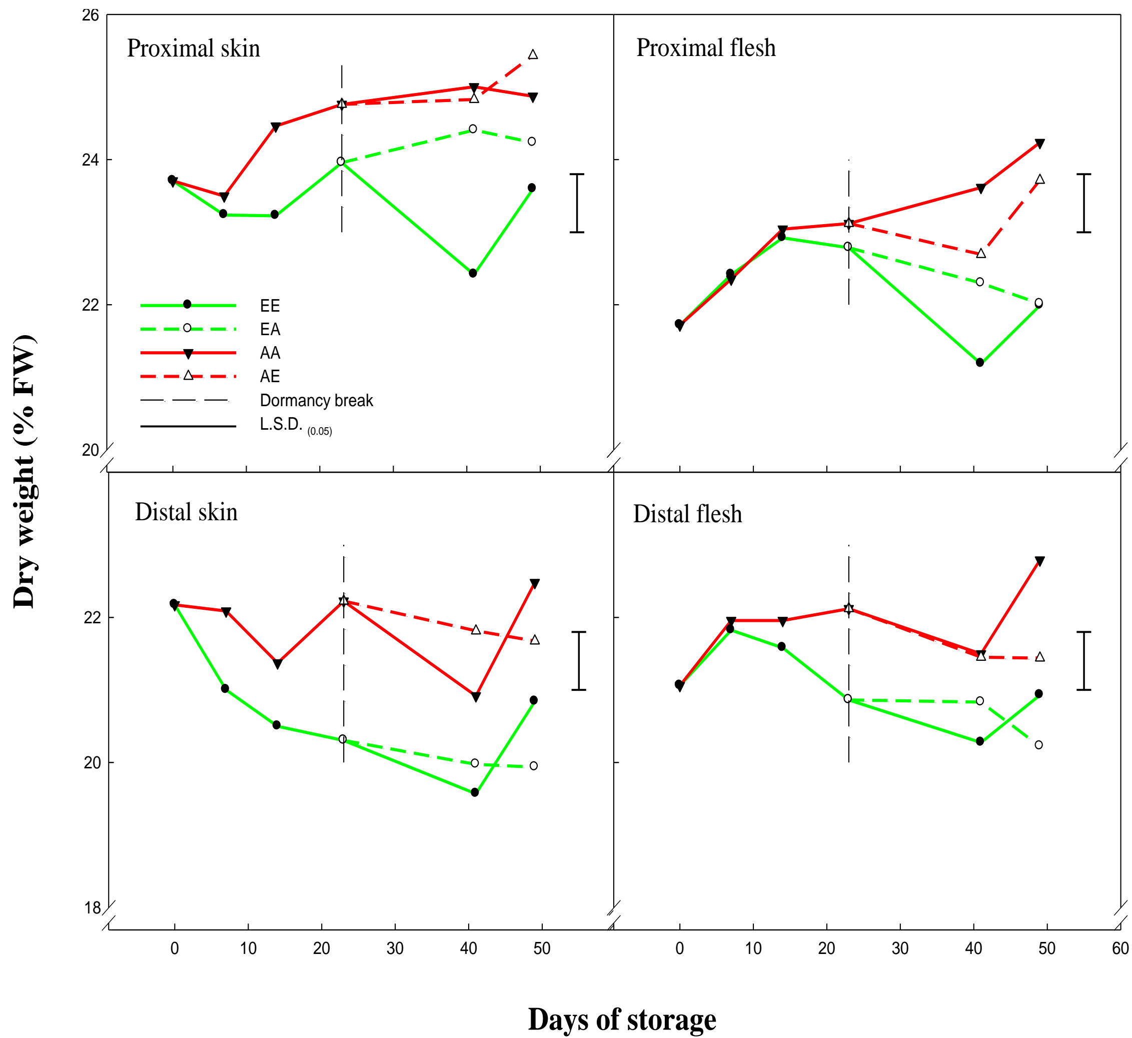


Figure 5.10 Dry weight (% FW) of the 'Covington' consignment 'NCCov II' ethylene or air. Each data point before dormancy break is the mean of six treatment boxes consisting of 3 roots per box (n=18). Each data point after dormancy break is the mean of three treatment boxes containing 3 roots per box (n=9). L.S.D. $_{(0.05)}$ bars are shown. The legend applies to all graphs. ANOVA table is in Appendix B: Table B8.

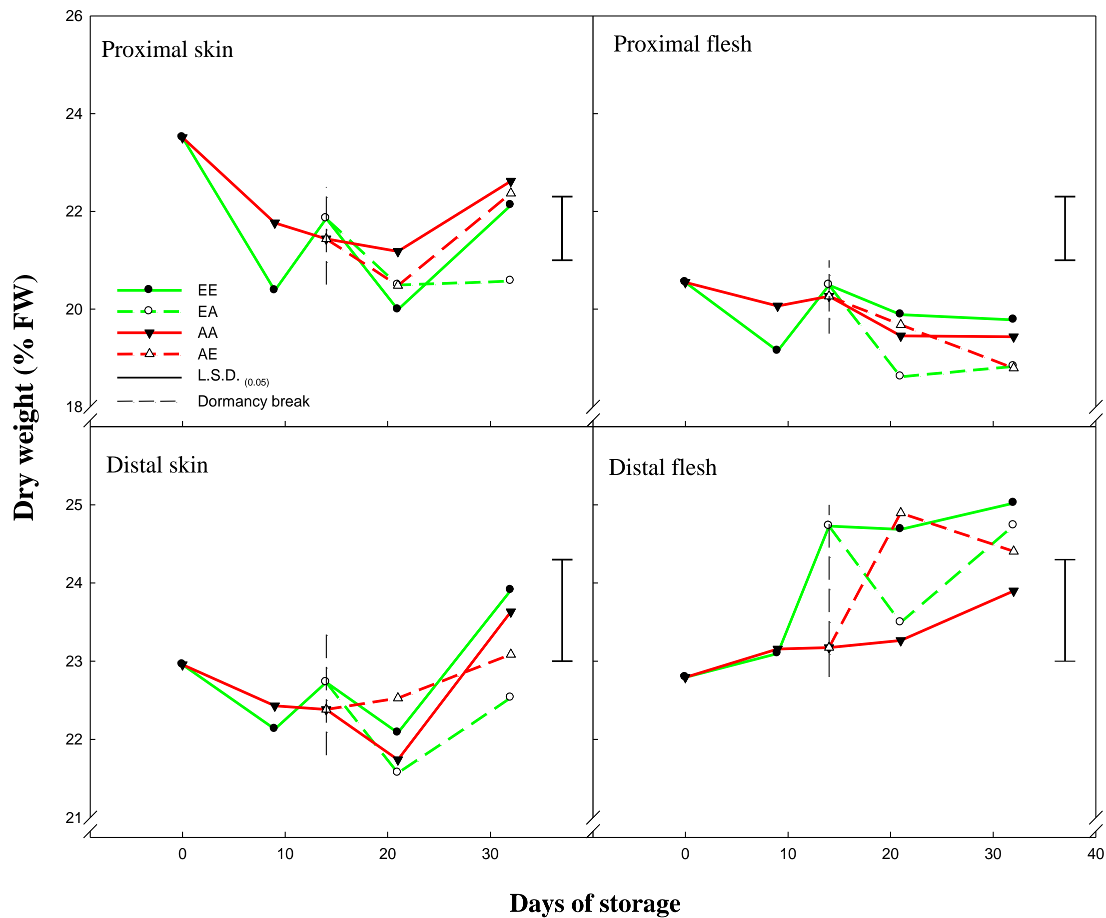


Figure 5.11 Dry weight (% FW) in the 'Covington' consignment 'NCCov III' in ethylene or air. Each data point before dormancy break is the mean of six treatment boxes consisting of 3 roots per box (n=18). Each data point after dormancy break is the mean of three treatment boxes consisting 3 roots per box (n=9). L.S.D. $_{(0.05)}$ bars are shown. The legend applies to all graphs. ANOVA table is in Appendix B: Table B9.

5.8.1.4 Effect of ethylene supplementation on the incidence of root decay

No incidence of decay was found in the freshly harvested produce 'NCCov II' within the evaluation period. On the other hand, the older roots, 'NCCov I' and 'NCCov III' were severely infected by diseases to various degrees depending on the treatment and the cultivar (**Figure 5.12**). Absolute values showed that in both consignments, ethylene supplementation increased the incidence of root decay over the control; albeit the difference was not statistically significant in 'NCCov I' (p-value > 0.05). In 'NCCov III',

however, decay in the continuous ethylene-treated roots (EE) was found to be *ca*. 2-fold greater than the control roots (AA) and 2.5-fold greater than the post-dormant ethylene supplementation (AE). In 'NCCov I', the earliest incidence of decay was after three weeks of storage whilst in 'NCCov III', the roots started decaying just after one week. The ethylene-induced decay was predominantly observed at the proximal ends (tip-rot) although in some roots the decay affected the other parts as well (**Figure 5.13**).

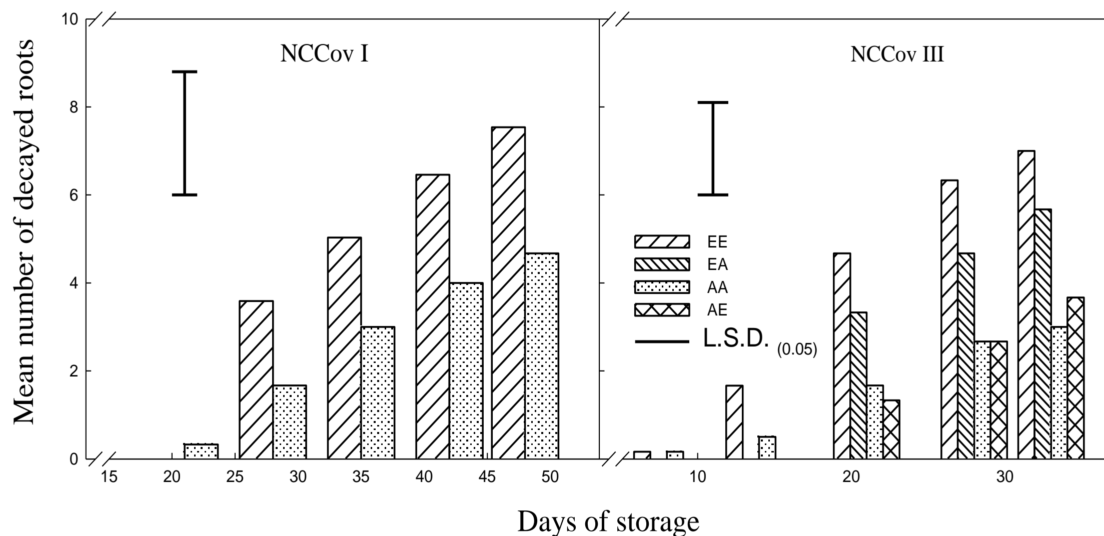


Figure 5.12 Decay in the 'Covington' consignments 'NCCov I' and 'NCCov III' stored in continuous ethylene (EE); ethylene, followed by air (EA); continuous air (AA); or air, followed by ethylene (AE). Each data point of 'NCCov I' is the mean of three treatment boxes containing 15 roots per box (n=45). Each data point of 'NCCov III' before dormancy break is the mean of six treatment boxes containing 10 roots each (n=60). Each data point of 'NCCov III' after dormancy break is the mean of three treatment boxes containing 10 roots (n=30). L.S.D. $_{(0.05)}$ bars are shown. The legend applies to all graphs. ANOVA tables are in Appendix B: Tables B10-B11.

A B

Figure 5.13 Proximal decay (tip rot) in 'NCCov I' (A) and 'NCCov III' (B) in ethylene treatment

5.8.1.5 Effects of ethylene supplementation on sprout growth

Dormancy break (measured at *ca.* 10% of roots in air having ≥1 mm sprouts) occurred after approximately 21, 23 and 14 days in 'NCCov I', 'NCCov II' and 'NCCov III', respectively. Sprout development in each treatment was measured in three terms: (a) the percentage of roots that sprouted; (b) the mean number of sprouts per root and (c) the maximum sprout length per root (**Table 5.4** and **Figure 5.14**). Contrary to expectations from the elevated respiration, both the continuous ethylene storage and the post-dormant swap from air into ethylene significantly suppressed sprout growth (**Figure 5.14 – 5.15**). Compared to air storage, continuous ethylene reduced the percentage of sprouted roots by *ca.* 14-fold; the number of sprouts per root by *ca.* 3-fold and the sprout length by *ca.* 38-fold. At the end of the respective storage periods, the percentage sprouted roots (air:ethylene) in the cultivars were: 'NCCov I' (49%:2.5%); 'NCCov II' (85.7%:28.3%); 'NCCov III' (60%:3.1%), respectively. Compared to air storage, continuous ethylene treatment reduced the number of sprouted eyes per root by *ca.* 20-fold, 4.6-fold and over 100-fold in 'NCCov I', 'NCCov II' and 'NCCov III', respectively. After dormancy break, sprout growth in roots stored in continuous ethylene was retarded, with the maximum

sprout length only reaching ≤ 1.0 mm in ‘NCCov I’, ≤ 8.0 mm in ‘NCCov II’ and ≤ 3.0 mm in ‘NCCov III’, respectively (**Table 5.1**). The corresponding maximum sprout length in the air treated roots were 96 mm, 18.6 mm and 47.5 mm for ‘NCCov I’, ‘NCCov II’ and ‘NCCov III’, respectively. There was a significant benefit in the post-dormant transfer of roots from air into ethylene as it achieved an equivalent effect as that of continuous ethylene supplementation in terms of inhibiting further sprout emergence or the elongation of previous sprouts. Although more roots sprouted in the post-dormant ethylene treatment compared to the continuous ethylene, both the number of sprouted eyes per roots and sprout elongation were suppressed in equal measure. Conversely, truncating the ethylene supplementation after dormancy break, and transferring the roots into air exacerbated sprout growth, triggering a large number of long sprouts.

Table 5.4 Range of sprout length (mm) in ‘Covington’ consignments ‘NCCov I’, ‘NCCov II’ and ‘NCCov III’ during storage in ethylene or air

‘NCCov I’

Treatment	Day	0	13	21	28	35	42	53
EE		0	0	≤1	≤1	≤1	≤1	≤1
AA		0	0	1 - 7	1-50	1-85	1-105	1-110

‘NCCov II’

Treatment	Day	0	8	15	22	30	38	48
EE		0	0	≤1	≤1	0.5-2.0	1-8	1-8
EA		-	-	-	-	0.5-18	1-45	1-70
AA		0	0	≤1	1-2	1-8	1-14	1-25
AE		-	-	-	-	1-6	1-6	1-10

‘NCCov III’

Treatment	Day	0	8	14	21	28	33
EE		0	≤1	≤1	1-5	1-6	1-3
EA		-	-	-	1-25	1-60	1-80
AA		0	≤1	1-4	1-35	1-54	1-58
AE		-	-	-	1-12	1-12	1-17

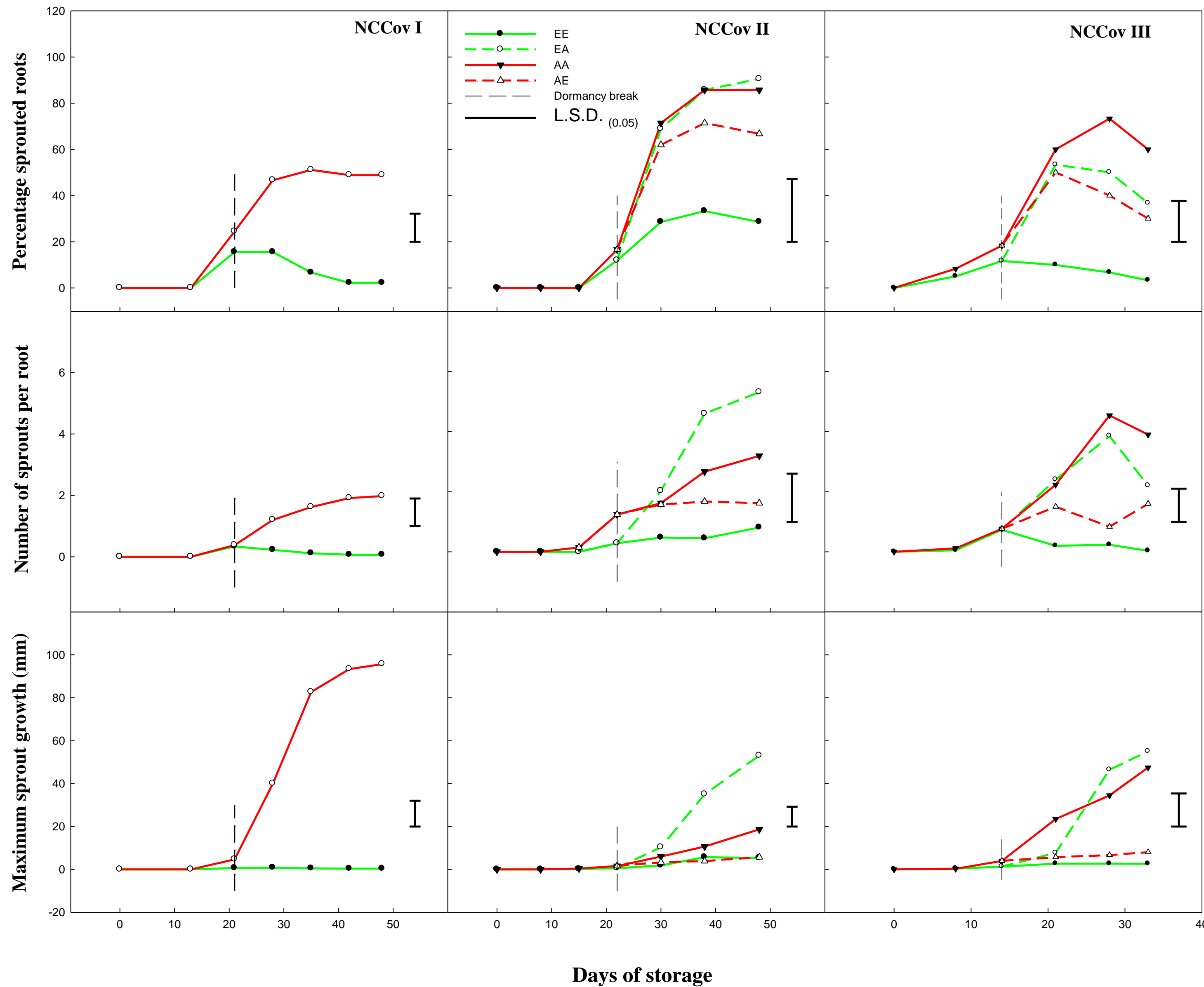


Figure 5.14 Sprout growth curves for the 'Covington' consignments 'NCCov I', 'NCCov II' and 'NCCov III' in continuous ethylene (EE); ethylene, followed by air (EA); continuous air (AA); or air, followed by ethylene (AE). Each data point of 'NCCov I' is the mean of three treatment boxes containing 15 roots per box (n=45). Each data point of 'NCCov II' and 'NCCOv III' before dormancy break is the mean of six treatment boxes containing 7 roots per box for 'NCCov II' (n=42) and 10 for 'NCCov III' (n=60). Each data point of 'NCCov II' and' NCCOv III' after dormancy break is the mean of three treatment boxes containing 7 roots per box for 'NCCov II' (n=21) and 10 for 'NCCov III' (n=30). L.S.D. $_{(0.05)}$ bars are shown. The legend applies to all graphs. ANOVA tables are in Appendix B: Tables B12-B20.

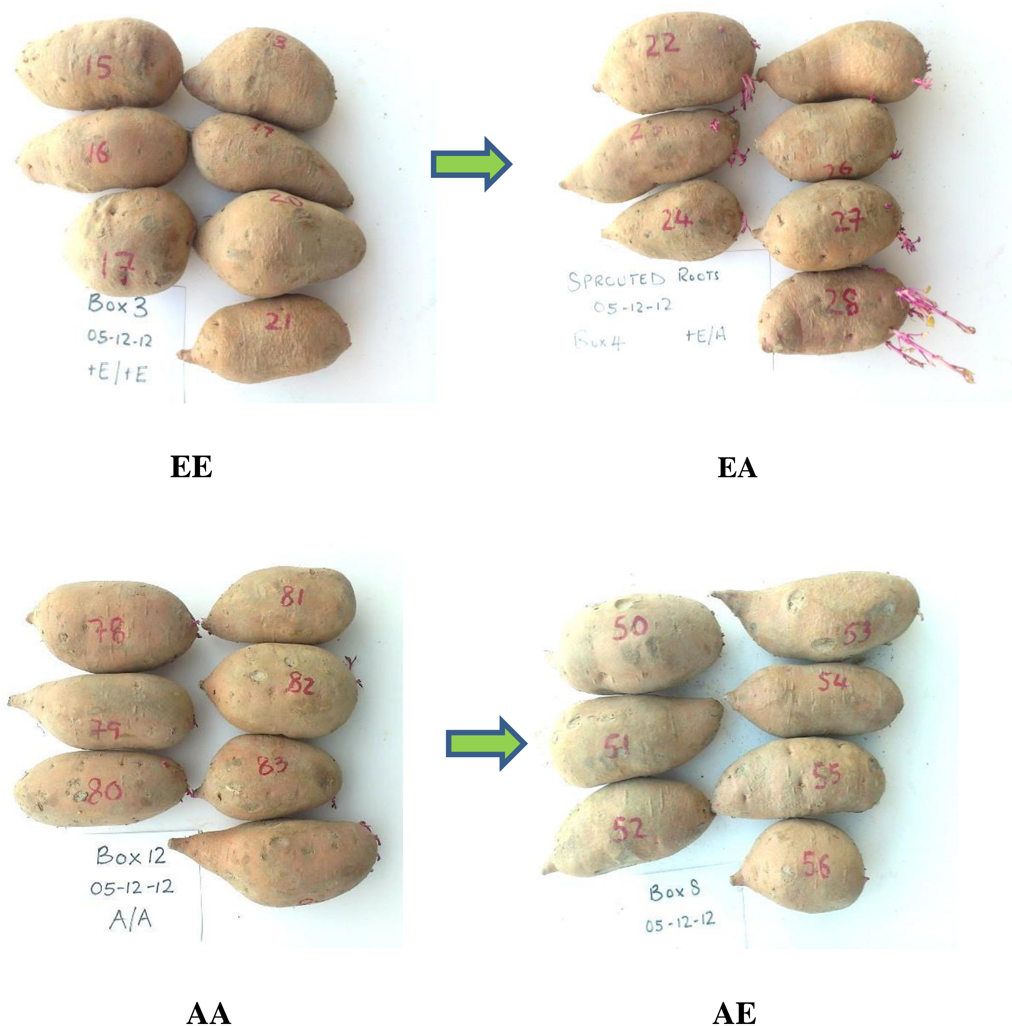


Figure 5.15 Effect of the transition of sweetpotato from ethylene to air or vice-versa on sprout growth

5.8.2 Biochemical variables

5.8.2.1 The effect of storage duration on non-structural carbohydrates

The non-structural carbohydrates were analysed in only the two Anderson East Farm consignments, namely, 'NCCov II' and 'NCCov III'. As stated in Section 5.6, the two consignments were part of the same harvest but 'NCCov II' was received as a fresh

consignment (10 days after harvest) for Experiment 5 whilst 'NCCov III' was held in storage (14°C) for *ca.* 5 months before Experiment 6 began.

The monosaccharides fructose and glucose; and the disaccharide sucrose, were the main sugars identified. Their spatial distribution in the root tissues and the changes when subjected to the respective ethylene and air treatments are shown in **Figures 5.16 - 5.17**. The spatial profiles of the sugars showed that they were unevenly distributed across the root tissues. In both consignments, the concentration of the sugars in the flesh tissues was about 1.5 times greater than in the skin tissues. Sucrose was the most abundant sugar (baseline concentration 17.5 and 56.9 mg g^{-1} FW for 'NCCov II' and 'NCCov III', respectively). Glucose (baseline concentration: 9.6 and 4.2 mg g^{-1} FW for 'NCCov II' and 'NCCov III', respectively) and fructose (baseline concentration: 9.9 and 4.2 mg g^{-1} FW for 'NCCov II' and 'NCCov III', respectively) occurred at approximately the same concentration, with the former having slightly higher concentration in most roots. There was a significant time-change effect, both within and between the two consignments, as the fructose and glucose concentrations declined while sucrose increased during the storage. Juxtaposed together, the decline between the fresh and the previously stored consignments was consistent. The mean baseline concentration of fructose, glucose and sucrose in the flesh tissues of 'NCCov II' were 12.6, 12.2 and 18.2 mg g^{-1} FW, respectively. Seven weeks later, the concentrations of fructose and glucose had dropped to 5.6, 10.3, while sucrose increased to 34.8 mg g^{-1} FW, respectively. When analysed five months later in 'NCCov III', the concentration of fructose and glucose in the flesh had reduced by almost 50% each to 5.5 and 6.6 mg g^{-1} FW, respectively, while sucrose had increased by *ca.* 70% to 60.2 mg g^{-1} FW. A similar observation was made in the skin tissues. It was noted, however, that after declining for some time, the concentration of fructose and glucose stabilized. In particular, their concentrations in the skin tissues in 'NCCov III' remained fairly constant. Glucose was consistently more abundant in the distal tissues of 'NCCov II' and 'NCCov III'. Glucose and sucrose were more stable in the distal root sections of 'NCCov II' when compared to the proximal sections. Conversely, sucrose concentration appeared to saturate at a maximum level.

5.8.2.2 Effect of ethylene supplementation on non-structural carbohydrates

The contents of fructose and glucose in 'NCCov II' were not significantly affected by the ethylene treatment or any of the post-dormant swap treatments (**Figure 5.16**). Absolute values, however, showed a marginal decline in glucose by the ethylene treatment compared to the air storage. The concentration of sucrose was significantly increased in the ethylene treatment. The sucrose content increased by about 2-fold in the distal tissues during the first two weeks of storage. No dramatic change in the concentration of the sugars was found in the 'NCCov II' at dormancy break. When roots were swapped from air into ethylene, the sucrose level increased sharply by *ca.* 1.3-fold in two weeks but dropped thereafter. Conversely, the sucrose level dropped in roots swapped from ethylene into air.

In 'NCCov III' (**Figure 5.17**) ethylene supplementation significantly suppressed the contents of both fructose and glucose within the flesh tissues whilst the sucrose level increased concomitantly. The fructose content significantly increased when roots were swapped from ethylene into air but there was no change in the corresponding swap treatment from air into ethylene. No significant difference was also detected in the swap treatments involving glucose. The concentration of sucrose generally dropped when roots were swapped from ethylene into air at dormancy break. Conversely, the transfer of roots from air into ethylene marginally increased the sucrose content. Dormancy break was associated with a transitory increase in the monosaccharides within the control flesh tissues to peak values. The peaking in fructose was less pronounced compared that in glucose. Fructose and glucose were virtually absent from the proximal skin tissues throughout storage, whilst in the distal tissues, they declined to the basal levels during the post-dormant period.

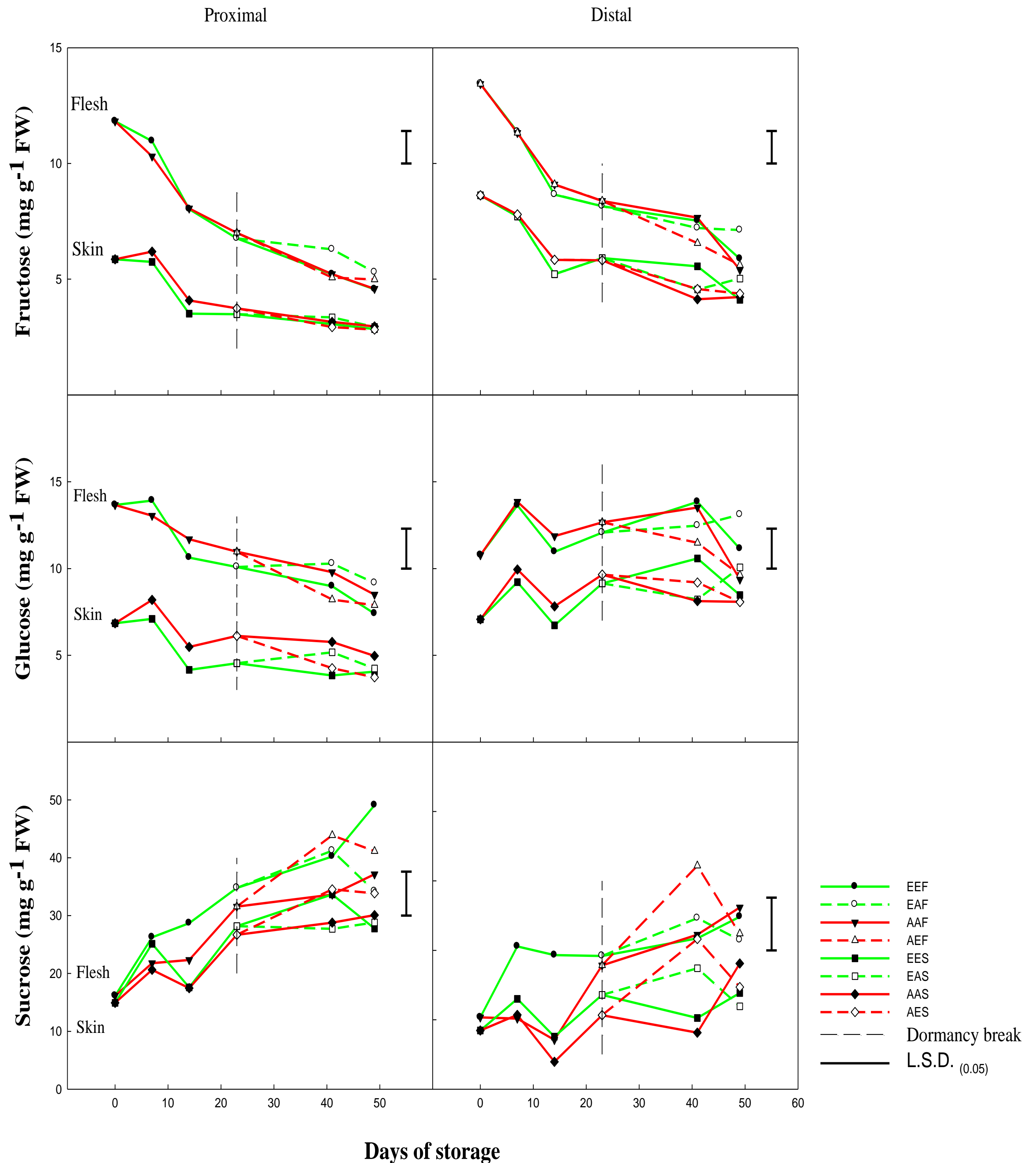


Figure 5.16 Effects of ethylene on sugars (mg g^{-1} FW) in the 'Covington' consignment 'NCCov II'. Each data point before dormancy break is the mean of six treatment boxes consisting of 3 roots per box (n=18). Each data point after dormancy break is the mean of three treatment boxes containing 3 roots per box (n=9). L.S.D. $_{(0.05)}$ bars are shown. ANOVA tables are in Appendix B: Table B21-B23.

