Reducing Postharvest Losses and Wastage in UK Potato Storage due to Sprouting

FINAL REPORT

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EXECUTIVE SUMMARY

- Sprout suppression was achieved using UV-C at doses of 10, 15 and 20 kJ m\(^2\) applied at harvest or at 10% eye movement in a range of cultivars (Maris Piper, Russet Burbank, VR808, Cabaret and Saturna).
- UV-C treatment at 10% eye movement was more successful at reducing sprout growth in potato than when applied at harvest. This may be due to a combined systemic and physical response to the UV-C treatment or alternatively the different stage of tuber dormancy.
- Evidence suggests that UV-C is effective for controlling sprouting when tubers are washed or unwashed.
- Multivariate analysis revealed that phenolic content was not affected by UV-C treatment long term however an immediate effect was noted within 72 h of treatment with the highest UV-C dose.
- Treatment at 10% eye movement resulted in different sugar and phenolic profiles compared with potatoes treated at harvest suggesting a different mechanism(s) for sprout suppression.
- Treatment at harvest rather than at 10% eye movement may minimise increases in sugar content and therefore avoid darkening upon frying.
- ABA, ABA-GE and Z (zeatin) increased between harvest and 10% eye movement corresponding with the transition from endodormancy to ecodormancy.
- Physical damage by UV-C was confirmed by quantification of CPD-DNA which was highest following treatment with 10 or 20 kJ m\(^2\).
- A method for the quantification of CIPC was developed and validated [objective 2]

OBJECTIVES

Objective 2. Further develop CIPC vapour as a potential sprout control method (Cranfield University to develop a method for the quantification of CIPC)

Objective 3. To develop an alternative or complementary sprout suppression technology based on a physical control
1. INTRODUCTION

Potatoes represent a significant proportion of the UK diet with three out of five meal occasions in the UK including potatoes in some form (Terry et al., 2011). Some six million tonnes of potatoes are grown in UK annually and around 60% of the crop is placed in storage for supply during November to July (Potato Council, 2010), before suitable crop becomes available again. Due to advances in storage technologies, the UK is nearer to self sufficiency for potatoes as compared to other similar fresh produce types; however sprouting still represents a major cause of postharvest loss during storage, retail and in the home (Terry et al., 2011). Ensuring adequate supply of affordable tubers whilst minimising inputs is central to Defra's food security policy.

The quality of stored potatoes is maintained by controlling the temperature of stores and the application of sprout growth suppressing treatments. Despite research into alternative treatments (e.g. ethylene, Defra LK09127; PCL projects R298 and R412; Foukaraki et al., 2009), the main treatment used is chlorpropham (CIPC), on which the potato industry is still heavily reliant. Although research has resulted in dramatic reductions in CIPC requirements, this has been limited to certain store types. Changes in legislation controlling how much suppressant can be used, as well as general retailer and consumer pressure to reduce pesticide use (and residues of pesticides on foods), are limiting the effective storage duration that can be obtained. Alongside reductions in CIPC use, research continues to explore alternatives to CIPC, but this has tended not to consider abiotic methods of sprout control. Low temperature storage is used, but gives rise to a high sugar concentration that impairs the eating and processing quality of potatoes, as well as requiring large amounts of electricity throughout long-term storage. Gamma irradiation can be effective but can have detrimental effects on potato quality (Ezekiel et al., 2008; Anon., 1988) and is currently prohibited from use.

The aim of this project was to identify the optimum dose and treatment timing of UV-C treatment on potato tubers as an alternative method of sprout suppression to reduce the dependence on the chemical sprout suppressant, CIPC.
2. MATERIALS AND METHODS

2.1 Experimental Design

In the first year trial, six cultivars\(^1\) of potato viz. Cabaret, Maris Piper, Russet Burbank, VR808, Hermes and Saturna were grown at various sites. Cabaret was grown by Sacker Potatoes (Grantham, Lincs.), Saturna, Hermes and VR808 were grown at Chennels (North Scarle, Lincs.) and Maris Piper and Russet Burbank were supplied by McCain. Harvest dates are detailed in Table 2.1. Tubers were not washed prior to treatment. In the final year trial, three potato cultivars viz. Maris Piper, Saturna and VR808 were harvested. Saturna and VR808 were both grown on the same site in Yorkshire and Maris Piper were grown in Cambridgeshire. Harvest dates can be found in Table 2.2. Tubers were divided into two groups; washed and unwashed. The tuber washing was carried out by hand at room temperature water to prevent CIPC contamination using a commercial system. The tubers were air dried prior to treatment and subsequent storage.

The experiments were completely randomised designs with four UV-C treatments (First year trial; 0, 5, 10 and 15 kJ m\(^{-2}\), final year trial; 0, 10, 20 and 30 kJ m\(^{-2}\)), three treatment timings (pre [at harvest], post [after one week storage at 9°C], eye [at 10% eye movement] in the first year and two (pre and eye) in the final year, one storage temperature (9°C) and four replicates each containing three tubers. In the first year, tubers (n = 20) were divided into paper bags depending on cultivar (n = 6), UV-C dose (n = 4), UV-C timing (n = 3) and replicate (n = 3). In the final year, more tubers per treatment were required (n = 40); therefore tubers were stored in commercial trays depending on cultivar (n = 3), UV-C dose (n = 4), UV-C timing (n = 2), washed/unwashed (n = 2), and replicate (n = 4). In the final year, tubers were taken for biochemical assessment immediately after treatment, after 24 h and after 72 h (n = 3). Treatment of tubers with UV-C (254 nm) was conducted at Sutton Bridge Crop Storage Research (SBCSR; Spalding, Lincs.) using a UV-C conveyor (Applied Food Technologies, Oxon., UK), transferred from Cranfield University, with control unit and extractor fans. The conveyer rotated the tubers to allow for an even distribution of UV-C exposure. The dose emitted by the twenty two surface mounted lamps (80 W, No. 709, 254 nm, Hanovia, Berks., UK) was measured using an optical radiometer (Multisense-100) (Ultra Violet Products, Camb., UK). A subset of tubers was withheld from UV-C exposure and acted as the control. Tubers were then stored at 9°C for long term storage at SBCSR. After
treatment at harvest (pre), a subset of tubers was returned to Cranfield University for biochemical and physiological assessment (First year; n = 96 [6 x cvs, 4 x doses, 4 x reps], 288 tubers. Final year; n = 96 [3 x cvs, 2 x washed/unwashed, 4 x doses, 4 x reps]). After treatment following one week in storage (post), a subset of the pre- and post-treated potatoes was taken for analysis (First year only; n = 192, 576 tubers [6 x cvs, 4 x doses, 2 x timings, 4 x reps]). At 10% eye movement (eye) a subset of the pre-, (post-) and eye-treated tubers was taken for analysis (First year; n = 288, 864 tubers [6 x cvs, 4 x doses, 3 x timings, 4 x reps], final year; n = 192, 576 [3 x cvs, 2 x washed/unwashed, 4 x doses, 2 x timings, 4 x reps]). This same sample number was then taken again 10 weeks after 10% eye movement in the first year trial (n = 288 bags, 864 tubers). In the final year, samples were taken 5 and 10 weeks after 10% eye movement (n = 192) and 3 weeks after 10% eye movement a sprout assessment was carried out however tubers were not analysed physiologically or biochemically and therefore were not removed from the trays. In total, in the first year, 864 pooled samples were analysed, each of which consisted of three tubers giving an overall total of 2592 tubers. In the final year, samples were taken immediately after harvest, after 24 h and 72 h and tuber skin was analysed separately to tuber flesh therefore analysis of all cultivar was not feasible. Therefore, only Maris Piper was analysed biochemically (flesh; n = 208, skin; n = 208) since this was a common cultivar between the two years’ data that showed significant differences between treatments. The washed tubers were only assessed for sprout length, sprout number and eye movement.

