Final Report

Reducing energy usage and wastage by improving ethylene control of potato sprouting

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1. SUMMARY

This project sought to reduce energy usage and cut wastage during potato storage by developing strategies for improved sprout control that reduce quality losses while opening the way for storage at higher temperatures for both processing and fresh marketed potatoes. Through development of alternative strategies to CIPC usage we sought to provide growers with a wider range of viable strategies if further restrictions on CIPC use were to be imposed in the future.

Continuous application of ethylene (4-10 ppm) to potatoes during storage has been developed as an alternative to CIPC prior to the initiation of this project, as it inhibits the growth of sprouts once they have initiated. However, an important constraint to ethylene use for sprout suppression is that varietal variability in ethylene sensitivity means that most varieties need to be held at low temperature for effective sprout control. In addition to this, ethylene can induce increased respiration and sugar accumulation in some varieties. This is not acceptable for processing varieties as sugar accumulation causes fried products to become unacceptably dark. Sugar accumulation also impacts on the increased potential to produce acrylamide, identified as a potential carcinogen, during potato processing.

During the project two approaches were taken;
- Firstly, using a range of advanced molecular biology techniques, comparison of ethylene sensitive cultivars with non-responders was used to enhance our understanding of the molecular mechanism by which ethylene exerts its control on sprout growth with the aim of identifying the key potato genes involved and developing markers for these genes.
- Secondly, by combining the use of ethylene with other plant growth regulators, storage strategies were developed to provide sufficient sprout control and good quality tubers (low sugar accumulation) for a wider range of cultivars and conditions than previously possible.

1.1. Developing an understanding of the molecular biology of ethylene effects on sprout growth

The GENPOP1 population (12601 ab1 x Stirling, developed by the James Hutton Institute) was selected for study within this project because it was well characterized genotypically, and also due to the differences in sprout growth response to ethylene between the parents that had been observed prior to the project initiation. During the course of the project development and project trials, these two lines were characterized over four seasons. The results underline that although there are common responses across seasons, with Stirling always exhibiting greater sensitivity to ethylene, the magnitude of the response varies very significantly between seasons. Similarly phenotyping of the complete GENPOP1 population indicated that the range in ethylene response varied between seasons.

The behaviour of the parents and progeny across seasons indicates a strong environmental contribution to ethylene response. An understanding of the environmental contribution could potentially be as useful as understanding the genetic contribution by providing a means of manipulating varietal behavior for storability.

Using the GENPOP1 population a number of QTLs have been identified for sprouting in air and in ethylene. None of these account for more than 9% variance, and most account for less

than 7%. This is lower than the variance accounted for by QTLs identified for other potato characteristics such as resistance to pathogens including nematodes (PCN), viruses (Potato Virus Y) and Oomycetes (such as *Phytophthora infestans*). This is probably an indication that the trait being considered is very complex; controlled by a large number of genes, which is not unexpected especially given that ethylene affects dormancy break as well as sprout growth. It is also consistent with the environmental contribution.

With low levels of variance accounted for and inconsistency across seasons, markers for the QTLs identified are unlikely to be of direct practical use within breeding. In practice the value of this analysis will be to combine with other information, for example, from microarray analysis in order to identify genes of interest.

Microarray analyses were carried out to study changes in gene expression induced by ethylene both for the parents (12601 ab1 and Stirling) and samples from the GENPOP1 population. For the parents, genelists have been produced highlighting those genes differentially expressed by treatments and by variety. For the GENPOP1 population RNA bulks from tuber bud samples from four sets of clones with similar responses to ethylene were constructed and patterns of gene expression were measured for each bulk by microarray analysis in season 2010. (The four sets of clones were *1: No significant effect of ethylene on sprouting. 2: Reduction in sprout length on ethylene treatment. 3: Increase in sprout length on ethylene treatment. 4: Reduction in sprout length on ethylene treatment and little sprout growth*.) In season 2011 this was repeated for comparison using material from one set of clones (4), which represent the most useful clone set as far as the industry is concerned. Differentially expressed genes from bulks with contrasting ethylene traits were genetically mapped in order to re-enforce the QTL analysis, and provide a rapid means of developing gene markers closely associated with ethylene responsiveness.

Although gene expression patterns did vary by season due to environmental effects a selection of 203 genes were identified that were expressed consistently in both season 2010 and season 2011 within the clone set 4. The expression of these genes was characterised across all four clone pools. For example 48 genes were identified for which expression increased with sprout length in presence of ethylene.

Although QTL information from this project does not allow us to identify the target genes directly, information on gene function and expression patterns will allow a manageable number of key genes to be identified for further study.

1.2. Developing storage strategies by combining ethylene treatment with other chemicals

Trials undertaken on three varieties; Maris Piper, Saturna and Russet Burbank indicated that the maximum inhibitory effect of ethylene on sprout growth during storage at 9°C required concentrations greater than 1 ppm, with no clear difference between 10 and 25 ppm. The trials did not indicate a clear difference in ethylene stimulation of sugar accumulation nor of acrylamide production across the concentrations tested. These two findings suggest that the industry standard of 10 ppm is appropriate.

With ethylene control sprouting is greater in tubers stored at 9°C than at 6°C. Ethylene effects are variety dependent and therefore treatment is not sufficiently effective for commercial use in all varieties. With respect to key UK varieties Markies, Russet Burbank and Sylvana are responsive to ethylene, with Cabaret, Maris Piper, Hermes and Saturna less responsive. This varietal difference is consistent across seasons. Results of trials undertaken during this project indicate that ethylene could provide a good strategy for sprout control in varieties such as Sylvana (up to 6 months), Markies (up to 4 months, but processing quality is a problem) and Russet Burbank (up to 4 months)

Ethylene has a detrimental effect on processing quality as it tends to stimulate sugar accumulation. This is independent of the effect on sprouting; demonstrated by the adverse effect of ethylene on processing quality of the chipping varieties Markies and Hermes which were relatively sensitive and insensitive to ethylene, in terms of inhibition of sprouting, respectively. Sugar accumulation is greater in tubers stored at 6°C than at 9°C. Raising the storage temperature of potatoes from 6 to 9°C mitigates the effect of ethylene induced accumulation of glucose and fructose seen at lower temperatures.

SmartFresh™ has no effect on sprouting behaviour but helps to ameliorate the ethylene induced sugar accumulation in some varieties (such as Sylvana and Markies) early in the storage season up to 2 months. Ethylene and SmartFresh™ responses in terms of sprout suppression and sugar accumulation in potato are not universal to all varieties tested. Verdi was the least responsive variety to ethylene induction of sugar accumulation and sprout suppression whilst Sylvana and Russet Burbank were sensitive to ethylene in-terms of sugar accumulation and sprout suppression. Hermes was relatively unresponsive to ethylene in-terms of sugar accumulation and sprout suppression,

During this project several chemicals have been tested for their synergistic behaviour with ethylene treatment to determine whether a combined treatment could provide a more reliable strategy for sprout control than ethylene alone. Spearmint (R-carvone) treatment is as effective as ethylene at 2 months but this effect declined significantly at 4 and 6 months. For varieties that are unresponsive to ethylene the addition of spearmint improves sprout control. Spearmint had little or no apparent effect on processing quality.

A wide range of other chemicals have been tested for their potential for sprout control in combination with ethylene. 1,4 dimethylnaphthalene and methyl jasmonate have been identified as chemicals with potential for development in the medium term.

The way forward

- Phenotyping of the GENPOP1 population provided us with QTLs for both ethylene control of sprouting and dormancy length - however, these were minor QTLs usually accounting for 5-6% variance. This is almost certainly a result of the complexity of the phenotypic characteristics that we were studying; the effect of ethylene on sprout length being affected by ethylene effects on breaking dormancy and by the inhibitory effect of ethylene on sprout growth. One way to take this work forward would be to design trials in such a way as to remove the dormancy effects to focus phenotyping only on sprout growth rates. The use of bromoethane, which rapidly results in co-ordinated exit from bud endodormancy may enable the ethylene response trait to be dissected into some of its component parts.

- Despite advances in analytical techniques it is still very complex to identify QTLs in a tetraploid population. Analysis of a diploid population would simplify the approach and may lead to the identification of candidate genes more rapidly. In fact a project "Controlling dormancy and sprouting in potato and onion" approved through the BBSRC HAPI programme will take this approach to look at potato dormancy. The recently developed method of *de novo* DNA sequence driven bulk segregant analysis to delimit chromosomal regions that underpin phenotypic traits may also be an approach worth considering in the future. Genome-wide association studies in potato may also be an approach that is becoming feasible but all of these approaches require accurate phenotypic analysis.

- Although it has not been possible to identify individual target genes any further studies would be able to build on the information such as genelists and QTLs available from this project to focus in on candidates.

- Environmental effects on ethylene response are clearly significant, as indicated by the different behaviour of the parents and the GENPOP1 progeny between seasons. An understanding of the environmental effects would provide tools to manipulate this response, which might be more straight forward than breeding for cultivars with the required response. This avenue could be explored using a carefully designed set of trials using for example different planting dates, locations with different climates and daylengths, irrigation regimes, preharvest ethylene inhibiting sprays for selected lines.

- Ethylene exerts major effects on the potato tuber life-cycle, clearly impacting on both tuber bud endodormancy release and rate of sprout growth. Considerable knowledge of the ethylene biosynthetic pathways, ethylene perception and response mechanisms is available from the study of model plant systems such as *Arabidopsis* and tomato. Perhaps surprisingly, very little research in potato has been published where ethylene biochemistry has been perturbed transgenically. In view of the complexity of ethylene responses in potato and their importance in tuber development, it would be timely to use a direct transgenic approach to dissect the mechanism of the potato ethylene response. Technically, such an approach is well within our capability and such an approach would complement the search for naturally occurring alleles that can be incorporated into a conventional potato breeding approach.

- Strategies for the use of Spearmint (R-Carvone) for sprout control together with ethylene should be developed for specific varieties.

- Likewise strategies to use 1-MCP treatment to improve processing quality should be developed.

- 1,4 DMN and methyl jasmonate should also be investigated as commercially viable chemicals to be used in combination with ethylene

- In order to achieve effective sprout control in potato using Plant Growth Regulators/plant hormone-based sprout suppressants it is critical to understand the physiological status of the tubers at the point of treatment. Better methods of

determining tuber maturity at harvest and during storage are required to allow for more accurate targeting of treatments to afford effective sprout control.

2. INTRODUCTION

2.1. Background

This project is a collaboration between the Natural Resources Institute, James Hutton Institute (formerly Scottish Crop Research Institute), Potato Council, Greenvale AP Ltd, Cygnet Potato Breeders Ltd, Pepsico International Ltd, Landseer Ltd and Greenwich University Enterprise Ltd supported by the Sustainable Arable Link Programme (DEFRA and RERAD).

Potato storage is among the most significant energy consuming processes in the food industry (9th highest energy consumer, Swain 2008). Control of sprouting is essential for efficient storage of the *c* 3.5m tonnes of crop stored in the UK, and without this potato tubers rapidly become unsuitable for both processing and fresh marketing. Until recently the most common strategy for controlling sprouting in both fresh marketed and processing potatoes has been to treat with the sprout inhibitor, chlorpropham (CIPC). Although alternatives have been identified for fresh marketed produce (see below), in the UK most potatoes destined for processing are still treated with CIPC. A new EU maximum residue limit (MRL) for CIPC was established in 2005 of 10 mg kg^{-1} for potatoes. Subsequently, as part of the review of EU 91/414, the risk of MRL exceedance was indentified in the UK and the Advisory Committee on Pesticides introduced application restrictions. An industry 'stewardship scheme' has been implemented in the UK to address this, but concerns remain about its continued availability as CIPC is on the B-list of chemicals currently being reviewed by EU for hazard assessment. Supermarket/consumer pressure is also pushing for reduced pesticide usage and lower residues and in some supply chains the use of CIPC is being discouraged by retailers. With no current alternative to CIPC the processing industry would be faced with adopting strategies that would be more costly and would increase energy usage and wastage: Thus it may be necessary to import potatoes for several months of the year, or, for some products, increase the short-term processing capacity so that the storage period of the UK crop could be cut, and products stored instead. This strategy would be less efficient operationally and the quality of the products would be compromised.

Continuous application of ethylene (4-10 ppm) to potatoes during storage has been developed as an alternative to CIPC, as it inhibits the growth of sprouts once they have initiated (Prange *et al*. 1998). Ethylene has hence been used as an alternative to CIPC in the UK fresh marketed potato sector. This development was brought about through previous research funded by DEFRA (HH2114STF) and additional industry funded trials (e.g. by Greenvale). Currently, over 150,000 tonnes of potatoes are treated with ethylene. However, an important constraint to ethylene use for sprout suppression is that varietal variability in ethylene sensitivity means that most varieties need to be held at low temperature for effective sprout control. In addition to this, ethylene can induce increased respiration and sugar accumulation in some varieties. This is not acceptable for processing varieties as sugar accumulation causes fried products to become unacceptably dark. Sugar accumulation also impacts on the increased potential to produce acrylamide, identified as a potential carcinogen, during potato processing.

This issue is of great economic importance; in 2006 approximately half of the UK's annual consumption of potato (5.56 million tonnes) was purchased as processed products (crisp, chips, frozen and dehydrated) and this trend is increasing steadily. The UK potato industry is

subject to severe competition from imports, half of the processed potato market (1.29 million tonnes) is currently met from imports (BPC 2007). More efficient and safer forms of sprout control would enable the UK potato industry to further increase its share of the UK market (retail value £3.6 billion per annum) and hence would contribute to the sustainability of UK agriculture. Further, any reduction in waste of at this late stage of the handling chain not only cuts the volume of waste for disposal, but also leads to a reduction in the additional energy usage involved in storing produce that ultimately cannot be used. Strategies that allow the use of higher storage temperatures will contribute to an abatement of greenhouse gases through an estimated saving of 14 MWh of electricity per year for every 1°C increase in storage temperature achieved (Cunnington, A., SBCSR; *pers. comm.*).

2.2. Project mission

This project seeks to reduce energy usage and cut wastage during potato storage. It will achieve this by developing strategies for improved sprout control that reduce quality losses while opening the way for storage at higher temperatures for both processing and fresh marketed potatoes. As well as reducing energy usage and wastage relative to current levels, by developing viable alternatives to CIPC this project will protect the potato industry from the potentially disasterous consequences if CIPC usage were further restricted or the chemical were banned. Without an alternative strategy, loss of CIPC could lead to huge losses and increases in energy usage as the industry tries to adapt.

The proposed work will develop a robust and practical alternative to CIPC. Firstly, treatments based on the use of ethylene will be developed further, extending the range of cultivars and conditions for which ethylene treatment is effective by combining the use of ethylene with other plant growth regulators. Secondly, using a range of advanced molecular biology techniques, comparison of ethylene sensitive cultivars with non-responders will enhance our understanding of the molecular mechanism by which ethylene exerts its control on sprout growth. This will lead to the identification of the key potato genes involved and the development of markers for these genes.

The work programme involves trials in which a range of varieties and breeding lines will be tested for their reaction to ethylene in terms of sprout growth and processing quality. We will then examine differential patterns of gene expression among cultivars and lines that differ in their reaction to ethylene, and identify the key genes involved using transcriptomic analysis, coupled with physiological and biochemical characterisation. QTL analysis will provide information on the number of genes controlling each trait and gene mapping studies will further support the involvement of candidate genes in the ethylene response. User-friendly gene markers will be developed and tested for the best candidate genes providing a resource for future breeding programmes.

2.3. Project objectives

The overall objective is to cut wastage during storage of processing potatoes by improving sprout control, focusing on strategies involving ethylene that reduce chemical usage and energy consumption.
Specific objectives are as follows:

Objective 1: To understand the basic science underlying ethylene control of sprouting and its influence on tuber quality, in particular by identifying the main controlling genes.

Objective 2: To develop and test markers for key genes regulating ethylene mediated sprout control that might be used to identify new cultivars with increased response to ethylene.

Objective 3: To optimise storage strategies for processing potatoes using ethylene to control sprout growth while maintaining good processing quality, by exploiting synergistic interactions between ethylene and other plant hormones/ antagonists.

Objective 4: To facilitate exploitation and technology transfer of project outputs.

2.4. Scientific background

Potato tubers must be stored for greater periods than their natural dormancy, to supply the processing industry with raw material year round. Once tuber dormancy is broken, sprouting occurs with loss in quality due both to disease-related and physiological processes (Burton *et al.*, 1992). To extend the storage time for potato tubers beyond that of natural dormancy (generally 1-15 weeks) two main strategies are employed by the potato processing industry; storage at low temperatures (expensive and effects quality) and by the use of chemical sprout suppressants such as CIPC (expensive and leaves chemical residues in the food product - the EU maximum residue level has recently been reduced to 10 ppm). A more environmentally–friendly alternative is being developed based on the observation that continuous application of ethylene (4 ppm) to potatoes during storage reduces the length of sprouts once they have initiated (Prange *et al.*, 1998). Ethylene has hence been used as an alternative to CIPC in the UK fresh market potato sector.

Although it is clear that continuous exposure to ethylene gas controls sprout growth, for some cultivars it can also result in darker fry colour. Additionally variation in sprout control between varieties can be reduced by lowering the storage temperature below 6°C although this often stimulates low-temperature sweetening, leading to the accumulation of reducing-sugars (fructose and glucose) (reviewed in Sowokinos, 2001). Strategies to counteract the adverse effects of ethylene through ramped application of ethylene or treatment with 1-methylcyclopropene (1-MCP), a competitive inhibitor of ethylene receptors, are presently being tested for fresh marketed varieties. 1-MCP, can ameliorate the effects of ethylene induced darkening of fry colour while having little/no effect on sprout control. This suggests that combining ethylene and 1-MCP treatments could lead to a practical tuber storage strategy for some cultivars. However, there is variability between varieties in response to 1-MCP (Daniels-Lake, 2008; Coleman, P., Greenvale AP, *pers. comm.*).

Although ethylene is effective in some cultivars under some storage conditions, many important commercial cultivars do not respond to ethylene. Furthermore, as ethylene tends to stimulate sugar accumulation, its uptake by the industry has been slow. Although it is widely accepted that ethylene can restrict the growth of emerging potato sprouts, there is a gap in our knowledge concerning the mechanism by which this process occurs and our current understanding cannot be advanced through an empirical approach alone.

A wealth of research investigating the mechanism by which plants perceive and mediate response to ethylene in model species such as tomato and *Arabidopsis* has identified the key genes and mechanisms (reviewed in Klee and Tieman, 2002; Binder, 2008). The key genes include those encoding ethylene receptors such as ETR1, ERS1 and downstream signalling components including CTR1, EIN2, EIN3/EIL and ERF-1. Ethylene is able to bind to receptors by the aid of a copper (I) cofactor. The receptors act as negative regulators of ethylene responses (Hua and Meyerowitz, 1998). Our current understanding indicates CTR1 actively suppresses signal transduction in the absence of ethylene. Ethylene binding to the receptors leads to a conformational change in CTR1 and a reduction in activity and a relinquishing of repression of ethylene signalling. Single amino acid changes in the ethylene binding domain of receptors frequently results in ethylene insensitivity, through an inability to recognise ethylene. Thus in principle, allelic variation and/or expression level variation in genes encoding any of the ethylene perception and signalling pathway may lead to insensitivity to ethylene, which supports our strategy to identify whether any alleles or expression level differences can be correlated with altered sensitivity to ethylene among potato varieties.

It is likely that ethylene is able to interact with other plant growth regulators (auxin and gibberellin). Ethylene is known to interact with early auxin response genes; IAA9 a transcript repressor of auxin signalling is regulated by ethylene (Lin *et al.* 2008). Although a lot is known about ethylene signalling in model plants, the challenge is to transfer this knowledge to crop species such as potato for practical benefit.

3. MATERIALS AND METHODS

Note: Seasons are referred to by the harvest year (Season 2009. Season 2010 and Season 2011).

3.1. Phenotyping parents (12601 ab1 and Stirling) of selected cross and their progeny (GENPOP1)

Trials to phenotype the parents and the 220 progeny of a characterised cross (12601 ab1 x Stirling) for sensitivity to ethylene control of dormancy break and control of sprout growth were carried out by NRI using facilities at East Malling Research (EMR).

The GENPOP population and parental material (12601ab and Stirling) were grown in 2010 and 2011 in two replicate three plant plots using a randomised block design at JHI, Invergowrie, Dundee. In 2010 the trial was planted in May and harvested by hand in late September. In 2011 the trial was planted in May and harvested in October. Netted samples were cured at ambient for two weeks before delivery to the storage facility at EMR, where field replicate samples were split into two separate storage replicates. Each storage replicate was divided into air and ethylene treated cabinets. Samples were cooled over 48 hours to ensure tubers had reached the storage temperature (9°C), before loading into 0.5 tonne CA cabinets, each cabinet contained approximately 2000 tubers. In addition, 3 kg bags of hydrated lime (Ca(OH)$_2$) were added to each cabinet to absorb excess carbon dioxide and within the control cabinets tubes of potassium permanganate coated clay beads were placed to remove any extraneous sources of ethylene. Each control cabinet received a flow of air equivalent to (1L kg^{-1} h^{-1}) while ethylene treated cabinets were supplied with a separate air-line (1L kg^{-1} h^{-1}) amended with 10 ppm ethylene supplied from a 20% ethylene gas cylinder (BOC). The storage atmosphere of each cabinet was monitored regularly for O$_2$, CO$_2$ and ethylene.

Potatoes were removed from store after 14, 28 and 70 days. On the first two occasions, individual tubers were washed prior to apical bud excision with surrounding cortex tissue removed under a dissecting stereo microscope, each bud was excised in the presence of 40μL RNA-later (Ambion). One bud per tuber per replicate was removed from each of 220 clones in the presence and absence of ethylene (10 ppm) on two sampling occasions; a total of 1,700 buds were extracted. Buds were place in individual RNase free 1.5mL centrifuge tubes and flash frozen in liquid nitrogen prior to storage at -80°C.

After 21, 35 and 77 days (1 week after bud sampling) the length of the apical bud/sprout was measured for five tubers per sample (approximately 4400 tubers assessed on each occasion).

3.2. Total RNA extraction from potato tuber buds

RNA extraction of bud material followed the Plant RNAeasy extraction protocol (Qiagen) with the addition of on-column DNase removal using DNAse I. RNA samples were quantified using a spectrophotometer and quality tested using an RNA 6000 nano chip on an Agilent 2100 Bioanalyzer (www.chem.agilent.com). RNA samples are aliquoted in 20 μg (1 μg/μl) batches and stored at –80°C prior to transfer to JHI for microarray analysis.

3.3. Marker based Anova and QTL analysis

3.3.1. Marker based anova analysis for season 2010

For the 2010 season, using the ethylene phenotyping data a preliminary analysis was carried out to investigate whether significant associations between SNP markers (and hence chromosomal locations, as all the SNP marker locations are known) and ethylene response could be detected. A REML model was used to look for significant associations with SNP markers, fitting treatment (i.e. the effect of ethylene) as a fixed effect and genotype (i.e. clone) as a random effect.

3.3.2. QTL analysis for seasons 2010 and 2011

In May 2012 a draft genetic map for the 12601ab1 x Stirling population was completed allowing a full QTL analysis of the genotype data. While the marker based ANOVA uses more data (190 genotypes * 2 treatments * 3 timepoints) and therefore has more power to detect effects than 190 genotypes as used in the QTL analysis, the QTL analysis is more robust than the REML model because it enables proper statistical tests (permutation tests) of significance to be carried out. Data from season 2010 and season 2011 trials were therefore analysed using the new map data.

3.4. Microarray analyses

3.4.1. Microarray analysis for 12601 ab1 and Stirling in season 2009

In order to investigate gene expression in potato tuber buds a microarray experiment was carried out using the POCI microarray to analyse global gene expression in potato (described in Kloosterman *et al.,* 2008), representing 42,034 unigene sequences and approximately 70% of the potato transcriptome. Gene expression in control air treated buds was compared with ethylene treated buds, and analysis was carried out using the parents of the GENPOP1 population, 12601ab and Stirling. Expression profiles were investigated at three time points; after 4 weeks, 7 weeks and 12 weeks of ethylene exposure compared with air controls, from tubers stored at 9^oC. There were four replicates per sample and so a total of 48 single colour arrays were used. The layout of the microarray processing order followed a split-plot format.

3.4.2. Microarray analysis of pooled samples of GENPOP1 that differ in ethylene response. Identification of differentially expressed genes and their relationship with QTLs.

For this analysis a new potato microarray was employed. A custom Agilent microarray was designed to the predicted transcripts from assembly v.3.4 of the DM potato genome (PGSC, 2011). Single 60mer probes were designed for a total of 52,848 transcripts, which includes alternative isoforms, using eArray (https://earray.chem.agilent.com/earray/) with default parameters in 8x 60k format. The complete design of the microarray can be found at ArrayExpress (http://www.ebi.ac.uk/arrayexpress/). Access to ordering the array design (AMADID 033033) from Agilent is available on request from the authors.

The new design, gives 25-30% greater coverage than the previously used POCI array and near "whole-transcriptome" coverage coupled with improved sensitivity (50 ng of total RNA/sample). Ethylene treated samples were compared with air-controls in an eight array experiment.

Sprout growth data was analysed and sprouting behaviour was categorised into tubers where apical growth was significantly shorter or longer in ethylene, where there was no effect and where tubers remained dormant 'super dormant'. Buds from eight tubers selected from each response group were selected for RNA extraction along with buds from parent material.

RNA labelling, array hybridisation, washing and scanning were as detailed in Stushnoff *et al.,* (2010). Data were extracted using Agilent FE software and imported into Genespring v 7.3 as previously described (Stushnoff *et al.,* 2010). Normalisation of data was performed using the default Lowess algorithm. Volcano plots were used to identify probes with significant differential expression between each pool type (Student's t-test p-value ≤0.05, fold-change ≥2x) for each trait.

3.5. Trial to optimise the ethylene concentration for sprout control.

In year one (season 2009) a trial was carried out to assess the effect of ethylene applied at a range of concentration on sprout growth and processing quality including the potential acrylamide production during processing.

This trial was conducted at SBCSR. It involved 4 treatments 0, 1, 10 and 25 ppm ethylene at 9°C, and three varieties Cabaret, Maris Piper, Russet Burbank. Originally a fourth variety, Verdi was to be included, but due to insufficient tuber numbers was replaced by Saturna. Cabaret was unfortunately treated with Maleic Hydrazide in the field.

Four ethylene treatments were applied at 9°C, untreated, and 1, 10 and 25 ppm continuous ethylene treatment. No ramping was used to gradually acclimatise the potato to ethylene. Variation was assessed by two within-store replicates. Each treatment was applied in duplicate 0.5 m^3 Controlled Atmosphere chambers with, 4 x 25 tuber samples of each variety per chamber. The base of each chamber was flooded to provide constant high humidity and lime was used to scrub carbon dioxide. Ethylene was monitored daily and controlled to within 10% of the specified concentration. Assessments were carried out after 1, 2, 3 and 4 months for longest length and number of sites of sprouting and at 4 months dry matter and sprout weight were also measured. Core samples from opposite eighths are taken and stored in a freezer at -20°C for HPLC analysis of sugars (glucose, fructose and sucrose) by NRI.

One tuber from each replicate sample per treatment was sliced to approx 1mm, and the slices spread out within a 20x20 cm plastic bag and rapidly frozen between 2 -20 °C plates in a -20 °C freezer. Frozen samples were delivered to Dr S. Elmore, Reading University for acrylamide analysis. Full details of the method are included within the Reading University report available as Appendix 13.

3.6. Small-scale synergy trials

3.6.1. Season 2009

In the first season (2009) these trials were conducted by NRI in facilities based at East Malling Research. Tubers were stored by SBCSR until the point of dormancy break and then transported to NRI. Using Maris Piper, the following treatments were tested with and without continuous application of 10 ppm ethylene:

- Topical application: (10 µL) of 50 µM solutions of paclobutrazole, diniconazole, epibrassinosteriod, (+)-(-) Abscissic Acid (ABA), Salicylic acid (SA) , 2-Chlorophenyl urea (2-CPU), Naphthyl acetic acid (NAA) and Indole acetic acid (IAA).

- Wicked application:1,4-dimethyl naphthalene (1,4 DMN at 3.6µL/6kg tubers), s-carvone (3.6 mL/6kg tubers), methyl jasmonate 500 µL/6kg tubers.

Application rates were based on previously published results.

Subsequent trials with Cabaret and Russet Burbank focussed on identification of dose response between 10 ppm ethylene and 1, 10 and 50µm: 1,4-DMN, s-carvone, methyl jasmonate, paclobutrazole, diniconazole, 24-epibrassinosteriod and ABA. Application was made at the point of bud opening, tubers were held previously in air at 6°C for 6 months prior to treatment. After treatment tubers were held at 9°C with or without ethylene treatment (10 ppm).

3.6.2. Season 2010

In the second season (2010), Maris Piper and Hermes tubers were sent by SBCSR and were from the same stock utilised in the main commercial variety trial. Tubers of all varieties were received in the last week of October. Treatments from year 1 that were near to commercial exploitation (1,4 DMN, s-carvone and methyl jasmonate were selected for further investigation).

The following treatments were tested with and without continuous application of 10 ppm ethylene:

- Wicked application: 1,4-dimethyl napthylene (1,4 DMN) at 3.6µL/6kg tubers, s-carvone (3.6 mL/6kg tubers), methyl jasmonate 500 µL/6kg tubers.

Tubers were stored in 25 L polypropylene containers with a flow of air. Each container received a flow of air equivalent to (1L kg^{-1} h^{-1}) while ethylene treated cabinets were supplied with a separate air-line (1L kg^{-1} h^{-1}) amended with 10 µL L^{-1} ethylene. Tubers were stored at 9°C. For Maris Piper, the treatments were applied at the point of bud movement, whereas tubers of Hermes were endodormant at the time of treatment. The storage atmosphere of each cabinet was monitored regularly for O_2, CO_2 and ethylene. Length of apical sprout growth was measured at intake and at monthly intervals over a 4 month period.

3.6.3. Season 2011

Small scale trials investigating the role of methyl jasmonate and 1,4 dimethyl naphthalene on suppression of sprout growth in potato cultivars Hermes and Maris Piper. Tubers were sent

by SBCSR at the end of October, and were washed and randomised before being allocated to treatments. Due to the degree of bud movement in Maris Piper, it was decided to restrict 1,4 DMN treatment to Hermes that was exhibiting complete endodormancy at the time of treatment.

Methyl jasmonate [2mM] was applied as either a vapour treatment wicked into a sealed chamber for 24 hours or applied as an emulsified spray using 0.01% v/v agral wetter. Tubers were allowed do dry in air before being placed in 25 L storage containers with a flow of air (1 L kg^{-1} h^{-1}) amended with or without ethylene 10 μL L^{-1} (ppm) ethylene and stored at 9°C. For Maris Piper, the treatments were applied at the point of bud movement, whereas tubers of Hermes were endodormant at the time of treatment.

Tubers were inspected every 14 days, over a three and a half month period, for signs of bud movement and sprout growth.

3.7. Trial to assess the effect of ethylene and ethylene+1-MCP treatment on sprout growth and sugar levels in commercial varieties (season 2009)

This trial was conducted at Sutton Bridge Crop Storage Research (SBCSR). The trial was conducted at two temperatures; 6 and 9°C, with three treatments (Air, 10 ppm ethylene, 1-MCP/10 ppm ethylene), 4 replicates and sampling at 4 assessment time points, approximately 1, 2, 3, and 4 months for 9°C and 1, 2, 3, and 5.5 months for 6°C stored samples. The latter time point was used because of limited manpower availability at the 4 month time point and the relatively limited sprout development at 6°C.

A range of UK processing varieties, Cabaret, Hermes, Maris Piper, Markies, Russet Burbank, Saturna, Sylvana and Verdi, were provided by commercial partners in sufficient quantity for the trials. On 2nd October 2009 Sylvana [Greenvale AP Ltd] arrived at SBCSR. Cabaret [Cygnet PB Ltd] arrived on 8th October, Saturna [G H Chennells (Farms) Ltd] on 19th October, Maris Piper [Proctor Bros (Long Sutton) Ltd] on 22nd October. Hermes, Markies and Verdi [R S Cockerill (York) Ltd] arrived on 23rd October and Russet Burbank [Greenvale AP Ltd] on 3rd November 2009. As soon as possible, potatoes were hand graded to remove soil, rots, damage, green and undersize tubers (< 45 mm) and loaded into 10 kg capacity plastic trays. The trayed crop was placed on pallets. Once in trays, crops underwent a controlled pull-down regime of 0.5 °C per day, at ambient relative humidity (RH), to a holding temperature of 9.0 °C. This was to minimise temperature stress and allow time for skin healing after handling. However, due to the late arrival of Russet Burbank the temperature of this crop was pulled down at an accelerated rate of 1.0 °C per day to facilitate the start of the experiment.

The tubers thus arrived at SBCSR over a period of 5-6 weeks, and were held at 9°C until use. Treatment with 1-MCP [SmartFresh^{TM}] was carried out at 9ºC at a rate of 625 ppb by Landseer on 10th November. Tubers destined for the 6ºC trial were then cooled at a rate of 0.5°C per day. Twelve-tonne controlled environment rooms were set at a target temperature of 9 or 6°C with a tolerance ±0.5°C and 95 % RH with a tolerance ±5 %.

Ethylene was manually added to store to achieve the desired ramping of concentration over 16 days after which an EMU2 TS Ethylene Management Unit [BioFresh Ltd] was used to attain and maintain a constant 10ppm. Assessments were carried out each month on longest sprout

length and number of sprouting sites. Core samples from opposite eighths were taken for sugar analysis by NRI. At each assessment distinct samples (25 tubers) were assessed.

Dry matter measurement and sprout weight were assessed at the last time point. Buds/sprouts and cortex were sampled from all eight varieties (+/- ethylene) and stored at -80°C – prior to RNA extraction for microarray/real-time PCR analysis.

3.8. Large scale trials, effects of spearmint (R-Carvone), ethylene and 1-MCP on sprout suppression (season 2010)

These trials were undertaken at Sutton Bridge Crop Storage Research (SBCSR).

Potato varieties
Seven processing varieties Cabaret, Hermes, Maris Piper, Markies, Russet Burbank, Saturna, Sylvana and Verdi were provided to SBCSR during 2010 by commercial partners in sufficient quantity for the trials as detailed below:

Maris Piper and Russet Burbank [McCain Foods GB Ltd]: intake, 22nd October 2010
Cabaret [Sacker Farms for Cygnet PB Ltd]: intake, 28th October 2010
Saturna and Hermes, [R S Cockerill (York) Ltd]: intake, 1st November 2010
Markies [McCain Foods GB Ltd]: intake, 2nd November 2010
Sylvana [Greenvale AP Ltd]: intake, 4th November 2010

As soon as possible after intake, potatoes were hand graded to remove soil, rots, damaged, green and undersize tubers (< 45 mm) and loaded into 10 kg capacity plastic trays. Intake samples were taken from the graded tubers. The trayed crop was placed on pallets for treatment and storage.

Treatments
The following treatments/treatment combinations were applied; untreated (unt), 10 ppm ethylene (eth), methyl cyclopropene (1-MCP) [SmartFresh^{TM}] /10ppm ethylene (eth+1-MCP), spearmint oil (spear), spearmint oil/10 ppm ethylene (eth+spear) to all seven processing varieties.

1-MCP treatment
Within 48 hours of sample intake, sub-samples were placed in a 5m^3 containment tent with internal circulation, contained within a 57 m^3 store, and treated for 24 hours with 665 ppm 1-MCP following the manufacturers recommendations. Following treatment samples the tent was vented to provide fresh air and exhaust residual treatment for a further 24 hours.

Spearmint Oil (R-Carvone; Biox M) treatment
Following pull-down to storage temperature, sub-samples were placed in a 5m^3 containment tent with internal circulation, contained within a 57 m^3 store at 9°C. Spearmint Oil (Biox M) was applied on the 16th November at 90 g/tonne using the manufacturers (Xeda International S.A.) recommended method of hot fogging and recommendations of internal recirculation. Following treatment, the tent was vented to provide fresh air and exhaust residual treatment for a further 24 hours.

Ethylene treatment

Following pull-down to storage temperature and application of all other treatments, ethylene was manually added to store to achieve the desired ramping of concentration to 10ppm over 16 days, starting on the 16th November 2010. An EMU2 TS Ethylene Management Unit [BioFresh Ltd] was used to maintain a constant 10ppm ethylene and was used from 2nd December 2010 for the duration of the trial.

Pull-down and storage

Once in trays, crops underwent a controlled pull-down regime of 0.5 °C per day, at ambient relative humidity (RH), to a holding temperature of 9.0 °C. This was to minimise temperature stress and allow time for skin healing after handling.

Twelve-tonne capacity controlled environment rooms were set at a target temperature of 9 or 6°C with a tolerance ±0.5°C and 95 % RH with a tolerance ±5 %.

Sample assessments

At 2 monthly intervals over 6 months of storage, sprout number and sprout length was recorded and processing quality was assessed by fry colour with Cabaret, Maris Piper, Russet Burbank, Sylvana processed as chips and Hermes, Markies and Saturna as crisps. Each sample assessment was of 25 tubers, replicated four times.

Opposite eighth tuber core samples were from taken from 5 tubers per sample, frozen at -20°C, on behalf of NRI. Sample dry matters were assessed at the start and completion of the trial.

3.9. Large scale trials, effects of ethylene and 1-MCP, including multiple applications, on sprout suppression (season 2011)

Potato varieties

Six commercial processing varieties: were provided during 2011 by commercial partners of the project in sufficient quantity for the trials. Sylvana, received 30/9/2011, and Maris Piper, received 12/10/2011, were both provided by Greenvale AP. Cabaret, received 14/10/2011, was provided by Cygnet PB, Hermes and Saturna, received 12/10/2011, were both provided by PepsiCo and Russet Burbank, received 13/10/2011, was provided by McCain Foods (GB) Ltd. As soon as possible after intake, potatoes were hand graded to remove soil, rots, damaged, green and undersize tubers (< 45 mm) and loaded into 10 kg capacity plastic trays. Intake samples were taken from the graded tubers. The trayed crop was placed on pallets for treatment and storage.

Treatments

The following treatments and treatment combinations were applied to all varieties.