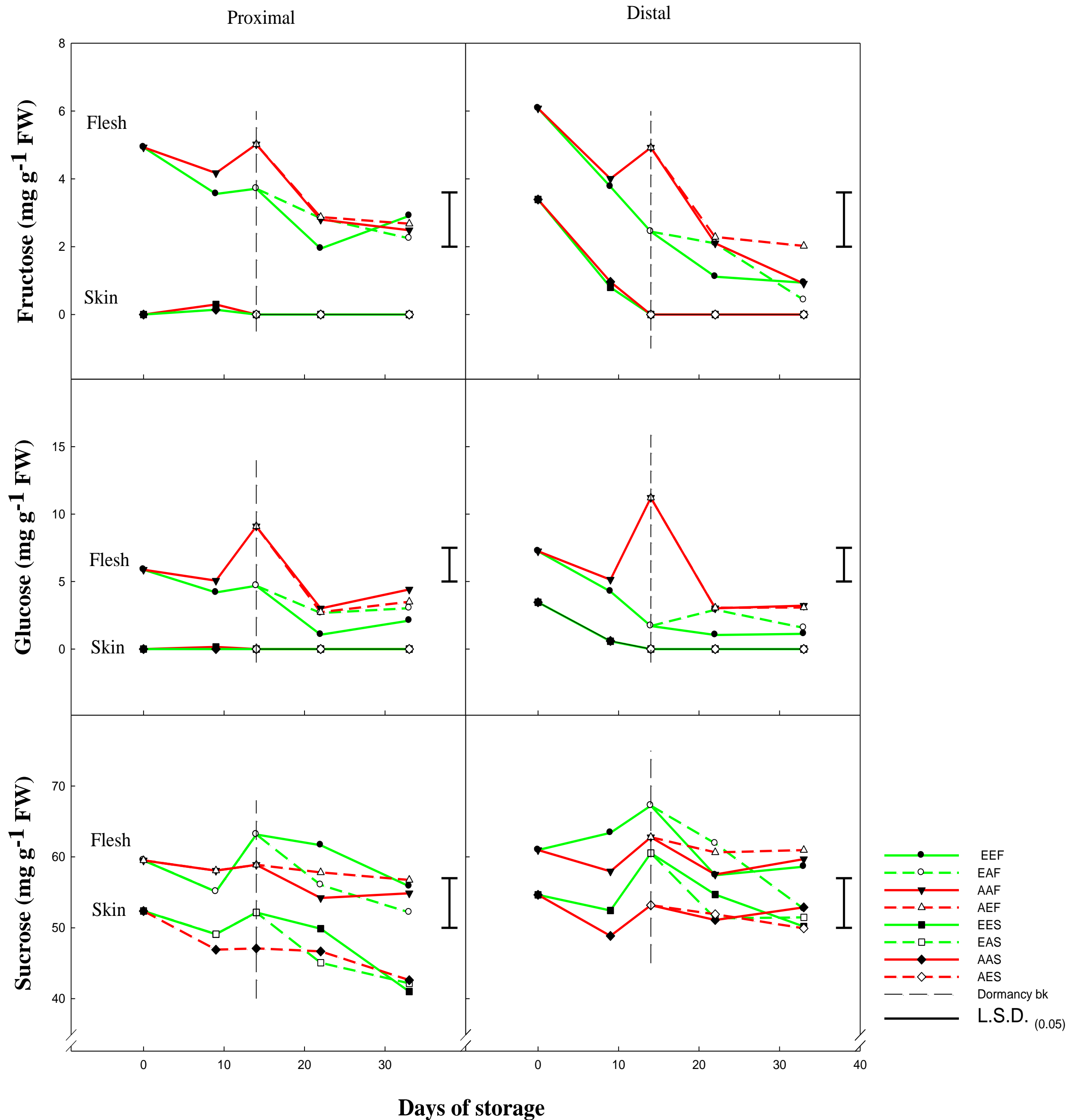


Figure 5.17 Effects of ethylene on sugars (mg g^{-1} FW) in 'Covington' consignment 'NCCov III'. Each data point before dormancy break is the mean of six treatment boxes consisting of 3 roots per box (n=18). Each data point after dormancy break is the mean of three treatment boxes consisting of 3 roots per box (n=9). L.S.D. $_{(0.05)}$ bars are shown. The legend applies to all graphs. Abbreviations in the legend are defined in the list of Notations. ANOVA tables are in Appendix B: B24-B26.

5.8.2.3 Effect of ethylene on the phenolic compounds in sweetpotato

The phenolic compounds in the two 'Covington' consignments 'NCCov II' and 'NCCov III' were identified and quantitated as described in Section 4.8.6. Pure commercial standards were calibrated against the samples; therefore the quantitated concentrations were expressed in absolute terms as mg/100 g FW. The major phenolic compounds found in the two 'Covington' consignments were caffeic acid, coumaric acid ('NCCov II' only), chlorogenic acid and the three isomers of chlorogenic acid (isochlorogenic acids A, B and C). Their relative spatial concentrations as mediated by the respective ethylene and air treatments during storage are presented below.

Chlorogenic acid and isochlorogenic acid A were the dominant phenolic compounds (**Tables 5.1 and 5.2**). **Figures 5.18-5.21** show the spatial distribution of the individual phenolic compounds in NCCov II and NCCov III, respectively. The individual phenolics were non-uniformly distributed across the spatial regions, being more significantly abundant (*ca*. 6-fold) in the skin than in the flesh tissues. With the exception of caffeic and coumaric acids, the contents of all the compounds were significantly higher (*ca.* 1.4-fold) in the proximal than in the distal root sections. The concentrations of the phenolic compounds within the flesh of the untreated control roots remained fairly constant during storage. Caffeic and coumaric acids were almost absent from the flesh tissues throughout storage; and in the skin, caffeic acid was slightly more abundant in the distal tissues. With storage time, the content of chlorogenic acid and its isomers tended to increase in the skin tissues. On the other hand, the skin content of caffeic acid was fairly stable during the dormancy period while coumaric acid concentration dropped significantly by *ca*. 66% within the same period. Dormancy break was marked with accentuated increases in the concentrations of chlorogenic acid and its isomers, which was generally more pronounced in the skin than in the flesh tissues. For caffeic and coumaric acids, only their contents in the control skin tissues increased after dormancy break.

Exogenous ethylene differentially affected the spatial concentrations of the individual phenolic compounds with distinctive effects between chlorogenic acid and its isomers on one hand, and caffeic and coumaric acids on the other hand. Significant differences between treatments were only observed after dormancy break. In the fresh 'Covington'

consignment, ‘NCCov II’, ethylene supplementation increased the proximal contents of isochlorogenic acids A and C by significant amounts (*ca*. 1.4-fold) compared to the control roots whilst no significant differences were found for chlorogenic acid and isochlorogenic acid B. With the exception of the proximal skin tissues, where the swap treatment from air into ethylene significantly increased the concentration of isochlorogenic acid A, all the other swap treatments in ‘NCCov II’ did not effect any significant changes in the concentrations of chlorogenic acid and its isomers. While ethylene supplementation tended to increase the concentrations of chlorogenic acid and its isomers (**Figures 5.18 and 5.21**), it depressed the skin contents of caffeic and coumaric acids by *ca*. 41 and 28%, respectively (**Figures 5.19 and 5.20**). The swap treatments tended to reciprocate the contents of caffeic and coumaric acids in the skin tissues.

In the previously stored ‘Covington’ consignment ‘NCCov III’, significant differences between the treatments were also observed but only after dormancy break. After dormancy release, exogenous ethylene significantly increased the concentration of isochlorogenic acid A in both root sections while it boosted chlorogenic acid content in only the proximal flesh tissues. Likewise, exogenous ethylene increased the skin concentration of isochlorogenic acid C in the distal tissues only. The swap treatment from ethylene to air significantly suppressed the content of all the isochlorogenic acid isomers in the distal skin tissues while in the proximal tissues, chlorogenic acid and the isomers showed delayed decline. None of the swap treatments from air into ethylene effected any significant change in the content of the individual phenolics except chlorogenic acid whose concentration in the distal skin tissues dropped significantly. As it was for the fresh consignment ‘NCCov II’, exogenous ethylene suppressed caffeic acid content in the skin tissues of ‘NCCov III’ by *ca*. 13%. The content of caffeic acid in the ethylene treatment, however, tended to increase after dormancy break (**Figures 5.19 and 5.20**).

Table 5.5 Baseline concentration of phenolic compounds in the tissues of 'NCCov II'

	Concentration (mg /100g FW)	
Phenolic Compound	Flesh	Skin
Chlorogenic acid	3.6	20.9
Isochlorogenic acid A	1.8	16.1
Isochlorogenic acid B	0.4	1.9
Isochlorogenic acid C	0	0.9
Caffeic acid	0	1.2
Coumaric acid	0	0.5
Total phenolics	5.8	41.5

Table 5.6 Baseline concentration of phenolic compounds in the tissues of 'NCCov III'

	Concentration (mg /100g FW)	
Phenolic Compound	Flesh	Skin
Chlorogenic acid	1.6	13.7
Isochlorogenic acid A	0.4	16.8
Isochlorogenic acid B	0.1	2.7
Isochlorogenic acid C	0	1.1
Caffeic acid	1.7	1.7
Total phenolics	3.8	36.0

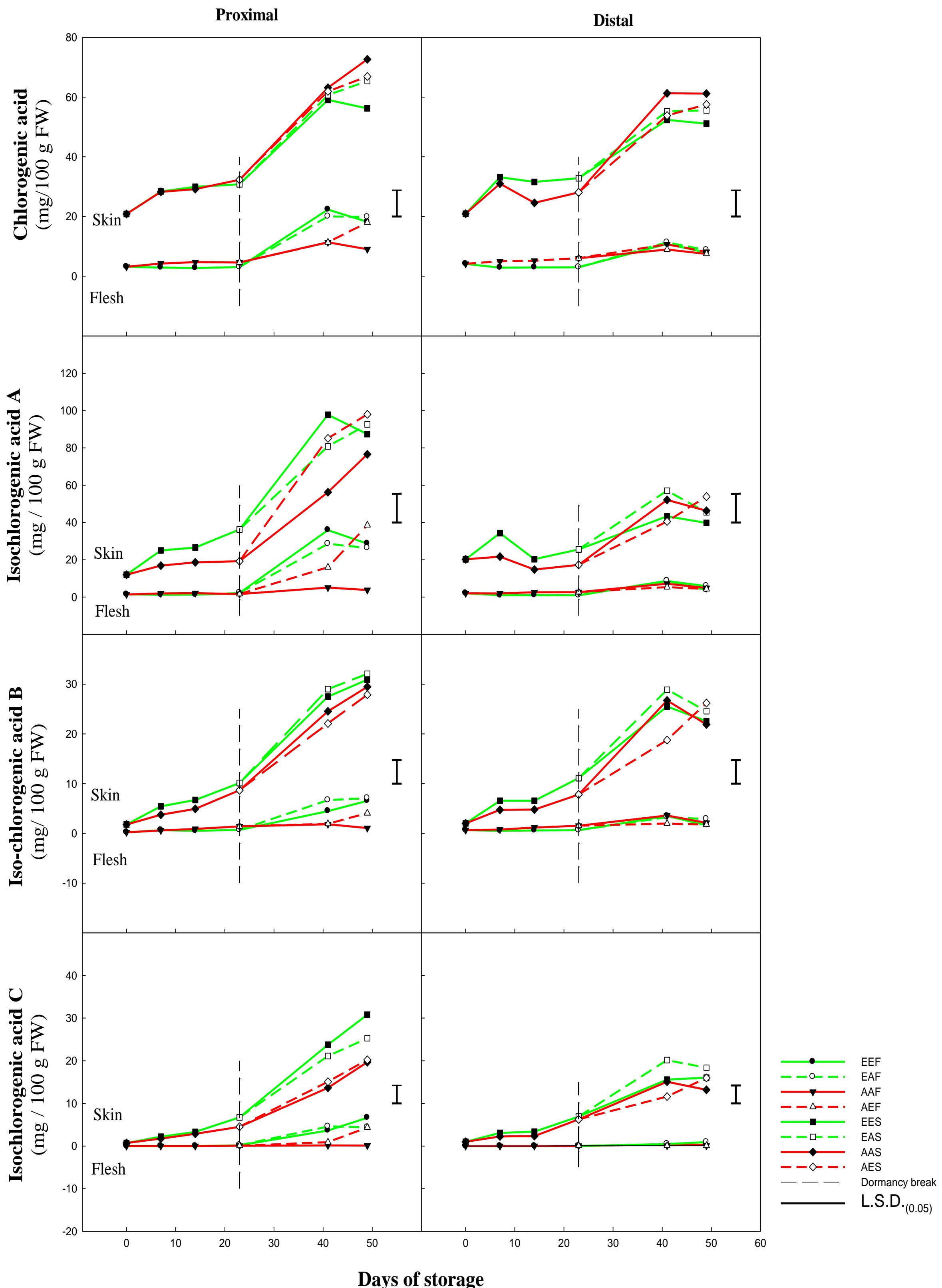


Figure 5.18 Effect of ethylene on chlorogenic acid and its isomers (mg /100 g FW) in the 'Covington consignment 'NCCov II'. Each data point before dormancy break is the mean of six treatment boxes consisting of 3 roots per box (n=18). Each data point after dormancy break is the mean of three treatment boxes consisting of 3 roots per box (n=9). L.S.D. $_{(0.05)}$ bars are shown. The legend applies to all graphs. The legend applies to all graphs. Abbreviations in the legend are defined in the list of Notations. ANOVA tables are in Appendix B: B27-B30.

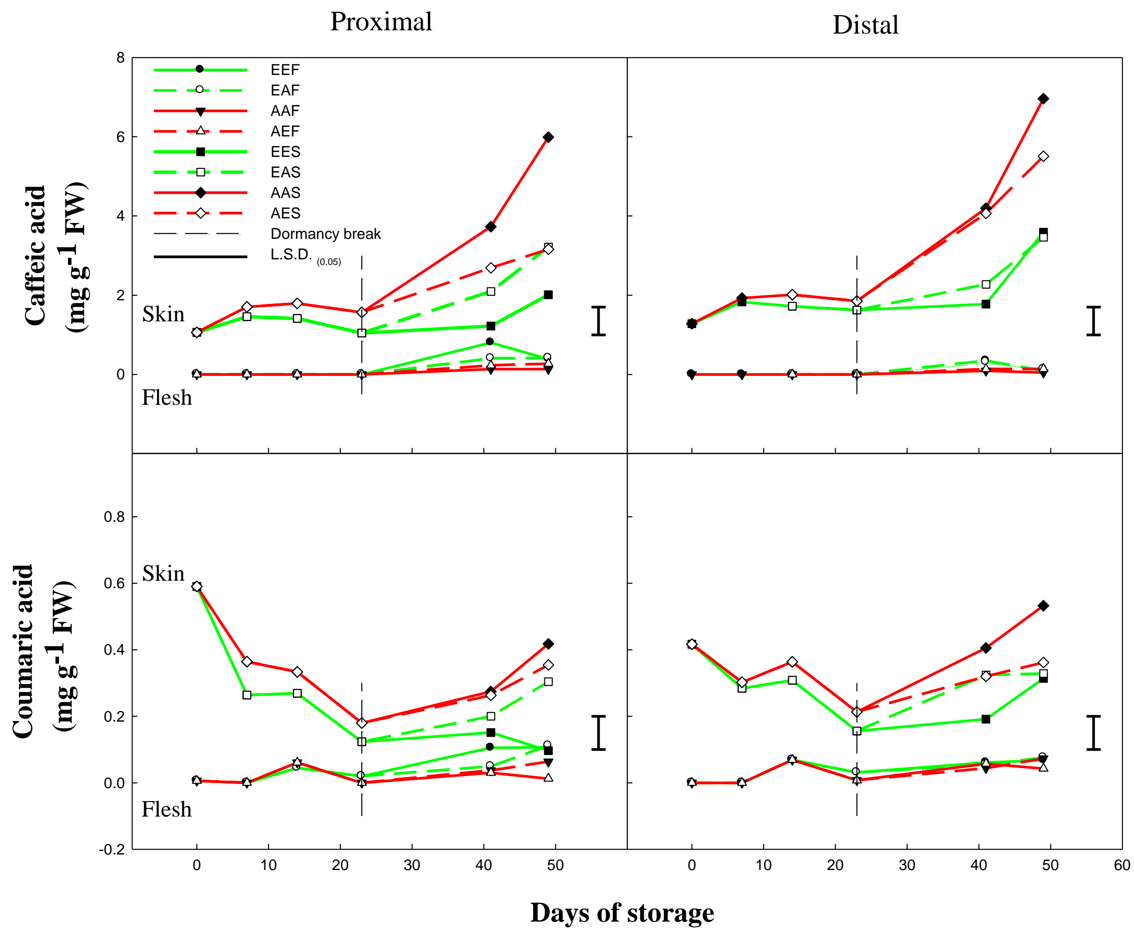


Figure 5.19 Effect of ethylene on caffeic acid and coumaric acid (mg /100 g FW) in the 'Covington' consignment 'NCCov II'. Each data point before dormancy break is the mean of six treatment boxes consisting of 3 roots per box (n=18). Each data point after dormancy break is the mean of three treatment boxes consisting of 3 roots per box (n=9). L.S.D. $_{(0.05)}$ bars are shown. The legend applies to all graphs. Abbreviations in the legend are defined in the list of Notations. ANOVA tables are in Appendix B, Table B31-B32.

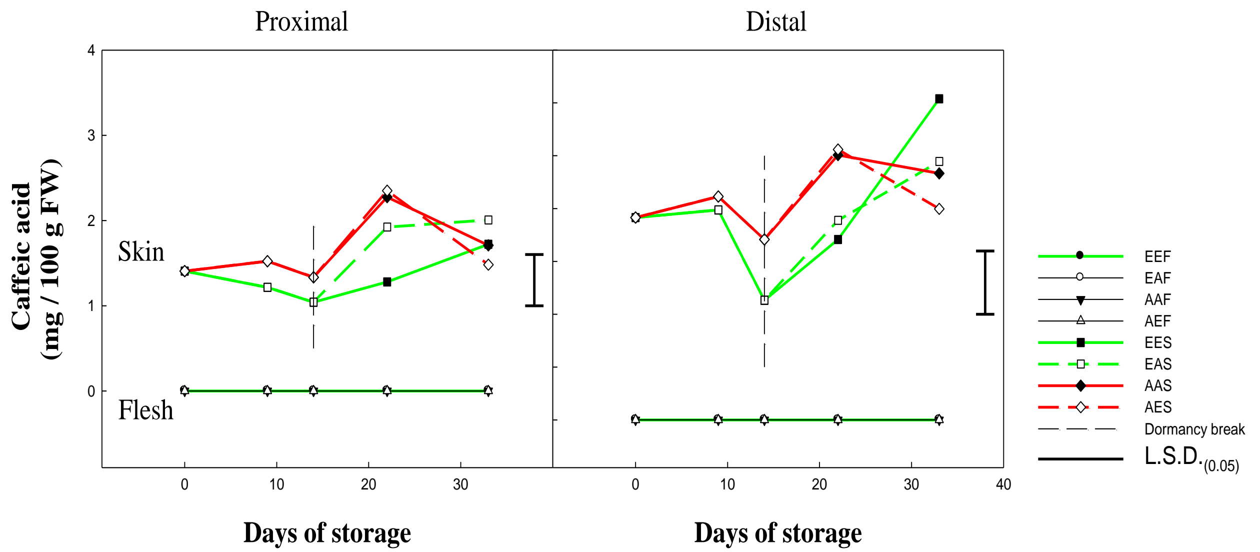


Figure 5.20 Effect of ethylene on caffeic acid (mg /100 g FW) in the 'Covington' consignment 'NCCov III'. Each data point before dormancy break is the mean of six treatment boxes consisting of 3 roots per box (n=18). Each data point after dormancy break is the mean of three treatment boxes consisting of 3 roots per box (n=9). L.S.D. $_{(0.05)}$ bars are shown. The legend applies to all graphs. Abbreviations in the legend are defined in the list of Notations. ANOVA table is in Appendix B, Table B33.

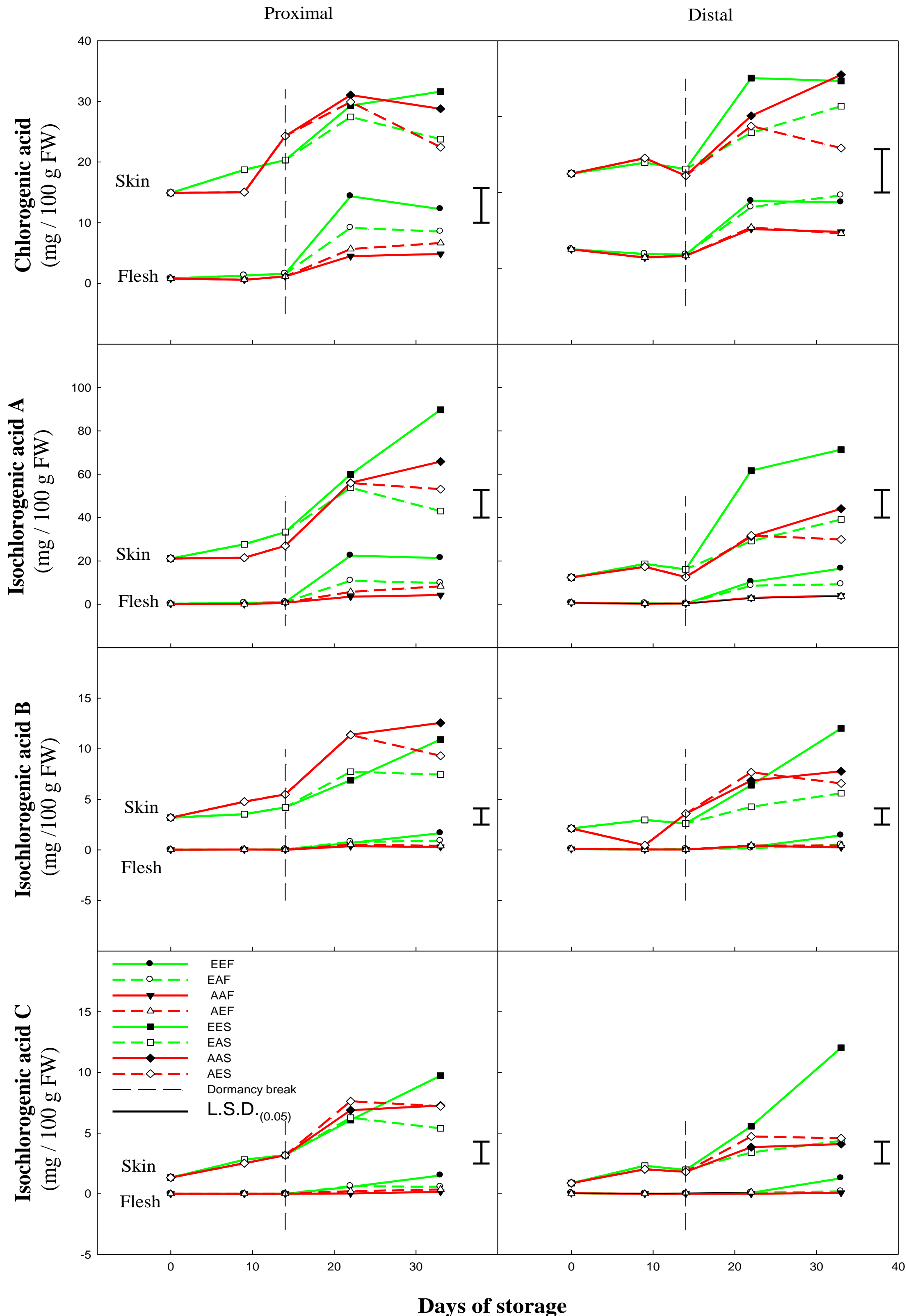


Figure 5.21 Effect of ethylene on chlorogenic acid and its isomers (mg /100 g FW) in the ‘Covington’ consignment ‘NCCov III’. Each data point before dormancy break is the mean of six treatment boxes consisting of 3 roots per box (n=18). Each data point after dormancy break is the mean of three treatment boxes consisting of 3 roots per box (n=9). L.S.D. $_{(0.05)}$ bars are shown. The legend applies to all graphs. Abbreviations in the legend are defined in the list of Notations. ANOVA tables are in Appendix B: B34-B37.

5.9 Discussion

The effects of different timings of exogenous ethylene supplementation (10 µl L^{-1}) on the physiological and biochemical profiles of the sweetpotato cv. North Carolina Covington were studied. Three consignments, designated as 'NCCov I', 'NCCov II' and 'NCCov III' were analysed in three separate experiments (Experiments 4, 5 and 6, respectively). 'NCCov I' was an old stock, having been previously stored for *ca*. 8 months. Both 'NCCov II' and 'NCCov III' were part of the same harvest but studied at different times (2012 and 2013, respectively) with a 5-month time lag between them. 'NCCov II' was a fresh consignment (10 days old on delivery) while the 'NCCov III' was 5 months old, having been initially held in storage at 14°C before the experiment. In all the experiments, the storage temperature was 25°C at a relative humidity *ca*. 60-95%. The effects of four concerted treatments *viz.* continuous ethylene supplementation throughout storage; continuous storage in air; and post-dormancy transition from ethylene into air or vice-versa, were studied. It was hypothesized that neither the main ethylene treatment nor the transitions after dormancy break has any significant effect on the physiology and biochemistry of the roots. The results obtained are now discussed in the following sections.

5.9.1 Effect of ethylene on the respiration rate

In all the three experiments, exogenous ethylene supplementation significantly boosted the respiration rates in the roots by about 1.5 - 2.0 fold compared to the basal respiration levels in the air storage. In each case, the ethylene treatment elicited a common response in the roots with a transient, climacteric-like increase to a peak level, followed by a typical non-climacteric decline. The swap treatments from either ethylene into air or vice-versa, also swapped the respiratory status, consolidating the observation that, exogenous ethylene is responsible for the induction of the increased respiration in sweetpotato roots.

Observations regarding ethylene effect on the respiration of other non-climacteric crops such as onions, potatoes and sweetpotatoes have been reported (Huelin and Barker, 1939; Reid and Pratt, 1972; Buescher *et al*., 1975; Katinoja, 1987; Saltveit, 1999; Cools *et al*., 2011; Foukaraki, *et al*., 2011, Cheema *et al.,* 2013). They all point to increased respiration

upon ethylene perception by the tissues and therefore, the result in this study was to be expected. In particular, the respiratory pattern observed in the current study has similarities with the finding about potato respiration as reported by Reid and Pratt (1972). Reid and Pratt (1972) noted that, after a time lag of about 8 h, the respiration of potato tubers (cultivar not named) incubated in jars and flushed with 10 µl L^{-1} ethylene rose to a peak level 5-10 times that of the control tubers after 25-30 h of storage and declined thereafter. Foukaraki (2012), also found that exogenous ethylene increased the respiration rates of potato cvs. Marfona and Russet Burbank, during storage over 30 weeks. It has also been published that a short 24 h treatment of onion cvs. Wellington and Sherpa, immediately before or after curing, increased their respiration rates considerably (Downes *et al*., 2010). Cheema *et al.* (2013) treated sweetpotato cvs. Bushbuck and Ibees with continuous ethylene (10 µl L^{-1}) during storage in 3.2 L glass jars over four weeks. Roots treated with ethylene had higher respiration rates than the air control roots. The experimental set up in the present study, involving swap treatments from ethylene into air or vice-versa is an innovative approach that enabled ethylene effect on respiration to be effectively evaluated by tracking the respiratory behaviour as the roots were transferred between air and ethylene at different time points during storage. The reciprocity of the root respiration when swapped between ethylene and air is, perhaps, more compelling evidence that ethylene induces higher respiration in sweetpotato.

The biochemical basis for the stimulation of respiration by ethylene has not been definitively elucidated. Suggestions offered mainly implicate stress response or ethylene-induced metabolism (Reid and Pratt, 1972). Evidence that alludes to stress response comes from the observation that the respiration of sweetpotato and other crops increase in response to biotic and abiotic stresses such as temperature, wound, and fungi (Salveit, 1999; Hyodo *et al*., 2003). At the same time, these stresses induce endogenous ethylene production (Pratt and Goeschl., 1969; Wang, 1990; Abeles *et al*., 1992), which can feed back into the respiratory process. In part, the stress hypothesis is supported by the current study since exogenous ethylene inflicted tissue injury by causing proximal tissue bursting. Tissue wounding by ethylene has also been reported in sweetpotatoes by Stahmann *et al.* (1966) and Kitinoja (1987), and in the latter case, the wounding induced fungal growth. These observations have led to ethylene being described as a “wound hormone” (Reid and Pratt, 1972). The respiratory activity of wounded plant tissue increases because of

increased requirement for healing substances such as lignin and suberin (De Baerdemaerker *et al*., 1999). It is thus likely that the ethylene-induced respiration may be a response related to wound stress.

Alternatively, Reid and Pratt (1972) proposed further that the increased respiration may be related to metabolic processes such as protein synthesis, starch hydrolysis or other glycolytic events, leading to an increase in the levels of high energy and intermediates. The situation whereby the respiration status immediately changed at the instance of the swap treatments suggests that ethylene is a signal; and the induced respiration may therefore involve a signalling and transcription mechanism in which transcription factors activate the transcription of genes upon ethylene perception, whilst the genes are repressed when ethylene is removed. Generally, elevated respiration of crops during storage has undesirable ramifications as it accelerates metabolic activities and leads to pre-mature senescence.

5.9.2 Effect of ethylene on sprout growth

Sprout growth inhibition by exogenous ethylene has been observed in potato, onion and sweetpotato in previous experiments (Prange *et al.,* 1998; Daniels-Lake *et al*., 2005; Downes *et al*., 2010; Cheema, *et al*., 2013; Cools *et al*., 2011; Foukaraki *et al*., 2011). The viability of ethylene as a practical alternative to chemical sprout suppressants such as chlorphropham (CIPC) and maleic hydrazide (MH) has found commercial application in the UK and Canada (Chope and Terry, 2008). Cheema *et al*. (2013) suggested that the sprout inhibition effect of ethylene may also provide a practical solution to alleviate sweetpotato sprouting in developing countries and for long distance transport. The most technically and economically suitable timing of exogenous ethylene supplementation, however, has not been determined; and the current practice whereby crops are treated with ethylene at beginning of storage is only coincidental. The ethylene supply company, Restrain, has proposed that supplementation of ethylene should be delayed until after dormancy break as against a suggestion to activate ethylene supplementation from the beginning of storage by another company, BioFresh (British Potato Council, 2006). Work done by Foukaraki *et al*. (2010) demonstrated that ethylene exposure after dormancy

break was as effective as continuous ethylene in inhibiting sprout growth in some UK-grown potato cultivars. To date, however, the relative advantages of these options have not been evaluated in sweetpotato.

Results from the present study on sweetpotato concurs with the finding by Foukaraki *et al*. (2010) on potato that both continuous ethylene supplementation from the beginning of storage and post-dormant swap from air storage into ethylene significantly suppressed sprout growth in practically equal measure. However, exogenous ethylene supplementation just after dormancy break had greater benefits in reducing the incidence of root decay and weight loss. It may thus offer more competitive advantage compared to ethylene supplementation throughout storage.

The transfer of roots from ethylene into air after dormancy break exacerbated sprout growth, as there was proliferous growth in terms of the number of roots that sprouted, the number of sprouted eyes and the sprout length. Thus once predisposed to ethylene, sweetpotato roots may have to remain in the ethylene or risk compromising its quality. This effect was also observed in apples by Elmer (1936) and in potatoes by Rylski *et al*. (1974), Kader (1985) and Foukaraki *et al*. (2010), suggesting common fundamental mechanism.

The instance where the high respiration rate induced by exogenous ethylene did not translate into sprout growth presents a complex dichotomy. Cheema (2010) observed a similar contradiction in sweetpotato cv. Bushbuck and initially suggested that the ethylene-induced effect on sprouting and respiration are independent phenomena. Later, Cheema *et al*. (2013) hypothesised that the respiratory energy released at the instance of ethylene supplementation is utilized in a different ethylene stimulated process rather than sprout growth. It is intriguing to note that sprout inhibition in the roots occurred only in the presence of ethylene but when removed from the ethylene medium, sprout growth was more vigorous even at the subsequently reduced respiration. This suggests the possible involvement of a signal transduction and gene transcription mechanism. Understanding the mechanism by which plants detect ethylene and use it to coordinate biological processes may be of great value in unravelling the diverse ethylene actions and effects. In this regard, advances in biochemical and transcriptional analytical methods, which enable detailed analysis of sprout development during storage (Chope *et al*., 2011)

may be employed to explain the sprout growth inhibition effect of ethylene. Genetic studies have discovered a number of key components in the ethylene signalling pathway, including receptors and their downstream transcription factors. Elucidation of the signal mechanism in ethylene response may offer a useful tool for investigating the control that ethylene exercises in plant tissues (Reid and Prat, 1972). A transcription factor known as Ethylene Insensitive 3 (EIN3) is believed to be the key regulator of ethylene responses by co-ordinating the expression of genes that direct ethylene-mediated growth. EIN3 functions by promoting the transcription of a variety of ethylene response factor genes that ultimately control the growth and physiological responses under ethylene control (Solano *et al.,* 1998). In the model plant Arabidopsis, the absence of ethylene is reported to cause rapid degradation of EIN3 (Alonso *et al*., 1999). While ethylene promotes the activity of EIN3, the latter is reported to also promote the expression of proteins that inhibit ethylene response via a feedback loop (Chang *et al*., 2013). In transcriptional studies by Cools *et al.* (2011), exogenous ethylene resulted in a negative feedback by down-regulating ethylene perception and ethylene signalling pathways in onion, which led to inhibited sprout growth. The authors found that exogenous ethylene, alongside 1-MCP, down-regulated both a transcript with similarity to an ethylene receptor and EIN3. They therefore suggested that this down-regulation of ethylene perception and signalling events may explain the sprout inhibition effect. It is possible that this is the fundamental mechanism underlying sprout inhibition in non-climacteric crops, including sweetpotato.

5.9.3 Effect of ethylene on weight loss

The weight loss data showed that ethylene supplementation was associated with higher root weight loss. Weight loss may result from two main causes: respiratory water loss and conversion of dry matter into energy. Furthermore, removal of decayed roots reduces the marketable weight. Evidence that ethylene induces higher respiratory water loss was shown in the graphical output by the ExpeData analytical software (**Figures 5.22-5.23**). Ethylene has been reported to cause tissue expansion with consequent loss of cell compartmentalization (Pratt and Goeschl, 1969). The loosened tissues could facilitate water loss. This was confirmed in the present study with an incidental observation whereby the ethylene treatments resulted in proximal tissue splitting (**Figure 5.24**).

Weight loss was significantly reduced when ethylene was applied after dormancy break compared to ethylene supplementation throughout storage.