1 Due to the delay in the project start date, it was not possible to assess 10 cultivars as stated in the project proposal.
**Table 2.1** Dates of treatment at harvest (pre), after one week of storage (9°C), at 10% eye movement and sprout growth assessment 10 weeks after 10% eye movement.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Type</th>
<th>Pre</th>
<th>Post</th>
<th>Eye</th>
<th>Sprout</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maris Piper</td>
<td>Chips</td>
<td>22.10.10</td>
<td>29.10.10</td>
<td>12.11.10</td>
<td>21.01.11</td>
</tr>
<tr>
<td>Russet Burbank</td>
<td>Chips</td>
<td>22.10.10</td>
<td>29.10.10</td>
<td>06.01.11</td>
<td>17.03.11</td>
</tr>
<tr>
<td>VR808</td>
<td>Crisps</td>
<td>27.10.10</td>
<td>03.11.10</td>
<td>21.12.10</td>
<td>01.03.11</td>
</tr>
<tr>
<td>Hermes</td>
<td>Crisps</td>
<td>27.10.10</td>
<td>03.11.10</td>
<td>06.12.10</td>
<td>14.02.11</td>
</tr>
<tr>
<td>Saturna</td>
<td>Crisps</td>
<td>27.10.10</td>
<td>03.10.10</td>
<td>03.12.10</td>
<td>11.02.11</td>
</tr>
<tr>
<td>Cabaret</td>
<td>Chips</td>
<td>28.10.10</td>
<td>04.10.10</td>
<td>26.11.10</td>
<td>04.02.11</td>
</tr>
</tbody>
</table>

*10% eye movement

**Table 2.2** Dates of treatment at harvest (Pre), at 10% eye movement (Eye) and sprout growth assessment 3 weeks, 5 weeks and 10 weeks after 10% eye movement.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Type</th>
<th>Pre</th>
<th>Eye</th>
<th>Sprout a</th>
<th>Sprout b</th>
<th>Sprout c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maris Piper</td>
<td>Chips</td>
<td>13/10/11</td>
<td>25/10/11</td>
<td>14/12/11</td>
<td>06/12/11</td>
<td>10/01/12</td>
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<tr>
<td>VR808</td>
<td>Crisps</td>
<td>20/10/11</td>
<td>19/12/11</td>
<td>09/01/12</td>
<td>23/01/12</td>
<td>27/02/12</td>
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<tr>
<td>Saturna</td>
<td>Crisps</td>
<td>19/10/11</td>
<td>16/11/11</td>
<td>05/12/11</td>
<td>16/12/11</td>
<td>25/01/12</td>
</tr>
</tbody>
</table>

*3 weeks after 10% eye movement (sprout assessment only)

5 weeks after 10% eye movement

10 weeks after 10% eye movement
2.2 Physiological measurements

2.2.1 Respiration rate

Three tubers were placed in 3 L jars with air tight lids and septum. The jars were sealed for 4 h at room temperature and gas samples removed with repeated full withdrawal-injection displacements using a 30 mL plastic syringe (Foukaraki et al. 2011). Gas samples were analysed using gas chromatography (GC model 8340, DP800 integrator, Carlos Erba Instruments, Herts., UK) with hot wire detection for CO\textsubscript{2} analysis (Chope et al., 2007). The GC was calibrated using 10.06\% CO\textsubscript{2} (10\% CO\textsubscript{2}, 2\% O\textsubscript{2}, 88\% N\textsubscript{2}; Certified Standard from BOC). The three tubers were weighed and respiration rate calculated in mmoles kg\textsuperscript{-1} h\textsuperscript{-1}. Due to the high number of samples, only cvs. Maris Piper, Cabaret and Hermes were analysed for respiration rate in the first year.

In the final year, a new piece of respiratory equipment was used to measure real time respiration rate. Three tubers were placed in 3 L jars with air tight lid and septum which led to a Sable Respirometry System (Model 1.3.8 Pro, Sable Systems International, Las Vegas, USA), which was calibrated with 10.06 \% CO\textsubscript{2} and 1.99\% O\textsubscript{2} (10 \% CO\textsubscript{2}, 2 \% O\textsubscript{2}, 88 \% N\textsubscript{2}; certified standard from BOC, Surrey, UK). Sequencing was controlled by a MUX flow multiplexer and air was subsampled at 1 L/min from each jar via a ‘push mode’ set up using an SS4-subsampler. Water vapour pressure (WVP) was determined using an RH-300 water vapour detector, after which, sampled air was passed through a water scrubber (Drierite). Subsequent analysis using a CA-10 carbon dioxide detector and a FC-10 oxygen detector, determined CO\textsubscript{2} (%) and O\textsubscript{2} (%), respectively, as well as flow rate (FR, mL/min) and barometric pressure (BP, kPa).

The UI-2 universal interface, connected to the subsampler, multiplexer and detectors, allowed data to be analysed and interpreted by the computer software. The subsampled air from each jar was analysed over a 5 min period, three times (cycles), to provide one average reading for each day. Before each sample was analysed, a 2 min baseline measurement was recorded which sampled air from the controlled temperature room at an identical flow rate of 1 L/min. This was to prevent cross contamination occurring between samples and allowed for
the initial O\textsubscript{2} and CO\textsubscript{2} levels (which would later be used for the software calculations) to be accurately determined.

In the software calculations, O\textsubscript{2}, and CO\textsubscript{2} values were converted from the initial % into fractional concentrations. Lag corrections were applied to O\textsubscript{2}, CO\textsubscript{2} and WVP values to ensure concentration changes, associated with the next sample, were aligned with the baseline markers. Since the air flow used to flush the samples was not dried chemically, BP and WVP corrections were applied to O\textsubscript{2} and CO\textsubscript{2} to compensate for water vapour dilution. Flow rate was converted from mL/min to mL/h by increasing the slope to (n=60). The mean CO\textsubscript{2} value was determined for the first baseline measurement recorded during each analysis to allow normal atmospheric CO\textsubscript{2} levels to be removed from the respiration rate calculations. The Sable System was able to calculate the levels of CO\textsubscript{2} in mL/h based on the sub-sampling flow rate. Finally, values were adjusted for tuber weight to determine the respiration rate in mL kg\textsuperscript{-1} h\textsuperscript{-1} for each chamber.

2.2.2 Sprout length, sprout number and dry weight

Sprout length and number was measured 10 weeks after eye movement for each cultivar. In the final year, sprout length, sprout number and eye movement was recorded 3, 5 and 10 weeks after 10% eye movement. Average sprout length was measured for each tuber in mm and average sprout number per tuber was recorded. Each replicate, cultivar, treatment dose and treatment timing consisted of three tubers. In the first year, each set of three tubers were processed by cutting a 1cm section through the centre including skin and flesh. Each section was halved and a half from each tuber pooled into one sample to give two identical pooled samples which were snap frozen in liquid nitrogen. One sample was kept at -40°C for biochemical analysis and one kept at -80°C as a back-up sample for any future analysis. In the final year the same applied, however, flesh and skin was snap frozen in liquid nitrogen separately. All -40°C samples were subsequently weighed before and after lyophilisation to calculate tuber dry weight.
2.3 Biochemical measurements

Due to the high number of samples, only cvs. Maris Piper, Cabaret and Hermes were analysed biochemically in the first year and Maris Piper only in the final year.

2.3.1 Sugar analysis

Sugars were extracted and measured as described by Foukaraki et al. (2010) with slight modifications. The sugars were extracted from 150 mg of potato using 3 mL of 62.5:37.5 HPLC grade methanol:water (v/v). The samples were placed in a shaking water bath at 55°C for 15 min and agitated every 5 min to prevent layering. Filtered extracts were diluted 1 in 5 before injection into the HPLC. Extracts were analysed using an Agilent 1200 series HPLC system (Agilent, Berks.). The extract (20 μL) was injected into a Rezex RCM monosaccharide Ca\(^{+}\) size exclusion column of 300 mm × 7.8 mm diameter, 8 μm particle size (Phenomenex, CA, USA; Part no. 00H-0130-K0) with a Carbo-Ca\(^{2+}\) guard cartridge of 4 mm × 3 mm diameter (Phenomenex; Part no. AJ0-4493). The mobile phase was HPLC grade water at a flow rate of 0.6 mL min\(^{-1}\). An evaporative light scattering detector (ELSD 2420, Waters, MA, USA) connected to the system via a UCI-50 universal chromatography interface detected the eluted carbohydrates. Sugar concentrations were calculated against authentic calibration standards of fructose, glucose and sucrose ranging from 0.05 to 2.5 mg mL\(^{-1}\) (Sigma, Dorset, UK).

