Ultrastructural and Physiological Aspects of Bruising in Potatoes

Teresa Edgell

A thesis submitted in partial fulfilment of the requirements of The Nottingham Trent University for the degree of Doctor of Philosophy

September, 2000

Declaration

The author has not been a registered candidate nor an enrolled student for another award of the Nottingham Trent University or other academic or professional institution during this research programme. Material contained in this thesis has not been used in any other submission for an academic award and is entirely the author's contribution. Due acknowledgement is made of assistance received. The author has attended appropriate lectures, seminars and conferences in partial fulfilment of the requirements of the degree.

Signed:..... J. Edgell
(Candidate)

Signed:..... A H COBB
(Director of Studies)

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Abstract

To establish what influences blackspot susceptibility in potato tubers, physiological and biochemical parameters have been investigated in cultivars, Pentland Dell, Maris Piper and Record, during long term storage.

Measurements of respiration, dopachrome formation, cation leakage and bruise susceptibility, were made at regular intervals through storage. Results indicated that the biochemical potential for producing melanin (dopachrome formation at 475 nm) did not correlate with measurements of bruise susceptibility, (bruise severity following impact). This indicated that changes in tuber biochemistry did not influence blackspot susceptibility through storage and that tuber physiology and structural integrity played an important role. Cultivar had a significant influence on tuber physiology and on blackspot susceptibility. Maris Piper was less susceptible to blackspot bruise formation than Pentland Dell and Record, this correlated with a low respiration rate, cation leakage and potential dopachrome formation.

Temperature at impact had a significant effect on bruise susceptibility. Tubers impacted at 5 °C displayed a greater percentage bruise formation than tubers impacted at 10 °C.

Time-course investigations following impact found that respiration and potassium, magnesium and calcium ion leakage increased immediately following impact whilst turgor declined. These responses occurred in particular following impact during early storage.

Tubers of Pentland Dell were examined following impact using transmission electron microscopy. An ultrastructural sequence of events was highlighted which occurred during blackspot formation, firstly a collapse of intracellular compartmentation, followed by an apparent increased ribosomal and mitochondrial abundance and a darkening of the cytoplasm with the development of melanin.

Ultrastructural investigations found that at harvest impacted tubers developed microscopic shatter, the tonoplast was disrupted showing vesicularisation, with increased numbers of mitochondrial sections and nuclear invaginations. Conversely following storage, impacted tubers developed blackspot bruising with melanin forming within the cytoplasm, along the cell wall and surrounding the starch grains.

Acknowledgements

I would like to acknowledge the Ministry of Agriculture, Fisheries and Food for financial support, ADAS Arthur Rickwood and Terrington for provision of tubers and the British Potato Council, formerly the Potato Marketing Board, for storage facilities. In particular, Jeremy Wiltshire and the staff at Sutton Bridge Experimental Station for their help and Chris Hole for advice on water relations.

I acknowledge the help of all the technical staff at NTU for their support and maintenance of equipment. In particular Dave Lacey for his help and advice on microscopy techniques.

I also wish to thank Andy Cobb for expert supervision, Ewen Brierley for all his help, Ev for his support and invaluable computing knowledge. Also to the various members of the lab who provided help during the 3 years research.

List of Contents

Publications from This Thesis

Edgell T & Cobb AH (1996). An ultrastructural study of potato bruising. Abstracts of Conference Papers, Posters and Demonstrations of the 13th Triennial Conference of the European Association for Potato Research. pp 534-535.

Edgell T, Brierley ER & Cobb AH (1998). An ultrastructural study of bruising in stored potato (*Solanum tuberosum* L.) tubers. *Annals of Applied Biology*, 132:143-150.

Edgell T & Cobb AH (1998). An ultrastructural comparison of blackspot bruise and shatter bruise in potato (Solanum tuberosum) tubers. *Aspects of Applied Biology*, 52: 315-320.

Abbreviations

3D = 3 dimensional
aq = aqueous
cm = centimetres
cv/s = cultivar/s
dwt = dry weight
Fig = Figure
fwt = fresh weight
g = grammes
hr/s = hour/s
IRGA = Infra-red gas analyser
J = Joules
LAH = lipolytic acyl hydrolase
LOX = lipoxygenase
m = meter
M = Molar
min/s = minute/s
ml = millilitres
mm = millimetres
mM = milli molar
MPa = Mega Pascals
nm = nano meters
P = turgor pressure
PPO = polyphenol oxidase
PVC = polyvinyl chloride
RH = relative humidity
rpm = revolutions per minute
RWC = relative water content
s = seconds
S.E. = standard error
TEM = transmission electron microscopy
v/v =volume to volume

w:v = weight : volume ratio

wt = weight

wks = week

μV = micro Volt

μm = micro moles

μM = micro Molar

μl = microlitre

ºC = degrees Centigrade

ºK = degrees Kelvin

Ψ = water potential

π = osmotic potential

1.0 Introduction

1.1 The Potato Crop

The potato plant (*Solanum tuberosum* L.) is an annual, herbaceous dicotyledon (Kleinkopf, 1983). It is a solanaceous plant belonging to the large genus *Solanum*. There are over 1000 species of *Solanum* across the world (Berrie, 1977), less than a tenth of which are tuber bearing (Burton, 1989). The Solonaceae family includes a number of other cultivated plants including *Solanum melongena* L., the aubergine, *Capsicum* spp, the chilli peppers and *Lycopersicon esculentum* Mill., the tomato (Hawkes, 1992).

Archaeological evidence and accounts chronicled by the Spanish following their 16th century conquest of Mexico and Western South America indicate that the potato has its origins in South America (Kleinkopf, 1983; Burton, 1989; Hawkes, 1992). The plant, known to the Spanish by the Inca word papa, was first cultivated in the highlands of the areas now known as south Peru and western Bolivia, where a number of similar wild species exist (Hawkes, 1992).

The earliest established date for the potato in continental Europe is 1587 in Belgium (Burton, 1989), although it is widely assumed that it was first introduced prior to this by the Spanish around 1570 (Hawkes, 1992). The Europeans used the Indian name batata for the potato which also included the sweet potato *Ipomea batatas* L. and from this came patata and finally potato (Hawkes, 1992). From Spain, the potato was sent to Venice and Belgium as a botanically interesting specimen from which it spread through continental Europe and eventually into Asia (Hawkes, 1992). It later became widely distributed throughout Europe becoming a staple food in many areas by the late 1700's (Kleinkopf, 1983). Evidence taken from herbaria indicates that this early potato of the 16th and 17th centuries was *S. tuberosum* ssp *andigena*, which would have produced only small tubers late in the season under the long summers of Western and Northern Europe. Artificial selection for earliness resulted in the subspecies *tuberosum* that would have produced an adequate crop. *S. tuberosum* ssp *tuberosum* is the plant that we cultivate today (Hawkes, 1992).

The first recorded mention of the potato in England is by Gerard in 1596 (Burton, 1989) and from England it spread to Ireland, Scotland, Wales and the British colonies, as well as northern Europe. At this time a number of different edible tuber bearing plants were known also as potatoes, including the sweet potato, *Ipomea batatas* and the Jerusalem artichoke, *Helianthus tuberosus* L. Once *S. tuberosum* became widespread throughout the British Isles, the name potato was adopted to describe this species and no others.

It is likely that the potato was first cultivated in the Peruvian Andes, long before the time of the Spanish colonists (Hawkes, 1992) and there is some evidence to suggest that the potato was grown as a crop in Italy by the beginning of the 17th century. However, the potato only became firmly established as a European food crop in the 18th century and was not popular in England until the latter half of the century. In Ireland, agricultural experiments were carried out by landlords and, once introduced, the potato was found to be a high yielding success. By the late 18th century potato production was well established and the Irish were dependent on the crop as a food source as well as an economy (Burton, 1989). Dependence upon the potato made the consequences of crop failure more devastating. Potato blight, *Phytophthora infestans* (Mont.) de Bary, spread throughout Ireland in the mid nineteenth century, resulting in a million deaths and 1.5 million emigrations out of a total population of 8 million. Crop failures caused a loss of confidence in the potato throughout Europe, although popularity and cultivation increased again during the late 19th century.

At the turn of this century world potato production was 135 million tonnes a year, 90 % of which was produced by Europe, by 1950 this had increased to 250 million tonnes a year (Horton & Anderson, 1992). Following World War 2 the production of potatoes declined in Europe, the former USSR (Horton & Anderson, 1992) and the United States (Walker, Schmiediche & Hijmans, 1999), but increased elsewhere. Since 1960 the total world production of potatoes has fallen by 20 % masking the increase in developing countries by 70 % (Horton & Anderson, 1992).

During the latter half of this century there has been a major growth in processing potatoes (Burton, 1989). During the war dried potato products

were important due to them being lighter and non-perishable compared with the fresh vegetable. By 1970 approximately half the potatoes grown in the USA were processed. In the UK between 1990 and 1996 an average of approximately 24 % of UK produced potatoes were processed (BPC, 1997).

Potato tubers are a good source of carbohydrate, protein, vitamins and in particular vitamin C and B group vitamins (Mondy and Ponampalam, 1986; Storey and Davies, 1992) and minerals including iron (Storey and Davies, 1992; Ereifej, Shibli, Ajlouni and Hussein, 1997). Approximately 10 % of the dry matter of tubers is protein (Burton, 1989) which makes an important contribution to total protein consumption, particularly in areas of Eastern Europe (Millard, 1986). Tubers also provide fibre and are a good source of lysine, although they are low in the sulphur containing amino acids such as methionine (Storey and Davies, 1992).

The potato is currently the world's fourth largest food crop (Preston, Glynn, Orr & Hofman, 1996) and therefore of considerable economic importance. It is grown throughout the world, wherever the growing season is long enough to produce good yields. The majority of potato production occurs in Europe and the former USSR (Storey and Davies, 1992), but areas of cultivation also include Africa, America, Asia and Oceania (BPC, 1997a). The average global production between 1991 and 1995 was estimated at approximately 268 million tonnes a year (BPC, 1997).

1.2 Harvest, Curing and Storage of the Potato Crop

Growth and harvest of the potato crop is restricted by climate. In Britain, harvest of the main crop takes place in September and October after the crop has finished bulking and before the temperature drops too low (Burton, 1989). Tubers destined for storage are then "cured" (stored at 12-15 °C for up to 2 wks to allow wound healing) before being placed into storage.

1.2.1 Harvest

At harvest potato tubers are mechanically lifted from the ground using a harvester. A number of factors must be taken into consideration if harvest is to be successful.

Firstly, the timing of harvest is fundamental to the quality of tubers, particularly if they are to be stored. Harvest should take place as early as possible following bulking and skin set, when both the soil and weather are suitable (i.e. dry weather and moist soil are preferable) (Ward, 1990; PMB, 1996). Harvesting before mid October reduces disease in store (PMB, 1996a). Harvest temperature is also important, since tuber susceptibility to damage increases at low temperatures (Smittle, Thornton, Peterson and Dean, 1974; Gray & Hughes, 1978; Rogers-Lewis, 1980; Brook, 1996; Peters, 1996). A temperature between 10 and 15 °C is recommended for lifting tubers with minimum damage (PMB, 1996).

The majority of damage to potato tubers occurs at harvest (McRae, 1980; Peters, 1996; Siim, 1996). A survey carried out by the Potato Marketing Board in the 1970's found a third of tubers were damaged by the time they reached the store (Gray & Hughes, 1978). A highly mechanised system is necessary to meet the demand for potatoes (Molema, Klooster, Verwijs, Hendriks & Breteler, 1997), hence tubers are mechanically lifted and transferred along conveyors from harvester to trailer invariably involving impacts and drops (McRae and Fleming, 1989).

In order to produce tubers with a minimum of damage, timing, tuber temperature and harvester operation must all be taken into consideration, however often a farmer's main priority is speed and consequently tubers incur damage.

1.2.2 Curing

Following harvest, tubers are transported to the potato store for curing and storage. Prior to loading into store, loose soil is removed from the tubers. This allows effective temperature control, removal of a source of microorganisms and an even spread of any chemicals which may be added during storage.

If tubers have been lifted under very wet conditions it may be necessary to dry the crop prior to curing (Rastovski, 1987). This involves ventilation and should be halted as soon as the tuber surface moisture has been removed (PMB, 1996).

Until the mid 1980's, "curing", or wound healing, involved leaving tubers in a closed store at approximately 15 ºC for 2 weeks, allowing relative humidity to reach 95 %, ideal for curing as tuber moisture loss is reduced (Cunnington, 1996; PMB, 1996). This process may now be modified, especially where tubers in store are up to 4.5 m deep (Ward, 1990). Tubers can be dry cured by reducing the humidity (Cunnington, 1996) and thus reducing the spread of disease such as bacterial soft rot (PMB, 1996). In addition, tubers are ventilated for a few hours each day when outdoor temperatures are around 15 ºC and humidity is low. This is to reduce temperature variations within the stored tubers and maintain a lower relative humidity around 65 – 80 % (Ward, 1990). Increased temperature during tuber curing (optimally between 12-15 ºC) allows wound healing by suberisation, thus minimising infection and weight loss during storage (Rastovski, 1987; PMB, 1996). The warmer the temperature of the tubers during curing, the faster suberisation will occur such that for 2 layers of wound periderm to form takes 4-9 wks at 5 ºC, but only 9-16 days at 10 ºC (Ward, 1990).

Curing and indeed storage are not recommended if some of the crop display signs of blight or soft rots, if there has been severe damage during harvest or if the crop is wet (Ward, 1990). This is because the increase in temperature and humidity may cause disease to spread (Ward, 1990; PMB, 1996) resulting in the loss of the entire crop.

Following curing, temperature in store is cooled to the holding temperature for storage as quickly as possible. The time for temperature to be reduced is taken into account in the curing time (Ward, 1990).

1.2.3 Storage

Once tubers have been harvested in the autumn no further UK crops are available until the following June, when the first earlies are ready for harvest. Tuber storage is therefore critical to supplying market demands with

quality tubers all year round. In the UK approximately 1.5 million tonnes of potatoes are stored annually for up to 10 months for the processing industry (PMB, 1997). Potato tubers are held in specialised stores which should be capable of temperature control and ventilation. A potato store should be designed so that handling of tubers is easy to allow application of chemicals and condensation should be kept to a minimum (Rastovski, 1987).