- untreated (unt),
- intake methyl cyclopropene (1-MCP) [SmartFresh™]
- intake and 2 month 1-MCP (1-MCPx2)
- intake, 2 and 4 month 1-MCP (1-MCPx3)
- 10 ppm ethylene (Eth)
- intake 1-MCP / 10 ppm ethylene (Eth+1-MCPx1)
- intake and 2 month 1-MCP / 10 ppm ethylene (Eth+1-MCPx2)

- intake, 2 and 4 month 1-MCP / 10 ppm ethylene (Eth+1-MCPx3)

1-MCP treatment

Within 48 hours of sample intake, sub-samples were placed in an 83 m^3 store, and treated for 24 hours with 665 ppm 1-MCP following the manufacturer's recommendations. Following treatment of samples the store was vented to provide fresh air and exhaust residual treatment for a further 24 hours.

Further sub-samples were additionally treated at 2 (Eth+1-MCPx2) or at 2 and 4 months storage (Eth+1-MCPx3).

Pull-down and storage

Nets were buried within bulk Russet Burbank in 1 tonne boxes. Crops underwent a controlled pull-down regime of 0.5 °C per day, at ambient relative humidity (RH), to a holding temperature of 9.0 °C. This was to minimise temperature stress and allow time for skin healing after handling.

Twelve-tonne controlled environment rooms were set at a target temperature of 9°C with a tolerance ±0.5°C and 95 % RH with a tolerance ±5 %. Store temperature and humidity records are archived at Sutton Bridge Crop Storage Research.]

Ethylene treatment

Following pull-down to storage temperature and application of other treatments, ethylene was manually added to store to achieve the desired ramping of concentration to 10ppm over 16 days, starting on the 7th November 2011. An EMU2 TS Ethylene Management Unit [BioFresh Ltd] was used to maintain a constant 10ppm ethylene and was used from 25th November 2010 for the duration of the trial.

Sample assessments

Each sample assessment was of 25 tubers with four times replication, at 2 (SO1), 4 (SO2) and 6 months (SO3) storage. At each assessment occasion sprout length was recorded and fry colour was assessed with Cabaret, Maris Piper, Russet Burbank, Sylvana processed as French Fries (chips) and Hermes, Saturna as crisps.

Chip assessment

Following washing and rumbling of all tubers in a sample, a single 7mm square longitudinal section chip was cut from 25 tubers of each of the three replicate sub-samples. Chips were fried for 90 seconds in oil which was at 190°C at the start of frying. The fry colour of individual chips was compared to a USDA standard colour chart (Munsell Color, Baltimore, Maryland, USA) under standardised lighting conditions. The USDA scale (000, 00, 0, 1, 2, 3, 4) was linearised to the SBCSR scale 1-7.

Crisp assessment

300 g of slices consisting of 2 to 4 slices per tuber from 30 tubers and with a thickness of 48-58 thousandths of an inch (~1.22-1.47 mm), were fried for 3 minutes in rapeseed oil at 177°C at the start of frying.

The sample fry colour was determined objectively using a HunterLab D-9000 colour quality meter fitted with a D25-L optical sensor (Mountsorrel, Leics., UK).

After frying, the sample was weighed and crisps assessed for defects. Defects were judged as an area equivalent to a circle of 5 mm diameter with a fry colour less than L 49 on the HunterLab scale. The weight of defects was recorded. The fry colour of the remaining sample was then determined using the HunterLab D-9000.

Opposite eighth tuber core samples were from taken from 5 tubers per sample, frozen at -20oC, before being transported to NRI.

Sample dry matters were assessed at the start and completion of the trial.

3.10. Method of sugar analysis

Samples of tuber cortex were taken from opposite eighths of potatoes using a cork borer (size No 5). Each replicate consisted of cores combined from 5 tubers, with four replicates per variety for each sampling occasion. Samples were frozen immediately at -20°C before subject to 48 hours freeze drying freeze drying before grinding in a pestle and mortar. Sugars were extracted from powdered potato samples (0.2 g) with 1.6 ml of 80:20 (ethanol : water) for 2 hours at 70°C in a shaking water bath. Samples were centrifuged at 10,000 g for 5 minutes, the supernatant was filtered through a 0.45µm PTFE syringe filter. 5 µl samples were injected onto an HPLC column (Agilant Zorbax carbohydrate analysis column) maintained at 30°C using 75 % acetonitrile running at 1.5 ml/min as the mobile phase. Sugars were detected using a refractive index detector (Agilent 1200 refractive index detector). Data was analysed by using data system EZChrom 3.3 (Agilent).

4. RESULTS

4.1. Phenotyping parents of selected cross (GENPOP1); 12601 ab1 and Stirling.

A key component part of this project is the phenotypic and genotypic analysis of a cross with differential response to ethylene. The population chosen for study during this project is a cross between 12601ab1 and Stirling. This population (GENPOP1) has been extensively genotyped by the James Hutton Institute (JHI). Phenotyping prior to the start of the project indicated that the parents had differential responses to ethylene (see 4.1.1). 12601 ab1 and Stirling were characterised for their sprouting characteristics and response to ethylene during four consecutive seasons (2008 to 2011).

4.1.1. Phenotyping 12601 ab1 and Stirling in season 2008

Figure 4.1 shows the percentage inhibition of sprout length by continuous exposure to ethylene for five potato varieties including 12601 ab1 and Stirling in season 2008 (prior to the start of this project) measured after 16 weeks of storage. The ethylene effect is complicated as ethylene tends to shorten dormancy as well as inhibit sprout growth. In general, it was noted that tubers stored in ethylene had a shorter period of dormancy compared to air-stored tubers, apical dominance was absent and the rate of sprout growth was retarded. Variation in the length of dormancy was observed between the breeding lines, Stirling and 12601 ab1. 12601ab1 had a longer dormancy period and the rate of sprout growth was slower. Although sprouts from these lines were at an early stage of development 12601 ab1 exhibited a lower degree of ethylene-induced sprout inhibition (16%) compared to Stirling (74%). Ethylene-induced inhibition for Mayan Gold, HB171 and DB226 was 57, 69 and 62 % inhibition, respectively, however, the rate of growth in DB226 sprouts was greater. No significant sign of rotting or weight loss was observed.
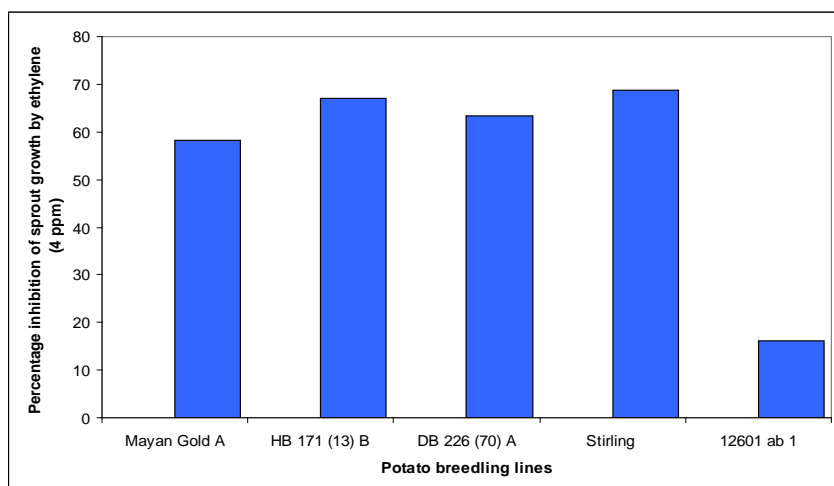
Figure 4.1. Control of sprout growth in potato (*S. tuberosum* and *S. phureja*) by the application of ethylene (4 ppm) to the storage atmosphere (16 weeks storage). *Cured potatoes sent from JHI in early November 2008 were placed into 25 L polypropylene-bins fed with a flow of humidified-air (1 L kg^{-1}h^{-1}), half the replicates were stored in air while the rest received air amended with 4 ppm ethylene. Tubers were stored at 6°C and removed approximately every 4 weeks; weighed and the number and length of sprouts of 20 tubers per treatment were measured.*

4.1.2.   Phenotyping 12601 ab1 and Stirling in season 2009

The cultivars were phenotyped in more detail during the first year of the project (2009). Unlike the behaviour in season 2008 the percentage inhibition of sprout length by ethylene was similar in the two cultivars; 57% and 54% inhibition of sprout length after 3 months storage respectively. Figure 4.2 shows more details of sprout growth with and without ethylene and in the presence of ethylene with the ethylene antagonist 1-MCP.

Sprout growth in untreated Stirling followed a general sigmoidal pattern, while ethylene treated tubers had a suppressed growth rate and maintained a linear growth curve. 1-MCP delayed the onset of dormancy break, but by three months sprout length between ethylene and 1-MCP + ethylene was comparable. This is consistent with previous observations that 1-MCP inhibits the ethylene effect of shortening dormancy, but not its inhibitory effect on sprout growth.   Sprout growth of 12601 ab1 was less vigorous than Stirling. Ethylene suppressed growth rates in 12601 ab1 tubers, and growth slowed down rapidly compared to untreated tubers. 1-MCP-treated and ethylene treated tubers had a delayed onset of sprout growth.
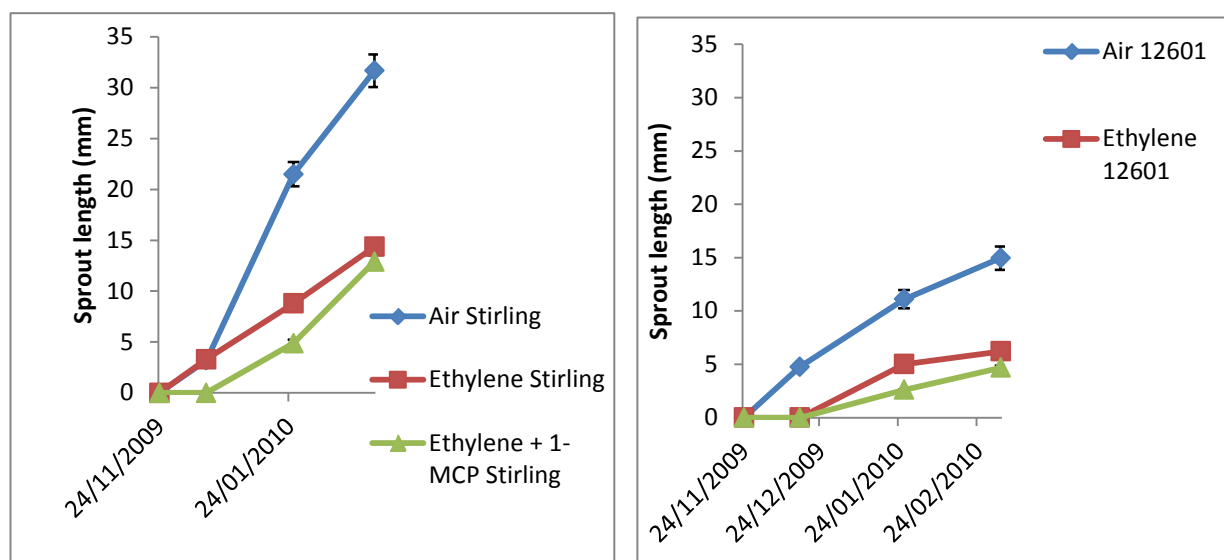

Fig 4.2. Sprout lengths of Stirling and 12601 ab1. Tubers were continuously treated with ethylene (10ppm) or 1-MCP (625 ppb) and ethylene (10 ppm). Sprout lengths were measured on 10 tubers per replicate with 4 replicates per treatment. Tubers were stored at 9°C and sprout lengths measured after 4, 7 and 12 weeks.

Sugar accumulation

After 4 weeks storage (inspection 1) in 10 ppm ethylene Stirling exhibited increased concentrations of glucose, while pre-treatment with 1-MCP reduced glucose and fructose concentration. The sugar content in 12601 ab1, a low sugar breeding line, remained low in the presence of ethylene while 1-MCP reduced the fructose and glucose content and lowered overall sugar content.

By the second inspection, the effect of 1-MCP on Stirling had disappeared and tubers treated with ethylene or ethylene + 1-MCP had increased concentrations of fructose and glucose. Sugars in 12601 ab1 remained low while ethylene treated tubers contained less glucose and ethylene + 1-MCP treated tubers less glucose and fructose.

By the final inspection (week 12) the glucose and fructose content of air-stored Stirling had risen, while the fructose and glucose content of 1-MCP +ethylene treated Stirling dropped significantly. Sugar concentrations in 12601 ab1 was generally lower than in previous inspections and no increase in sugar content was observed in tubers treated with ethylene while tubers pre-treated with 1-MCP had lower amounts of glucose and fructose.
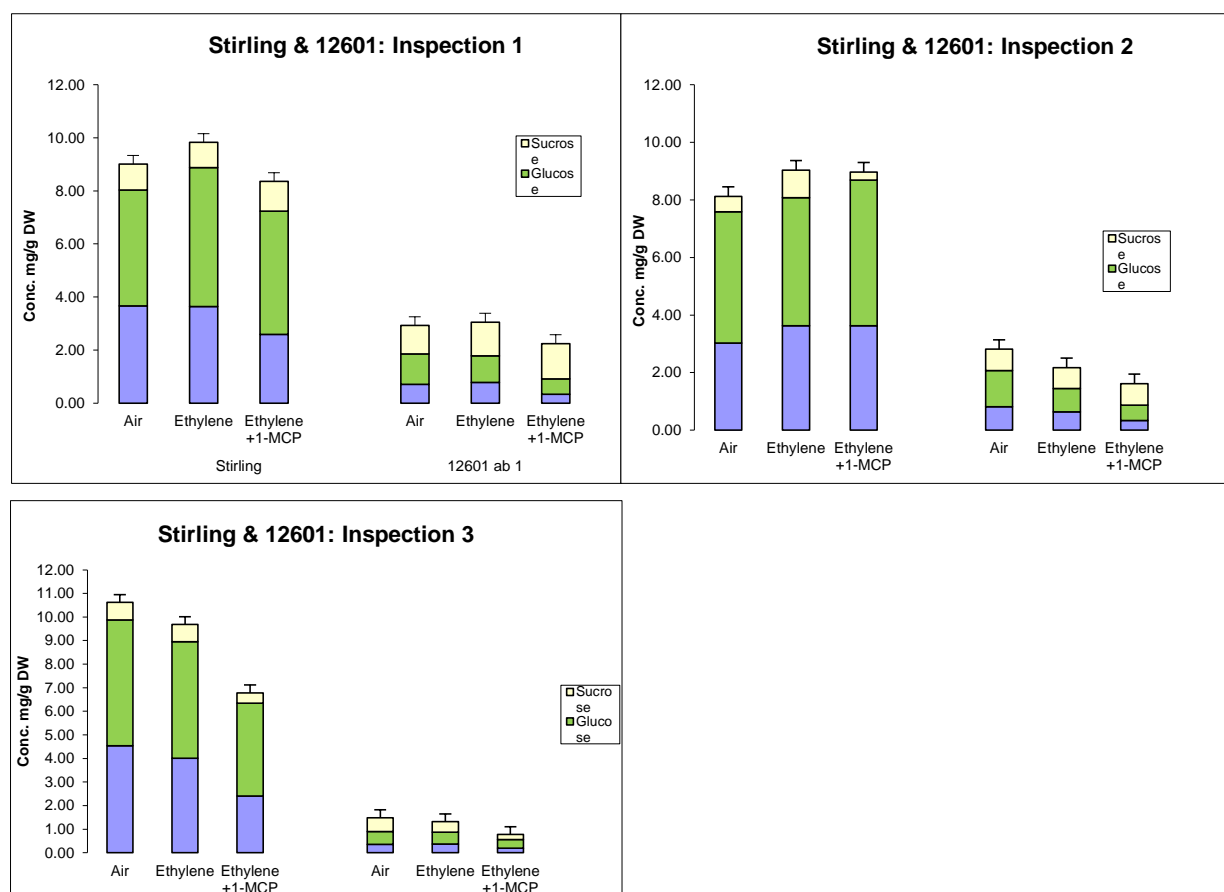

Figure 4.3. Sugars were measured on composite samples made from 5 tubers per rep with a total of 4 reps per treatment. $LSD_{0.05}$ for total sugar content for the interaction of treatment x variety x inspection was 0.929 on 103 df. Sugars were analysed from freeze dried powders from the first three inspections. Data from this trial is presented on a dry weight basis (mg g^{-1}DW).

4.1.3. Phenotyping 12601 ab1 and Stirling in season 2010

Whereas in season 2009 Stirling and 12601 ab1 exhibited a similar response to ethylene in terms of sprout growth, in season 2010 there was a clearer distinction with Stirling responsive to ethylene and 12601ab1 less responsive (Figure 4.4), confirming the observations made prior to the start of the trial (4.1.1). For Sterling sprout length was inhibited by 38% after three months storage whereas 12601 ab1 had longer sprouts in the presence than absence of ethylene. The response of 12601 ab1 can be explained by ethylene treatment resulting in a shortening of dormancy, as well as inhibiting sprout growth.

Figure 4.4. length of apical sprouts for Stirling and 12601 ab1 tubers after 14, 28 and 70 days storage at 9°C in the presence and absence of 10 ppm ethylene. Each data point is the mean of measurements on 5 tubers in each of two replicates. Error bars are standard errors. (*not visible for 12601 ab1 due to small size*)

4.1.4. Phenotyping 12601 ab1 and Stirling in season 2011

In season 2011 ethylene inhibition of sprout growth was much more effective than in the previous season for both cultivars (Figure 4.5).
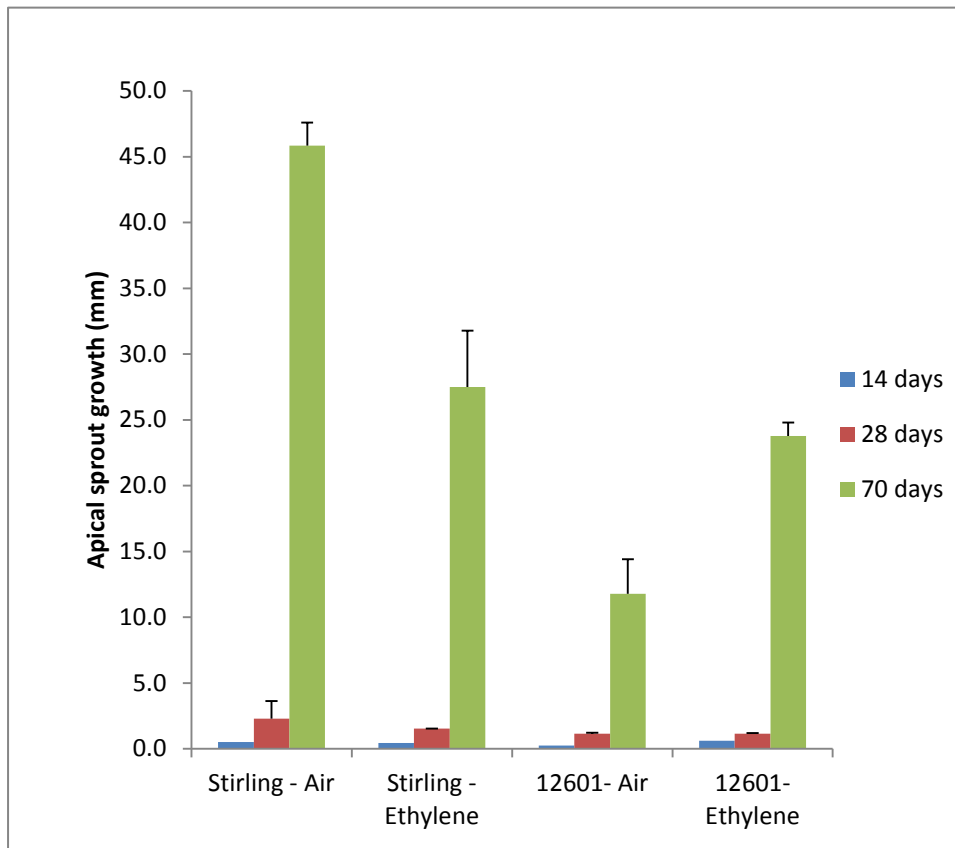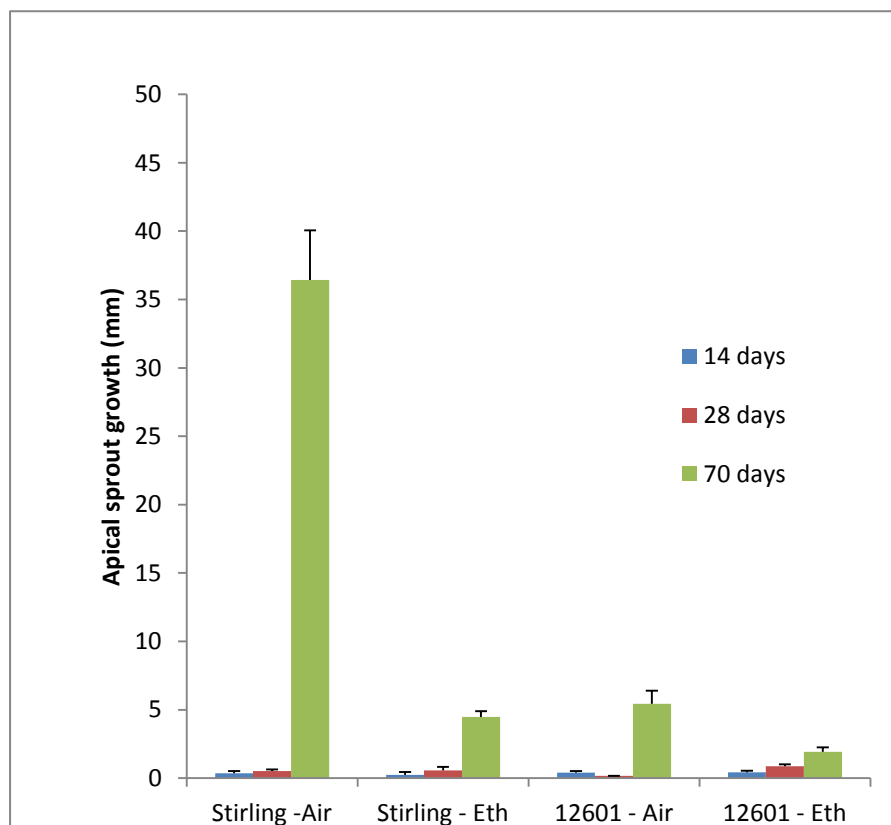

Figure 4.5. Length of apical sprouts for Stirling and 12601 ab1 tubers after 14, 28 and 70 days storage at 9°C in the presence and absence of 10 ppm ethylene. Each data point is the mean of measurements on 5 tubers in each of two replicates. Error bars are standard errors.

4.1.5. Comparison of 12601ab1 and Stirling phenotypes over seasons

There are consistent differences between Stirling and 12601 ab1 in their sprouting phenotype; Stirling has a shorter dormancy, exhibits a greater rate of sprout growth and is more sensitive to ethylene in terms of sprout growth inhibition than 12601 ab1. However the magnitude of these differences varies by season (Table 4.1).

In the years prior to the phenotyping of the GENPOP1 population a significant difference was observed in 2008, but this was less pronounced in 2009. During the two seasons for which the GENPOP1 population was genotyped, a big difference was observed in 2010 but this was smaller in 2011, with both varieties being more sensitive to ethylene.

Observations are complicated by the opposing effects of ethylene; on dormancy length (ethylene promotes break of dormancy) and on sprout growth (ethylene inhibits sprout growth).

**Table 4.1. Inhibition of sprout growth for Stirling and 12601 ab1 by continuous exposure to ethylene**

| Season | %Inhibition of sprout growth by ethylene | | Temperature and storage period |
	Stirling	12601 ab1	
2008	74	16	100 days, 6°C, 4 ppm ethylene
2009	57	54	100 days, 9°C, 10 ppm ethylene
2010	38	<0	70 days, 9°C, 10 ppm ethylene
2011	90	60	70 days, 9°C, 10 ppm ethylene

4.2. Phenotyping GENPOP1 (progeny of the 12601 ab1 x Stirling cross)

4.2.1. Phenotyping GENPOP1 in season 2010

As expected, the progeny exhibited a wider range of responses than their parents. The sprouting behaviour of the different clones was sufficiently characterised after 70 days storage at 9°C in a flow of air (1L $kg^{-1}h^{-1}$) to allow for distinct patterns in ethylene response to be recorded. In over a third of the population ethylene reduced sprout growth (more than 70% inhibition in 10 progeny), approximately a third showed little response. In addition, some tubers remained dormant throughout storage, while others, in the same way as parental line 12601 ab1, showed enhanced sprout growth after exposure to ethylene, most likely a result of ethylene stimulating the breaking of dormancy.

Representative examples of responses for individual lines are given in Figure 4.6
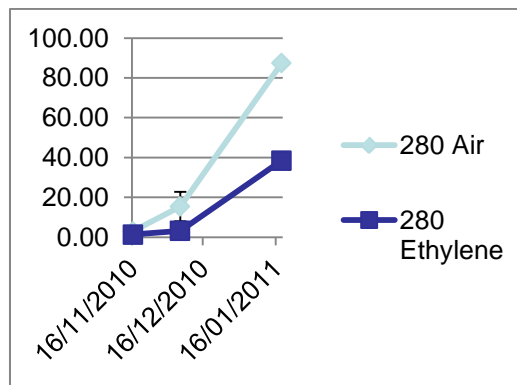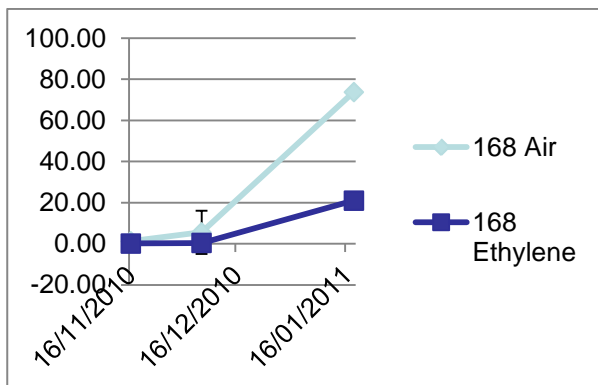
Lines with a short dormancy period followed by a rapid increase in growth. Sprout growth sensitive to ethylene.
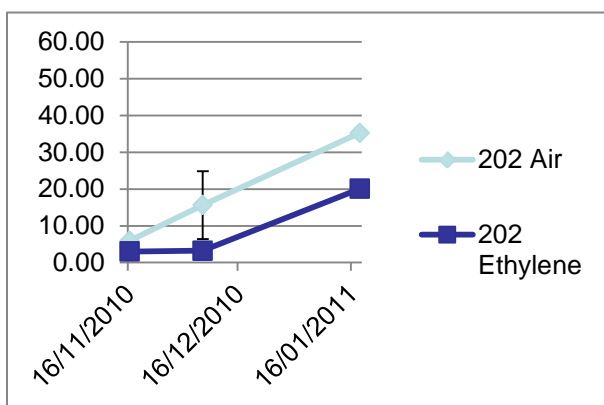
Non-dormant, rapid sprout growth in air
Moderate inhibition of sprout growth by ethylene (10 ppm)
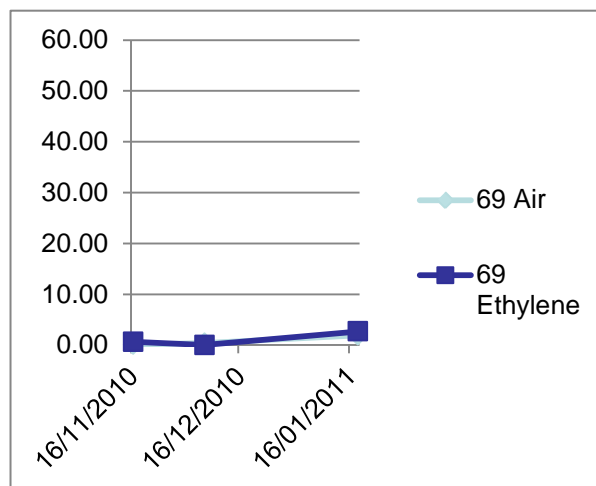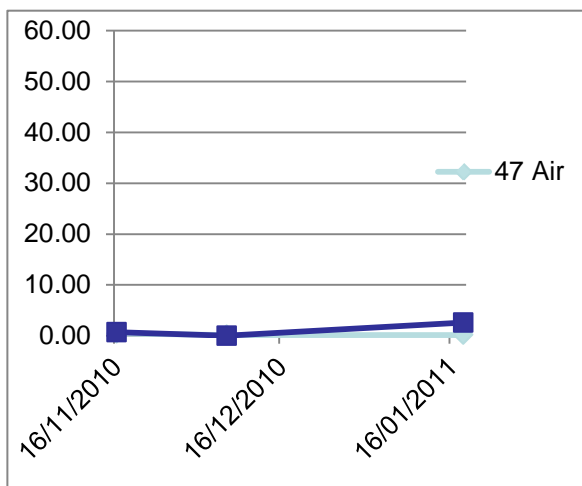
Extended dormancy and very low rates of sprout growth, no effect of ethylene on sprout growth

Figure 4.6 Example responses of progeny lines to ethylene in terms of sprout growth.
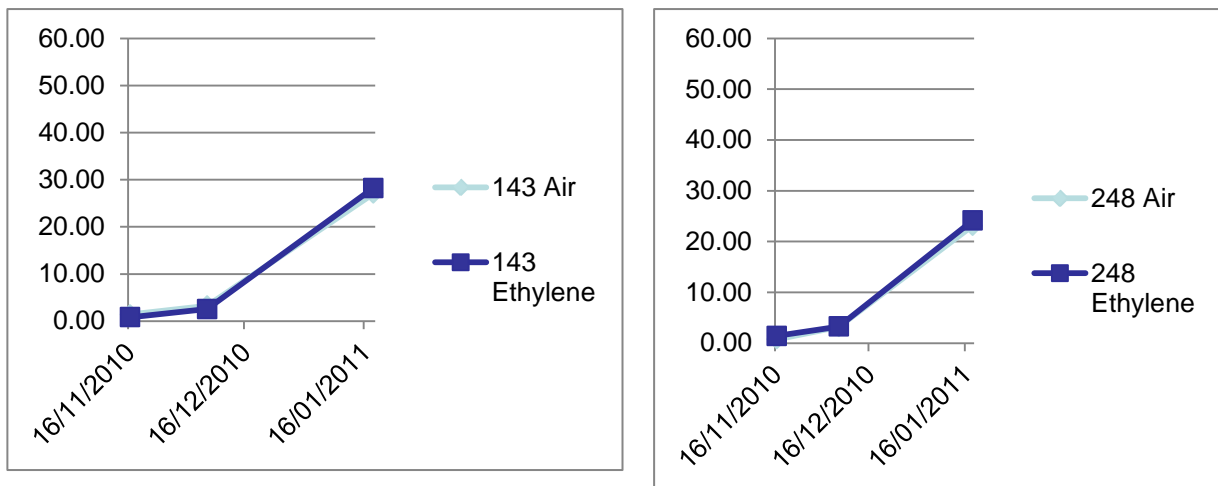
Lines with greater rates of sprout growth but exhibiting insensitivity to ethylene
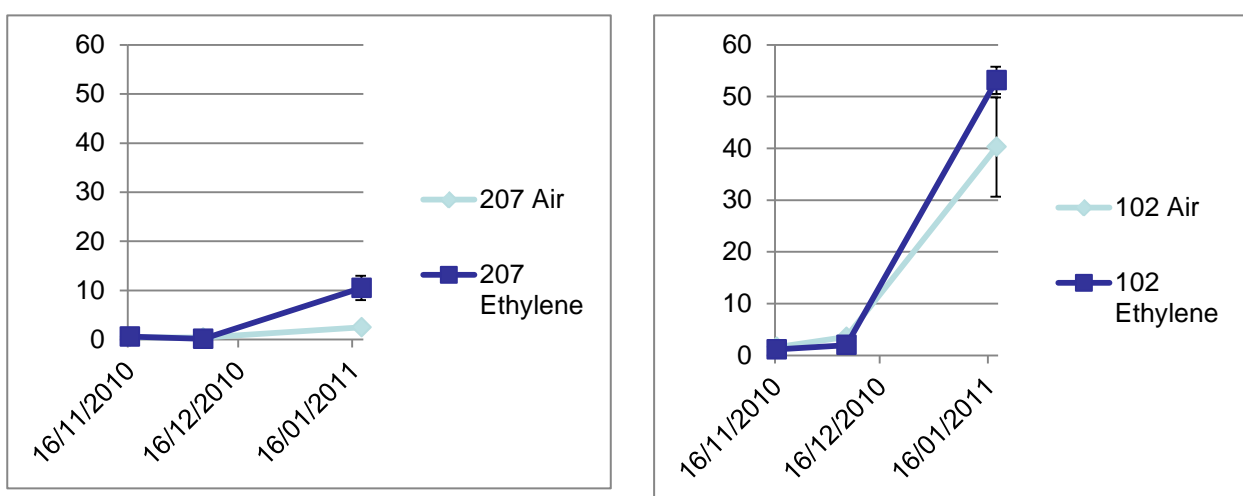
Lines where ethylene treated tubers have longer sprouts than air-treated controls suggests ethylene enhanced dormancy break with insensitivity to ethylene during sprout growth

Figure 4.6 cont. Example responses of progeny lines to ethylene in terms of sprout growth

The mean sprout lengths in air versus ethylene treatment and the significance of the effect of ethylene at inspection 3 (10 weeks at 9°C) is shown in Appendix 1.

Genotypes from the population were assigned to one of four groupings depending on their response to ethylene at inspection 3 (10 weeks at 9°C). These categories were: (1) No significant effect on sprouting (Figure 4.7a) (2) Reduction in sprout length on ethylene treatment (Figure 4.7b) (3), Increase in sprout length on ethylene treatment, (4) Reduction in sprout length and little sprout growth.

Examples of the different sprouting responses of these groupings are shown in Figure 4.7, and the overall response summarised in Figure 4.8.
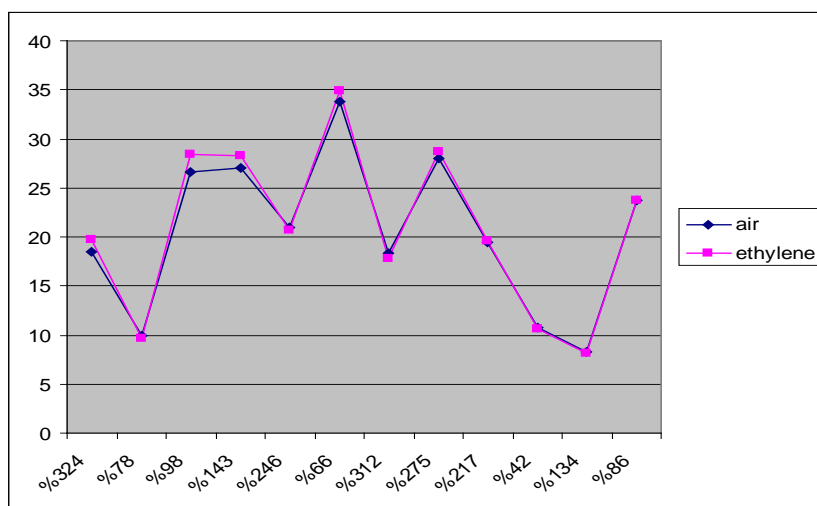
a. No effect of ethylene
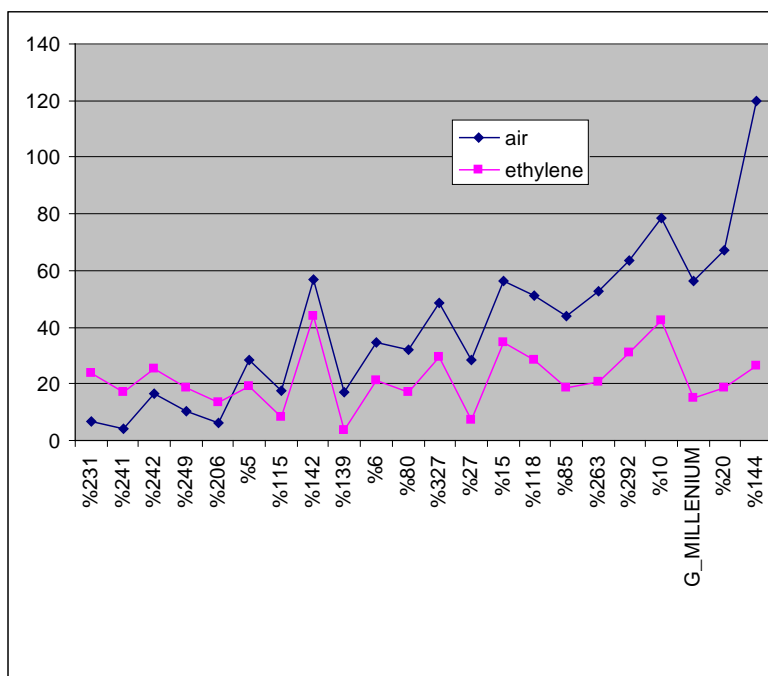
b. Significant effect of ethylene

Figure 4.3 Examples of sprout behaviour in different potato genotypes (y axis sprout length (mm), x-axis clone name). 4.3a. No effect of ethylene, 4.3b. Significant effect of ethylene.
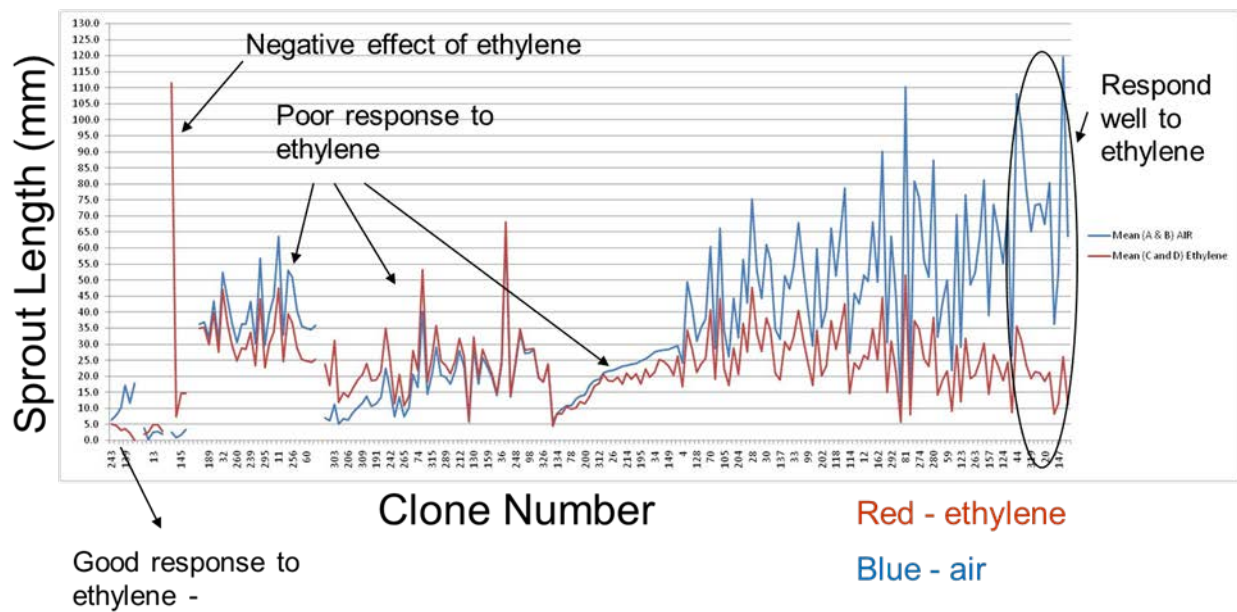
Figure 4.8 Response to ethylene of all progeny tested in terms of sprout length.