2.3.2 Phenolic analysis

Flavonol concentrations were extracted and quantified according to Giné Bordonaba and Terry (2008) with slight modifications. Potato (200 mg) was mixed with 1.5 mL of 70:29:5:0.5 methanol:water:hydrochloric acid (v/v/v) and vortexed to mix thoroughly. The vials were held at room temperature for 1 h before being incubated at 37°C for 1.5 h in a shaking water bath and agitated every 15 min. Cooled samples were filtered through a 0.2 μm Millex-GV filter unit and stored at −40°C.

Undiluted extracts were analysed using an Agilent 1200 series HPLC system (Agilent, Berks., UK). The extracts (10 μL) were injected into an Agilent ZORBAX Eclipse XDB-C18 column, 4.6 mm × 150 mm, 5 μm particle size (Part no. 993967-902), with an Agilent
ZORBAX Eclipse XDB guard column, 1.0 mm × 17 mm (Part no. 5185-5921). The mobile phase consisted of acetonitrile (A) and HPLC grade water with 0.5% formic acid (B). The gradient involved a linear increase/decrease of solvent B: 95–62%, 13 min; 62-30%, 5 min; 30-95%, 1 min; at a flow rate of 1 mL min$^{-1}$ and a column temperature of 25°C. An Agilent 1200 DA G1315B/G1365B photodiode array detected eluted flavonols at a wavelength of 340 nm. The data was presented in Agilent ChemStation Rev. B.02.01 software and flavonol concentration calculated against authentic calibration standards (caffeic acid, chlorogenic acid and ferulic acid; Sigma).

2.3.3 Hormone analysis

Hormone analysis was only carried out on samples taken 0, 24 or 72h after treatment at harvest (n=48) or at 10% eye movement (n=48). An Agilent 1290 Infinity LC system comprised of the following was used to obtain chromatographic separation: Agilent 1290 Infinity Thermostatted column compartment (TCC) operated at 30°C, Agilent UHPLC 1290 Infinity Autosampler (ALS), and an Agilent 1290 Infinity Binary Pump with Jet Weaver V35 mixer. Column ZORBAX RRHD Eclipse Plus C18 column (2.1 x 50 mm 1.8 µm). Compounds were separated at a flow rate of 0.6 mL min$^{-1}$ using a linear gradient of solvent A (water + 0.1 % formic acid) and solvent B (acetonitrile + 0.1 % formic acid) programmed as the following profile; 0 min, 10 % B + 90 % A; 2 min, 15 % B + 85 % A; 12 min, 50 % B + 50 % A; 12.10 min 100 % B and hold for 3 min; 15.20-20.00 min 10 % B + 90 % A. Samples (5 µL) were injected with needle wash and of total volume. Standards and internal standards were prepared with the solvent A and prepared at seven levels of concentrations as follow: 5, 10, 25, 50, 75, 100 and 150 ng mL$^{-1}$.

MS and MS/MS experiments were carried out on an Agilent 6540 Ultra High Definition Accurate Mass Q-TOF LC-MS System. All the samples were analysed using ESI Agilent Jet Stream source in negative and positive mode. For both negative and positive mode the following settings were applied: Nebulizer gas temperature (N2) 325°C, at a flow of 8 L min$^{-1}$, sheath gas temperature (N2) 350°C at flow rate of 11 L min$^{-1}$. Capillary voltage ±4000V, Nozzle voltage ±500 V, fragmentor ±175 V. Full scan data was acquired on the range of 100-1000 m/z, at acquisition rate of 3 spectra s$^{-1}$, data were recorded in centroid mode, using a maximum time between MS spectra of 3 s. Accurate-mass internal mass calibration was
performed automatically using a dual-nebulizer ion source combined with an automated calibrant delivery system, which introduce continuously the internal reference ion solution at approximately 50 µL min\(^{-1}\), the reference ion masses were 119.036320 and 966.000725 m/z in negative mode; 121.050873 and 922.009798 m/z in positive mode. In product ion scan experiments (MS/MS) products ions were produced by collision induced dissociation (CID) of selected precursor ions using targeted MS/MS experiments, with collision energy optimized for each compound and ranged from of 10-24 V with an isolation window of 4 m/z (medium) for all compounds. Product ion scan was acquired on the mass range of 100-500 m/z, with an acquisition rate of 3 spectra s\(^{-1}\).

The LC and MS systems and data acquisition were controlled by Agilent MassHunter Data Acquisition software B.04.00, data analysis was processed using Agilent MassHunter quantitative analysis software B.05.00 (Ordaz Ortiz et al., unpublished).

2.4 Cyclobutane pyrimidine dimer quantification

Cyclobutane pyrimidine dimer is the predominant type of DNA damage which occurs in response to UV light although this is not specific to UV-C. DNA was extracted from 100mg of frozen potato skin macerated using a Cryomill (Retsch, Haan, Germany). DNA was extracted from the skins of potatoes cv. Maris Piper from the final year trial treated at harvest (pre) with 0, 10, 20 or 30 kJ m\(^{-2}\). Samples were taken immediately after treatment (0h), after 24 h and 72h totalling 24 samples of which there were 4 biological replicates (4 x doses, 4 x replicates, 3 x sampling times [0, 24, 72h]; n = 48). To each 100 mg of frozen skin sample, 500 µl of CTAB buffer (20g/L cetyltrimethylammonium (ctrimonium) bromide [CTAB], 1.4M NaCl, 0.1M Tris/HCl and 20mM EDTA adjusted to pH 8 using sodium hydroxide) was added and incubated for 30 mins at 65°C. The samples were centrifuged for 10 min at maximum speed and the liquid layer transferred to a fresh tube. Chloroform (200µl) was added and gently mixed by inversion before being centrifuged at maximum speed for 10mins. The upper layer was carefully removed and transferred to a fresh tube and 0.6 volumes of isopropanol was added and mixed by inversion. The samples were incubated overnight at 4°C to allow the DNA to precipitate. The samples were centrifuged at maximum speed for 10 mins and the liquid carefully poured away to leave the DNA pellet. The DNA pellet was washed with 70% (v/v) ethanol, centrifuged and carefully poured away without
dislodging the DNA pellet. The pellet was air dried and resuspended in 100 µl of sterile water. The DNA concentration was quantified using a Picodrop spectrophotometer (Picodrop Limited, Essex, UK) and each sample adjusted to 2 µg/ml. The CPD damage was then quantified using an OxiSelect UV-Induced DNA Damage ELISA Kit (CPD) Quantification (Cell Biolabs, CA, USA) according to the manufacturer’s instructions.

2.5 DNA fragmentation

Genomic DNA was extracted and purified using a Wizard Genomic DNA Purification Kit (A1120) according to the manufacturer’s guidelines. DNA was extracted from 12 samples (40 mg each); 3 x sampling times after harvest UV-C treatment (Pre) (0, 24 and 72h) and 4 x doses with each sample containing an equal mixture of skin sample from four individual potato replicates. The extracted DNA was quantified using a Picodrop spectrophotometer (Picodrop Limited, Essex, UK). The genomic DNA (0.5 ng) was run on a 1.8% agarose gel for 4 h at 60V.

2.6 CIPC quantification [objective 2]

A method to quantify CIPC in potato samples was developed and validated as part of Objective 2 of the project. CIPC was extracted according to Corsini et al. (1978) and quantified according to Mondy et al. (1992) with slight modifications. The flesh and skin of three potatoes treated with CIPC as per commercial practise in the last three months were analysed to validate the method. Fresh frozen potato (25 g) was ground under liquid nitrogen. Petroleum ether (250 ml) was added to the ground frozen potato powder in a 500 ml round bottomed flask and allowed to sit at room temperature for 20 h. The samples were rota evaporated to dryness at 35 °C and resuspended in 5 ml cold hexane. A sample of the hexane was stored at -40°C until further analysis.

Undiluted extracts were analysed using an Agilent 1200 series HPLC system (Agilent, Berks., UK). The extracts (20 µL) were injected into a Waters Spherisorb NH2 column, 4.6 mm × 150 mm, 5 µm particle size (Part no. PSS831113). The mobile phase consisted of an isocratic run of 10% dichloromethane in hexane at a flow rate of 1.3 mL min⁻¹ and a column temperature of 70°C. An Agilent 1200 DA G1315B/G1365B photodiode array detected eluted CIPC at a wavelength of 238 nm. The data was presented in Agilent ChemStation
Rev. B.02.01 software and CIPC concentration calculated against an authentic calibration standard kindly donated by Grace Gillard. To validate the method, limit of detection (LOD), limit of quantification (LOQ) and relative standard deviation (R.S.D) was calculated according to Downes and Terry (2010).