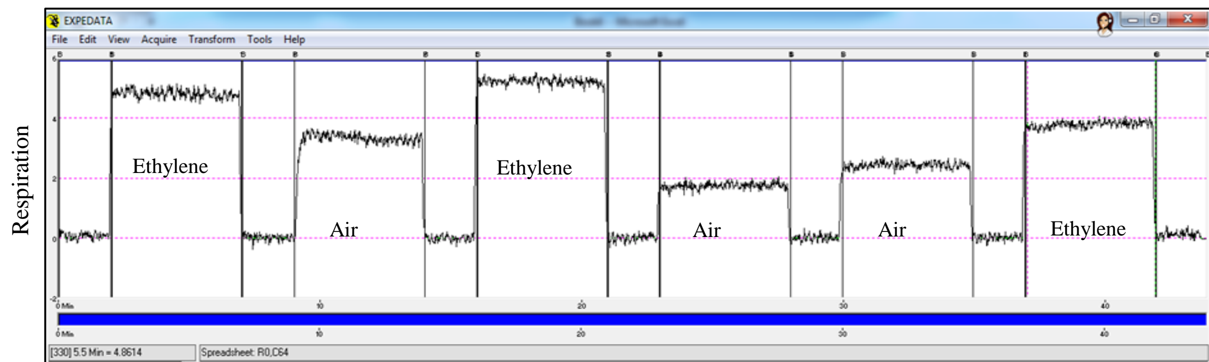


Figure 5.22 Typical ExpeData graph for the respiration of sweetpotatoes in storage jars flushed with ethylene or air. The roots have approximately the same weight.

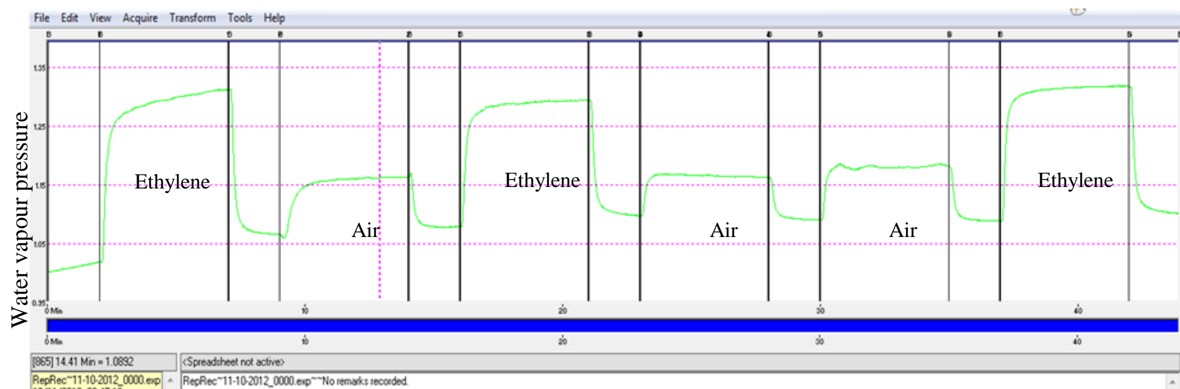
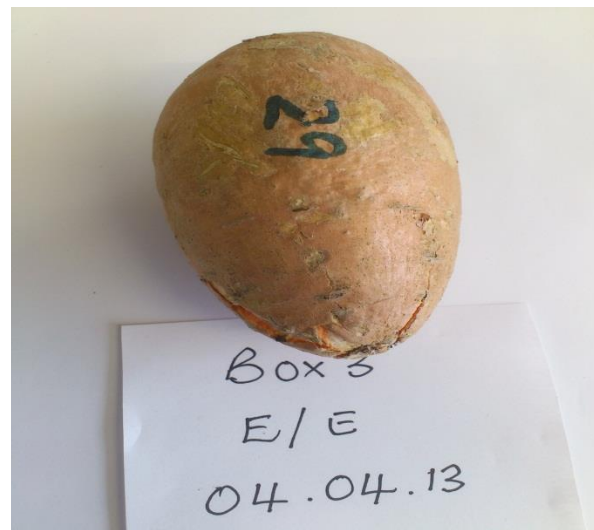


Figure 5.23 Typical ExpeData graph for the water vapour pressure in storage jars containing sweetpotato roots treated with ethylene or air. The roots have approximately equal weights

NCCov II



NCCov III

Figure 5.24 Proximal tissue splitting in ethylene-treated sweetpotato roots

5.9.4 Effect of ethylene on diseases

It was found in the current study that the ethylene treatment induced higher incidence of diseases than the air-stored roots. The ethylene-related diseases mostly affected the proximal ends (tip rot). A similar incidence was also observed by Kitinoja (1987) during the curing of sweetpotato cvs. Jersey, Garnet and Jewel in ethylene (2 µl L^{-1}) at 30ºC. The author reported that the ethylene-cured roots developed callus at the root ends and this was associated with heavy fungal infection and higher rate of water loss than air-cured

roots. Ethylene-induced tip rot was also observed by Arancibia *et al*. (2013) when an analogue of ethylene, ethephon was applied to sweetpotato cv. Beauregard 1-7 days before harvest. The role of ethylene in pathogen-infected plants investigated by Hoffman *et al*. (1999) shows that ethylene may either reduce or enhance disease symptoms depending on the pathogen. The pathogens responsible for the proximal decay could not be investigated in this study. The visual symptoms, however, were soft and black rots, which could be associated with *Rhizopus stolonifer* and *ceratocystis frimbriata*. There is also the possibility of bacterial soft rot caused by *Erwinia chrysanthemii*. The bursting of proximal tissues as observed in the current study (**Figure 5.24**) may be symptomatic evidence of expanded and loosened tissues (Pratt and Goeschl, 1969), which could be the underlying cause of the reduced resistance to pathogens. Such internal mechanical stress could also elicit higher respiratory response, as was observed in the ethylene-treated roots. 1-MCP was found in experiment one to curb proximal diseases in the organically grown 'Covington'. The greater incidence of diseases recorded in the ethylene-treated roots implies that ethylene supplementation, as a means to forestall sweetpotato sprouting, may have to be leveraged with 1-MCP treatment or other integrated methods of diseases control. The incidence of diseases was found to be *ca*. 2.5 times lower when the ethylene supplementation was delayed until just after dormancy break (**Figure 5.12**).

5.9.5 Effect of ethylene on sugars

Carbohydrates play important roles in tissue metabolism such as energy storage and its release for other metabolic activities during respiration. The present study investigated the spatial distribution of sugars in sweetpotato cv. North Carolina Covington when treated with different timings of ethylene supplementation as described in this chapter. The aim was to understand the effect of exogenous ethylene on the nutritional content of carbohydrates in sweetpotato and the mechanisms by which ethylene interacts with carbohydrates in effecting physiological changes in the crop.

The baseline contents of sucrose, fructose and glucose measured in the 'Covington' consignments (Section 5.7.2.1) ranged between 1.8 -5.7 % FW, 0.4-1.0 % FW and 0.4-0.9 % FW, respectively. These values are comparable to sugar contents measured in raw

dessert sweetpotato by Picha (1985) who found the concentrations of sucrose, fructose and glucose to be in the range 1.3-4.1 %, 0.3-1.2% and 0.2-1.5% FW while raw staple types had respective concentrations 1.3-2.5%, 0.4-0.7% and 0.4-1.0% on fresh weight basis. The concentration of sugars in sweetpotato, however, is cultivar dependent (Zhang *et al*., 2002). Likewise, the changes in the sugars concentration during storage have been reported to differ among genotypes (Zhang *et al*., 2002; Morrison *et al*., 1993). Data presented by Zhang *et al*. (2002) showed increase in fructose concentration with storage time in four of six cultivars studied whilst it declined in two cultivars. In all the cultivars, Zhang *et al*. (2002) found that glucose and sucrose increased with storage time over the first 60 days. On the other hand, Morrison *et al.* (1993) found that changes in the individual and total sugars in sweetpotatoes during storage were negligible. In the present study on the two consignments of the cv. North Carolina Covington, the concentrations of fructose and glucose declined with time while sucrose level increased in 'NCCov II' but remained fairly constant in 'NCCov III'. This result may be explained by the starch-sugar inter-conversion phenomenon as described by Wills *et al*. (1998). At any given temperature, starch and sugars are in dynamic equilibrium with some sugars degrading to carbon dioxide during respiration (Wills *et al*., 1998). In sweetpotato and potato, the starch-sugar balance at ambient temperatures is biased towards accumulation of starch whilst at low storage temperatures, there is higher accumulation of sugars. For each crop, there is a critical temperature above which the balance favours starch accumulation. The critical temperature at which the inter-conversion between starch and sugars occurs in sweetpotato is 15°C (Wills *et al*., 1998). Thus storage of sweetpotatoes at temperatures ≥15°C is likely to shift the balance in favour of starch accumulation with concomitant reduction in the monosaccharides. Thus in the present study, the high storage temperature (25°C) may account for the decline in the contents of fructose and glucose.

Crops are normally characterised by non-homogenous distribution of the composite substances, including the carbohydrates, suggesting differential spatial metabolisms. In the sweetpotato consignments studied ('NCCov II' and 'NCCov III'), the sugars were unevenly distributed across the root tissues and sections, which suggests their relative importance for specific localised functions in the spatial regions such as proximal sprouting. Glucose was consistently more abundant in the distal sections of both consignments but the content of fructose and sucrose in the root ends varied depending

on individual roots. The high content of glucose in the distal than the proximal sections is probably linked to their relative rates of utilization in respiration and the effect of solute potential. Sprouts normally initiate from the proximal sections and therefore the proximal tissues are expected to be metabolically more active with higher requirements for soluble sugars for sprout growth.

Ethylene supplementation differentially affected the content of sugars in 'NCCov II' and 'NCCov III'. In 'NCoV III', fructose and glucose concentrations were significantly reduced by the ethylene treatment. Concomitantly, the disaccharide sucrose significantly increased in the roots of both consignments. The swap treatment from ethylene to air decreased the sucrose content while fructose increased. In the fresh consignment 'NCCov II', however, suppression of the monosaccharides by ethylene was statistically inconsequential. Reduction of the monosaccharides in sweetpotato by exogenous ethylene was also reported by Cheema *et al*. (2013) who analysed the single time point concentrations 4 weeks after the storage of sweetpotato cvs. Buchbuck and Ibees (10 µl L^{-1}, 25°C). Sucrose was also found by Cheema *et al.* (2013) to have increased by exogenous ethylene but it was not significant. A seemingly contradictory result was earlier reported by Chegeh and Picha (1993) who found no change in the fructose and glucose content in cured and non-cured cvs. Beauregard and Jewel when stored for 15 days at 21°C in a range of ethylene concentrations from 0 -1000 µl L^{-1}. Chegeh and Picha (1993) however, found an increase in the sucrose level in ethylene treatment. In the current study in which the dynamics of the non-structural carbohydrates were assessed at multiple time points over a longer storage period, it was found that ethylene supplementation (10 µl L^{-1}) significantly suppressed the monosaccharides in the cv. North Carolina Covington whilst it increased sucrose concomitantly. The evidence for ethylene suppression of fructose and glucose was more pronounced in the 5 month old consignment 'NCCov III' than the fresh consignment 'NCCov II', suggesting that the effect takes longer time to become significant. This may explain why Chegeh and Picha (1993) found no change in the monosaccharides in the relatively fresh produce, which had been stored for about two weeks.

In potatoes, a contradictory effect of ethylene has been reported that sugars accumulate when treated with ethylene (Foukaraki *et al*., 2010). According to Rutherford and Whittle

(1982), soluble sugars are required to provide energy for sprout growth and this requirement may stimulate hydrolysis of starch and sucrose and lead to the accumulation of monosaccharides. Cheema (2010) explained that the content of sugars in potatoes is low compared to sweetpotato. Thus in potato, sugars are mobilised from starch hydrolysis to provide substrates for the ethylene-induced respiration whilst in sweetpotato, the already available sugars are utilised in the respiration resulting in a reduction of their levels. Effort to reduce the consequential darkening of the fry colour in potato caused by ethylene-induced sugar accumulation involves treatment combination with 1-MCP (Prange *et al*., 2005; Foukaraki *et al*., 2011). The result in the present study, however, suggests that the ethylene-induced sugar accumulation problem may not exist in sweetpotato.

Dormancy break in 'NCCov III' was marked with significant abrupt increases in the fructose and glucose contents in the control roots. Such peaks in sugars were noted to precede sprouting in non-climacteric onions (Benkeblia *et al.,* 2005). Chope *et al*. (2011) stated that changes in the concentrations of key metabolites could be used to predict events such as the onset of sprouting. Soluble sugars are utilized to provide energy for sprout growth (Rutherford and Whittle 1982). The peaking of monosaccharides sugars in 'NCCov III' suggests that the dormancy break event requires a certain level of energy substrates for sprout growth and this may be provided by stimulating increased starch hydrolysis and invertase activity to yield the required amount of monosaccharides. In 'NCCov II', peaking of the monosaccharides was not observed. This may probably be because at the dormancy break point, the monosaccharides concentrations were already at the required levels for sprout growth. Dormancy break occurred in 'NCCov II' when the fructose and glucose concentrations in the proximal flesh tissues were *ca.* 7 and 11.0 mg g^{-1} FW, respectively (**Figure 5.25**). Interestingly, the peak levels of fructose and glucose at which dormancy was broken in 'NCCov III' (*ca.* 5.0 and 9.0 mg g^{-1} FW, respectively) were equivalent to the respective concentrations at dormancy release in 'NCCov II'. This may suggest that the threshold concentrations for dormancy to be broken are *ca*. 5.0 and 9.0 mg g^{-1}FW for fructose and glucose, respectively. In 'NCCov III', exogenous ethylene suppressed the monosaccharides below the presumed level at which dormancy was broken whilst in 'NCCov II', their levels in the ethylene treatment were just higher than the presumed threshold concentration. Thus the low levels of

monosaccharides (because they were suppressed by ethylene) may explain why even though the ethylene treated roots sprouted, the sprouts fail to grow. The ability to sprout and grow, however, depends also on many other factors including temperature. After reaching the peak levels, the monosaccharides concentrations in the control roots of 'NCCov III' dropped significantly, suggesting that the sugars were being utilized by the growing sprouts. Transfer of roots from ethylene into air boosted the monosaccharides to the equivalent levels in the control roots or higher. This probably explains why sprouts in this treatment grew faster.

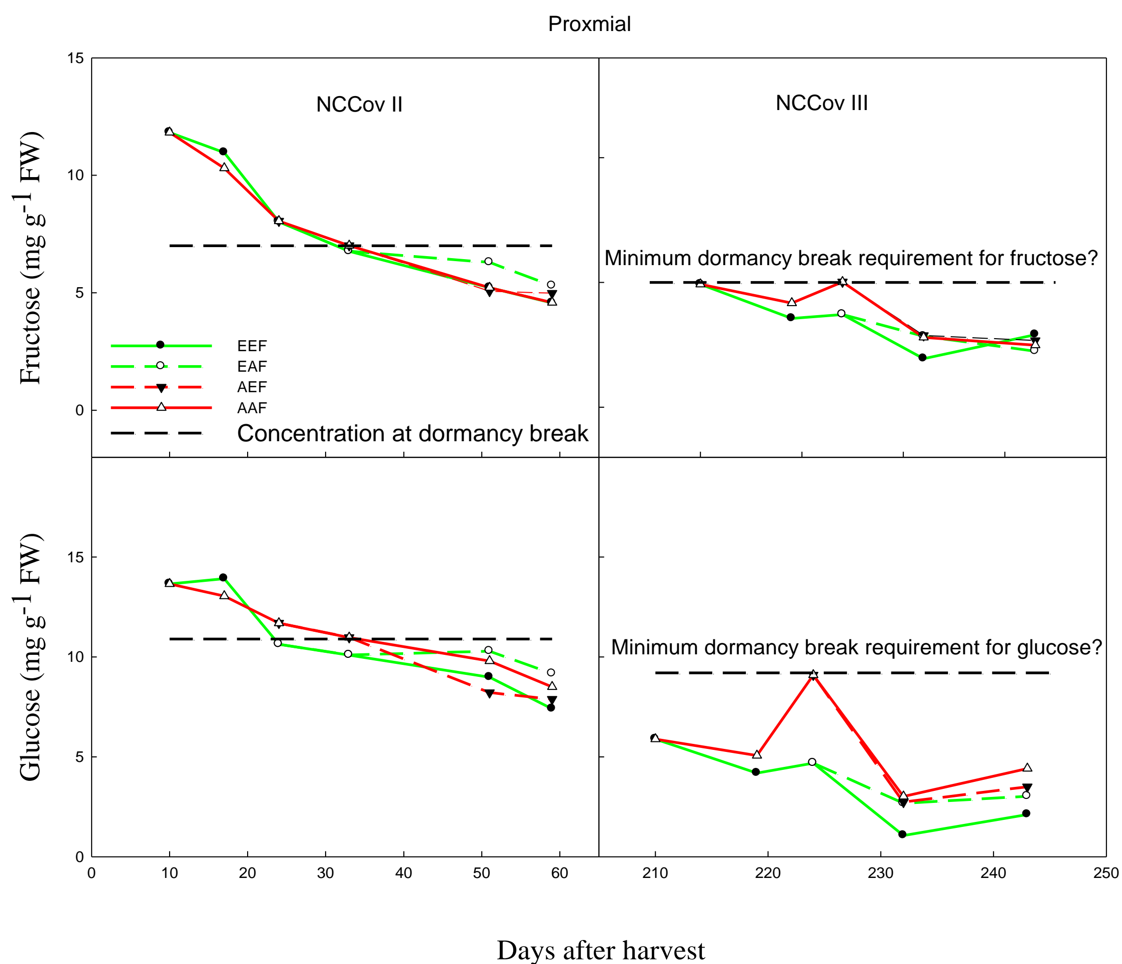


Figure 5.25 Changes in the proximal concentrations of fructose and glucose in 'NCCov II' and 'NCCov III' during storage at 25°C. Roots of 'NCCov III' were held in storage at *ca*. 14-15°C between day 10 and day 210 after harvest (Similar pattern was observed in the distal tissues).

5.9.6 Effect of ethylene on phenolic compounds

The major phenolic compounds in sweetpotato roots and their concentrations have been previously reported (Kim *et al.,* 1971; Walter, Purcell and McCollum, 1979; Walter and Schadel, 1981; Truong *et al.*, 2004; Truong *et al.*, 2007; Teow *et al.*, 2007 and Jung *et al.*, 2011). There are wide variations in the phenolic contents between genotypes. The content of phenolic compounds in the 'Covington' consignments as measured in the present study (**Tables 5.1 and 5.2**) were within the range of the reported values, although as it is to be expected, differences always exist depending on genotype and growing conditions (Islam *et al*, 2003). Many assays on phenolics composition in sweetpotato mainly aim to quantify the concentrations during nutritional studies in order to create consumer awareness about their health value. Equal attention has not been given to studies on the spatio-temporal distribution of the individual compounds and the implication for their roles in metabolic activities. As stated by Chope *et al.* (2011), physiological changes such as sprouting are associated with changes in key metabolites. That said, it is important to understand how the metabolism of the individual phenolics are related to mechanisms like dormancy break and sprout growth. Understanding the bioactivity of the phenolic compounds could also reveal important information about their relationship with ethylene in effecting changes in plants.

The major phenolic compounds found in the Covington consignments were caffeic acid, coumaric acid ('NCCov II' only) and chlorogenic acid and its isomers (iso-chlorogenic acids A, B and C). These compounds have been reported to be present in considerable quantities in many genotypes of sweetpotato (Truong *et al.*, 2004; Nanantu *et al.*, 2007; Jung *et al.*, 2011; Walter *et al.*, 1979; Truong *et al.*, 2007; Teow *et al.*, 2007). The finding in the present study that chlorogenic acid and isochlorogenic acid A are the dominant phenolics in sweetpotato is consistent with reports by Padda (2006) and Teow *et al.* (2007). The concentrations of all the phenolic compounds were significantly higher in the skin than in the flesh tissues as is also reported by Kim *et al.* (1971) and Walter and Schadel (1981). Chlorogenic acid content was the highest in the flesh tissues of both 'Covington' consignments 'NCCov II' and 'NCCov III'. Chlorogenic also dominated in the skin of 'NCCov II' but isochlorogenic A was the dominant compound in the skin of 'NCCov III'. There was *ca*. 8-fold higher concentration of the total phenolics in the skin

than in the flesh tissues and 1.4-fold greater concentration in the proximal than the distal root sections. Chlorogenic acid is the most important cinnamic acid derivative and its role in plants as antifungal agent against phytopathogens is well documented (Harbone, 1994). Phenolic compounds are also known to be important structural components of plant cell walls, being utilized for lignin biosynthesis (Nanantu *et al*., 2007; Bhuiyan *et al.,* 2009). The greater accumulation of phenolics in the skin tissues is more likely to be associated with their roles as the first line chemical defense against infection (Scwalb and Feucht, 1999) and in the mechanical fortification of lignified cell walls. Although the root firmness was not quantitatively measured in the current study, evidence from textural feel showed that the proximal root sections were firmer than the distal sections; and this may be explained by the relatively higher accumulation of phenolic compounds, leading to greater structural stability. Within the edible flesh tissues, all the phenolic compounds remained fairly stable in the untreated control roots throughout storage. Thus ambient storage of sweetpotato may not significantly change the nutritional integrity. Caffeic acid was almost absent from all flesh tissues; and in the skin tissues, it was found in greater abundance in the distal than in the proximal sections. Walter *et al.* (1979) who first used HPLC to analyse sweetpotato phenolics did not find caffeic acid in the root tissues but the presence of caffeic acid in the skin was reported by Sondheimer (1958). These conflicting results probably arise from the relatively low content of caffeic acid in sweetpotato. In the phenylpropanoid pathway, both caffeic and coumaric acids are intermediate precursors for downstream biosynthesis of chlorogenic acid and its isomers and they appear to be drawn down as soon as they are synthesised.

Common to all the phenolic compounds was that, dormancy break was marked with accentuated increase in their concentrations both in the ethylene and air treatments. It thus suggests the possible involvement of phenolics in the initiation of sprout growth in sweetpotato. This is supported by the growing evidence that polyamines, which are usually conjugated to phenolic compounds for distribution throughout plants, have a regulatory role in plant morphogenesis involving, DNA replication and cell division, differentiation and proliferation, as well as stabilization of macromolecular structures (Galston and Sawhney, 1990; Seiler and Raul, 2005). Protacio and Flores (1992) found that polyamines were necessary for potato tuber formation in-vitro in the early stages of morphogenesis involving active cell division. The authors proposed that

hydroxycinnamic acid esters (chlorogenic acid and its derivatives) serve as storage pools for polyamines and are conjugated to form hydroxycinnamic acid amides (HCAs). The possible involvement of phenolics in sprout initiation and growth may therefore be linked to their conjugating function, which enables polyamines to be transported to the meristematic tissues. Both polyamines and ethylene are synthesised from S-adenosyl methionine (SAM) such that the balance between the two can be influenced by the levels of each (Evans and Malmberg, 1989; Valero *et al.,* 2002). Dormancy break in potato has been found to be associated with increased biosynthesis of polyamines and the production of polyamines such as spermidine and spermine is accentuated by exogenous ethylene (Jeon *et al*., 2002).Thus dormancy break, which subsequently leads to the proliferation of new tissues, is likely to also stimulate the biosynthesis of polyphenols to facilitate polyamines transport.

Ethylene-induced biosynthesis of phenolics has been reported in sweetpotato (Buescher, 1979; Buescher *et al*., 1975 and Walter *et al*., 1980) as a consequence of increased phenylalanine ammonia lyase (PAL) activity, which increases accumulation of phenolic compounds (Saltveit, 2000). PAL is a key regulatory enzyme which catalyses the initial reaction in the biosynthesis of phenylpropanoid products to chlorogenic acid and isochlorogenic acids.

The synthesis of the individual phenolic compounds, however, was found to be differentially affected by exogenous ethylene depending on the compound-tissue-section matrix. The ethylene-induced boost in the total phenolics appeared to be dominated by its increasing effect on the concentrations of isochlorogenic acids A. Chlorogenic acid was not significantly affected by exogenous ethylene except in the flesh of 'NCCov III' where the proximal content increased. Likewise, there was no significant effect of exogenous ethylene on isochlorogenic acid B in 'NCCov II' while in 'NCCoov III', it only showed a delayed increase in the distal skin tissues.

Whilst ethylene tended to boost the concentrations of chlorogenic acid and its derivatives, a contrary observation was made whereby the content of caffeic and coumaric acids declined in ethylene supplementation. This was evident in the chromatographic peaks, which showed reciprocity between chlorogenic acid and caffeic acid accumulation. Data presented by Islam (2003) on polyphenolic extracts in sweetpotato cell suspension culture

showed that, during the stationary phase of cell growth in a high-anthocyanin producing medium, accumulation of caffeic acid increased 3-fold whilst concomitantly, chlorogenic acid level dropped by about 1.5-fold. Caffeic and coumaric acids are closely related to the hydroxycinnamic esters (chlorogenic acid and the derivatives Isochlorogenic acids A, B and C) in the phenylpropanoid pathway. As intermediate precursors in the phenylpropanoid pathway, both coumaric and caffeic acids are feedstock pools from which the hydroxycinnamic esters are synthesised. It appears that while ethylene increases PAL activity, and thereby boosts the total phenolics, coumaric and caffeic acids are utilized faster for the downstream synthesis of the phenolics than they are replenished from the upstream reactions in the phenylpropanoid pathway. The evidence whereby the content of coumaric and caffeic acids decline in ethylene treatment, however, disputes the generalised notion that ethylene enhances the biosynthesis of all polyphenols and suggests that ethylene effect is specific to individual compounds.

5.9.7 Conclusions

The effects of different timings of exogenous ethylene supplementation (10 µl L^{-1}) on the spatio-temporal changes in the physiological and biochemical variables in sweetpotato cv. North Carolina Covington during storage at 25°C was studied. The timings included continuous ethylene supplementation from the beginning to end of the storage and swap treatments from previous storage in air into the ethylene or vice-versa after dormancy break. The swap treatment used in the current study is a novel approach to track the dynamic effects of exogenous ethylene on sweetpotato. Evidence from the study supports previous findings that ethylene elevates respiration in sweetpotato roots. Coincident with the increased respiration was ethylene-suppressed sprout growth. Whilst the reduced sprout growth may be advantageous for sweetpotato stored for the consumer market, the benefits were discounted by the fact that exogenous ethylene also accelerated weight loss and the incidence of diseases. A possible remedial measure identified in the study was to reduce the length of exposure to the ethylene through appropriate timing of the ethylene supplementation. Applying exogenous ethylene immediately after dormancy break practically achieved the same benefit in reducing sprout growth as that of ethylene supplementation from the beginning of storage. Alongside, the post-dormant ethylene

application significantly reduced the incidence of diseases. The post-dormant ethylene supplementation did not compromise the crop's quality in terms of changes in carbohydrate content as in this treatment, no significant differences were found between the individual sugars and the main ethylene treatment (from the beginning of storage). The current study has also shown that contrary to its effect on potato, ethylene supplementation reduces the content of monosaccharides in sweetpotato whilst it increases the sucrose level. Dormancy break in the previously stored consignment 'NCCov III' was associated with peaking of the monosaccharides fructose and glucose in the control roots. At the same time, the ethylene treatment suppressed this increase in the monosaccharides. The increased monosaccharides may reflect mobilisation of soluble sugars needed for sprout growth but the fact that ethylene suppressed these increases may explain why sprout growth in ethylene-treated roots was inhibited.

Coincident with dormancy break was a dramatic increase in all the phenolic compounds suggesting the possible involvement of phenolics in the sprouting mechanism. The study further demonstrated that although ethylene supplementation tended to enhance the total phenolics in sweetpotato, it did not increase all the individual phenolic compounds proportionately. Isochlorogenic acid A content was identified to be most enhanced by exogenous ethylene and contributed greatly to the perceived increase in the total phenolics. On the other hand, the level of caffeic and coumaric acids in the skin tissues declined in the presence of ethylene. This disputes the general perception that ethylene boosts the biosynthesis of all phenolic compounds and suggest that the effect is dependent on the type of compound and the matrix effect of the tissue.

CHAPTER SIX: EFFECT OF EXOGENOUS ETHYLENE ON THE HORMONAL CHANGES IN SWEETPOTATO ROOTS IN RELATION TO DORMANCY DURING STORAGE

6.1 Introduction

Dormancy is exhibited by many species in the plant kingdom, including seeds, vegetative buds, corms, roots and tubers (Suttle and Hulstrand, 1994). It was demonstrated in experiments 4-6 (Chapter 5) that sweetpotato has a relatively short dormancy period of just about two to three weeks after harvest. This has been confirmed by other authors (Rees *et al*., 2001; Cheema, *et al*., 2013). The short dormancy in sweetpotato impacts negatively on its marketing and contribution to food security. There can be substantial sprout growth aftedormancy break, which may lead to the rejection or downgrading of a lot if 10% of the sprouts are more than 19 mm (USDA, 2005). The control of dormancy to permit long-term storage of sweetpotato in the food chain is therefore important.

Effective control of dormancy, however, is contingent on adequate understanding of the physiological and biochemical basis for the dormancy status and the transition to the meristematic growth phase. Although a number of both endogenous and exogenous conditions have been cited to control dormancy, to date, the precise mechanistic pathways for the phenomenon in sweetpotato and many other crops are not clarified. Hypotheses postulated to explain the regulatory triggers of dormancy range from effects of external factors including temperature and water stress (Hartmann *et al.,* 2002) to changes in endogenous metabolites. Other conflicting views about the dormancy mechanism were discussed in Section 2.3.4. Currently, there is considerable interest in the involvement of plant growth regulators (PGRs) in the dormancy of root and tubers as they are known to mediate many plant functions and biosynthetic pathways. Four principal plant hormones, including ethylene, abscisic acid (ABA), gibberellins (GAs) and cytokinins, have been implicated in dormancy regulation (Hemberg, 1985). Yet the precise roles played by the individual hormones and the synergy between them are, to date, elusive. Much of the present understanding of endogenous hormonal activity comes indirectly from pharmacological effects resulting from the application of exogenous growth regulators

and correlative studies comparing gross changes in endogenous hormonal levels with sprouting behaviour (Suttle, 1998). Evidence from these studies suggest that ethylene and ABA are actively involved in the inception and maintenance of the dormancy phase, while gibberellins and cytokinins are associated with the termination of dormancy and subsequent sprout growth (Kader, 1985; Suttle, 1998).

While research is on-going to understand the precise role of endogenous ethylene in mediating the dormancy regime, many studies on potato have confirmed that when exogenously applied, ethylene may shorten or extend dormancy depending on a mix of factors, most importantly, the concentration, timing and duration of exposure (Rylski *et al.,* 1974; Kader, 1985; Prange *et al.,* 1998; Foukaraki *et al*., 2010). This was found to be equally true for sweetpotato in Chapter 5 of the present study. These contradictory effects make understanding of the natural role of endogenous ethylene even more complicated. There are suggestions that ethylene may not act in isolation but in concert with other growth regulators in a complex chain of interactions (Libermann, 1979). A study by Suttle (1998) on potato microtuber endodormancy established the involvement of endogenous ethylene in tuber dormancy. The author suggested that the involvement of ethylene may be restricted to the initial period. In other studies, the sustained synthesis and action of ABA was found to be essential for the establishment and maintenance of microtuber dormancy (Suttle and Hulstrand, 1994). The nature and extent of interaction between ethylene and ABA in mediating dormancy, however, is unknown (Suttle, 1998).

Cytokinins alongside gibberellins have also been implicated to be growth promoters (Rossouw, 2008). Earlier, Hemberg (1985) and Suttle (2004a, 2005) found that exogenously applied cytokinins can break dormancy in potato tubers. The most active cytokinins include zeatin (Z), zeatin riboside (ZR), dihydro-zeatin riboside (DHZR) and isopentenyl adenine (IPA). In sweetpotato, the major cytokinin is reported to be ZR (Matsuo *et al*., 1983, Hyzyo, 1973) and may be expected to play a major role in dormancy break and sprout growth. The technology of exogenous ethylene supplementation to inhibit sprouting in sweetpotato is likely to find commercial application as it has in potato. How exogenous ethylene interacts with endogenous growth promotion or inhibition hormones, however, has not been reported. Such understanding may improve the ethylene application system. In this chapter, the changes in the concentrations of endogenous ABA

and ZR in response to exogenous ethylene supplementation were studied. Results from experiment 6 (Chapter Five) suggested that there is dominant activity in terms of sprouting in the proximal root section. Also Wang *et al*. (2006) reported that the concentration of ABA, ZR, DHZR and indole-acetic acid (IAA) are all higher in the proximal than the distal ends. Matsuo *et al*. (1988) also supported the finding that ZR is higher in the proximal section of sweetpotato. Biological activity in the proximal section is therefore expected to dictate the postharvest life of sweetpotato. Therefore only the proximal flesh and skin tissues were investigated in this study.

6.2 Aim and objectives of the study

6.2.1 Aim

The aim of this experiment was to understand the synergy between exogenous ethylene and endogenous hormones in mediating dormancy in sweetpotato.

6.2.2 Specific objectives

1. To investigate if exogenous ethylene acts independently or in concert with ABA and ZR in controlling dormancy, its release and subsequent sprout growth.

2. To understand the mechanism by which exogenous ethylene inhibits or promotes sprout growth in sweetpotato in relation to changes in the endogenous levels of ABA and ZR.