Eight genotypes were identified for each of the four categories of sprout response. RNA was extracted from buds 7 days after the start of treatment (air or air +ethylene) prior to any sprouting were used for microarray analysis.

4.2.2. Comparison of GENPOP1 phenotypes in seasons 2010 and 2011

Similar to the response of the parents, in season 2011 the progeny tended to be more sensitive to ethylene in terms of sprout growth inhibition, and therefore the range in response was smaller. Nevertheless the relative response across lines was reasonably consistent. Figure 4.9 shows a highly significant correlation between sprout length in the presence of ethylene in season 2010 and 2011. Figure 4.10 shows an even stronger correlation between sprout length in air for the two seasons.
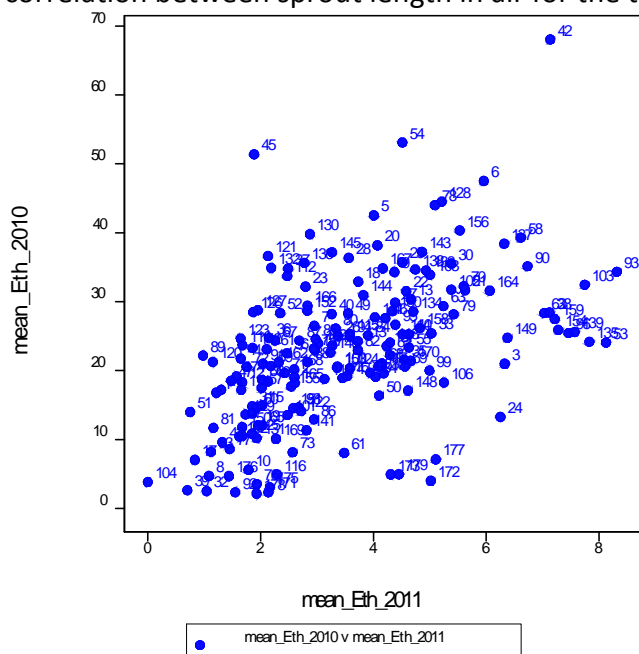
Figure 4.9. Season1 (2010) vs season 2 (2011) – sprout length on ethylene treatment – inspection 3 (21/1/12)
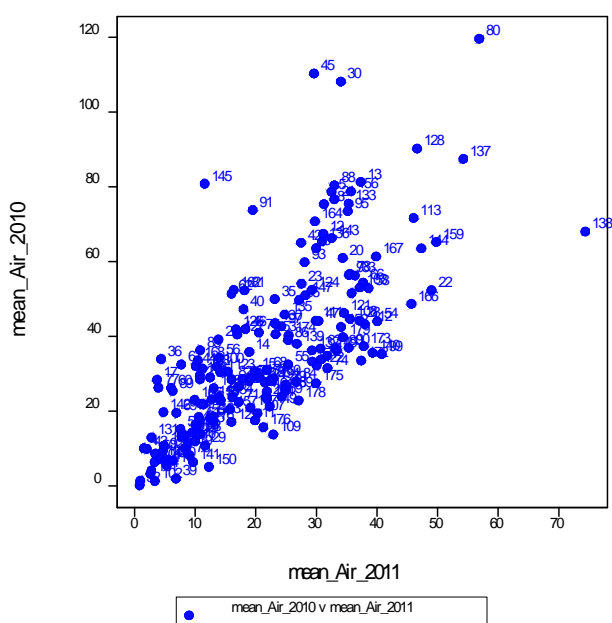
Figure 4.10. Season1 (2010) vs season 2 (2011)– sprout length on air treatment – inspection 3 (21/1/12)

4.3. Using Marker based Anova and QTL analysis to identify key chromosome locations relating to sprouting and ethylene response for GENPOP1

Access to genetic tools and therefore the methods available for analysis have improved during the course of this project. Advances in potato genetics have led to the development of the Potato Infinium SNP Platform by the US SOLCAP consortium (http://solcap.msu.edu/potato_infinium.shtml). This platform enables high throughput genotyping to be carried out and was not initially envisaged as part of the current LINK project. However, it was decided that it was sensible to use the outcomes from genotyping the 12601ab1 x Stirling, using this platform. Using this "SNP Chip" approximately 5000 polymorphic gene-based markers have been obtained for the 12601ab1 x Stirling tetraploid population.

4.3.1. Marker based Anova for season 2010

For season 2010, a preliminary analysis was carried out using the ethylene phenotyping data to investigate whether significant associations between SNP markers (and hence chromosomal locations, as all the SNP marker locations are known) and ethylene response could be detected. Data on sprout length for 190 lines in the presence and absence of ethylene and over three time points were used. A REML model was used to look for significant associations with SNP markers, fitting treatment (i.e. the effect of ethylene) as a fixed effect and genotype (i.e. clone) as a random effect.

For SNP by treatment interactions, there are few noteworthy markers. However, three closely linked markers, all from chromosome III, seem of most interest. The most significant is solcap_snp_c1_6864. This marker appears on scaffold 154. Interestingly, this hits a "hot spot" in the microarray gene list on 2 adjacent scaffolds. These scaffolds cover 2.7 Mbp of DNA and contain 212 genes. Several of these genes have roles in dormancy release/ cell cycle control such as AP2 CBF factor, C3H4 zinc finger, retinoblastoma binding protein, beta-1,3-glucanase.

4.3.2. QTL analysis for seasons 2010 and 2011

In May 2012 a draft genetic map for the 12601ab1 x Stirling population was completed allowing a full QTL analysis of the genotype data. While the marker based ANOVA uses more data (190 genotypes * 2 treatments * 3 timepoints) and therefore has more power to detect effects than 190 genotypes as used in the QTL analysis, the QTL analysis is more robust than the REML model because it enables proper statistical tests (permutation tests) of significance to be carried out. Data from season 2010 and season 2011 trials were therefore analysed using the new map data. The characteristics considered for this analysis were sprout length during storage in air, and sprout length during storage with ethylene. The significant QTLs identified are shown in Tables 4.2 a-d. QTL interval mapping was used to relate sprouting traits to the latest SNP map. QTLs are considered present if % variance explained is greater than 5% and possible if greater than 4%.

The most significant regions are on chromosome V, VI and XI.

The ethylene response is complicated because, as indicated above, ethylene promotes dormancy break and also inhibits sprout growth. However although the possibility of

adjusting sprouting scores for maturity (time for dormancy break) was considered, analysis of available data indicated that this was not justified. Genotype means for the 190 offspring in the mapping population were regressed on the maturity scores previously measured for this population (Bradshaw *et al.*, 2008). The variances explained by regression of the sprouting scores on the maturity score are low (mostly 0 – 2%) and so the conclusion is that the relationship between maturity and sprouting is not strong enough to require adjustment of sprouting scores for maturity

Table 4.2

Chromosome	Season	Characteristic analysed	Location of QTL	% Variance explained
III	2011	Sprout growth in air and ethylene	22 cM	5.7
V	2010	Sprout growth in air	29 cM	5.3
	2010	Sprout growth in air	12 cM	5.8
	2011	Sprout growth in ethylene	25-30 cM	8.6
VI	2010	Sprout growth in air	76 cM	6.0
	2011	Sprout growth in air	77 cM	6.3
VIII	2010	Sprout growth in air	48 cM	5.9
	2010	Sprout growth in ethylene	48 cM	5.7
XI	2010	Sprout growth in air	41 cM	5.2
	2010	Sprout growth in ethylene	29 cM	5.6
	2011	Sprout growth in air	31 cM	5.1
	2011	Sprout growth in ethylene	18 cM	5.1

The QTL on chromosome V for sprout growth in ethylene is supported by REML data. It is close to maturity marker (23 cM) but shows a different segregation pattern

The QTL on chromosome VI for sprout length in air was identified in both seasons. This was supported by ANOVA data from 2011 but was not present in the 2010 ANOVA analysis

A detailed report on the genetic analysis aspects of this project is available as Appendix 2.

In summary; a number of QTLs have been identified for sprouting in air and in ethylene. None of these accounts for more than 9% variance, and most are less than 7%. At this level they are unlikely to represent markers that could be of practical use. Furthermore, few of these QTLs are consistent across seasons. In practice the value of this analysis will be to combine with information from microarray analysis in order to help us focus on genes of interest

4.4. Microarray analysis for parents (12601 ab1 and Stirling) and GENPOP1 population.

4.4.1. Microarray analysis for 12601 ab1 and Stirling in season 2009

In order to investigate gene expression in potato tuber buds and how this relates to ethylene effects on sprout growth a microarray experiment was carried out using the POCI microarray to analyse global gene expression in potato (described in Kloosterman *et al.*, 2008), representing 42,034 unigene sequences and approximately 70% of the potato transcriptome. In season 2009 for 12601 ab1 and Stirling gene expression in control air treated buds was compared with ethylene treated buds, and analysis was carried out at three time points; after 4 weeks, 7 weeks and 12 weeks of ethylene exposure compared with air controls, from tubers stored at 9^{0}C.

After filtering for inconsistent data, 32,441 probes were analysed for the 12601ab genotype, whereas 32,050 probes remained for Stirling. Volcano plots were subsequently used to filter this data at each time point on the basis of fold-change, comparing air and ethylene treated samples. Gene lists were compiled for genes (probes) showing greater than two-fold change in expression level and a T-test p-value of less than 0.05.

Figure 4.11 shows the relationships between significant genelists at the three time points studied (4, 7 and 12 weeks of ethylene treatment), while Figure 4.12 shows the overlap of the genelists for each genotype and time point. Initial microarray analysis found key differences in the transcript profiles of Stirling and 12601.ab.1 apical buds/sprouts where growth rates respond differentially to exposure to ethylene. Early events after exposure to ethylene provide the greatest difference in microarray profiles between the two parents, with no differences found at 12 weeks, suggesting that the differences in gene expression are related to differences in ethylene response.

The size of the genelists was then reduced using stringent (Benjamini and Hochberg) multiple-testing correction. For genotype 12601ab, 79 genes were differentially expressed at 4 weeks, 26 at 7 weeks and 0 at 12 weeks. For the Stirling genotype, 365 genes were differentially expressed at 4 weeks, 33 at 7 weeks and 0 at 12 weeks. The lists are available as Appendix 3A and 3B. Ethylene and cytokinin related genes are highlighted in these lists and the expression patterns of ethylene related genes is shown in Figure 4.13. Multivariate analysis was carried out to highlight genes that underpin genotype, treatment and time point differences. Genes were identified that exhibited different expression responses to ethylene treatment. These are available as Appendices 4-7

Appendix 4: genes which showed significant differential expression (SDE) between air and ethylene but showed no SDE between genotypes.

Appendix 5: genes which showed SDE between air and ethylene treatments and showed SDE between genotypes.

Appendix 6: genes which showed SDE between air and ethylene treatment and showed SDE for treatment by genotype interaction but did not show any SDE between genotypes.

Appendix 7: genes which showed SDE for treatment by genotype interaction but did not show any SDE between genotypes or between treatments.

Appendix 5 is of most interest for identifying target genes for developing markers for ethylene sensitivity

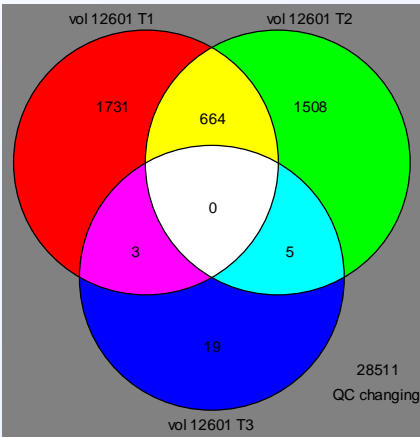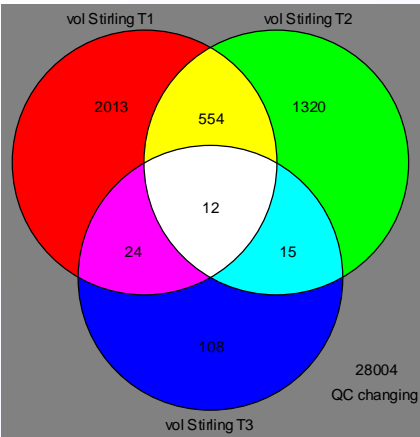Figure 4.11 Relationships between significant genelists for Stirling and for 12601 ab1 at the three time points studied (4, 7 and 12 weeks of ethylene treatment).
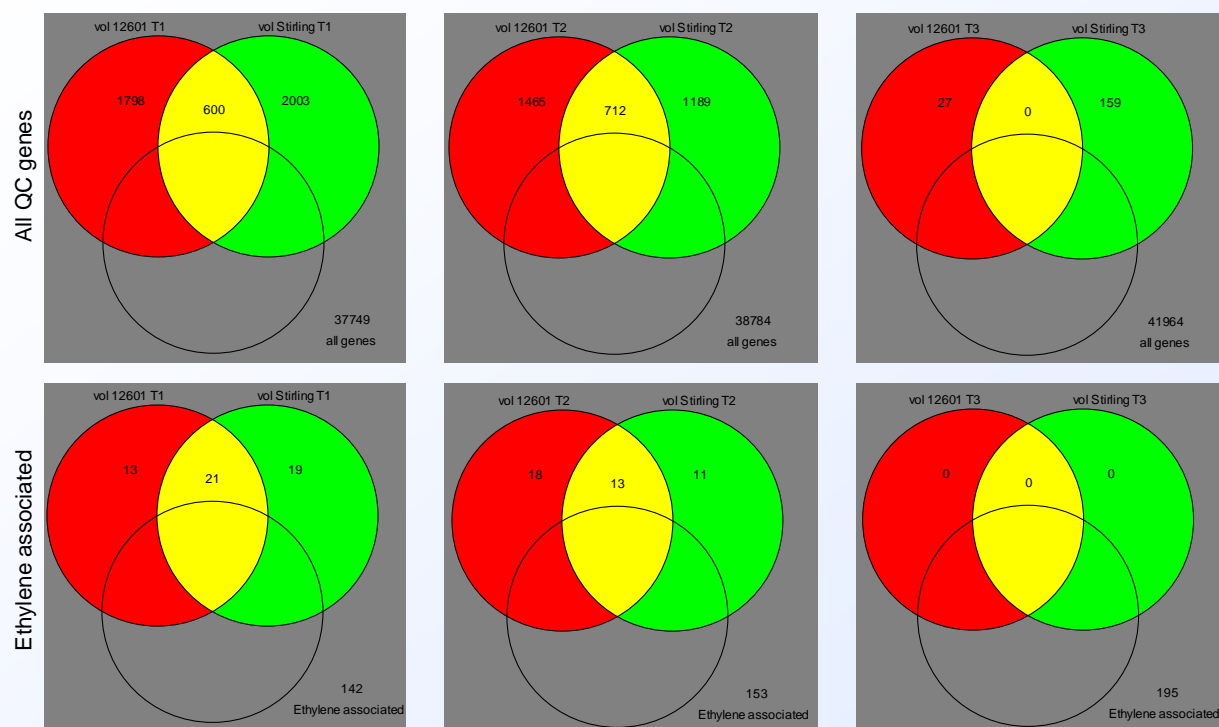
Figure 4.12
Figure 4.12 Overlap of the genelists for each genotype (Stirling and 12601 ab1) and time point
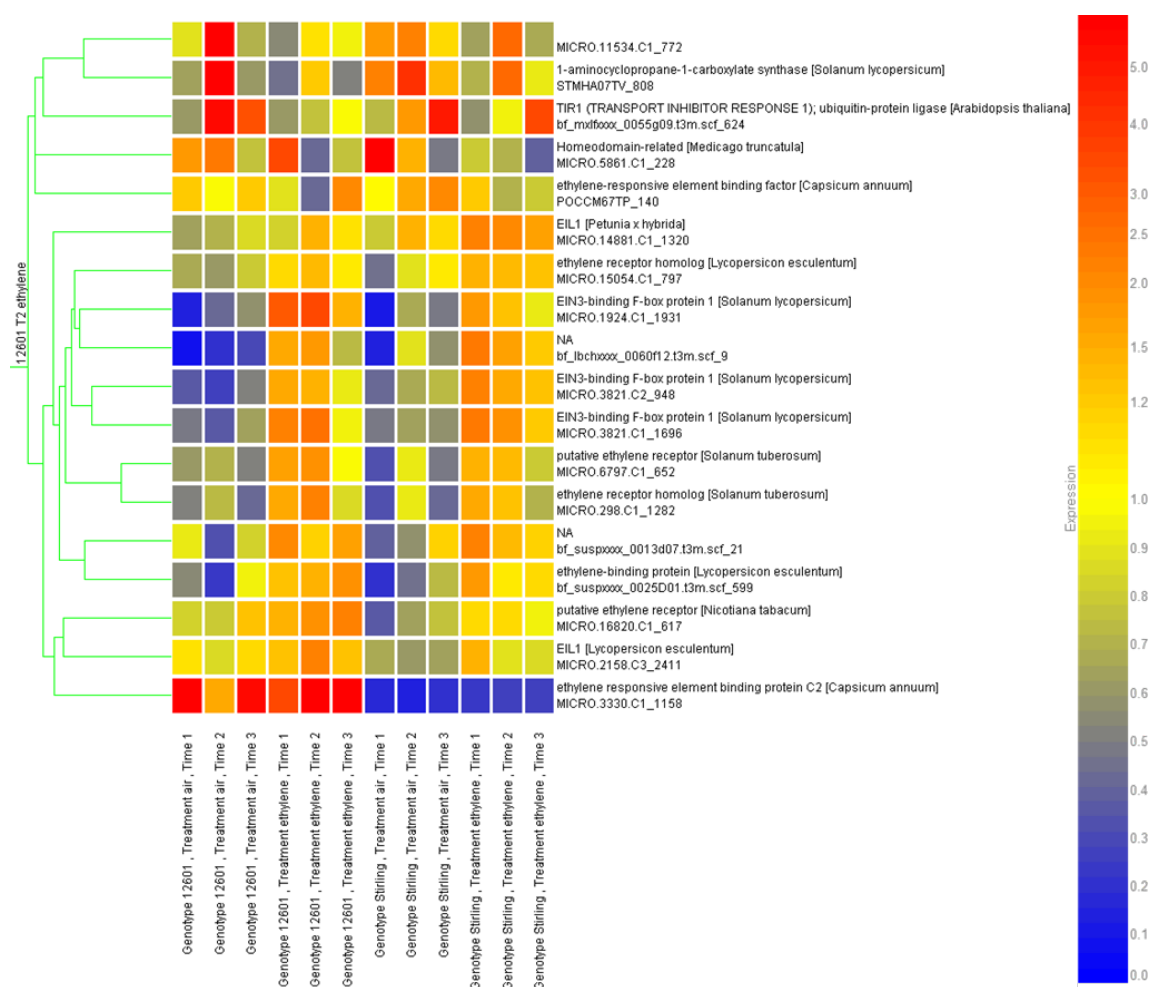(4, 7 and 12 weeks).

Figure 4.13 – Expression patterns that are differentially expressed in potato buds on treatment with ethylene.

4.4.2. Microarray analysis of pooled samples of GENPOP1 that differ in ethylene response. Identification of differentially expressed genes and their relationship with QTLs (Season 2010).

In season 2010 microarray analysis was conducted on pooled tissue samples (eight progeny per pooled sample) from each of the four response categories within GENPOP1 described in 4.2.1. For this analysis a new potato microarray that was not available for the season 2009 analysis was employed. A custom Agilent microarray was designed to the predicted transcripts from assembly v.3.4 of the DM potato genome (PGSC, 2011). Single 60mer probes were designed for a total of 52,848 transcripts, which includes alternative isoforms, using eArray (https://earray.chem.agilent.com/earray/) with default parameters in 8x 60k format. The complete design of the microarray can be found at ArrayExpress (http://www.ebi.ac.uk/arrayexpress/). Access to ordering the array design (AMADID 033033) from Agilent is available on request from the authors.

The new design, gives 25-30% greater coverage than the previously used POCI array and near "whole-transcriptome" coverage coupled with improved sensitivity (50 ng of total RNA/sample). Ethylene treated samples were compared with air-controls in an eight array experiment.

For each of the four categories (pools) described as above genes that were differentially expressed on ethylene treatment compared with air treatment were identified (Table 4.3).

Table 4.3 Number of differentially expressed genes from pooled bud microarray experiment.

Pool	Expressed at higher level	Expressed at lower level
1. No significant effect	507	72
2. Ethylene reduces sprouting	343	360
3. Ethylene increases sprouting	285	410
4. Ethylene reduces sprouting and little sprout growth	15	894

Gene lists for treatments where there was a response to ethylene are available as Appendix 8 – (up- refers to genes up-regulated on ethylene treatment, down – down –regulated on ethylene treatment – group numbers as in Table 4.3). Also shown in Appendix 8 are common genes that are up- or down- regulated on ethylene treatment for groups 2, 3 and 4 (common up and common down).

The microarray probes were annotated with their coordinates on the 12 potato chromosomes enabling the identification of "hot spots" consisting of genomic regions over-represented in the numbers of differentially expressed genes. For example the location on chromosome III of differentially expressed genes represented in group (2) Reduction in sprout length on ethylene treatment, are shown in Figure 4.14.
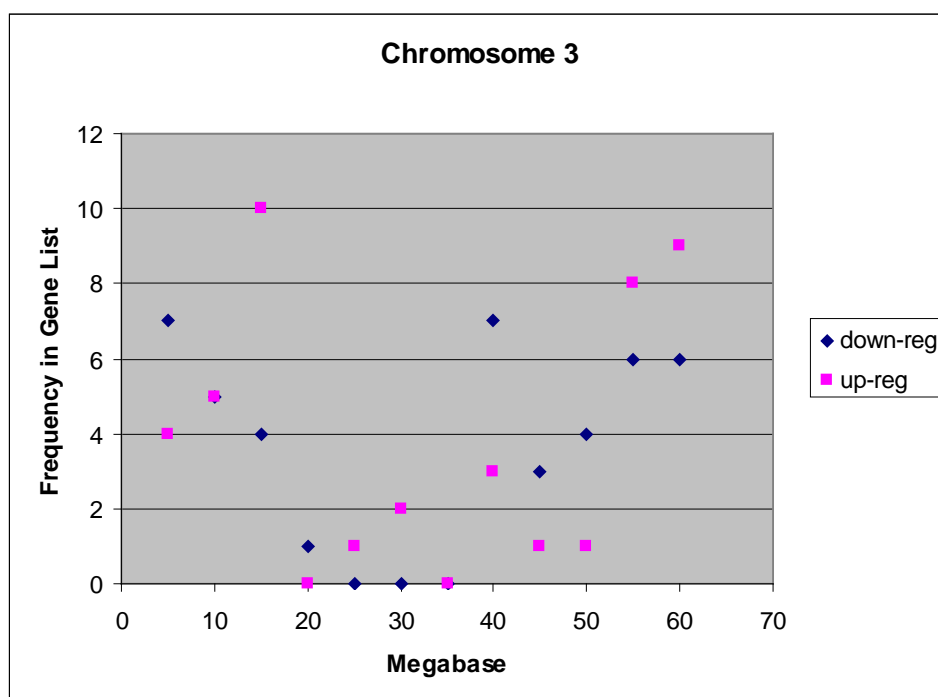
Figure 4.14 – Location on chromosome III of differentially expressed genes from pool (2) clones -Reduction in sprout length on ethylene treatment. Note regions enriched in differentially expressed clones.

4.4.3. Microarray analysis of pooled samples of GENPOP1 that differ in ethylene response. Identification of differentially expressed genes and their relationship with QTLs (joint analysis of seasons 2010 and 2011).

For season 2011 the microarray analysis was repeated for selected samples. Due to differences in ethylene response in season 2010 and 2011, individuals that behaved differently in the two seasons were removed from the pools (1-4) as described previously. Appendix 9 lists genes that were differentially expressed on ethylene treatment compared with air treatment for genotypes in group 4 (that is, genotypes that are responsive to ethylene and show very little sprout growth). As for the previous season, the chromosomal location of the differentially expressed genes was investigated and the number of differentially expressed genes in each chromosomal location is shown in Figure 4.15. For comparison, the data sets from season 2010 and season 2011-2012 (labelled (1) and (2), respectively) were compared. Also shown in Figure 4.15 is the location of QTL linked to ethylene responsiveness (see Table 4.2). In some cases there was coincidence of a high number of differentially genes and QTL (for example the QTL at 55MBp on linkage group 6) possibly providing eQTL support for the QTL.

A comparison of the microarray data from pool 4 genotypes revealed that 203 genes were common to these lists in season 2010 and season 2011.

Chromosome III (QTL at about 40)
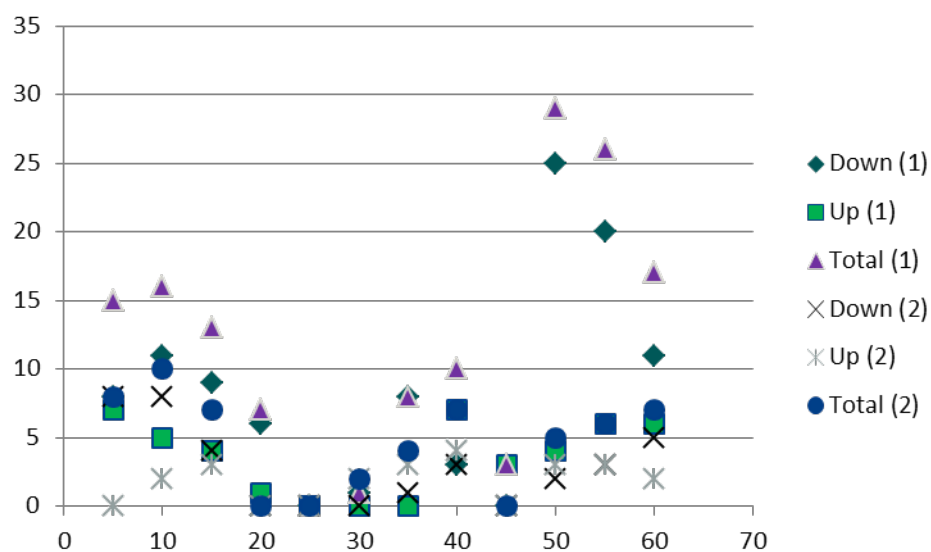
Chromosome V (QTL at about 10)

Figure 4.15 Number of genes differentially expressed after 4 weeks storage in the presence or absence of ethylene in each chromosomal location for pooled samples from buds of eight progeny within group 4 (genotypes that are responsive to ethylene and show very little sprout growth). Chromosome III and V

Chromosome VI (QTL at 55)

Chromosome VIII (QTL at about 33)

Figure 4.15 cont. Number of genes differentially expressed after 4 weeks storage in the presence or absence of ethylene in each chromosomal location for pooled samples from buds of eight progeny within group 4 (genotypes that are responsive to ethylene and show very little sprout growth). Chromosome VI and VIII
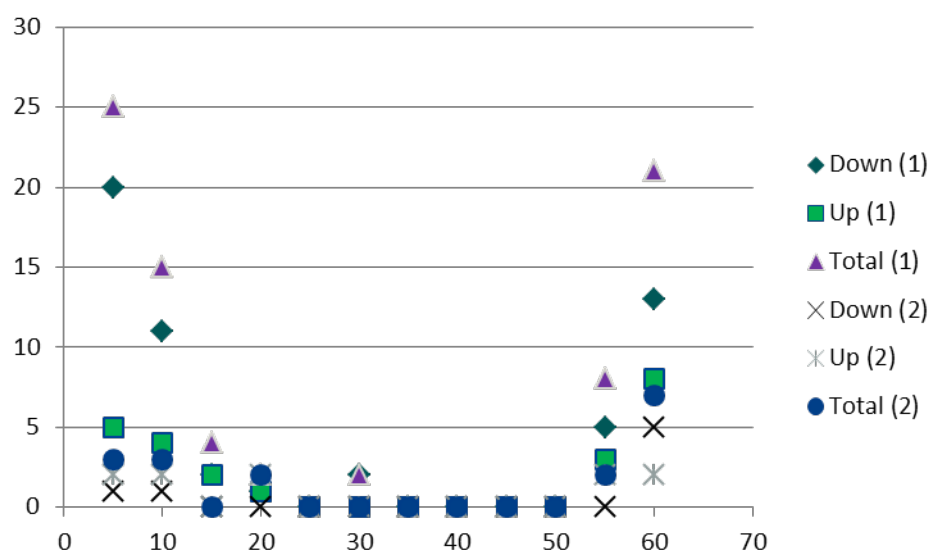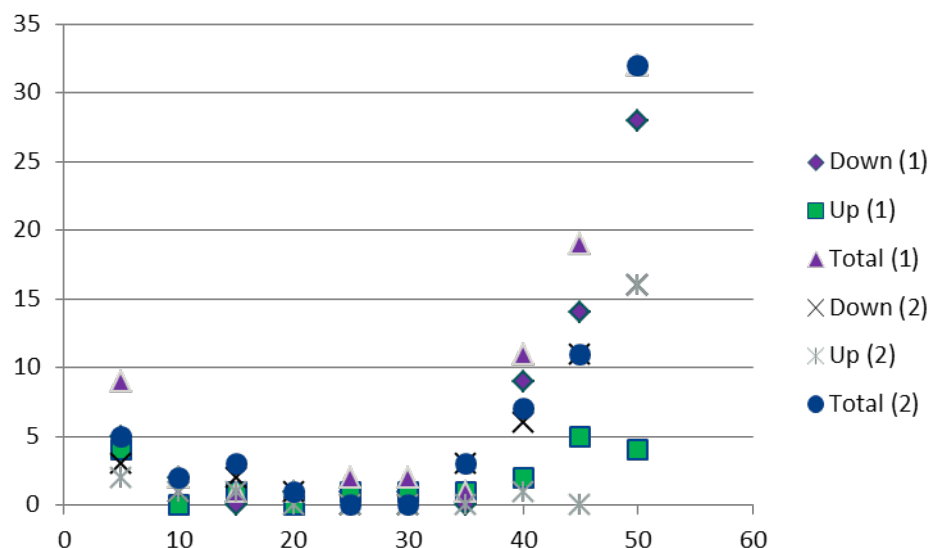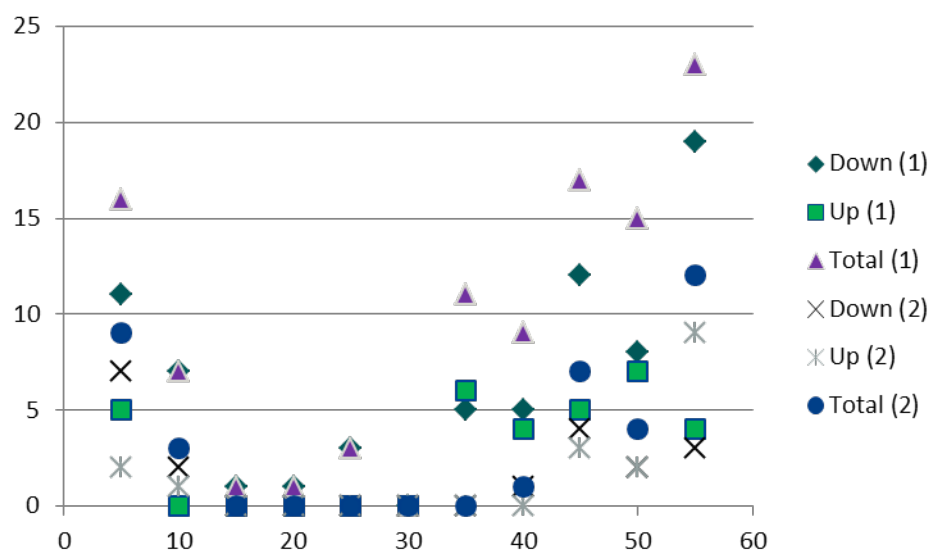
Chromosome XI (QTL at about 9)
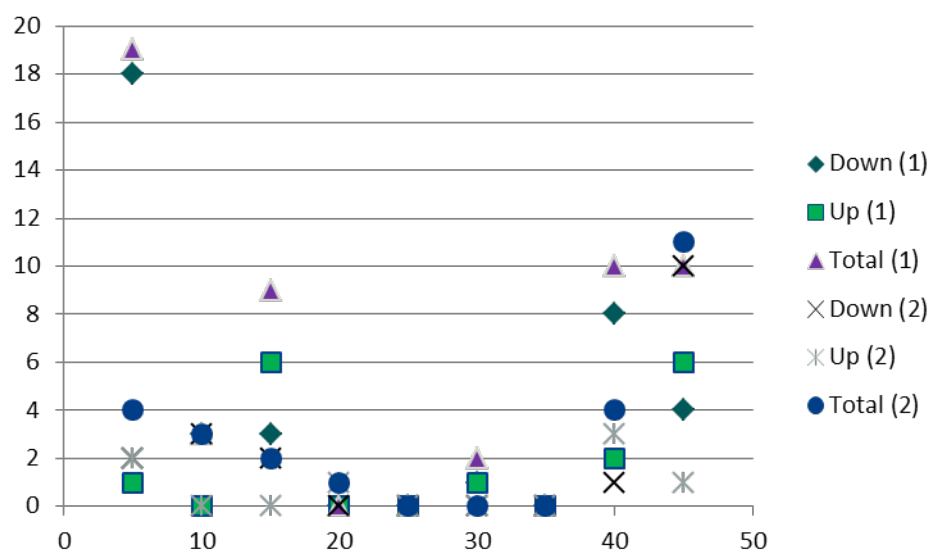
Figure 4.15 cont. Number of genes differentially expressed after 4 weeks storage in the presence or absence of ethylene in each chromosomal location for pooled samples from buds of eight progeny within group 4 (genotypes that are responsive to ethylene and show very little sprout growth). Chromosome XI

A further microarray experiment was carried out to investigate gene expression patterns related to the bud response to ethylene. In this experiment pools were constructed based on bud responses to ethylene. Pool A – contained the shortest buds following ethylene treatment (less than 1.1 mm), Pool B were next shortest, Pool C longer, whereas pool D contained the longest ethylene treated buds (greater than 3.8 mm). Microarray analysis was carried out for these four pools.

In the previous section 203 genes were identified as showing differential expression in both season 2010 and season 2011 within pool 4 genetypes (genotypes that are responsive to ethylene and show very little sprout growth). The behaviour of these 203 genes in the ethylene response genotype pools is shown in Figure 4.16. Interestingly distinctive patterns of gene expression can be seen in the different genotype classes. The k-means clustering algorhythmn of Genespring was used to distinguish four patterns of expression. The associated gene list for each pattern is available as Appendix 10.

Figure 4.16 Expression patterns relating to sprout length in tubers stored with ethylene for 203 genes that were identified as having consistent behaviour over two seasons in group 4 progeny.
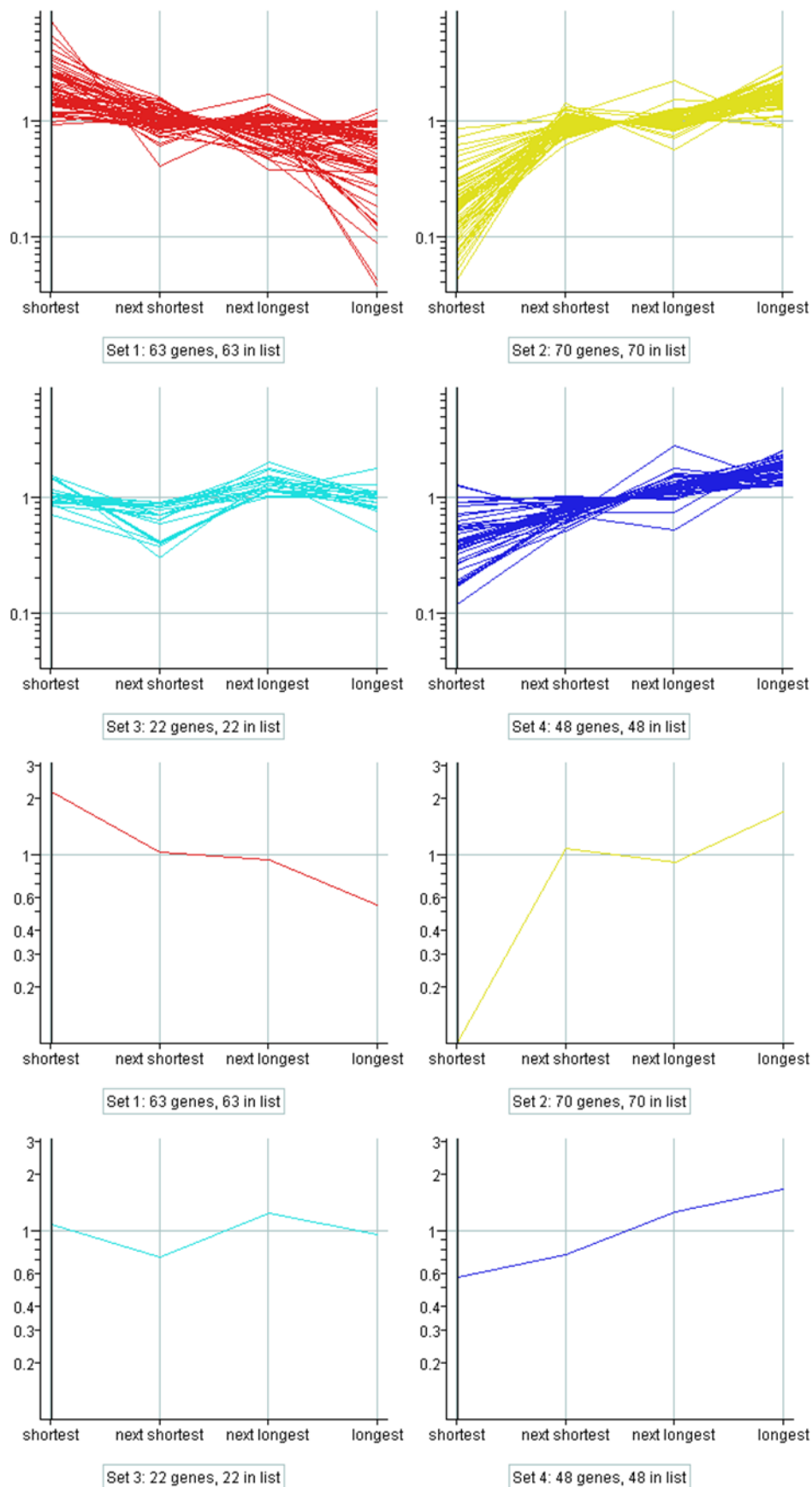
4.5. Trial to optimise the ethylene concentration for sprout control.