2.6 Statistics

All statistical analyses were carried out using Genstat for Windows 12th Edition, Version 12.1.0.3338 (VSN International Ltd., Herts., UK) unless otherwise stated. There was an imbalance in treatment numbers since treatments were staggered throughout storage at harvest, after one week storage and at 10% eye movement. To adjust for this imbalance a common baseline was used to compare with other factors. Least significant difference values (LSD; p=0.05) were calculated from each analysis, for comparison of appropriate treatment means, using critical values of t for two-tailed tests. Results are significant to p<0.05 unless otherwise stated. Multivariate analysis (Principle Component Analysis) was used to identify clustering of data according to specific factors. Pat Bellamy (former Head of Statistics at Cranfield University) was consulted for all statistical analysis.
3. **RESULTS – First year trial**

3.1 Physiological Analysis

3.1.1 Respiration rate

Due to high treatment numbers, only cvs. Cabaret, Hermes and Maris Piper were measured. Although respiration rate was measured immediately after each treatment timing and for each dose, there was no significant difference for all cultivars measured. Respiration rate of cv Cabaret at harvest was 0.12 mmoles CO$_2$ kg$^{-1}$ h$^{-1}$ which then decreased to 0.10 mmoles CO$_2$ kg$^{-1}$ h$^{-1}$ one week later then decreased again to 0.05 mmoles CO$_2$ kg$^{-1}$ h$^{-1}$ at 10% eye movement. Potatoes cv. Hermes also reduced from 0.39 to 0.20 to 0.12 mmoles CO$_2$ kg$^{-1}$ h$^{-1}$ from harvest to after one week in storage to 10% eye movement. Potatoes cv. Maris Piper did not follow the same trend with respiration rate starting at 0.05 mmoles CO$_2$ kg$^{-1}$ h$^{-1}$ at harvest then increasing to 0.12 mmoles CO$_2$ kg$^{-1}$ h$^{-1}$ after one week then 0.0849 at 10% eye movement.

3.1.2 Average Sprout Length and Number

Sprout growth of the tubers was assessed 10 weeks after the average eye movement of each cultivar had reached 10%. At this point, both average sprout length (mm) and average number of sprouts per tuber was recorded. Each cultivar was assessed separately since they were grown under different environmental conditions.

There was an overall significant effect of treatment dose on cv. Cabaret with tubers treated with 10 kJ m$^{-2}$ having the shortest average sprout length (12.13 mm) compared with the control (19.63 mm). There was also an interaction between treatment timing and treatment dose whereby tubers treated with 10 kJ m$^{-2}$ at harvest (pre) had the shortest sprouts (7.63 mm) compared with the control (18.04 mm). In addition, tubers treated at 10% eye movement with 15 kJ m$^{-2}$ had significantly shorter sprouts (9.79 mm) than the control tubers (21.70 mm) (Figure 3.1). There were no significant differences between sprout numbers for Cabaret tubers. No significant differences between sprout length or number was observed for UV-C treated tubers cvs. Hermes (Figure 3.1) or Saturna (Figure 3.1).
There was a main effect of treatment timing and treatment dose on the sprout length of cv. Maris Piper as well as a significant interaction between the two factors. Treatment at 10% eye movement resulted in, on average, shorter sprouts (22.65 mm) compared with those treated at harvest (pre; 24.28 mm) and even more so than those treated after one week of storage (post; 28.11 mm). The treatment dose of 10 kJ m\(^{-2}\) resulted in the shortest sprout length at an average of 21.69 mm compared with the control (28.47 mm). The interaction between the two factors of treatment timing and dose was due to the shorter sprouts of tubers treated with 10 kJ m\(^{-2}\) but only when applied at harvest (pre) or at 10% eye movement (Figure 3.1). The treatment dose also had an overall effect on sprout number whereby tubers treated with 10 and 15 kJ m\(^{-2}\) UV-C had less sprouts (1.89 and 1.78, respectively) than the control (3.06) however this was irrespective of treatment timing (Figure 3.2).

Tubers cv. VR808 had on average a lower number of sprouts when treated with 10 kJ m\(^{-2}\) UV-C. No significant difference in sprout length was found (Figure 3.1). There was also an interaction between treatment timing and dose where tubers treated with 10 kJ m\(^{-2}\) specifically at 10% eye movement had a lower number of sprouts (1.75) compared with the control (2.917) (Figure 3.2).

There was a main effect of treatment timing and dose on sprout length of tubers cv. Russet Burbank. Application of the treatment at eye movement (37.1 mm) resulted in overall shorter sprouts than at harvest (pre) and after one week storage (post) (50.8 and 54.1 mm, respectively). In addition at dose of 10 kJ m\(^{-2}\) resulted in shorter sprouts (37.6 mm) compared with the control (60.9 mm) and other treatment doses; 5 kJ m\(^{-2}\) (45.8 mm) and 10 kJ m\(^{-2}\) (45.1 mm) (Figure 3.1).
Figure 3.1 Average sprout length (mm) of six potato cvs. 10 weeks after the emergence of 10% eye movement treated with 0, 5, 10 or 15 kJ m$^{-2}$ UV-C applied at harvest (pre), after one week storage at 9°C (post) or at 10% eye movement.
Figure 3.2 Average sprout number per tuber of potato cvs. Maris Piper and VR808 10 weeks after the emergence of 10% eye movement treated with 0, 5, 10 or 15 kJ m\(^{-2}\) UV-C applied at harvest (pre), after one week storage at 9°C (post) or at 10% eye movement.

3.1.3 Dry weight

There was a main effect of time on the dry weight of cvs. Cabaret, Hermes and Maris Piper where dry weight increased steadily throughout storage from an average of 217.65 mg g\(^{-1}\) FW to 238.5 mg g\(^{-1}\) FW 10 weeks after 10% eye movement. There was a significant interaction between time, treatment dose and timing for cv. Maris Piper since tubers treated with 5 kJ m\(^{-2}\) at eye movement had significantly lower dry weight (206.3 mg g\(^{-1}\) FW) compared with the control and other treatment doses (Figure 3.3)
Figure 3.3 Dry weight (mg g⁻¹ FW) of potato cvs. Maris Piper, Cabaret and Hermes throughout storage at 9°C treated with 0, 5, 10 or 15 kJ m⁻² UV-C applied at harvest, after one week storage at 9°C or at 10% eye movement.
3.2 Biochemical Analysis

3.2.1 Phenolic acids

No negative effects on phenolic content were found as a result of UV-C treatment and phenolics were found in the range of those previously reported (Burns and Terry, unpublished). There was an overall effect of time on chlorogenic acid where there was an increase in the first week for cvs. Maris Piper and Cabaret which then decreased following the initiation of sprouting. This pattern in chlorogenic acid content was not observed for cv. Hermes where the opposite trend was observed (Figure 3.4); indeed, the opposite trend was observed for caffeic and ferulic acid content in cvs Hermes and Cabaret (Figure 3.5 and 3.6). The caffeic acid and ferulic acid content over time differed in cv. Maris Piper where caffeic acid decreased by half and ferulic acid doubled from harvest to 10 weeks after 10% eye movement. There was a main effect of treatment timing on chlorogenic and caffeic acid in cvs. Hermes and Maris Piper. For cv. Hermes, tubers treated one week after harvest (post) had approx. 16-fold lower chlorogenic acid and caffeic acid compared with the other treatment timings and this same trend was observed at 10% eye movement but only in tubers treated at harvest. For cv. Maris Piper, there was an approx. 40 and 90-fold decrease in chlorogenic and ferulic acid, respectively, in tubers 10 weeks after being treated at 10% eye movement. This same trend was not observed in caffeic acid although after one week of storage, tubers treated at harvest had approx. a quarter to a half less caffeic acid than the other treatments (Figure 3.5).
Figure 3.4 Chlorogenic acid (mg g\(^{-1}\) DW) of potato cvs. Maris Piper, Cabaret and Hermes throughout storage at 9°C treated with 0, 5, 10 or 15 kJ m\(^{-2}\) UV-C applied at harvest, after one week storage at 9°C or at 10% eye movement.
Figure 3.5 Caffeic acid (mg g⁻¹ DW) of potato cvs. Maris Piper, Cabaret and Hermes throughout storage at 9°C treated with 0, 5, 10 or 15 kJ m⁻² UV-C applied at harvest, after one week storage at 9°C or at 10% eye movement.
<table>
<thead>
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<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
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<td>0.002</td>
<td>0.004</td>
<td>0.006</td>
<td>0.008</td>
</tr>
<tr>
<td>Cabaret</td>
<td>0</td>
<td>0.002</td>
<td>0.004</td>
<td>0.006</td>
<td>0.008</td>
</tr>
<tr>
<td>Hermes</td>
<td>0</td>
<td>0.002</td>
<td>0.004</td>
<td>0.006</td>
<td>0.008</td>
</tr>
</tbody>
</table>