Conditions within the potato store are carefully controlled in order to provide tubers suitable for particular markets. Throughout storage temperature control is very important for maintaining good quality tubers. Temperature influences tuber respiration, reducing sugar accumulation, bud growth, sprouting and moisture loss as well as microbial attack (Rastovski, 1987). Temperature in store is controlled by ventilation systems (which also helps to keep tubers dry and so prevent rots). A number of factors must be taken into consideration when choosing a storage temperature. High temperatures (>10 °C) throughout storage increase the risk of bacterial soft rot, silver scurf, sprouting and softening. However, temperatures that are too low (<4 °C) increase the likelihood of gangrene, skinspot and chilling damage (PMB, 1996), as well as increasing the reducing sugar content of the potatoes by a process known as "low temperature sweetening".

It has been suggested that tubers stored at low temperatures undergo membrane damage including that of the amyloplast envelope (Ohad, Freidberg, Ne'eman & Schramm, 1971). This intracellular membrane damage may be responsible for the increase in starch to sugar conversion resulting in low temperature sweetening. A comparison of reducing sugar content of Pentland Dell tubers stored at 5 °C and 10 °C by Hart, Pallett & Cobb (1986) found a rapid increase in tubers stored at 5 °C during the first 8 wks of storage, whereas reducing sugars in tubers stored at 10 °C did not increase until 18 wks into the storage period (a result of senescent sweetening which occurs as tubers break dormancy, rather than low temperature storage). Low temperature sweetening is one of the main disadvantages to storing at low temperature because it renders tubers unacceptable for processing. It is the result of an increase in reducing sugars, which, with amino acids, are substrates of the non-enzymic Maillard reaction. This process is responsible for the discolouration that can occur

during processing (Wiltshire & Cobb, 1996). The reducing sugars react with amino acids resulting in a dark colouration known as a dark fry colour (Hill, Reimholz, Shroder, Nielsen & Stitt, 1996).

Tubers which have undergone low temperature sweetening can be reconditioned where the tuber temperature is raised to 15-20 ºC for about 2-3 wks during or at the end of storage (Ward, 1990; PMB, 1996). This lowers the amount of reducing sugars that are used up by glycolysis and respiration or upgraded to starch (Wiltshire & Cobb, 1996) enabling the tubers to be used for processing. Successful reconditioning is dependent to some extent on cv, for example Record is more suitable for reconditioning than Pentland Dell (Ward, 1990; Wiltshire & Cobb, 1996). Tubers that have undergone senescent sweetening however, cannot be reconditioned (PMB, 1996). Again, sugar formation due to senescence has been associated with a reduction in membrane quality, in particular that of the amyloplast envelope (Van Es & Hartmans, 1987).

Tubers tend to be stored at different temperatures according to their eventual destination and use (Rastovski, 1987). Thus, tubers grown for general ware will be stored at 7 ºC, while those for pre-packing will be kept between 3-6 ºC. Tubers intended to be used for processing (e.g., crisps and chips) will be stored between 8-10 ºC (PMB, 1996).

Before unloading from store tuber temperature should be raised to about 10 ºC to reduce damage through handling (PMB, 1994). In addition to the temperature, CO_2 accumulation during storage resulting from tuber respiration has a negative affect on fry colour. A short burst of ventilation can easily prevent this effect (PMB, 1997).

Controlled atmosphere storage is becoming increasingly important. Under this system, the air components oxygen and carbon dioxide are governed in particular to aid the breaking of tuber dormancy and to increase progeny tuber numbers in seed potatoes (BPC, 1997).

Humidity can also be controlled in order to reduce moisture losses throughout storage, helping tubers to maintain a high level of turgidity. However, tubers within a store must be kept dry. Moisture caused by condensation, both stack condensation onto the potatoes themselves and structural condensation on to the surfaces of the store, can drip onto the

potatoes and must be dealt with. This can be done by ventilating the store or by covering the tubers with loose straw or synthetic quilts to absorb any moisture (PMB, 1997b).

During long-term storage of potato tubers, chemicals are applied to prevent disease and sprout growth. At lifting, tubers are naturally dormant in that they will not begin to sprout even when placed under ideal conditions. They remain dormant for up to 15 wks (PMB, 1992; Wiltshire & Cobb, 1996) following which they would naturally begin to sprout. This is unacceptable to the market and must therefore be suppressed. Sprouting can be prevented in several ways. Following the natural period of dormancy, sprouting increases with temperature (PMB, 1992). Storing tubers at temperatures below 4 °C enables the provision of sprout free tubers (Ward, 1990; PMB, 1992). However, this results in low temperature sweetening and is therefore unacceptable for use with tubers destined for the processing market because of the resultant darkening of fry colour (PMB, 1992; Wiltshire & Cobb, 1996). Low temperature is only used to control sprout growth in tubers used for seed in which chemical treatments cannot be used (PMB, 1992).

Another method that can be employed to control sprout growth is gamma irradiation. This is used commercially in Japan and is a possibility for other countries. While there may be some adverse effects on the crop, such as an increase in the onset of senescent sweetening, the main problem with this method of sprout control is consumer acceptance.

Currently, chemical control is the most common method of sprout suppression in Britain. Chlorpropham (CIPC) is the most widely used sprout suppressant (PMB, 1996), inhibiting mitosis and hence sprout growth (PMB, 1992; Wiltshire & Cobb, 1996). It is usually applied as a fog but is also available as granules (PMB, 1992; PMB, 1997). Propham (IPC) is also used in conjunction with CIPC and speeds up initial sprout control (Wiltshire & Cobb, 1996). However this chemical is soon to be withdrawn from the market. Both CIPC and IPC are applied following curing as they slow wound healing, which takes place during the curing process. CIPC is applied as soon as there are signs of sprout growth and late treatment results in less satisfactory control (PMB, 1992). Following application, sprout growth is

inhibited for 3-5 months (Ward, 1990) after which further doses can be applied.

An alternative to CIPC and IPC is tecnazene, which inhibits cell division and elongation, acting as a sprout suppressant as well as a fungicide. It does not affect wound healing and is applied to tubers as dust, spray, fog or in granules following which sprouting is controlled for 4-6 months (Ward, 1990). Tecnazene, however, will not control sprouting once dormancy has broken or is about to break (Ward, 1990; PMB, 1992).

Maleic hydrazide, or "Fazor", has been used as a growth regulator on potatoes abroad for many years (Ward, 1990) and has recently been put into use in Britain as a sprout suppressant (Ward, 1990; PMB, 1992). It is applied to the crop as a foliar spray (Wiltshire & Cobb, 1996) normally 3-5 weeks before haulm destruction. This chemical does not provide complete control but does control volunteers.

In addition to sprouting, disease must also be minimised in the potato store. Bacterial diseases are responsible for the greatest losses during storage. Unfortunately, there is currently no effective chemical for control of bacterial disease (Ward, 1990; PMB, 1992). The chemicals iodophor and dichlorophen are marketed as having fungicidal and bactericidal activities (Ward, 1990) despite having no control over bacterial based soft rotting under commercial trials done at Sutton Bridge Experimental Station, UK (PMB, 1992).

Fungicides are used to treat a number of fungal diseases in store. Thiabendazole (TBZ) is the most widely used chemical, suitable for treatment of gangrene and dry rot and of the surface blemishes skin spot and silver scurf (Ward, 1990; PMB, 1992). It is sold on its own as a fungicide (PMB, 1996), with tecnazene (TCNB) providing sprout suppression and with 2-aminobutane which improves control. Resistance to TBZ has been recorded in three pathogens in the UK, *Helminthosporium solani* (silver scurf), *Polyscytalum pustulans* (skin spot) and *Fusarium sulphureum* (dry rot) (PMB, 1996). Alternatively, the closely related chemical, carbendazim, can be used with TCNB as a fungicide/sprout suppressant combination (PMB, 1992). Other options include Fungazil containing the active ingredient imazalil which is active against skinspot, silver scurf, gangrene and dry rot or

alternatively Gambit, containing fenpiclonil, which is used against skinspot, silver scurf, gangrene and black scurf (BPC, 1997). Historically, copper containing compounds e.g. Bordeaux mixture, were used to protect the potato against *Phytophthora infestans* (BPC, 1997). Today, a number of other groups of fungicide are used including the phenylamides, carbamates, phthalonitrile, dithiocarbamate, cinnamic acid derivative, cyanoacethemide, diarylamine and organotins. The number of fungicides available for protection against this organism indicates it's continuing threat to the potato industry.

1.2.4 Tuber Physiology Through Storage

It is important in dealing with the stored tuber to remember that it is a living organ, a stem exhibiting nodes and buds from which the tuber is able to grow into a new plant (Wiltshire & Cobb, 1996).

At harvest tuber respiration is high. This declines following a few wks in store and subsequently remains constant or continues to decline very slowly while the tuber is naturally dormant (Schippers, 1977). Following the dormant phase the tuber buds begin to grow with a further increase in respiration and reducing sugars to fuel sprout growth (Bailey, Philips & Pit, 1978; Wiltshire & Cobb, 1996), unless prevented by artificial control.

The tuber metabolises it's carbohydrate reserves to respire, even whilst dormant, requiring the conversion of starch to sugar (Burton, 1966). This results in some weight loss during storage (Wiltshire & Cobb, 1996). Phosphorylase is thought to be the most important enzyme involved in starch degradation, converting starch into hexose-phosphates, which are then converted to sucrose with the help of sucrose phosphate synthase (SPS). Sucrose may then be hydrolysed to glucose and fructose by invertase (Hill, et al., 1996).

Protein and nucleic acid synthesis also occurs continuously in tuber buds throughout storage (Wiltshire & Cobb, 1996)

When stored at low temperatures tubers respond with an increase in respiration and sugar content (Amir, Kahn & Unterman, 1977). Sugar accumulation may have been important for protecting the tuber from the cold in the time when the potato grew as a wild plant in the Andes. This method of

cold acclimation occurs in a number of plants (Hill et al., 1996). During cold storage there is also a decline in cell membrane integrity resulting in a loss of intracellular compartmentation (Turnbull & Cobb, 1992).

After a prolonged period in store, senescent sweetening may occur. This increase in sugars is the result of the starch to sugar conversion for fuelling sprout growth. Turnbull and Cobb (1992) also noted an increase in mitochondrial abundance as storage progressed.

1.3 Tuber bruising

The darkening seen following impact is a result of melanin formation, an insoluble black pigment that is a product of the polymerisation of the dihydroxy-phenyl compounds (Lerner & Fitzpatrick, 1950; Burton, 1970). The meaning of melanin can be derived from the Greek word melas which translates as black (Lerner & Fitzpatrick, 1950). Bruise formation may be a natural response to damage by pests, disease, damage and stress (Gubb, Hughes, Jackson and Callow, 1989).

Potato tuber bruising is a major economic problem to the potato industry, accounting for annual losses of up to £30 million in the UK (PMB, 1996) and approximately $299 million in the U.S. (Brook, 1996). Bruising damage varies between and within cvs, throughout storage and differs from one year to the next and with changing growth and storage conditions (Gray and Hughes, 1978).

The tubers are most likely to suffer from impacts at harvest through collisions with stones, soil, machinery and other tubers (Evans, 1995). Additional damage occurs in the transport and loading of tubers into store as well as through crushing pressure brought about by the weight of tubers in store (Storey & Davies, 1992).

1.3.1 Structural Characterisation

Potato tubers may exhibit a number of different types of bruise:

Shatter bruise is severe, resulting in splits within the tissue, often extending from the periderm. This includes cracks between cells, membrane

damage and possible cell wall failure (Brook, 1996), allowing a deep blackening in the surrounding areas (McRae & Fleming, 1989).

Crushing bruise, due to the damage brought about by the weight of tubers in store, develops as a large area of discolouration close to the periderm (McRae & Fleming, 1989). These areas of damage tend to be softened and flattened or indented because of the pressure from adjacent tubers and the surfaces of storage vessels (Gray & Hughes, 1978; Lulai, Glynn & Orr, 1996). Lulai et al. (1996) found that pressure bruised areas had higher rates of water vapour loss than control areas of tuber.

Blackspot is a small blue-black (or sometimes brown) bruise occurring primarily in the region of the vascular ring (Boyd, 1951; Burton, 1970; Hughes, 1974; Baritelle, Hyde, Thornton, Bajema, 2000), 1-2mm below the periderm, beneath a layer of cortical cells which are often resistant to discolouration (Kunkel, Gardener and Holstad, 1986), see Figure 1.1. The vascular ring is more susceptible to mechanical injury than cortical cells (Burton, 1989; Brook, 1996), although large bruises may extend into the cortex (Brook, 1996). With blackspot bruising the damage is internal with no obvious splitting or cracking of the tissue. The periderm remains unbroken. Consequently, this type of bruise is difficult to grade out developing 1-3 days following impact (Hughes, 1974a).

It has also been known for a bruised area of tuber to turn white following impact instead of the normal black (McRae and Fleming, 1989; McGarry, Hole, Drew & Parsons, 1996; Baritelle, et al., 2000), this presumably occurs due to a lack of chemical potential for melanin formation. Following impact, damage may also give rise to dry, cork- lined cavities in the vascular region of the tuber (Burton, 1989).

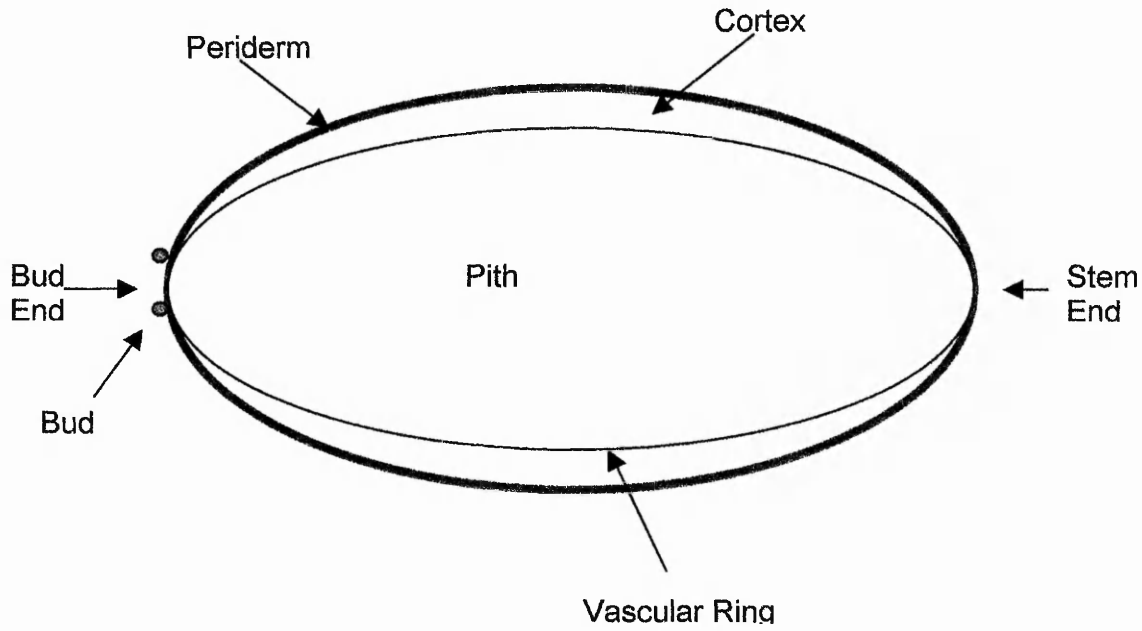


Figure 1.1. Internal anatomy of a potato tuber

1.3.2 Melanin Development

Bruising is the formation of melanin resulting from the enzymatic action of polyphenol oxidase (PPO) with it's substrates when brought together in the presence of oxygen following cellular damage.