6.3 Hypothesis

6.3.1 Null Hypothesis

It was hypothesised that:

HO: Exogenous ethylene has no significant effect on endogenous levels of ABA and ZR.

6.3.2 Alternate Hypothesis

HA: Exogenous ethylene significantly affects endogenous levels of ABA and ZR.

6.4 Materials and methods

Selected samples (proximal skin and flesh tissues) of the sweetpotato consignment 'NCCov II' in experiment 5 (Chapter 5), which were analysed for sugars and phenolic compounds at the periodic outturns were also assayed for the corresponding levels of endogenous hormones. A method was developed for the extraction and quantification of the hormones as described in Section 3.5.5 of Chapter 3. A preliminary assay identified ABA and ZR as the major endogenous hormones in majority of the roots and therefore the study was limited to the effect of the exogenous ethylene treatment on these two hormones.

6.5 Results for Experiment Seven

6.5.1 Effect of exogenous ethylene supplementation on abscisic acid (ABA)

The concentrations of absisic acid (ABA) in both tissues were initially high (487.0 and 345.2 ng g^{-1} DW in the skin and flesh, respectively). With storage time, the concentrations declined in both the continuous ethylene treated and control roots (**Figure 6.**1). The ABA levels in the control roots declined consistently over time to 70 and 87 ng g^{-1} DW in the skin and flesh tissues, respectively, without any dramatic change at the dormancy break point or during the subsequent sprout growth. Dormancy was broken when the ABA level fell to an average of 185.3 ng g^{-1} DW in the control roots while in the ethylene treated roots, dormancy break occurred when the ABA level fell to a minimum in the skin tissues at *ca.* 120 ng g^{-1} DW. In the flesh, ABA level stabilized at a significantly lower value of *ca*. 66.9 ng g^{-1} DW just before dormancy break. Comparing the treatments, the continuous ethylene supplementation significantly suppressed ABA in the flesh tissues. Even though the same observation was made in the skin tissues, this was less significant.

6.5.2 Effect of exogenous ethylene supplementation on zeatin riboside (ZR)

The concentration of ZR in the control roots remained fairly constant before and after dormancy break (mean concentration 44.6 and 29.5 ng g^{-1} DW in the skin and flesh tissues, respectively), although it showed a late increase in the skin after 40 days of storage (**Figure 6.1**). On the contrary, the concentration of ZR within the ethylene treated tissues changed significantly over time, increasing consistently from 48.4 - 192.4 ng g^{-1} DW in the skin, while in the flesh tissues, it increased from 60.3 ng g^{-1} DW to a peak level of 391.9 ng g^{-1} DW 18 days after dormancy break, and then declined thereafter. Compared to the control roots, the content of ZR in the ethylene treated roots was 6-fold greater in the flesh and 4-fold greater in the skin tissues. One week before the dormancy break, the concentration of ZR declined in both ethylene-treated tissues to *ca*. 84 ng g^{-1} DW at which point, the roots sprouted. Again the post-dormant increase in the flesh tissues was more dramatic.

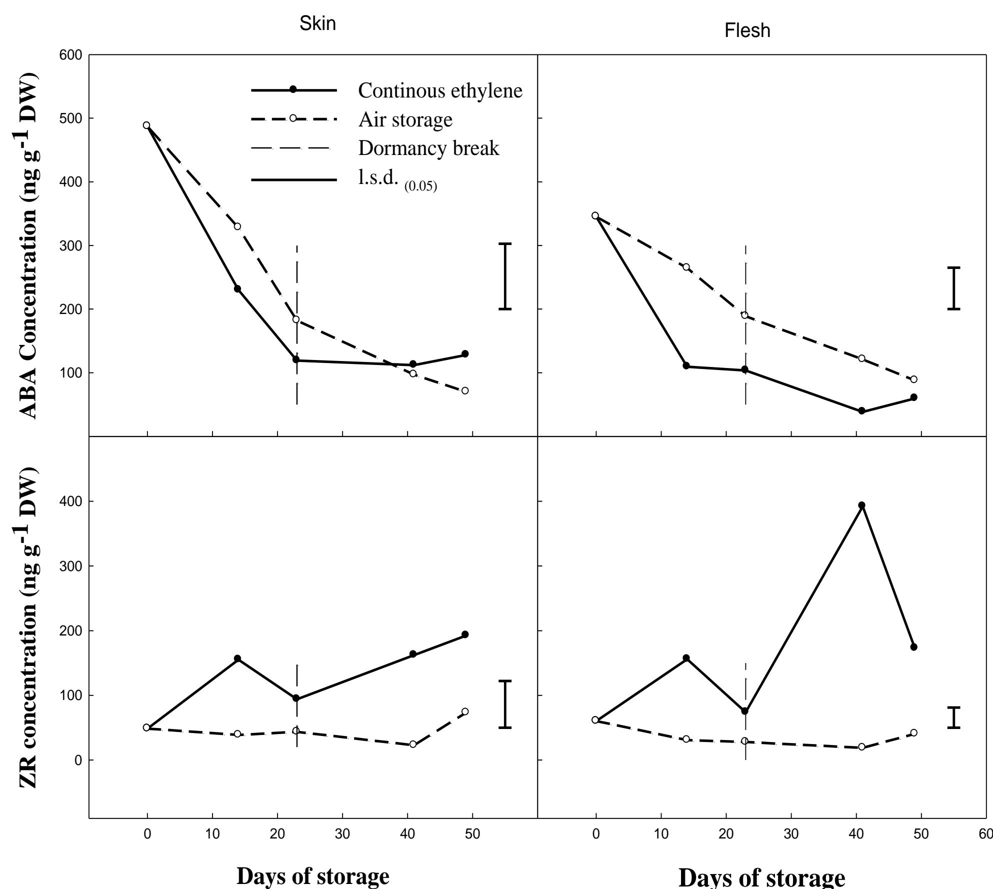


Figure 6.1 Effect of ethylene on the concentration of ABA and ZR in sweetpotato consignment NCCov II. Each data point before dormancy break is the mean of six treatment boxes consisting of 3 roots per box (n=18). Each data point after dormancy break is the mean of three treatment boxes consisting of 3 roots per box (n=9). L.S.D. $_{(0.05)}$ bars are shown. The legend applies to all graphs. ANOVA tables are in Appendix C: C1-C4.

6.5.3 Discussion

Several observations in many crops support the evidence that ABA levels are initially high during the dormancy period but declines towards dormancy break. Suttle (1995) found that ABA level in potato declined consistently without any threshold point to mark the inception of sprouting. Similar observation was made by Classeens and Vreugdenhil (2000). In a more recent study, Foukaraki (2010) also noted a similar pattern of decline in ABA content in ethylene-treated potato cv. Marfona. In a different study by Chope *et*

al. (2006), when onion cvs. Red Baron, Sherpa and Wellington were cured, the ABA concentration decreased during storage until sprouting occurred at the minimum level. The finding in the present study involving the storage of sweetpotato is in agreement with above authors. The ABA concentration declined continuously in both treatments and tissues without any interruption by the dormancy break event. The level of ABA in a plant tissue depends on the rate of biosynthesis and catabolism of the hormone (Nambara and Marion-Poll, 2005). The decline in ABA can be explained by the fact that ABA can be degraded by the enzyme abscisic acid 8'-hydroxylase into phaseic acid (PA). Phaseic acid level, however, was not measured in the study. ABA can also be inactivated by glucose conjugation (ABA-glucose ester). The activity of ABA is dependent on the concentration (Hartmann *et al*., 2002); and the threshold concentration has been suggested to be most important for the induction of dormancy (Hilhorst and Toorop, 1997; Biemelt *et al*., 2000). Put together, it may be inferred that while ABA is necessary for the initiation of dormancy, dormancy break and subsequent sprout development is dependent more on the biosynthesis of growth stimulatory hormones than the instantaneous level of ABA. Indeed according to Hill (1980) and Gardner *et al*. (1985), it is the ratio of ABA to GAs that determines dormancy break. This may explain why ABA alone did not show any marked change at dormancy break. Gibberellins, however, could not be measured in this study.

The effect of exogenous ethylene on the content of ABA in sweetpotato was investigated in the present study. This has not been reported before in available literature. Knowledge about the interaction between exogenous ethylene and ABA may deepen understanding of the ethylene-induced dormancy mechanism. Both ethylene and ABA are classified as growth inhibitors and therefore, their actions were expected to be complementary. Ethylene and ABA have been observed to interact in the regulation of other physiological processes, including the expression of certain wound-induced genes and the regulation of leaf abscission (O'Donnell *et al*., 1996). Coleman (1998) noted that a short 24 h treatment with 1.74 µmol L^{-1} ethylene decreased ABA concentration in potato by 4-fold, but this was reversed to normal levels a few hours later. In a recent study, Suttle *et al*. (2013) found that while wounding of potato tubers induced increased biosynthesis of ABA, the application of exogenous ethylene completely suppressed the wound-induced content of ABA and dramatically down-regulated ABA metabolic genes. Consistent with the above observations, it was demonstrated in the present study that exogenous ethylene

significantly decreased the content of ABA in the flesh tissues of sweetpotato. This, together with the aforementioned findings suggests that although they both play a role in dormancy by inhibiting gowth, the interaction between exogenous ethylene and ABA results in the suppression of ABA. In other words, their pathways leading to dormancy may be different. ABA regulates transpirational water use by restricting conductance through the stomata. In the sweetpotato root, ABA may also restrict water availability to the peripheral meristematic buds, and in so doing, retard growth-related activities. Faust *et al*. (1991) related dormancy to the water status in the buds, being found in a more bound state whilst towards the release from dormancy, it becomes free. They suggested that the change in the water status controls the induction of dormancy and later, its release. These proposed changes may be controlled by the levels of ABA. Suppression of ABA by exogenous ethylene may have a limiting effect on transpirational water loss regulation and this, in part, may explain why the application of exogenous ethylene was associated with greater loss of moisture.

Zeatin riboside (ZR) is a growth stimulatory hormone and is associated with dormancy break and sprout growth. Again no literature provides information about the changes in the content of ZR in sweetpotato during transition from the dormancy phase through dormancy break to sprout growth, except Wang *et al*. (2006) who reported the changes in endogenous hormones during the bulking of the roots. Data provided by Wang *et al*. (2006) demonstrated that the content of ZR increases during tuberization of the roots to a peak level at maturity. In potatoes, Hyzyo (1973) and Matsuo *et al*. (1983) reported that the endogenous level of ZR reaches a maximum just after root formation. The present study investigated for the first time, the changes in ZR during postharvest storage of sweetpotato. It was found that after harvest, the content of ZR in the ‘Covington’ consignment ‘NCCov II’ remained at a stable level for at least 40 days during storage in air (**Figure 6.1**). The ZR level in air storage tended to increase thereafter but this was long after dormancy had broken. This suggests the involvement and coordination of ZR with other growth promoting factors in stimulating sprouting. The application of exogenous ethylene significantly increased the content of ZR both before and after dormancy break. Exogenous ethylene was also reported to increase ZR in the basal ends of onion (Downes, 2010). Cytokinins, including ZR, play a role in cell division and the initiation of sprouts and therefore, the ethylene-induced boost of ZR level was expected

to also lead to increased sprout growth. It was noted in experiments 4, 5, and 6 (Chapter 5), however, that sprout elongation was retarded as long as ethylene was present until the ethylene supplementation was truncated, which subsequently provoked the most proliferous sprout growth. Many authors have equally observed that short-term exposure of potato to exogenous ethylene causes premature dormancy break and leads to excessive sprout growth whilst long-term ethylene supplementation inhibits sprout elongation (Rylski *et al*., 1974; Prange *et al*., 1998; Foukaraki, 2010). No explanation has yet been given in available literature regarding this intriguing phenomenon. An important conclusion, which may be drawn from these observations, however, is that short-term application of ethylene may not induce sprout growth directly but most likely, through its interactions with growth promoting substances. It is also important to note that changes in plants are not only stimulated by corresponding changes in the contents of certain plant hormones but also sensitivity of the tissues to the hormones may be the controlling factor rather than the hormonal levels (Trewavas, 1992; Hanke, 2013). Thus the ethylene boost of the content of ZR may not necessarily lead to sprout growth if the tissues are insensitive to ZR. Coleman (1987) stated that cytokinin sensitivity is prevalent at the beginning and end of dormancy and tubers will be less sensitive to cytokinin application between these two periods. In relation to this, the mechanism of sprout growth inhibition by exogenous ethylene appears to be unravelled when the recent finding by Hanke (2013) is taken into consideration. Hanke (2013) found that in potato cv. Majestic, the dormancy period is regulated by tissue sensitivity to growth-promoting cytokinins. The author noted that during potato tuber dormancy, the tissues are insensitive to cytokinins as a result of the accumulation of a cytokinin-sequestering protein. The level of the sequestering protein was found to correlate directly to the duration of dormancy. It was also found that expression of the cytokinin-sequestering protein in other plants such as rice prolonged seed dormancy. Relating the observations by Hanke (2013) to the current study, it is likely that, ZR in sweetpotato is also complexed with the cytokinin-sequestering protein, whose level may be regulated by ethylene. Thus exogenous ethylene may enhance the biosynthesis of both ZR and the cytokinin-sequestering protein such that, the tissues become insensitive to ZR as long as ethylene is present to also stimulate the protein. Removal of ethylene may inactivate or cause a decline in the level of the cytokinin-sequestering protein and subsequently enhance tissue perception of ZR. The excessive

growth after the short-term exogenous ethylene may therefore be the consequence of the high level of ZR already accumulated when ethylene was present. Further studies are, however, required to verify if there is a relationship between exogenous ethylene and the cytokinin-sequestering protein.

6.6 Conclusions

The experiment in this chapter investigated the interaction between exogenous ethylene, ABA and ZR in the control of sweetpotato dormancy. It was hypothesised that the application of exogenous ethylene does not affect the concentrations of ABA and ZR in sweetpotato. The study showed to the contrary, that applying exogenous ethylene suppresses ABA content and increases ZR. ABA suppression by exogenous ethylene may explain why the latter was associated with increased water loss, since ABA is reported to regulate water loss. Whilst the suppression of ABA, which also produces the same sprout inhibition effect as ethylene, presents a complex dichotomy, the ethylene-induced biosynthesis of ZR provides a possible context for explaining why short-term exposure of sweetpotato to ethylene provokes dormancy break and increased sprout growth. It appears that although its level is boosted by exogenous ethylene, the tissues are insensitive to ZR as long as ethylene is present. It is hypothesized that ZR may form a complex with an ethylene-regulated substance, possibly a sequestering protein, which inhibits tissue sensitivity to ZR, until ethylene is removed.

CHAPTER SEVEN: GENERAL DISCUSSION AND CONCLUSIONS

7.1 Introduction

Sweetpotato is produced mainly in the tropical and sub-tropical climates. In tropical developing countries, sweetpotato is valued as an important food security crop because of the relatively high yield, adaptability to a wide range of climatic and soil conditions, and the promising health benefits that may be derived from its consumption. Year round availability of sweetpotato is an essential factor for the crop's contribution to sustained food supply. In tropical regions, however, ambient storage promotes early sprouting, alongside diseases and physiological disorders. Sprout growth negatively affects the marketing of the produce and therefore, the profitability of sweetpotato business. Although low temperature storage (13-15°C, 95% relative humidity) has been demonstrated to be capable of insuring year round availability of sweetpotato (Picha, 1986), farmers in tropical developing countries often do not have access to cold storage infrastructure because of economic and technological constraints. Technological options for sprout inhibition such as pre-harvest spraying with maleic hydrazide (MH) and chlorpropham (CIPC) are also limited by environmental and health concerns associated with the chemical residues.

In this study, the prospects of the use of ethylene supplementation and the anti-ethylene gas 1-methylcyclopropene (1-MCP) to improve the storage of sweetpotato were investigated. Earlier studies by other authors relating to exogenous ethylene supplementation demonstrated its efficacy in inhibiting sprouting in potato and onion without adverse physiological disorders. This has led to the commercial acceptance and use of ethylene for the storage of potato and onion in the UK and Canada. Treatment combination of ethylene and 1-MCP has also been reported to alleviate deleterious effects of applying ethylene alone (Prange *et al*., 2005; Downes *et al*., 2010; Cools *et al*., 2011; Foukaraki *et al.*, 2010; Foukaraki *et al.*, 2011). Sweetpotato shares biological characteristics with potato and onion, being classified together as non-climacteric crops. Therefore it was reasonable to assume that findings relating to the use of ethylene and 1-MCP on onion and potato may also apply to sweetpotato. There is, however, limited

literature about the prospects of ethylene supplementation on sweetpotato, except for a recent study by Cheema *et al*. (2013) who found that continuous ethylene treatment (10µl L^{-1}) could also be a practical sprout control method for sweetpotato in the tropics. With respect to ethylene treatment, however, the ideal timing has not been investigated. Neither have the ethylene mechanism for sprout control and the detailed effects on the physiological and biochemical profiles in sweetpotato been fully investigated. Increasing implication of ethylene as a major plant hormone in plants has created the need for intensified efforts to elucidate its mode of action (Reid and Pratt, 1972). To elucidate the effects of ethylene and 1-MCP on sweetpotato, changes in the spatio-temporal profiles of key metabolites that affect growth and development were analysed in relation to concomitant physiological changes when treated with the respective plant growth regulators. These metabolites include sugars, phenolic compounds and endogenous hormones whilst the physiological and pathological variables were respiration, weight loss, dry weight, sprouting and the incidence of decay. Experiments performed involved treatment of different sweetpotato consignments with 1-MCP (1.0 µl L^{-1}, 24h) and different timings of ethylene supplementation (10 µl L^{-1}) as described in Chapters 4 and 5. In order to be able to administer ethylene at different timings, a novel laboratory scale apparatus, which allows variable ethylene concentrations to be generated for supplementation of crops during storage trials was designed. Also due to the lack of information on individual phenolic compounds in sweetpotato, a method was developed to separate and quantify the compounds in a relatively shorter time compared to other traditional methods of measuring phenolics. The following is a summary of the major findings.

7.2 Respiration

It was evident in all the experiments that sweetpotato exhibited non-climacteric pattern of respiration. The baseline respiration ranged between 13-20 mL CO_2 kg^{-1} h^{-1} but either remained stable or dropped with time to as low as 6 mL CO_2 kg^{-1} h^{-1} depending on cultivar and age after harvest. In general, the old consignments such as 'Organic Covington', 'NCCov I' and 'NCCov III' had fairly stable respiration levels whilst the fresh produce 'TIS-2'and 'NCCov II' had high initial values, which declined with time to basal

respiration levels. This is consistent with the non-climacteric pattern of respiration. According to Cantwell and Suslow (2013) typical sweetpotato respiration rates are 12 and 15 mL CO_2 kg^{-1} h^{-1} for cured and non-cured roots, respectively. The respiration values obtained in the present study are fairly comparable to the above stated range by Cantwell and Suslow (2013). It must be noted, however, that the respiration rate is not stable and depends on the age after harvest, and therefore in stating root respiration rate, it is important to indicate whether it is for fresh produce or previously stored produce.

Many studies show that 1-MCP reduces the respiration of fruit and vegetables (Blankenship and Dole, 2003) such as apple (Xuan and Streif, 2005), peach and nectarine (Shushang *et al*., 2003) and banana (Zhang *et al*., 2006). However, 1-MCP effect has also been reported to be temperature dependent and in most studies, it has worked effectively at temperatures 20-25°C. 1-MCP application at lower temperatures has been effective for only a few crops, and there exists a relationship between temperature, concentration and treatment time (Blankenship and Dole, 2003). In broccoli (*Brassica oleracea*), 1-MCP application produced better results at 20°C than at 5°C (Ku and Wills, 1999). Lowering the storage temperature at the same 1-MCP concentration in apples was also found to reduce the effect on firmness, which led to the hypothesis that lower temperatures might reduce the affinity of the binding site to 1-MCP (Mir *et al.*, 2001). It appears from Experiments 1 to 3 (Chapter 4) of the current study that, at storage temperatures (up to 20°C), 1-MCP (1.0 µl L^{-1}, 24h) does not significantly affect the respiration levels in sweetpotato although there were noticeable changes in other biochemical variables such as phenolics metabolism. When sweetpotato cvs. Bushbuck and Ibees were stored at a relatively higher temperature of 25°C, Cheema *et al*. (2013) found that 1-MCP marginally lowered the respiration rate compared to air storage. The foregoing results suggest that 1-MCP effect on sweetpotato respiration tends to increase with temperature.

The set up in experiments 5 and 6, involving swap treatments from ethylene into air or vice-versa, is a new approach to evaluate ethylene effect on respiration. In contrast to 1-MCP, when ethylene was applied to the three consignments of ‘North Carolina Covington’ at 25°C, their respiration rates were significantly boosted 1.5-2.0 fold. The reciprocity of root respiration when swapped between ethylene and air is a compelling evidence that ethylene induces higher respiration in sweetpotato. The observation that

ethylene promotes tissue respiration is consistent between different reports (Huelin and Barker, 1939; Reid and Pratt, 1972; Buesher, 1975; Kitinoja, 1987; Foukaraki, 2012; Cheema *et al*., 2013).

The biochemical basis for the stimulation of respiration by ethylene, however, has not been definitively elucidated. There are reports that biotic and abiotic stresses such as temperature, wound, and fungi can provoke higher respiration in sweetpotato (Saltveit, 1999; Hyodo *et al*., 2002). Ethylene has been reported to cause tissue wounding in sweetpotato (Stahmann *et al*., 1966; Pratt and Goeschl, 1969; Kitinoja, 1987). This observation is supported by the present study where tissue splitting was inadvertently discovered on the surface of some ethylene treated roots; and may be symptomatic evidence of internal injury. These wounds healed a few days later and the proximal tissues were able to sprout. The process of wound healing requires lignification in the wounded sites. It is thus possible to speculate that, internal injury resulting from tissue expansion could signal the respiratory response to mobilize healing substances. It has also been proposed that the increased respiration may be related to metabolic processes such as protein synthesis, starch hydrolysis or other glycolytic events (Reid and Pratt, 1972). In the present study, the formation of glucose and fructose through starch hydrolysis was not evident as the levels of the monosaccharides declined in the ethylene treatment. Some phenolic compounds such as iso-chlorogenic acid A and C were, however, synthesised in response to ethylene treatment. Saltveit (2000) stated that some wound-induced proteins are enzymes of phenolics metabolism such as phenylalanine ammonia-lyase (PAL), which catalyses the biosynthesis of plant phenylpropanoid products to phenols. Increased PAL activity leads to the accumulation of phenolic compounds like chlorogenic acid and isochlorogenic acids (Whittaker and Lee, 1995; Saltveit, 2000). Biggs (1985) found that accumulation of phenolic compounds is a necessary step in the biosynthesis of suberin. Thus the increased respiration may be part of a cascade of processes signalled by ethylene to synthesize phenolics and other healing substances.

7.3 Sprouting

It was found in the present work that low storage temperature (15-20ºC) is probably sufficient to suppress sprouting in sweetpotato as during Experiments 1, 2 and 3, involving the sweetpotato consignments 'Organic Covington', 'Portuguese-derived Covington' and 'TIS-2', neither the 1-MCP treated roots nor the control roots sprouted during the experimental period. This lack of 1-MCP effect on sprouting may be due to the same reason of low temperature as explained in Section 7.2. Picha (1986) stated that storage of sweetpotato at 13-15ºC and 95% relative humidity is capable of extending the shelf-life up to a year. As far as sprouting is concerned, this was confirmed in the present study. Indeed, low temperature storage at 14ºC is the current practice by Barfoots of Botley, an importer and distributor of fresh sweetpotatoes in the UK (Justin Creasy, Personal Communication). When the potency of the roots was tested by storing some control roots at 25ºC, they sprouted within two weeks. It was thus concluded that the effect of 1-MCP on sweetpotato sprouting may better be evaluated at higher storage temperatures. Evaluating the effect on sprouting at 25ºC, Cheema *et al*. (2013) reported that 1-MCP (625 nl L^{-1}, 24 h) was able to suppress sprout growth in sweetpotato cvs. Ibees and Bushbuck.

In Experiments 4, 5 and 6, the three North Carolina-derived 'Covington' consignments were stored at 25ºC while being supplemented with either ethylene (10 µl L^{-1}) or air at different timings as described in Section 5.4. In all the consignments, continuous ethylene supplementation significantly inhibited sprout growth compared to air storage. This result is in agreement with the findings by previous authors who evaluated the effects of exogenous ethylene on non-climacteric crops like potato, onion and sweetpotato (Prange *et al*., 2008; Foukaraki, *et al*., 2010; Cools *et al*., 2011 and Cheema, *et al*., 2013). The sprout growth inhibition effect of continuous ethylene treatment was observed inadvertently in a trial where ethylene was intended to be used to induce dormancy break in potato (Prange and Daniels-Lake, 2005). Since then, successive trials have led to the commercial use of continuous ethylene treatment for sprout growth inhibition in potato and onion storage. In work done by Cheema *et al*. (2013), continuous ethylene supplementation also inhibited sprout growth in sweetpotato. The authors therefore suggested that ethylene treatment may offer a practical solution for sprout suppression in

sweetpotato during storage in the tropics. An aspect of ethylene supplementation on sweetpotato, which has not been investigated, however, is the ideal timing of exposure. In the UK, two ethylene management systems have been proposed. While the company, BioFresh, recommends exposing the crop to ethylene at the start of storage, another company, Restrain, suggests that the ethylene should be applied just after dormancy break (British Potato Council, 2006). The first reported trial involving different timings of ethylene supplementation was by Foukaraki *et al.* (2010) who found that, in potato, post-dormant ethylene supplementation achieved the same sprout inhibition effect as continuous ethylene from the beginning of storage. To the best of the author's knowledge, the study presented herein, is the first to also investigate the optimal timing of ethylene supplementation on sweetpotato. As found for potato by Foukaraki *et al.* (2010), it was also found in Experiments 4, 5 and 6 that ethylene supplementation, either continuously from the beginning of storage or just after dormancy break significantly inhibited sprout growth compared to air storage. The post-dormant supplementation with ethylene had additional benefits of reducing ethylene-induced rot symptoms and weight loss. These, added to the reduced cost of treatment, means that applying the ethylene just after dormancy break will be able to meet the expectations of technical and economic efficiency.

The study also showed that, as long as ethylene was perceived by the roots, the sprouts failed to elongate to any significant level, but when the ethylene treatment was truncated after dormancy break, and the roots were immediately transferred into air, there was vigorous sprout growth. Sprout growth following short-term ethylene treatment of potato and onion has been previously reported (Kader, 1985; Bufler 2009; Downes, 2010). To date, however, there is no conclusive explanation in available literature for the mechanism by which ethylene inhibits sprout growth and why sprout growth is even more vigorous when ethylene is removed. More intriguing is also the situation whereby exogenous ethylene induces increased respiration without concomitant sprout growth. Cheema *et al.* (2013) hypothesised that the respiratory energy released at the instance of ethylene supplementation is utilized in a different ethylene stimulated process rather than sprout growth. Respiration rate is usually indicative of the rate of metabolic activities within a crop. That said, the ethylene-induced respiration, while inhibiting sprout growth raises queries about the conceptual view of dormant tissues as being in a quiescent state. It

suggests, to the contrary, that exogenous ethylene imposes a form of paradormancy or a pseudo-dormancy whereby there is increased metabolic activity, which leads to dormancy break without the sprouts being able to grow. Indeed, contrary to the traditional view of dormant tissues being in a quiescent state, many biochemical activities are associated with ethylene-induced dormancy and it appears that there is a competitive demand by these activities for the respiratory energy at the expense of sprout growth. Ethylene induced activities include autocatalytic production of copious amounts of ethylene itself (Yang, 1987), which makes use of the respiratory energy in adenine (ATP). Also, as indicated in the results of Experiments 4 and 5, exogenous ethylene stimulates the biosynthesis of phenolic compounds (Saltveit, 1999), possibly for the biosynthesis of the healing substance suberin and lignin for wound repair (Biggs, 1985) or in defence against invading pathogens, which may attack the roots through the loosened tissues. The energy demand for the diverse ethylene induced metabolisms may limit the supply for growth. It was also found in Experiment 6 that among the metabolic processes induced by exogenous ethylene, there was increased biosynthesis of the growth hormone zeatin riboside (ZR). Yet sprout growth was inhibited as long as the exogenous ethylene was present and also inducing other metabolic activities. It may therefore be speculated that, although ethylene triggered increased biosynthesis of ZR, it also inhibited tissue sensitivity to ZR. Ethylene inhibition of the action of cytokinins during tuberization of potatoes has been reported by Dimalla and Staden (1977) and it is possible that ethylene elicits the same effect when applied during the dormancy regime. According to Turnbull and Hanke (1985), tissue sensitivity to cytokinins is important for the regulation of dormancy, while Suttle (2004) added that, an increase in cytokinin sensitivity and content appear to be the principal factors leading to the loss of dormancy. Also Suttle (2001) found that potato tubers reacted to cytokinins in a time dependent manner. More recently, Hanke (2013) found that during potato and rice dormancy, there exists a cytokinin-sequestering protein which inhibits tissue sensitivity to cytokinins. It is thus possible that ethylene also stimulates the biosynthesis of a cytokinin-sequestering protein in sweetpotato. Removal of exogenous ethylene may therefore activate tissue sensitivity to the accumulated ZR, which leads to proliferous sprout growth.

Advances in biochemical and transcriptional analytical methods may also be employed to explain the sprout growth inhibition effect of ethylene (Chope *et al*., 2011). Genetic

studies have found a number of receptors and transcription factors in the ethylene signalling pathway. Elucidation of the signal mechanism involved in ethylene response may offer a useful tool for investigating the control that ethylene exercises in plant tissues (Reid and Prat, 1972). It is thought that a transcription factor known as Ethylene Insensitive 3 (EIN3) is the key regulator of ethylene response and co-ordinates the expression of genes that direct ethylene-mediated growth (Chang *et al*., 2013). Transcriptional studies by Cools *et al.* (2011) showed that in onion, exogenous ethylene down-regulated both a transcript with similarity to an ethylene receptor and the transcriptional regulator EIN3. They therefore suggested that this down-regulation of ethylene perception and signalling events may explain the sprout inhibition effect of exogenous ethylene.

Experiment 6 showed that ABA was suppressed by exogenous ethylene. This is consistent with findings in related studies on potato (Coleman, 1998; Suttle *et al*., 2013). It suggests that although they are both growth inhibitors, the mechanism by which exogenous ethylene inhibits sprout development may be different from that of ABA. It is, however, not known if endogenous ethylene has the same interaction with ABA. Suppression of ABA by exogenous ethylene may be the underlying cause of increased water loss by limiting the regulation of transpirational water loss by ABA.

7.4 Decay and weight loss

Evidence from the present study and a report by Villordon Arthur (2012, Personal communication) indicate that 1-MCP decreases the incidence of root decay in a cultivar-dependent manner. In contrast, exogenously applied ethylene was demonstrated to be associated with higher rot symptoms in all the roots tested. Ethylene-induced rots were noticed to initiate from the proximal root ends where tissue splitting occurred. The proximal tissue splitting in sweetpotato can be a major drawback in its use to inhibit sprouting. Though incidentally discovered and therefore not quantified, the tissue splitting affected only a few roots and the magnitude was cultivar-dependent. The resulting proximal rot is possibly associated with loosening of the tissues with a reduction in mechanical resistance to invading pathogens. Interestingly, 1-MCP was found to be

more effective against roots with proximal rots. The efficacy of 1-MCP in alleviating rot symptoms is likely to be related to its antagonistic role to ethylene. Thus a combined treatment between 1-MCP and ethylene may inhibit ethylene-induced rots. This, however, is subject to verification in a future research.

In the 1-MCP experiments, marketable root weight was measured after removal of decayed roots. In part, weight loss was also as a result of water loss or respiratory conversion of carbohydrates into energy (De Baerdemaeker *et al.*, 1999). Weight reduction due to moisture loss, however, was not estimated independently in the 1-MCP experiments. According to van Oirschot (2000), about 90% of weight loss during the marketing of wholesome roots is attributed to water loss. Weight loss due to respiratory conversion of carbohydrates is related to changes in the dry matter content (De Baerdemaeker *et al.*, 1999). The results in the current study showed that the dry matter proportion (% FW) between the 1-MCP treated and the control roots was not significant. It may thus be concluded that decay was the greatest cause of total marketable weight loss. This is consistent with data presented by van Oirschot (2000), which showed that between 5-15% of total weight loss in sweetpotato was due to respiratory losses, the rest being due to microbial decay. 1-MCP effect on the marketable weight was cultivar dependent and reflects the removal of decayed roots, as it maintained higher marketable weight in ‘Organic’ and ‘Portuguese-derived Covington’ but not in ‘TIS-2’.