**Figure 3.6** Ferulic acid (mg g\(^{-1}\) DW) of potato cvs. Maris Piper, Cabaret and Hermes throughout storage at 9°C treated with 0, 5, 10 or 15 kJ m\(^{-2}\) UV-C applied at harvest, after one week storage at 9°C or at 10% eye movement.
3.2.2 Sugars

Sugar concentrations were in the same range as that previously reported (Foukaraki et al., 2011). Sucrose and glucose content of tubers 10 weeks after 10% eye movement was half as low in tubers treated at eye movement compared with those treated at harvest (Figure 3.7). This result only applied to cvs. Cabaret and Maris Piper, the two cultivars which had shorter sprout growth in response to UV-C treatment at 10% eye movement. For cv. Hermes, the sucrose content was half as low at eye movement in tubers treated at harvest (pre) and at 10% eye movement compared with the tubers treated one week after harvest (post). This said, the levels returned to initial sucrose levels 10 weeks after eye movement. The trend in sucrose content over time differed between each cultivar (Figure 3.7), however, glucose and fructose content showed the same trend between cultivars (Figure 3.8). Glucose and fructose content (Figure 3.9) doubled during the first week of storage then subsequently decreased at the onset of sprouting.
Figure 3.7 Sucrose (mg g\(^{-1}\) DW) of potato cvs. Maris Piper, Cabaret and Hermes throughout storage at 9°C treated with 0, 5, 10 or 15 kJ m\(^{-2}\) UV-C applied at harvest, after one week storage at 9°C or at 10% eye movement.
Figure 3.8 Glucose (mg g⁻¹ DW) of potato cvs. Maris Piper, Cabaret and Hermes throughout storage at 9°C treated with 0, 5, 10 or 15 kJ m⁻² UV-C applied at harvest, after one week storage at 9°C or at 10% eye movement.
Figure 3.9 Fructose (mg g\(^{-1}\) DW) of potato cvs. Maris Piper, Cabaret and Hermes throughout storage at 9°C treated with 0, 5, 10 or 15 kJ m\(^{-2}\) UV-C applied at harvest, after one week storage at 9°C or at 10% eye movement.
3.3 Multivariate analysis

Principle Component Analysis was used to identify clustering of variables according to specific factors. For the multivariate analysis, sprout data, phenolic data and sugar data was used however, respiration rate was not included as it was not measured at the final sampling 10 weeks after eye movement. Figure 3.10, which represents data from cv. Maris Piper tubers, showed that tubers not treated with UV-C (represented by 0) had higher sprout length and sprout incidence as well as lower dry weight and glucose. Figure 3.11, also representing data from cv. Maris Piper tubers, showed that tubers treated at eye movement had higher fructose but lower sucrose and phenolic acids however this included untreated tubers and was not linked to sprouting.

![PCP biplot (66.9%)](image)

**Figure 3.10** Principle Component Analysis of sprout data, phenolic data and sugar data of cv. Maris Piper 10 weeks after 10% eye movement labelled according to the factor of time; 0, harvest (pre); 1, one week of storage (post); 2, 10% eye movement (eye); 3, 10 weeks after 10% eye movement (sprout assessment).
3.4 Validation of CIPC method

The CIPC concentration of three potatoes was analysed in the flesh and skin to validate the method. The concentrations of CIPC in the three potato samples are presented in Figure 3.12. The LOD and LOQ were calculated for 10 repeat sample injections. The LOD was 0.032 μg g⁻¹ FW and the LOQ was 0.108 μg g⁻¹ FW. These values are very low which indicates the accuracy of the method. Any values above the LOD are classed as either semi-quantitative or fully quantitative when above the LOQ. These results show that the concentration of CIPC in the potato skin was above the LOQ and is therefore fully quantitative. The level of CIPC in the potato flesh was very low and the results in Figure 3.12 lie between the LOD and LOQ therefore the CIPC content in the flesh can only be
described as semi-quantitative. These values are in the range of those previously reported (Ezekiel and Singh, 2007). The R.S.D value was calculated as 1.15% which lies well below the recommended level of 20% (Downes and Terry, 2010).

![CIPC concentration (ug g⁻¹ FW) in three potatoes separated into skin and flesh.](image)

**Figure 3.12** CIPC concentration (ug g⁻¹ FW) in three potatoes separated into skin and flesh.

4. **RESULTS – Final year trial (Year 2)**

4.1 **Physiological analysis**

4.1.1 **Sprout length, sprout number and eye movement**

There were three timing in which sprout length, sprout growth and eye movement was recorded; 3, 5 and 10 weeks after 10% eye movement. For potato cv. VR808, sprout growth was rapid following the eye movement treatment therefore eye movement measurements 3, 5 and 10 weeks after 10% eye movement was 100%. Eye movement 3 weeks after 10% eye movement in cvs. Maris Piper and Saturna had not yet reached 100% however, differences between treatments were not observed at this stage. Beyond 3 weeks, eye movement was 100%.

Sprout number was reduced 3 weeks after 10% eye movement in potatoes cv. VR808 with those treated with 10 or 20 kJ m⁻² having a lower number of sprouts (2.86 and 3.44
sprouts, respectively) compared with the control (4.42 sprouts) and those treated with 30 kJ m$^{-2}$ (3.69 sprouts) (Figure 4.1). There was also a significant difference between washed and unwashed tubers with the washed tubers having a higher number of sprouts (3.97 sprouts) compared with the unwashed tubers (3.23 sprouts).

![Figure 4.1](image)

**Figure 4.1** Average sprout number per tuber of washed/unwashed potato cv. VR808 3 weeks after the emergence of 10% eye movement treated with 0, 10, 20 or 30 kJ m$^{-2}$ UV-C applied at harvest (pre) or at 10% eye movement (eye).

Average sprout length of cv Maris Piper was significantly lower in tubers treated with 10 or 20 kJ m$^{-2}$ UV-C at 8.79 and 8.29 mm compared with the control tubers (11.45 mm) or those treated with 30 kJ m$^{-2}$ (10.82 mm) 5 weeks after 10% eye movement. Although there was no significant interaction between dose and UV-C timing, there was an overall effect of treatment timing with tubers treated at 10% eye movement (8.94 mm) was significantly shorter than the tubers treated at harvest (Pre, 10.73 mm). Sprout length 10 weeks after 10% eye movement of cv. Maris Piper was also significantly different between treatments as well as a significant interaction between treatment dose and timing. The lowest sprout length was in tubers cv. Maris Piper treated with 10 kJ m$^{-2}$ or 20 kJ m$^{-2}$ applied at 10% eye movement and in particular in the tubers which were unwashed (Figure 4.2). Sprout number was also significantly affected by treatment in cv. Maris Piper 5 and 10 weeks after 10% eye
movement. Again, the most efficacious treatments were 10 or 20 kJ m\(^{-2}\) applied at 10% eye movement (Figure 4.2).

### Table: Sprout Length and Average Sprout Number

<table>
<thead>
<tr>
<th>Treatment Timing</th>
<th>Pre unwashed</th>
<th>Pre washed</th>
<th>Eye unwashed</th>
<th>Eye washed</th>
<th>0 kJ m(^{-2})</th>
<th>10 kJ m(^{-2})</th>
<th>20 kJ m(^{-2})</th>
<th>30 kJ m(^{-2})</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maris Piper 5 weeks after 10% eye movement</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sprout length (mm)</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>10</td>
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<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 4.2** Sprout length (mm) and average sprout number per tuber of washed/unwashed potato cv. Maris Piper 5 and 10 weeks after the emergence of 10% eye movement treated with 0, 10, 20 or 30 kJ m\(^{-2}\) UV-C applied at harvest (pre) or at 10% eye movement (eye).