Tubers of three varieties; Maris Piper, Saturna and Russet Burbank, were tested for the effect of ethylene at 1, 10 and 25 ppm on sprout growth, sugar concentrations and acrylamide production at 9°C using the methods described in section 3.5.

Tubers were sampled at approximately monthly intervals, (acrylamide production was assessed after one and four months only). Data for length of longest sprout are presented in Figures 4.17a – 4.20a, acrylamide in Figures 4.17b – 4.20b and sugar concentrations (sucrose, glucose and fructose) in Figures 4.17c – 4.20c.

For Maris Piper, Saturna and Russet Burbank ethylene reduced sprout length even at the lowest concentration of 1 ppm from two months onwards. For Maris Piper and Saturna the inhibition of sprout growth increased with increasing ethylene concentration, with greater inhibition at 10 ppm than 1 ppm but no clear advantage of increasing from 10 to 25 ppm. In the case of Russet Burbank the rate of sprout growth was slower, and although ethylene had a clear inhibitory effect no differences between concentrations used could be detected up to the four months of storage studied.

After 1 month storage (SO1) ethylene has increased the acrylamide content, markedly at the highest treatment dose of 25 ppm. However at four months storage (SO4) acrylamide content had reduced to similar levels with ethylene treatment.

Saturna and Maris Piper showed only limited increases in fructose and glucose in response to rising concentrations of ethylene early in the storage period. On the other hand Russet Burbank with characteristics of longer dormancy responded to increasing ethylene concentration by accumulating increasing amounts of fructose and glucose.
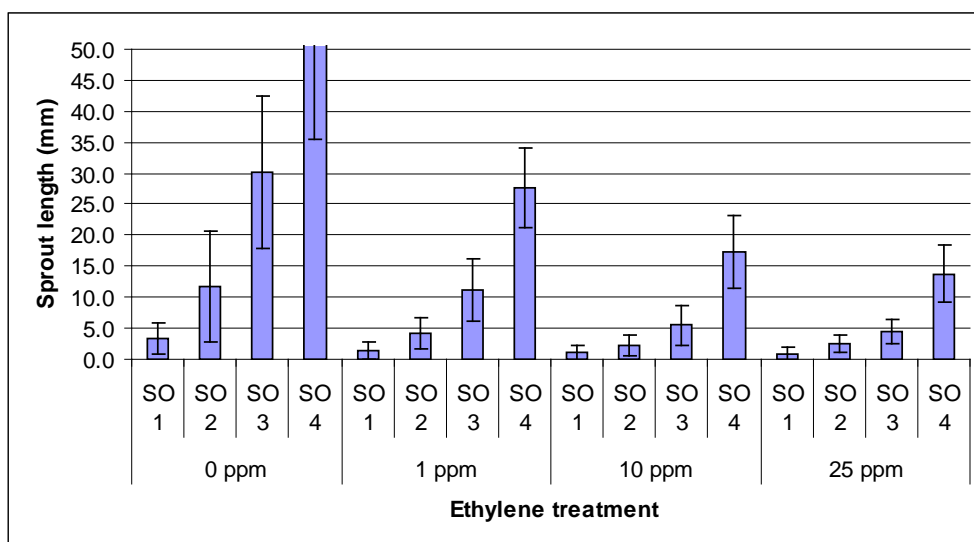
Figure 4.17a Maris Piper, length of longest sprout
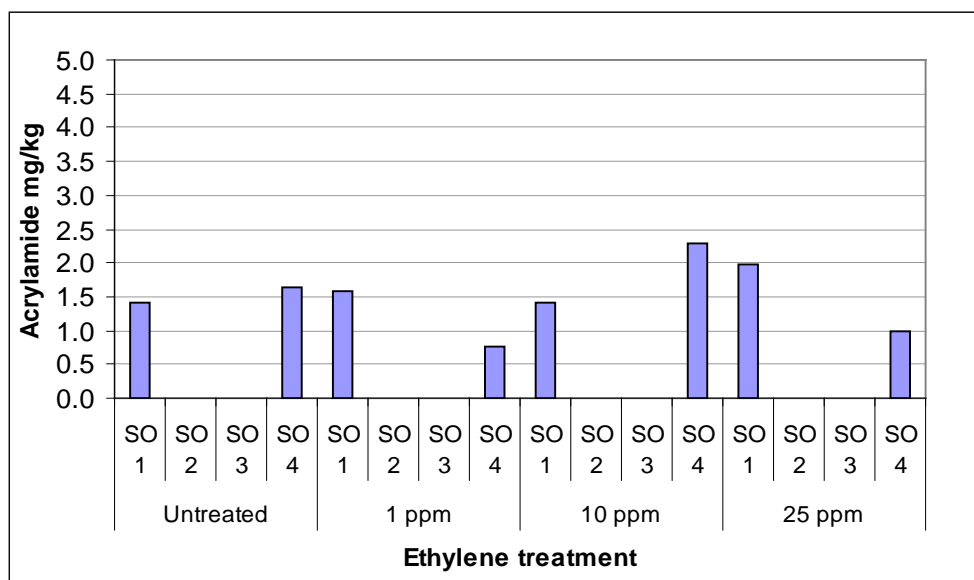
Figure 4.17b Maris Piper, acrylamide content measured at SO1 and SO4 only
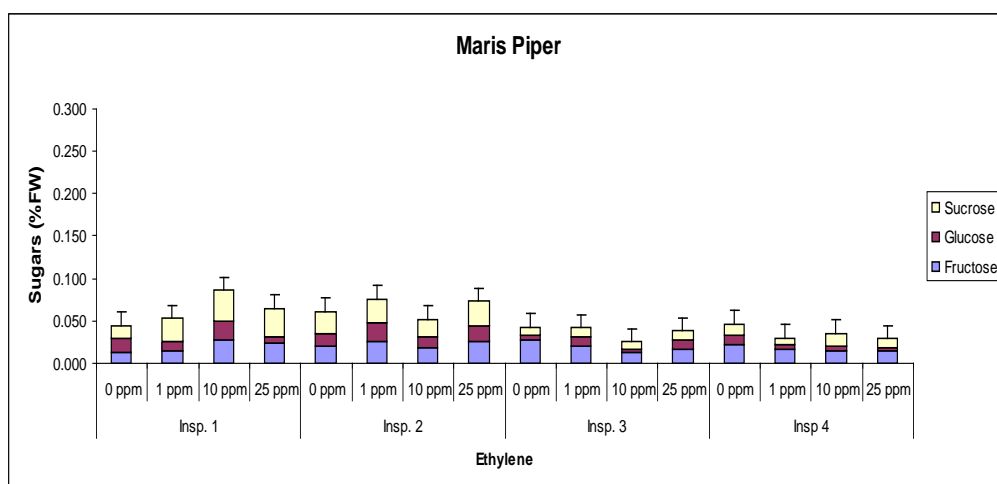
Figure 4.17c Ethylene effects on sugar concentrations in Maris Piper
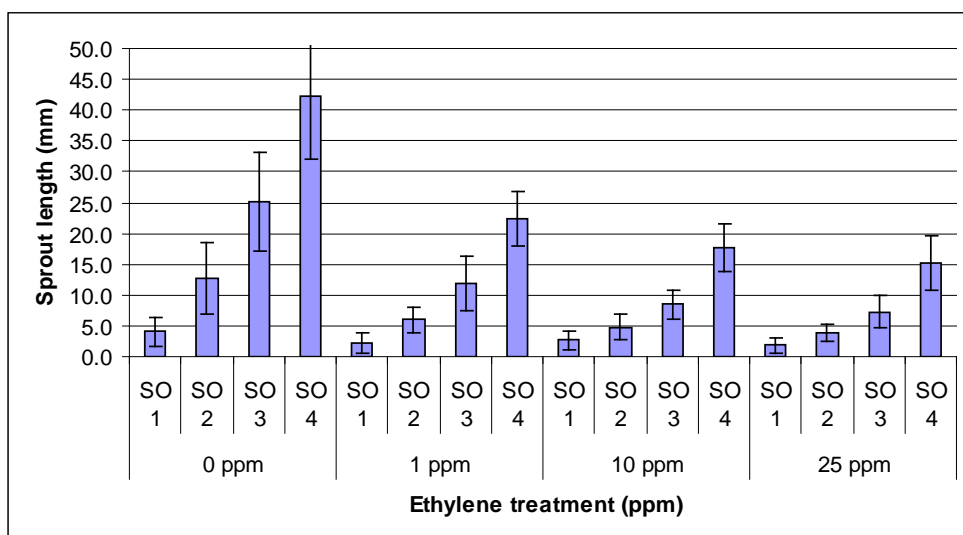
Figure 4.18a Saturna, length of longest sprout
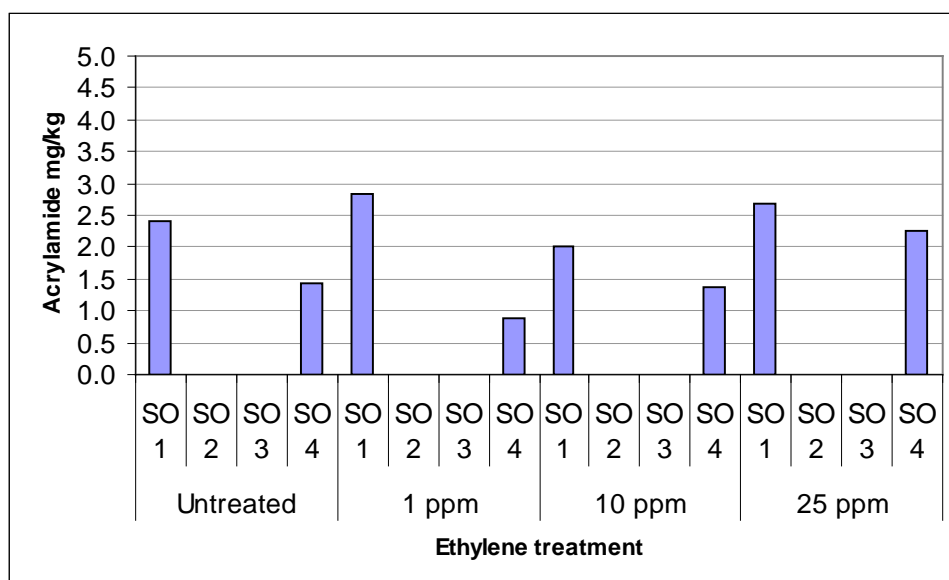
Figure 4.18b Saturna acrylamide content measured at SO1 and SO4 only
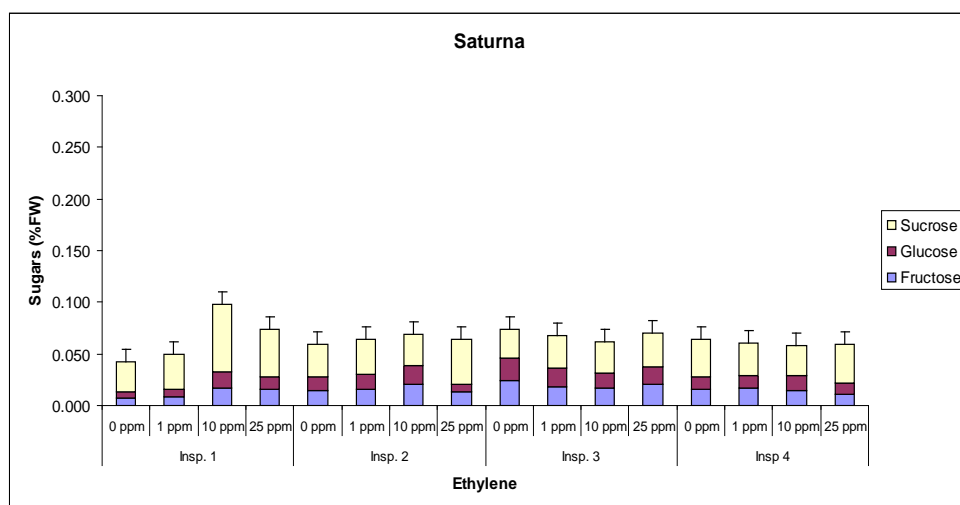
Figure 4.18c Ethylene effects on sugar concentrations in Saturna
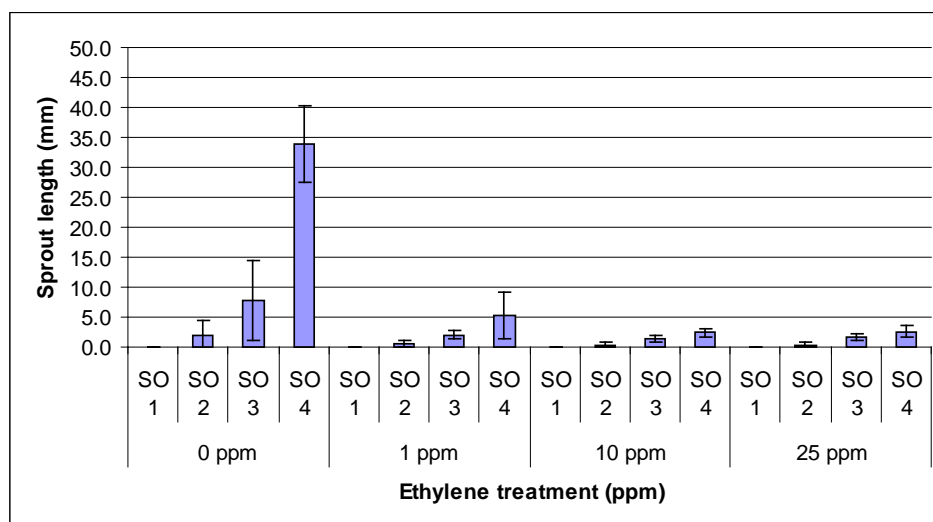
Figure 4.19a Russet Burbank length of longest sprout.
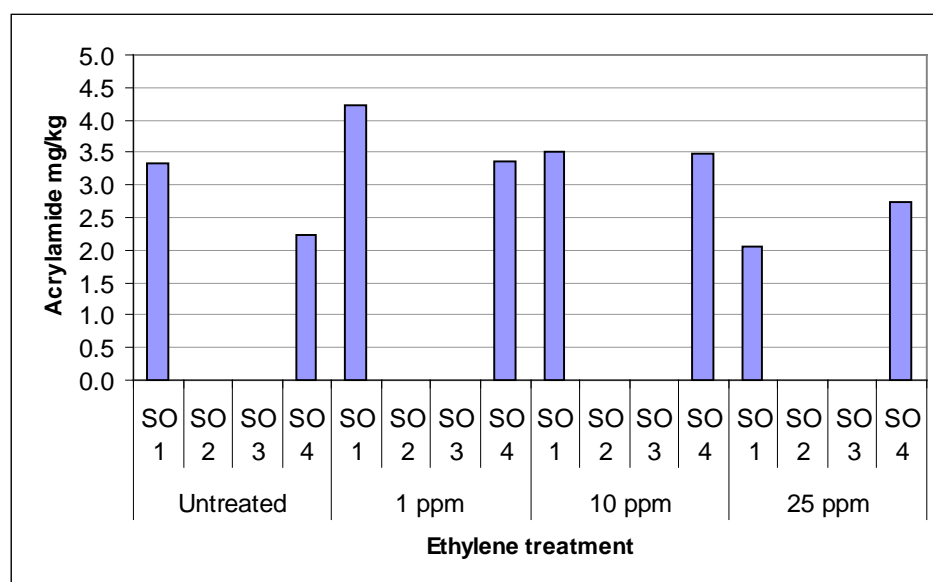
Figure 4.19b Russet Burbank acrylamide content measured at SO1 and SO4 only .
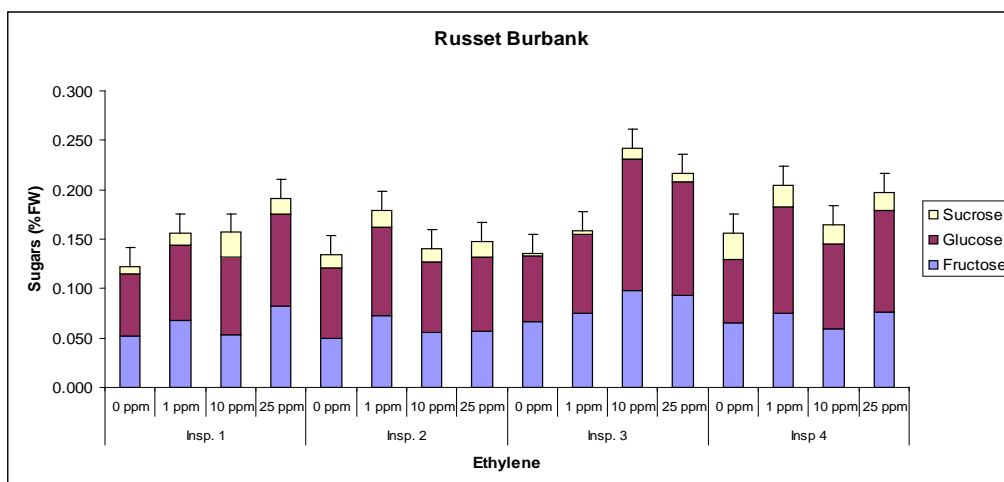
Figure 4.19c Ethylene effects on sugar concentrations in Russet Burbank

4.5.1. Main findings

- Trials undertaken on three varieties; Maris Piper, Saturna and Russet Burbank indicated that the maximum inhibitory effect of ethylene on sprout growth during storage at 9°C required concentrations greater than 1 ppm, with no clear difference between 10 and 25 ppm.

- There is no clear difference in ethylene stimulation of sugar accumulation across the concentrations tested (1, 10 and 25 ppm).

- It was not possible to carry out statistical analyses of the acrylamide data as a single measurement was made at each variety/time point/ethylene concentration. Acrylamide levels appeared to differ between varieties. In Maris Piper and Saturna, higher acrylamide levels were recorded in the 25ppm ethylene treatment at SO1 than in the untreated controls. The difference between control and ethylene treated tubers was less pronounced after 4 months storage.

4.6. Small scale trials to assess synergistic effects of a range of treatments added to ethylene

The use of volatile oils and plant growth regulators that may provide synergistic interactions with ethylene to extend the range of varieties responsive to ethylene-induced sprout suppression was tested.

4.6.1. Small scale tests on effects on sprout growth and interaction with ethylene of a range of plant growth regulators (PGRs) and volatile oils (Season 2009)

Figure 4.21 shows the sprout lengths measured for Maris Piper tubers stored for 2 months at 9°C with a range of treatments. Continuous treatment with ethylene for two months storage at 9°C reduced sprouting to 4.2 mm down from 22.7 mm in the controls, although this demonstrates a significant degree of sprout suppression, growth exceeded the threshold of commercial acceptibilty (3mm). In comparison 1,4 dimethyl naphthalene (1,4 DMN), s-carvone (Caraway) and methyl jasmonate used on their own or in conjunction with ethylene suppressed sprout growth below 3mm during the initial 2 month period. Similarly, the combination of ethylene with the gibberellin synthesis inhibitor paclobutrazole or diniconazole (inhibitor of ABA breakdown) suppressed sprout growth below 3 mm. Brassinosteriod and ABA alone or in combination with ethylene reduced sprout growth but the efficacy was low compared to other treatments.

The inclusion of salicyclic acid, the synthetic cytokinin :2-chorophenyl- urea, auxins (IAA and NAA) and gibberellin (GA$_3$) in the initial trial provided information regarding the effectiveness of PGR's in overiding the sprout suppressive action of ethylene affording opportunities to identify particular pathways that ethylene may interact with. In the abscence of ethylene all stimulated sprout growth beyond that observed in the untreated controls. Ethylene overcame the stimulation caused by salicylic acid and synthetic cytokinin and to a lesser extent the auxins; IAA and NAA and the gibberellin; GA$_3$. It is interesting to note that salicylic acid stimulation of sprout growth, in the absence of ethylene was as strong as GA$_3$. Salicylic acid has been reported to increase ethylene production in potato slices (Liang *et al.* 1997) and this in-turn may have led to an earlier stimulation of dormancy break, whereas in the presence of continuous ethylene sprout growth of salicylic acid treated tubers was suppressed.

Figure 4.21 sprout lengths for Maris Piper tubers stored for 2 months at 9°C with a range of treatments with and without continuous exposure to 10 ppm ethylene.

Further assessments of sprout growth of Maris Piper stored at 9°C were made after 4 months at 9°C (Figure 4.22). 1,4 DMN in conjunction with ethylene provided the strongest suppression of sprout growth. Diniconazole, the inhibitor of ABA catabolism inhibited sprout growth both in the presence and absence of ethylene
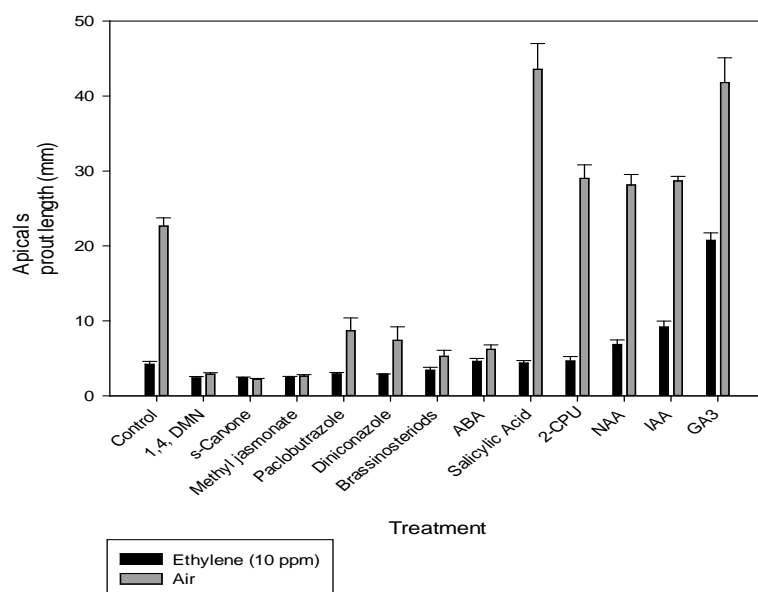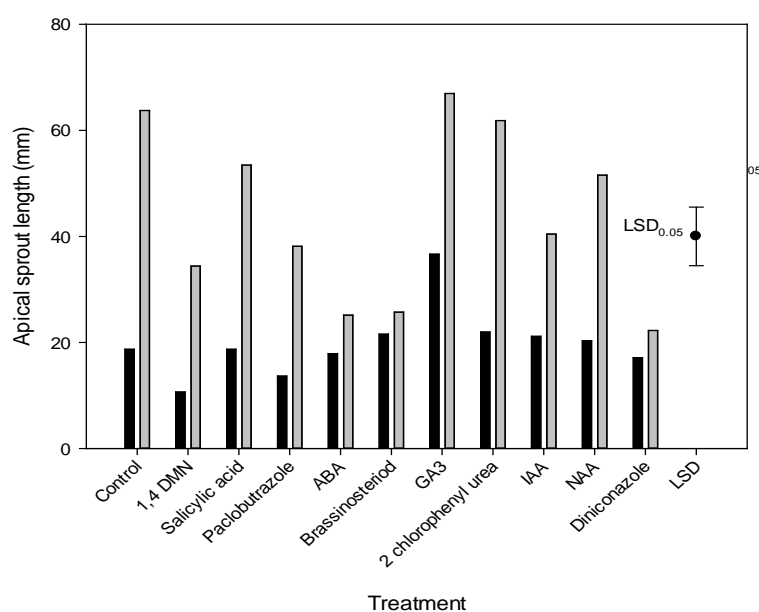

Figure 4.22 sprout lengths for Maris Piper tubers stored for 4 months at 9°C with a range of treatments with and without continuous exposure to 10 ppm ethylene.

Subsequent synergy trials with Cabaret and Russet Burbank focussed on identification of dose response between 10 ppm ethylene and 1, 10 and 50µm paclobutrazole, diniconazole, brassinosteroids and ABA. Application was made at the point of bud opening, tubers were held previously in air at 6°C for 6 months prior to treatment. After treatment tubers were held at 9°C with or without ethylene treatment (10 ppm) and assessed after 2 and 4 months storage.

The results are illustrated in Figure 4.23a and b. A strong variety response was observed after 2 months storage; Russet Burbank was more responsive to treatments than Cabaret and this may underlie the endo/eco-dormancy state of tuber at the point of treatment. After two months storage, untreated Cabaret sprouts reached 13.5 mm, while ethylene suppressed sprouting to less than 8mm. Increasing the concentration of paclobutrazole from 10-50 µM, reduced sprout growth to <6 mm, while the addition of ethylene in this case reduced sprout growth further (<4 mm). After 2 months storage, sprout growth in Russet Burbank had reached 4 mm in air, while ethylene treated tubers averaged 3.4 mm. Treatment with 50 µM paclobutrazole lowered sprout growth to <3 mm in both air and ethylene treated tubers.

Air-stored Cabaret treated with 50 µM diniconazole reduced sprout growth marginally (11.3 mm) and in combination with ethylene sprout growth was further lowered to 5.2 mm. Sprout growth in Russet Burbank treated with ethylene was not significantly lower than untreated tubers. Diniconazole applied at 50µM had a reduced sprout growth to below 3 mm, no incremental reduction was observed in the presence of ethylene.

ABA applied in combination with ethylene treatment of Cabaret reduced sprout growth at higher concentrations (10-50 µM) to around 6 mm. Russet Burbank failed to respond to ethylene or ABA and showed signs of mild stimulation in response to high concentrations of ABA.

24-Epibrassinosteriod applied at 50µm to Cabaret reduced sprout growth (<6 mm) but no reduction was observed at lower concentrations moreover, no synergistic interactions with ethylene were noted. Interestingly, 24-epibrassinosteriod stimulated sprout growth in Russet Burbank.

Cabaret

Figure 4.23a Suppression of sprout growth in potato cv. Cabaret in the presence of 10 ppm ethylene combined with a single application of either ABA, diniconazole, paclobutrazole or 24-epibrassinosteroid applied at harvest. Tubers were stored for 2 months at 9°C in air or in the continuous presence of ethylene. Values are the mean of 20 tubers ± SE.

Russet Burbank

Figure 4.23b Suppression of sprout growth in potato cv. Russet Burbank in the presence of 10 ppm ethylene combined with a single application of either ABA, diniconazole, paclobutrazole or 24-epibrassinosteroid applied at harvest. Tubers were stored for 2 months at 9°C in air or in the continuous presence of ethylene. Values are the mean of 20 tubers ± SE.

After 4 months storage at 9°C (Fig 4.24 a and b) an increase in sprout growth was observed in all treatments with air stored Cabaret averaging 14.9 mm, while ethylene treatment only marginally suppressed sprout growth (12.6 mm). Cabaret tubers treated with 10-50 μM paclobutrazole suppressed sprout growth (<11 mm), and this was further reduced to 7.3-8 mm when paclobutrazole was combined with ethylene. Diniconazole applied at 50 μM to air stored Cabaret was the most effective sprout suppressant lowering growth to 7.3 mm which was greater than the combined effect of ethylene and diniconazole (9.5 mm). ABA (50 μM) combined with ethylene reduced sprouting to 9.8 mm.

Sprout growth in Russet Burbank stored in air at 9°C reached 16.7 mm after 4 months storage and this was reduced to 3.3 mm when tubers were pre-treated with 50 μM paclobutrazole, a further reduction to 2.2 mm was observed when paclobutrazole was combined with ethylene. Of the other treatments, Diniconazole (50 μM) lowered sprouting to 8.7 mm in air-stored tubers, while ethylene alone lowered sprout growth to 5.1 mm this was further reduced to 3.1 mm when diniconazole and ethylene were combined. ABA and 24-epibrassionosteriod had little effect on sprout growth after 4 months storage.
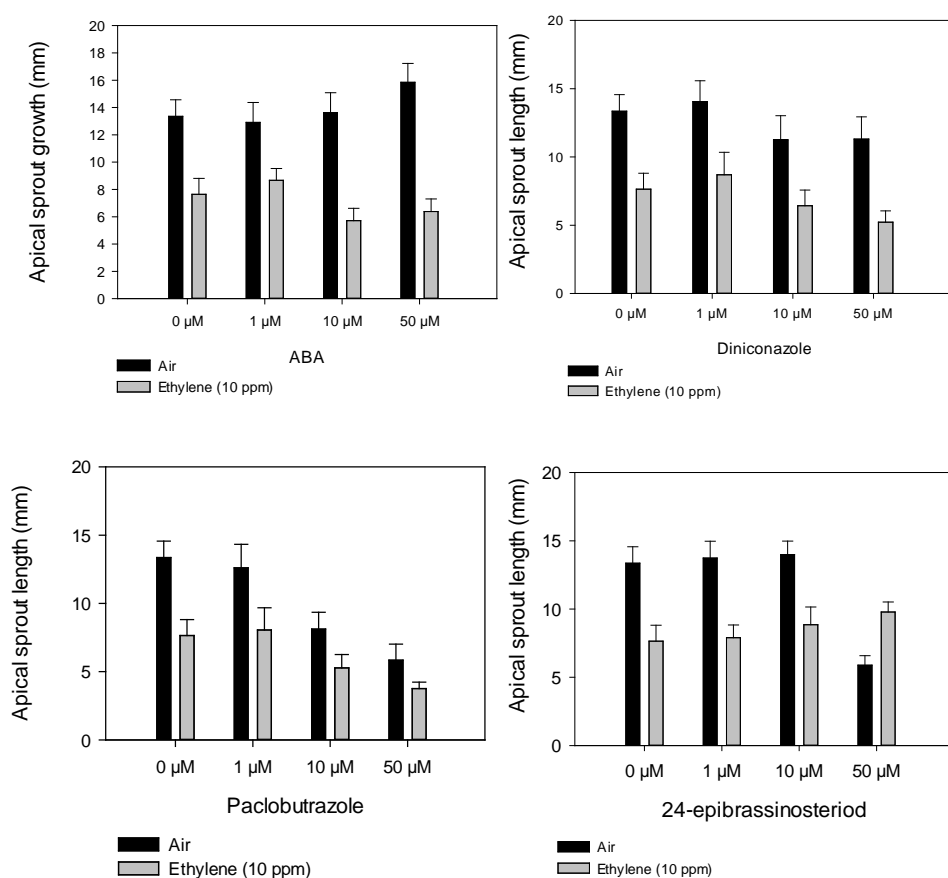
Cabaret

Figure 4.24a Suppression of sprout growth in potato cv. Cabaret in the presence of 10 ppm ethylene combined with a single application of either ABA, diniconazole, paclobutrazole or 24-epibrassinosteroid applied at harvest. Tubers were stored for 4 months at 9°C in air or in the continuous presence of ethylene. Values are the mean of 20 tubers ± SE.
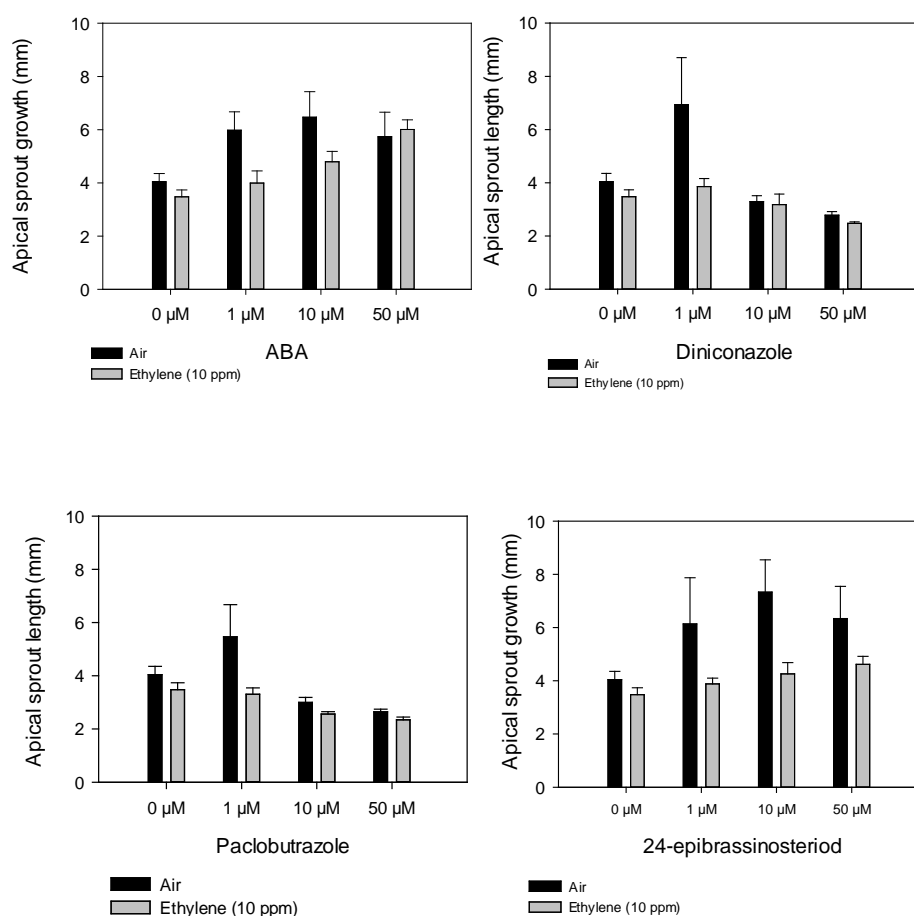
Figure 4.24b Suppression of sprout growth in potato cv. Russet Burbank in the presence of 10 ppm ethylene combined with a single application of either ABA, diniconazole, paclobutrazole or 24-epibrassinosteroid applied at harvest. Tubers were stored for 4 months at 9°C in air or in the continuous presence of ethylene. Values are the mean of 20 tubers ± SE.

A second set of repeat synergy trials with 10 ppm ethylene combined with either s-Carvone, 1,4-DMN or methyl jasmonate applied as volatiles at the point of eye-movement to Cabaret stored at 9°C (Fig 4.25a) were carried out. Once bud break was initiated, these treatments were less effective at suppressing subsequent sprout growth, with the exception of 2 mM methyl jasmonate where sprout growth was lowered to 10.6 mm compared to 36 mm in air stored controls.