In the ethylene experiments, weight loss was measured with respect to moisture and dry matter loss in the wholesome roots (physiological weight loss). The results showed that, the ethylene treatments caused a significantly greater loss of root weight compared to the air control checks. This contrasts with Cheema *et al.* (2011) who found that in the cvs. Bushbuck and Ibees, weight loss was greatest in air treatment but this was associated with the high rate of sprout growth in air. In the present study, the weight loss through dry matter loss was only significant for the fresh consignment ‘NCCOv II’ but not for the others; showing that, in wholesome roots, the contribution of water loss to the total weight loss is much greater. When it is considered that ethylene caused greater root decay, it shows that the total loss of marketable weight due to ethylene treatment can be substantial and offset the benefits of inhibited sprout growth. This highlights the importance of appropriate timing of the ethylene application, as it was shown that, enabling the ethylene

supplementation after dormancy break reduced both the incidence of decay and physiological weight loss of wholesome roots.

7.5 Non-structural carbohydrates

There has not been much literature about the effect of 1-MCP and ethylene on the non-structural carbohydrates in sweetpotato. In the study reported herein, 1-MCP treatment was shown to have no significant effect on the non-structural carbohydrates sucrose, glucose and fructose although 1-MCP is reported to reduce ethylene-induced accumulation of the sugars in potato (Prange *et al.,* 2005; Foukaraki *et al.,* 2011). When stored at 25ºC, data presented by Cheema (2010) indicated that 1-MCP reduced the accumulation of the monosaccharides (glucose and fructose) in sweetpotato cv. Bushbuck. It is unclear if the difference between the present study and that of Cheema is due to cultivar or storage temperature and has to be confirmed in future studies.

In contrast to potato, in which ethylene is reported to increase the biosynthesis of the monosaccharides (Prange *et al.,* 2005; Foukaraki *et al.,* 2011), evidence from the present work supports the finding by Cheema *et al*. (2013) that ethylene appears to induce an opposite effect in sweetpotato. Cheema *et al*. (2013) explained that unlike potato where starch is hydrolised to sugars during the ethylene induced respiration, sweetpotato contains relatively higher concentration of the sugars which are utilized in respiration. With respect to the involvement of the sugars in the dormancy mechanism, a remarkable observation was made in which there was a transitory increase in the monosaccharides during dormancy break in the control roots of the consignment 'NCCov III'. This may probably be related to increased hydrolysis of starch to provide energy for sprout growth. Analysis of the spatial contents of the sugars did not show any significant treatment effect by 1-MCP or ethylene between the proximal, middle or distal sections.

7.6 Phenolic compounds

The spatial distribution of phenolic compounds consistently showed greater abundance in the skin than the flesh tissues. Also, there was proximal dominance of the phenolic

compounds compared to the middle and distal section. This is in agreement with Jung *et al*. (2011) who found greater abundance of phenolic compounds in the proximal root sections. Sprouting of the roots initiates from the proximal ends suggesting a possible involvement of phenolics with sprout development. The effect of 1-MCP on the spatial distribution showed that it greatly suppressed the content of phenolic compounds in the middle and distal sections but much less in the proximal sections. This left a significantly higher balance of phenolic compounds in the proximal root sections. This is the first report regarding the effect of 1-MCP on the phenolic compounds in the tissues of sweetpotato. Phenolic compounds have been associated with defence against diseases. Also diseases in sweetpotato mostly initiated from the proximal or distal ends and therefore, the end-to-end concentration of phenolic compounds may be important for their defensive role. In particular, ethylene-induced rots started from the proximal sections. Thus the dominance of phenolics in the proximal tissues, as stimulated by 1-MCP, may provide a context to explain its mechanistic suppression of ethylene-induced rot symptoms. The higher balance of phenolic compounds in the proximal sections may also play a role in sprout growth as phenolics are found to be conjugated to growth related polyamines.

The total phenolics content was generally boosted by exogenous ethylene. The study revealed further, for the first time, that although ethylene supplementation may enhance the total phenolics in sweetpotato, it does not increase the individual phenolic compounds proportionately. Isochlorogenic acid A was found to be greatest contributor to the phenomenal increase in the total phenolics. Contrary to the hydroxycinnamic acid esters, ethylene supplementation caused a decline in the levels of the hydroxycinnamic acids caffeic and coumaric acids in the skin tissues. This disputes the general thought that ethylene boosts the biosynthesis of all phenolic compounds and suggests that the effect is dependent on the type compound and the matrix effect of the tissue. The possible involvement of phenolic compounds in the dormancy mechanism is suggested by the observation that there was a dramatic increase in all the phenolic compounds after dormancy break, being more evident in the skin than the flesh tissues. Thus if phenolic compounds play a role in dormancy it may be more related to their concentrations in the skin tissues. The phenolic acids in the edible flesh tissues were fairly stable throughout

the storage suggesting that ambient storage of sweetpotato may not significantly change the sensory attributes and nutritional integrity.

7.7 Project conclusions

Based on the three-year study of the effects of 1-MCP and exogenous ethylene supplementation on the physiology and biochemistry of sweetpotato, the following conclusions, in relation to the set objectives in Section 1.2 are presented.

7.7.1 Effect of 1-MCP on the physiology and biochemistry of sweetpotato

- The application of 1-MCP (1 µl L^{-1}, 24h) has no significant effect on the respiration of sweetpotato roots stored at 15-20°C.
- 1-MCP treatment is able to curb the incidence of decay in a cultivar-dependent manner, being more effective against proximal tip rots.
- In sweetpotato, application of 1-MCP causes a decline in the phenolic compounds in the middle and distal root sections but has little or no effect on the proximal sections. This differential effect accentuates proximal dominance in phenolics concentration and may explain why 1-MCP is more effective against proximal rots.
- 1-MCP treatment does not affect the concentration of sugars in sweetpotato across the root sections.

7.7.2 Design of ethylene supplementation apparatus

- A laboratory-scale apparatus was designed to permit the simultaneous supplementation of multi-chamber storage boxes with variable ethylene concentrations and flow rates. This apparatus will enhance future research aimed at investigating the effects of ethylene on sweetpotato and other crops.

7.7.3 Effect of ethylene supplementation on the physiology and biochemistry of sweetpotato

- Ethylene supplementation (10 µl L^{-1}) significantly boosts sweetpotato respiration by *ca.* 1.5-2.0 fold.
- Ethylene-induced respiration in sweetpotato is characterized by initial transient surge to a peak value, followed by non-climacteric decline.
- Despite the increased respiration, sprout growth is inhibited by exogenous ethylene.
- Continuous ethylene supplementation is associated with increased weight loss, which is accounted for mainly by moisture loss.
- Ethylene supplementation can cause proximal tissue splitting.
- Exogenous ethylene is associated with significantly higher incidence of root decay. Ethylene-related decay initiates from the proximal tip (tip rot) and progresses to the distal end.
- The supplementation of sweetpotatoes with ethylene causes a significant decline in the monosaccharides glucose and fructose whilst it increases the content of sucrose. This is the direct opposite of ethylene effect on the sugars in potato.
- Exogenous ethylene differentially mediates the biosynthesis of individual phenolic compounds within the tissues and root sections. The ethylene-related boost in the total phenolics appears to be dominated by an increase in the concentration of isochlorogenic acid A.
- Caffeic and coumaric acids in the skin tissues decline in exogenous ethylene, which disputes the generalised thought that ethylene enhances the biosynthesis of all polyphenols and suggests that the effect is dependent on the compound-tissue matrix.

7.7.4 Effect of different timings of ethylene supplementation on sweetpotato

- Applying exogenous ethylene immediately after dormancy break is as effective as ethylene supplementation throughout the storage as they practically inhibit sprout growth in equal measure. The post-dormant supplementation with ethylene

has additional benefits of reducing ethylene-induced rot symptoms and weight loss. These, added to the reduced cost of treatment, means that applying the ethylene just after dormancy break will be able to meet the expectations of technical and economic efficiency.

- Short-term exposure of sweetpotato to ethylene such as truncating ethylene supplementation after dormancy break, leads to adverse effects of proliferous sprout development.

7.7.5 The mechanisms by which exogenous ethylene mediates dormancy in sweetpotato

- Exogenous ethylene supplementation imposes a form of dormancy in the root tissues in a process by which, contrary to the general understanding of dormant tissues as being in a quiescent state, there is increased respiratory activity. This results in dormancy break but the sprouts become paradormant probably because of competitive demand for the energy for other ethylene-induced processes. Exogenous ethylene induces the biosynthesis of growth-promoting ZR but at the same time, it probably inhibits tissue sensitivity to ZR until the ethylene is removed. When the ethylene treatment is truncated, the tissues become sensitive to the highly accumulated ZR, which leads to proliferous sprout growth.

- ABA levels are suppressed by exogenous ethylene suggesting that although they both control dormancy, their pathways are independent.

- Concomitant with the increased respiration, exogenous ethylene results in reduced concentration of the monosaccharides fructose and glucose in sweetpotato as they are utilized for the respiratory processes.

7.8 Implications for sweetpotato storage in the tropics

The present study has demonstrated the effectiveness of ethylene supplementation as a means to inhibit sprout growth in sweetpotato during storage in the tropics. Ethylene application, however, may undesirably promote high incidence of root decay and increased weight loss. Therefore integrated storage, involving treatment with a suitable insecticide alongside the ethylene application may be useful in curbing root decay. Also, the associated weight loss may be minimised by applying the ethylene immediately after dormancy break. Depending on the cultivar, treatment with 1-MCP during low temperature storage was shown in this study to be capable of reducing both the incidence of decay and weight loss. Reports by other authors have demonstrated that 1-MCP is also effective in inhibiting sprouting. 1-MCP may therefore be applied in combination with ethylene to achieve the best results. The handling of ethylene gas, however, may present a difficulty for tropical farmers. It may therefore be necessary to adopt appropriate technologies to generate ethylene such as the application of ethylene analogues like calcium carbide. The use of calcium carbide in facilitating the ripening of climacteric fruits is common in the tropics. Alternatively, the storage of ethylene-generating fruits like ripening apples or bananas may produce the required ethylene for treatment and has to be confirmed through research.

7.9 Suggestions for future work

a) To evaluate the effect of 1-MCP on sweetpotato roots stored at 25°C. This is to confirm if 1-MCP effect on sweetpotato is temperature dependent.
b) To investigate the effect of exogenous ethylene on sweetpotato roots pre-treated with 24 h 1-MCP (1 µl L^{-1}). The aim is to verify if prior treatment with 1-MCP could reduce the incidence of decay and weight loss caused by exogenous ethylene.
c) To study the relationship between ethylene and the cytokinin sequestering protein as found by Hanke (2013). This may unravel the sprout inhibition effect of ethylene and explain why truncating ethylene supplementation leads to increased sprout growth.

d) To determine the microoganisms associated with ethylene-induced tip rot in sweetpotato so that the roots could be treated with the suitable chemical.

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APPENDIX A

ANOVA TABLES FOR CHAPTER 4

EFFECTS OF 1-METHYLCYCLOPROPENE (1-MCP) ON THE PHYSIOLOGY AND BIOCHEMISTRY OF SWEETPOTATO ROOTS DURING STORAGE

Table A1. ANOVA table for the effect of 1-MCP on decay in 'Organic Covington' (**Figure 4.3**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
Treatment	1	12.5952	12.5952	11.50	0.028
Subject*Time stratum					
Time	6	62.5714	10.4286	44.54	<0.001
Time*Treatment	6	9.2381	1.5397	6.58	0.039
Residual	24	5.6190	0.2341		
Total	41	94.4048			

Table A2. ANOVA table for the effect of 1-MCP on decay in 'Portuguese-derived Covington' (**Figure 4.3**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
Treatment	1	17.5208	17.5208	5.26	0.084
Subject*Time stratum					
Time	7	48.9792	6.9970	13.99	0.005
Time*Treatment	7	16.6458	2.3780	4.76	0.058
Residual	28	14.0000	0.5000		
Total	47	110.4792			

Table A3. ANOVA table for the effect of 1-MCP on decay in 'TIS-2' (**Figure 4.3**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
Treatment	1	148.15	148.15	1.39	0.304
Subject*Time stratum					
Time	4	22384.26	5596.06	122.41	<0.001
Time*Treatment	4	384.26	96.06	2.10	0.197
Residual	16	731.48	45.72		
Total	29	24074.07			

Table A4. ANOVA table for the effect of 1-MCP on percentage weight loss in ‘Organic Covington’ (**Figure 4.5**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
Treatment	1	1239.25	1239.25	9.64	0.036
Subject*Time stratum					
Time	6	5868.14	978.02	76.31	<.001
Time*Treatment	6	560.96	93.49	7.29	0.019
Residual	22	281.96	12.82		
Total	39	8404.10			

Table A5. ANOVA table for the effect of 1-MCP on percentage weight loss in ‘Portuguese-derived Covington’ (**Figure 4.5**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
Treatment	1	164.348	164.348	7.96	0.048
Residual	4	82.626	20.657	5.46	
Subject*Time stratum					
Time	8	3768.477	471.060	124.40	<.001
Time*Treatment	8	170.809	21.351	5.64	0.048
Residual	31	117.382	3.787		
Total	52	4293.748			

Table A6. ANOVA table for the effect of 1-MCP on percentage weight loss in ‘TIS-2’ (**Figure 4.5**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
Treatment	1	108.34	108.34	3.87	0.120
Subject*Time stratum					
Time	4	24705.90	6176.47	236.60	<.001
Time*Treatment	4	150.94	37.73	1.45	0.296
Residual	15	391.58	26.11		
Total	28	23541.12			

Table A7. ANOVA table for the effect of 1-MCP on the respiration of ‘Organic Covington’ (**Figure 4.6**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	1	24.592	24.592	2.62	0.113
Time	3	2.180	0.727	0.08	0.972
Treatment*Time	3	37.313	12.438	1.33	0.280
Residual	40	375.434	9.386		
Total	47	439.520			

Table A8. ANOVA table for the effect of 1-MCP on the respiration of 'Portuguese-derived Covington' (**Figure 4.6**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	284.585	284.585	39.83	<0.001
Baseline*Time	4	730.927	182.732	25.57	<0.001
Baseline*Treatment	1	0.750	0.750	0.10	0.747
Baseline*Time*Treatment	4	26.498	6.625	0.93	0.451
Residual	113	807.416	7.145		
Total	123	1810.758			

Table A9. ANOVA table for the effect of 1-MCP on the respiration of 'TIS-2' (**Figure 4.6**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	71.06	71.06	1.28	0.261
Baseline*Time	3	1142.60	380.87	6.86	<0.001
Baseline*Treatment	2	149.48	74.74	1.35	0.265
Baseline*Time*Treatment	3	315.78	105.26	1.90	0.135
Residual	104	5776.20	55.54		
Total	113	7455.12			

Table A10. ANOVA table for the effect of 1-MCP on the dry weight (% FW) of 'Organic Covington' (**Figure 4.7**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	9.490	9.490	2.55	0.111
Baseline*Time	2	51.462	25.731	6.91	0.001
Baseline*Section	4	7.658	1.914	0.51	0.726
Baseline*Tissue	2	778.285	389.142	104.46	<0.001
Baseline*Treatment	1	19.057	19.057	5.12	0.024
Baseline*Time*Section	4	24.402	6.101	1.64	0.164
Baseline*Time*Tissue	2	15.380	7.690	2.06	0.129
Baseline*Section*Tissue	4	3.493	0.873	0.23	0.919
Baseline*Time*Treatment	2	28.443	14.221	3.82	0.023
Baseline*Section*Treatment	2	2.170	1.085	0.29	0.748
Baseline*Tissue*Treatment	1	0.037	0.037	0.01	0.921
Baseline*Time*Section*Tissue	4	2.366	0.592	0.16	0.959
Baseline*Time*Section*Treatment	4	2.138	0.534	0.14	0.966
Baseline*Time*Tissue*Treatment	2	4.701	2.351	0.63	0.533
Baseline*Section*Tissue*Treatment	2	1.247	0.624	0.17	0.846
Baseline*Time*Section*Tissue*Treatment	4	1.783	0.446	0.12	0.975
Residual	336	1251.735	3.725		
Total	377	2203.845			

Table A11. ANOVA table for the effect of 1-MCP on the dry weight (%FW) of 'Portuguese-derived Covington' (**Figure 4.8**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	34.332	34.332	6.09	0.014
Baseline*Time	4	39.163	9.791	1.74	0.143
Baseline*Tissue	2	617.597	308.799	54.74	<0.001
Baseline*Treatment	1	39.179	39.179	6.95	0.009
Baseline*Outturn*Tissue	4	25.828	6.457	1.14	0.336
Baseline*Outturn*Treatment	4	22.534	5.634	1.00	0.409
Baseline*Tissue*Treatment	1	2.704	2.704	0.48	0.489
Baseline*Outturn*Tissue*Treatment	4	5.978	1.494	0.26	
0.900					
Residual	248	1399.048	5.641		
Total	269	2147.296			

Table A12. ANOVA table for the effect of 1-MCP on the dry weight (% FW) of 'TIS-2' (**Figure 4.9**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	130.249	130.249	35.31	<0.001
Baseline*Outturn	3	311.531	103.844	28.15	<0.001
Baseline*Tissue	2	1750.291	875.145	237.21	<0.001
Baseline*Treatment	1	7.878	7.878	2.14	0.145
Baseline*Outturn*Tissue	3	20.282	6.761	1.83	0.142
Baseline*Outturn*Treatment	3	5.263	1.754	0.48	0.700
Baseline*Tissue*Treatment	1	2.807	2.807	0.76	0.384
Baseline*Outturn*Tissue*Treatment	3	8.459	2.820	0.76	0.515
Residual	204	752.607	3.689		
Total	221	2952.779			

Table A13. ANOVA table for the effect of 1-MCP on the total phenolics concentration in the skin of 'Organic Covington' (**Figure 4.10**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	112098.0	112098.0	7.08	0.009
Baseline*Treatment	1	21437.0	21437.0	1.35	0.246
Baseline*Section	4	482624.0	120656.0	7.62	<0.001
Baseline*Time	2	1309346.0	654673.0	41.33	<0.001
Baseline*Treatment*Section	2	1178.0	589.0	0.04	0.964
Baseline*Treatment*Time	2	74416.0	37208.0	2.35	0.099
Baseline*Section*Time	4	168293.0	42073.0	2.66	0.035
Baseline*Treatment*Section*Time	4	4279.0	1070.0	0.07	0.992
Residual	168	2660866.0	15838.0		
Total	188	4834537.0			

Table A14. ANOVA table for the effect of 1-MCP on the total phenolics concentration in the flesh of 'Organic Covington' (**Figure 4.10**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	384.13	384.13	6.73	0.010
Baseline*Treatment	1	628.20	628.20	11.01	0.001
Baseline*Section	4	2793.12	698.28	12.24	<0.001
Baseline*Time	2	1561.18	780.59	13.68	<0.001
Baseline*Treatment*Section	2	49.73	24.86	0.44	0.648
Baseline*Treatment*Time	2	1101.55	550.77	9.65	<0.001
Baseline*Section*Time	4	182.12	45.53	0.80	0.528
Baseline*Treatment*Section*Time	4	643.11	160.78	2.82	0.027
Residual	168	9586.08	57.06		
Total	188	16929.22			

Table A15. ANOVA table for the effect of 1-MCP on the concentration of chlorogenic acid in the skin of 'Organic Covington' (**Figure 4.12**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	5421.8	5421.8	7.11	0.008
Baseline*Time	2	23624.0	11812.0	15.49	<0.001
Baseline*Section	4	30662.0	7665.5	10.05	<0.001
Baseline*Treatment	1	228.1	228.1	0.30	0.585
Baseline*Outturn*Section	4	1981.6	495.4	0.65	0.628
Baseline*Outturn*Treatment	2	11360.7	5680.3	7.45	<0.001
Baseline*Section*Treatment	2	1648.4	824.2	1.08	0.342
Baseline*Outturn*Section*Treatment	4	3770.4	942.6	1.24	0.298
Residual	168	128141.2	762.7		
Total	188	206838.2			

Table A16. ANOVA table for the effect of 1-MCP on the concentration of chlorogenic acid in the flesh of 'Organic Covington' (**Figure 4.12**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	391.33	391.33	18.30	<0.001
Baseline*Time	2	444.50	222.25	10.39	<0.001
Baseline*Section	4	1147.21	286.80	13.41	<0.001
Baseline*Treatment	1	149.96	149.96	7.01	0.009
Baseline*Time*Section	4	53.61	13.40	0.63	0.644
Baseline*Time*Treatment	2	522.50	261.25	12.22	<0.001
Baseline*Section*Treatment	2	15.57	7.79	0.36	0.695
Baseline*Time*Section*Treatment	4	173.00	43.25	2.02	0.094
Residual	168	3593.06	21.39		
Total	188	6490.73			

Table A17. ANOVA table for the effect of 1-MCP on the concentration of isochlorogenic acid A in the skin of 'Organic Covington' (**Figure 4.13**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	24997.0	24997.	6.69	0.011
Baseline*Time	2	137319.0	68660.	18.37	<0.001
Baseline*Section	4	141056.0	35264.	9.43	<0.001
Baseline*Treatment	1	16775.0	16775.	4.49	0.036
Baseline*Time*Section	4	8347.0	2087.	0.56	0.693
Baseline*Time*Treatment	2	19433.0	9717.	2.60	0.077
Baseline*Section*Treatment	2	647.0	324.	0.09	0.917
Baseline*Time*Section*Treatment	4	6534.0	1633.	0.44	0.782
Residual	168	628029.0	3738.		
Total	188	983137.0			

Table A18. ANOVA table for the effect of 1-MCP on the concentration of isochlorogenic acid A in the flesh of 'Organic Covington' (**Figure 4.13**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	90.155	90.155	12.73	<0.001
Baseline*Time	2	220.068	110.034	15.54	<0.001
Baseline*Section	4	266.030	66.508	9.39	<0.001
Baseline*Treatment	1	95.995	95.995	13.55	<0.001
Baseline*Time*Section	4	29.985	7.496	1.06	0.379
Baseline*Time*Treatment	2	74.409	37.204	5.25	0.006
Baseline*Section*Treatment	2	10.323	5.161	0.73	0.484
Baseline*Time*Section*Treatment	4	88.881	22.220	3.14	0.016
Residual	168	1189.930	7.083		
Total	188	2065.775			

Table A19. ANOVA table for the effect of 1-MCP on the concentration of isochlorogenic acid B in the skin of 'Organic Covington' (**Figure 4.14**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	1947.4	1947.4	8.03	0.005
Baseline*Time	2	6918.5	3459.2	14.26	<0.001
Baseline*Section	4	9784.5	2446.1	10.09	<0.001
Baseline*Treatment	1	460.4	460.4	1.90	0.170
Baseline*Time*Section	4	862.5	215.6	0.89	0.472
Baseline*Time*Treatment	2	2752.8	1376.4	5.68	0.004
Baseline*Section*Treatment	2	15.4	7.7	0.03	0.969
Baseline*Time*Section*Treatment		371.0	92.7	0.38	0.821
Residual	168	40743.2	242.5		
Total	188	63855.6			

Table A20. ANOVA table for the effect of 1-MCP on the concentration of isochlorogenic acid B in the flesh of 'Organic Covington' (**Figure 4.14**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	3.1145	3.1145	6.96	0.009
Baseline*Time	2	3.2407	1.6203	3.6	0.029
Baseline*Section	4	7.1421	1.7855	3.99	0.004
Baseline*Treatment	1	5.2043	5.2043	11.62	<0.001
Baseline*Time*Section	4	1.2757	0.3189	0.7	0.585
Baseline*Time*Treatment	2	3.4553	1.7276	3.86	0.023
Baseline*Section*Treatment	2	0.1584	0.0792	0.18	0.838
Baseline*Time*Section*Treatment	4	3.3888	0.8472	1.89	0.114
Residual	168	75.2106	0.4477		
Total	188	102.1904			

Table A21. ANOVA table for the effect of 1-MCP on the concentration of isochlorogenic acid C in the skin of 'Organic Covington' (**Figure 4.15**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	2852.8	2852.8	12.53	<0.001
Baseline*Time	2	5257.5	2628.8	11.55	<0.001
Baseline*Section	4	11036.0	2759.0	12.12	<0.001
Baseline*Treatment	1	97.9	97.9	0.43	0.513
Baseline*Time*Section	4	1225.0	306.3	1.35	0.255
Baseline*Time*Treatment	2	2407.0	1203.5	5.29	0.006
Baseline*Section*Treatment	2	130.4	65.2	0.29	0.751
Baseline*Time*Section*Treatment	4	427.3	106.8	0.47	0.758
Residual	168	38245.5	227.7		
Total	188	61679.4			

Table A22. ANOVA table for the effect of 1-MCP on the concentration of isochlorogenic acid C in the flesh of 'Organic Covington' (**Figure 4.15**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	90.6292	90.6292	281.79	<0.001
Baseline*Time	2	0.2523	0.1262	0.39	0.676
Baseline*Section	4	1.5364	0.3841	1.19	0.315
Baseline*Treatment	1	0.0577	0.0577	0.18	0.673
Baseline*Time*Section	4	0.1876	0.0469	0.15	0.965
Baseline*Time*Treatment	2	0.2298	0.1149	0.36	0.700
Baseline*Section*Treatment	2	0.0197	0.0099	0.03	0.970
Baseline*Time*Section*Treatment	4	0.3758	0.0940	0.29	0.883
Residual	168	54.0318	0.3216		
Total	188	147.3203			

Table A23. ANOVA table for the effect of 1-MCP on the concentration of caffeic acid in the skin of 'Organic Covington' (**Figure 4.16**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	7847.9	7847.9	9.08	0.003
Baseline*Time	2	11989.2	5994.6	6.93	0.001
Baseline*Section	4	13112.9	3278.2	3.79	0.006
Baseline*Treatment	1	6366.7	6366.7	7.36	0.007
Baseline*Time*Section	4	1910.9	477.7	0.55	0.697
Baseline*Time*Treatment	2	4017.6	2008.8	2.32	0.101
Baseline*Section*Treatment	2	1346.1	673.0	0.78	0.461
Baseline*Time*Section*Treatment	4	4177.2	1044.3	1.21	0.309
Residual	168	145250.8	864.6		
Total	188	196019.1			

Table A24. ANOVA table for the effect of 1-MCP on the concentration of caffeic acid in the flesh of 'Organic Covington' (**Figure 4.16**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	0.34293	0.34293	8.13	0.005
Baseline*Time	2	0.71515	0.35758	8.48	<0.001
Baseline*Section	4	1.08556	0.27139	6.43	<0.001
Baseline*Treatment	1	0.19717	0.19717	4.67	0.032
Baseline*Time*Section	4	0.14354	0.03589	0.85	0.495
Baseline*Time*Treatment	2	0.09931	0.04966	1.18	0.311
Baseline*Section*Treatment	2	0.12419	0.06209	1.47	0.232
Baseline*Time*Section*Treatment	4	0.03540	0.00885	0.21	0.933
Residual	168	7.08810	0.04219		
Total	188	9.83135			

Table A25. ANOVA table for the effect of 1-MCP on chlorogenic acid concentration in 'Portuguese-derived Covington' (**Figure 4.17**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	27.9	27.9	0.06	0.810
Baseline*Time	3	5374.9	1791.6	3.72	0.012
Baseline*Tissue	2	420355.2	210177.6	436.70	<0.001
Baseline*Treatment	1	250.6	250.6	0.52	0.471
Baseline*Time*Tissue	3	3253.3	1084.4	2.25	0.084
Baseline*Time*Treatment	3	382.3	127.4	0.26	0.851
Baseline*Tissue*Treatment	1	407.7	407.7	0.85	0.359
Baseline*Time*Tissue*Treatment	3	222.7	74.2	0.15	0.927
Residual	186	89519.7	481.3		
Total	203	499890.4			

Table A26. ANOVA table for the effect of 1-MCP on the concentration of isochlorogenic acid A in 'Portuguese-derived Covington' (**Figure 4.17**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	38123.0	38123.0	14.59	<0.001
Baseline*Time	3	132761.0	44254.0	16.94	<0.001
Baseline*Tissue	2	1822647.0	911323.0	348.82	<0.001
Baseline*Treatment	1	9.0	9.0	0.00	0.953
Baseline*Time*Tissue	3	123728.0	41243.0	15.79	<0.001
Baseline*Time*Treatment	3	2399.0	800.0	0.31	0.821
Baseline*Time*Treatment	1	25.0	25.0	0.01	0.922
Baseline*Time*Tissue*Treatment	3	2463.0	821.0	0.31	0.815
Residual	185	483331.0	2613.0		
Total	203	2530178.0			

Table A27. ANOVA table for the effect of 1-MCP on the concentration of isochlorogenic acid B in 'Portuguese-derived Covington' (**Figure 4.17**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	2066.21	2066.21	38.51	<0.001
Baseline*Time	3	5691.95	1897.32	35.37	<0.001
Baseline*Tissue	2	29052.42	14526.21	270.77	<0.001
Baseline*Treatment	1	83.89	83.89	1.56	0.213
Baseline*Time*Tissue	3	5343.16	1781.05	33.20	<0.001
Baseline*Time*Treatment	3	70.92	23.64	0.44	0.724
Baseline*Tissue*Treatment	1	124.78	124.78	2.33	0.129
Baseline*Time*Tissue*Treatment	3	100.71	33.57	0.63	0.599
Residual	186	9978.60	53.65		
Total	203	51653.81			

Table A28. ANOVA table for the effect of 1-MCP on the concentration of isochlorogenic acid C in 'Portuguese-derived Covington' (**Figure 4.17**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	1439.52	1439.52	31.73	<0.001
Baseline*Time	3	3519.11	1173.04	25.86	<0.001
Baseline*Tissue	2	20543.84	10271.92	226.43	<0.001
Baseline*Treatment	1	1.84	1.84	0.04	0.841
Baseline*Outturn*Tissue	3	3468.29	1156.10	25.48	<0.001
Baseline*Outturn*Treatment	3	33.56	11.19	0.25	0.864
Baseline*Tissue*Treatment	1	1.94	1.94	0.04	0.836
Baseline*Outturn*Tissue*Treatment	3	30.08	10.03	0.22	0.882
Residual	186	8437.83	45.36		
Total	203	36902.33			

Table A29. ANOVA table for the effect of 1-MCP on the concentration of caffeic acid in 'Portuguese-derived Covington' (**Figure 4.17**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	1150.38	1150.38	59.71	<0.001
Baseline*Time	3	3515.00	1171.67	60.81	<0.001
Baseline*Tissue	2	23796.25	11898.13	617.56	<0.001
Baseline*Treatment	1	77.07	77.07	4.00	0.047
Baseline*Time*Tissue	3	3516.78	1172.26	60.85	<0.001
Baseline*Time*Treatment	3	146.34	48.78	2.53	0.058
Baseline*Tissue*Treatment	1	77.07	77.07	4.00	0.047
Baseline*Time*Tissue*Treatment	3	146.34	48.78	2.53	0.058
Residual	186	3583.53	19.27		
Total	203	35350.73			

Table A30. ANOVA table for the effect of 1-MCP on the concentration of fructose in the tissues of 'Organic Covington' (**Figure 4.18**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	51.7390	51.7390	64.79	<0.001
Baseline*Time	2	119.3116	59.6558	74.70	<0.001
Baseline*Tissue	2	482.1881	241.0941	301.90	<0.001
Baseline*Treatment	1	0.0060	0.0060	0.01	0.931
Baseline*Time*Tissue	2	8.9629	4.4815	5.61	0.004
Baseline*Treatment*Tissue	1	0.0016	0.0016	0.00	0.965
Baseline*Time*Treatment	2	2.6702	1.3351	1.67	0.189
Baseline*Time*Treatment*Tissue	2	0.2119	0.1059	0.13	0.876
Residual	364	290.6886	0.7986		
Total	377	955.7798			

Table A31. ANOVA table for the effect of 1-MCP on the concentration of glucose in the tissues of 'Organic Covington' (**Figure 4.18**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	61.841	61.841	25.46	<0.001
Baseline*Time	2	58.047	29.024	11.95	<0.001
Baseline*Tissue	2	1977.920	988.960	407.15	<0.001
Baseline*Treatment	1	1.025	1.025	0.42	0.516
Baseline*Time*Tissue	2	13.146	6.573	2.71	0.068
Baseline*Time*Treatment	2	12.117	6.058	2.49	0.084
Baseline*Treatment*Tissue	1	0.041	0.041	0.02	0.897
Baseline*Time*Treatment*Tissue	2	4.155	2.078	0.86	0.426
Residual	364	884.157	2.429		
Total	377	3012.450			

Table **A32**. ANOVA table for the effect of 1-MCP on the concentration of sucrose in the tissues of 'Organic Covington' (**Figure 4.18**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	556.7	556.7	3.37	0.067
Baseline*Time	2	20.8	10.4	0.06	0.939
Baseline*Tissue	2	10701.7	5350.9	32.41	<0.001
Baseline*Treatment	1	253.8	253.8	1.54	0.216
Baseline*Time*Tissue	2	5.3	2.7	0.02	0.984
Baseline*Treatment*Tissue	1	18.5	18.5	0.11	0.738
Baseline*Time*Treatment	2	323.7	161.9	0.98	0.376
Baseline*Time*Treatment*Tissue	2	8.4	4.2	0.03	0.975
Residual	364	60101.1	165.1		
Total	377	71990.1			