Sprout number and eye movement was not affected by the UV-C treatments in potato cv. Saturna. This said, there was a significant effect of dose and timing on sprout length 5 and 10 weeks after 10% eye movement. Sprout length 5 weeks after 10% eye movement was 2-fold lower in all treated tubers compared with the control and was lower in tubers treated at
harvest (Pre; 3.71 mm) compared with those treated at 10% eye movement (5.29 mm). At 10 weeks after 10% eye movement, the sprout length of tubers cv. Saturna was shortest in tubers treated with 20 or 30 kJ m⁻² although there was no overall difference between whether this treatment was applied at harvest or at 10% eye movement (Figure 4.3).

![Saturna 5 weeks after 10% eye movement](image1)

![Saturna 10 weeks after 10% eye movement](image2)

**Figure 4.3** Sprout length (mm) of washed/unwashed potato cv. Saturna 5 and 10 weeks after the emergence of 10% eye movement treated with 0, 10, 20 or 30 kJ m⁻² UV-C applied at harvest (pre) or at 10% eye movement (eye).

4.1.2 Respiration rate

There was no significant difference in respiration rate between treatment dose or timing for tubers cv. Maris Piper, Saturna or VR808 which was also observed in the first year trial.

4.1.3 Dry weight

Dry weight in the final year trial was only measured in the flesh and skin of potatoes cv. Maris Piper. The dry weight of the flesh did not change significantly during storage however, the skin decreased significantly during storage from 245.9 mg g⁻¹ FW at harvest to 220.3 mg g⁻¹ FW after 13 weeks storage (Figure 4.4). There was a significant interaction between treatment timing and storage time due to the significantly higher skin dry weight in tubers treated at 10% eye movement (246.9 mg g⁻¹ FW) compared with those treated at
harvest (213.1 mg g\(^{-1}\) FW) however this was only significant after two weeks of storage. Dry weight was also measured in cv. Maris Piper 0, 24 and 72h after treatment; dry weight was significantly lower in the flesh of tubers treated with 10 kJ m\(^{-2}\) 24 h after treatment (211.8 mg g\(^{-1}\) FW) compared with the untreated tubers (241.1 mg g\(^{-1}\) FW) (Figure 4.5). There was an overall significant difference between tubers treated at harvest (skin, 234.3 mg g\(^{-1}\) FW; flesh, 259.8 mg g\(^{-1}\) FW) and 10% eye movement (skin, 223.3 mg g\(^{-1}\) FW; flesh, 241.9 mg g\(^{-1}\) FW) for both flesh and skin.

![Graph showing dry weight of unwashed potato skin and flesh cv. Maris Piper treated with 0, 10, 20 or 30 kJ m\(^{-2}\) UV-C applied at harvest (pre) or at 10% eye movement (eye) and stored for 13 weeks at 9°C.]

**Figure 4.4** Dry weight (mg g\(^{-1}\) FW) of unwashed potato skin and flesh cv. Maris Piper treated with 0, 10, 20 or 30 kJ m\(^{-2}\) UV-C applied at harvest (pre) or at 10% eye movement (eye) and stored for 13 weeks at 9°C.
4.2 Biochemical analysis

In the final year trial, sugar and phenolic concentrations were measured separately in the tuber skin and flesh for cv. Maris Piper only. Also, sugar and phenolic content was measured immediately after treatment and after 24 and 72 h.

4.2.1 Phenolics

Chlorogenic acid was almost 2-fold higher in the flesh of cv. Maris Piper following treatment with 30kJ m$^{-2}$ irrespective of the timing although this was not mirrored in the potato skin. The chlorogenic acid content of the flesh treated at 10% eye movement was
approximately 5-fold lower than tubers treated at harvest, throughout storage and in the days after treatment although this is unlikely to be due to the treatments themselves since the control was also affected similarly (Figure 4.6 and 4.7).

Caffeic acid was below the limit of detection in the flesh of tubers cv. Maris Piper therefore only the skin levels have been reported. There was no significant difference in caffeic acid between tubers treated with different doses of UV-C. Caffeic acid did increase 2-fold 72h after treatment however no difference between the doses was observed (Figure 4.9). Caffeic acid also increased in the skins during storage, peaking at 8 weeks storage (Figure 4.8).

**Figure 4.6** Chlorogenic acid (µg g⁻¹ DW) of unwashed potato skin and flesh cv. Maris Piper treated with 0, 10, 20 or 30 kJ m⁻² UV-C applied at harvest (pre) or at 10% eye movement (eye) and stored for 13 weeks at 9°C.
Figure 4.7 Chlorogenic acid (µg g⁻¹ DW) of unwashed potato skin and flesh cv. Maris Piper 0, 24 and 72 h after treatment with 0, 10, 20 or 30 kJ m⁻² UV-C at harvest (pre) or at 10% eye movement (eye).
**Figure 4.8** Caffeic acid (µg g\(^{-1}\) DW) of unwashed potato skin cv. Maris Piper treated with 0, 10, 20 or 30 kJ m\(^{-2}\) UV-C applied at harvest (pre) or at 10% eye movement (eye) and stored for 13 weeks at 9°C.

**Figure 4.9** Caffeic acid (µg g\(^{-1}\) DW) of unwashed potato skin cv. Maris Piper 0, 24 and 72 h after treatment with 0, 10, 20 or 30 kJ m\(^{-2}\) UV-C at harvest (pre) or at 10% eye movement (eye).
4.2.2 Sugars

Glucose and fructose concentrations increased steadily throughout storage in both the skin and flesh and flesh only, respectively, of tubers cv. Maris Piper (Figure 4.10 and 4.12). Skin glucose and fructose content increased by approximately 2-fold within 24 h of treatment with 10 kJ m$^{-2}$ (irrespective of treatment timing) which mirrored that of the control tubers (Figure 4.11 and 4.13). This same trend was not observed in the glucose and fructose content of tubers treated with 20 or 30 kJ m$^{-2}$ which remained steady within 72 h of treatment. The glucose and fructose content of the tuber flesh also increased by approximately 4-fold and 2-fold in response to 10 or 20 kJ m$^{-2}$ UV-C treatment, respectively, however this was only observed in the tubers treated at 10% eye movement. Glucose and fructose levels then returned back to baseline levels within 72 h of treatment. There was no effect of UV-C dose on sucrose content (Figure 4.15). Sucrose content increased in the tuber skin during storage from 8.0 – 13.1 mg g$^{-1}$ DW yet decreased in the tuber flesh from 11.7 – 9.1 mg g$^{-1}$ DW during the 13 weeks at 9°C (Figure 4.14).
Figure 4.10 Fructose content (mg g\(^{-1}\) DW) of unwashed potato skin and flesh cv. Maris Piper treated with 0, 10, 20 or 30 kJ m\(^{-2}\) UV-C applied at harvest (pre) or at 10% eye movement (eye) and stored for 13 weeks at 9°C.
**Figure 4.11** Fructose content (mg g\(^{-1}\) DW) of unwashed potato skin and flesh cv. Maris Piper 0, 24 and 72 h after treatment with 0, 10, 20 or 30 kJ m\(^{-2}\) UV-C at harvest (pre) or at 10% eye movement (eye).
Figure 4.12 Glucose content (mg g\textsuperscript{-1} DW) of unwashed potato skin and flesh cv. Maris Piper treated with 0, 10, 20 or 30 kJ m\textsuperscript{-2} UV-C applied at harvest (pre) or at 10% eye movement (eye) and stored for 13 weeks at 9°C.
Figure 4.13 Glucose content (mg g⁻¹ DW) of unwashed potato skin and flesh cv. Maris Piper 0, 24 and 72 h after treatment with 0, 10, 20 or 30 kJ m⁻² UV-C at harvest (pre) or at 10% eye movement (eye).
Treated at harvest  
Treated at 10% eye movement

![Line graphs showing sucrose content (mg g\(^{-1}\) DW) of unwashed potato skin and flesh cv. Maris Piper treated with 0, 10, 20 or 30 kJ m\(^{-2}\) UV-C applied at harvest (pre) or at 10% eye movement (eye) and stored for 13 weeks at 9°C.]