During impact, damage to intracellular membranes results in the release of the enzyme lipolytic acyl hydrolase (LAH) from the vacuole and the beginning of the sequence of events resulting in melanin formation (see Figure 1.2). LAH activity is particularly high in potato tubers (Galliard, 1978; Turnbull, 1995). It causes cell membrane degradation by attacking the lipid component of the membranes, including phospholipids, galactolipids and monoacylglycerols (Galliard, 1978). Free fatty acids, the products of LAH activity, stimulate further LAH degradation of membrane bound lipids, and additionally can have a "detergent effect" on the cellular membranes (Galliard, 1978).

Tuber impact

↓

Membrane damage

↓

Release of LAH (patatin) from vacuole

↓

Degradation of cell membrane phospholipids

↓

Production of polyunsaturated fatty acids
(linoleic and linolenic acid -substrates for LOX)

↓

LOX generates oxygen free radicals and ethane

↓

Membrane peroxidation

↓

Release of PPO

↓

Conversion of (major substrate) tyrosine and chlorogenic acid (minor substrate) to DOPA
(addition of a hydroxyl group)

↓ Oxidation

Dopaquinone

↓ Non-enzymic oxidation

Dopachrome (red/orange)

↓ Non enzymic oxidation

5,6 dihydroxy indole (purple)

↓Condensation and polymerisation

Melanin

Figure 1.2. Stages in bruise formation. Key; LAH, Lipolytic acyl hydrolase; FA, Fatty acids; LOX, Lipoxygenase; DOPA, 3,4-dihydroxyphenyl alanine (After Evans, 1995; Brook, 1996).

Further damage is brought about by peroxidation of the intracellular membranes. Following the action of LAH, polyunsaturated fatty acids such as linoleic (18:2) and linolenic (18:3) acid become available for peroxidation by the enzyme lipoxygenase (LOX) (Galliard, 1978) the activity of which has been shown to increase in tuber tissue following wounding (Galliard, 1978). This enzyme generates fatty acid hydroperoxides (Galliard, 1978), oxygen free radicals and eventually ethane (Galliard, 1978) causing further membrane peroxidation. Intracellular membrane damage brings about the union between the copper containing enzyme PPO (also known as tyrosinase) (Lerner & Fitzpatrick, 1950) and it's substrate phenols, primarily the monophenol tyrosine and the diphenol chlorogenic acid (caffeic acid may also play a minor role as PPO substrate). It has been suggested that prior to impact PPO and its substrates are kept apart by intracellular compartmentation, the former being associated with the internal membranes of plastids and the latter located within the vacuole (Hunt, Eannetta, Yu, Newman & Steffens, 1993; McGarry, Hole, Drew & Parsons, 1996).

Figure 1.3 shows the formation of melanin from tyrosine. Firstly, tyrosine is oxidised by PPO resulting in the formation of 3,4-dihydroxyphenyl alanine (DOPA) (Mulder, 1949; Lerner & Fitzpatrick, 1950; Burton, 1970). Further enzymic and non-enzymic oxidative reactions result in the formation of 5,6 dihydroxy indole which undergoes condensation and polymerisation into melanin, the pigment responsible for the dark discolouration found in bruised tissue (Hughes, 1980; Brook, 1996). Intracellular membrane damage caused by impact, allows PPO and its substrates to be brought together, thus initiating melanin formation in the presence of oxygen. Hughes (1980) suggested that the presence of starch grains in tuber cells may be responsible for rupturing intracellular membranes on impact and consequentially starch content of tuber cells (and so percentage dry matter) is thought to be an important component of bruise susceptibility.

1.3.3 Tuber Physiology Associated with Bruising

Following impact there are effects on a number of tuber processes, as might be expected in response to stress and injury. One process is an

increase in metabolism (Burton, 1970), such that respiration rates have been found to increase upon impact (Burton, 1970). Additionally, a number of enzymes have been found to increase following tuber impact, including peroxidase (Burton, 1970) and phenylalanine ammonia lyase (PAL) (Belknap, Rickey & Rockhold, 1990). Phenol content has been found by some workers to increase following impact (Burton, 1970). However no clear correlations between activity of enzymes known to be involved in post-damage metabolism (including PPO, ascorbate peroxidase and lipoxygenase) and bruise susceptibility could be demonstrated in enzyme studies in potato tubers (Croy, Baxter, Deakin, Edwards, Gatehouse, Gates, Harris, Hole, Johnson & Raemaekers, 1998).

The antioxidant ascorbic acid has been found to temporarily increase in concentration following impact (Burton, 1970), an effect that would quench oxygen free radicals.

1.3.4 Factors Influencing Bruising

Tuber susceptibility to bruising is dependent on the morphological, biochemical and physiological characteristics of the tuber, which is why various cvs of potato demonstrate different susceptibilities to bruising. Differences between tubers include the biochemical potential of the tissue to produce coloured oxidation products, the susceptibility of the tissue to structural damage (Hughes, 1974), as well as the intensity of impact. While a number of factors have been shown to be important to bruising, the relative importance of each factor is still unclear (Hughes, 1974a; Hughes, 1980). A large number of specific factors are known to affect the degree of bruise formation. The principal influences are described below.

Handling temperature: It has been established that low temperature at harvest or during handling increases blackspot damage (Hughes, 1974; Burton, 1989). Temperature affects metabolic processes as well as chemical composition of tubers e.g. respiration, sprouting and sweetening.

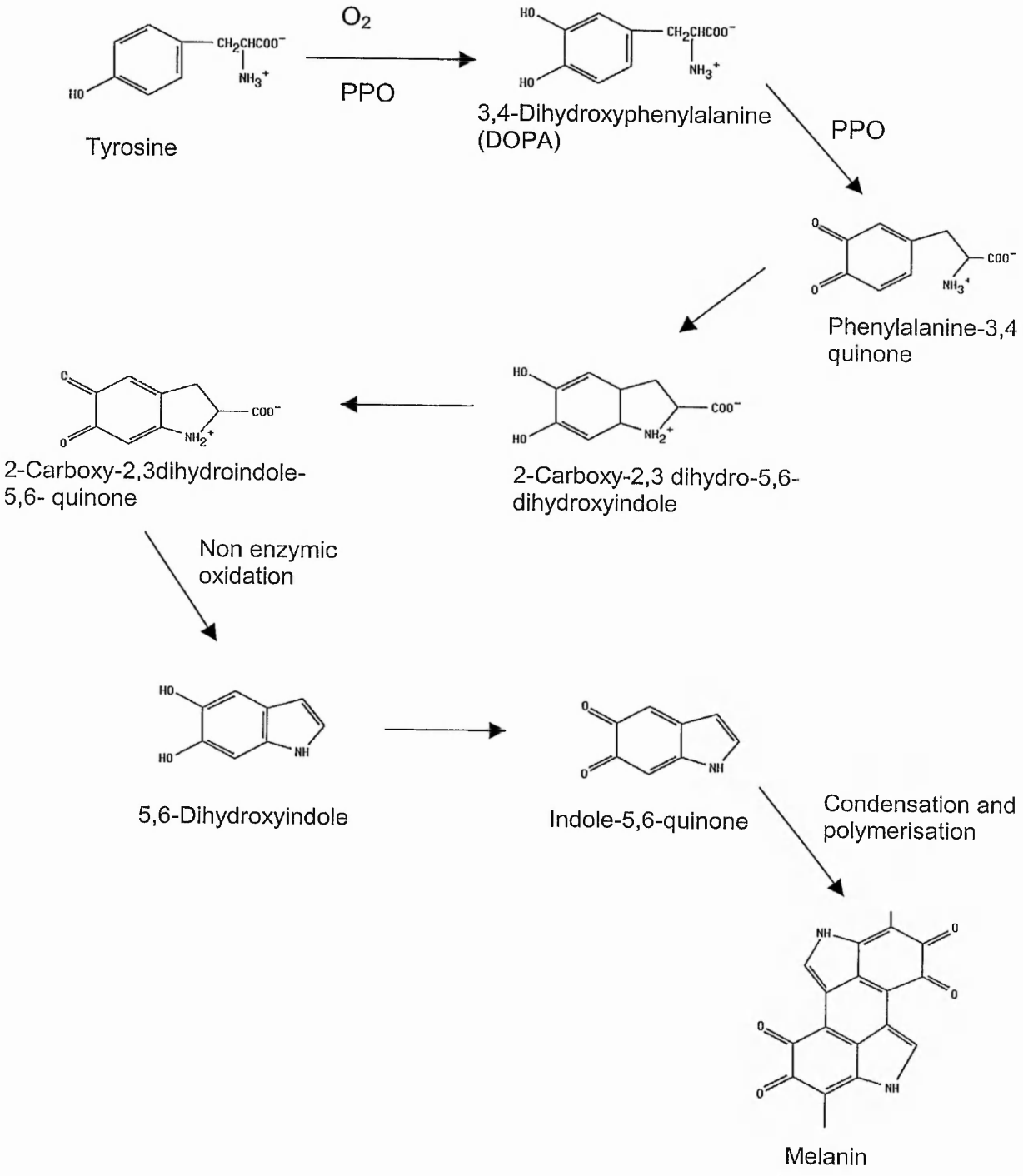


Figure 1.3. Pathway of melanin formation from tyrosine. After Burton, 1970.

Hughes (1974) suggests that an increase in blackspot, as a result of impact at low temperature, may be due to the affect of temperature on the rheological properties of the tissue.

Bruise formation, however, occurs at a faster rate when the temperature is higher and consequently as a test of bruising damage, a sample of potatoes can be placed in a "hot box" to accelerate bruise formation at 25 ºC 95 % RH for 24 hrs (Evans, 1995).

Tuber turgidity: Boyd (1951) concluded that tubers must have lost turgidity before blackening would occur. However, both fully turgid tubers and wilted, flaccid tubers are susceptible to bruising (Burton, 1989). Turgid tubers tend to shatter bruise more easily, whereas flaccid tubers are more prone to blackspot (Smittle, Thornton, Peterson and Dean, 1974; Brook, 1996). A direct relationship has been found between turgidity and blackspot within varieties, although not between varieties where other factors may also influence susceptibility, with the damage inflicted by a uniform weight increasing with a decrease in tuber turgor (Gray & Hughes, 1978; Thomson, Evert & Kelman, 1995). Kunkel and Gardner (1965) investigated the effect of tuber specific gravity on blackspot as an indication of the effect of turgor. Tuber hydration levels were reduced by severing the roots of potato plants. They concluded that increased tuber water content was correlated with increased blackspot resistance. Turgor influences bruise susceptibility because it affects the amount of deformation which can take place before cell walls and membranes are damaged (Hughes, 1974). A tuber with low turgidity can undergo a greater deformation upon impact than a highly turgid tuber (Gray & Hughes, 1978). Ilker and Szczesniak (1990) report that tubers that are turgid will undergo cell wall failure when compressed, whereas less turgid tuber's cells were put under less strain and therefore intercellular debonding was the result of compression (Ilker & Szczesniak, 1990).

Potassium content: The importance of potassium for reducing blackspot development was first noted in the 1920's by Dutch investigators who suggested that, "potash deficient tubers which had lost turgidity", were susceptible to "blue-spotting" (Boyd, 1951). Where soil potassium content is deficient, there is an increase in bruise susceptibility (Mulder, 1949). This

may be due to the affect of potassium on wall extensibility or osmotic regulation (Burton, 1989). Alternatively, the effect may be indirect e.g. by reducing the level of PPO activity (Burton, 1989) or through an increase in tyrosine content (Mulder, 1949; Mondy & Munshi, 1993; Brook, 1996). Mulder (1949) noted that tubers deficient in potassium are more prone to discolouration. An increase in potassium content of the deficient soils results in a reduction in blackspot susceptibility (Hughes, 1974).

Biochemical properties: Phenol content, in particular the PPO substrate tyrosine, correlates well with enzymatic discolouration (Stark, Corsini, Hurkey & Dwelle, 1985; Dean, Jakowaik, Nagle, Pavek & Corsini, 1993). Tyrosine content is a primary factor in varietal differences in blackspot susceptibility, with high concentrations of tyrosine relating to elevated melanin formation (Brook, 1996). The PPO substrates chlorogenic acid and caffeic acid are less abundant, with tyrosine being present at a concentration up to 2 orders of magnitude higher (Cobb, 1998), chlorogenic and caffeic acid have not been found to correlate with blackspot susceptibility (Dean et al., 1993). Increased phenol content may increase the amount of melanin produced, assuming that PPO activity and oxygen are not limiting factors. An increase in PPO activity in tubers following wounding and stress has been reported (Cheung & Henderson, 1972; Galliard, 1978). However, no correlation has been found between the concentration of PPO within tuber tissue or it's affinity for its substrates (Stark et. al, 1985) and susceptibility to melanin formation. While the biochemical properties of potato tubers may result in some of the differences seen in melanin formation in different cvs, the internal damage required to bring enzymes and substrates together may be one of the reasons why tuber biochemical properties alone cannot explain differences in bruising susceptibility (Hughes, 1974).

Starch content: This is thought to be a determining factor in bruise susceptibility. Starch granules rupture membranes during the deformation on impact (Gray & Hughes, 1978; Hughes, 1980). Determination of tuber dry matter, which is closely correlated to starch content, is often used as a key marker to determine the potential bruise sensitivity of a crop. However, starch content decreases during storage (Burton, 1989) whilst blackspot susceptibility increases.