Sprout growth of Russet Burbank tubers was particularly vigorous when stored in a flow of air (1 L kg^{-1} h^{-1}), While 1,4 DMN showed a dose responsive suppression of sprout growth the effect of methyl jasmonate was less clear. Sprout suppression of Russet Burbank by ethylene was very strong and combining treatments with other PGR's did not increase effectiveness.
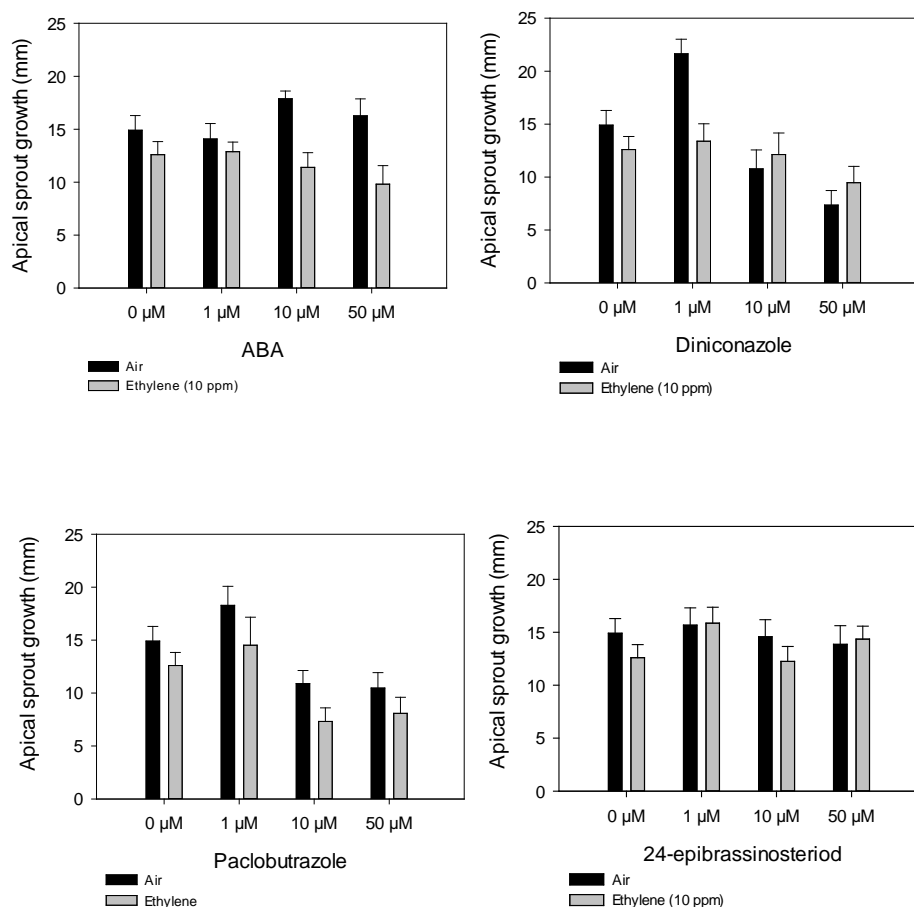
Cabaret
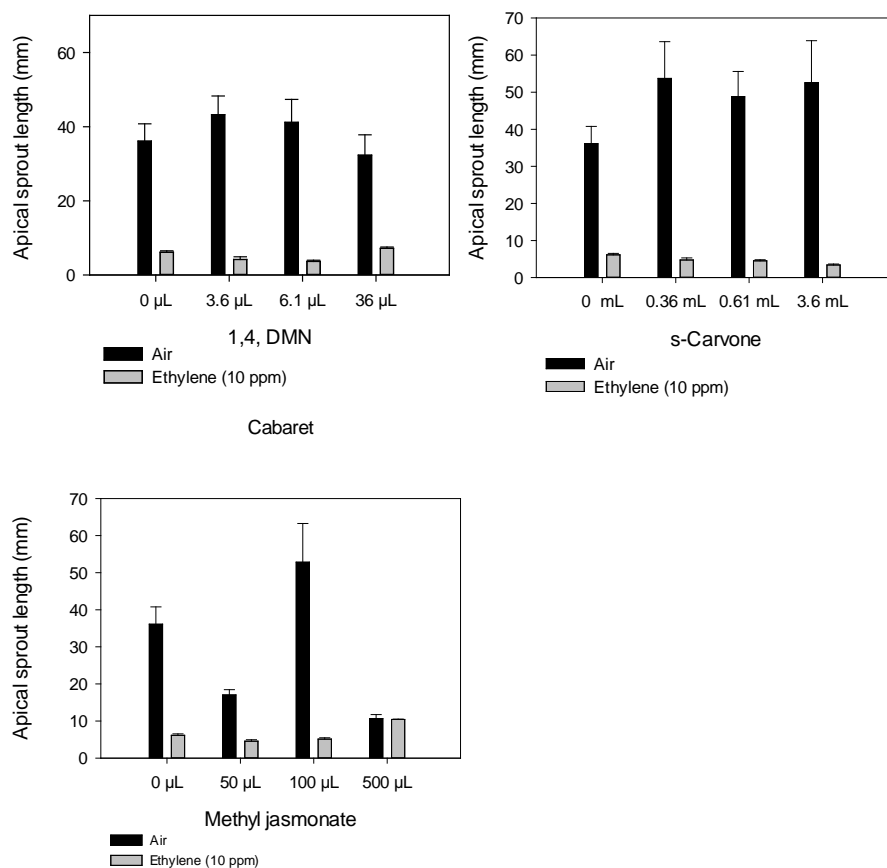
Figure 4.25a. Suppression of sprout growth in potato cv. Cabaret in the presence of 10 ppm ethylene combined with a single application of either 1,4, dimethyl naphthalene, s-carvone or methyl jasmonate applied at harvest. Tubers were stored for 2 months at 9°C in air or in the continuous presence of ethylene. Values are the mean of 20 tubers ± SE.
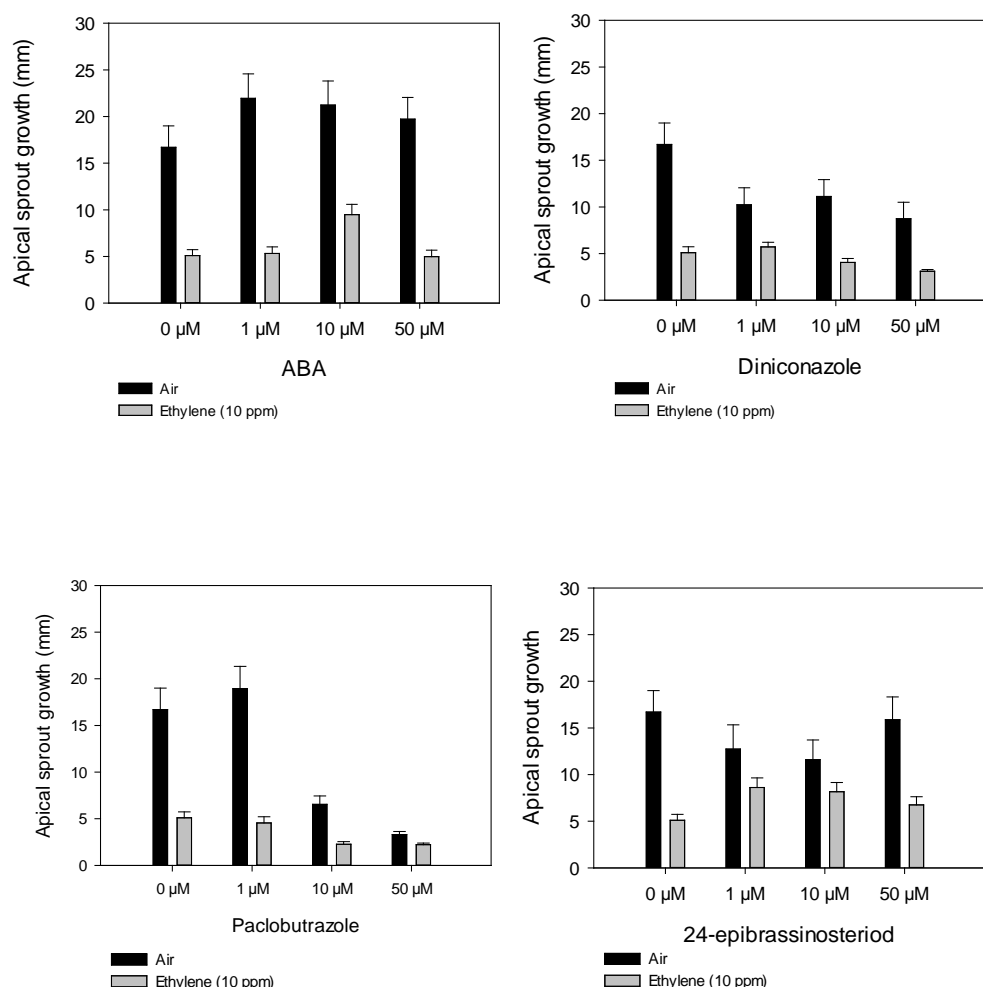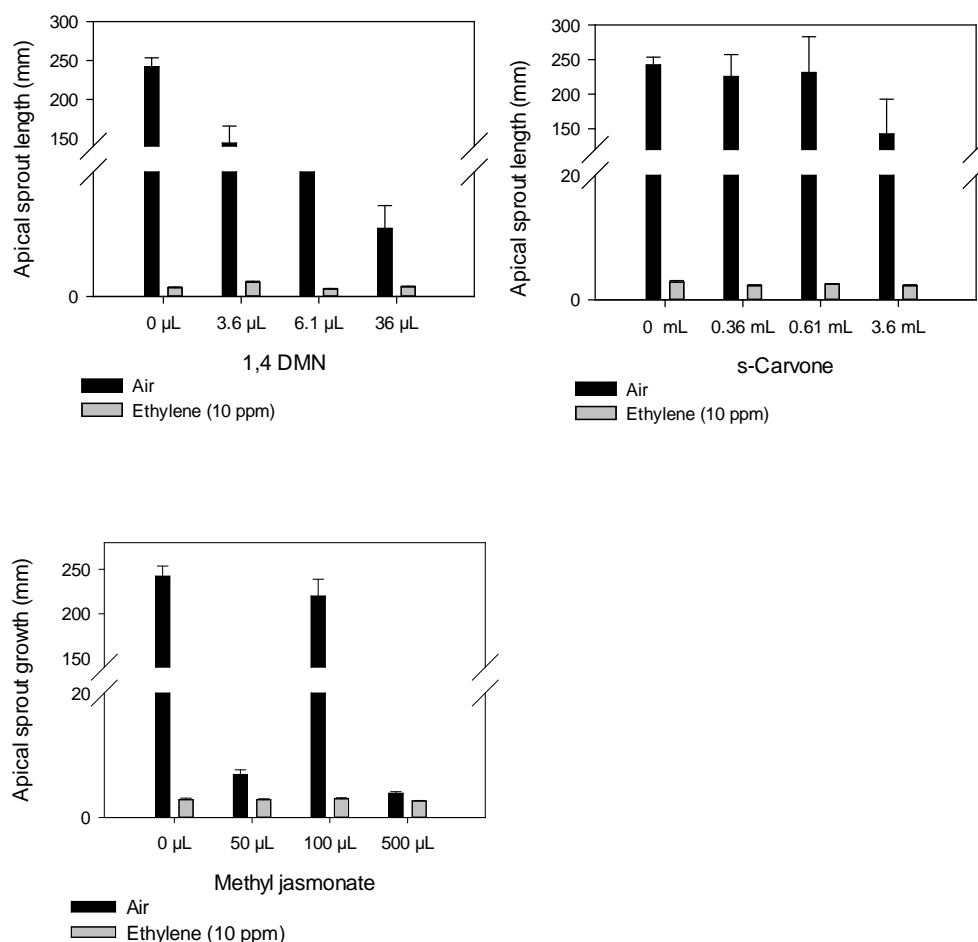
Figure 4.25b. Suppression of sprout growth in potato cv. Russet Burbank in the presence of 10 ppm ethylene combined with a single application of either 1,4, dimethyl naphthalene, s-carvone or methyl jasmonate applied at harvest. Tubers were stored for 2 months at 9°C in air or in the continuous presence of ethylene. Values are the mean of 20 tubers ± SE.

4.6.2.   Summary of season 2009 findings

Combining 10 ppm ethylene with s-carvone, 1,4-dimethyl naphthalene and methyl jasmonate showed signs of improved sprout control in Maris Piper when applied early in the storage season. Application of treatments after prolonged storage (3-4 months) to Cabaret and Russet Burbank tubers at bud break was less effective.   Repeat application may increase the efficacy of treatments. The addition of 50 µM paclobutrazole and diniconazole with 10 ppm ethylene afforded greater sprout control than ethylene alone, however, identifying an appropriate method of application may require further investigation

4.6.3. Small scale tests on effects on sprout growth and interaction with ethylene of s-carvone, 1,4 DMN and methyl jasmonate (Season 2010)

Following on from season 2009 results, small scale trials to assess synergistic effects of treatments added to ethylene to control sprout growth at different temperatures, while maintaining low sugar were repeated for s-carvone, 1,4 DMN and methyl jasmonate. Trials were carried out on Maris Piper and Hermes known to have different sprouting characteristics, including in their response to ethylene. Trials were only conducted for three months, but provide useful information on strategies that merit larger scale trials.

Maris Piper: 1,4 DMN, methyl jasmonate and s-carvone

Air-treated Maris Piper tubers produced the longest sprouts during initial sampling. Treatment with 1,4 DMN increased dormancy, however, following dormancy break the rate of sprouting was high and apical sprout lengths were similar to air-treated tubers by early February. Ethylene's ability to break dormancy and overcome the effects of 1,4 DMN on dormancy extension were apparent and growth rates of ethylene and ethylene combined with 1,4 DMN were similar, both treatments reduced sprout length over the 3 month storage period compared to air stored tubers

Methyl jasmonate reduced sprout growth compared to air-treated and ethylene-treated tubers. For the first two months of storage, methyl jasmonate or methyl jasmonate combined with ethylene reduced sprout growth beyond ethylene alone. While the combined treatment suppressed sprout growth for a further month, sprouting of methyl jasmonate treated-tubers increased to rates similar to ethylene treated tubers, which in turn was significantly better than air stored tubers.

S-carvone treatments failed to reduce sprout growth when applied alone to Maris Piper. S-carvone applied in combination with ethylene was no better than when ethylene was applied alone.

1,4 DMN - Maris Piper

Methyl Jasmonate - Maris piper

s-Carvone - Maris Piper

Figure 4.26 Apical sprout growth for Maris Piper tubers stored at 9°C and subjected to combinations of ethylene with either 1,4 DMN, methyl jasmonate or s-carvone treatment.

Hermes: 1,4 DMN and ethylene

Initially, 1,4 DMN reduced sprout growth through extension of dormancy. Combining treatments with ethylene counteracted the dormancy inducing effects on 1,4 DMN. After initiation of sprouting, 1,4 DMN treated sprouts developed at the same rate as control tubers, while tubers under continuous ethylene treatment had reduced rates of sprout growth.

Figure 4.27 Apical sprout growth for Hermes tubers stored at 9°C and subjected to combinations of ethylene and either 1,4 DMN, methyl jasmonate or s-carvone treatment.

4.6.4. Summary of season 2010 findings

- The efficacy of sprout suppressant treatments differed significantly between the two cultivars studied; Maris Piper and Hermes.
- Ethylene treatment inhibited sprout growth in Maris Piper by approximately 40% for the whole three month period studied. For Hermes the initial effect of ethylene treatment was small, probably due to the counteracting effect of ethylene in shortening dormancy, but after three months the inhibitory effect of ethylene was likewise approximately 40%.
- The combination of ethylene and methyl jasmonate was significantly more effective in reducing sprout growth compared to ethylene alone in Maris Piper (>70% inhibition for the whole three month period studied). However this result was not found for Hermes, for which the combination of methyl jasmonate with ethylene was less effective than ethylene alone.
- 1,4 DMN applied to Maris Piper controlled sprout growth by more than 90% over a 2 month period but was less effective when combined with ethylene, presumably due to ethylene counteracting the dormancy extension induced by 1,4 DMN. The effect of 1,4 DMN was not maintained to 3 months of storage.
- s-Carvone provided poor control of sprout growth in both Maris Piper and Hermes

4.6.5. Small scale tests on effects on sprout growth and interaction with ethylene of 1,4 DMN and methyl jasmonate (Season 2011)

A single application of 1,4 DMN applied to Hermes at harvest was as effective as a continuous supply of ethylene for the first two months of storage, thereafter sprout growth rates were comparable with untreated controls (Figure 4.28). While the addition of ethylene to the storage atmosphere lowered the rate of sprout growth the combination of ethylene and 1,4 DMN had the greatest effect on lowering the rate of sprout growth in Hermes.

A reduction in sprout growth in potato c.v. Hermes was observed where tubers were pre-treated with methyl jasmonate spray, and a further reduction in sprout growth was seen where ethylene and methyl jasmonate (spray) were combined. However, these results were not replicated when methyl jasmonate was applied as a vapour prior to storage. Sprout growth in Hermes was reduced through continual application of ethylene (Figure 4.28)

A similar response was observed with Maris Piper suggesting that more direct application to the apical bud had a longer lasting effect (Figure 4.29).
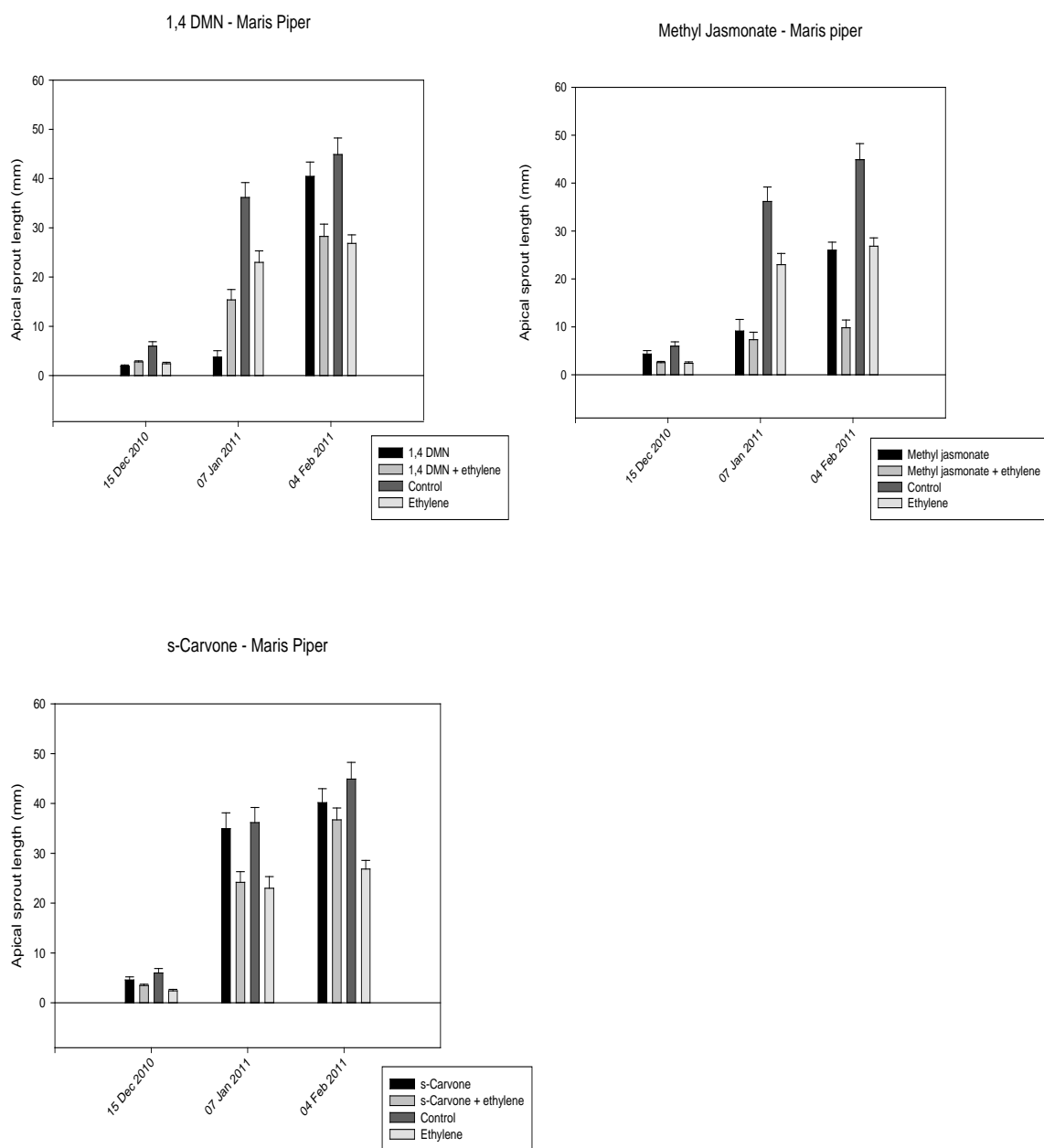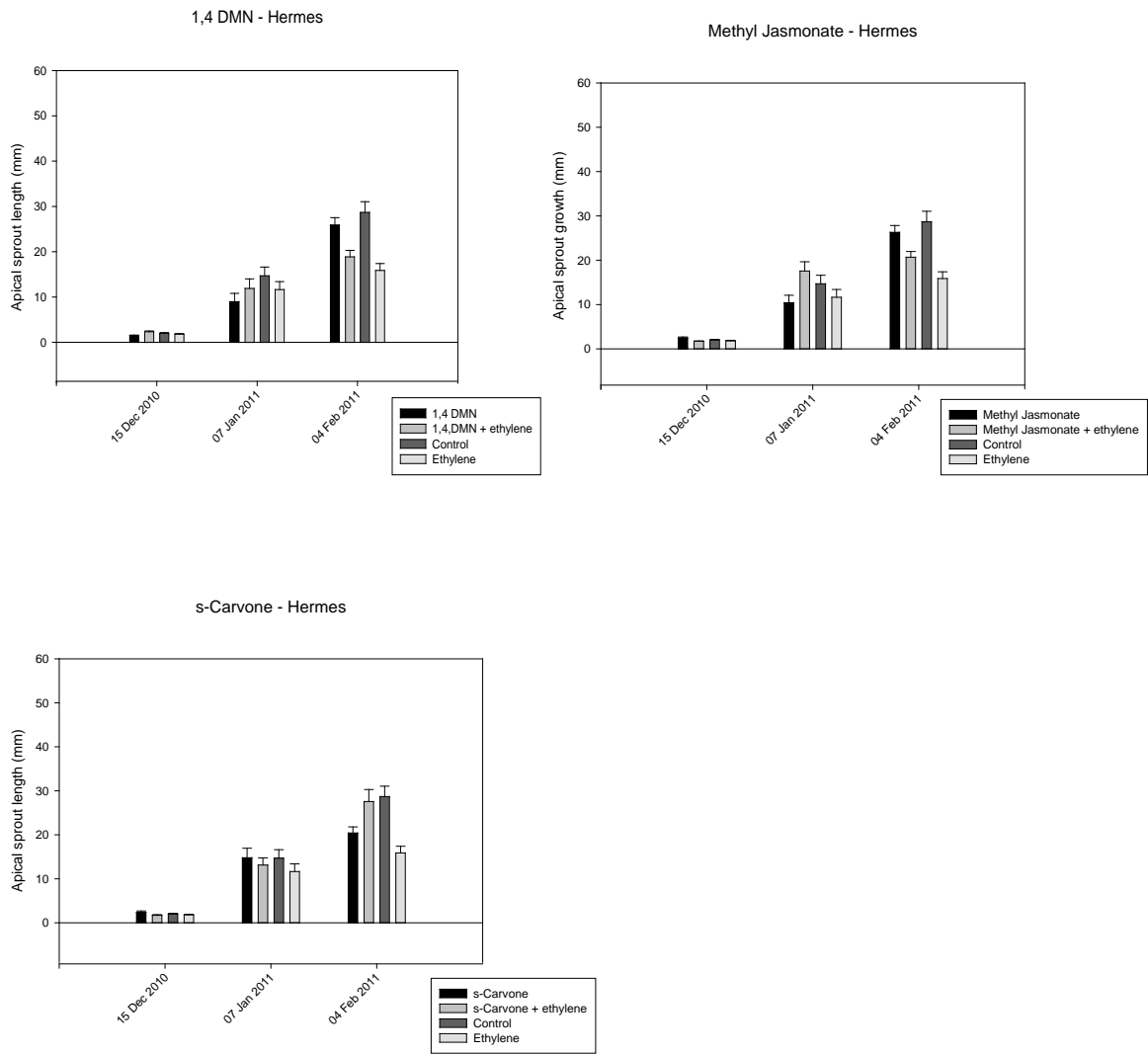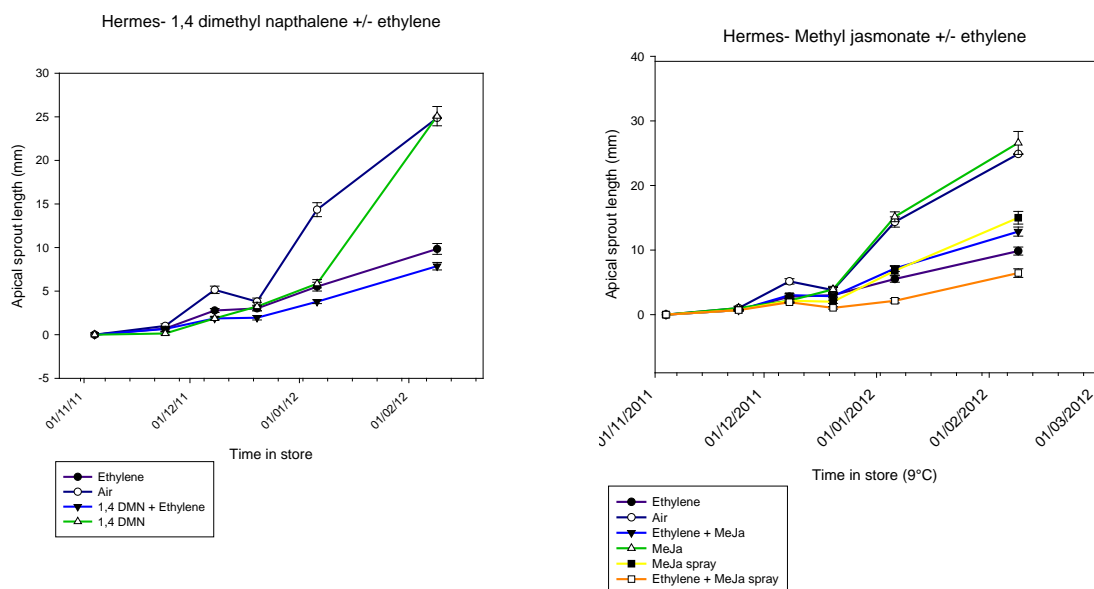
Figure 4.28 Apical sprout growth for Hermes tubers stored at 9°C and subjected to combinations of ethylene and either 1,4 DMN or methyl jasmonate treatment.
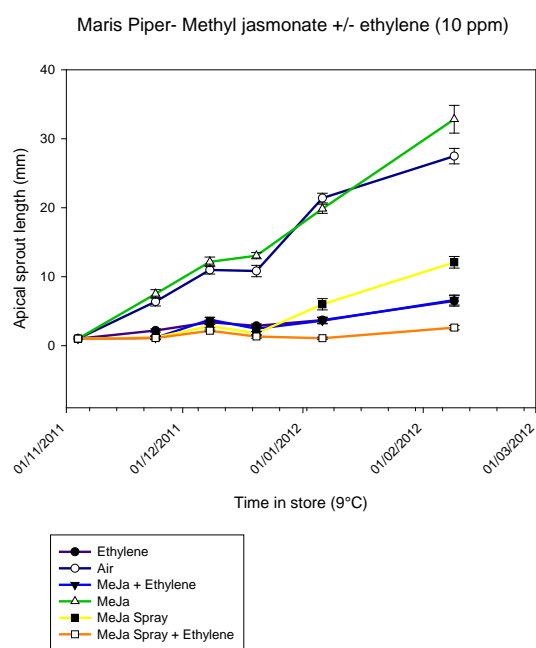
Figure 4.29 Apical sprout growth for Maris Piper tubers stored at 9°C and subjected to combinations of ethylene and methyl jasmonate treatment.

4.6.6. Summary of season 2011 findings

- Methyl jasmonate applied as a spray improved sprout control of Maris Piper and Hermes stored in the presence of 10 ppm ethylene.
- In Hermes 1,4 DMN applied in combination with ethylene reduced the rate of sprout growth compared to ethylene alone.

4.7. Trial to assess the effect of ethylene and ethylene + 1-MCP treatment on sprout growth and sugar levels in commercial varieties

In season 2009 a trial was carried out to determine the effect of standard ethylene treatments on a range of commercial varieties.

The ethylene concentrations during manual ramping are shown in Figure 4.30. After the ramp ethylene concentrations were established and maintained at 10 ppm using a EMU2 TS Ethylene Management Unit [BioFresh Ltd] system. This took a further 3-4 days so that the tubers were effectively subjected to a ramping over 17 – 18 days.
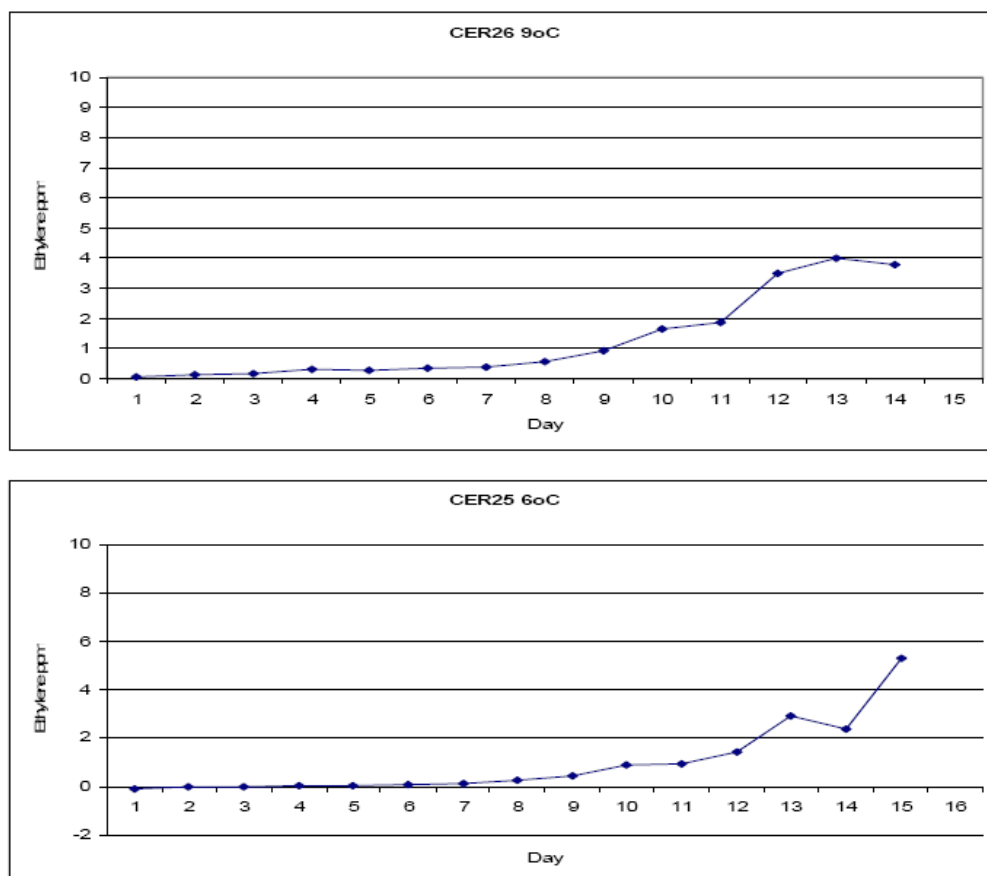
Figure 4.30 ethylene concentrations during establishment of conditions at the start of the trial.

Sprouting and sugar concentrations for the eight commercial varieties at 6 and 9°C assessed after 2 and 4 months storage are illustrated in Figure 4.31. Lines inserted at a sprout length of 3 mm and a reducing sugar concentration (glucose + fructose) of 0.2 % are considered to be the limit of commercial acceptance. The sprouting assessments for all varieties for all assessment periods are not shown here but are available on request to the report authors. The main observations for each variety are as follows:

- **Russet Burbank** – *Occasion 2*
Sprout growth at 6°C was minimal and sprout growth in tubers stored at both 6 and 9°C was below 3 mm. Sprout growth in tubers was greater at 9°C. Sprout growth was reduced by ethylene with 1-MCP having no additional effect.

Tubers stored at 9°C had lower total sugars compared to 6°C and sugar accumulation wasn't induced by ethylene.

- **Russet Burbank** – *Occasion 4*

Untreated tubers at 9°C had sprouted to an average of more than 5mm, however, ethylene maintained acceptable sprout control (<3mm) in tubers stored at both 6°C and 9°C. 1-MCP provided no additional effect in terms of sprouting. After 4 months storage, tubers stored at 9°C had lower sugars than those at 6°C. Ethylene increased the proportion of glucose and fructose in tubers stored at 6°C.

- **Sylvana** - *Occasion 2*

At 9°C ethylene provided significant (and commercially acceptable <3mm) sprout control compared to untreated tubers, while tubers stored at 6°C exhibited minimal sprouting in all treatments. Storage at 9°C lowered fructose and glucose concentration compared to tubers stored at 6°C. Ethylene elevated glucose and fructose content of tubers, while SmartFresh™ was able to overcome the early effects of ethylene-induced sugar accumulation on tubers stored at 6 and 9°C.

- **Sylvana** - *Occasion 4*

Ethylene was very effective in suppressing sprouting at 6 and 9°C. 1-MCP provided no additional effect in terms of sprouting. While ethylene increased the amount of fructose and glucose in tubers, ethylene-induced accumulation of fructose and glucose content was lower in tubers stored at 9°C.

- **Saturna** - *Occasion 2*

Ethylene reduced sprouting at 9°C although sprout growth was above 3mm. 1-MCP provided no additional effect in terms of sprouting. In general, tubers stored at 9°C had lower total sugars compared to tubers stored at 6°C. Only a minimal increase in sugars was observed in response to ethylene.

- **Saturna** - *Occasion 4*

Ethylene significantly reduced sprouting in tubers at 9°C although sprouts were still greater than 5 mm. 1-MCP provided no additional effect in terms of sprouting. Tubers stored at 9°C had lower total sugars compared to 6°C. No increase in fructose and glucose content was observed in the presence of ethylene. However, unexpectedly an increase in sugars was observed in SmartFresh™ and ethylene treated tubers stored at 6°C.

- **Verdi** - *Occasion 2*

With all treatments sprout growth at 6°C was minimal whereas at 9°C sprouts in untreated tubers stored were greater than 5mm. Ethylene did apparently reduce sprout growth at 9°C although the effect was not significant. 1-MCP provided no additional effect in terms of sprouting. Verdi's sugar content remained low during storage at 6 and 9°C and exposure to ethylene did not increase sugar content.

- **Verdi** - *Occasion 4*

Sprouting was evident and greater than 3mm at 6°C and greater than 10 mm at 9°C. Ethylene did not significantly affect sprouting nor did 1-MCP provide any additional effect. Sugars were

unresponsive to ethylene: generally, very low fructose and glucose content was observed in tubers from all treatments.

- **Hermes** - *Occasion 2*

Sprouting was minimal at 6°C and greater than 3mm at 9°C. Although ethylene apparently reduced sprouting it was not significant and was still greater than commercial acceptable (3mm). 1-MCP provided no significant additional effect in terms of sprouting. Glucose and fructose content was unaffected by ethylene in tubers stored at 9°C while at 6°C Hermes responds to ethylene by increasing glucose and fructose content.

- **Hermes** - *Occasion 4*

At 6°C sprouting is lower than at 9°C but no treatment significantly affected sprout length at either temperature. At both temperatures sprouting was commercially unacceptable. Ethylene at 9°C caused a slight rise in fructose and glucose content of tubers. SmartFresh^{TM} reduced ethylene effects on sugar accumulation. Ethylene increases glucose and fructose content in tubers stored at 6°C but this response was reduced when potatoes were stored at 9°C

- **Markies** - *Occasion 2*

Tubers stored at 6°C showed minimal amounts of sprout growth. Sprout lengths were greater at 9°C but were commercially acceptable. Markies appeared responsive to ethylene-mediated sprout suppression. Markies stored at 9°C maintained low fructose and glucose content and sugar accumulation was not responsive to ethylene, while at 6°C, glucose and fructose content was elevated by ethylene but effects on sugar accumulation were controlled by SmartFresh^{TM}.

- **Markies** - *Occasion 4*

At 6°C sprouting was low with all treatments but both ethylene and 1-MCP/ethylene treatment significantly reduced sprouting. Ethylene caused strong suppression of sprouting at 9°C, controls exceeded the commercial threshold of acceptability for sprout length being greater than 5 mm. 1-MCP provided no significant additional effect in terms of sprouting. Fructose and glucose content remained low in tubers stored at 9°C and storage at higher temperatures prevented ethylene increasing the content of fructose and glucose within the tuber. In contrast at 6°C, glucose and fructose content increased and were further elevated by ethylene nevertheless pre-treating tubers with 1-MCP was able to mitigate the effects of ethylene and in particular lower the concentration of fructose.

- **Maris Piper** - *Occasion 2*

Storage at 6°C provided effective control of sprouting, while ethylene treatments provided additional suppression of sprouting. Sprouting was particularly vigorous at 9°C and even though ethylene very significantly reduced sprouting sprout length still slightly exceeded commercially acceptable limits. A small increase in fructose and glucose content was recorded in tubers stored at 6°C in response to ethylene, while at 9°C reducing sugar content was low in all treatments.

- **Maris Piper** - *Occasion 4*

Storage at 6°C suppressed sprout growth with all treatments within commercially acceptable limits. Ethylene reduced the length of sprouts. At 9°C the untreated controls sprouted

vigorously, generating the longest sprouts of all varieties. However, ethylene markedly and significantly reduced sprout growth treatment although sprouts were just larger than commercial acceptable. 1-MCP provided no significant additional effect in terms of sprouting. Glucose and fructose content were elevated slightly by storage at 6°C below the amount regarded as critical in leading to a darkening of fry colour. Ethylene had a very limited effect on raising sugar content. At 9°C reducing sugar content was minimal and no treatment effects were observed.

- **Cabaret** - *Occasion 2*

Sugars in tubers stored at 6 and 9°C were below threshold concentrations and no treatment effects were observed. The analysis of ethylene effects of sprouting are difficult to interpret because the consignment of potatoes had been accidentally field-treated with maleic hydrazide.