**Figure 4.14** Sucrose content (mg g\(^{-1}\) DW) of unwashed potato skin and flesh cv. Maris Piper treated with 0, 10, 20 or 30 kJ m\(^{-2}\) UV-C applied at harvest (pre) or at 10% eye movement (eye) and stored for 13 weeks at 9°C.
Figure 4.15 Sucrose content (mg g\(^{-1}\) DW) of unwashed potato skin and flesh cv. Maris Piper 0, 24 and 72 h after treatment with 0, 10, 20 or 30 kJ m\(^{-2}\) UV-C at harvest (pre) or at 10% eye movement (eye).

4.3 Hormones

Flesh samples were analysed for hormone content 0, 24 or 72 h after treatment either at harvest (Pre) or at 10% eye movement (Eye). Zeatin (Z), zeatin riboside (ZR2), dihydrophaseic acid (DPA), phaseic acid (PA) abscisic acic (ABA) and ABA metabolite (ABA-GE) were found in flesh samples cv. Maris Piper. ABA, ABA-GE, and Z increased from harvest to 10% eye movement coinciding with dormancy to dormancy break, respectively. ABA, ABA-GE and Z increased from 10.73, 152.4 and 990.0 to 18.59, 176.0 and 1307.0 ng g\(^{-1}\) DW, respectively. In contrast, DPA decreased during the first few weeks
after harvest by approximately one half. The ZR2 concentrations were very low with an average of 4.32 ng g\(^{-1}\) DW and no differences were observed between doses or over time. Also, the levels of PA were even lower at 1.98 ng g\(^{-1}\) DW therefore results are not included.

Differences in response to the variable UV-C doses were observed in ABA concentration in the 24 and 72 h after treatment at harvest. ABA remained constant at around 13-14 ng g\(^{-1}\) DW in potato tubers treated with 10 or 20 kJ m\(^{-2}\). In contrast, the untreated tubers and tubers treated with 30 kJ m\(^{-2}\) increased between 24 and 72h by approximately 30\% (Figure 4.16). There was also a significant difference in DPA between UV-C doses as tubers treated at harvest with 20 kJ m\(^{-2}\) contained significantly higher DPA compared with the control (Figure 4.17).

![Graph showing ABA concentration in tuber flesh cv. Maris Piper 0, 24 and 72 h after treatment with 0, 10, 20 or 30 kJ m\(^{-2}\) UV-C (average of at harvest or at 10% eye movement data).](image)

**Figure 4.16** ABA concentration in tuber flesh cv. Maris Piper 0, 24 and 72 h after treatment with 0, 10, 20 or 30 kJ m\(^{-2}\) UV-C (average of at harvest or at 10% eye movement data).
**Figure 4.17** DPA concentrations in tuber flesh cv. Maris Piper after treatment with 0, 10, 20 or 30 kJ m$^{-2}$ UV-C (average of 0, 24 and 72 h data) at harvest or at 10% eye movement.

4.4 CPD analysis and DNA fragmentation

There were significant differences in CPD-DNA according to UV-C dose and a significant interaction between dose and time after treatment. DNA damage was highest in the tubers treated with 20 kJ m$^{-2}$ at harvest (Figure 4.18) followed by tubers subjected to 10kJ m$^{-2}$ at 11.6 and 12.55 ng, respectively (0.58 and 0.63% of the total DNA, respectively). The DNA damage of tubers treated with 30kJ m$^{-2}$ was less than that of the two other treatment doses yet still higher than the control.

DNA fragmentation was assessed by running purified DNA from tuber skin on a 1.8% agarose gel. The results showed that treatment with 30 kJ m$^{-2}$ was not sufficient to result in apoptosis since no DNA fragmentation was observed in the gel (Figure 4.19).
**Figure 4.18** CPD-DNA (ng) in unwashed potato skin cv. Maris Piper treated with 0, 10, 20 or 30 kJ m\(^{-2}\) UV-C at harvest (pre).

**Figure 4.19** DNA fragmentation assay showing intact genomic DNA. DNA purified from tuber skin of potatoes 0, 24 and 72h after treatment with 0, 10, 20 or 30 kJ m\(^{-2}\). No fragmentation indicates no cell apoptosis as a result of the UV treatments.
4.5 Multivariate analysis

Principle component analysis (PCA) of chlorogenic acid, caffeic acid, sucrose, glucose, fructose, dry weight, and sprout length and number of tuber flesh cv. Maris Piper analysed 5 and 10 weeks after 10% eye movement in order to include the sprout length and number in the data set. Tubers treated at harvest and at 10% eye movement were clustered separately along PC1 and PC2 (Figure 4.20). Tubers treated at 10% eye movement were clustered towards the top right of the PCA indicating lower sugar content and phenolic acid content compared with tubers treated at harvest. PCA was also carried out on the whole flesh and skin data set including tuber data at harvest, at 10% eye movement plus 5 and 10 weeks after 10% eye movement. Since there was no sprout data for the first two data points, only phenolic acid, sugar data, and dry weight could be included. Data clustered according to tuber flesh and skin with the clustering of the skin indicating higher sucrose and phenolic acid content and the clustering of the flesh indicating higher dry weight than the skin and a small sample of flesh samples with high fructose and glucose content (Figure 4.21).

Hormone analysis was carried out on the flesh samples taken 0, 24 and 72h after treatment at harvest and at 10% eye movement. Principle component analysis on this data set, including hormone concentrations, phenolic acids, sugars and dry weight, clustered the data according to treatment timing (Pre and Eye) although the control clustered with the treatment doses suggesting the differences were due to the time after harvest rather than a dose response. The analysis confirms the results of the ANOVA data showing an increase in ABA and ABA-GE and a decrease in DPA between harvest (pre) and dormancy break at 10% eye movement (Figure 4.22).
Figure 4.20 Principle component analysis of dry weight, phenolic acids, sugars and sprout growth of tubers flesh cv. Maris Piper treated with UV-C at harvest (Pre) or 10% eye movement (Eye), stored at 9°C and analysed 5 and 10 weeks after 10% eye movement.

Figure 4.21 Principle component analysis of dry weight, phenolic acids and sugars of tuber flesh (F) and skin (S) cv. Maris Piper treated with UV-C at harvest or 10% eye movement and stored for 13 weeks at 9°C.
5. DISCUSSION

The extension of potato storage through sprout suppression is reliant on the application of the chemical CIPC. The aim of this project was to extend potato storage life through the use of non-chemical means to possibly reduce chemical residues from CIPC. Results from this study have shown that treatment with the higher doses of UV-C; 10, 15 and 20 kJ m\(^{-2}\) reduced sprout length in a number of cultivars yet 30 kJ m\(^{-2}\) was too high resulting in increased sprouting. It has been found previously (Burns and Terry, unpublished) that treatment with 5.4 kJ m\(^{-2}\) was a successful potato sprout suppressant. However, the tubers treated by Burns and Terry (unpublished) were washed whereas the tubers herein treated with 5 kJ m\(^{-2}\) (first year only) were not washed prior to treatment. In the final year, both washed and unwashed tubers were assessed yet there was little effect of soil debris on the effect of UV-C on sprout growth. This said, tubers cv. Maris Piper treated at 10% eye movement with 10 or 20 kJ m\(^{-2}\) UV-C was especially effective when the tubers were unwashed suggesting

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**Figure 4.22** Principle component analysis of dry weight, hormones, phenolic acids and sugars of tuber flesh cv. Maris Piper 0, 24 and 72h after treatment with 0, 10, 20 or 30 kJ m\(^{-2}\) UV-C at harvest (Pre) or at 10% eye movement (Eye).
washing may in fact have a deleterious effect on sprout growth in some cultivars. Tubers cv. VR808 were also negatively affected by the washing procedure resulting in a higher sprout number. The success of the lower UV-C dose found by Burns et al. (unpublished) may therefore be due to other factors such as skin thickness, soil composition and other factors relating to growing site and weather conditions. This study suggests that it is not essential for the potatoes to be washed prior to treatment and that UV-C can be used on both fresh loose potatoes which are often not washed before sale as well as packaged washed potatoes. The amount of soil on the potatoes lessened throughout storage as the skins dried out and may therefore have been less severe when treated at 10% eye movement. Even so, treatment at harvest, when the soil was at its wettest and thickest, reduced sprout growth compared with the control in several cultivars.