Tuber rheology: The rheology and mechanical strength of the tuber skin and tissue affects the amount of structural damage sustained as a result of impact (Hughes, 1974; Gray & Hughes, 1978). Rheological properties such as elasticity, "firmness", cell strength (in particular that of the cell wall) and tissue strength (or force to rupture point), including intercellular adhesion and turgor, are all important and have been shown to differ between cultivars (Hughes, 1974; Gray & Hughes, 1978). This may bring about some of the observed differences in bruise susceptibility. Following application of an external force such as compression or impact, cells deform and may rupture. Cell size has been positively correlated with bruise susceptibility. However, data is inconclusive and there is little evidence to determine whether an increase in tuber cell size causes increased bruise susceptibility (McGarry et al., 1996).

Tuber size and shape: Large, heavy tubers bruise more easily than small ones when dropped from the same height as the increased weight will result in an increase in impact force, radius of curvature at the point of impact is also important. Impact sites with a small radius of curvature concentrate the impact forces over a small area causing a higher tissue stress and increased degree of bruising (Brook, 1996).

Region of tuber: It is well substantiated that tuber susceptibility to melanin formation varies throughout the tuber (Hughes, 1974). Tubers are more susceptible to bruising at the stem end than the bud end (Hughes, 1974a; Burton, 1989). The vascular tissue is more susceptible to bruising than the surrounding tissue. Indeed, an impact may cause no bruising to the cortex above or the pith beneath, while the vascular tissue will bruise following impact (Burton, 1989). Both the stem end of the tuber and the vascular tissue are areas of high dry matter (i.e. starch) content and elevated tyrosine.

Age/Time in storage: Tubers become more susceptible to blackspot during storage (Burton, 1989) and as they mature. This is due to changes in a number of the above factors through storage e.g. turgor and membrane integrity.

1.4 Aims of the Investigation

This project is part of a wider study, the aims of which were to investigate some of the biochemical and physiological aspects of bruising in stored potato tubers. With so many documented variables involved in potato susceptibility to blackspot bruise, previous work has been unable to establish a clear picture of which factors are important in influencing bruise susceptibility and what is occurring within the impacted tuber.

This study aims to establish the key influences determining blackspot susceptibility in potato tubers using two approaches. The first will be to monitor tuber physiological, biochemical and ultrastructural characteristics through storage alongside measurements of bruise susceptibility with the aim of identifying which variables correlate and therefore might be key influences in determining bruise susceptibility. The second approach will be to look at the effect of impact on tuber biochemistry, physiology and ultrastructure over time to provide an understanding of how a tuber is effected by impact and possible areas of weakness. In particular, this project aims to establish an ultrastructural sequence of events following tuber impact, which will be, attempted by light and electron microscope studies.

Measurements of a large number of tuber characters which are thought to influence blackspot bruising will be measured including respiration which provides information on tuber metabolism, water relations, dry matter content, and the biochemical potential for forming melanin. Links between loss of cell compartmentation through storage and bruise susceptibility will be investigated by measuring the leakage of various cations from tuber tissue through storage and following impact. The use of different tuber cultivars, sites and physiological age of seed tubers will be employed with the aim of highlighting tubers with a range of susceptibilities to blackspot and correlating this with particular tuber characteristics.

The work reported in this thesis is part of a wider study with Dr E.R. Brierley and Sutton Bridge Experimental Station whose observations on various factors are referred to here and acknowledged as Brierley (1998) unpublished and SBEU (1998) unpublished, respectively.

2.0 Potato Tuber Physiology Through Storage and Its Influence on Blackspot Bruise Susceptibility

2.1 Introduction

Plant physiology may be described as the study of plant functions, including metabolism and growth, and how these interact within the plant (Taiz & Zeiger, 1991). The physiology of a plant will influence how it responds to the external environment. Consequently, differences in the functioning of the potato tuber may potentially affect its susceptibility to bruise upon impact. Variations in tuber physiology between potato cvs (Wiltshire & Cobb, 1996), may account for the differences in susceptibility to blackspot and melanin formation.

During storage a number of aspects of tuber physiology change. Following entrance into store the tuber becomes dormant resulting in a reduction in metabolism to a basal level (Schippers, 1977; Hemberg, 1985). Metabolism increases again towards the end of storage as the tubers break dormancy and start sprouting (Schippers, 1977; Williams & Cobb, 1993; Wiltshire & Cobb, 1996). A number of factors within store affect tuber physiology. Firstly, the holding temperature which influences the metabolic and respiration rates (Burton, 1966) along with reducing sugar content (Wiltshire & Cobb, 1996).

Secondly, the application of chemical sprout suppressants to tubers undergoing medium to long-term storage prevents sprout growth at the end of its natural dormant period (Gunn, 1990), enforcing dormancy and affecting physiology.

2.1.1 Tuber Respiration

Aerobic respiration is the process by which organic compounds e.g. starch are broken down to produce the high energy compound adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NADH), involving the uptake of O_2 and release of H_2O and CO_2. In the majority of higher organisms, including the potato, this is the main method employed to fuel and sustain cellular life and structure (Dizengremel, 1985). Measurements of respiration act as a marker for the metabolic state of the tuber and methods

of determining the rate of aerobic respiration involve measuring the rate of O_2 uptake or CO_2 release.

Whole tuber respiration can be measured using an Infra-Red Gas Analyser (IRGA). This measures the gaseous concentration of CO_2 and H_2O using infra-red absorption following the circulation of ambient air through an air-tight chamber containing the respiring material. Water shares a similar infra-red absorption band to CO_2 broadening the CO_2 infra-red response. It is therefore removed from the air using Drierite® (Hammond Drierite Company Ltd containing 97% anhydrous calcium sulphate and 3% cobalt chloride) before measurement to prevent interference.

Oxygen electrodes provide another means to measure respiration. This involves enclosing a small quantity of respiring tissue within a water-jacketed chamber above a platinum electrode. The water jacket enables temperature control, the electrode and tissue are separated by a membrane. An electrical charge, passed between the platinum cathode and a silver anode, is affected by oxygen in the reaction solution above the membrane. Consequently, any change in oxygen brought about by respiration of the tissue within the reaction solution can be detected and measured. The main limitation of this method is that large quantities of tissue cannot be used, hence respiration of entire tubers would not be possible. Respiration of tuber tissue slices was reported to be five times greater than that of the whole tuber, rising in response to wounding (Thomson et al., 1995). The oxygen electrode method would therefore be unsuitable for looking at damage response as only sections of tissue could be used. It does however allow close control of temperature which is an important factor when measuring respiration.

Other methods for measuring respiration involve the incubation of tubers and taking gas samples at a set interval in order to determine the change in either CO_2 or O_2 concentrations. These methods are not as convenient as IRGA and oxygen electrodes.

It is important to note that the external environment including temperature, external oxygen concentration and injury can affect the respiration rate and must be controlled if accurate measurements of respiration are to be taken.

2.1.1.1 Tuber Respiration During Storage: Respiration of dormant tubers is very low (Dizengremel, 1985), falling rapidly after harvest and entry into storage (Dizengremel, 1985; Williams & Cobb, 1993) and increasing again at the break of dormancy and initiation of sprouting as tuber metabolism increases (Schippers, 1977; Williams & Cobb, 1993; Wiltshire & Cobb, 1996).

It has been suggested that increase in tuber susceptibility to blackspot is proportional to an increase in respiration (Brook, 1996), although whether this is a direct relationship appears not to have been discussed.

2.1.2 Turgidity of Potato Tubers

All plant processes are influenced to some extent by water availability (Kramer and Boyer, 1995). For most plants water constitutes more than 90% of the fresh weight, 60 – 90 % of this water is within the cell vacuole while the remainder permeates the cell wall (Turner & Burch, 1983). Water is important for transport both through the plant and plant cells. It is used as an all-purpose solvent for growth and as a major reactant of important chemical reactions within cells, for example those involved in photosynthesis. Water is also essential for maintaining cell turgidity, a term used to describe the internal hydrostatic pressure in excess of ambient atmospheric pressure observed in plant and bacterial cells. This pressure arises from the internal forces exerted on the cell wall (Zimmerman, 1977; Turner and Burch, 1983). Maintaining turgidity is vital for support and expansion growth within a plant (Turner & Burch, 1983).

Turgidity may be calculated using the following equation

$$\Psi = P + \pi + \tau + g$$

Where; Ψ = water potential

P = pressure potential (turgor)

π = osmotic potential

τ = matrix potential

g = gravity

Water potential is a term traditionally used in plant physiology. It is the chemical potential of water, i.e. the difference between the potential of a solution in a standard state (pure water at ambient pressure and at the same temperature as the sample) and it's potential in the given state (water potential of water in a standard state plus the osmotic pressure (π), the turgor pressure (P), the matrix potential (τ) and gravity (g)) expressed as joules per mole (Taiz and Zeiger, 1991). This can best be explained by demonstrating the transport of water across a semi-permeable membrane which is permeable to water, but not a solute dissolved within it. Thus, if on one side of the membrane (A) was pure water and on the other (B) was water plus a dissolved solute, water would pass from side A, through the membrane to side B, from a solution with a high water potential, to that of a lower water potential. An increase in turgor would bring about a subsequent increase in the water potential.

Osmotic potential can be defined as the concentration of water, due to the presence of dissolved solutes (Turner & Burch, 1983). An increase in the osmotic potential or solute content brings about a decrease in water potential.

Matrix potential is particularly important in soils. It involves the short-range interfacial forces occurring between liquid and solid phases. Within the plant cell wall, the only part of the cell that can be affected by matrix potential, only a very small fraction of the total water will be influenced. For nearly all living plants, the cells would be dead before a significant amount of water was affected by matrix potential. For this reason, matrix potential is invariably omitted from water potential calculations within plants.

For short vertical heights, such as those found in field crops, the effect of gravity is very small and is therefore also omitted from the calculation (Turner & Burch, 1983), leaving the formula

$$\Psi = P + \pi$$

Progress in determining tuber turgidity has been limited by the difficulties in finding an accurate method. This problem has resulted in a poor understanding of the interrelationship between turgor and many cellular phenomena (Zimmerman,1977). There are a number of methods which have been employed to measure the water status of plant material, these are described below.

Thermocouple Psychrometry: The thermocouple psychrometer measures water potential (see Figure 2.1). This involves the enclosure of a section of plant tissue or other subject material and a thermocouple which are held at a constant temperature until thermal and vapour equilibration is complete. The thermocouple is then cooled to allow water vapour to condense upon it. Upon cessation of cooling, water transfers from the thermocouple and will be taken up by the tissue, this will result in the temperature of the thermocouple decreasing. The amount of water taken up by the tissue will be dependent upon the turgidity of the tissue and hence the degree of cooling by the thermocouple is related to water potential and can be expressed as a voltage output from which a measurement of water potential can be calculated. For calculations of turgidity, the osmotic potential of the solution must be calculated, in addition to the water potential. This is done by destroying the cellular construction of the tissue and taking a reading of the water potential. Turgor is then calculated using the formula

$$\Psi = P + \pi$$

(Boyer & Potter, 1973; Kramer & Boyer, 1995)

The limitations of this method are it's sensitivity to small changes in temperature, sample chamber contamination and also the time taken for equilibration within the chamber.

A further problem identified by Tyree and Jarvis (1982), is that osmotic potential measured on crushed or frozen tissue will result in the mixing of the apoplastic water with that of the cells. For measurements of turgor it is only the solution within the cells that is important. In potato tubers intercellular spaces account for only 1 % of tissue resulting in only a slight error in the measurement.

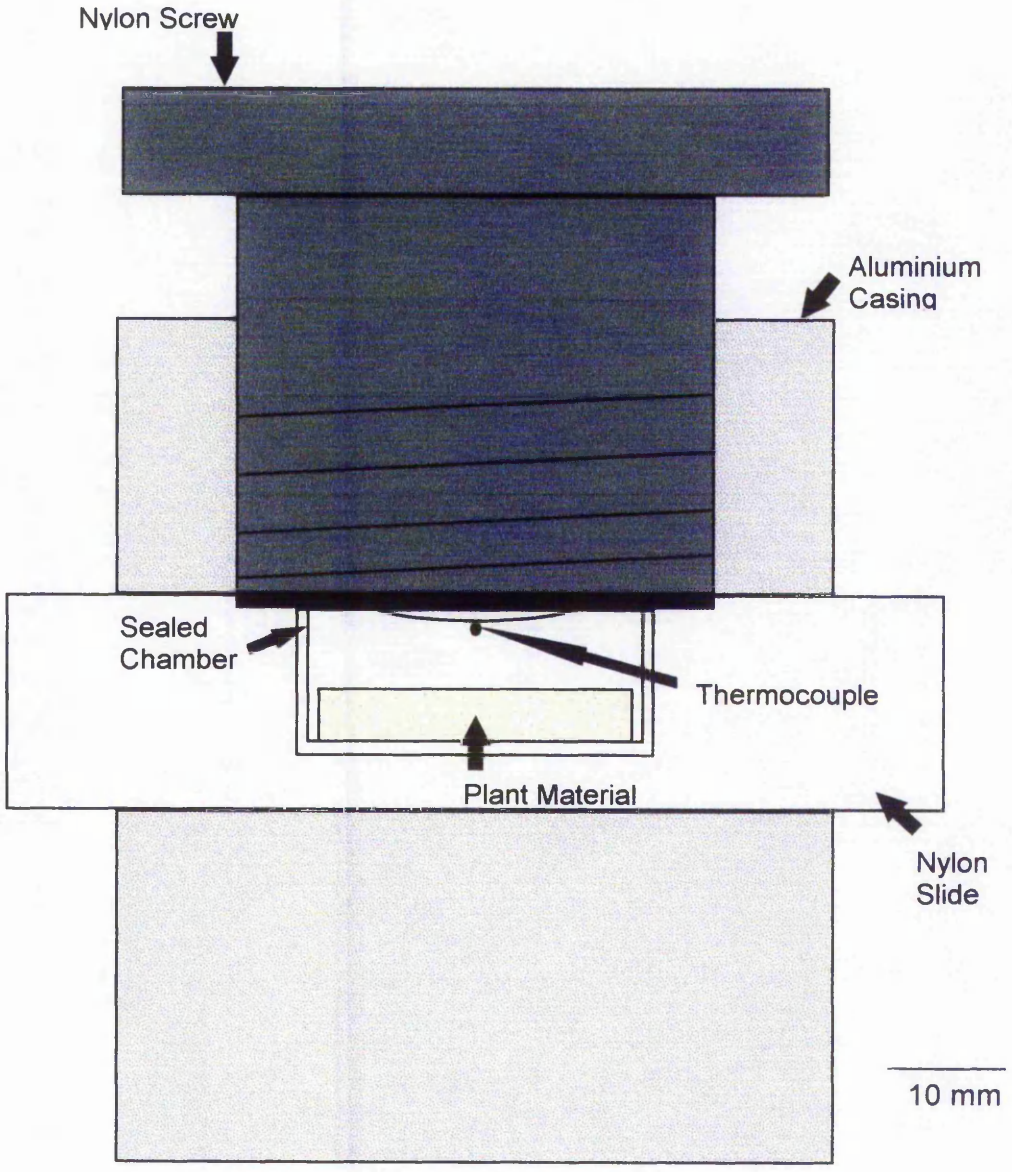


Figure 2.1. Cross section of a Wescor C52 psychrometer sample chamber (Wescor, Inc. Utah, USA).