Table A33. ANOVA table for the effect of 1-MCP on the concentration of maltose in the tissues of 'Organic Covington' (**Figure 4.18**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	10.8982	10.8982	14.63	<0.001
Baseline*Time	2	46.4755	23.2377	31.20	<0.001
Baseline*Tissue	2	8.4829	4.2414	5.70	0.004
Baseline*Treatment	1	1.9817	1.9817	2.66	0.104
Baseline*Time*Tissue	2	1.6710	0.8355	1.12	0.327
Baseline*Time*Treatment	2	0.9147	0.4573	0.61	0.542
Baseline*Treatment*Tissue	1	0.0256	0.0256	0.03	0.853
Baseline*Time*Treatment*Tissue	2	0.1360	0.0680	0.09	0.913
Residual	364	271.0934	0.7448		
Total	377	341.6790			

Table A34. ANOVA table for the effect of 1-MCP on fructose concentration in the sections of 'Organic Covington' (**Figure 4.19**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	51.739	51.739	24.59	<0.001
Baseline*Time	2	119.312	59.656	28.35	<0.001
Baseline*Treatment	1	0.006	0.006	0.00	0.958
Baseline*Section	4	27.465	6.866	3.26	0.012
Baseline*Time*Treatment	2	2.670	1.335	0.63	0.531
Baseline*Time*Section	4	1.939	0.485	0.23	0.921
Baseline*Treatment*Section	2	0.306	0.153	0.07	0.930
Baseline*Time*Treatment*Section	4	1.126	0.281	0.13	0.970
Residual	357	751.218	2.104		
Total	377	955.780			

Table A35. ANOVA table for the effect of 1-MCP on glucose concentration in the sections of 'Organic Covington' (**Figure 4.19**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	61.841	61.841	7.82	0.005
Baseline*Time	2	58.047	29.024	3.67	0.026
Baseline*Treatment	1	1.025	1.025	0.13	0.719
Baseline*Section	4	46.391	11.598	1.47	0.212
Baseline*Time*Treatment	2	12.117	6.058	0.77	0.466
Baseline*Time*Section	4	4.292	1.073	0.14	0.969
Baseline*Treatment*Section	2	0.380	0.190	0.02	0.976
Baseline*Time*Treatment*Section	4	5.642	1.411	0.18	0.949
Residual	357	2822.716	7.907		
Total	377	3012.450			

Table A36. ANOVA table for the effect of 1-MCP on sucrose concentration in the sections of 'Organic Covington' (**Figure 4.19**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	556.7	556.7	2.88	0.090
Baseline*Time	2	20.8	10.4	0.05	0.948
Baseline*Treatment	1	253.8	253.8	1.31	0.252
Baseline*Section	4	1229.1	307.3	1.59	0.176
Baseline*Time*Treatment	2	323.7	161.9	0.84	0.433
Baseline*Time*Section	4	525.5	131.4	0.68	0.606
Baseline*Treatment*Section	2	75.7	37.9	0.20	0.822
Baseline*Time*Treatment*Section	4	76.0	19.0	0.10	0.983
Residual	357	68928.8	193.1		
Total	377	71990.1			

Table A37. ANOVA table for the effect of 1-MCP on maltose concentration in the sections of 'Organic Covington' (**Figure 4.19**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	10.8982	10.8982	16.04	<0.001
Baseline*Time	2	46.4755	23.2377	34.20	<0.001
Baseline*Treatment	1	1.9817	1.9817	2.92	0.089
Baseline*Section	4	27.7279	6.9320	10.20	<0.001
Baseline*Time*Treatment	2	0.9147	0.4573	0.67	0.511
Baseline*Time*Section	4	5.2687	1.3172	1.94	0.104
Baseline*Treatment*Section	2	1.7797	0.8898	1.31	0.271
Baseline*Time*Treatment*Section	4	4.0369	1.0092	1.49	0.206
Residual	357	242.5957	0.6795		
Total	377	341.6790			

Table A38. ANOVA table for the effect of 1-MCP on fructose concentration in the tissues of 'Portuguese-derived Covington' (**Figure 4.20**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	187.671	187.671	24.47	<0.001
Baseline*Time	3	828.144	276.048	36.00	<0.001
Baseline*Treatment	1	1.016	1.016	0.13	0.716
Baseline*Tissue	2	2278.222	1139.111	148.55	<0.001
Baseline*Time*Treatment	3	12.322	4.107	0.54	0.658
Baseline*Time*Tissue	3	210.461	70.154	9.15	<0.001
Baseline*Treatment*Tissue	1	0.024	0.024	0.00	0.955
Baseline*Time*Treatment*Tissue	3	2.068	0.689	0.09	0.966
Residual	186	1426.252	7.668		
Total	203	4946.178			

Table A39. ANOVA table for the effect of 1-MCP on glucose concentration in the tissues of 'Portuguese-derived Covington' (**Figure 4.20**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	232.969	232.969	27.48	<0.001
Baseline*Time	3	1169.907	389.969	46.00	<0.001
Baseline*Treatment	1	0.921	0.921	0.11	0.742
Baseline*Tissue	2	3454.453	1727.226	203.72	<0.001
Baseline*Time*Treatment	3	9.644	3.215	0.38	0.768
Baseline*Time*Tissue	3	148.303	49.434	5.83	<0.001
Baseline*Treatment*Tissue	1	0.820	0.820	0.10	0.756
Baseline*Time*Treatment*Tissue	3	1.190	0.397	0.05	0.987
Residual	186	1576.978	8.478		
Total	203	6595.184			

Table A40. ANOVA table for the effect of 1-MCP on sucrose concentration in the tissues of 'Portuguese-derived Covington' (**Figure 4.20**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	2716.9	2716.9	17.43	<0.001
Baseline*Time	3	169.7	56.6	0.36	0.780
Baseline*Treatment	1	411.1	411.1	2.64	0.106
Baseline*Tissue	2	961.9	480.9	3.09	0.048
Baseline*Time*Treatment	3	1643.8	547.9	3.51	0.016
Baseline*Time*Tissue	3	478.5	159.5	1.02	0.384
Baseline*Treatment*Tissue	1	195.4	195.4	1.25	0.264
Baseline*Time*Treatment*Tissue	3	141.7	47.2	0.30	0.823
Residual	186	28995.8	155.9		
Total	203	35714.6			

APPENDIX B

ANOVA TABLES FOR CHAPTER 5

EFFECT OF DIFFERENT TIMINGS OF EXOGENOUS ETHYLENE SUPPLEMENTATION ON THE PHYSIOLOGY AND BIOCHEMISTRY OF SWEETPOTATO ROOTS DURING STORAGE

Table GS. GenStat structure used to create ANOVA tables for statistical analysis of dry weight, sugars and phenolics in Experiments 5 and 6 ('NCCov II' and 'NCCov III')

GenStat analysis structure: Tissue*Section*Baseline/(P1*T1/(T2*P2))

T1 = Outturns before swap treatments

T2 = Outturns after swap treatments

P1 = Treatments before swap

P2 = Treatments after swap

P = Proximal sections

D = Distal sections

F = Flesh tissue

S = Skin tissue

Outturn	Treatment	Baseline	Section	Tissue	T1	T2	P1	P2
0	B4 storage	Yes	P	F	0	0	B4 storage	B4 swap
0	B4 storage	Yes	P	S	0	0	B4 storage	B4 swap
0	B4 storage	Yes	D	F	0	0	B4 storage	B4 swap
0	B4 storage	Yes	D	S	0	0	B4 storage	B4 swap
1	Air	No	P	F	1	0	Air P1	B4 swap
1	Air	No	P	S	1	0	Air P1	B4 swap
1	Air	No	D	F	1	0	Air P1	B4 swap
1	Air	No	D	S	1	0	Air P1	B4 swap
1	Ethy	No	P	F	1	0	Ethy P1	B4 swap
1	Ethy	No	P	S	1	0	Ethy P1	B4 swap
1	Ethy	No	D	F	1	0	Ethy P1	B4 swap
1	Ethy	No	D	S	1	0	Ethy P1	B4 swap
2	Air	No	P	F	2	0	Air P1	B4 swap
2	Air	No	P	S	2	0	Air P1	B4 swap
2	Air	No	D	F	2	0	Air P1	B4 swap
2	Air	No	D	S	2	0	Air P1	B4 swap
2	Ethy	No	P	F	2	0	Ethy P1	B4 swap
2	Ethy	No	P	S	2	0	Ethy P1	B4 swap
2	Ethy	No	D	F	2	0	Ethy P1	B4 swap
2	Ethy	No	D	S	2	0	Ethy P1	B4 swap
3	Air	No	P	F	3	0	Air P1	B4 swap
3	Air	No	P	S	3	0	Air P1	B4 swap
3	Air	No	D	F	3	0	Air P1	B4 swap

3	Air	No	D	S	3	0	Air P1	B4 swap
3	Ethy	No	P	F	3	0	Ethy P1	B4 swap
3	Ethy	No	P	S	3	0	Ethy P1	B4 swap
3	Ethy	No	D	F	3	0	Ethy P1	B4 swap
3	Ethy	No	D	S	3	0	Ethy P1	B4 swap
4	Air Air	No	P	F	0	1	Air P1	Air P2
4	Air Air	No	P	S	0	1	Air P1	Air P2
4	Air Air	No	D	F	0	1	Air P1	Air P2
4	Air Air	No	D	S	0	1	Air P1	Air P2
4	Air Ethy	No	P	F	0	1	Air P1	Ethyl P2
4	Air Ethy	No	P	S	0	1	Air P1	Ethyl P2
4	Air Ethy	No	D	F	0	1	Air P1	Ethyl P2
4	Air Ethy	No	D	S	0	1	Air P1	Ethyl P2
4	Ethy Air	No	P	F	0	1	Ethy P1	Air P2
4	Ethy Air	No	P	S	0	1	Ethy P1	Air P2
4	Ethy Air	No	D	F	0	1	Ethy P1	Air P2
4	Ethy Air	No	D	S	0	1	Ethy P1	Air P2
4	Ethy Ethy	No	P	F	0	1	Ethy P1	Ethyl P2
4	Ethy Ethy	No	P	S	0	1	Ethy P1	Ethyl P2
4	Ethy Ethy	No	D	F	0	1	Ethy P1	Ethyl P2
4	Ethy Ethy	No	D	S	0	1	Ethy P1	Ethyl P2
5	Air Air	No	P	F	0	2	Air P1	Air P2
5	Air Air	No	P	S	0	2	Air P1	Air P2
5	Air Air	No	D	F	0	2	Air P1	Air P2
5	Air Air	No	D	S	0	2	Air P1	Air P2
5	Air Ethy	No	P	F	0	2	Air P1	Ethyl P2
5	Air Ethy	No	P	S	0	2	Air P1	Ethyl P2
5	Air Ethy	No	D	F	0	2	Air P1	Ethyl P2
5	Air Ethy	No	D	S	0	2	Air P1	Ethyl P2
5	Ethy Air	No	P	F	0	2	Ethy P1	Air P2
5	Ethy Air	No	P	S	0	2	Ethy P1	Air P2
5	Ethy Air	No	D	F	0	2	Ethy P1	Air P2
5	Ethy Air	No	D	S	0	2	Ethy P1	Air P2
5	Ethy Ethy	No	P	F	0	2	Ethy P1	Ethyl P2
5	Ethy Ethy	No	P	S	0	2	Ethy P1	Ethyl P2
5	Ethy Ethy	No	D	F	0	2	Ethy P1	Ethyl P2
5	Ethy Ethy	No	D	S	0	2	Ethy P1	Ethyl P2

Table B1. ANOVA table for the effect of ethylene on the respiration of the Covington consignment 'NCCov I' (**Figure 5.7**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	1	871.94	871.94	13.27	<0.001
Time	4	711.40	177.85	2.71	0.034
Treatment*Time	4	699.43	174.86	2.66	0.036
Residual	110	7229.22	65.72		
Total	119	9511.99			

Table B2. ANOVA table for the effect of ethylene on the respiration of the Covington consignment 'NCCov II' (**Figure 5.7**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	180.314	180.314	18.76	<0.001
Baseline.P1	1	1387.892	1387.892	144.41	<0.001
Baseline.T1	3	3672.335	1224.112	127.37	<0.001
Baseline.P1.T1	3	982.423	327.474	34.07	<0.001
Baseline.T1.T2	1	20.145	20.145	2.10	0.149
Baseline.P1.P2	2	300.749	150.374	15.65	<0.001
Baseline.P1.T1.T2	1	6.092	6.092	0.63	0.427
Baseline.T1.T2.P2	1	6.077	6.077	0.63	0.428
Baseline.P1.T1.T2.P2	1	1.056	1.056	0.11	0.741
Residual	176	1691.534	9.611		
Total	190	8196.183			

Table B3. ANOVA table for the effect of ethylene on the respiration of the Covington consignment 'NCCov III' (**Figure 5.7**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	636.04	636.04	12.22	<0.001
Baseline.P1	1	1711.30	1711.30	32.88	<0.001
Baseline.T1	2	749.77	374.88	7.20	0.001
Baseline.P1.T1	2	76.88	38.44	0.74	0.480
Baseline.T1.T2	1	209.02	209.02	4.02	0.047
Baseline.T1.P2	1	211.21	211.21	4.06	0.046
Baseline.P1.T1.T2	1	66.92	66.92	1.29	0.259
Baseline.P1.T1.P2	1	19.74	19.74	0.38	0.539
Baseline.T1.T2.P2	1	215.63	215.63	4.14	0.044
Baseline.P1.T1.T2.P2	1	40.94	40.94	0.79	0.377
Residual	138	7181.84	52.04		
Total	150	10890.30			

Table B4. ANOVA table for the effect of ethylene on the weight loss of the Covington consignment 'NCCov I' (**Figure 5.8**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
Treatment	1	1271.44	1271.44	6.26	0.014
Subject*Time stratum					
Time	6	18468.87	3078.14	290.34	<0.001
Time*Treatment	6	533.12	88.85	8.38	<0.001
Residual	376	3986.27	10.60		
Total	468	31645.54			

Table B5. ANOVA table for the effect of ethylene on the weight loss of the Covington consignment 'NCCov II' (**Figure 5.8**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
Treatment	3	276.8946	92.2982	22.96	<0.001
Subject*Time stratum					
Time	6	3078.5573	513.0929	1117.14	<0.001
Time*Treatment	18	177.8684	9.8816	21.51	<0.001
Residual	473	217.2456	0.4593		
Total	580	3985.9545			

Table B6. ANOVA table for the effect of ethylene on the weight loss of the Covington consignment 'NCCov III' (**Figure 5.8**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
Treatment	3	36.5116	12.1705	5.92	<0.001
Subject*Time stratum					
Time	4	6184.7492	1546.1873	2113.36	<0.001
Time*Treatment	12	110.7482	9.2290	12.61	<0.001
Residual	379	277.2861	0.7316		
Total	514	4734.3023			

Table B7. ANOVA table for the effect of ethylene on the dry weight of the Covington consignment 'NCCov I' (**Figure 5.9**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	1.212	1.212	0.76	0.386
Baseline*Time	3	29.633	9.878	6.16	<.001
Baseline*Tissue	2	4.051	2.026	1.26	0.285
Baseline*Treatment	1	2.528	2.528	1.58	0.211
Baseline*Time*Tissue	3	1.317	0.439	0.27	0.844
Baseline*Time*Treatment	3	4.866	1.622	1.01	0.389
Baseline*Tissue*Treatment	1	0.908	0.908	0.57	0.453
Baseline*Time*Tissue*Treatment	3	0.190	0.063	0.04	0.990
Residual	198	317.610	1.604		
Total	215	362.314			

Table B8. ANOVA table for the effect of ethylene on the dry weight of the Covington consignment 'NCCov II' (**Figure 5.10**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tissue	1	57.346	57.346	18.26	<0.001
Section	1	812.675	812.675	258.84	<0.001
Tissue*Section	1	122.426	122.426	38.99	<0.001
Baseline	1	1.400	1.400	0.45	0.504
Baseline*Tissue	1	12.866	12.866	4.10	0.043
Baseline*Section	1	11.827	11.827	3.77	0.053
Baseline*P1	1	174.841	174.841	55.69	<0.001
Baseline*T1	3	5.626	1.875	0.60	0.617
Baseline*Tissue*Section	1	1.680	1.680	0.54	0.465
Baseline*Tissue*P1	1	9.919	9.919	3.16	0.076
Baseline *Section*P1	1	1.913	1.913	0.61	0.435
Baseline*Tissue* T1	3	8.305	2.768	0.88	0.450
Baseline*Section *T1	3	44.368	14.789	4.71	0.003
Baseline*P1*T1	3	40.016	13.339	4.25	0.005
Baseline*T1*T2	1	16.352	16.352	5.21	0.023
Baseline.T1.P2	1	8.427	8.427	2.68	0.102
Baseline*Tissue*Section*P1	1	2.101	2.101	0.67	0.414
Baseline*Tissue*Section*T1	3	2.517	0.839	0.27	0.849
Baseline*Tissue *P1*T1	3	6.616	2.205	0.70	0.551
Baseline Section *P1*T1	3	10.788	3.596	1.15	0.330
Baseline*Tissue *T1*T2	1	0.117	0.117	0.04	0.847
Baseline*Section *T1*T2	1	0.033	0.033	0.01	0.918
Baseline*P1*T1*T2	1	1.155	1.155	0.37	0.544
Baseline*Tissue *T1*P2	1	1.328	1.328	0.42	0.516
Baseline*Section*T1*P2	1	4.870	4.870	1.55	0.213
Baseline*P1*T1*P2	1	0.154	0.154	0.05	0.825
Baseline*T1*T2*P2	1	2.785	2.785	0.89	0.347
Baseline*Tissue*Section* P1*T1	3	1.317	0.439	0.14	0.936
Baseline*Tissue*Section* T1*T2	1	1.133	1.133	0.36	0.548
Baseline*Tissue* P1*T1*T2	1	2.046	2.046	0.65	0.420
Baseline*Section* P1*T1*T2	1	0.196	0.196	0.06	0.803
Baseline*Tissue*Section* T1*P2	1	0.634	0.634	0.20	0.653
Baseline*Tissue* P1*T1*P2	1	5.594	5.594	1.78	0.182
Baseline*Section* P1*T1*P2	1	6.117	6.117	1.95	0.163
Baseline*Tissue* T1*T2*P2	1	0.016	0.016	0.01	0.942
Baseline*Section* T1*T2*P2	1	4.522	4.522	1.44	0.230
Baseline*P1*T1*T2*P2	1	13.274	13.274	4.23	0.040
Baseline*Tissue*Section* P1*T1*T2	1	0.107	0.107	0.03	0.854
Baseline*Tissue*Section* P1*T1*P2	1	1.350	1.350	0.43	0.512
Baseline*Tissue*Section* T1*T2*P2	1	0.250	0.250	0.08	0.778
Baseline*Tissue* P1*T1*T2*P2	1	0.040	0.040	0.01	0.911
Baseline*Section* P1*T1*T2*P2	1	5.142	5.142	1.64	0.201
Baseline*Tissue*Section* P1*T1*T2*P2	1	0.073	0.073	0.02	0.879
Residual	704	2210.326	3.140		
Total	763	3599.145			

Table B9. ANOVA table for the effect of ethylene on the dry weight of the Covington consignment 'NCCov III' (**Figure 5.11**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tissue	1	8.705	8.705	3.07	0.080
Section	1	1059.767	1059.767	373.97	<0.001
Tissue*Section	1	355.866	355.866	125.58	<0.001
Baseline	1	16.219	16.219	5.72	0.017
Baseline*Tissue	1	22.870	22.870	8.07	0.005
Baseline*Section	1	40.242	40.242	14.20	<0.001
Baseline*P1	1	1.599	1.599	0.56	0.453
Baseline*T1	2	27.149	13.575	4.79	0.009
Baseline*Tissue*Section	1	0.158	0.158	0.06	0.813
Baseline*Tissue* P1	1	11.496	11.496	4.06	0.044
Section*Baseline*P1	1	16.176	16.176	5.71	0.017
Tissue*Baseline*T1	2	2.514	1.257	0.44	0.642
Baseline*Section*T1	2	29.120	14.560	5.14	0.006
Baseline*P1*T1	2	31.681	15.841	5.59	0.004
Baseline*T1*T2	1	38.514	38.514	13.59	<0.001
Baseline*T1P2	1	16.463	16.463	5.81	0.016
Baseline*Tissue*Section* P1	1	0.308	0.308	0.11	0.742
Baseline*Tissue*Section* T1	2	11.343	5.672	2.00	0.136
Baseline*Tissue* P1*T1	2	0.756	0.378	0.13	0.875
Baseline*Section* P1*T1	2	1.148	0.574	0.20	0.817
Baseline*Tissue* T1*T2	1	27.341	27.341	9.65	0.002
Baseline*Section* T1*T2	1	1.369	1.369	0.48	0.487
Baseline*P1*T1*T2	1	0.787	0.787	0.28	0.598
Baseline*Tissue* T1*P2	1	2.860	2.860	1.01	0.315
Baseline*Section* T1*P2	1	4.117	4.117	1.45	0.229
Baseline*P1*T1*P2	1	8.919	8.919	3.15	0.077
Baseline*T1*T2*P2	1	0.389	0.389	0.14	0.711
Baseline*Tissue*Section* P1*T1	2	4.419	2.209	0.78	0.459
Baseline*Tissue*Section* T1*T2	1	2.245	2.245	0.79	0.374
Baseline*Tissue* P1*T1*T2	1	2.911	2.911	1.03	0.311
Baseline*Section* P1*T1*T2	1	0.986	0.986	0.35	0.555
Baseline*Tissue*Section* T1*P2	1	0.015	0.015	0.01	0.942
Baseline*Tissue* P1*T1*P2	1	0.774	0.774	0.27	0.601
Baseline*Section* P1*T1*P2	1	3.726	3.726	1.31	0.252
Baseline*Tissue* T1*T2*P2	1	7.738	7.738	2.73	0.099
Baseline*Section* T1*T2*P2	1	4.104	4.104	1.45	0.229
Baseline*P1*T1*T2*P2	1	5.880	5.880	2.08	0.150
Baseline*Tissue*Section* P1*T1*T2	1	0.291	0.291	0.10	0.749
Baseline*Tissue*Section* P1*T1*P2	1	2.451	2.451	0.86	0.353
Baseline*Tissue*Section* T1*T2*P2	1	1.278	1.278	0.45	0.502
Baseline*Tissue* P1*T1*T2*P2	1	2.567	2.567	0.91	0.342
Baseline*Section* P1*T1*T2*P2	1	0.019	0.019	0.01	0.934
Baseline*Tissue*Section* P1*T1*T2*P2	1	0.243	0.243	0.09	0.770
Residual	563	1595.444	2.834		
Total	614	3342.337			

Table B10. ANOVA table for the effect of ethylene on the decay of the Covington consignment 'NCCov I' (**Figure 5.12**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
Treatment	1	17.160	17.160	3.29	0.144
Subject*Time stratum					
Time	6	243.758	40.626	31.39	<.001
Time*Treatment	6	16.168	2.695	2.08	0.198
Residual	24	31.057	1.294		
Total	41	329.024			

Table B11. ANOVA table for the effect of ethylene on the decay of the Covington consignment 'NCCov III' (**Figure 5.12**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
Treatment	3	49.3750	16.4583	5.31	0.026
Subject*Time stratum					
Time	5	251.9028	50.3806	63.20	<0.001
Time*Treatment	15	37.3750	2.4917	3.13	0.025
Residual	40	31.8889	0.7972		
Total	71	395.3194			

Table B12. ANOVA table for the effect of ethylene on the percentage sprouted roots of the Covington consignment 'NCCov I' (**Figure 5.14**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
Treatment	1	6772.49	6772.49	72.73	0.001
Residual	4	372.49	93.12	3.88	
Subject*Time stratum					
Time	6	6317.46	1052.91	43.90	<0.001
Time*Treatment	6	4294.18	715.70	29.84	<0.001
Residual	24	575.66	23.99		
Total	41	18332.28			

Table B13. ANOVA table for the effect of ethylene on the percentage sprouted roots of the Covington consignment 'NCCov II' (**Figure 5.14**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
Treatment	3	6550.0	2183.3	3.06	0.092
Subject*Time stratum					
Time	6	78858.1	13143.0	92.74	<.001
Time*Treatment	18	9674.4	537.5	3.79	0.018
Residual	48	6802.7	141.7		
Total	83	107599.6			

Table B14. ANOVA table for the effect of ethylene on the percentage sprouted roots of the Covington consignment 'NCCov III' (**Figure 5.14**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
Treatment	3	9137.50	3045.83	21.93	<.001
Residual	8	1111.11	138.89	1.76	
Subject*Time stratum					
Time	5	20756.94	4151.39	52.62	<.001
Time*Treatment	15	7670.83	511.39	6.48	<.001
Residual	40	3155.56	78.89		
Total	71	41831.94			

Table B15. ANOVA table for the effect of ethylene on the number of sprouts per root of the Covington consignment 'NCCov I' (**Figure 5.14**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
Treatment	1	8.47503	8.47503	12.95	0.023
Subject*Time stratum					
Time	6	7.10032	1.18339	14.11	0.017
Time*Treatment	6	6.96868	1.16145	13.84	0.017
Residual	24	2.01354	0.08390		
Total	41	27.17513			

Table B16. ANOVA table for the effect of ethylene on the number of sprouts per root of the Covington consignment 'NCCov II' (**Figure 5.14**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
Treatment	3	21.2349	7.0783	4.27	0.045
Residual	8	13.2750	1.6594	4.12	
Subject*Time stratum					
Time	6	96.9898	16.1650	40.10	<0.001
Time*Treatment	18	44.0967	2.4498	6.08	0.015
Residual	48	19.3508	0.4031		
Total	83	194.9473			

Table B17. ANOVA table for the effect of ethylene on the number of sprouts per root of the Covington consignment 'NCCov III' (**Figure 5.14**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
Treatment	3	29.8049	9.9350	11.13	0.003
Subject*Time stratum					
Time	5	52.1307	10.4261	49.99	<0.001
Time*Treatment	15	33.1376	2.2092	10.59	<0.001
Residual	40	8.3433	0.2086		
Total	71	130.5599			

Table B18. ANOVA table for the effect of ethylene on the maximum sprout length per root of the Covington consignment 'NCCov I' (**Figure 5.14**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
Treatment	1	21082.88	21082.88	1148.86	<0.001
Residual	4	73.40	18.35	0.63	
Subject*Time stratum					
Time	6	18096.99	3016.16	102.91	<0.001
Time*Treatment	6	17975.37	2995.89	102.22	<0.001
Residual	24	703.43	29.31		
Total	41	57932.07			

Table B19. ANOVA table for the effect of ethylene on the maximum sprout length per root of the Covington consignment 'NCCov II' (**Figure 5.14**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
Treatment	3	2041.77	680.59	44.20	<0.001
Residual	8	123.19	15.40	0.74	
Subject*Time stratum					
Time	6	4893.83	815.64	38.98	<0.001
Time*Treatment	18	4459.27	247.74	11.84	<0.001
Residual	48	1004.48	20.93		
Total	83	12522.54			

Table B20. ANOVA table for the effect of ethylene on the maximum sprout length per root of the Covington consignment 'NCCov III' (**Figure 5.14**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
Treatment	3	5101.54	1700.51	11.38	0.003
Residual	8	1195.42	149.43	3.49	
Subject*Time stratum					
Time	5	9607.43	1921.49	44.86	<0.001
Time*Treatment	15	7380.36	492.02	11.49	<0.001
Residual	40	1713.42	42.84		
Total	71	24998.16			

Table B21. ANOVA table for the effect of ethylene on fructose concentration in the Covington consignment 'NCCov II' (**Figure 5.16**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tissue	1	1895.209	1895.209	585.25	<0.001
Section	1	443.176	443.176	136.85	<0.001
Tissue*Section	1	19.635	19.635	6.06	0.014
Baseline	1	606.709	606.709	187.35	<0.001
Baseline *Tissue	1	64.538	64.538	19.93	<0.001
Baseline*Section	1	5.683	5.683	1.75	0.186
Baseline*P1	1	0.468	0.468	0.14	0.704
Baseline*T1	3	1620.867	540.289	166.84	<0.001
Baseline*Tissue*Section	1	0.816	0.816	0.25	0.616
Baseline*Tissue* P1	1	2.851	2.851	0.88	0.348
Baseline*Section* P1	1	0.397	0.397	0.12	0.726
Baseline*Tissue* T1	3	128.312	42.771	13.21	<0.001
Baseline*Section* T1	3	7.588	2.529	0.78	0.505
Baseline*P1*T1	3	17.812	5.937	1.83	0.140
Baseline*T1*T2	1	24.413	24.413	7.54	0.006
Baseline*T1*P2	1	3.353	3.353	1.04	0.309
Baseline*Tissue*Section* P1	1	1.732	1.732	0.53	0.465
Baseline*Tissue*Section* T1	3	8.007	2.669	0.82	0.481
Baseline*Tissue* P1*T1	3	2.845	0.948	0.29	0.831
Baseline*Section* P1*T1	3	2.391	0.797	0.25	0.864
Baseline*Tissue* T1*T2	1	7.846	7.846	2.42	0.120
Baseline*Section* T1*T2	1	2.093	2.093	0.65	0.422
Baseline*P1*T1*T2	1	0.127	0.127	0.04	0.843
Baseline*Tissue*T1*P2	1	3.078	3.078	0.95	0.330
Baseline*Section*T1*P2	1	0.378	0.378	0.12	0.733
Baseline*P1*T1*P2	1	1.848	1.848	0.57	0.450
Baseline*T1*T2*P2	1	0.464	0.464	0.14	0.705
Baseline*Tissue*Section* P1.T1	3	1.038	0.346	0.11	0.956
Baseline*Tissue*Section* T1*T2	1	1.672	1.672	0.52	0.473
Baseline*Tissue* P1*T1*T2	1	0.853	0.853	0.26	0.608
Baseline*Section* P1*T1*T2	1	0.950	0.950	0.29	0.588
Baseline*Tissue*Section* T1*P2	1	0.792	0.792	0.24	0.621
Baseline*Tissue* P1*T1*P2	1	0.689	0.689	0.21	0.645
Baseline*Section* P1*T1*P2	1	0.666	0.666	0.21	0.650
Baseline*Tissue* T1*T2*P2	1	1.868	1.868	0.58	0.448
Baseline*Section* T1*T2*P2	1	3.722	3.722	1.15	0.284
Baseline*P1*T1*T2*P2	1	5.920	5.920	1.83	0.177
Baseline*Tissue*Section* P1*T1*T2	1	2.402	2.402	0.74	0.389
Baseline*Tissue*Section* P1*T1*P2	1	1.748	1.748	0.54	0.463
Tissue*Section*Baseline*T1*T2*P2	1	0.511	0.511	0.16	0.691
Baseline*Tissue* P1*T1*T2*P2	1	0.675	0.675	0.21	0.648
Section*Baseline*P1*T1*T2*P2	1	5.408	5.408	1.67	0.197
Baseline*Tissue*Section* P1*T1*T2*P2	1	0.240	0.240	0.07	0.786
Residual	701	2270.049	3.238		
Total	760	7121.317			

Table B22. ANOVA table for the effect of ethylene on glucose concentration in the Covington consignment 'NCCov II' (**Figure 5.16**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tissue	1	3618.332	3618.332	433.27	<0.001
Section	1	1055.281	1055.281	126.36	<0.001
Baseline	1	6.303	6.303	0.75	0.385
Tissue*Section	1	149.651	149.651	17.92	<0.001
Baseline*Tissue	1	10.681	10.681	1.28	0.258
Baseline*Section	1	172.395	172.395	20.64	<0.001
Baseline*P1	1	9.141	9.141	1.09	0.296
Baseline*T1	3	736.459	245.486	29.40	<0.001
Baseline*Tissue*Section	1	5.803	5.803	0.69	0.405
Tissue* P1	1	14.452	14.452	1.73	0.189
Baseline*Section*Baseline*P1	1	21.334	21.334	2.55	0.110
Tissue*Baseline*T1	3	92.761	30.920	3.70	0.012
Baseline*Section* T1	3	240.325	80.108	9.59	<0.001
Baseline*P1*T1	3	88.963	29.654	3.55	0.014
Baseline*T1*T2	1	59.738	59.738	7.15	0.008
Baseline*T1*P2	1	21.689	21.689	2.60	0.108
Baseline*Tissue*Section* P1	1	1.340	1.340	0.16	0.689
Baseline*Tissue*Section* T1	3	10.344	3.448	0.41	0.744
Baseline*Tissue* P1*T1	3	5.810	1.937	0.23	0.874
Baseline*Section* P1*T1	3	22.727	7.576	0.91	0.437
Baseline*Tissue* T1*T2	1	16.332	16.332	1.96	0.162
Baseline*Section* T1*T2	1	5.517	5.517	0.66	0.417
Baseline*P1*T1*T2	1	9.051	9.051	1.08	0.298
Baseline*Tissue* T1*P2	1	4.260	4.260	0.51	0.475
Baseline*Section* T1*P2	1	16.441	16.441	1.97	0.161
Baseline*P1*T1*P2	1	1.917	1.917	0.23	0.632
Baseline*T1*T2*P2	1	8.299	8.299	0.99	0.319
Baseline*Tissue*Section* P1*T1	3	5.165	1.722	0.21	0.892
Baseline*Tissue*Section* T1*T2	1	9.078	9.078	1.09	0.297
Baseline*Tissue* P1*T1*T2	1	1.945	1.945	0.23	0.630
Baseline*Section* P1*T1*T2	1	4.518	4.518	0.54	0.462
Baseline*Tissue*Section* T1*P2	1	5.688	5.688	0.68	0.409
Baseline*Tissue* P1*T1*P2	1	0.122	0.122	0.01	0.904
Section*Baseline*P1*T1*P2	1	0.167	0.167	0.02	0.888
Baseline*Tissue* T1*T2*P2	1	0.887	0.887	0.11	0.745
Baseline*Section* T1*T2*P2	1	12.646	12.646	1.51	0.219
Baseline*P1*T1*T2*P2	1	30.174	30.174	3.61	0.058
Baseline*Tissue*Section* P1*T1*T2	1	3.288	3.288	0.39	0.531
Baseline*Tissue*Section* P1*T1*P2	1	1.472	1.472	0.18	0.675
Baseline*Tissue*Section* T1*T2*P2	1	10.816	10.816	1.30	0.255
Baseline*Tissue* P1*T1*T2*P2	1	11.343	11.343	1.36	0.244
Baseline*Section* P1*T1*T2*P2	1	12.439	12.439	1.49	0.223
Baseline*Tissue*Section* P1*T1*T2*P2	1	0.208	0.208	0.02	0.874
Residual	702	5862.546	8.351		
Total	761	12278.495			