- **Cabaret** – *Occasion 4*

Tubers stored at 9°C had low concentrations of sugars that mainly comprised of sucrose. No treatment differences were observed at 9°C while slightly higher sugars were recorded in tubers stored at 6°C; ethylene elevated slightly glucose and fructose content. All sugar contents were below threshold for deviations in fry colour.
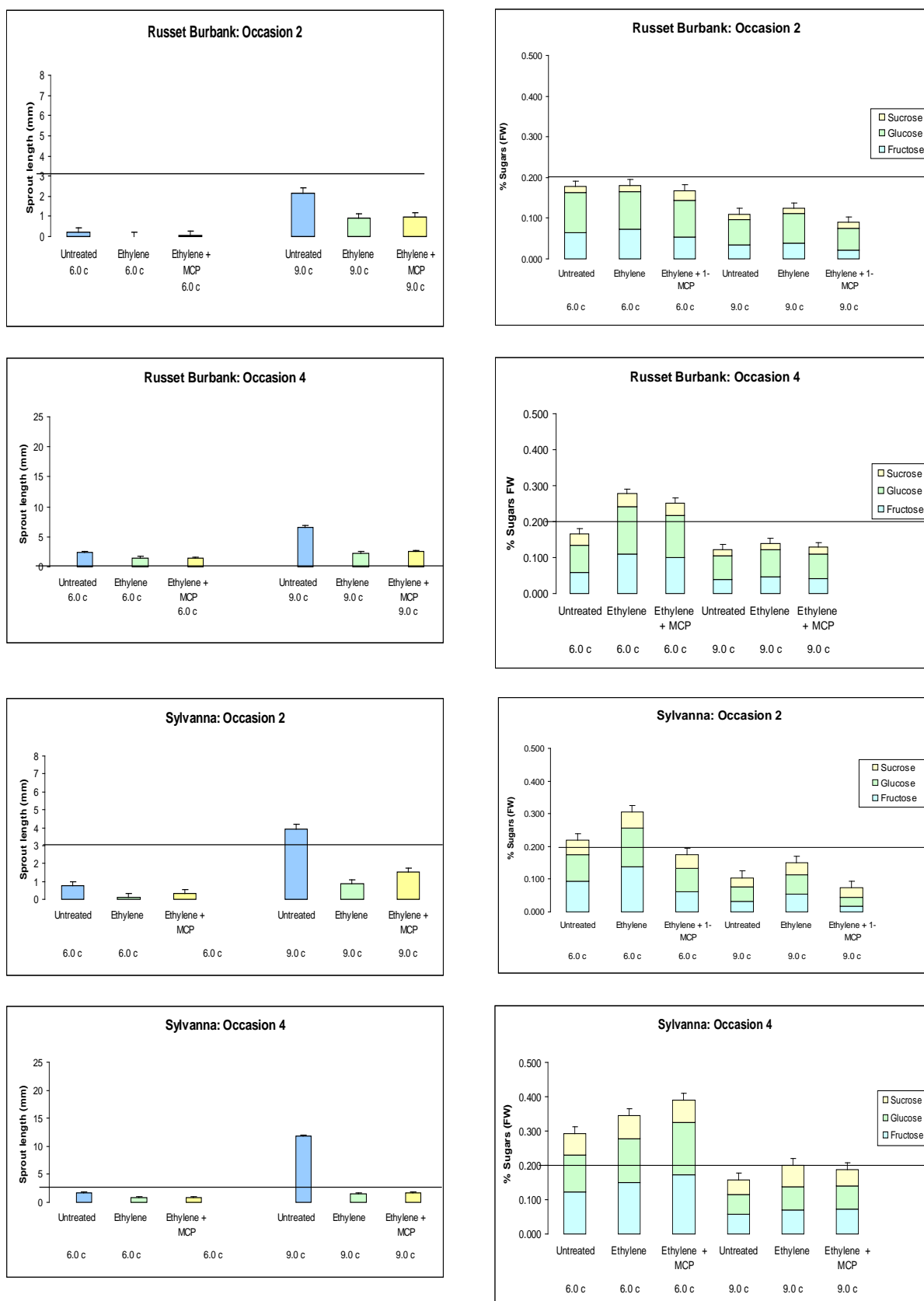
Figure 4.31 sprouting and sugar concentrations after 2 months (occasion 2) and 5.5 months (occasion 4) of storage at 6 and 9ºC for key commercial potato varieties. Each data point is the mean +/- s.e. of 4 replicates. Sprout length was measured on 25 tubers for each sample. Sugars were measured for 5 tubers for each per replicate. Lines inserted at a sprout length of 3 mm and a reducing sugar concentration (glucose + fructose) of 0.2 % are considered to be the limit of commercial acceptance.
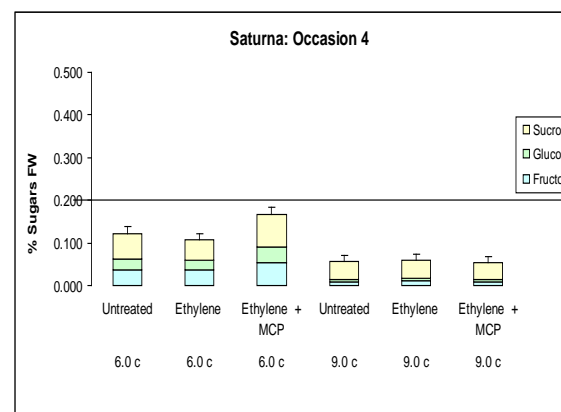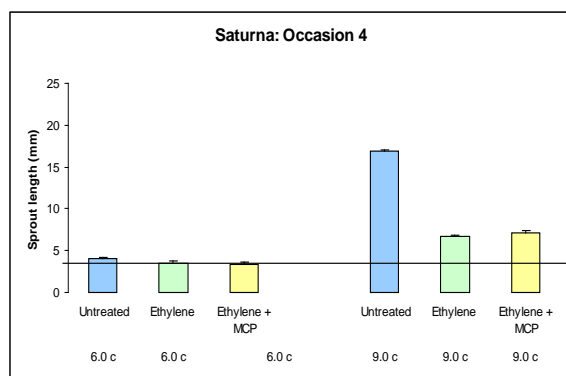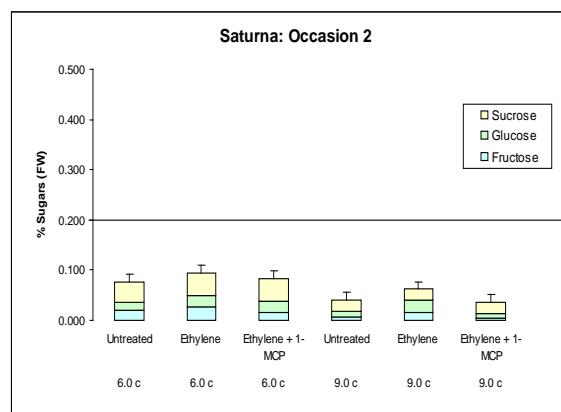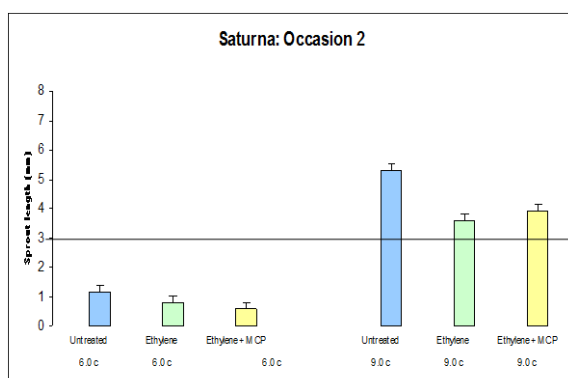
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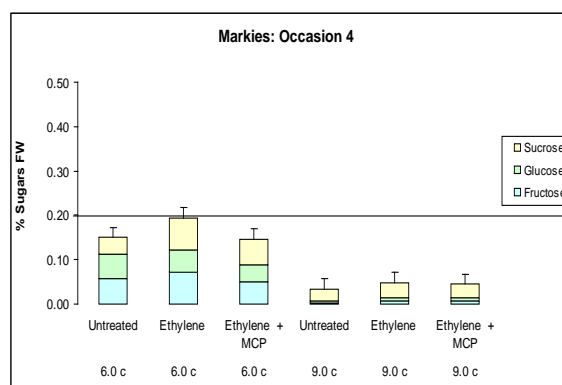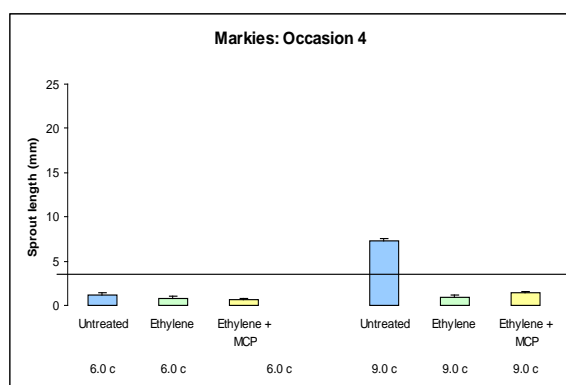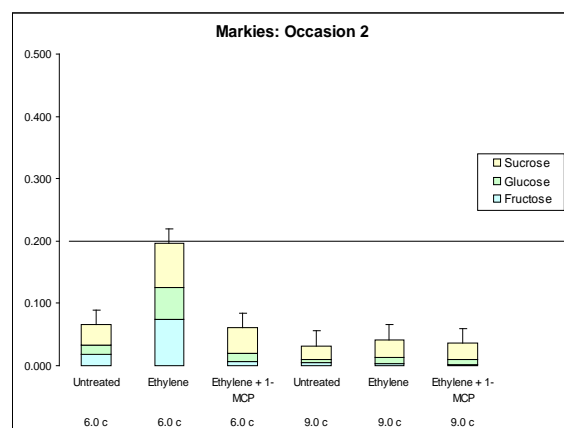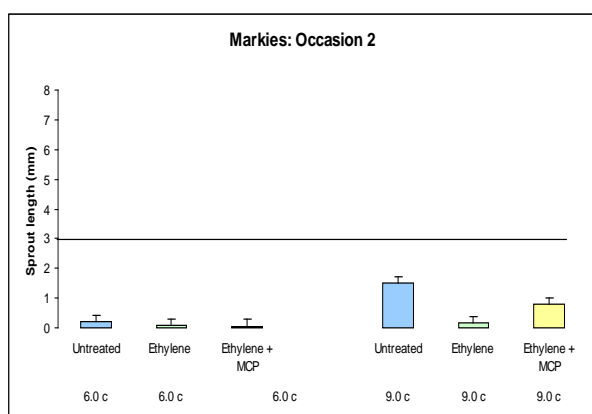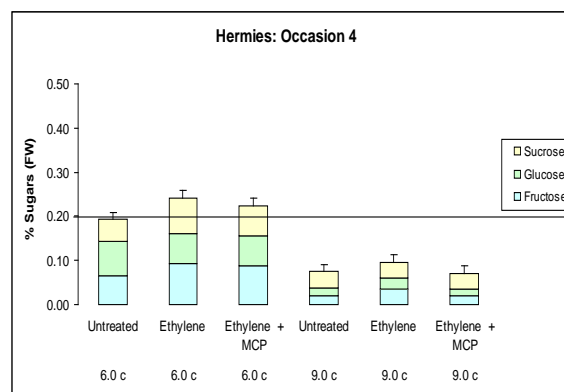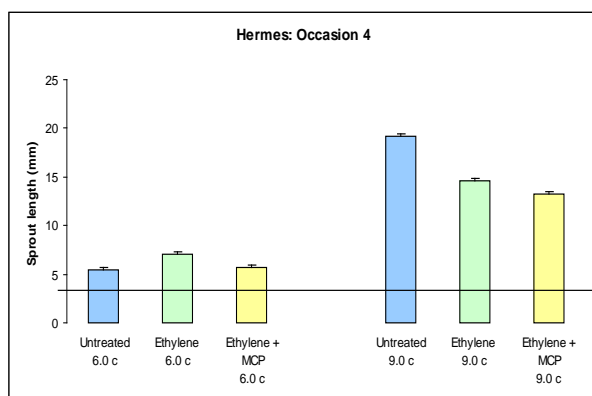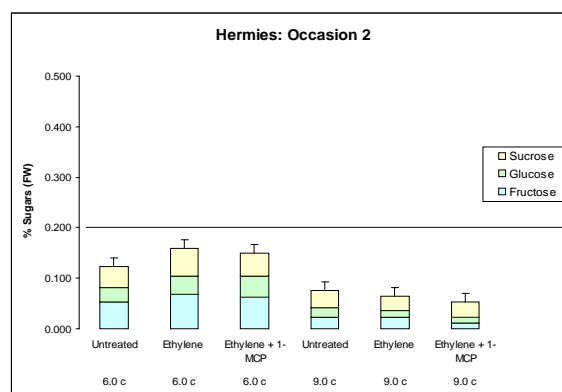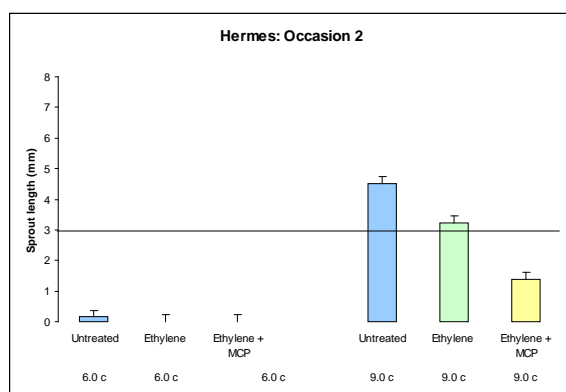
Figure 4.31 cont.
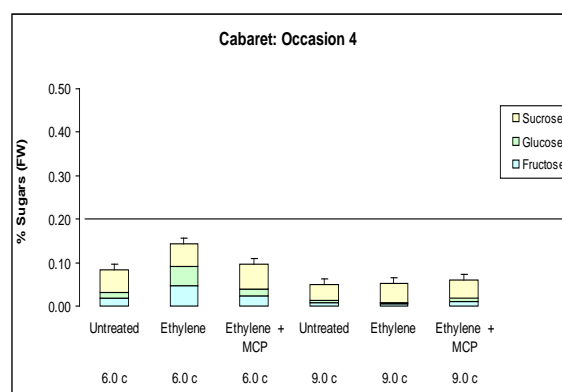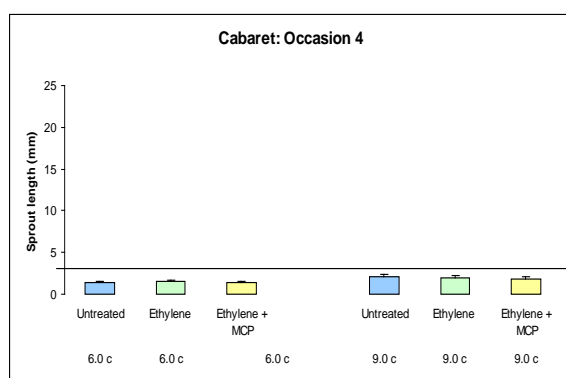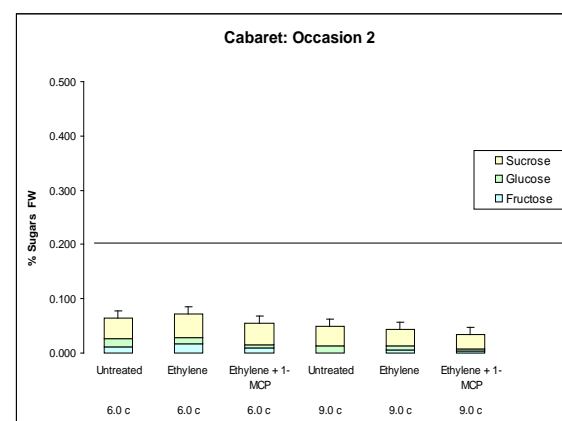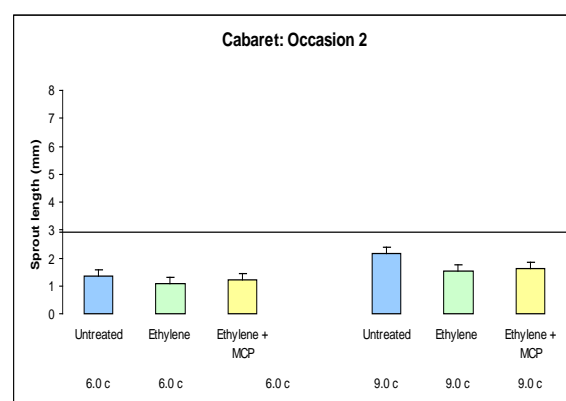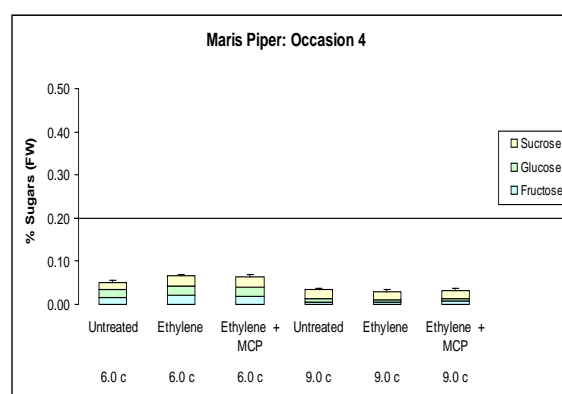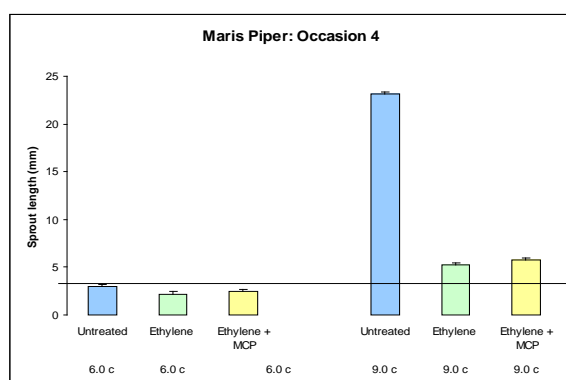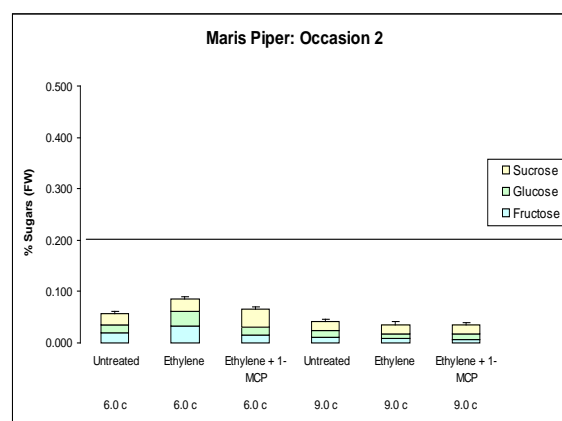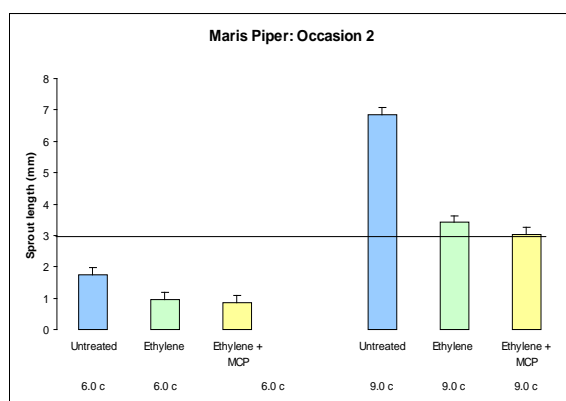
Figure 4.31 cont.

Table 4.4 Reducing sugar concentrations and sprout length for key commercial varieties.

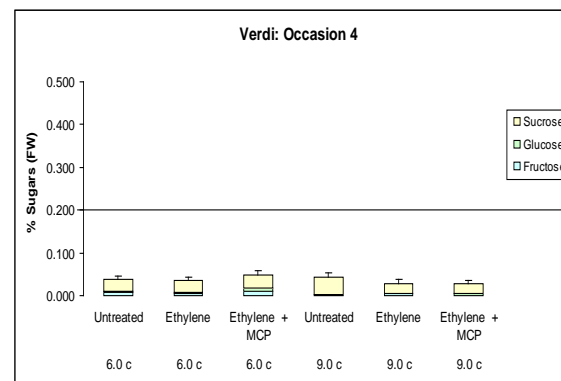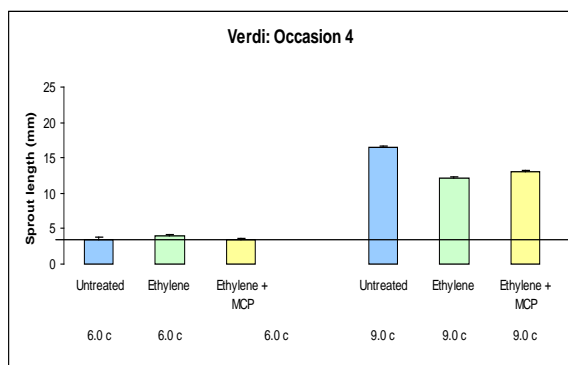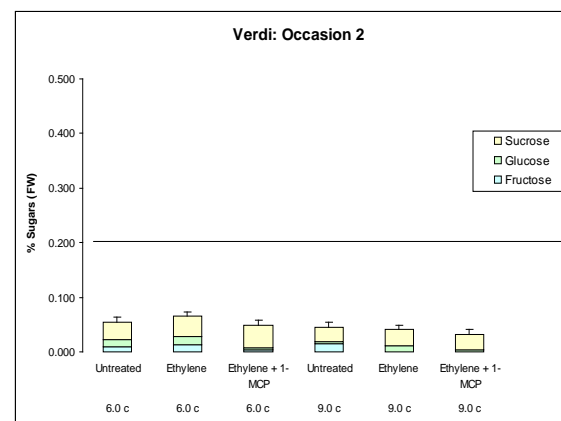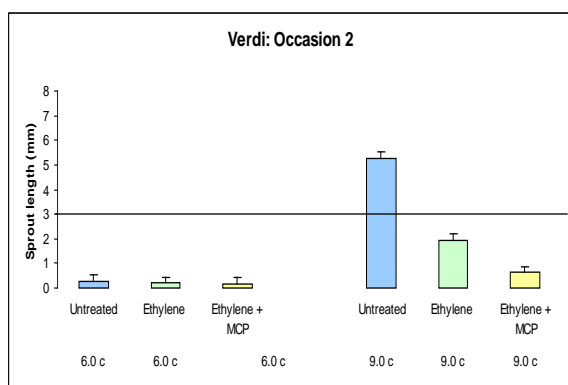|	Temp °C	Treatment	Occasion 2 total reducing sugar % fw	Occasion 2 sprout length (mm)	Occasion 4 total reducing sugar % fw	Occasion 4 sprout length (mm)
			<0.2%	<2 mm	<0.2%	<2 mm
			0.2-0.3%	2-3 mm	0.2-0.3%	2-3 mm
			>0.3%	>3 mm	>0.3%	>3 mm
Cabaret	6	Untreated	0.03	1.36	0.03	1.36
Cabaret	6	Ethylene	0.03	1.09	0.09	1.47
Cabaret	6	Eth + 1-MCP	0.02	1.23	0.04	1.35
Cabaret	9	Untreated	0.01	2.17	0.01	2.11
Cabaret	9	Ethylene	0.01	1.52	0.01	1.94
Cabaret	9	Eth + 1-MCP	0.01	1.60	0.02	1.84
Hermes	6	Untreated	0.08	0.15	0.14	5.45
Hermes	6	Ethylene	0.10	0.00	0.16	7.09
Hermes	6	Eth + 1-MCP	0.10	0.00	0.16	5.68
Hermes	9	Untreated	0.04	4.50	0.04	19.21
Hermes	9	Ethylene	0.04	3.24	0.06	14.63
Hermes	9	Eth + 1-MCP	0.02	1.39	0.04	13.23
Maris Piper	6	Untreated	0.04	1.75	0.03	2.99
Maris Piper	6	Ethylene	0.06	0.95	0.04	2.21
Maris Piper	6	Eth + 1-MCP	0.03	0.86	0.04	2.48
Maris Piper	9	Untreated	0.02	6.84	0.01	23.11
Maris Piper	9	Ethylene	0.02	3.41	0.01	5.26
Maris Piper	9	Eth + 1-MCP	0.02	3.04	0.01	5.79
Markies	6	Untreated	0.03	0.20	0.11	1.21
Markies	6	Ethylene	0.13	0.08	0.12	0.76
Markies	6	Eth + 1-MCP	0.02	0.05	0.09	0.59
Markies	9	Untreated	0.01	1.50	0.01	7.32
Markies	9	Ethylene	0.01	0.15	0.01	0.90
Markies	9	Eth + 1-MCP	0.01	0.78	0.01	1.37
Russet Burbank	6	Untreated	0.16	0.22	0.13	2.37
Russet Burbank	6	Ethylene	0.17	0.01	0.24	1.48
Russet Burbank	6	Eth + 1-MCP	0.14	0.06	0.22	1.43
Russet Burbank	9	Untreated	0.10	2.17	0.11	6.60
Russet Burbank	9	Ethylene	0.11	0.89	0.12	2.29
Russet Burbank	9	Eth + 1-MCP	0.08	0.94	0.11	2.51
Saturna	6	Untreated	0.04	1.16	0.06	4.00
Saturna	6	Eth + 1-MCP	0.04	0.56	0.06	3.36
Saturna	6	Ethylene	0.05	0.78	0.09	3.54
Saturna	9	Untreated	0.02	5.29	0.01	16.85
Saturna	9	Ethylene	0.04	3.59	0.02	6.65
Saturna	9	Eth + 1-MCP	0.01	3.90	0.02	7.19

Sylvana	6	Untreated	0.18	0.75	0.23	1.71
Sylvana	6	Ethylene	0.26	0.09	0.28	0.82
Sylvana	6	Eth + 1-MCP	0.13	0.34	0.33	0.77
Sylvana	9	Untreated	0.08	3.94	0.12	11.77
Sylvana	9	Ethylene	0.11	0.87	0.14	1.48
Sylvana	9	Eth + 1-MCP	0.04	1.54	0.14	1.69
Verdi	6	Untreated	0.02	0.29	0.01	3.51
Verdi	6	Ethylene	0.03	0.20	0.01	4.02
Verdi	6	Eth + 1-MCP	0.01	0.18	0.02	3.39
Verdi	9	Untreated	0.02	5.28	0.00	16.51
Verdi	9	Ethylene	0.01	1.95	0.01	12.15
Verdi	9	Eth + 1-MCP	0.00	0.64	0.00	12.98

Ethylene @ 10 ppm
Ethylene (10 ppm) + 1-MCP = Eth + 1-MCP

4.7.1. Main findings

- Sprouting increased with time and was greater in tubers stored at 9°C than at 6°C
- SmartFresh[TM] has no effect on sprouting behaviour
- Sugar accumulation increased in most varieties with time and was greater in tubers stored at 6°C than at 9°C
- Raising the storage temperature of potatoes from 6 to 9°C mitigates the effect of ethylene-induced accumulation of glucose and fructose seen at lower temperatures.
- Ethylene and SmartFresh[TM] responses in terms of sprout suppression and sugar accumulation in potato are not universal to all varieties tested.
- SmartFresh[TM] helps to ameliorate the ethylene induced sugar accumulation in Sylvana and Markies early in the storage season.
- Verdi was the least responsive variety to ethylene induction of sugar accumulation and sprout suppression whilst Sylvana and Russet Burbank were sensitive to ethylene in-terms of sugar accumulation and sprout suppression. Hermes was relatively unresponsive to ethylene in-terms of sugar accumulation and sprout suppression.

4.8. Large scale trials: effects of spearmint (r-carvone), ethylene and 1-MCP on sprout suppression

Following on from results obtained in year 1 (season 2009), larger scale trials were carried out at SBCSR in season 2010 to assess the interactions between ethylene and 1-MCP and also the effect of spearmint (r-carvone) with and without ethylene.

The longest sprout length of each variety and treatment was assessed after 2, 4 and 6 months of storage. The results are shown in Figure 4.32a for chipping varieties and Figure 4.32b for crisping varieties. Processing quality for crisping varieties, measured as the fry colour and fry defects are shown in Figures 4.33 and 4.35, respectively. Processing quality for chipping varieties is shown in Figure 4.41. Sugar concentrations measured by HPLC are given in Figure 4.34 and 4.37 for comparison.
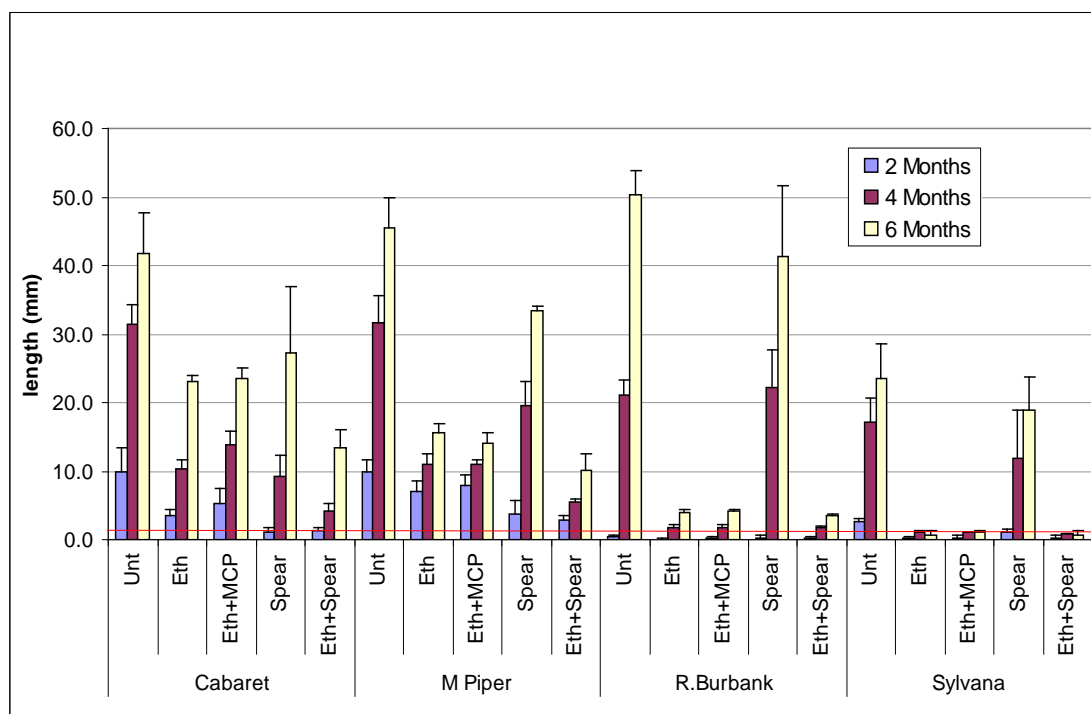
Figure 4.32a length of longest sprout at 2, 4 and 6 months following treatment: chipping varieties
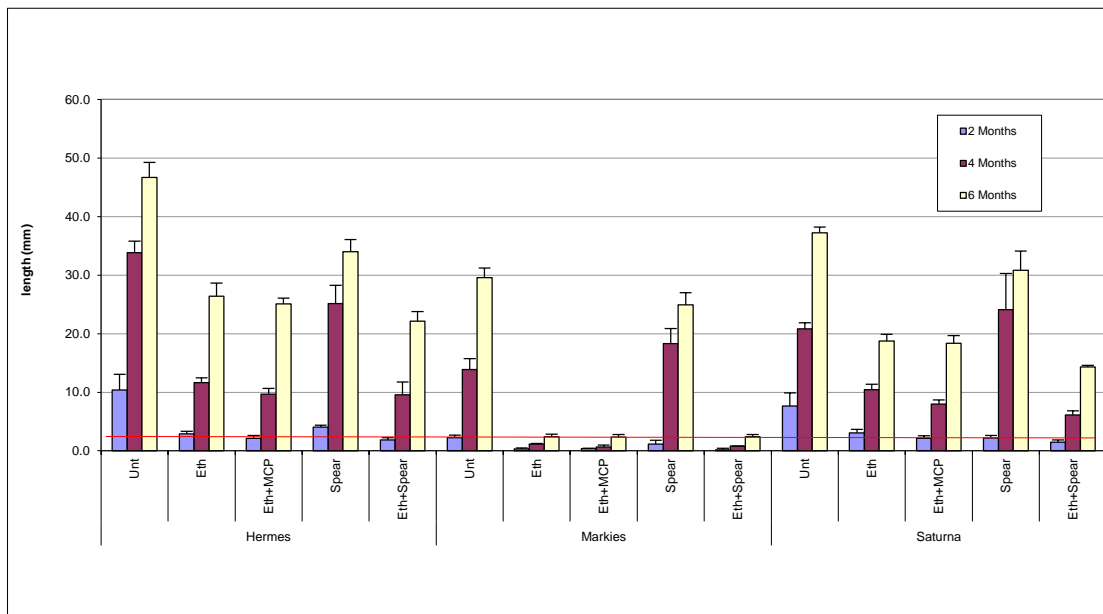
Figure 4.32b length of longest sprout at 2, 4 and 6 months following treatment: crisping varieties
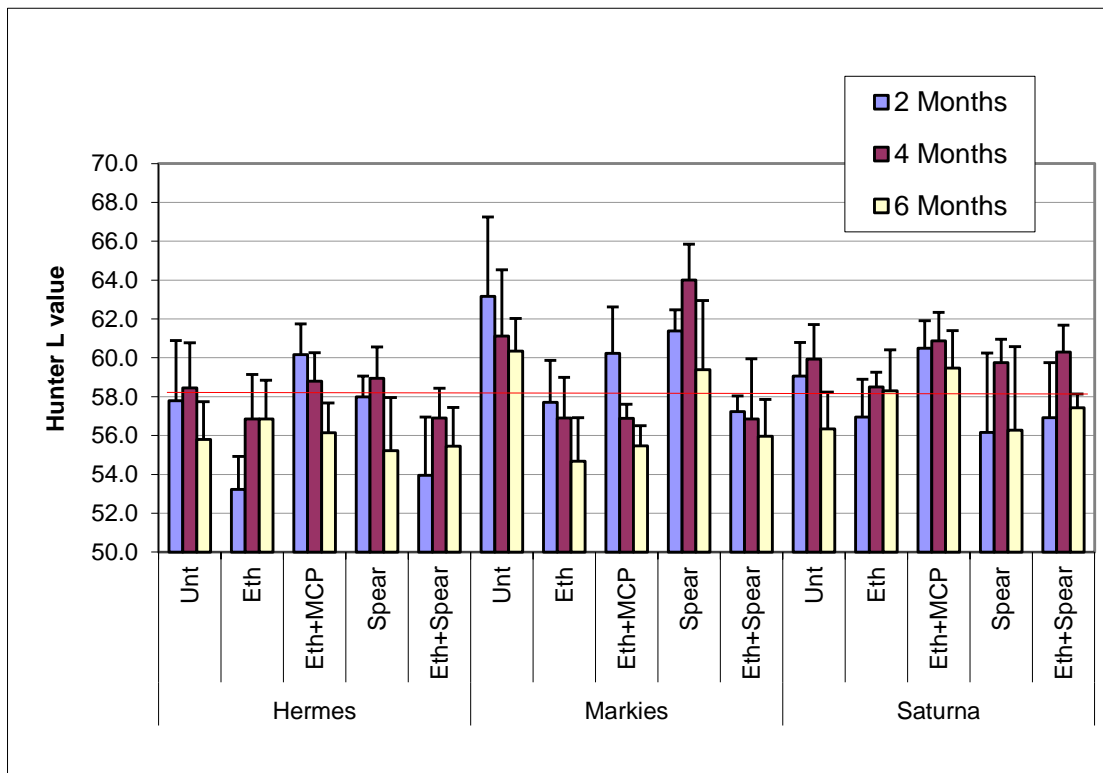
Figure 4.33 Processing quality, fry colour: crisping varieties

The Hunter L value is a standard colour scale for crisp fry colour. Lower values indicate increasing darkness and values of less than 58/59 are considered commercially unsuitable. +/- s.d.
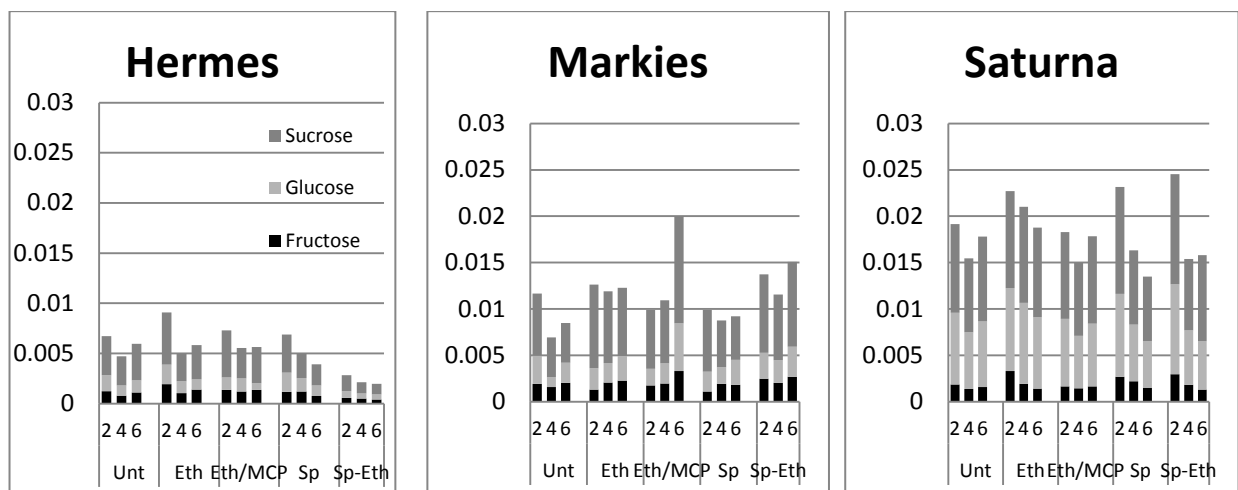
Figure 4.34 Sugar concentrations (Fructose, glucose and sucrose) expressed as % sugars/fresh weight of tubers stored for 2, 4, 6 months and subjected to the range of treatments shown in Figure 4.12. (Full are available in Appendices 2a and 2b).
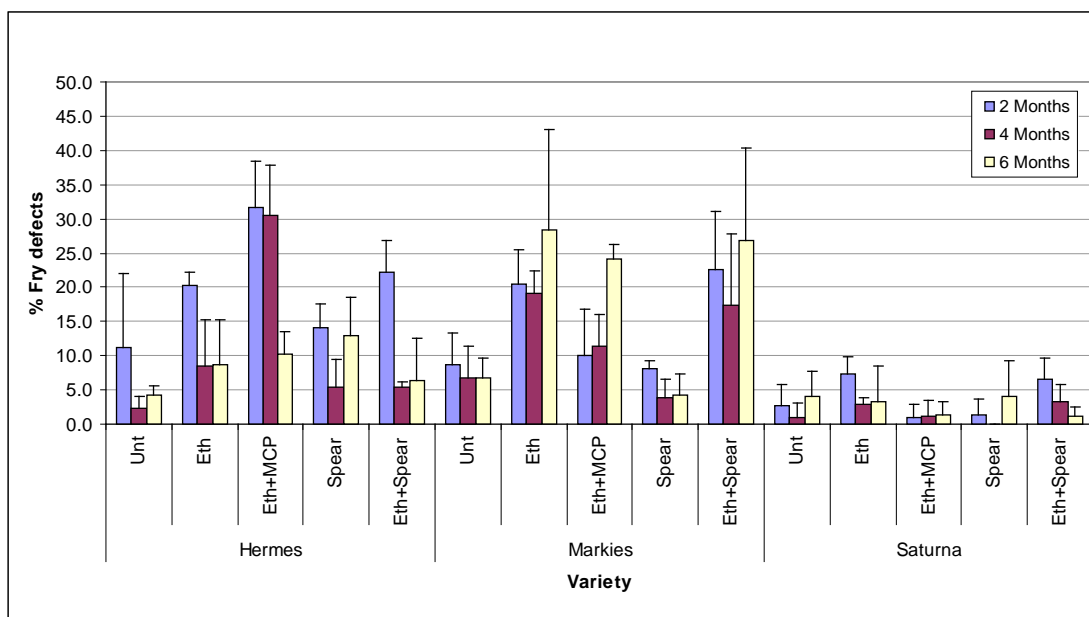
Figure 4.35 Processing quality, crisp fry defects

Defect levels < 10% attract a bonus payment, with increased defect levels increasingly unacceptable for commercial use. +/- s.d.
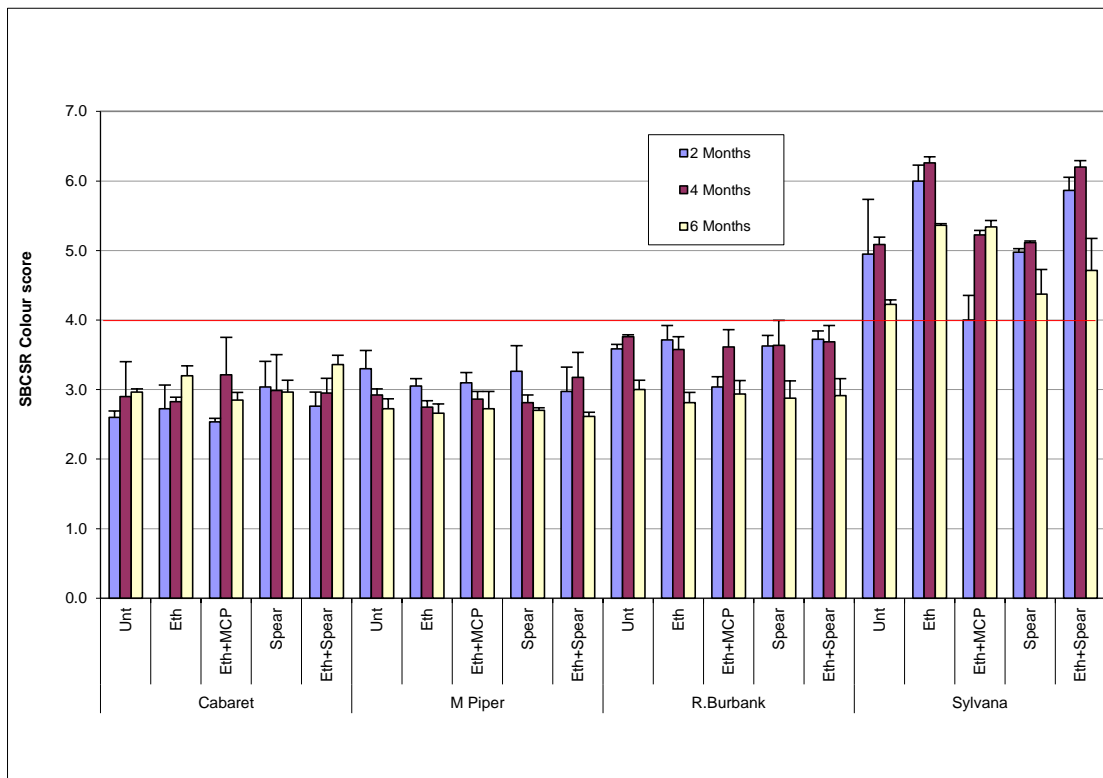
Figure 4.36 Processing quality, fry colour: Chipping varieties

SBSCR values 1-7 correspond to USDA 000, 00, 0, 1, 2, 3 and 4 respectively. SBSCR values greater than 4 (USDA 1) are considered unacceptable. +/- s.d.
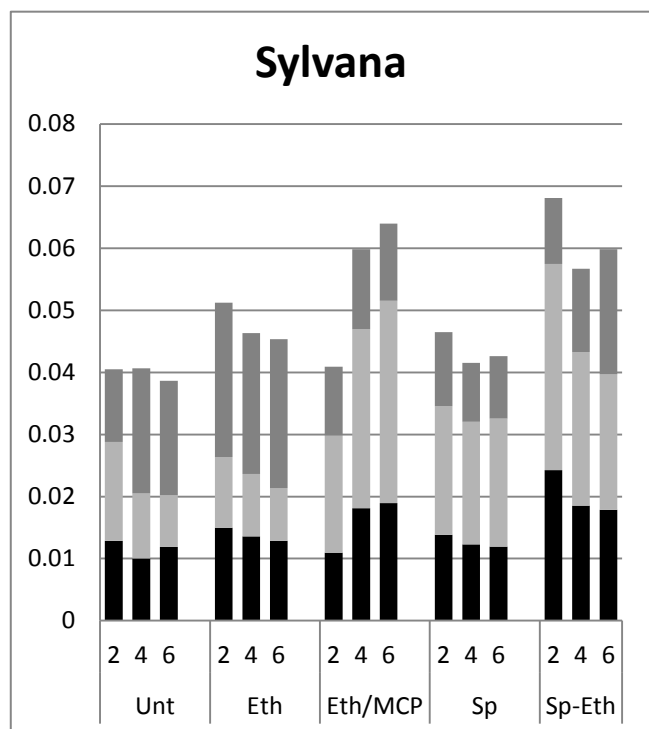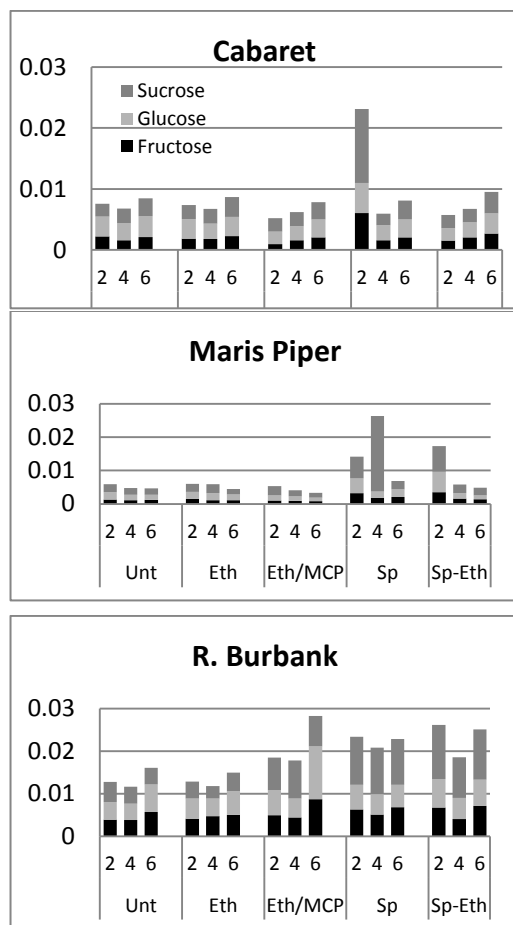
Figure 4.37 Sugar concentrations (Fructose, glucose and sucrose) expressed as % sugars/fresh weight of tubers stored for 2, 4, 6 months and subjected to the range of treatments shown in Figure 4.15. (Full data is not shown, but is available on request from report authors).