Pressure Probe: This technique measures the water potential of individual cells. It consists of a microcapillary tube connected to a small pressure chamber. Both the capillary tube and the chamber contain silicone oil. The chamber contains a pressure transducer which converts the pressure within the chamber into a voltage output. The tip of the microcapillary is inserted into a cell and the oil cell sap boundary is pushed up the microcapillary by the turgor pressure. This boundary meniscus is adjusted to be at the tip of the microcapillary, close to the cell. The pressure within the cell is converted to voltage via the silicone in the microcapillary tube and the pressure transducer (Steudle, 1990).

This method has been used widely, in particular for turgor measurements in large single celled algae (Tyree & Jarvis, 1982) and it allows the direct measurement of cell water relations in intact tissue (Steudle, 1990). However it has limitations. Damage to surrounding cells can be caused, which will have an affect on the turgor of cells being measured (Tyree & Jarvis, 1982). The number of cells which would have to be measured in order to give a good estimation of water potential within the tissue would be quite large and hence it is not always a practical method for determining turgidity in cells within large areas of tissue. Nor can the pressure probe be used to measure turgidity in cells below 20 μ meters in diameter, which is smaller than the cells of a number of vascular plants (Tyree & Jarvis, 1982).

Compensation methods: Here the determination of tissue water potential is established by finding a solution that is isotonic with the tissue water potential (Slavik, 1974). This method, involves the incubation of tissue (uniform in size and surface area) within a graded series of solutions of known osmotic potential. Net water movement between tissue and solution is calculated by determining weight change following tissue incubation. Molarity of the solution (usually sucrose or mannitol) is plotted against net water transfer (signified by a change in weight) to determine at what molarity net water movement is zero, at this point the osmotic potential of the test solution is equal to the water potential of the tissue (Slavik, 1974). Water potential is estimated from the molarity of the solution using the ideal gas law,

$$\Psi = -M \times i \times R \times T$$

where; M = molarity of solution

i = ionisation constant (1 for sucrose)

R = ideal gas constant, 0.008314

T = temperature, °K

Following this osmotic potential is estimated by determining the osmotic potential of the solution at incipient plasmolysis (i.e. detachment of peripheral cytoplasm from the cell wall). At this point turgor pressure is zero and hence water potential is equal to osmotic potential. From these calculations of water potential and osmotic potential, turgor is calculated using the formula

$$\Psi = P + \pi$$

2.1.2.1 The Effect of Turgidity on Blackspot Bruise: Water stress in potato plants is known to result in a decrease in tuber quality and even yield if the stress continues for a long period of time (Kleinkopf, 1983). A link between tuber turgidity and susceptibility to blackspot bruising has been proposed by many workers (Boyd, 1951; Kunkel and Gardener, 1965; Smittle et al., 1974; Hughes, 1980; Li, 1985; Brook, 1996), with a loss of water during the growing season, harvest or storage leading to increased blackspot bruise susceptibility (Hughes, 1980).

While blackspot susceptibility in potato tubers increases as the tuber dehydrates, the opposite effect is seen with shatter bruise (Smittle, et al., 1974), such that as turgor increases, tension within the tuber increases and cell walls become more susceptible to structural failure upon impact (Hughes, 1980). Hence, freshly dug tubers, which are more turgid than stored tubers are more susceptible to cracking and splitting damage (i.e. shatter bruise) upon impact (Hughes, 1980a). Flaccid cells deform when impacted. Hence, rather than the cell walls shattering, they are more likely to damage their internal membrane system (Gray and Hughes, 1978) resulting in blackspot bruising. Flaccid tubers with a high starch content are most susceptible to blackspot bruising, e.g. tubers which have lost water during storage (Hughes, 1980).

Work on the turgidity of potato tubers has been varied and actual measurements of turgidity have not been performed. In many cases,

measurements of specific gravity have been used as an indication of tuber water content or simply changes in tuber weight (Kunkel and Gardner, 1965). While the relationship between specific gravity and tuber turgidity is highly significant, this relationship may be indirect, reflecting changes in cell size, cell wall properties or direct through it's indication of starch content which itself can play a role in bruising through damage to the cell membranes upon impact. This has led to problems in comparing the results from different investigations.

The relationship between turgidity and blackspot susceptibility needs to be established and quantified to enable a more accurate picture to be drawn. Previous work in this area has not been able to accurately quantify the turgidity of potato tubers. An aim of this study has been to establish a method which will enable tuber turgidity to be measured and to investigate the relationship between tuber turgidity and bruising.

2.1.3 Cation Leakage

Compartmentation is essential to the functioning of a cell, enabling various cellular processes to be isolated in separate organelles and structures. Consequently, the contents of the various organelles differs. It has been suggested that as prolonged storage progresses there is a degradation of certain membranes within the tuber tissue (Kumar & Knowles, 1993). Monitoring the leakage of cations, known to be located in particular cellular organelles from tuber tissue enables us to determine the physiological integrity of intracellular membranes. Consequently, the release of the cations calcium, magnesium, potassium and sodium from tuber tissue through storage has been monitored using ion chromatography in order to determine the extent of membrane degradation within the cell during storage.

2.1.4 Determination of Bruise Susceptibility

It is well established that as storage progresses, tuber susceptibility to bruising changes (Burton, 1989), typically with an increase in blackspot susceptibility with storage (Sawyer & Collins, 1960; Smittle, et al., 1974), although opposing evidence has been put forward (McGarry, et al., 1996). Hence, regular determination of blackspot susceptibility throughout storage

enables us to determine when significant alterations in tuber vulnerability to blackspot occur. Correlating susceptibility with any major changes in other physiological aspects of the tuber should enable us to determine which physiological factors have a significant influence on bruising through storage.

There is no standard method for measuring bruise susceptibility. However, two general approaches have been employed, either impacting tubers and grading the damage or destroying the cell structure for example by homogenisation or abrasive peeling, followed by an assessment of colour formation. To impact tubers and then grade the damage exhibited (Kunkel & Gardner, 1965) several factors must be considered. Firstly, the method of impact. Drop tests, in which tubers free fall from a given height on to a hard surface (McGarry, et al, 1996), are unacceptable because the impact energy received by the tuber will depend upon the tuber mass and tuber curvature at the point of impact. In addition, the area of tuber impacted is a factor which itself will influence susceptibility (Hughes, 1974) such that if a tuber falls on its stem end it is more likely to bruise than if it lands on its bud end (Hughes, 1974; Burton, 1989). Tubers may also be impacted within a revolving drum, resulting in numerous impacts. Again, damage will be dependant upon the shape and mass of indiyidual tubers and therefore this method does not produce reproducible results. The use of these methods commercially, however, can allow a relatively fast estimate of how susceptible the tubers are to damage (McGarry, et al., 1996).

In order to get over the problems of differences in tuber mass, shape and orientation, alternative methods can be employed such as the falling bolt and pendulum. These methods require that tubers are held in place while an external object is allowed to impact the tuber, allowing for standardisation of the area of tuber impacted as well as the energy of impact (McGarry, et al., 1996). It is still important that tuber curvature at the point of impact is standardised and that tubers of approximately the same size are chosen because tuber size is thought to influence bruise susceptibility (McGarry, et al., 1996).

Impact energy is also important, Molema, et al., (1997) found that a reduction in the impact energy from 0.6 J to 0.067 J resulted in an average reduction of volume of discoloured tissue of 95 %. A falling bolt is set up as a

metal bolt of known weight, held a specific height above a potato so that on release of the bolt it will fall down a guiding tube, without touching the sides, to impact the potato at a given point with a known amount of energy. The potato may be held in position in a number of ways. Maas (1966) used a spring-loaded hinged platform but noted that hand holding tubers was quicker.

With the pendulum method of impact, the device is more complex and therefore more costly to build. It may however be able to measure the amount of deformation as well as control secondary impacts and therefore produce more accurate results.

In the commercial setting, following impact tubers are generally stored in a "hot box" at 25 °C for 24hrs before grading the damage they exhibit. One such method of grading is to calculate the percentage of tubers impacted showing melanin formation (PMB, 1981). This method reveals the inherent differences between tubers, so that within a population of potatoes with identical backgrounds, some may bruise while others may not. Alternatively, tubers have been visually assessed according to a damage assessment scale. These qualitative methods may give the worker some idea of the differences between tubers, but they are subject to personal judgement. This can be a problem, particularly where evaluation is taking place over time or by different workers.

Alternatively, the tuber tissue is sliced or homogenised to bring together PPO and its substrates. The degree of colour formation is then assessed. One such method is known as abrasive peeling in which layers of tissue can be removed with an abrasive peeler before incubating tubers in darkness for 24 hrs at 20 °C followed by an assessment of colour formation, for example using a photovolt reflection meter or by a visual assessment of discolouration (Stark, et al., 1985). Qualitative estimates should only be used in conjunction with a quantitative method. Again, assessments following abrasive peeling employed a subjective visual rating and for this reason the homogenisation method will be a more accurate assessment of the biochemical properties of the tubers.

Dean et al. (1993) outlined a method for determining the optical density at 475 nm of tuber tissue homogenised in a 1:1 ratio of phosphate

buffer and filtered before incubation for 24 hrs at 22 °C followed by centrifugation. This method measures the amount of dopachrome (a precursor to melanin) formation that has occurred and allows a quantitative value to be produced.

These tests give a good indication of the biochemical potential of the tuber tissue to produce melanin, and produce results that often correlate well with tyrosine content and enzyme activity (Stark, et al., 1995). However, they are not a measurement of tuber susceptibility to bruising because factors such as periderm thickness, turgor, dry matter content and cell wall strength are not taken into consideration.

In this investigation two approaches have been undertaken to determine bruise susceptibility. The first involving impacting tubers throughout storage and then determining the percentage bruised tubers and the degree of bruising observed. The second method determines the potential colour formation as described by Dean, et al. (1993). This involves homogenising the tissue and consequently takes account of the biochemical aspects of the tissue, in particular enzyme activity and substrate availability, ignoring the affects of cell ultrastructure and turgidity.

2.1.5 Aims of Physiological Investigations

Blackspot susceptibility is affected by storage and is generally thought to increase as storage progresses, although some evidence has been presented to the contrary (McGarry, et al., 1996). In order to determine the affect of various physiological factors upon tuber susceptibility to blackspot, physiological characteristics have been investigated throughout storage over three years in conjunction with measurements of bruise susceptibility. The aim of this work has been to establish which physiological factors are most likely to influence blackspot susceptibility.

2.2 Materials and Methods

2.2.1 Plant Material

2.2.1.1 1995/6 Storage Season: Potato tubers of cv. Pentland Dell and Record were harvested by hand on 18/10/95 and 25/9/95 respectively, from ADAS, Arthur Rickwood, UK. They were cured at 15 °C for 2 wks before being placed into store in trays at the Potato Marketing Board Experimental Station, Sutton Bridge, UK. Tubers were stored at 10 °C and 5 °C, at 95 % relative humidity for 36 wks. Applications of MSS CIPC 50M were made as follows:

Table 2.1 Application of MSS CIPC 50 M to stored potato crops during the 1995/6 storage season

Date	Tuber Populations Treated	Application Rate (ml tonne^{-1})
10 - 11 - 95	All	42.5
15 - 12 - 95	10°C stored tubers	42.5
23 - 01 - 96	10°C stored tubers	42.5

For the two cvs Pentland Dell and Record at both 5 and 10 °C, 20 tubers of each were taken for analyses from store at 6 wk intervals and transported to The Nottingham Trent University in cold boxes, packed with vermiculite, which had been allowed to equilibrate to tuber temperature. Of these 20 tubers, 5 whole tubers were used for measuring respiration, 5 for relative water content, 5 for cation analysis and 5 for colour formation.

Tubers were stored overnight in incubators at 5 and 10 °C according to their storage regime until required for investigations.

2.2.1.2 1996/7 Storage Season: Potato tubers of cv. Pentland Dell, Record and Maris Piper, grown from seed tubers of 0 (young) and 150 (old) day degrees physiological age were harvested by hand on 21/10/96 from ADAS Arthur Rickwood, UK (free draining peaty loam, pH 6.8, irrigated) and on 7/10/96 from ADAS Terrington, UK (silty clay, pH 8.2, not irrigated). They were cured as in 2.2.1.1 and stored at the Potato Marketing Board

Experimental Station, Sutton Bridge, UK. at 10 °C and 95 % relative humidity for up to 36 wks. MSS CIPC 50 M applications were made (Table 2.2).

Table 2.2 Application of MSS CIPC 50 M to stored potato crops during the 1996/7 storage season

Date	Tuber Populations Treated	Application Rate (ml tonne^{-1})
15 - 11 - 96	All	42.5
23 - 12 - 96	All	42.5
20 - 02 - 97	All	42.5
08 - 05 - 97	All	42.5

There were 12 combinations of tuber cultivar, physiological age and site which were assigned a number for convenience (Table 2.3).

A total of 40 tubers were taken of each of the 12 treatments for assessment at harvest and after 12, 24 and 36 wks of storage. Tubers were transported as in 2.2.1.1 for analyses. Five of the 40 tubers were used to measure respiration, 5 to measure colour formation, 15 to measure bruise susceptibiity at 5 °C and 15 to measure bruise susceptibility at 10 °C. Tubers were then stored in incubators as in 2.2.1.1.

2.2.1.3 1997/8 Storage Season: Tubers of cv. Pentland Dell, Record and Maris Piper, grown from seed tubers of 0 (young) and 500 (old) day degrees physiological age, were harvested by hand on 23.9.97 from ADAS Terrington, UK (silty clay, pH 8.2, not irrigated) and on 7.10.97 from ADAS Arthur Rickwood, UK (free draining peaty loam, pH 6.8, irrigated). They were cured as in 2.2.1.1 and stored at the Potato Marketing Board Experimental Station, Sutton Bridge, UK. at 10 °C and 95 % relative humidity for up to 40 wks. MSS CIPC 50 M applications were made (Table 2.4).