UV-C treatment at harvest and at 10% eye movement resulted in reduced sprout growth, yet the mechanism by which this sprout suppression was achieved could differ depending on the timing. Sprout suppression as a result of UV-C treatment at harvest could not have been due to direct physical damage of the eye tissue since eye opening had yet to occur. However, the CPD ELISA assay showed periderm DNA damage which may have triggered sprout suppression through a hormonal response to that damage. Treatment with UV-C at eye movement may also result in sprout suppression through systemic hormonal changes however, sprout suppression was achieved in a greater number of cultivars when applied at eye movement which may have been due to a combined systemic and local effect on exposed meristematic (sprout) tissue. It must be noted that this improved treatment effect at eye movement may be a result of the difference in the amount of soil attached to the tubers at different stages of storage although differences between washed and unwashed tubers was minimal. In addition, respiration rate was not affected by UV-C irradiation suggesting the effect of UV-C irradiation may not be immediate. In the final year trial, a time course after treatment (0, 24 and 72h) at harvest and at 10% eye movement was carried out using Maris Piper to look at immediate effects in addition to the long term effects throughout storage. There were significant differences in biochemistry and especially the hormones between the tubers at harvest and 2 weeks later at 10% eye movement as demonstrated by the multivariate analysis which may be an important factor into to the success of the treatment at eye movement over treatment at harvest.
It has not previously been investigated whether the sprout suppression of potato as a result of UV-C irradiation (Burns and Terry, unpublished) is a result of systemic changes or direct DNA damage, especially to actively growing tissue. Potato skin is robust therefore it is possible that UV-C irradiation does not penetrate the potato skin but only effects the meristematic and periderm tissue. Physical damage was measured in the final year trial by quantification of cyclobutane pyrimidine dimer production, the main product of DNA UV damage, in tuber skin following treatment at harvest. The analysis of CPD-DNA damage in the tubers treated at 10% eye movement are not yet complete but will be carried out prior to publication as this data is important to understand whether the improved sprout suppression when treated at 10% eye movement is related to a difference in DNA damage. This work is in addition to the agreed project milestones. Results to date showed that treatment with 10 or 20 kJ m\(^{-2}\) at harvest resulted in higher CPD-DNA damage compared with the control. CPD-DNA damage is usually repaired by nucleotide excision repair or base excision repair; however, severe irreversible damage can result in apoptosis. The lower CPD-DNA damage found in the tubers treated with 30 kJ m\(^{-2}\) in the final year trial may have been too extensive and therefore resulted in damage beyond that of dimer production. The failure of the higher treatment to reduce sprout growth may have been due to this extensive damage. Extensive damage can result in 6,4 pyrimidine-pyrimidones which are more mutagenic and can lead to apoptosis. It was hypothesised that the 30 kJ m\(^{-2}\) may have resulted in apoptosis and therefore a stress response since an increase in phenolic content was also observed. This apoptosis may have resulted in dormancy break and therefore an increase in sprout growth. This said, extracted DNA taken from the potato skin was purified and run on a 1.8% agarose gel to identify fragmentation which is a clear indicator of apoptotic activity. Results showed that no apoptosis was present immediately after treatment (0h), after 24 h and 72 h after treatment. In the first year, application of 15 kJ m\(^{-2}\) (highest dose in year 1) was also less effective at reducing sprout growth compared with the lower doses, however, in the final year 20 kJ m\(^{-2}\) was an effective sprout suppressant suggesting preharvest factors may affect the optimal dose. This said, 10kJ m\(^{-2}\) was effective in both years and on a range of cultivars especially cv. Maris Piper.

The difference in the response of each cultivar to the UV-C doses may be due to eye depth which is most shallow in cv. Maris Piper. The NIAB (National Institute of Agricultural
Botany) scores for eye depth range from 1-9 with 9 being the most shallow. Maris Piper was scored high at 8 whereas Hermes, Saturna and Cabaret were scored 3, 4 and 6, respectively. In the first year trial, sprout growth and number of Hermes and Saturna were not affected by the UV-C dose which may be due to the deep eye tissue whereas the affect of UV-C on cv. Maris Piper, which has shallow eye depth, was successful in both years. The efficacy of UV-C as a sprout suppressant may therefore only be appropriate for certain cultivars with a shallow eye depth.

Destefano-Beltran et al (2006) measured ABA concentration throughout the storage of Russet Burbank tubers and found that ABA increased over 100 ng g⁻¹ FW (approximately 15 ng g⁻¹ DW) in the first 25 days of storage before subsequently decreasing steadily by approximately one half after 150 days (3°C). This is in agreement with the results herein where an increase in ABA was observed in the first 12 days of storage up until 10% eye movement. In the tubers treated with 10 or 20 kJ m⁻² (doses which resulted in sprout suppression), the ABA concentration remained constant in the first 12 days of storage whereas the ABA content of tubers treated with 30 kJ m⁻² or untreated increased by around 30%. Sustained ABA biosynthesis and action are required for tuber dormancy induction and maintenance (Suttle and Hultstrand, 1994). Therefore, the consistent ABA content of the tubers treated with 10 or 20 kJ m⁻² may be an indication of dormancy maintenance and therefore extension of sprout suppression prior to a later sharp increase and subsequent decrease around the initiation of sprout growth. These results therefore suggest that the effect that the UV-C has on the potato skin cells may result in the maintenance of ABA concentrations and result in the extension of endodormancy or the break of endodormancy and subsequent ecodormancy (sprout suppression) phase. ABA is metabolised by potato tubers into DPA and PA (Suttle, 1995) therefore it follows that an increase in DPA in the first few weeks of storage would result in a decrease in DPA. The concentration of DPA was found to be higher in the tubers treated at harvest with 20 kJ m⁻² presumably due to the breakdown of excessive ABA in order to maintain the consistent levels described above.

An increase in cytokinin content and sensitivity was found to be a main factor leading to the loss of dormancy (Suttle 2004a) and in particular exogeneous cis-zeatin has been found to be effective at breaking tuber dormancy. Moreover, endogenous cis-zeatin increased prior to the onset of sprout growth, unlike its riboside (Suttle and Banowetz, 2000). An increase in
zeatin was observed between harvest and 10% eye movement which agrees with the above. This said, no difference in zeatin between treatment doses was observed suggesting zeatin unlike ABA, may not be affected by UV-C treatment.

In conclusion, this study has shown that treatment at harvest or at 10% eye movement with UV-C at a dose of 10 kJ m^{-2} suppressed sprout length and sprout number in a range of cultivars grown in two separate seasons. Although treatment at harvest and at 10% eye movement were successful, multivariate analysis suggests that treatment at eye movement may prevent deleterious characteristics. Results of the ELISA assay suggest that 10 and 20 kJ m^{-2} results in CPD damage to the potato skin DNA and that this may be having an effect on the biochemistry and in particular hormonal changes in the tuber flesh. The hormone quantification results suggest that ABA may be involved in UV-C induced sprout suppression.

Patent and Publication plan

- A patent and paper including all results from Objective 3 will be published in the coming months with the preliminary title of ‘The effect of differential UV-C doses on the biochemistry and sprouting of potato tubers during long term storage’ Cools, K., Alamar Gavidia, M.C. and Terry, L.A.

Future work

- CPD-DNA quantification was only carried out on the samples treated at harvest. Repetition of the ELISA assay on the tubers treated at 10% eye movement would be beneficial for publication and will be carried out in addition to the project milestones.

- Full metabolomic profiling and/or transcriptional analysis of potatoes treated with differential UV-C doses to elucidate the mechanism by which UV-C inhibits sprout growth.

- Eye tissue (electron) microscopy to identify changes in the cell structure since higher UV-C dose resulted in an increase in sprout number.
6. REFERENCES


Foukaraki S.G., Chope G.A. and Terry L.A. (2010) Ethylene exposure after dormancy break is as effective as continuous ethylene to control sprout growth in some UK grown potato cultivars. 28th International Horticultural Congress 22-27th August 2010, Lisbon, Portugal


Appendix A

Photographs of potato tubers cvs. Cabaret, Hermes, Russet Burbank, Saturna, VR808 and Maris Piper 10 weeks after 10% eye movement from the first year trial.

**Cabaret**
Hermes
Russet Burbank
Saturna
Maris Piper