Efficacy of treatments in terms of sprout control

For all the varieties, all treatments reduced sprouting compared with the untreated control after two months storage, but not necessarily sufficiently for commercial practice (sprout lengths greater than 2 mm are unacceptable).

Effect of ethylene on sprout growth
This trial was carried out with a gradual ramp of ethylene designed to minimise the effect on respiration and fry colour. The effect of ethylene on sprouting of potatoes was variety dependent with, Markies, Russet Burbank and Sylvana being very responsive (Figure 4.11a and b); at 4 and 6 months ethylene, with or without an additional treatment, reduced sprouting to approximately 10% compared with the control. The control of sprout growth in Sylvana would be considered commercially acceptable for any ethylene treatment up to 6 months and acceptable for Markies and Russet Burbank up to 4 months

Cabaret, Maris Piper, Hermes and Saturna had a generally similar pattern of response in terms of sprouting behaviour in the presence of ethylene (Figures 4.11a and b). For all these varieties, at 4 and 6 months ethylene, with or without an additional treatment, reduced sprouting to approximately 50% compared with the control. With this level of inhibition sprout length was still greater than 2mm, so that this would not be a commercially acceptable treatment.

Effect of spearmint (+/- ethylene) on sprout growth
Spearmint treatment significantly reduced sprouting in all varieties at 2 months compared to untreated controls, and was about as effective as any other treatment at that time point (Figure 4.11a and b). However, this effect declined significantly and at 4 and 6 months control was poor. The manufacturer's recommendation is that repeat applications are made to effectively control sprouting. There was some evidence of a combined effect of spearmint and ethylene as sprouting at 4 and 6 months was less than where either treatment was applied individually.

For Sylvana, Markes and Russet Burbank which are very sensitive to ethylene there is no evidence of an advantage in using spearmint with ethylene. However, for the less ethylene sensitive varieties some advantage could be seen at 2 months.

For Cabaret spearmint or spearmint with ethylene gave commercially acceptable sprout control at two months. For Hermes and Saturna only the combination of spearmint with ethylene gave acceptable sprout control at two months, whereas for Maris Piper neither treatment was sufficiently effective. Sprout growth would not be considered commercially acceptable for any treatment or treatment combination at 4 or 6 months. Nevertheless there was some evidence of a combined effect of spearmint and ethylene as sprouting at 4 and 6 months was less than when either treatment was applied individually.

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Effect of 1-MCP
1-MCP had no effect on sprouting. There was no difference in the length of the longest sprout in the presence of ethylene with or without 1-MCP (Figure 4.11a and b).

Table 4.5 The effect of treatments on sprout length at 2, 4 and 6 months of storage for seven key varieties. Red indicates that the longest sprout length was greater than 2 mm, green shading indicates that the longest sprout length was less than 2 mm.

	Untreated			Ethylene			Ethylene +1-MCP			Spearmint			Spearmint +Ethylene		
Months of storage	2	4	6	2	4	6	2	4	6	2	4	6	2	4	6
Sylvana	Red	Red	Red	Green	Green	Green	Green	Green	Green	Green	Red	Red	Green	Green	Green
Markies	Red	Red	Red	Green	Green	Red	Green	Green	Red	Green	Red	Red	Green	Green	Red
R. Burbank	Green	Red	Red	Green	Green	Red	Green	Green	Red	Green	Red	Red	Green	Green	Red
Hermes	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Green	Red	Red
Cabaret	Red	Red	Red	Red	Red	Red	Red	Red	Red	Green	Red	Red	Green	Red	Red
Saturna	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Green	Red	Red
M. Piper	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red

Table 4.6: The effect of treatments on processing quality at 2, 4 and 6 months of storage for seven key varieties. Tan shading indicates that the processing quality is unacceptable, while white indicates commercially acceptable processing quality.

	Untreated			Ethylene			Ethylene +1-MCP			Spearmint			Spearmint +Ethylene		
Months of storage	2	4	6	2	4	6	2	4	6	2	4	6	2	4	6
Sylvana	Tan	Tan	Tan	Tan	Tan	Tan	Tan	Tan	Tan	Tan	Tan	Tan	Tan	Tan	Tan
Markies				Tan	Tan	Tan		Tan					Tan	Tan	
R. Burbank															
Hermes				Tan	Tan	Tan		Tan					Tan	Tan	
Cabaret															
Saturna				Tan	Tan						Tan	Tan	Tan		Tan
M. Piper															

Effects of treatments in terms of processing quality

The deterioration of processing quality induced by ethylene was independent of the effect on sprouting. This can be seen by the adverse effect of ethylene on processing quality of the chipping varieties Markies and Hermes which were relatively sensitive and insensitive to ethylene, in terms of inhibition of sprouting, respectively.

The chip fry colour was acceptable for Cabaret, Maris Piper and Russet Burbank, for all treatments and sampling occasions (Figure 4.15). There was no apparent general effect of any treatment on fry colour. Sylvana had been exposed to cool storage conditions prior to intake with the consequence of poor processing quality for all treatments and assessment occasions. Nevertheless the fry colour can be seen to be adversely affected by ethylene, with or without spearmint, an effect mitigated by 1-MCP but only at 2 months.

The crisp fry colour for Hermes, Markies and Saturna was adversely affected by ethylene, with or without spearmint (Figure 4.12). The adverse effect of ethylene on processing quality was mitigated by 1-MCP but only at 2 months. Spearmint had little or no apparent effect on processing quality.

The short-term beneficial effect of 1-MCP may be because as ethylene receptors are resynthesised, 1-MCP activity diminished below an active concentration and/or that later during storage, respiration, even in the presence of ethylene, gradually reached a baseline against which the beneficial effect of 1-MCP is too small to be measured.

Effect of treatments on sugar concentrations.

- Markies, Maris Piper and Russet Burbank maintained low concentrations of glucose and fructose in the presence of ethylene over 6 months of storage.
- Saturna contained moderate amounts of fructose, glucose and sucrose. Ethylene increased sucrose content but glucose and fructose content was unchanged.
- Sylvana contained higher amounts of fructose, glucose and sucrose. Ethylene increased sucrose content but fructose and glucose remained stable. Fructose and glucose content increased in the presence of 1-MCP/ethylene after 4 and 6 month storage.
- Spearmint/ethylene treatments increased fructose and glucose content initially in Maris Piper and Sylvana but concentrations dropped during storage
- Spearmint increased sucrose content in Russet Burbank while in Saturna spearmint caused an initial increase in sucrose which declined during storage

4.8.1. Main findings

- For all the varieties (Sylvana, Markies, Russet Burbank, Hermes, Cabaret, Saturna and Maris Piper) all treatments (ethylene, Ethylene+ 1-MCP, Spearmint, Spearmint + ethylene) reduced sprouting compared with the untreated control after two months storage, but not necessarily sufficiently for commercial practice (sprout lengths greater than 2 mm are unacceptable).
- The effectiveness of ethylene treatment was variety dependent; Markies, Russet Burbank and Sylvana being very responsive, with Cabaret, Maris Piper, Hermes and Saturna less responsive.
- Spearmint treatment was about as effective as any other treatment at 2 months but this effect declined significantly at 4 and 6 months. For varieties that are unresponsive to ethylene the addition of spearmint improved sprout control.
- Ethylene has a detrimental effect on processing quality. This is independent of the effect on sprouting; demonstrated by the adverse effect of ethylene on processing quality of the chipping varieties Markies and Hermes which were relatively sensitive and insensitive to ethylene, in terms of inhibition of sprouting, respectively.
- The adverse effect of ethylene on processing quality was mitigated by 1-MCP but only at 2 months. Spearmint had little or no apparent effect on processing quality.

4.9.   Effect of repeat 1-MCP treatments on quality

In season 2011 trials were carried out to determine whether repeat applications of 1-MCP could be used to improve processing quality during long-term storage. The longest sprout length of each variety and treatment was measured at 2, 4 and 6 months of storage with the exception of untreated samples at 6 months which, because of clearly extensive sprouting, were photographed. These photographs are archived at Sutton Bridge Crop Storage Research.

The results of sprout length measurement and fry colour of each variety are shown in Figure 4.38 – 4.43 a and b. For these figures a sprout length of 2mm was generally considered commercially acceptable. For processing as French fries, SBSCR values greater than 4, which corresponds to USDA 1, were considered unacceptable. For processing as crisps Hunter L score less than 58 were considered commercially acceptable. Error bars are +/- standard deviation.

Cabaret

Cabaret sprouting

Figure 4.38a Cabaret; length of longest sprout (no data for SO3 Untreated 1-MCPx1 and 1-MCPx2)

Cabaret fry colour

Figure 4.38b Cabaret; Fry colour(no data for SO3 Untreated 1-MCPx1 and 1-MCPx2)

No treatment provides commercially acceptable levels of sprouting at any storage duration. For all treatments fry colour is acceptable until storage for 4 months after which it deteriorates. There is no difference between untreated and ethylene treatment at 2 and 4 month assessment periods.

At 2 months 1-MCP slightly improves fry colour even in the absence of ethylene (Figure 4.38b). At 4 months the lowest fry colour values have 1-MCP as a treatment whereas at 6 months the highest values are found where 1-MCP as a treatment.

Maris Piper

Maris Piper sprouting
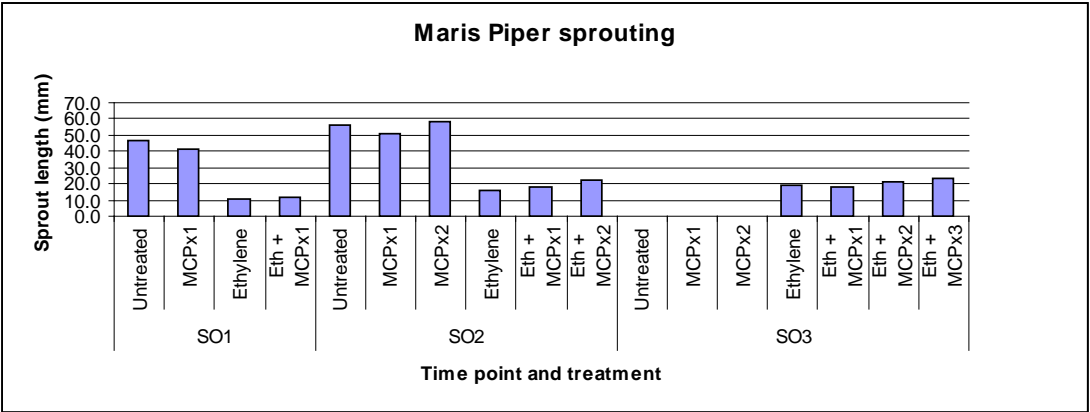
Figure 4.39a Maris Piper; length of longest sprout (no data for SO3 Untreated 1-MCPx1 and 1-MCPx2)

Maris Piper fry colour
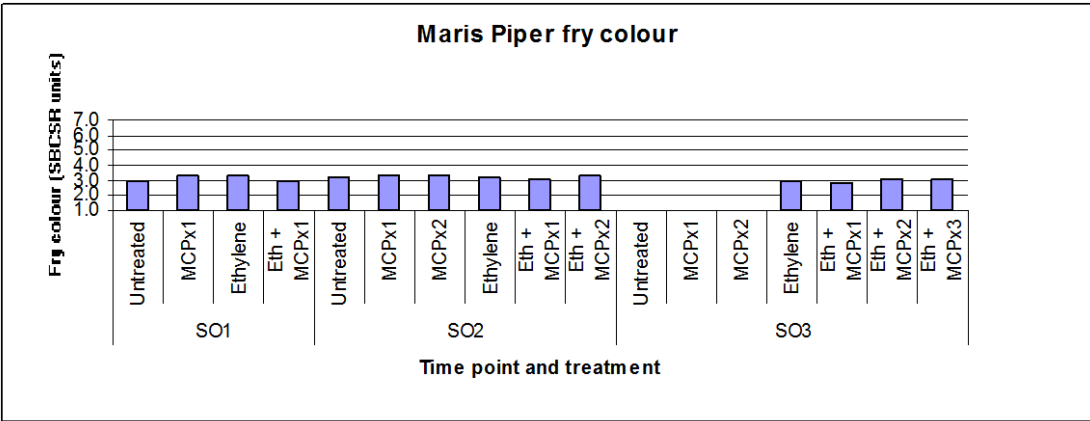
Figure 4.39b Maris Piper; Fry colour (no data for SO3 Untreated 1-MCPx1 and 1-MCPx2)

No treatment provides commercially acceptable levels of sprouting at any storage duration Fry colour is acceptable for all treatments until storage for the duration of storage months. There is no difference between untreated and ethylene treatment at 2 and 4 month assessment periods.

Russet Burbank
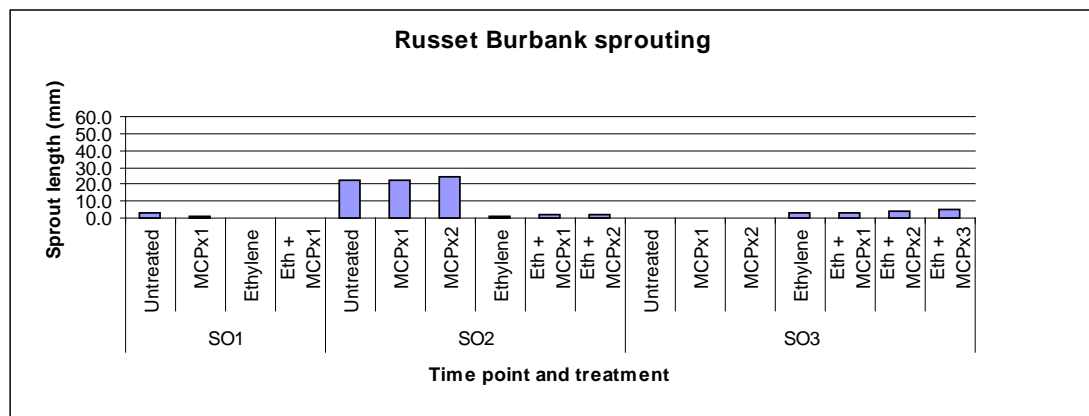
Figure 4.40a Russet Burbank; length of longest sprout (no data for SO3 Untreated 1-MCPx1 and 1-MCPx2)
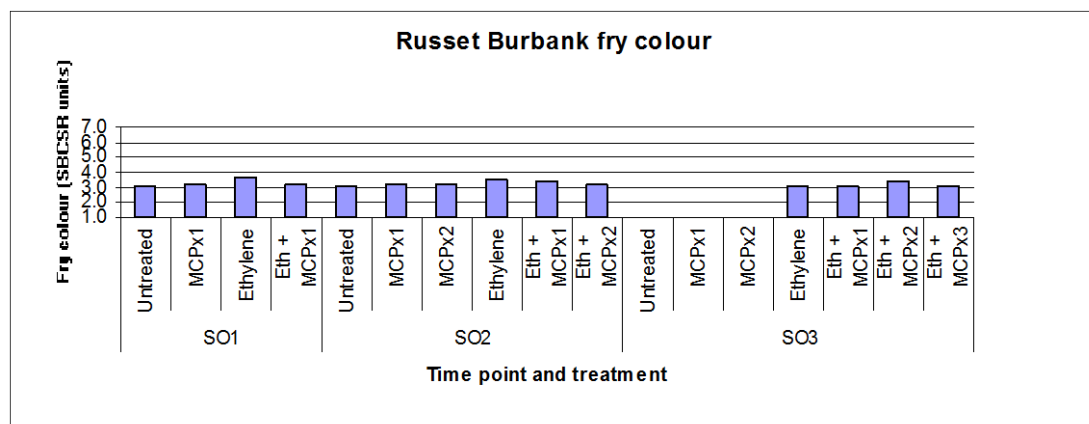
Figure 4.40b Russet Burbank; Fry colour (no data for SO3 Untreated 1-MCPx1 and 1-MCPx2)

Ethylene provides commercially acceptable levels of sprouting at 2 and 4 months of storage and good control of sprouting at six months albeit slightly longer sprouts than would be commercially unacceptable. Fry colour is acceptable with all treatments for the duration of the trial and the highest fry colour values are with ethylene treatment, perhaps reduced when in combination with 1-MCP treatment, however, there was insufficient data to a verify this statistically.

Sylvana
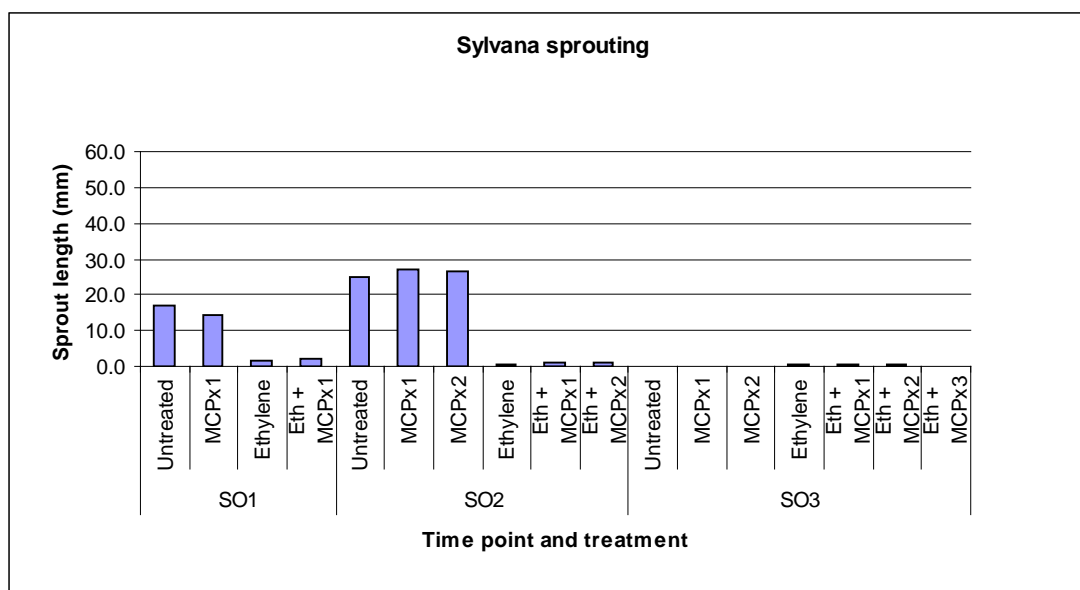
Figure 4.41a Sylvana; length of longest sprout (no data for SO3 Untreated 1-MCPx1 and 1-MCPx2)
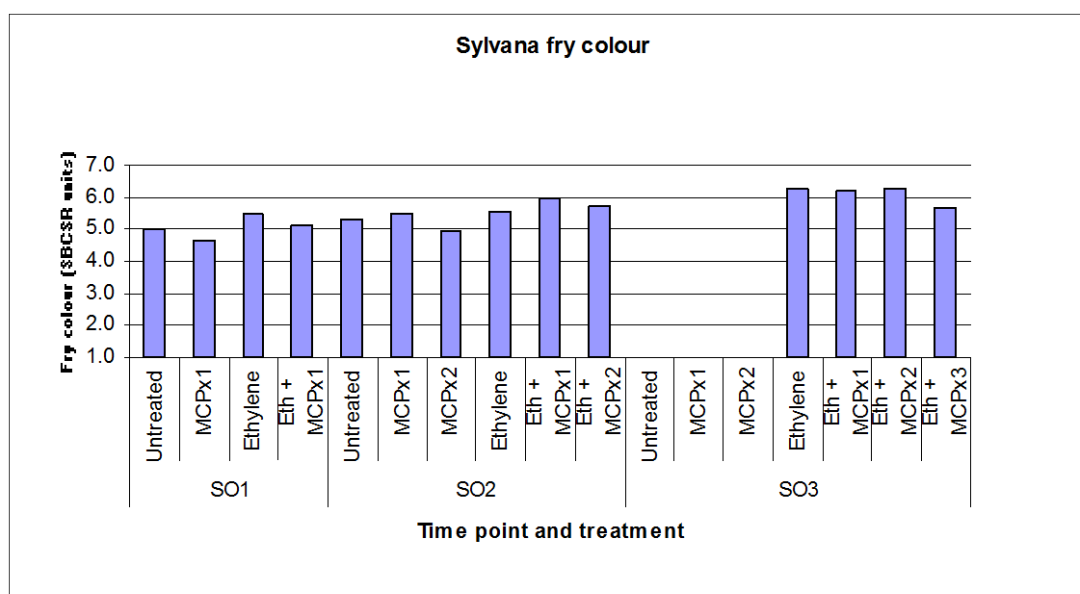
Figure 4.41b Sylvana; Fry colour (no data for SO3 Untreated 1-MCPx1 and 1-MCPx2)

Ethylene provides commercially acceptable levels of sprouting at 2, 4 and 6 months of storage. Fry colour is unacceptable with all treatments for the duration of the trial. The highest fry colour values are with ethylene treatment, perhaps reduced when in combination with 1-MCP treatment after 2 months storage.

Hermes
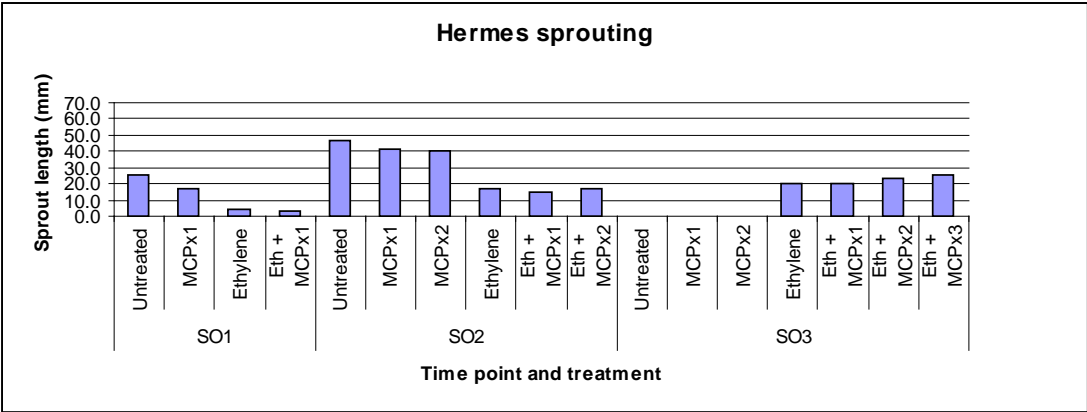
Figure 4.42a Hermes; length of longest sprout (no data for SO3 Untreated 1-MCPx1 and 1-MCPx2)
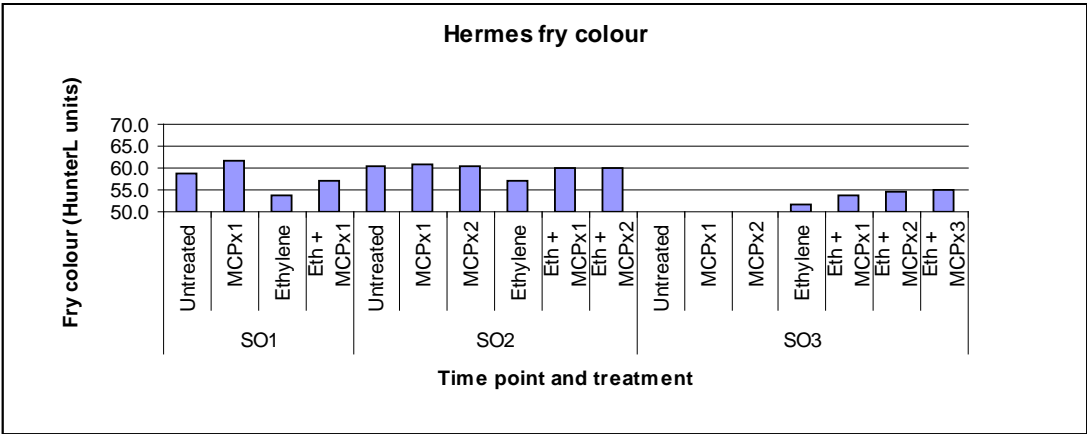
Figure 4.42b Hermes; Fry colour (no data for SO3 Untreated 1-MCPx1 and 1-MCPx2)

No treatment provides commercially acceptable levels of sprouting at any storage duration. Ethylene causes a commercially unacceptable darkening of fry colour, an effect mitigated by 1-MCP at 2 and 4 months storage. No treatment provides commercially acceptable colour after this storage duration. At 2 months 1-MCP appears to improve fry colour even in the absence of ethylene (Figure 4.42b). At 4 and 6 months the best fry colour values include 1-MCP as a treatment.
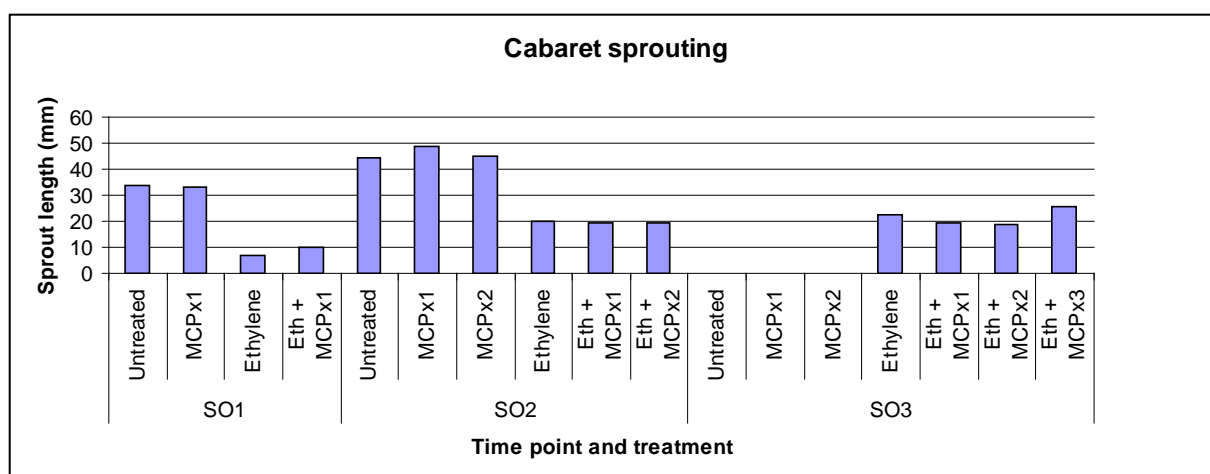
Saturna
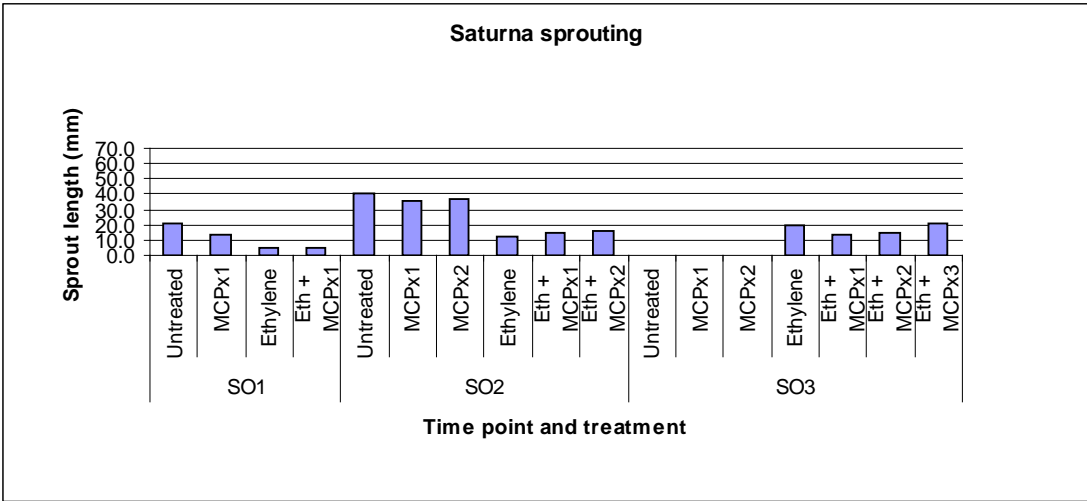
Figure 4.43a Saturna; length of longest sprout (no data for SO3 Untreated 1-MCPx1 and 1-MCPx2)
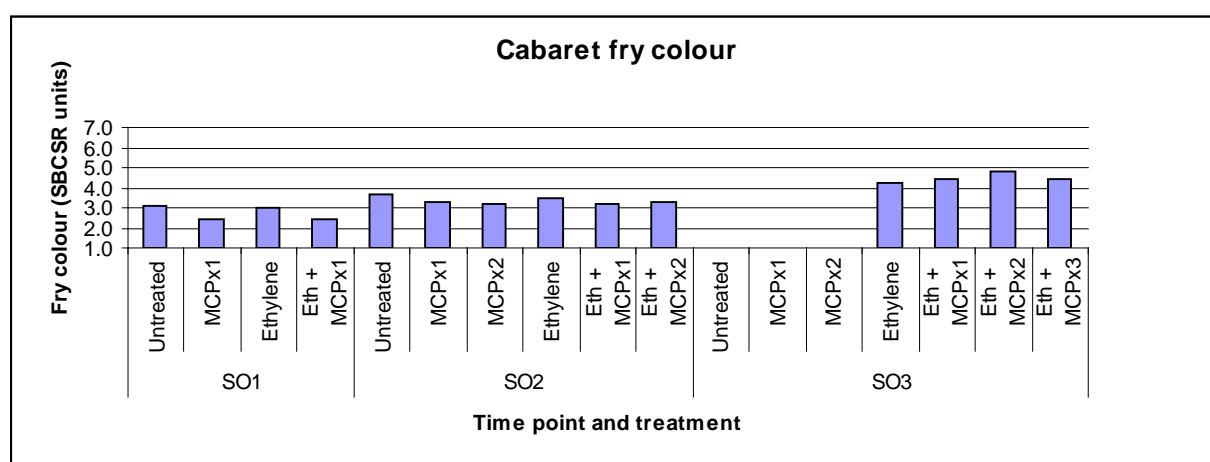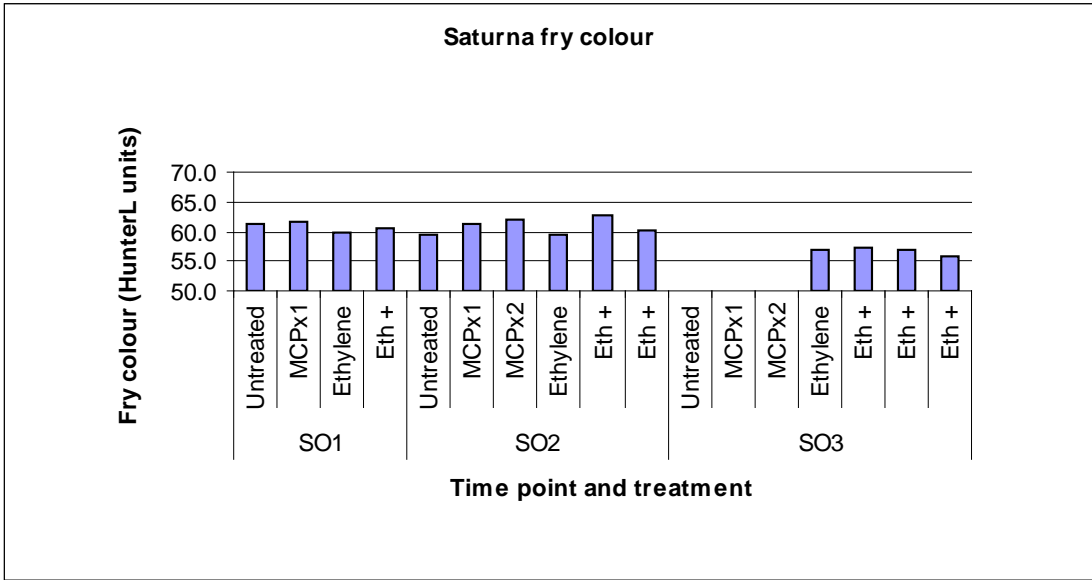

Figure 4.43b Saturna; Fry colour (no data for SO3 Untreated 1-MCPx1 and 1-MCPx2)

No treatment provides commercially acceptable levels of sprouting at any storage duration. Fry colour is acceptable with all treatments at 2 and 4 months storage. No treatment provides commercially acceptable fry colour after this storage duration. At 2 and 4 months the best fry colour values are found in samples with 1-MCP as a treatment and the worst whit ethylene as sole treatment.

Varietal response to ethylene treatments, sprouting

As in previous years of the trial the effect of ethylene on sprouting of potatoes is variety dependent. For example sprouting was commercially acceptable in cv. Sylvana treated with ethylene for the duration of the trial and for cv Russet Burbank up to four months whereas no treatment provided commercially acceptable at any time point for other varieties (Table 4.7).

Ethylene inhibits sprout growth in all varieties (Table 4.7) and there is little difference between the varieties in the response at 2 months to ethylene with % sprouting inhibited by 75-90%. However, a difference becomes apparent after 4 months treatment with ethylene exerting significant control on sprouting in Russet Burbank and Sylvana compared to the other varieties in which the effect of ethylene has somewhat weakened. These results confirm the findings of the second year trial in which ethylene modestly reduced sprouting of Cabaret, Hermes, Maris Piper and Saturna at 4 months whereas it reduced sprouting of Russet Burbank and Sylvana to approximately 10% of untreated control at the same time point.