Table 2.3 Treatment numbers assigned to combinations of tuber cv, physiological age and site.

Treatment	Cultivar	Physiological Age	Site
1	Pentland Dell	Young	Arthur Rickwood
2	Pentland Dell	Old	Arthur Rickwood
3	Maris Piper	Young	Arthur Rickwood
4	Maris Piper	Old	Arthur Rickwood
5	Record	Young	Arthur Rickwood
6	Record	Old	Arthur Rickwood
7	Pentland Dell	Young	Terrington
8	Pentland Dell	Old	Terrington
9	Maris Piper	Young	Terrington
10	Maris Piper	Old	Terrington
11	Record	Young	Terrington
12	Record	Old	Terrington

Table 2.4 Application of MSS CIPC 50 M to stored potato crops during 1997/8 storage season.

Date	Tuber Populations Treated	Application Rate (ml tonne^{-1})
13.11.97	All	42.5
28.1.98	All	42.5
2.7.98	All	42.5

The 12 combinations of tuber cv, physiological age and site were assigned a number for convenience (Table 2.3).

Fifty five tubers of each of the 12 treatments were taken for assessment at harvest and at 10 wk intervals throughout 40 wks of storage. Tubers were transported as in 2.2.1.1 for analyses. Five tubers of each treatment were used to measure respiration, 5 to measure cation leakage, 5 for colour formation and a total of 40 for bruise susceptibility, 10 tubers for analysis at 5 °C at both 0.5 and 0.7 J and at 10 °C at 0.5 and 0.7 J. An additional 5 tubers were transported of treatments 1, 3, 7 and 9 for

assessment of tuber turgidity. Tubers were then stored overnight in incubators as in 2.2.1.1.

2.2.2 Tuber Respiration

2.2.2.1 Respiration Through Storage: Measurements of respiration were carried out on tubers taken for analyses throughout storage season 1995/6 and 1996/7 and 1997/8 using the method outlined in 2.2.2.2.

2.2.2.2 Measurement of Tuber Respiration: Five tubers were accurately weighed and placed at room temperature, in a gas-tight perspex chamber, 2.57 litres in volume, which was then sealed using petroleum jelly. Ambient air was taken from outdoors, passed through a 4 m PVC tube and into a buffer box before entrance to the IRGA. CO_2 and H_2O were removed from the air using soda lime and drierite. The air flow through the tuber chamber via PVC tubes was at a rate of 200 µm s^{-1}. CO_2 concentration of outflow air was analysed and compared to that of ambient air using an Infra-Red Gas Analyser (IRGA), Model LCA 4, Analytical Development Company Ltd., Hertfordshire, UK. Once the chamber had equilibrated, readings were taken every 2 mins for 20 mins. Respiration was then calculated for the 10 measurements:

Respiration rate = CO_2 differential x flow rate ÷ total tuber wt (g)

An average rate of respiration was calculated from these 10 measurements for each treatment.

2.2.3 Tuber Turgidity

A method for determining tuber turgidity was developed and used to assess the turgidity of tubers. Compensation methods were undertaken on locally purchased Pentland Dell tubers (2.2.3.1).

Measurements of relative water content were carried out on tubers of Pentland Dell and Record from storage season 1995/6 at 6 wk intervals from 12 to 36 wks storage in Pentland Dell and between 15 and 39 wks storage in cv. Record (2.2.3.2).

A method for determining tuber turgidity using psychrometers was developed (2.2.3.4). This method was used to assess turgidity of tubers from

treatments 1,3,7 and 9 at 10 wk intervals at harvest and throughout the 1997/8 storage season (2.2.1.3). At each sample date 5 tubers of each treatment were used for turgor analysis.

2.2.3.1 Compensation Method: Tuber cores, 10 mm in diameter were taken from locally purchased Pentland Dell. The periderm was removed and stem end 2 cm was quartered longitudinally. Sections were gently dried with tissue paper, weighed and placed in sucrose solutions of 0.01, 0.1, 0.2, 0.3, 0.4 and 0.5 M. Five sections were incubated in each of the solutions at 25 °C for up to 48 hrs and weighed at 1, 5, 24, 36 and 48 hrs after drying gently on tissue paper. Percentage weight change compared with time 0 was calculated and plotted against the molarity of the incubation solution. The time taken for maximum percentage weight change was determined.

This investigation was repeated using sucrose solutions buffered with 0.02 M Phosphate buffer (K_2HPO_4 +KH_2PO_4) at pH 6.5.

To determine water potential of the tuber tissue, cores (10 mm in diameter) were taken from locally purchased Pentland Dell. The periderm was removed and stem end 2 cm was quartered longitudinally. Sections were gently dried with tissue paper, weighed and placed in sucrose solutions at 0.05 M intervals between 0.1 M and 1.0 M. Three tuber sections were incubated in each of the solutions and weighed following a 24 hr incubation at 25 °C. Weight change was determined (g g^{-1}fwt) and the mean and standard error for each sucrose solution was calculated. The results were plotted against molarity of the sucrose solution and a third order polynomial trendline was calculated. The molarity at which there was no net water movement between incubation solution and tuber tissue was determined. At this point the osmotic potential of the test solution was equal to the water potential of the tissue (Slavik, 1974). Osmotic potential of the solution (MPa) was calculated as follows

Osmotic potential (MPa) = m x i x R x T

Where; m = molarity of solution

i = ionisation constant, 1 for sucrose

R = ideal gas constant, 0.008314

T = temperature, °K

Osmotic potential was then calculated as the point at which tuber tissue underwent plasmolysis, i.e. where no further change in tuber weight occurred with increased molarity of the incubation solution. From the water potential (Ψ) and osmotic potential (π), turgor (P) was calculated using $\Psi = P + \pi$.

This investigation was repeated.

2.2.3.2 Calculation of Relative Water Content: A 1 mm thick slice from the tuber sections was accurately weighed and placed in water at room temperature for 24 hrs after which time the weight had become stable. The slice was then reweighed and relative water content (RWC) was calculated as follows:

original wt (g) ÷ final weight (g) x 100 = RWC

2.2.3.3 Method development for Determining Tuber turgidity of Potato Tubers Using a Psychrometer:

Equilibration Time Required for Tissue to Reach Vapour Equilibration. A 2 mm thick section of tuber core, 5 mm in diameter was taken, following the removal of the periderm. The section was inserted into a sample holder which was immediately enclosed within a Wescor C52 sample chamber (Wescor, Inc, Utah, U.S.A). The C52 sample chamber was left to equilibrate at 25 ºC following enclosure within a polystyrene box. Measurements were taken after 0, 10, 30, 60, 120, 180, 240 and 360 min of incubation. The thermocouple was cooled for 12 s before reading microvolt output using a dewpoint microvoltmeter (HR33T, Wescor, Inc. Utah, USA).

This was repeated using filter paper discs, saturated with 0.1, 0.25 and 0.5 M NaCl solution to determine the length of time required for vapour equilibration.

This investigation was repeated 4 times and an average length of time to reach vapour equilibration was calculated for potato tissue and for saturated filter paper discs.

Calibration of C52 Sample Chambers. Filter paper discs were saturated in NaCl solutions in concentrations of 0.5 M, 0.25 M, 0.125 M and 0.0625 M and immediately placed within a sample holder and enclosed within a C52 sample chamber. The chambers were incubated for 3 hrs at 25 ºC, the thermocouple was cooled for 12 s before a reading of microvolt output was taken as above. This was repeated 3 times for each solution with both

chambers. The mean of the results (in the form of microvolt output) for each of the 2 chambers were compared with actual water potentials of the solutions at the given temperature and calibration curves were plotted. A line of best fit was drawn using Excel, the equation for which and the R^2 were calculated.

2.2.3.4 Method for Determining Turgor of Potato Tuber Tissue: A tuber core, 6 mm in diameter was taken longitudinally. The periderm from the stem end of the core was removed and a 2 mm section was cut and immediately placed in a sample holder, which was closed within a Wescor C52 sample chamber (Wescor, Inc, Utah, USA).

A second section of tuber 2 mm thick (for analysis of osmotic potential) was cut and frozen in liquid N_2 and stored at –70 °C. Longitudinal cores, 6 mm in diameter, were taken through the tuber.

The C52 sample chamber was carefully placed within an open polystyrene box and left to equilibrate at 25 °C for 3 hrs. A lid was placed on the box which was then removed from the incubator for an immediate reading of water potential. The thermocouple was cooled for 12 s before reading microvolt output using a dewpoint microvoltmeter (HR33T, Wescor, Inc.Utah, USA).

The frozen tissue was then centrifuged in a MSE Micro Centaur (Jennings & Co., East Bridgford, UK) at 13000 rpm for 2 min and the fluid fraction was absorbed onto a filter paper disc before placing in a sample holder and taking a measurement of water potential (tuber osmotic potential, π) as previously outlined.

µV output was converted into water potential using the following equations (obtained from the calibrations performed in 2.2.3.3);

Chamber 4539 $y = 0.2664x + 0.2538$

Chamber 4555 $y = 0.3708x + 0.2039$

Turgor pressure was calculated using the formula

$$\Psi = P + \pi$$

Where: Ψ = water potential, measured using the intact tissue

π = osmotic potential, measured using the fluid fraction of the tissue

P = turgor

2.2.3.5 Testing the Psychometric Method: Tuber cores, 6 mm in diameter, were taken longitudinally through locally purchased tubers of the cultivar Maris Piper and were incubated for 24 hrs in 0.01, 0.1, 0.2 or 0.4 M solutions of sucrose. Measurements of water potential and osmotic potential were taken using the method outlined in 2.2.3.4 and turgor was calculated. Turgor was plotted against concentration of incubation solution.

2.2.3.6 Tuber Turgidity Through Storage: Five tubers of treatments 1, 3, 7 and 9 were taken for analyses throughout storage season 1997/8 as outlined in 2.2.1.3 and tuber turgidity was determined as outlined in 2.2.3.4 following measurements of water potential and osmotic potential. Results of water potential, osmotic potential and turgor were plotted over time.

2.2.4 Cation Leakage

2.2.4.1 Method for Determining Cation Concentrations: Cation concentrations were determined by ion chromatography, using a Dionex model DX - 100 system fitted with an advanced computer interface and cation self regulating suppresser (Dionex Corporation, 1992). Each sample was filtered through a 0.22 µ meter pore size membrane and injected into a 25 µl fill loop. The sample was subsequently injected into the mobile phase for separation of the cations using an IonPac CS12 4 mm analytical column and CG12 4 mm guard column. The mobile phase consisted of 20 mM methyl - sulphonic acid with a flow rate of 0.95 ml min^{-1}. Cations were detected by conductivity.

2.2.4.2 Calibration of Cation Measurements: Dilutions of a 1000 µM stock solution of KCl, $CaCl_2$, MgCl, NH_4Cl and NaCl were prepared using deionised water to give solutions of 1000, 667, 333, 134, 13.4, 1.34 and 0 µM. Cation concentration was measured as in 2.2.4.1. 5 replicas of each solution were measured. Results produced by the Dionex were in the form of chromatogram, with values of each cation displayed as area under the graph. These values were used to calibrate the Dionex with the values of known cation concentration.

2.2.4.3 Determination of Tuber Incubation Time for Cation Analysis: Longitudinal sections of tubers, 10 mm in diameter were taken for analysis of cations. The periderm was removed from the stem end and a 10 mm cross section was taken. The tuber section was accurately weighed, diced into discs 1.0 mm in diameter and rinsed in deionised water to wash cut surfaces. The tissue was then incubated in 10 ml of deionised water in a shaking water bath at 25 ºC for 0, 10, 30, 60 and 120 min. The bathing solution was poured off and stored at –70 ºC prior to analysis. Solutions were defrosted and cation concentration was determined using the method outlined in 2.2.4.1. This method was repeated for 5 tubers, means and S.E. were calculated.

2.2.4.4 Cation Leakage Through Storage: Tubers taken throughout the 1995/6 and 1997/8 storage seasons were analysed for cation leakage as in 2.2.4.3 using a 30 min incubation time.

2.2.5 Potential Colour Formation Through Storage

Tubers taken throughout the 3 storage seasons were analysed for potential colour formation (2.2.5.1).

2.2.5.1 Measurement of Potential Colour Formation: Five tubers were used from each treatment. A core, 10 mm in diameter, was taken through the tuber from the stem end. The periderm was removed and the basal 10 mm was used for analysis. Tuber sections were accurately weighed, individually homogenised in 0.02 M phosphate buffer (Na_2HPO_4 and NaH_2PO_4) pH 6.5, (1:1 w:v), filtered through 2 layers of muslin and incubated at 21 °C for 24 hrs. The homogenate was then centrifuged using a MSE Micro Centaur (Jennings & Co., East Bridgford, UK) at 13000 rpm for 5 min. The supernatant was diluted 3:1 with distilled water. Absorbance was measured spectrophotometrically using a Perkin Elmer Lambda 12 spectrophotometer (Perkin-Elmer Limited, Beaconsfield, England) at 475 nm to give a value of colour (dopachrome) formation.

2.2.6 Determination of Bruise Susceptibility

Throughout the 1996/7 storage season, 15 tubers of each treatment were impacted at 5 °C and 10 °C (2.2.6.1) before storage at 25 °C for 48 hrs. The periderm was carefully removed, the presence of a bruise was noted and

bruise height, width and depth were measured, to enable a cylindrical approximation of the bruise to be calculated;

Volume = $\pi \times r^2 \times h$

where; r = average bruise radius (height + width of bruise) ÷ 4

h = depth of bruise

The percentage of impacted tubers which bruised from each treatment was calculated and plotted.

For convenience all values of bruise volume were logged on 1 graph. Data fell into 4 categories and were grouped accordingly as follows,

Category 1 - Bruise = 0 mm^3 (no visible discolouration)

Category 2 - Bruise <124.9 mm^3 (small)

Category 3 - Bruise 125-374.9 mm^3 (medium)

Category 4 - Bruise >375 mm^3 (large)

This was repeated for all 4 sample dates. The % of tubers from treatments 1 to 12 falling into each category, was calculated and the results were plotted. Statistical analyses were performed on the actual bruise volume data and not the percentage of tubers falling into categories 1-4.

This was repeated for storage season 1997/8 with the following alterations. Twenty tubers were impacted from each treatment rather than 15, 10 of each impact temperature both at 0.7 J and at 0.5 J.