Table B23. ANOVA table for the effect of ethylene on sucrose concentration in the Covington consignment 'NCCov II' (**Figure 5.16**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tissue	1	8747.03	8747.03	97.30	<0.001
Section	1	2029.84	2029.84	22.58	<0.001
Baseline	1	4388.25	4388.25	48.81	<0.001
Tissue*Section	1	24.56	24.56	0.27	0.601
Baseline*Tissue	1	346.54	346.54	3.85	0.050
Baseline*Section	1	649.98	649.98	7.23	0.007
Baseline*P1	1	835.13	835.13	9.29	0.002
Baseline*T1	3	16292.46	5430.82	60.41	<0.001
Baseline*Tissue*Section	1	0.01	0.01	0.00	0.992
Baseline*Tissue* P1	1	449.10	449.10	5.00	0.026
Baseline*Section* P1	1	4.27	4.27	0.05	0.828
Baseline*Tissue* T1	3	1358.16	452.72	5.04	0.002
Baseline*Section* T1	3	1036.01	345.34	3.84	0.010
Baseline*P1*T1	3	1636.94	545.65	6.07	<0.001
Baseline*T1*T2	1	11.32	11.32	0.13	0.723
Baseline*T1*P2	1	819.52	819.52	9.12	0.003
Baseline*Tissue*Section* P1	1	2.81	2.81	0.03	0.860
Baseline*Tissue*Section* T1	3	39.86	13.29	0.15	0.931
Baseline*Tissue* P1*T1	3	273.88	91.29	1.02	0.385
Baseline*Section* P1*T1	3	326.12	108.71	1.21	0.305
Baseline*Tissue* T1*T2	1	0.03	0.03	0.00	0.986
Baseline*Section* T1*T2	1	2.91	2.91	0.03	0.857
Baseline*P1*T1*T2	1	3.20	3.20	0.04	0.850
Baseline*Tissue* T1*P2	1	67.38	67.38	0.75	0.387
Baseline*Section* T1*P2	1	271.35	271.35	3.02	0.083
Baseline*P1*T1*P2	1	188.59	188.59	2.10	0.148
Baseline*T1*T2*P2	1	60.00	60.00	0.67	0.414
Baseline*Tissue*Section* P1*T1	3	287.33	95.78	1.07	0.363
Baseline*Tissue*Section* T1*T2	1	53.52	53.52	0.60	0.441
Baseline*Tissue* P1*T1*T2	1	83.56	83.56	0.93	0.335
Baseline*Section* P1*T1*T2	1	7.26	7.26	0.08	0.776
Baseline*Tissue*Section* T1*P2	1	39.45	39.45	0.44	0.508
Baseline*Tissue* P1*T1*P2	1	54.25	54.25	0.60	0.438
Baseline*Section* P1*T1*P2	1	72.11	72.11	0.80	0.371
Baseline*Tissue* T1*T2*P2	1	101.34	101.34	1.13	0.289
Baseline*Section* T1*T2*P2	1	71.27	71.27	0.79	0.374
Baseline*P1*T1*T2*P2	1	1119.39	1119.39	12.45	<0.001
Baseline*Tissue*Section* P1*T1*T2	1	5.15	5.15	0.06	0.811
Baseline*Tissue*Section* P1*T1*P2	1	7.92	7.92	0.09	0.767
Baseline*Tissue*Section* T1*T2*P2	1	93.44	93.44	1.04	0.308
Baseline*Tissue* P1*T1*T2*P2	1	126.52	126.52	1.41	0.236
Baseline*Section* P1*T1*T2*P2	1	233.61	233.61	2.60	0.107
Baseline*Tissue*Section* P1*T1*T2*P2	1	309.32	309.32	3.44	0.064
Residual	700	62927.55	89.90		
Total	759	104913.28			

Table B24. ANOVA table for the effect of ethylene on fructose concentration in the Covington consignment 'NCCov III' (**Figure 5.17**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tissue	1	545.022	545.022	134.59	<0.001
Section	1	33.279	33.279	8.22	0.004
Baseline	1	186.288	186.288	46.00	<0.001
Tissue*Section	1	0.666	0.666	0.16	0.685
Baseline*Tissue	1	7.207	7.207	1.78	0.183
Baseline*Section	1	30.870	30.870	7.62	0.006
Baseline*P1	1	83.198	83.198	20.55	<0.001
Baseline.T1	2	263.303	131.652	32.51	<0.001
Baseline*Tissue* P1	1	5.514	5.514	1.36	0.244
Section*Baseline*P1	1	20.171	20.171	4.98	0.026
Baseline*Tissue* T1	1	66.806	66.806	16.50	<0.001
Baseline*Section* T1	2	13.976	6.988	1.73	0.180
Baseline*P1*T1	2	45.848	22.924	5.66	0.004
Baseline*T1*T2	1	22.435	22.435	5.54	0.019
Baseline*T1*P2	1	3.116	3.116	0.77	0.381
Baseline*Tissue*Section* P1	1	5.235	5.235	1.29	0.256
Baseline*Section* P1*T1	2	10.593	5.297	1.31	0.272
Baseline*Section* T1*T2	1	53.208	53.208	13.14	<0.001
Baseline*P1*T1*T2	1	5.863	5.863	1.45	0.230
Baseline*Section* T1*P2	1	1.490	1.490	0.37	0.545
Baseline*P1*T1*P2	1	10.425	10.425	2.57	0.110
Baseline*T1*T2*P2	1	39.325	39.325	9.71	0.002
Baseline*Section* P1*T1*T2	1	1.471	1.471	0.36	0.547
Baseline*Section* P1*T1*P2	1	4.284	4.284	1.06	0.304
Baseline*Section* T1*T2*P2	1	1.124	1.124	0.28	0.599
Baseline*P1*T1*T2*P2	1	13.255	13.255	3.27	0.071
Baseline*Section* P1*T1*T2*P2	1	0.735	0.735	0.18	0.670
Residual	327	1324.194	4.050		
Total	357	2091.594			

Table B25. ANOVA table for the effect of ethylene on glucose concentration in Covington consignment 'NCCov III' (**Figure 5.17**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tissue	1	2633.601	2633.601	272.99	<0.001
Section	1	1.649	1.649	0.17	0.679
Baseline	1	184.972	184.972	19.17	<0.001
Tissue*Section	1	12.408	12.408	1.29	0.257
Baseline*Tissue	1	6.820	6.820	0.71	0.401
Baseline*Section	1	69.696	69.696	7.22	0.007
Baseline*P1	1	243.218	243.218	25.21	<0.001
Baseline*T1	2	428.501	214.251	22.21	<0.001
Baseline*Tissue*Section	1	7.667	7.667	0.79	0.373
Baseline*Tissue* P1	1	249.308	249.308	25.84	<0.001
Baseline*Section* P1	1	18.621	18.621	1.93	0.165
Baseline*Tissue* T1	2	403.008	201.504	20.89	<0.001
Baseline*Section* T1	2	7.082	3.541	0.37	0.693
Baseline*P1*T1	2	229.562	114.781	11.90	<0.001
Baseline*T1*T2	1	1.810	1.810	0.19	0.665
Baseline*T1*P2	1	10.632	10.632	1.10	0.294
Baseline*Tissue*Section* P1	1	16.370	16.370	1.70	0.193
Baseline*Tissue*Section* T1	2	0.001	0.000	0.00	1.000
Baseline*Tissue* P1*T1	2	225.506	112.753	11.69	<0.001
Baseline*Section* P1*T1	2	40.368	20.184	2.09	0.124
Baseline*Tissue* T1*T2	1	1.810	1.810	0.19	0.665
Baseline*Section* T1*T2	1	5.954	5.954	0.62	0.432
Baseline*P1*T1*T2	1	1.408	1.408	0.15	0.703
Baseline*Tissue* T1*P2	1	10.632	10.632	1.10	0.294
Baseline*Section* T1*P2	1	0.478	0.478	0.05	0.824
Baseline*P1*T1*P2	1	3.510	3.510	0.36	0.547
Baseline*T1*T2*P2	1	0.476	0.476	0.05	0.824
Baseline*Tissue*Section* P1*T1	2	42.622	21.311	2.21	0.111
Baseline*Tissue*Section* T1*T2	1	5.954	5.954	0.62	0.432
Baseline*Tissue* P1*T1*T2	1	1.408	1.408	0.15	0.703
Baseline*Section* P1*T1*T2	1	0.137	0.137	0.01	0.905
Baseline*Tissue*Section* T1*P2	1	0.478	0.478	0.05	0.824
Baseline*Tissue* P1*T1*P2	1	3.510	3.510	0.36	0.547
Baseline*Section* P1*T1*P2	1	0.230	0.230	0.02	0.877
Tissue* T1*T2*P2	1	0.476	0.476	0.05	0.824
Baseline*Section* T1*T2*P2	1	0.401	0.401	0.04	0.838
Baseline*P1*T1*T2*P2	1	2.368	2.368	0.25	0.620
Baseline*Tissue*Section* P1*T1*T2	1	0.137	0.137	0.01	0.905
Baseline*Tissue*Section* P1*T1*P2	1	0.230	0.230	0.02	0.877
Baseline*Tissue*Section* T1*T2*P2	1	0.401	0.401	0.04	0.838
Baseline*Tissue* P1*T1*T2*P2	1	2.368	2.368	0.25	0.620
Baseline*Section* P1*T1*T2*P2	1	0.020	0.020	0.00	0.963
Baseline*Tissue*Section* P1*T1*T2*P2	1	0.020	0.020	0.00	0.963
Residual	566	5460.338	9.647		
Total	617	10316.236			

Table B26. ANOVA table for the effect of ethylene on sucrose concentration in the Covington consignment 'NCCov III' (**Figure 5.17**)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Tissue	1		13490.50	13490.50	174.58	<0.001
Section	1		3198.55	3198.55	41.39	<0.001
Baseline	1		280.27	280.27	3.63	0.057
Tissue*Section	1		303.32	303.32	3.93	0.048
Baseline*Tissue	1		85.92	85.92	1.11	0.292
Baseline*Section	1		91.14	91.14	1.18	0.278
Baseline*P1	1		461.90	461.90	5.98	0.015
Baseline*T1	2		2884.35	1442.17	18.66	<0.001
Baseline*Tissue*Section	1		12.65	12.65	0.16	0.686
Baseline*Tissue* P1	1		85.86	85.86	1.11	0.292
Baseline*Section* P1	1		24.76	24.76	0.32	0.572
Baseline*Tissue* T1	2		3.93	1.97	0.03	0.975
Baseline*Section* T1	2		99.32	49.66	0.64	0.526
Baseline*P1*T1	2		696.79	348.40	4.51	0.011
Baseline*T1*T2	1		443.76	443.76	5.74	0.017
Baseline*T1*P2	1		211.42	211.42	2.74	0.099
Baseline*Tissue*Section* P1	1		0.05	0.05	0.00	0.979
Baseline*Tissue*Section* T1	2		250.89	125.44	1.62	0.198
Baseline*Tissue* P1*T1	2		1.74	0.87	0.01	0.989
Baseline*Section* P1*T1	2		241.10	120.55	1.56	0.211
Baseline*Tissue* T1*T2	1		20.55	20.55	0.27	0.606
Baseline*Section* T1*T2	1		106.67	106.67	1.38	0.241
Baseline*P1*T1*T2	1		220.19	220.19	2.85	0.092
Baseline*Tissue* T1*P2	1		53.21	53.21	0.69	0.407
Baseline*Section* T1*P2	1		70.99	70.99	0.92	0.338
Baseline*P1*T1*P2	1		8.75	8.75	0.11	0.737
Baseline*T1*T2*P2	1		26.84	26.84	0.35	0.556
Baseline*Tissue*Section* P1*T1	2		145.47	72.73	0.94	0.391
Baseline*Tissue*Section* T1*T2	1		30.50	30.50	0.39	0.530
Baseline*Tissue* P1*T1*T2	1		37.88	37.88	0.49	0.484
Baseline*Section* P1*T1*T2	1		0.59	0.59	0.01	0.930
Baseline*Tissue*Section* T1*P2	1		1.00	1.00	0.01	0.909
Baseline*Tissue* P1*T1*P2	1		3.94	3.94	0.05	0.821
Baseline*Section* P1*T1*P2	1		2.46	2.46	0.03	0.858
Baseline*Tissue* T1*T2*P2	1		108.58	108.58	1.41	0.236
Baseline*Section* T1*T2*P2	1		28.55	28.55	0.37	0.544
Baseline*P1*T1*T2*P2	1		8.83	8.83	0.11	0.735
Baseline*Tissue*Section* P1*T1*T2	1		0.30	0.30	0.00	0.950
Baseline*Tissue*Section* P1*T1*P2	1		31.66	31.66	0.41	0.522
Baseline*Tissue*Section* T1*T2*P2	1		58.53	58.53	0.76	0.384
Baseline*Tissue* P1*T1*T2*P2	1		97.24	97.24	1.26	0.262
Baseline*Section* P1*T1*T2*P2	1		83.44	83.44	1.08	0.299
Baseline*Tissue*Section* P1*T1*T2*P2	1		16.67	16.67	0.22	0.643
Residual	563		43505.91	77.28		
Total	614		67236.80			

Table B27. ANOVA table for the effect of ethylene on chlorogenic acid concentration in the Covington consignment 'NCCov II' (**Figure 5.18**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tissue	1	213261.3	213261.3	1774.44	<0.001
Section	1	1104.5	1104.5	9.19	0.003
Baseline	1	6924.2	6924.2	57.61	<0.001
Tissue*Section	1	1.5	1.5	0.01	0.912
Tissue*Baseline	1	3313.0	3313.0	27.57	<0.001
Section*Baseline	1	109.4	109.4	0.91	0.340
Baseline*P1	1	5.1	5.1	0.04	0.837
Baseline*T1	3	63920.1	21306.7	177.28	<0.001
Tissue*Section*Baseline	1	1.7	1.7	0.01	0.904
Tissue*Baseline*P1	1	73.8	73.8	0.61	0.434
Section*Baseline*P1	1	0.0	0.0	0.00	0.989
Tissue*Baseline*T1	3	18761.8	6253.9	52.04	<0.001
Section*Baseline*T1	3	2603.8	867.9	7.22	<0.001
Baseline*P1*T1	3	52.0	17.3	0.14	0.933
Baseline*T1*T2	1	25.4	25.4	0.21	0.646
Baseline*T1*P2	1	255.4	255.4	2.12	0.145
Tissue*Section*Baseline*P1	1	477.7	477.7	3.97	0.047
Tissue*Section*Baseline*T1	3	166.6	55.5	0.46	0.709
Tissue*Baseline*P1*T1	3	2129.0	709.7	5.90	<0.001
Section*Baseline*P1*T1	3	287.5	95.8	0.80	0.496
Tissue*Baseline*T1*T2	1	230.4	230.4	1.92	0.167
Section*Baseline*T1*T2	1	153.1	153.1	1.27	0.259
Baseline*P1*T1*T2	1	212.5	212.5	1.77	0.184
Tissue*Baseline*T1*P2	1	490.8	490.8	4.08	0.044
Section*Baseline*T1*P2	1	56.3	56.3	0.47	0.494
Baseline*P1*T1*P2	1	13.8	13.8	0.11	0.735
Baseline*T1*T2*P2	1	4.7	4.7	0.04	0.842
Tissue*Section*Baseline*P1*T1	3	40.3	13.4	0.11	0.953
Tissue*Section*Baseline*T1*T2	1	5.2	5.2	0.04	0.835
Tissue*Baseline*P1*T1*T2	1	14.1	14.1	0.12	0.732
Section*Baseline*P1*T1*T2	1	64.5	64.5	0.54	0.464
Tissue*Section*Baseline*T1*P2	1	45.0	45.0	0.37	0.541
Tissue*Baseline*P1*T1*P2	1	15.0	15.0	0.13	0.724
Section*Baseline*P1*T1*P2	1	78.5	78.5	0.65	0.419
Tissue*Baseline*T1*T2*P2	1	68.4	68.4	0.57	0.451
Section*Baseline*T1*T2*P2	1	28.1	28.1	0.23	0.629
Baseline*P1*T1*T2*P2	1	143.3	143.3	1.19	0.275
Tissue*Section*Baseline*P1*T1*T2	1	0.2	0.2	0.00	0.967
Tissue*Section*Baseline*P1*T1*P2	1	0.7	0.7	0.01	0.938
Tissue*Section*Baseline*T1*T2*P2	1	99.8	99.8	0.83	0.363
Tissue*Baseline*P1*T1*T2*P2	1	9.5	9.5	0.08	0.779
Section*Baseline*P1*T1*T2*P2	1	23.7	23.7	0.20	0.657
Tissue*Section*Baseline*P1*T1*T2*P2	1	54.4	54.4	0.45	0.501
Residual	702	84370.2	120.2		
Total	761	398435.2			

Table B28. ANOVA table for the effect of ethylene on isochlorogenic acid A concentration in the Covington consignment 'NCCov II' (**Figure 5.18**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tissue	1	198313.3	198313.3	543.23	<0.001
Section	1	20059.2	20059.2	54.95	<0.001
Baseline	1	9510.0	9510.0	26.05	<0.001
Tissue*Section	1	3006.5	3006.5	8.24	0.004
Baseline*Tissue	1	4037.1	4037.1	11.06	<0.001
Baseline*Section	1	2758.0	2758.0	7.55	0.006
Baseline*P1	1	4727.8	4727.8	12.95	<0.001
Baseline*T1	3	133545.3	44515.1	121.94	<0.001
Baseline*Tissue*Section	1	771.7	771.7	2.11	0.146
Baseline*Tissue* P1	1	1199.0	1199.0	3.28	0.070
Baseline*Section* P1	1	1613.5	1613.5	4.42	0.036
Baseline*Tissue* T1	3	39355.5	13118.5	35.93	<0.001
Baseline*Section* T1	3	30460.9	10153.6	27.81	<0.001
Baseline*P1*T1	3	292.7	97.6	0.27	0.849
Baseline*T1*T2	1	218.8	218.8	0.60	0.439
Baseline*T1*P2	1	2254.9	2254.9	6.18	0.013
Baseline*Tissue*Section* P1	1	3.9	3.9	0.01	0.918
Baseline*Tissue*Section* T1	3	4831.2	1610.4	4.41	0.004
Baseline*Tissue* P1*T1	3	2258.1	752.7	2.06	0.104
Baseline*Section* P1*T1	3	1550.3	516.8	1.42	0.237
Baseline*Tissue* T1*T2	1	195.9	195.9	0.54	0.464
Baseline*Section* T1*T2	1	1184.5	1184.5	3.24	0.072
Baseline*P1*T1*T2	1	2255.0	2255.0	6.18	0.013
Baseline*Tissue* T1*P2	1	41.8	41.8	0.11	0.735
Baseline*Section* T1*	1	2238.2	2238.2	6.13	0.014
Baseline*T1*T2*P2	1	65.3	65.3	0.18	0.672
Baseline*Tissue*Section* P1*T1	3	150.3	50.1	0.14	0.938
Baseline*Tissue*Section* T1*T2	1	104.1	104.1	0.29	0.594
Baseline*Tissue* P1*T1*T2	1	103.3	103.3	0.28	0.595
Baseline*Section* P1*T1*T2	1	363.0	363.0	0.99	0.319
Baseline*Tissue*Section* T1*P2	1	186.8	186.8	0.51	0.475
Baseline*Tissue* P1*T1*P2	1	96.6	96.6	0.26	0.607
Baseline*Section* P1*T1*P2	1	992.8	992.8	2.72	0.100
Baseline*Tissue* T1*T2*P2	1	116.5	116.5	0.32	0.572
Baseline*Section* T1*T2*P2	1	381.8	381.8	1.05	0.307
Baseline*P1*T1*T2*P2	1	954.6	954.6	2.61	0.106
Baseline*Tissue*Section* P1*T1*T2	1	84.0	84.0	0.23	0.632
Baseline*Tissue*Section* P1*T1*P2	1	49.7	49.7	0.14	0.712
Baseline*Tissue*Section* T1*T2*P2	1	1651.1	1651.1	4.52	0.034
Baseline*Tissue* P1*T1*T2*P2	1	12.9	12.9	0.04	0.851
Baseline*Section* P1*T1*T2*P2	1	235.8	235.8	0.65	0.422
Baseline*Tissue*Section* P1*T1*T2*P2	1	133.2	133.2	0.36	0.546
Residual	704	257004.9	365.1		
Total	763	734706.7			

Table B29. ANOVA table for the effect of ethylene on the concentration of isochlorogenic acid B in the Covington consignment 'NCCov II' (**Figure 5.18**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tissue	1	27495.04	27495.04	808.96	<.001
Section	1	133.59	133.59	3.93	0.048
Baseline	1	2220.69	2220.69	65.34	<.001
Tissue*Section	1	17.38	17.38	0.51	0.475
Baseline*Tissue	1	1404.78	1404.78	41.33	<.001
Section*Baseline	1	17.49	17.49	0.51	0.473
Baseline*P1	1	397.02	397.02	11.68	<.001
Baseline*T1	3	21280.61	7093.54	208.71	<.001
Baseline*Tissue*Section	1	0.49	0.49	0.01	0.905
Baseline*Tissue* P1	1	137.90	137.90	4.06	0.044
Baseline*Section* P1	1	40.58	40.58	1.19	0.275
Baseline*Tissue* T1	3	12552.18	4184.06	123.10	<.001
Baseline*Section* T1	3	341.72	113.91	3.35	0.019
Baseline*P1*T1	3	142.00	47.33	1.39	0.244
Baseline*T1*T2	1	44.30	44.30	1.30	0.254
Baseline*T1*P2	1	95.18	95.18	2.80	0.095
Baseline*Tissue*Section* P1	1	15.91	15.91	0.47	0.494
Baseline*Tissue*Section* T1	3	59.76	19.92	0.59	0.624
Baseline*Tissue* P1*T1	3	40.77	13.59	0.40	0.753
Baseline*Section* P1*T1	3	96.71	32.24	0.95	0.417
Baseline*Tissue* T1*T2	1	44.71	44.71	1.32	0.252
Baseline*Section* T1*T2	1	243.90	243.90	7.18	0.008
Baseline*P1*T1*T2	1	50.96	50.96	1.50	0.221
Baseline*Tissue* T1*P2	1	48.64	48.64	1.43	0.232
Baseline*Section* T1*P2	1	8.56	8.56	0.25	0.616
Baseline*P1*T1*P2	1	7.11	7.11	0.21	0.648
Baseline*T1*T2*P2	1	103.13	103.13	3.03	0.082
Tissue.Section.Baseline.P1.T1	3	6.02	2.01	0.06	0.981
Baseline*Tissue*Section* T1*T2	1	56.12	56.12	1.65	0.199
Baseline*Tissue* P1*T1*T2	1	60.52	60.52	1.78	0.183
Baseline*Section* P1*T1*T2	1	14.42	14.42	0.42	0.515
Baseline*Tissue*Section* T1*P2	1	0.27	0.27	0.01	0.929
Baseline*Tissue* P1*T1*P2	1	5.58	5.58	0.16	0.685
Baseline*Section* P1*T1*P2	1	3.86	3.86	0.11	0.736
Baseline*Tissue* T1*T2*P2	1	30.61	30.61	0.90	0.343
Baseline*Section* T1*T2*P2	1	16.54	16.54	0.49	0.486
Baseline*P1*T1*T2*P2	1	67.01	67.01	1.97	0.161
Baseline*Tissue*Section* P1*T1*T2	1	4.82	4.82	0.14	0.707
Baseline*Tissue*Section* P1*T1*P2	1	27.13	0.80	0.372	
Baseline*Tissue*Section* T1*T2*P2	1	83.37	83.37	2.45	0.118
Baseline*Tissue* P1*T1*T2*P2	1	15.73	15.73	0.46	0.496
Baseline*Section* P1*T1*T2*P2	1	40.03	40.03	1.18	0.278
Baseline*Tissue*Section* P1*T1*T2*P2	1	22.49	22.49	0.66	0.416
Residual	704	23927.58	33.99		
Total	763	91365.19			

Table B30 ANOVA table for the effect of ethylene on the concentration of isochlorogenic acid C in the Covington consignment 'NCCov II' (**Figure 5.18**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tissue	1	13658.35	13658.35	492.92	<0.001
Section	1	364.09	364.09	13.14	<0.001
Baseline	1	1018.35	1018.35	36.75	<0.001
Tissue*Section	1	27.24	27.24	0.98	0.322
Baseline*Tissue	1	734.50	734.50	26.51	<0.001
Baseline*Section	1	29.92	29.92	1.08	0.299
Baseline*P1	1	587.41	587.41	21.20	<0.001
Baseline*T1	3	11912.94	3970.98	143.31	<0.001
Baseline*Tissue*Section	1	3.57	3.57	0.13	0.720
Baseline*Tissue* P1	1	212.95	212.95	7.69	0.006
Section*Baseline*P1	1	121.17	121.17	4.37	0.037
Baseline*Tissue* T1	3	7533.86	2511.29	90.63	<0.001
Baseline*Section* T1	3	805.99	268.66	9.70	<0.001
Baseline*P1*T1	3	488.01	162.67	5.87	<0.001
Baseline*T1*T2	1	245.94	245.94	8.88	0.003
Baseline*T1*P2	1	16.53	16.53	0.60	0.440
Baseline*Tissue*Section* P1	1	3.59	3.59	0.13	0.719
Baseline*Tissue*Section* T1	3	123.25	41.08	1.48	0.218
Baseline*Tissue* P1*T1	3	104.45	34.82	1.26	0.288
Baseline*Section* P1*T1	3	160.05	53.35	1.93	0.124
Baseline*Tissue* T1*T2	1	86.70	86.70	3.13	0.077
Baseline*Section* T1*T2	1	215.66	215.66	7.78	0.005
Baseline*P1*T1*T2	1	3.62	3.62	0.13	0.718
Baseline*Tissue* T1*P2	1	1.65	1.65	0.06	0.807
Baseline*Section* T1*P2	1	181.60	181.60	6.55	0.011
Baseline*P1*T1*P2	1	3.97	3.97	0.14	0.705
Baseline*T1*T2*P2	1	78.23	78.23	2.82	0.093
Baseline*Tissue*Section* P1.T1	3	8.59	2.86	0.10	0.958
Baseline*Tissue*Section* T1*T2	1	61.51	61.51	2.22	0.137
Baseline*Tissue* P1*T1*T2	1	4.18	4.18	0.15	0.698
Baseline*Section* P1*T1*T2	1	1.87	1.87	0.07	0.795
Baseline*Tissue*Section* T1*P2	1	29.37	29.37	1.06	0.304
Baseline*Tissue* P1*T1*P2	1	4.32	4.32	0.16	0.693
Baseline*Section* P1*T1*P2	1	22.50	22.50	0.81	0.368
Baseline*Tissue* T1*T2*P2	1	5.58	5.58	0.20	0.654
Baseline*Section* T1*T2*P2	1	0.13	0.13	0.00	0.944
Baseline*P1*T1*T2*P2	1	0.44	0.44	0.02	0.900
Baseline*Tissue*Section* P1*T1*T2	1	8.06	8.06	0.29	0.590
Baseline*Tissue*Section* P1*T1*P2	1	70.03	70.03	2.53	0.112
Baseline*Tissue*Section* T1*T2*P2	1	53.65	53.65	1.94	0.165
Baseline*Tissue* P1*T1*T2*P2	1	0.15	0.15	0.01	0.942
Baseline*Section* P1*T1*T2*P2	1	18.59	18.59	0.67	0.413
Baseline*Tissue*Section* P1*T1*T2*P2	1	15.80	15.80	0.57	0.450
Residual	704	19507.09	27.71		
Total	763	58492.12			

Table B31. ANOVA table for the effect of ethylene on the concentration of caffeic acid in the Covington consignment 'NCCov II' (**Figure 5.19**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tissue	1	949.3355	949.3355	1213.83	<0.001
Section	1	11.6562	11.6562	14.90	<0.001
Baseline	1	19.7725	19.7725	25.28	<0.001
Tissue*Section	1	19.1644	19.1644	24.50	<0.001
Baseline*Tissue	1	14.1305	14.1305	18.07	<0.001
Baseline*Section	1	0.2389	0.2389	0.31	0.581
Baseline*P1	1	38.5153	38.5153	49.25	<0.001
Baseline*T1	3	189.3451	63.1150	80.70	<0.001
Baseline*Tissue*Section	1	0.5440	0.5440	0.70	0.405
Baseline*Tissue* P1	1	53.8469	53.8469	68.85	<0.001
Baseline*Section* P1	1	0.5982	0.5982	0.76	0.382
Baseline*Tissue* T1	3	108.8527	36.2842	46.39	<0.001
Baseline*Section* T1	3	2.3061	0.7687	0.98	0.400
Baseline*P1*T1	3	26.8745	8.9582	11.45	<0.001
Baseline*T1*2	1	34.0160	34.0160	43.49	<0.001
Baseline*T1*P2	1	14.4702	14.4702	18.50	<0.001
Baseline*Tissue*Section* P1	1	0.0643	0.0643	0.08	0.774
Baseline*Tissue*Section* T1	3	7.3697	2.4566	3.14	0.025
Baseline*Tissue* P1*T1	3	43.5399	14.5133	18.56	<0.001
Baseline*Section* P1*T1	3	2.8842	0.9614	1.23	0.298
Baseline*Tissue* T1*T2	1	45.3496	45.3496	57.98	<0.001
Baseline*Section* T1*T2	1	1.7081	1.7081	2.18	0.140
Baseline*P1*T1*T2	1	2.3143	2.3143	2.96	0.086
Baseline*Tissue* T1*P2	1	20.9491	20.9491	26.79	<0.001
Baseline*Section* T1*P2	1	3.4854	3.4854	4.46	0.035
Baseline*P1*T1*P2	1	2.6052	2.6052	3.33	0.068
Baseline*T1*T2*P2	1	2.9217	2.9217	3.74	0.054
Baseline*Tissue*Section* P1*T1	3	1.1427	0.3809	0.49	0.691
Baseline*Tissue*Section* T1*T2	1	2.0335	2.0335	2.60	0.107
Baseline*Tissue* P1*T1*T2	1	0.4010	0.4010	0.51	0.474
Baseline*Section* P1*T1*T2	1	0.0311	0.0311	0.04	0.842
Baseline*Tissue*Section* T1*P2	1	5.5993	5.5993	7.16	0.008
Baseline*Tissue* P1*T1*P2	1	2.5534	2.5534	3.26	0.071
Baseline*Section* P1*T1*P2	1	0.2162	0.2162	0.28	0.599
Baseline*Tissue* T1*T2*P2	1	1.6330	1.6330	2.09	0.149
Baseline*Section* T1*T2*P2	1	0.8918	0.8918	1.14	0.286
Baseline*P1*T1*T2*P2	1	2.2301	2.2301	2.85	0.092
Baseline*Tissue*Section* P1*T1*T2	1	0.0564	0.0564	0.07	0.788
Baseline*Tissue*Section* P1*T1*P2	1	0.0244	0.0244	0.03	0.860
Baseline*Tissue*Section* T1*T2*P2	1	0.3183	0.3183	0.41	0.524
Baseline*Tissue* P1*T1*T2*P2	1	4.4727	4.4727	5.72	0.017
Baseline*Section* P1*T1*T2*P2	1	0.1946	0.1946	0.25	0.618
Baseline*Tissue*Section* P1*T1*T2*P2	1	0.0048	0.0048	0.01	0.937
Residual	705	551.3809	0.7821		
Total	764	2188.4859			