Table 4.7 Percent of sprouting in ethylene treatment compared to untreated tubers

	AVERAGE SPROUT LENGTH UNDER ETHYLENE AT 2 MONTHS (MM)	% OF SPROUTING UNDER ETHYLENE AT 2 MONTHS	AVERAGE SPROUT LENGTH UNDER ETHYLENE AT 4 MONTHS (MM)	% OF SPROUTING UNDER ETHYLENE AT 4 MONTHS
CABARET	7.1	20.9	19.8	44.5
HERMES	3.7	14.4	16.6	35.5
MARIS PIPER	10.9	23.3	15.5	27.5
RUSSET BURBANK	0.5	17.8	1.2	5.5
SATURNA	4.6	22.1	12.2	30.5
SYLVANA	1.7	10.0	0.7	2.8

Varietal response to treatments, processing quality

This trial was carried out with a gradual ramp of ethylene designed to minimise the effect on respiration and fry colour. The chip fry colour was acceptable for Maris Piper and Russet Burbank, for all treatments and sampling occasions. There was no apparent general effect of any treatment on fry colour. Sylvana had been exposed to cool storage conditions prior to intake with the consequences of poor processing quality for all treatments and assessment occasions. Nevertheless the fry colour can be seen to be adversely affected by ethylene. This effect was mitigated by 1-MCP at 2 months only.

The chip fry colour for Cabaret, Hermes, and Saturna was adversely affected by ethylene, an effect mitigated by 1-MCP at 2 months only. For all the fry colour was unacceptable after 4 months storage.

1-MCP
There is no difference in the length of the longest sprout in the presence of ethylene with or without 1-MCP. 1-MCP mitigates the deterioration of effect of processing quality induced by ethylene, with values for ethylene with 1-MCP better than with ethylene alone (Table 4.8). However, this reduction is apparent only at 2 months.

Table 4.8 Mean scores for variety sprout length and fry colour quality

		Cabaret		Maris Piper		Russet Burbank		Sylvana		Hermes		Saturna	
		sprout length (mm)	Fry colour (SBCSR units)	sprout length (mm)	Fry colour (SBCSR units)	sprout length (mm)	Fry colour (SBCSR units)	sprout length (mm)	Fry colour (SBCSR units)	sprout length (mm)	Fry colour (Hunter L score)	sprout length (mm)	Fry colour (Hunter L score)
SO1	Untreated	34.0	3.1	47.1	3.0	2.8	3.1	16.9	5.0	25.9	58.6	20.8	61.3
	1-MCPx1	33.1	2.5	41.1	3.3	1.3	3.2	14.6	4.7	17.2	61.7	13.6	61.6
	Ethylene	7.1	3.0	10.9	3.3	0.5	3.7	1.7	5.5	3.7	53.9	4.6	59.9
	Eth + 1-MCPx1	9.7	2.4	11.2	3.0	0.4	3.2	2.1	5.1	3.6	57.1	4.4	60.4
SO2	Untreated	44.5	3.6	56.3	3.2	22.0	3.1	24.9	5.4	46.8	60.5	39.9	59.5
	1-MCPx1	48.8	3.3	50.9	3.3	22.3	3.3	26.9	5.5	40.8	60.9	35.5	61.2
	1-MCPx2	45.3	3.2	58.2	3.3	24.0	3.2	26.4	5.0	40.6	60.6	36.3	62.1
	Ethylene	19.8	3.4	15.5	3.2	1.2	3.5	0.7	5.6	16.6	57.1	12.2	59.4
	Eth + 1-MCPx1	19.7	3.2	18.2	3.1	1.5	3.5	0.9	6.0	14.3	59.9	14.2	62.9
	Eth + 1-MCPx2	19.3	3.3	22.6	3.3	2.5	3.2	1.0	5.8	16.6	60.1	16.5	60.4
SO3	Untreated												
	1-MCPx1												
	1-MCPx2												
	Ethylene	22.3	4.2	18.8	2.9	2.7	3.1	0.3	6.3	20.6	51.6	19.2	57.0
	Eth + 1-MCPx1	19.3	4.4	18.2	2.9	3.4	3.1	0.3	6.2	20.4	53.8	13.3	57.4
	Eth + 1-MCPx2	18.9	4.9	21.0	3.1	4.4	3.4	0.4	6.3	23.8	54.6	14.4	57.0
	Eth + 1-MCPx3	25.4	4.5	23.3	3.0	4.8	3.1	0.2	5.7	25.9	55.2	20.3	56.0

Highlighted value are considered commercially unacceptable

4.9.1. Main findings

- The effect of ethylene on sprouting of potatoes is variety dependent and consistent with observations of previous years.
- 1-MCP mitigates the deterioration of effect of processing quality induced by ethylene, with values for ethylene with 1-MCP better than with ethylene alone. However, this reduction is apparent only at 2 months even with repeat application of 1-MCP.

5. DISCUSSION

5.1. Stability of sprout characteristics and ethylene effects

The GENPOP1 population was selected for study within this project because it was well characterized genotypically, but also due to the differences in sprout growth response to ethylene between the parents (12601 ab1 and Stirling) that had been observed prior to the project initiation. During the course of the project development and project trials, these two lines were characterized over four seasons. The results underline that although there are common responses across seasons, with Stirling always exhibiting greater sensitivity to ethylene, the magnitude of the response varies very significantly between seasons; differences were greater in seasons 2008 and 2010 than in seasons 2009 and 2011. Similarly phenotyping of the complete GENPOP1 population indicated that the range in ethylene response was greater in season 2010 than in season 2011, with lines generally showing much stronger response to ethylene in terms of sprout growth inhibition in the latter season. In season 2010 the inhibitory effect of ethylene on sprout growth was so weak in some lines that it was more than counteracted by the ethylene stimulation of dormancy break, so that the overall effect was for ethylene to cause an increase in sprout length.

The behaviour of the parents and progeny across seasons indicates a strong environmental contribution to ethylene response. An understanding of the environmental contribution could potentially be as useful as understanding the genetic contribution by providing a means of manipulating varietal behaviour for storability. It would be very useful to obtain information from commercial partners on their observations of seasonal differences in ethylene response, and to relate this to season characteristics. It would be particularly interesting to find out where seasonal differences are common across varieties in the UK, which would indicate a climatic component, and where it is possible to identify the effects of growing practices, planting and harvesting dates.

5.2. Using the 12601 ab1 x Stirling cross to identify markers for ethylene sensitivity of sprout growth through QTL analysis

QTL methodologies can be used as a powerful approach to understanding the genetic architecture of complex plant traits. This can provide information concerning the number of genes that strongly influence the trait of interest, and usually identifies the chromosomal location of these genes. In this project an aim was to conduct a genome-wide analysis for "response to ethylene" QTL.

During the course of this project a number of QTLs have been identified for sprouting in air and in ethylene. None of these account for more than 9% variance, and most account for less than 7%. This is lower than the variance accounted for by QTLs identified for other potato characteristics such as resistance to pathogens including nematodes (PCN), viruses (Potato virus Y) and fungi (such as *Phytophthora infestans*). This is probably an indication that the trait being considered is very complex; controlled by a large number of genes, which is not unexpected especially given that ethylene affects dormancy break as well as sprout growth. It

is also consistent with the environmental contribution as discussed in 5.1. This would also explain lack of consistency in QTL identification across seasons.

With low levels of variance accounted for and inconsistency across seasons, markers for the QTLs identified are unlikely to be of direct practical use within breeding. In practice the value of this analysis will be to combine with other information for example from microarray analysis in order to identify genes of interest, as discussion in the following section.

5.3. Microarray analyses and identification of target genes

Microarray analyses were carried out both for the parents (12601 ab1 and Stirling) and samples from the GENPOP1 population. Microarray analysis of 12601 ab1 and Stirling was carried out in season 2009 to examine the molecular basis of their different sensitivity to ethylene. When genes differentially expressed in the presence/absence of ethylene were considered, differential response between the two varieties was greatest at the start of storage and the two varieties showed no differentiation by week 17. This suggests that examination of the genes differentially expressed would provide useful information on the genetic basis of their differential response to ethylene. Genelists were produced for the genes in terms of their different expression by treatments and by variety. The most useful of these is likely to be that in appendix 5 which lists the genes which showed significant differential expression between air and ethylene treatments between genotypes.

Recent advances have demonstrated that using a genetical genomics approach is a powerful means to re-enforce QTL analysis, and in some cases provides a rapid way of developing gene markers closely associated with the target trait. Using this approach, for season 2010 RNA bulks from tuber bud samples from four sets of clones with similar responses to ethylene were constructed (1: No significant effect of ethylene on sprouting. 2: Reduction in sprout length on ethylene treatment. 3: Increase in sprout length on ethylene treatment. 4: Reduction in sprout length on ethylene treatment and little sprout growth.) and patterns of gene expression were measured for each bulk by microarray analysis. In season 2011 this was repeated for comparison using material from one set of clones (4). Variation of gene expression within these bulks may be due either to polymorphisms located near to or within the gene (cis-eQTL) or indirectly from a distant location on the genome (trans-eQTL) (Kloosterman *et al.*, 2008). Differentially expressed genes from bulks with contrasting ethylene traits were genetically mapped in order to re-enforce the QTL analysis, and provide a rapid means of developing gene markers closely associated with ethylene responsiveness. In some cases there was coincidence of a high number of differentially genes and QTL (for example the QTL at 55MBp on linkage group 6) providing eQTL support for that QTL.

Although gene expression patterns did vary by season due to environmental effect a selection of 203 genes were identified that were expressed consistently in both season 2010 and season 2011 within the clone set 4. The expression of these genes was characterised across all four clone pools. For example 48 genes were identified for which expression increased with sprout length in presence of ethylene.

The approach described above illustrates the point that although QTL information from this project does not allow us to identify the target genes directly, information on gene function and expression patterns will allow a manageable number of key genes to be identified for further study.

5.4. Optimising ethylene concentration for sprout control

Trials undertaken on three varieties; Maris Piper, Saturna and Russet Burbank indicated that the maximum inhibitory effect of ethylene on sprout growth during storage at 9°C required concentrations greater than 1 ppm, with no clear difference between 10 and 25 ppm. The trials did not indicate a clear difference in ethylene stimulation of sugar accumulation nor of acrylamide production across the concentrations tested. These two findings suggest that the industry standard of 10 ppm is appropriate.

5.5. Small-scale trials to assess synergistic effects of sprout suppressant treatments

During this project several chemicals have been tested for their synergistic behaviour with ethylene treatment to determine whether a combined treatment could provide a more reliable strategy for sprout control than ethylene alone. Following small-scale trials, larger scale trials were conducted on the use of R-carvone and 1-methyl cylopropene (1-MCP), to improve sprout control and processing quality, respectively (see section 5.6), while 1,4 dimethylnaphthalene (1,4 DMN) and methyl jasmonate have been identified has having most potential in the medium-term, and several other PGRs are identified as of longer-term interest.

1,4 DMN

The emphasis of the synergy trials in seasons 2010 and 2011 was to evaluate additional treatments that had potential for near-market commercial exploitation. 1,4, DMN marketed under 1,4SIGHT® is currently undergoing registration for use in Europe. A single application of 1,4 DMN was made to tubers that were either stored in air or continuous ethylene.

In some varieties (Russet Burbank, Hermes, Maris Piper) application of 1,4 DMN to tubers stored in air resulted in reduced sprout lengths over the first 2 months of storage compared to tubers stored in air alone. However, this effect was not observed in Cabaret. After longer periods of storage there was no detectable effect of the single application of 1,4 DMN and sprout lengths were similar to those of tubers stored in air.

The combination of 1,4 DMN and continuous ethylene produced mixed results. In Maris Piper (2010), ethylene's ability to break dormancy and overcome the effect of 1,4 DMN on dormancy extension was apparent after 2 months storage. After initiation of sprouting growth rates of sprouts on tubers in continuous ethylene or ethylene combined with 1,4DMN were similar. After 3 months storage, both treatments reduced sprout length compared to air stored tubers. In Hermes, ethylene's ability to break dormancy and overcome the effect of 1,4 DMN on dormancy extension was less apparent after 2 months storage. After 3 months storage (2010), both treatments reduced sprout length compared to air stored tubers. In 2011, the combination of 1,4DMN and ethylene had the greatest effect of lowering the rate of sprout growth in Hermes. However, in all cases, the sprouts were longer than would be commercially acceptable (ie greater than 2mm).

Overall, the results indicate that further work is required to optimise the use of 1,4 DMN in combination with ethylene to maximise sprout suppression in varieties with differing responses to ethylene. .

Methyl jasmonate

The use of methyl jasmonate vapours has been reported previously to reduce sprouting in topped radishes (*Raphinus sativus*) and a patent for the use of jasmonates (Lulai *et al* 2007 pat No 5,436,226) including methyl jasmonate has been filed for use as a potato sprout suppressant in the USA and has had the additional benefit of improving the processing fry quality of treated potatoes. Jasmonates regulate many processes in plants and its role in wound response is best understood. Cross-talk between ABA and ethylene has been reported with MEJa inducing both ethylene production through stimulation of ACC oxidase acitivity, (Hudgins and Franceshi 2004) and ABA biosynthesis via induction of the phenylpropanoid pathway.

While results from these trials have proven variable both between seasons and by the method of application, sufficient evidence exists to warrant for further work on identifying the optimum timing and appropriate method of application.

Diniconazole and paclobutrazole

Results from season 2009 identified two triazoles: diniconazole and paclobutrazole as potential alternative sprout control treatments. Paclobutrazole (2RS, 3RS)-4,4-dimethyl-2-(1,2,4-triazol-1-y)pentan-3-ol, is a potent plant growth retardant that inhibits the three steps in the oxidation of the gibberellin pre-cursor *ent*-kaurene to *ent*-kaurenoic acid (Hedden and Graebe, 1985) leading to reduced intermodal growth. Earlier work also suggested that paclobutrazole also inhibited ABA activity in the fungus *Cercospora rosicola* (Norman *et al.* 1986). Clearly paclobutrazole reduced sprout growth in a dose dependant manner and importantly when combined to ethylene led to further improvements in sprout growth. Recent work has found evidence of prolonged sprout suppressive effects of ethylene when seedlings (millet) were pre-treated with paclobutrazole (Kim *et al.* 2012) and that ethylene affects GA concentrations leading to modulation of ethylene growth inhibition kinetics.

Diniconazole is reported to inhibit ABA catabolism. Continuous exposure to diniconazole led to a two fold increase in ABA concentration in tuber meristems and a decline in dihydrophaseic acid, through the inhibition of metabolism of ABA to phaseic acid and dihydrophaseic acid (Suttle *et al.* 2012). Results from our studies indicate a reduction in sprout growth after application which is in contrast to Suttle *et al.* (2012) where continuous application of diniconazole had no effect on subsequent sprouting.

Brassinosteroids

Brassinosteroids (BR) are a relatively new class of plant hormones known to act as signalling agents for a range of plant physiological responses. Recent studies have shown that BR's are able to stimulate plant growth and that along with IAA are able to stimulate ethylene production (Swarup *et al*. 2002) and more recently it has been shown that BRs induce *AtACS4* an auxin responsive ACC-synthase gene in *Arabidopsis*. The combination of brassinolide with

the auxin IAA, synergistically enhances the rate of ethylene production in *Arabidopsis* compared to where IAA is used singularly (Arteca and Arteca 2008).

In Mung bean BR's have been reported to stimulate ethylene production in the absence of exogenously applied IAA (Swarup *et al.* 2002). Application of 24-epibrassinolide to potato cv.Neviskii led to elevated concentrations free and conjugated ABA content in buds and delay and a delay sprouting by 33-36 days and a sharp rise in ethylene production by over 150-300 fold (Korableva *et al*. 2002). In trials conducted in this project 24-epibrassinoteroid when applied soon after harvest reduced initial sprout growth for the first two months of storage but the effect was short-lived, application to more mature tubers was less effective. Combining BR's with ethylene had little benefit probably due to ethylene's role in stimulating dormancy break.

Hormonal interactions

It is clear that there exists a complex interaction between hormones in regulating dormancy break and sprout growth in potato. ABA availability is considered key in maintaining dormancy while auxins are responsible for stimulating cell division and gibberellins and auxins for co-ordinating cell expansion. Ethylene's role in regulating the availability and perception of these hormones is still in its infancy and more work is needed to elucidate the full mechanism. Auxins and brassinosteroids are known to enhance endogenous ethylene production and there exists a complex web of hormone 'cross talk' responsible for the production of ethylene and its subsequent ability to regulate other plant hormones.

In order to achieve effective sprout control in potato using PGR's/plant hormone based sprout suppressants it is critical to understand the physiological status of the tubers at the point of treatment. Better methods of determining tubers maturity at harvest and during storage are required to allow for more accurate targeting of treatments to afford effective sprout control.

5.6.   Large scale trials to assess synergistic effects of sprout suppressant treatments

Following small-scale trials, larger scale trials were conducted on the use of R-carvone and 1-methyl cylopropene (1-MCP), to improve sprout control and processing quality respectively. R-Carvone was studied because it is commercially available for use on potatoes in GB (BIOX-M)

There are clear varietal differences in response to treatments. The varieties can essentially be classified into two groups in terms of their response to ethylene; Markies, Russet Burbank and Sylvana being very responsive, with Cabaret, Maris Piper, Hermes and Saturna less responsive These varietal differences are not surprising.   As long as varietal differences are well understood effective focused sprout control strategies can be developed individually for major varieties.

Carvone

Two isomers of carvone were tested in this project. S-carvone (caraway oil) was found to have low efficacy for the two contrasting varieties Maris Piper and Hermes. R-carvone (spearmint oil), found to be effective up to two months for two chipping and crisping varieties. Repeat

applications may provide an effective long-term strategy as long as any tainting is not significant.

1-MCP

During the transition of potato tubers from dormancy to sprouting, processing quality deteriorates, cellular metabolism shifts to net starch and protein breakdown, leading to the formation of soluble sugars and amino acids. Sucrose, formed in parenchyma cells, is transported towards the developing sprout. Within the developing sprout sucrose is hydrolysed to glucose and fructose to support growth and development. These reducing sugars, together with amino acids, are involved in the Maillard reactions and have a significant effect on processing fry colour (Roe *et al*. 1990, Blenkinsop *et al* 2002).

A major constraint to the use of ethylene for processing tubers is that it stimulates sugar accumulation and therefore has a detrimental effect on processing quality. Although the results are clearly variety dependent, 1-MCP has positive effects on processing quality in many cases, even though the 1-MCP effects are seen for 2 months only. 1-MCP acts by binding irreversibly to ethylene receptors, thereby inactivating them. The length of time that 1-MCP effects last is dependent on commodity and probably related to the rate at which ethylene receptors are resynthesised. For potato tubers repeated treatment every two to three months might be needed to maintain 1-MCP effects. This was tested in season 2011, but the findings suggested that even with repeat applications 1-MCP could only improve processing quality for 2 months.

5.7. Additional conclusions from statistical analysis of three years of trials.

The data on sprout growth and sugar accumulation over the three years of trials were subjected to a statistical analysis by a consultant statistician. A report on the full analysis is available in Appendix 11, and the key points are available in Appendix 12. In this section the observations of practical significance are highlighted.

Grouping varieties in terms of sprout growth and its control by ethylene and carvone.

Analysis of the data from 2010, including trials on the efficacy of ethylene, 1-MCP and R-Carvone (Spearmint) indicates that as far as sprout growth is concerned the varieties tested fall into three groups, as illustrated by figure 5.1.

> Group A: Markies, Russet Burbank and Sylvana respond to ethylene treatment in terms of sprout growth. The addition of 1-MCP or Carvone does not affect sprout growth inhibition and has no additional effect to inhibit sprout growth. There is not significant/consistent effect on number of sprouts.

> Group B: Cabaret and Maris Piper are less responsive to ethylene in terms of sprout growth inhibition than Group A, but there is a synergistic effect of R-Carvone. There is not a significant/consistent effect on the number of sprouts.

Group C: Saturna and Hermes are less responsive to ethylene in terms of sprout growth inhibition than Group A. There is a synergistic effect of R-Carvone, but it is less pronounced than for Group B. There is not a significant/consistent effect on the number of sprouts.

Russet Burbank

Sylvana

Markies

Legend:
- 2 months at 9°C
- 4 months at 9°C
- 6 months at 9°C
- LSD 0.05

Cabaret

Maris Piper
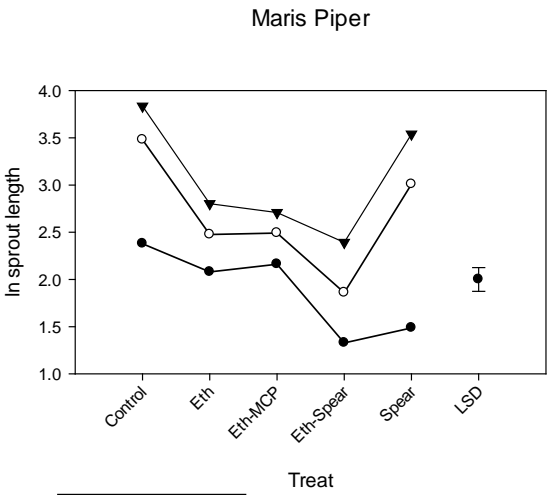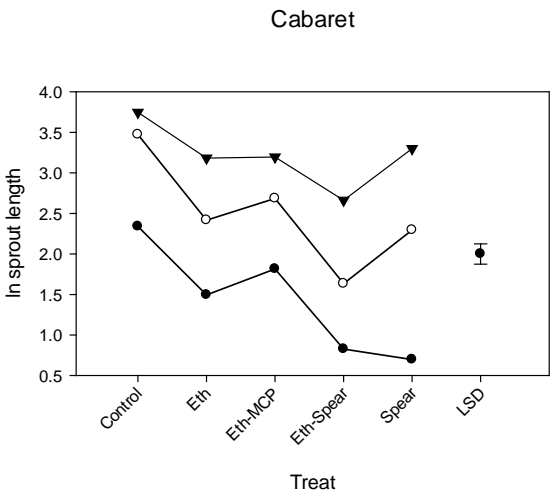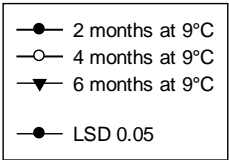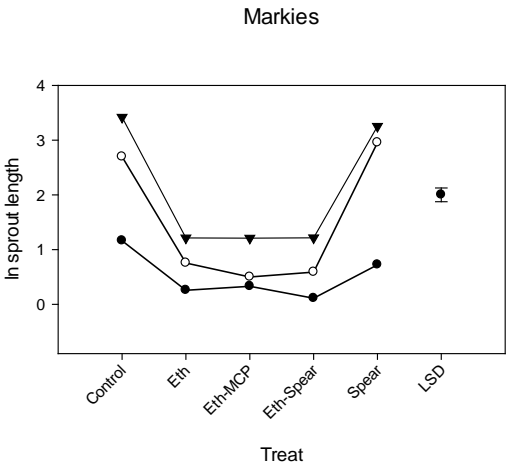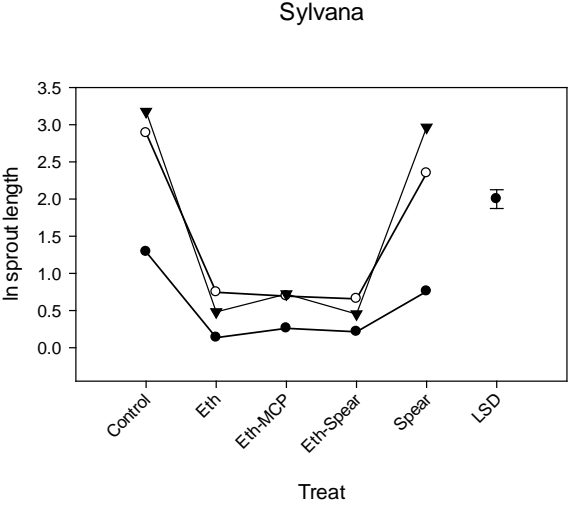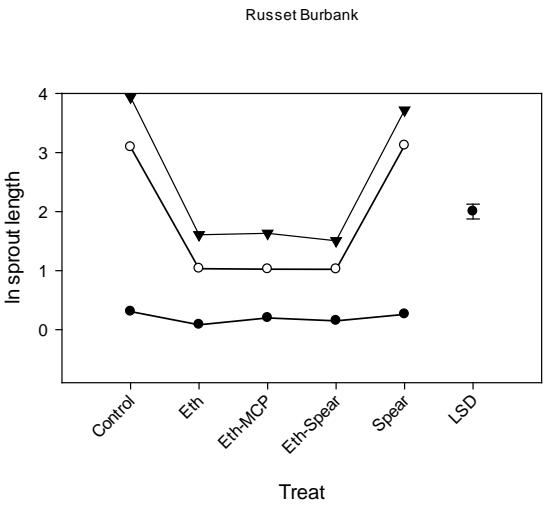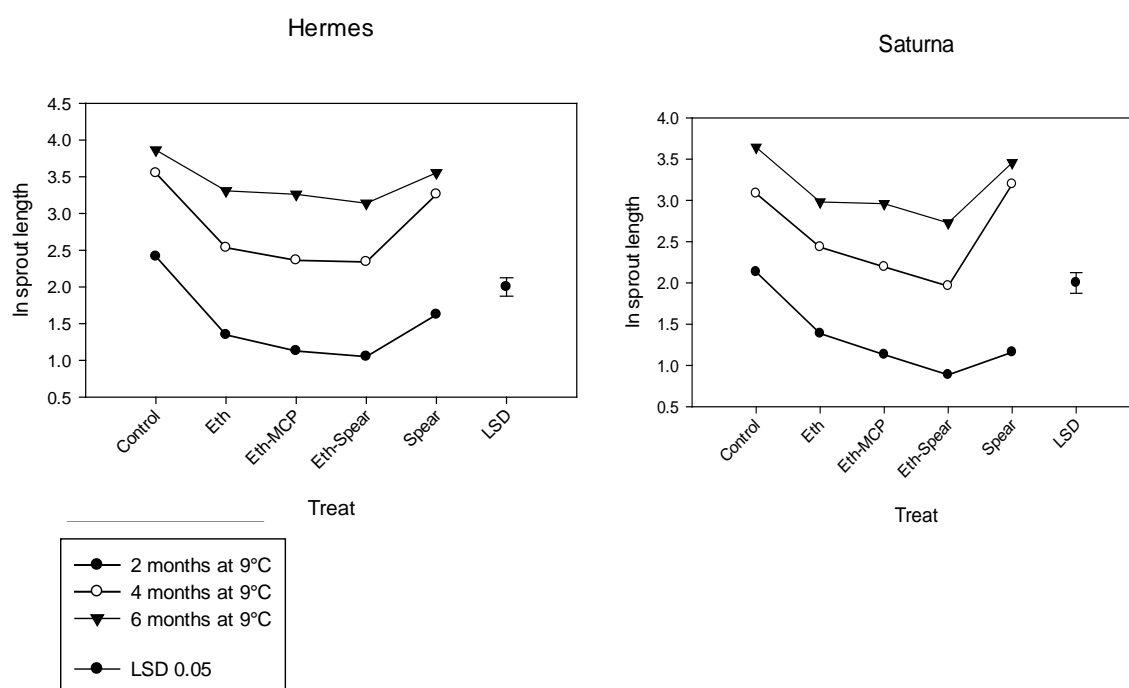
Figure 5.1. Sprout length (mm) subject to natural log transformation. Tubers were stored at 9°C for six months. Tubers were either pre-treated with 1-MCP (625 ppb) or Spearmint prior to treatment with ethylene (10 ppm).

The observation that the treatments affected sprout length, but not sprout number indicates that the control is at the level of sprout growth rather than dormancy break.

With respect to the effects of ethylene and ethylene +1-MCP the varieties essentially divided into the same groups in the first year (2009) although the effects of ethylene were generally less pronounced.

Characterisation of varieties in terms of the stimulation of sugar accumulation by ethylene, and the effect of 1-MCP

In 2009 trials were conducted at both 6 and 9°C. At 9°C, although ethylene was effective in suppressing sprout growth it did not tend to stimulate sugar accumulation.

There was a some tendency for ethylene to stimulate sugar accumulation particularly at 6°C. This was more marked in some varieties, notably Russet Burbank, and Cabaret. Likewise 1-MCP tended to reduce this effect, although this was only significant for some varieties, notably Markies and Sylvana (both within Group A above).

6. CONCLUSIONS AND THE WAY FORWARD

Objective 1: To understand the basic science underlying ethylene control of sprouting and its influence on tuber quality, in particular by identifying the main controlling genes.

Objective 2: To develop and test markers for key genes regulating ethylene mediated sprout control that might be used to identify new cultivars with increased response to ethylene.

With respect to objectives 1 and 2, the project supports the following conclusions:

- Variation in response of potato tubers to ethylene in terms of sprout growth inhibition has a genetic component and an environmental component.
- Considerable variation in the response to ethylene was observed between 12601 ab1 and Stirling and also among the progeny (GENPOP1), indicating that this is a useful population for this study.
- Phenotyping of 12601 ab1 and Stirling over four seasons and for the GENPOP1 population over two seasons indicated that there were seasonal differences in the inhibitory effect of ethylene treatment, but that in all but a few cases the relative response of lines was conserved.
- Using the GENPOP1 population a number of QTLs have been identified for sprouting in air and in ethylene. None of these accounts for more than 9% variance, and most are less than 7%.
- The low level of variance accounted for by the identified QTLs is partly due to the environmental effects on ethylene sensitivity, and also due to the complex nature of ethylene effects; for example the observations are consistent with the previously postulated role of ethylene to stimulate dormancy break.
- Although the main controlling genes have not been identified during the course of this project the information available from microarray analyses of gene expression at the point of sprouting in the presence and absence of ethylene for the parents and progeny of GENPOP1 can be used to focus on candidate genes. The list of most likely candidates can be reduced to less than 100 genes.
- Markers for sensitivity to ethylene have not been identified during this project. The ethylene response is more complex than envisaged at the start of the project, so that even with considerable advances in technologies available for genetic analysis, the approach of using QTLs to directly identify markers has not been successful. Nevertheless, the potential for developing key genes within the next three years is high.

The way forward

Phenotyping of the GENPOP1 population provided us with QTLs for both ethylene control of sprouting and dormancy length - however, these were minor QTL usually accounting for 5-6% variance. This is almost certainly a result of the complexity of the phenotypic characteristics that we were studying; the effect of ethylene on sprout length being affected by ethylene effects on breaking dormancy and by the inhibitory effect of ethylene on sprout growth. One way to take this work forward would be to design trials in such a way as to remove the dormancy effects to focus phenotyping only on sprout growth rates.   The use of bromoethane, which rapidly results in co-ordinated exit from bud endodormancy may enable the ethylene response trait to be dissected into some of its component parts. Thus treatment

of tubers from the population with bromoethane would remove dormancy effects and subsequent ethylene treatment would enable the effects on sprout growth alone to be measured. If this approach were to be successful, it would require no interaction between the bromoethane and ethylene treatments, and so pilot experiments would be required prior to a large scale phenotypic screen.

Despite advances in analytical techniques it is still very complex to identify QTLs in a tetraploid population. Analysis of a diploid population would simplify the approach and may lead to the identification of candidate genes more rapidly. In fact a project "Controlling dormancy and sprouting in potato and onion" approved through the BBSRC HAPI will take this approach to look at potato dormancy. The recently developed method of *de novo* DNA sequence driven bulk segregant analysis to delimit chromosomal regions that underpin phenotypic traits may also be an approach worth considering in the future. This method has recently been used to identify candidate genes involved in water use efficiency in potato (Kaminski *et al*., 2012). Genome-wide association studies in potato may also be an approach that is becoming feasible but all of these approaches require accurate phenotypic analysis.

Although it has not been possible to identify individual target genes any further studies would be able to build on the information such as genelists and QTLs available from this project to focus in on candidates. The BBSRC-HAPI project mentioned above will benefit from this information.

Environmental effects on ethylene response are clearly significant, as indicated by the different behaviour of the parents and the GENPOP1 progeny between seasons. An understanding of the environmental effects would provide tools to manipulate this response, which might be more straight forward than breeding for cultivars with the required response. This avenue could be explored using a carefully designed set of trials using for example different planting dates, locations with different climates and daylengths, irrigation regimes, preharvest ethylene inhibiting sprays for selected lines.

Ethylene exerts major effects on the potato tuber life-cycle, clearly impacting on both tuber bud endodormancy release and rate of sprout growth. Considerable knowledge of the ethylene biosynthetic pathways, ethylene perception and response mechanisms is available from the study of model plant systems such as *Arabidopsis* and tomato. Perhaps surprisingly, very little research in potato has been published where ethylene biochemistry has been perturbed transgenically. In view of the complexity of ethylene responses in potato and their importance in tuber development, it would be timely to use a direct transgenic approach to dissect the mechanism of the potato ethylene response. Technically, such an approach is well within our capability and such an approach would complement the search for naturally occurring alleles that can be incorporated into a conventional potato breeding approach.

Objective 3: To optimise storage strategies for processing potatoes using ethylene to control sprout growth while maintaining good processing quality, by exploiting synergistic interactions between ethylene and other plant hormones/ antagonists.

With respect to objective 3, the project supports the following conclusions

Ethylene
- The maximum inhibitory effect of ethylene on sprout growth during storage at 9°C requires concentrations greater than 1 ppm, with no clear difference between 10 and 25 ppm (three contrasting varieties tested). With no clear difference in ethylene stimulation of sugar accumulation and acrylamide production across the concentrations tested (1, 10 and 25 ppm) 10 ppm is an appropriate concentration of ethylene to use for sprout control.
- With ethylene control sprouting is greater in tubers stored at 9°C than at 6°C. Ethylene treatment is not sufficiently effective for commercial use in all varieties. The effectiveness of ethylene treatment is variety dependent, and we have characterised the varieties into three groups on this basis; Markies, Russet Burbank and Sylvana being very responsive (Group A), with Cabaret, Maris Piper (Group B), Hermes and Saturna (Group C) less responsive. Ethylene could provide a good strategy for sprout control in varieties such as Sylvana (up to 6 months), Markies (up to 4 months, but processing quality is a problem) and Russet Burbank (up to 4 months)

Ethylene, processing quality and effectiveness of 1-MCP
- Ethylene has a detrimental effect on processing quality but the extent of this varies with variety.
- This is independent of the effect on sprouting; demonstrated by the adverse effect of ethylene on processing quality of the chipping varieties Markies and Hermes which were relatively sensitive and insensitive to ethylene, in terms of inhibition of sprouting, respectively. Sugar accumulation is greater in tubers stored at 6°C than at 9°C. Raising the storage temperature of potatoes from 6 to 9°C mitigates the effect of ethylene induced accumulation of glucose and fructose seen at lower temperatures.
- SmartFresh^{TM} has no effect on sprouting behaviour but helps to ameliorate the ethylene-induced sugar accumulation in some varieties (such as Sylvana and Markies) early in the storage season up to 2 months.
- Ethylene and SmartFresh^{TM} responses in terms of sprout suppression and sugar accumulation in potato are not universal to all varieties tested. Verdi was the least responsive variety to ethylene induction of sugar accumulation and sprout suppression whilst Sylvana and Russet Burbank were sensitive to ethylene in-terms of sugar accumulation and sprout suppression. Hermes was relatively unresponsive to ethylene in-terms of sugar accumulation and sprout suppression.

R-Carvone (Spearmint)
- Spearmint treatment is as effective as ethylene at 2 months but this effect declined significantly at 4 and 6 months. For varieties that are unresponsive to ethylene the addition of spearmint improves sprout control. We have characterised these less responsive varieties into two groups in terms of their response to spearmint; for Group B (Cabaret and Maris Piper) there is a strong additive effect on sprout control for tubers treated with both ethylene and spearmint, while for Group C (Saturna and Hermes) the additive effect of Spearmint on sprout growth is less pronounced. Spearmint had little or no apparent effect on processing quality for all varieties.

Other potential sprout suppressant treatments

- A wide range of chemicals have been tested for their potential for sprout control in combination with ethylene. 1,4 dimethylnaphthalene and methyl jasmonate have been identified as chemicals with potential for development in the medium term.

The way forward

Strategies for the use of Spearmint (R-Carvone) for sprout control together with ethylene should be developed for specific varieties.

Ethylene offers an excellent route to control sprouting in specific varieties which are responsive to treatment providing any detrimental effects on potato processing quality can be mitigated.

Strategies to use 1-MCP treatment, in combination with ethylene, to improve processing quality should be developed.

Additionally, screening of varieties for ethylene sensitivity should be considered at an early stage in their development.

A more detailed study to further understand the factors influencing ethylene sensitivity, to maximise its potential for use across all ware potato storage, is required.

1,4 DMN and methyl jasmonate should also be pushed forward as commercially viable chemicals to be used in combination with ethylene.

In order to achieve effective sprout control in potato using PGR's/plant hormone based sprout suppressants it is critical to understand the physiological status of the tubers at the point of treatment. Better methods of determining tubers maturity at harvest and during storage are required to allow for more accurate targeting of treatments to afford effective sprout control.

7. REFERENCES

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8. LIST OF APPENDICES

Because of the size of some of the appendices they have not been included in this final report but they are available via Potato Council. Please contact the R&D team to request a copy of particular appendices.

8.1. **Appendix 1: Inspection 3 sprout data for the progeny of 12601 ab1 x Stirling season 2010.**

8.2. **Appendix 2: Results of genetic analysis for GENPOP1 for seasons 2010 and 2011**

8.3. **Appendix 3A and 3B: Genes expressed by 12601 ab1 and Stirling during sprout initiation, inspection 1 and 2 respectively (season 2009).**

8.4. **Appendix 4: genes which showed significant differential expression (SDE) between air and ethylene but showed no significant differential expression between genotypes.**

8.5. **Appendix 5: genes which showed significant differential expression between air and ethylene treatments and showed significant differential expression between genotypes.**

8.6. **Appendix 6: genes which showed significant differential expression between air and ethylene treatment and showed SDE for treatment by genotype interaction but did not show any significant differential expression between genotypes.**

8.7. **Appendix 7: genes which showed significant differential expression for treatment by genotype interaction but did not show any significant differential expression between genotypes or between treatments.**

8.8. **Appendix 8: Gene lists for genes that respond to ethylene treatment. (up- refers to genes up-regulated on ethylene treatment, down – down –regulated on ethylene treatment – group numbers as in Table 4.3). Also common genes that are up- or down- regulated on ethylene treatment for groups 2, 3 and 4 (common up and common down).**

8.9. **Appendix 9: Genes that were differentially expressed on ethylene treatment compared with air treatment for genotypes in group 4 (that is, genotypes that are responsive to ethylene and show very little sprout growth).**

8.10. **Appendix 10: List of genes that behave distinctly in terms of the expression patterns relating to sprout length with ethylene treatment**

8.11. **Appendix 11: Report on a statistical analysis of sprout and sugar data over three seasons.**

8.12. **Appendix 12: A summary of the findings from the statistical analysis of sprout and sugar data over three seasons.**

8.13. Appendix 13: Acrylamide analysis in heated potato flour. A report by the Flavour Centre of Reading University

9. ACKNOWLEDGEMENTS