2.2.6.1 Investigations of Bruising: A 235 g bolt with a smooth hemispherical head, 32 mm in diameter was attached via a hook to a length of string which ran over a pulley. The bolt head was marked with water-based ink and was suspended 30 cm above the tuber in a Perspex tube (38 mm in diameter), such that the bolt did not touch the side of the tube (Fig 2.2). The bolt was released and allowed to impact the basal end of the tuber, delivering approximately 0.7 J of energy at the point of impact according to the formula

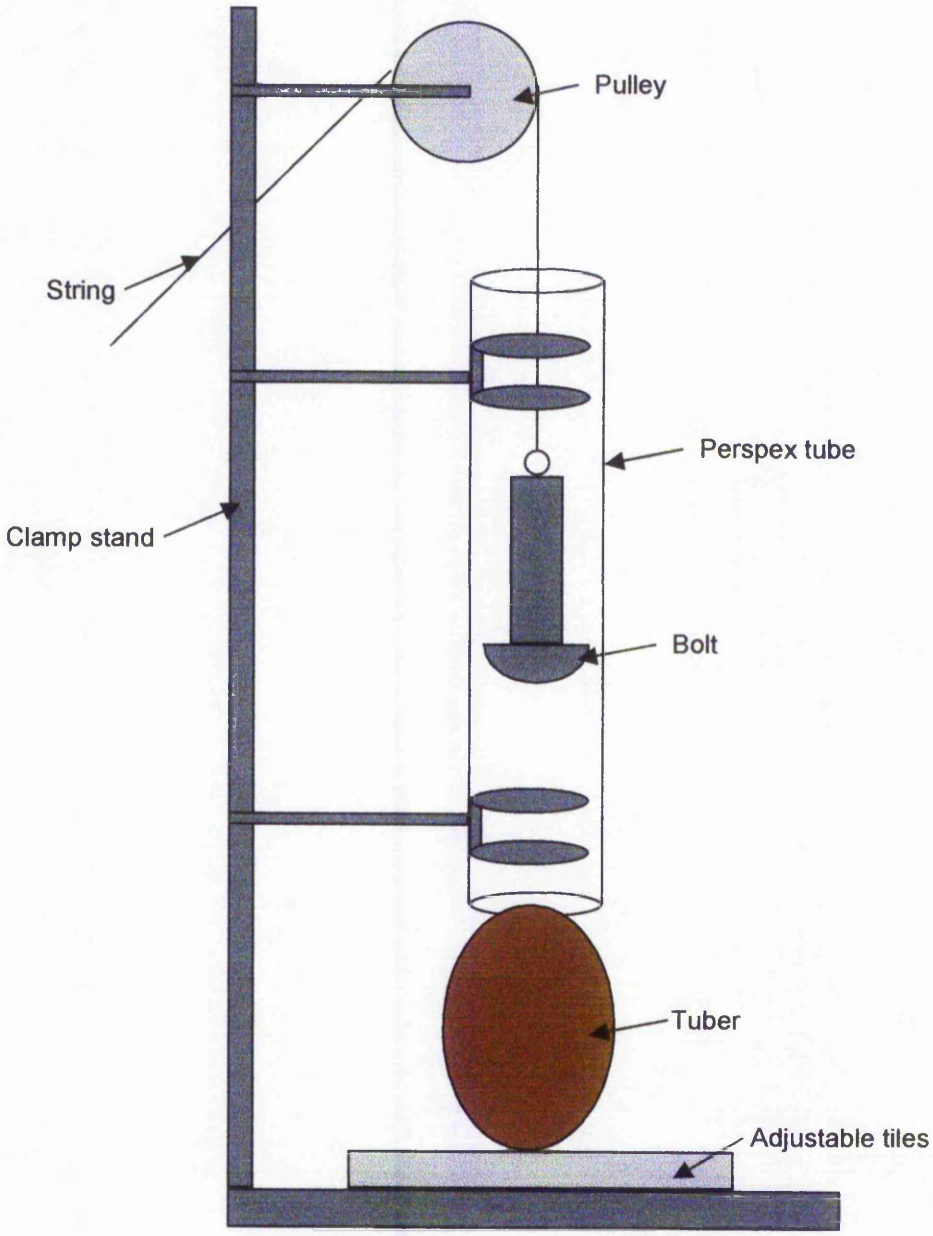


Figure 2.2. Diagram of the falling bolt used to impact tubers. Not to scale

$$E = m \times g \times h$$

Where: E = energy (Joules)

m = mass (kg)

g = gravity (9.8)

h = height (m)

The point of impact was marked by the ink.

2.2.7 Statistical Analyses

Where appropriate, data was analysed statistically using the SPSS version 8.0 (SPSS Inc.) general linear model. This is based on the 2-way anova. Where data did not show a linear formation, logged values were used. Alternatively, individual t-tests were performed when comparison of only 2 sets of data was required. All lines of best fit and R^2 were calculated using Excel (Microsoft). No statistical analyses were performed on respiration data.

2.2.8 Additional Measurements

In addition to the above, sub-samples of tubers from storage seasons 1996/7 and 1997/8 were analysed for sucrose, glucose and fructose content, and dry matter at the Sutton Bridge Experimental Station.

2.2 Results

Table 2.5. Treatment numbers assigned to combinations of tuber cv, physiological age and site for storage seasons 1996/7 and 1997/8.

Treatment	Cultivar	Physiological Age	Site
1	Pentland Dell	Young	Arthur Rickwood
2	Pentland Dell	Old	Arthur Rickwood
3	Maris Piper	Young	Arthur Rickwood
4	Maris Piper	Old	Arthur Rickwood
5	Record	Young	Arthur Rickwood
6	Record	Old	Arthur Rickwood
7	Pentland Dell	Young	Terrington
8	Pentland Dell	Old	Terrington
9	Maris Piper	Young	Terrington
10	Maris Piper	Old	Terrington
11	Record	Young	Terrington
12	Record	Old	Terrington

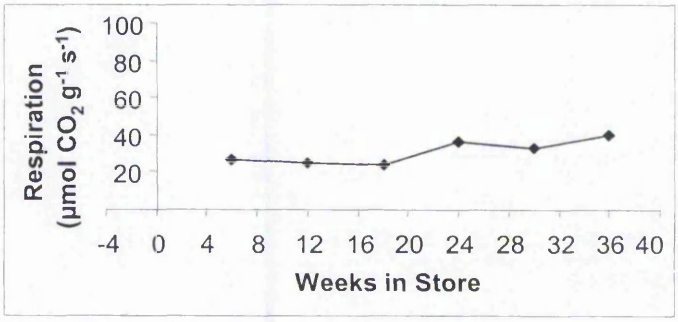
2.3.1 Respiration During Storage

No measurements of respiration were made at harvest for the storage season 1995/6 (Fig 2.3). Respiration remained stable for up to 18 wks in store in Pentland Dell and for up to 21 wks in Record. This was followed by a subsequent rise continuing until the end of storage (Fig 2.3) in both cvs at both temperatures. Tubers stored at 5 °C generally had a slightly lower respiration rate than those stored at 10 °C throughout storage.

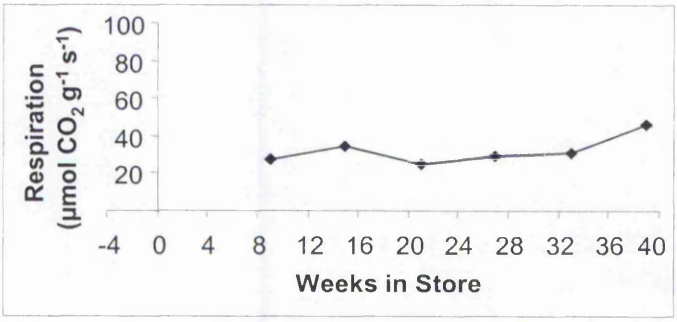
During storage season 1996/7, respiration of tubers from all 12 treatments decreased after harvest, reaching a minimum between 12 and 24 wks in store (Fig 2.4). This was followed by an increase at the end of storage. No consistent difference was seen between tubers of 0 and 150 day degrees physiological age (Fig 2.4). Tubers grown at Arthur Rickwood (treatments 1-6) had a lower respiration rate up until 12 wks in store than those grown at Terrington (treatments 7-12), after which the difference disappeared.

a.

b.



c.



d.

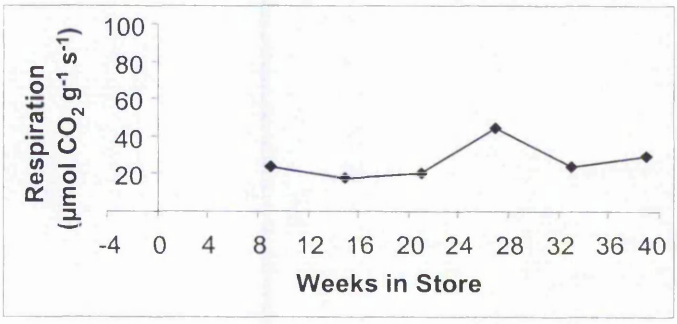


Figure 2.3. Respiration of tubers throughout storage season 1995/6. a. Pentland Dell stored at 10 °C, b. Pentland Dell stored at 5 °C, c. Record stored at 10 °C and d. Record stored at 5°C.

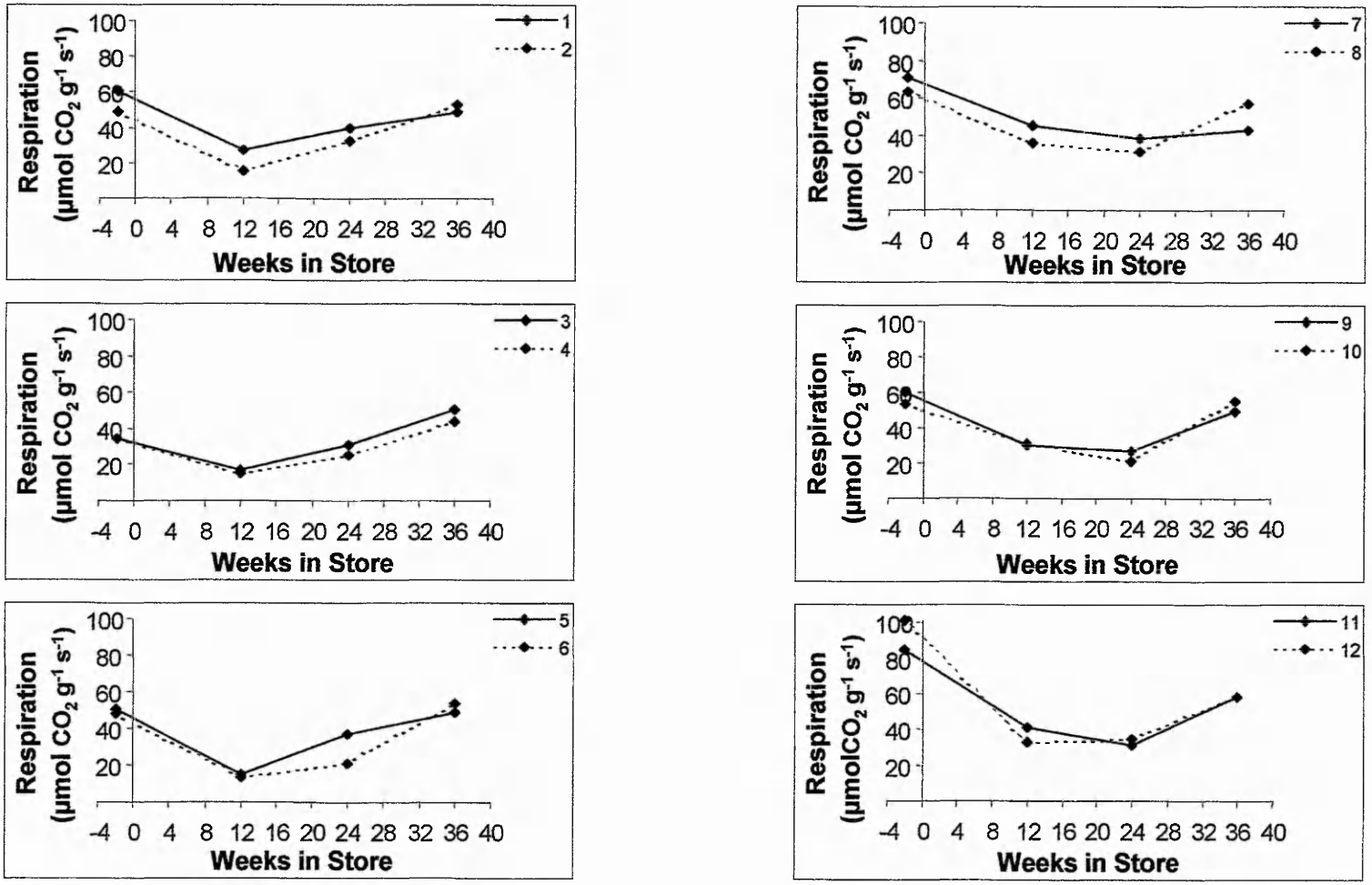


Figure 2.4. Tuber respiration from treatments 1 - 12 during the 1996/7 storage season.

During storage season 1997/8, tuber respiration of all 12 treatments declined immediately following the onset of storage, a low level was maintained during mid storage, reaching a minimum by 20 wks (Fig 2.5). Respiration rates then increased until 40 wks with the exceptions of treatments 2 and 7, which declined slightly between 30 and 40 wks. No consistent differences were seen between tubers of 0 and 500 day degrees physiological age or between tubers grown at Arthur Rickwood and Terrington (Fig 2.5).

No consistent differences were observed between the respiration rates over the 3 storage seasons or between cvs. No statistical analyses were performed on the respiration data due to a limited number of replications.

2.3.2 Tuber Turgidity

2.3.2.1 Determination of Tuber Turgidity Using Compensation Methods: The length of time taken for tuber sections to fully equilibrate in sucrose solutions was approximately 24 hrs (Fig 2.6a and b). This was the same for sections incubated with and without 0.02 M phosphate buffer. Addition of buffer to the sucrose solutions increased the stability of tuber section weights between 36 and 48 hrs. Tuber sections incubated in buffered sucrose solutions showed a greater weight change than those incubated without buffering ($P<0.05$).