Table B32. ANOVA table for the effect of ethylene on the concentration of coumaric acid in the Covington consignment 'NCCov II' (**Figure 5.19)**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tissue	1	12.52508	12.52508	509.58	<.001
Section	1	0.05523	0.05523	2.25	0.134
Baseline	1	0.39548	0.39548	16.09	<.001
Tissue*Section	1	0.04018	0.04018	1.63	0.201
Baseline*Tissue	1	0.76699	0.76699	31.20	<.001
Baseline*Section	1	0.14605	0.14605	5.94	0.015
Baseline*P1	1	0.21892	0.21892	8.91	0.003
Baseline*T1	3	0.95733	0.31911	12.98	<.001
Baseline*Tissue*Section	1	0.12403	0.12403	5.05	0.025
Baseline*Tissue*P1	1	0.47220	0.47220	19.21	<.001
Baseline*Section*P1	1	0.00195	0.00195	0.08	0.778
Baseline*Tissue*T1	3	0.42594	0.14198	5.78	<.001
Baseline*Section*T1	3	0.06701	0.02234	0.91	0.436
Baseline*P1*T1	3	0.02177	0.00726	0.30	0.829
Baseline*T1*T2	1	0.13581	0.13581	5.53	0.019
Baseline*T1*P2	1	0.10517	0.10517	4.28	0.039
Baseline*Tissue*Section*P1	1	0.02041	0.02041	0.83	0.362
Baseline*Tissue*Section*T1	3	0.09327	0.03109	1.26	0.285
Baseline*Tissue*P1*T1	3	0.10818	0.03606	1.47	0.222
Baseline*Section*P1*T1	3	0.01714	0.00571	0.23	0.874
Baseline*Tissue*T1*T2	1	0.06035	0.06035	2.46	0.118
Baseline*Section*T1*T2	1	0.00008	0.00008	0.00	0.954
Baseline*P1*T1*T2	1	0.00694	0.00694	0.28	0.595
Baseline*Tissue*T1*P2	1	0.20727	0.20727	8.43	0.004
Baseline*Section*T1*P2	1	0.00767	0.00767	0.31	0.577
Baseline*P1*T1*P2	1	0.00263	0.00263	0.11	0.743
Baseline*T1*T2*P2	1	0.00777	0.00777	0.32	0.574
Baseline*Tissue*Section*P1*T1	3	0.01763	0.00588	0.24	0.869
Baseline*Tissue*Section*T1*T2	1	0.00047	0.00047	0.02	0.890
Baseline*Tissue*P1*T1*T2	1	0.02468	0.02468	1.00	0.317
Baseline*Section*P1*T1*T2	1	0.00300	0.00300	0.12	0.727
Baseline*Tissue*Section*T1*P2	1	0.00012	0.00012	0.00	0.945
Baseline*Tissue*P1*T1*P2	1	0.00058	0.00058	0.02	0.878
Baseline*Section*P1*T1*P2	1	0.02166	0.02166	0.88	0.348
Baseline*Tissue*T1*T2*P2	1	0.01001	0.01001	0.41	0.524
Baseline*Section*T1*T2*P2	1	0.02342	0.02342	0.95	0.329
Baseline*P1*T1*T2*P2	1	0.00127	0.00127	0.05	0.820
Baseline*Tissue*Section*P1*T1*T2	1	0.00970	0.00970	0.39	0.530
Baseline*Tissue*Section* P1*T1*P2	1	0.02572	0.02572	1.05	0.307
Baseline*Tissue*Section*T1*T2*P2	1	0.01067	0.01067	0.43	0.510
Baseline*Tissue*P1*T1*T2*P2	1	0.01902	0.01902	0.77	0.379
Baseline*Section*P1*T1*T2*P2	1	0.03574	0.03574	1.45	0.228
Baseline*Tissue*Section*P1*T1*T2*P2	1	0.01949	0.01949	0.79	0.374
Residual	704	17.30390	0.02458		
Total	763	34.42084			

Table B33. ANOVA table for the effect of ethylene on caffeic acid concentration in the Covington consignment 'NCCov III' (**Figure 5.20**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tissue	0	0.000			
Section	1	33.936	33.936	30.70	<0.001
Baseline	1	0.768	0.768	0.69	0.405
Section*Baseline	1	0.024	0.024	0.02	0.882
Baseline*P1	1	8.135	8.135	7.36	0.007
Baseline*T1	2	58.593	29.297	26.51	<0.001
Baseline*Section* P1	1	0.074	0.074	0.07	0.796
Section*Baseline*T1	2	3.646	1.823	1.65	0.194
Baseline*P1*T1	2	1.954	0.977	0.88	0.414
Baseline*T1*T2	1	0.068	0.068	0.06	0.804
Baseline*T1*P2	1	1.010	1.010	0.91	0.340
Baseline*Section* P1*T1	2	1.305	0.653	0.59	0.555
Baseline*Section* T1*T2	1	4.759	4.759	4.31	0.039
Baseline*P1*T1*T2	1	23.632	23.632	21.38	<0.001
Baseline*Section* T1*P2	1	1.639	1.639	1.48	0.224
Baseline*P1*T1*P2	1	0.007	0.007	0.01	0.937
Baseline*T1*T2*P2	1	0.218	0.218	0.20	0.657
Baseline*Section* P1*T1*T2	1	0.496	0.496	0.45	0.503
Baseline*Section* P1*T1*P2	1	2.444	2.444	2.21	0.138
Baseline*Section* T1*T2*P2	1	0.125	0.125	0.11	0.737
Baseline*P1*T1*T2*P2	1	3.757	3.757	3.40	0.066
Baseline*Section* P1*T1*T2*P2	1	0.280	0.280	0.25	0.615
Residual	277	306.173	1.105		
Total	302	378.050			

Table B34. ANOVA table for the effect of ethylene on chlorogenic acid concentration in the Covington consignment 'NCCov III' (**Figure 5.21**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tissue	1	38704.26	38704.26	763.61	<0.001
Section	1	1631.23	1631.23	32.18	<0.001
Baseline	1	1041.14	1041.14	20.54	<0.001
Tissue*Section	1	1378.66	1378.66	27.20	<0.001
Baseline *Tissue	1	179.44	179.44	3.54	0.060
Baseline*Section	1	105.64	105.64	2.08	0.149
Baseline*P1	1	357.63	357.63	7.06	0.008
Baseline*T1	2	7355.85	3677.93	72.56	<0.001
Baseline*Tissue*Section	1	10.72	10.72	0.21	0.646
Baseline*Tissue*P1	1	153.05	153.05	3.02	0.083
Baseline*Section*P1	1	0.42	0.42	0.01	0.928
Baseline*Tissue*T1	2	187.49	93.74	1.85	0.158
Baseline*Section*T1	2	306.72	153.36	3.03	0.049
Baseline*P1*T1	2	315.74	157.87	3.11	0.045
Baseline*T1*T2	1	13.07	13.07	0.26	0.612
Baseline*T1*P2	1	53.61	53.61	1.06	0.304
Baseline*Tissue*Section* P1	1	46.01	46.01	0.91	0.341
Baseline*Tissue*Section* T1	2	212.63	106.31	2.10	0.124
Baseline*Tissue* P1*T1	2	128.69	64.35	1.27	0.282
Baseline*Section* P1*T1	2	92.16	46.08	0.91	0.403
Baseline*Tissue* T1*T2	1	4.62	4.62	0.09	0.763
Section*Baseline*T1*T2	1	92.67	92.67	1.83	0.177
Baseline*P1*T1*T2	1	16.48	16.48	0.33	0.569
Baseline*Tissue* T1*P2	1	29.36	29.36	0.58	0.447
Baseline*Section* T1*P2	1	59.62	59.62	1.18	0.279
Baseline*P1*T1*P2	1	554.27	554.27	10.94	0.001
Baseline*T1*T2*P2	1	56.94	56.94	1.12	0.290
Baseline*Tissue*Section* P1*T1	2	122.30	61.15	1.21	0.300
Baseline*Tissue*Section* T1*T2	1	66.22	66.22	1.31	0.254
Baseline*Tissue* P1*T1*T2	1	29.73	29.73	0.59	0.444
Baseline*Section* P1*T1*T2	1	0.24	0.24	0.00	0.945
Baseline*Tissue*Section* T1*P2	1	23.13	23.13	0.46	0.500
Baseline*Tissue* P1*T1*P2	1	308.33	308.33	6.08	0.014
Baseline*Section* P1*T1*P2	1	0.99	0.99	0.02	0.889
Baseline*Tissue* T1*T2*P2	1	20.71	20.71	0.41	0.523
Baseline*Section* T1*T2*P2	1	55.43	55.43	1.09	0.296
Baseline*P1*T1*T2*P2	1	40.34	40.34	0.80	0.373
Baseline*Tissue*Section* P1*T1*T2	1	60.04	60.04	1.18	0.277
Baseline*Tissue*Section* P1*T1*P2	1	32.15	32.15	0.63	0.426
Baseline*Tissue*Section* T1*T2*P2	1	39.81	39.81	0.79	0.376
Baseline*Tissue* P1*T1*T2*P2	1	104.43	104.43	2.06	0.152
Baseline*Section* P1*T1*T2*P2	1	10.49	10.49	0.21	0.649
Baseline*Tissue*Section* P1*T1*T2*P2	1	14.98	14.98	0.30	0.587
Residual	560	28384.05	50.69		
Total	611	81062.52			

Table B35. ANOVA table for the effect of ethylene on isochlorogenic acid A concentration in the Covington consignment 'NCCov III' (**Figure 5.21**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tissue	1	144687.9	144687.9	564.15	<0.001
Section	1	9453.7	9453.7	36.86	<0.001
Baseline	1	6338.6	6338.6	24.71	<0.001
Tissue*Section	1	5753.3	5753.3	22.43	<0.001
Baseline*Tissue	1	2594.8	2594.8	10.12	0.002
Section*Baseline	1	175.0	175.0	0.68	0.409
Baseline*P1	1	5116.5	5116.5	19.95	<0.001
Baseline*T1	2	51404.8	25702.4	100.22	<0.001
Baseline*Tissue*Section	1	28.1	28.1	0.11	0.741
Baseline*Tissue* P1	1	215.3	215.3	0.84	0.360
Baseline*Section* P1	1	60.0	60.0	0.23	0.629
Tissue*Baseline*T1	2	15385.0	7692.5	29.99	<0.001
Baseline*Section* T1	2	1210.6	605.3	2.36	0.095
Baseline*P1*T1	2	1925.2	962.6	3.75	0.024
Baseline*T1*T2	1	1246.9	1246.9	4.86	0.028
Baseline*T1*P2	1	4676.3	4676.3	18.23	<0.001
Baseline*Tissue*Section* P1	1	303.7	303.7	1.18	0.277
Baseline*Tissue*Section* T1	2	421.4	210.7	0.82	0.440
Baseline*Tissue* P1*T1	2	115.9	58.0	0.23	0.798
Baseline*Section* P1*T1	2	417.7	208.8	0.81	0.443
Baseline*Tissue* T1*T2	1	608.7	608.7	2.37	0.124
Baseline*Section* T1*T2	1	42.1	42.1	0.16	0.686
Baseline*P1*T1*T2	1	112.7	112.7	0.44	0.508
Baseline*Tissue* T1*P2	1	793.9	793.9	3.10	0.079
Baseline*Section* T1*P2	1	28.1	28.1	0.11	0.741
Baseline*P1*T1*P2	1	8212.0	8212.0	32.02	<.001
Baseline*T1*T2*P2	1	113.3	113.3	0.44	0.507
Baseline*Tissue*Section* P1*T1	2	774.8	387.4	1.51	0.222
Tissue*Section*Baseline*T1*T2	1	2.2	2.2	0.01	0.925
Baseline*Tissue* P1*T1*T2	1	128.9	128.9	0.50	0.479
Baseline*Section* P1*T1.T2	1	14.8	14.8	0.06	0.810
Baseline*Tissue*Section* T1*P2	1	284.5	284.5	1.11	0.293
Baseline*Tissue* P1*T1*P2	1	3907.3	3907.3	15.23	<.001
Baseline*Section* P1*T1*P2	1	8.3	8.3	0.03	0.857
Baseline*Tissue* T1*T2*P2	1	9.6	9.6	0.04	0.847
Baseline*Section* T1*T2*P2	1	437.5	437.5	1.71	0.192
Baseline*P1*T1*T2*P2	1	1418.3	1418.3	5.53	0.019
Baseline*Tissue*Section* P1*T1*T2	1	52.8	52.8	0.21	0.650
Baseline*Tissue*Section* P1*T1*P2	1	112.5	112.5	0.44	0.508
Baseline*Tissue*Section* T1*T2*P2	1	606.0	606.0	2.36	0.125
Baseline*Tissue* P1*T1*T2*P2	1	1157.7	1157.7	4.51	0.034
Baseline*Section* P1*T1*T2*P2	1	280.2	280.2	1.09	0.296
Baseline*Tissue*Section* P1*T1*T2*P2	1	600.1	600.1	2.34	0.127
Residual	559	143366.5	256.5		
Total	610	408026.0			

Table B36. ANOVA table for the effect of ethylene on isochlorogenic acid B concentration in the Covington consignment 'NCCov III' (**Figure 5.21**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tissue	1	4793.686	4793.686	1231.96	<0.001
Section	1	153.947	153.947	39.56	<0.001
Baseline	1	153.507	153.507	39.45	<0.001
Tissue*Section	1	129.118	129.118	33.18	<0.001
Baseline*Tissue	1	112.238	112.238	28.84	<0.001
Section*Baseline	1	3.266	3.266	0.84	0.360
Baseline*P1	1	36.994	36.994	9.51	0.002
Baseline*T1	2	930.659	465.330	119.59	<0.001
Baseline*Tissue*Section	1	1.554	1.554	0.40	0.528
Baseline*Tissue* P1	1	75.497	75.497	19.40	<0.001
Baseline*Section* P1	1	20.224	20.224	5.20	0.023
Baseline*Tissue* T1	2	570.598	285.299	73.32	<0.001
Baseline*Section* T1	2	25.757	12.879	3.31	0.037
Baseline*P1*T1	2	0.073	0.037	0.01	0.991
Baseline*T1*T2	1	44.672	44.672	11.48	<0.001
Baseline*T1*P2	1	30.595	30.595	7.86	0.005
Baseline*Tissue*Section* P1	1	32.280	32.280	8.30	0.004
Baseline*Tissue*Section* T1	2	14.932	7.466	1.92	0.148
Baseline*Tissue* P1*T1	2	6.075	3.037	0.78	0.459
Baseline*Section* P1*T1	2	10.147	5.073	1.30	0.272
Baseline*Tissue* T1*T2	1	18.918	18.918	4.86	0.028
Baseline*Section* T1*T2	1	7.566	7.566	1.94	0.164
Baseline*P1*T1*T2	1	54.707	54.707	14.06	<0.001
Baseline*Tissue* T1*P2	1	10.498	10.498	2.70	0.101
Baseline*Section* T1*P2	1	27.417	27.417	7.05	0.008
Baseline*P1*T1*P2	1	67.042	67.042	17.23	<0.001
Baseline*T1*T2*P2	1	5.092	5.092	1.31	0.253
Baseline*Tissue*Section* P1*T1	2	18.680	9.340	2.40	0.092
Baseline*Tissue*Section* T1*T2	1	4.738	4.738	1.22	0.270
Baseline*Tissue* P1*T1*T2	1	18.301	18.301	4.70	0.031
Baseline*Section* P1*T1*T2	1	1.456	1.456	0.37	0.541
Baseline*Tissue*Section* T1*P2	1	24.222	24.222	6.23	0.013
Baseline*Tissue* P1*T1*P2	1	46.315	46.315	11.90	<0.001
Baseline*Section* P1*T1*P2	1	2.035	2.035	0.52	0.470
Baseline*Tissue* T1*T2*P2	1	0.207	0.207	0.05	0.818
Baseline*Section* T1*T2*P2	1	0.078	0.078	0.02	0.888
Baseline*P1*T1*T2*P2	1	72.962	72.962	18.75	<0.001
Baseline*Tissue*Section* P1*T1*T2	1	0.525	0.525	0.13	0.714
Baseline*Tissue*Section* P1*T1*P2	1	0.967	0.967	0.25	0.618
Baseline*Tissue*Section* T1*T2*P2	1	0.036	0.036	0.01	0.923
Baseline*Tissue* P1*T1*T2*P2	1	47.456	47.456	12.20	<0.001
Baseline*Section* P1*T1*T2*P2	1	0.177	0.177	0.05	0.831
Baseline*Tissue*Section* P1*T1*T2*P2	1	0.014	0.014	0.00	0.953
Residual	558	2171.243	3.891		
Total	609	9531.317			

Table B37. ANOVA table for the effect of ethylene on isochlorogenic acid C concentration in the Covington consignment 'NCCov III' (**Figure 5.21**)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tissue	1	5187.323	5187.323	1020.73	<0.001
Section	1	43.028	43.028	8.47	0.004
Baseline	1	457.607	457.607	90.05	<0.001
Tissue*Section	1	81.127	81.127	15.96	<0.001
Baseline*Section	1	8.445	8.445	1.66	0.198
Baseline*P1	1	0.824	0.824	0.16	0.687
Baseline*T1	2	1968.226	984.113	193.65	<0.001
Baseline*Tissue* P1	1	26.803	26.803	5.27	0.022
Baseline*Section* P1	1	9.424	9.424	1.85	0.174
Baseline*Section* T1	2	38.562	19.281	3.79	0.023
Baseline*P1*T1	2	15.189	7.595	1.49	0.226
Baseline*T1*T2	1	43.626	43.626	8.58	0.004
Baseline*T1*P2	1	65.573	65.573	12.90	<0.001
Baseline*Tissue*Section*P1	1	110.511	110.511	21.75	<0.001
Baseline*Section*P1*T1	2	48.560	24.280	4.78	0.009
Baseline*Tissue*T1*T2	1	18.645	18.645	3.67	0.056
Baseline*Section*T1*T2	1	8.689	8.689	1.71	0.192
Baseline*P1*T1*T2	1	73.662	73.662	14.49	<0.001
Baseline*Tissue*T1*P2	1	79.195	79.195	15.58	<0.001
Baseline*Section*T1*P2	1	4.597	4.597	0.90	0.342
Baseline*P1*T1*P2	1	57.771	57.771	11.37	<0.001
Baseline*T1*T2*P2	1	56.072	56.072	11.03	<0.001
Baseline*Tissue*Section* T1*T2	1	4.461	4.461	0.88	0.349
Baseline*Tissue* P1*T1*T2	1	4.760	4.760	0.94	0.334
Baseline*Section* P1*T1*T2	1	19.521	19.521	3.84	0.051
Baseline*Tissue*Section* T1*P2	1	21.132	21.132	4.16	0.042
Baseline*Tissue* P1*T1*P2	1	26.026	26.026	5.12	0.024
Baseline*Section* P1*T1*P2	1	8.114	8.114	1.60	0.207
Baseline*Tissue* T1*T2*P2	1	3.539	3.539	0.70	0.405
Baseline*Section* T1*T2*P2	1	3.814	3.814	0.75	0.387
Baseline*P1*T1*T2*P2	1	43.728	43.728	8.60	0.004
Baseline*Tissue*Section* P1*T1*P2	1	6.030	6.030	1.19	0.277
Baseline*Tissue* P1*T1*T2*P2	1	28.445	28.445	5.60	0.019
Baseline*Section* P1*T1*T2*P2	1	0.166	0.166	0.03	0.857
Residual	364	1849.840	5.082		
Total	402	4763.223			

APPENDIX C

ANOVA TABLES FOR CHAPTER 6

EFFECTS OF EXOGENOUS ETHYLENE SUPPLEMENTATION ON THE HORMONAL CHANGES IN SWEETPOTATO ROOTS IN RELATION TO DORMANCY DURING STORAGE

Table C1. ANOVA table for the effect of ethylene on the concentration of ABA in the skin tissues of 'NCCov II' **(Figure 6.1)**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	1111847	1111847	97.99	<0.001
Baseline*Treatment	1	8805	8805	0.78	0.385
Baseline*Time	3	378501	126167	11.12	<0.001
Baseline*Treatment*Time	3	68293	22764	2.01	0.134
Residual	30	340407	11347		
Total	38	915260			

Table C2. ANOVA table for the effect of ethylene on the concentration of ABA in the flesh tissues of 'NCCov II' **(Figure 6.1)**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	514731	514731	110.55	<0.001
Baseline*Treatment	1	138070	138070	29.65	<0.001
Baseline*Time	3	160276	53425	11.47	<0.001
Baseline*Treatment*Time	3	36477	12159	2.61	0.065
Residual	39	181581	4656		
Total	47	646506			

Table C3. ANOVA table for the effect of ethylene on the concentration of ZR in the skin tissues of 'NCCov II' **(Figure 6.1)**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	25002	25002	4.31	0.043
Baseline*Treatment	1	203318	203318	35.01	<0.001
Baseline*Time	3	37698	12566	2.16	0.103
Baseline*Treatment*Time	3	20168	6723	1.16	0.335
Residual	52	301978	5807		
Total	60	533855			

Table C4. ANOVA table for the effect of ethylene on the concentration of ZR in the flesh tissues of 'NCCov II' **(Figure 6.1)**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Baseline	1	29705	29705.	27.68	<0.001
Baseline*Treatment	1	515605	515605.	480.49	<0.001
Baseline*Time	3	230374	76791.	71.56	<0.001
Baseline*Treatment*Time	3	269960	89987.	83.86	<0.001
Residual	43	46142	1073.		
Total	51	461972			

APPENDIX D

Correlation between total phenolic compounds and antioxidant activity (Chapter 4)

Antioxidant capacity assay was carried out for only the baseline roots of 'Organic Covington' (Day 0 - with no treatment). This was measured in nine (9) individual roots and the mean taken. In general, there was *ca*. 5.5 times higher antioxidant capacity in the skin (mean: 12.09 μmol TE g^{-1}FW) than flesh (mean: 2.21 μmol TEg^{-1}FW). The antioxidant capacity was highly correlated to the total phenolics (R^2=0.95) (**Figure D1**). At the initial stage before storage, there was higher antioxidant activity in distal sections of sweetpotato skin, followed by the proximal and middle sections, respectively.

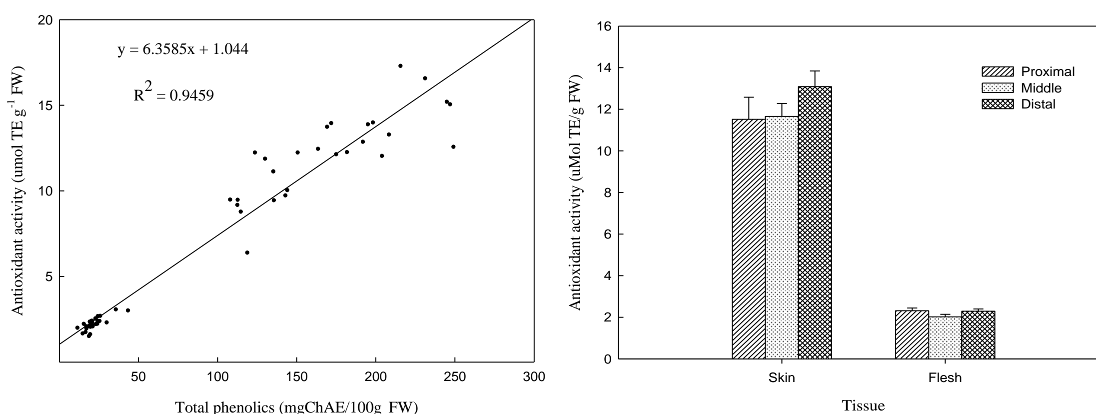

Figure D1. Corrrelation between antioxidant capacity and total phenolics in 'Organic Covington'.

APPENDIX E

CRANFIELD HEALTH POSTGRADUATE CONFERENCE

Cranfield, UK, 26 September, 2013 (Oral presentation).

Understanding the biochemical and physiological changes in sweetpotato roots as mediated by ethylene and 1-methylcyclopropene (1-MCP) during storage

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Keywords: respiration, decay, weight loss, phenolics, sugars

Abstract

The effects of exogenously applied ethylene and the ethylene binding inhibitor 1-methylcyclopropene (1-MCP) as potential treatments to prolong the postharvest life of sweetpotato [*Ipomoea batatas* (L.) Lam] were examined. In one set of experiments, 1-MCP (1.0 µl L^{-1}, 24 h) was applied to the sweetpotato roots pre-storage while in the other studies, the roots were subjected to four concerted regimes of ethylene (10 µl L^{-1}) *viz.* (1) continuous ethylene supplementation throughout the storage; (2) truncated ethylene supplementation after dormancy break, followed by storage in air; (3) post-dormant ethylene supplementation after previous air storage and (4) continuous air storage. The effects of the treatments on the physiological quality attributes as well as the spatial profiles of the non-structural sugars and individual phenolic compounds in the roots were assessed during storage. The 1-MCP treatment significantly reduced weight loss and the incidence of root decay while it also accentuated proximal dominance in phenolics biosynthesis. Root respiration was significantly boosted in both continuous ethylene supplementation or when the roots were transferred into ethylene after dormancy break; and both treatment regimes effectively inhibited sprout growth in equal measure. Truncating ethylene supplementation after dormancy break promoted vigorous sprout growth. The studies showed distinctive effects of ethylene on the metabolism of the individual sugars and phenolic compounds in both the distal and proximal regions.

VI INTERNATIONAL CONFERENCE ON MANAGING QUALITY IN CHAINS

Cranfield, *UK, 2-5 September, 2013* (Poster presentation)

Cranfield Health

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A Laboratory-Scale Design to Generate Variable Ethylene Concentrations for Supplementation of Crops During Storage

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Background

Exogenous ethylene supplementation of non-climacteric crops such as potatoes and onions has been shown to inhibit sprout growth[1]. The ethylene concentration and timing of supplementation are critical, and uniquely suited to individual crops. However, facilities to flexibly manipulate these parameters during storage trials are often not readily available. A simple design which allows parallel flushing of small capacity multi-chambers with variable ethylene levels is described.

Materials and methods

Certified ethylene in nitrogen (BOC, Surrey, UK) of stock concentration 5000 μL L^{-1} was diluted to 10 μL L^{-1} with air from a Hydrovane Compressor. This was accomplished by connecting two calibrated mass flow control valves (Sierra, The Netherlands) in line with the ethylene and air stream, respectively, to regulate their flow and blend them at a T-junction downstream. The calibrated maximum flow rates of the ethylene and air valves were 0.03 and 13.5 L min^{-1}, respectively. A multi-channel gas mixer (MFC-4, Sable Systems, NV, US) was connected to the valves to control the actual flow rates. The desired ethylene concentration K (μL L^{-1}) was achieved with the derived model:

$$K = \{(x\% \text{ of } 30)/(y\% \text{ of } 13500)\} * 5000$$

Where $x\%$ is the % full-scale setting of the ethylene flow control valve
$y\%$ is the % full-scale setting of the air flow control valve

The diluted ethylene and pure air from the compressor (controlled by a third valve) were applied continuously to sweet potato roots (*Ipomoea batatas* (L.) Lam.) in 81.2 L storage boxes via flexible nylon tubing and gas distribution manifolds (HNL Engineering Ltd., UK). Headspace gas samples in the storage boxes were periodically analysed with a gas chromatograph to ascertain the concentration.

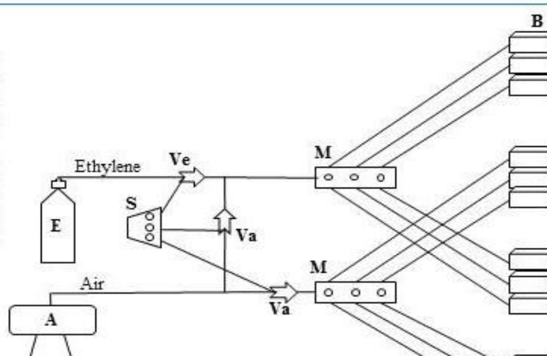

Figure 1. Schematic diagram of the experimental design
A. Air compressor **E.** Ethylene cylinder **Ve.** Ethylene flow control valve **Va.** Air flow control valve **S.** MFC-4 Sable gas mixer **M.** Gas distribution manifolds **B.** Crop storage boxes

Results

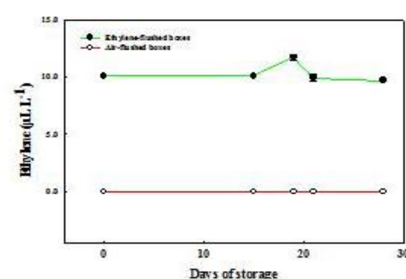

Figure 2. Ethylene concentration in sweetpotato storage boxes over time. Each data point is the mean for six replicate boxes. Error bars are shown.

Figure 3a. Condition of sweetpotato flushed continuously with 10 μL L^{-1} ethylene for 10 weeks.

Figure 3b. Sprout growth of sweetpotato flushed continuously with air for 10 weeks.

Conclusions

- Ethylene concentration of 10 μL L^{-1} was achieved (with little variation).
- By selecting appropriate flow rates for the ethylene/air channels, the ethylene concentration could be varied for continuous supplementation of the crop storage boxes.
- The system offers easy-to-manage ethylene application for research.

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Reference

1. 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.

Acknowledgements

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SCI BIORESOURCES YOUNG RESEARCHERS CONFERENCE

Reading, UK, 2 July 2013 (Oral presentation).

Effects of exogenous ethylene on the biochemistry and physiology of sweetpotato roots during storage

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Abstract

The effects of continuous ethylene treatment (10 μL L^{-1}) on sweetpotato quality during storage at 25°C were examined since exogenous ethylene has been reported to improve the storage of some other non-climacteric storage organs. Alongside the continuous ethylene and air control treatments, roots were also transferred, at first indication of sprouting, from ethylene to air and vice-versa. At period intervals before and after dormancy break, samples were randomly collected from all treatments and the weight, disease incidence, respiration, sprouting incidence and severity evaluated. Spatial variations in the individual phenolic compounds were also assessed at the proximal (stem end) and distal (root end) segments for both skin and flesh tissues using HPLC. Root respiration was promoted whilst sprout growth was reduced in roots held in continuous ethylene or when moved from air into ethylene. Concomitantly, ethylene treatment was associated with increased weight loss. The implications of ethylene application on the storage of sweetpotato and the downstream effects on the physiological and biochemical changes are discussed.

XI INTERNATIONAL CONTROLLED AND MODIFIED ATMOSPHERE RESEARCH CONFERENCE

Trani, Italy, 3-7 June 2013 (Oral Presentation)

Effects of ethylene on the biochemical and physiological changes in sweetpotato roots during storage

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Keywords: respiration, weight loss, sprouting, sugars, phenolics

Abstract

The effects of continuous ethylene treatment (10 µL L^{-1}) on sweetpotato quality during storage at 25°C were examined since exogenous ethylene has been reported to improve the storage of some other non-climacteric storage organs. Alongside the continuous ethylene and air control treatments, roots were also transferred, at first indication of spouting, from ethylene to air and vice-versa. At periodic intervals before and after dormancy break, samples were randomly collected from all treatments and the weight, disease incidence, respiration, sprouting incidence and severity evaluated. Spatial variations in non-structural carbohydrate levels (maltose, fructose, glucose, sucrose) and individual phenolics were also assessed at the proximal (stem end) and distal (root end) segments for both skin and flesh tissues using HPLC. Root respiration was promoted whilst sprout growth was reduced in roots held in continuous ethylene or when moved from air into ethylene. Concomitantly, ethylene treatment was associated with increased weight loss. The implications of ethylene application on the storage of sweetpotato and the downstream effects on the physiological and biochemical changes are discussed.

ISHS ACTA HORTICULTURAE 1012

VII INTERNATIONAL POSTHARVEST SYMPOSIUM

Kuala Lumpur, Malaysia, 25-29 June 2012 (Oral presentation)

Biochemical and physiological changes in stored sweetpotatoes as mediated by 1-methylcyclopropene (1-MCP)

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Keywords: respiration, decay, weight loss, phenolics, sugars

Abstract

Many countries in Africa are currently promoting the consumption of sweetpotato (*Ipomoea batatas* (L.) Lam) as a public health tool for improved child and maternal nutrition. The difficulties in long-term storage of sweetpotato roots, however, pose a major challenge in its role as a food security crop. This study investigated the effects of 1-methylcyclopropene as a potential treatment to prolong the postharvest life of sweetpotato since the ethylene inhibitor and indeed ethylene have been shown to affect other non-climacteric crops which also produce very low ethylene levels. Two sweetpotato cultivars, Organic Covington and Portuguese-derived Covington were treated with 1-MCP (1.0 µL L^{-1}) for 24 h and stored at 15°C. At periodic intervals, samples were randomly collected from storage and the decay, saleable weight, dry matter, sprouting and respiration evaluated. The spatial variations of non-structural sugars (maltose, fructose, glucose, sucrose) and individual phenolics (chlorogenic acid, iso-chlorogenic acids, caffeic acid, coumaric acid and ferulic acid) in the organic Covington were assessed at the proximal (stem end), middle, and distal (root end) segments for both skin and flesh tissues using high performance liquid chromatography. 1-MCP treatment significantly reduced weight loss and decay development in both cultivars. Phenolic compounds and dry matter were more abundant in the skin than flesh for all segments with the proximal sections

accumulating higher amounts with time. The proximal dominance in phenolics accumulation was significantly more pronounced in the 1-MCP-treated roots. No sprouting was recorded in both cultivars for treated and control roots. The implications of the use of 1-MCP on sweet potato and the accompanying physiological and biochemical changes are discussed.