Figure 2.6c shows the results of the compensation method following a 24 hr incubation period. Tuber weight declined with increasing sucrose concentration of the incubation solution. Net water movement ceased at a sucrose solution of 0.25 M and the graph levels off at approximately 0.86 M sucrose. Table 2.6 shows the sucrose concentration at which the water potential and osmotic potential of the tissue could be calculated, and also the calculated result of water potential and osmotic potential are shown along with the calculation of turgor for this tissue. Results are shown for both replicates in this investigation.

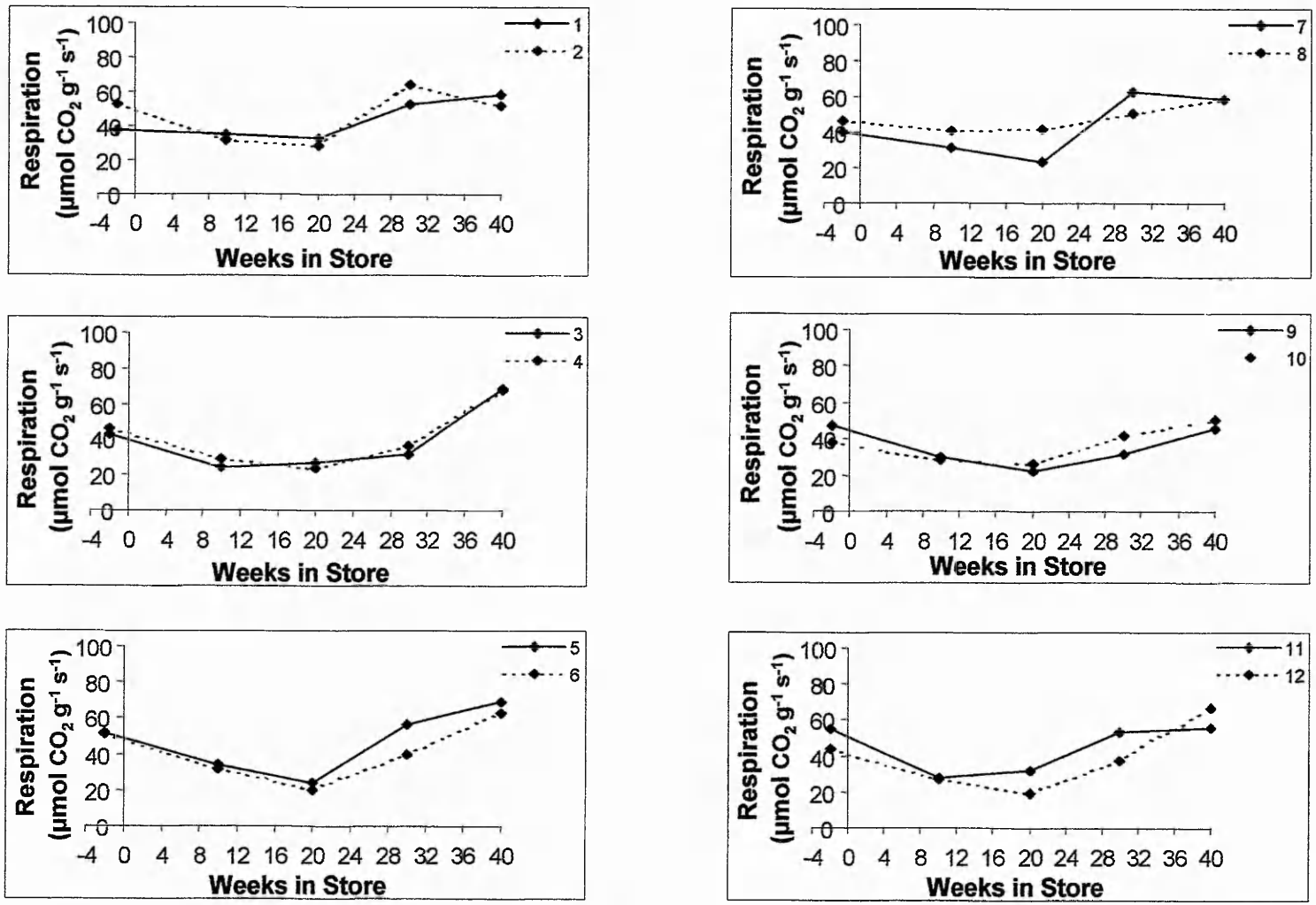


Figure 2.5. Tuber respiration from treatments 1 - 12 through 1997/8 storage season.

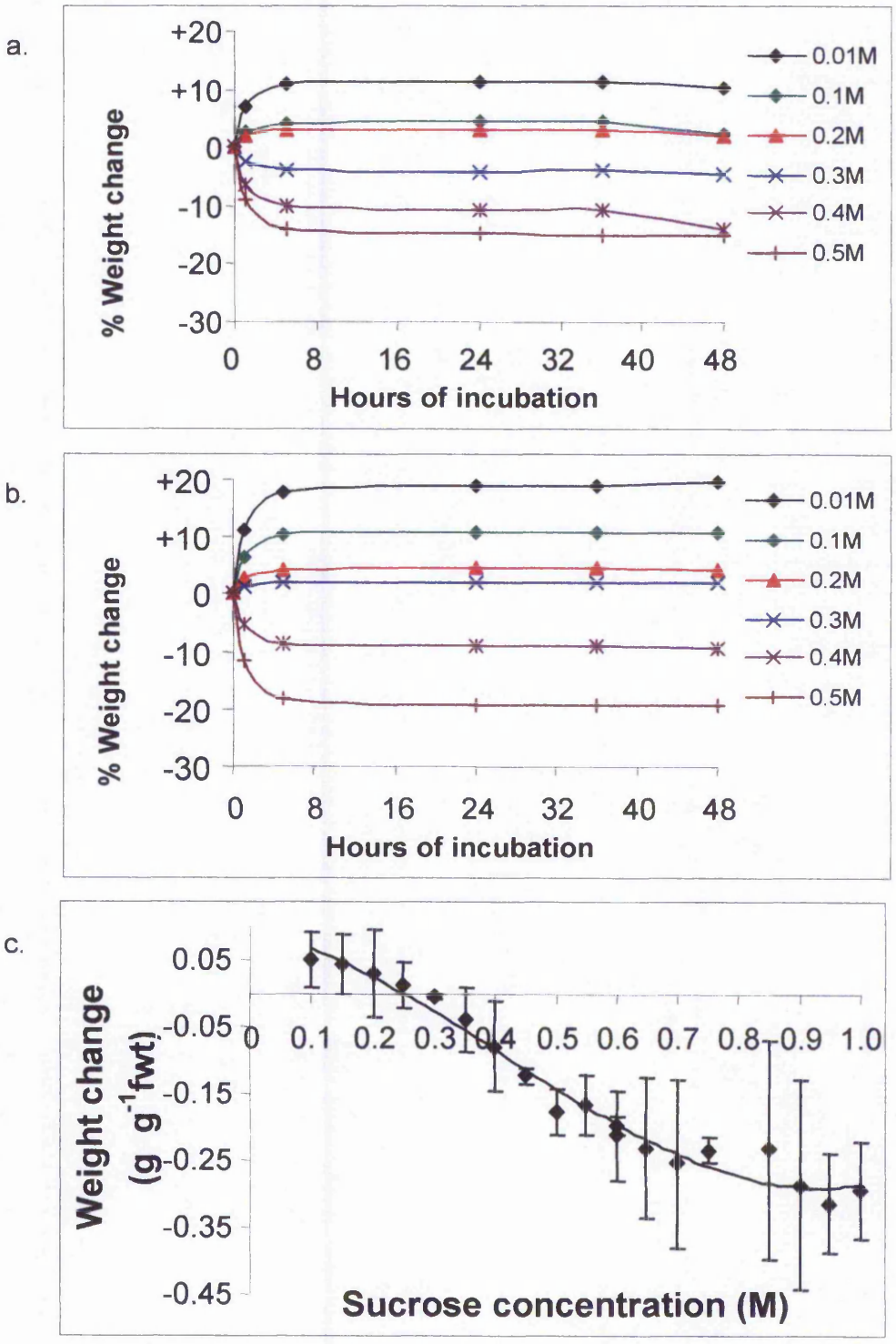


Figure 2.6. a. Weight equilibration of tuber tissue sections incubated in sucrose solutions over time. b. Weight equilibration of tuber tissue sections incubated in buffered sucrose solutions over time. c. Weight change of tuber tissue in solutions of differing sucrose concentration. Values are means ± S.E., where n = 5.

Table 2.6. Calculation of turgor using the compensation method including sucrose concentration at which ψ and π were calculated.

	Rep 1		Rep 2	
	Sucrose Concentration (M)	Calculated Result	Sucrose Concentration (M)	Calculated Result
ψ	0.25	0.618	0.38	0.940
π	0.86	-2.127	0.74	-1.830
P		2.745		2.77

2.3.2.2 Relative Water Content Through Storage: There was no significant change in RWC during the 24 wks for which measurements were taken during the storage season 1995/6 (Fig 2.7). There was no significant difference between Pentland Dell and Record ($P>0.05$), nor any between 5 °C and 10 °C stored tubers ($P>0.05$).

2.3.2.3 Determination of Tuber Turgidity Using a Psychrometer: The time taken for vapour equilibrium to be reached within the sample chambers, for both 0.1 M, 0.25 M (Fig 2.8a) and 0.5 M NaCl solutions and tuber tissue (Fig 2.8b) was 180 min, after which there was no significant difference ($P>0.05$) in chamber reading with time up to 360 min.

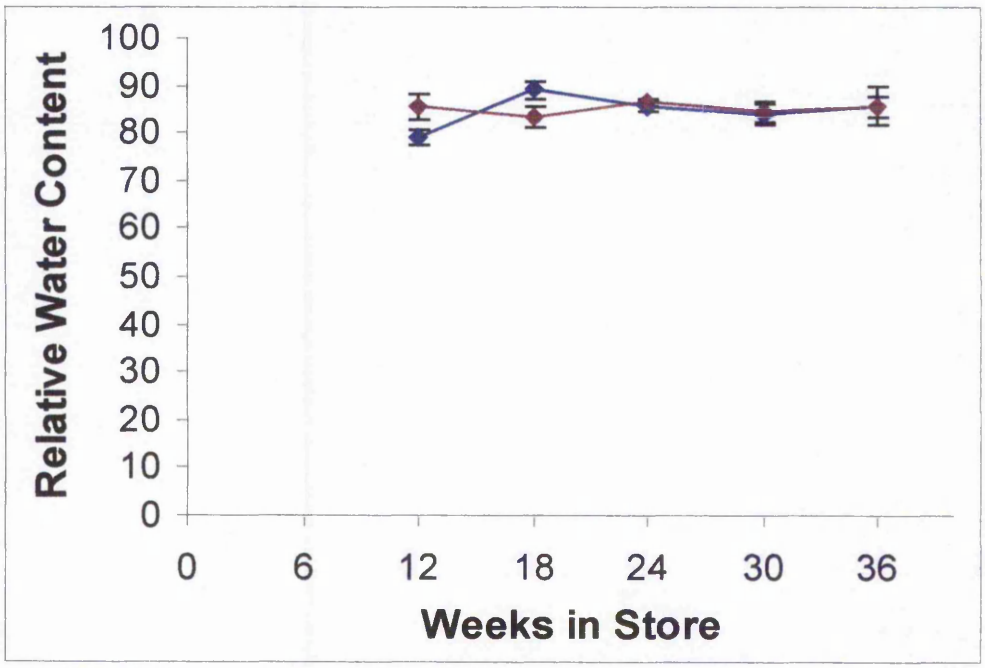
Calibration curves for the two C52 chambers are shown in Fig 2.8c showing the decline in chamber reading with the decline of water potential. The equations of the calibration curves and R^2 values of the best fit lines are shown in Table 2.7.

Table 2.7 The R^2 values and best fit line equations of the calibration curves for the two Wescor C52 sample chambers.

Chamber	R^2 of calibration curve	Best fit line equation
4539	0.9999	y = 0.2664x + 0.2538
4555	0.9999	y = 0.3708x + 0.2039

2.3.2.4 Testing the Psychrometric Method: There was a significant ($P<0.05$) decline in turgor from 1.44 Bars to 0.52 Bars when tuber sections were incubated in sucrose solutions ranging from 0.01 M to 0.4 M (Fig 2.8d).

a.



b.

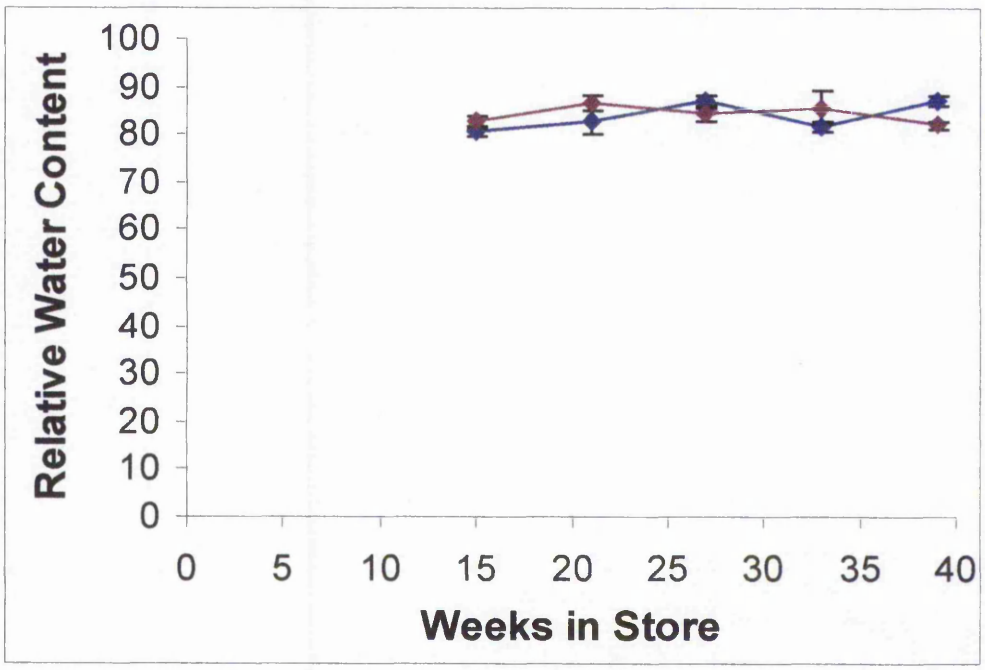


Figure 2.7. Relative water content of tubers (represented as a percentage of the maximum) from 1995/6 storage season at —◆— 5°C and —◆— 10°C in a. Pentland Dell and b. Record. Values are means ± S.E., where n = 5.

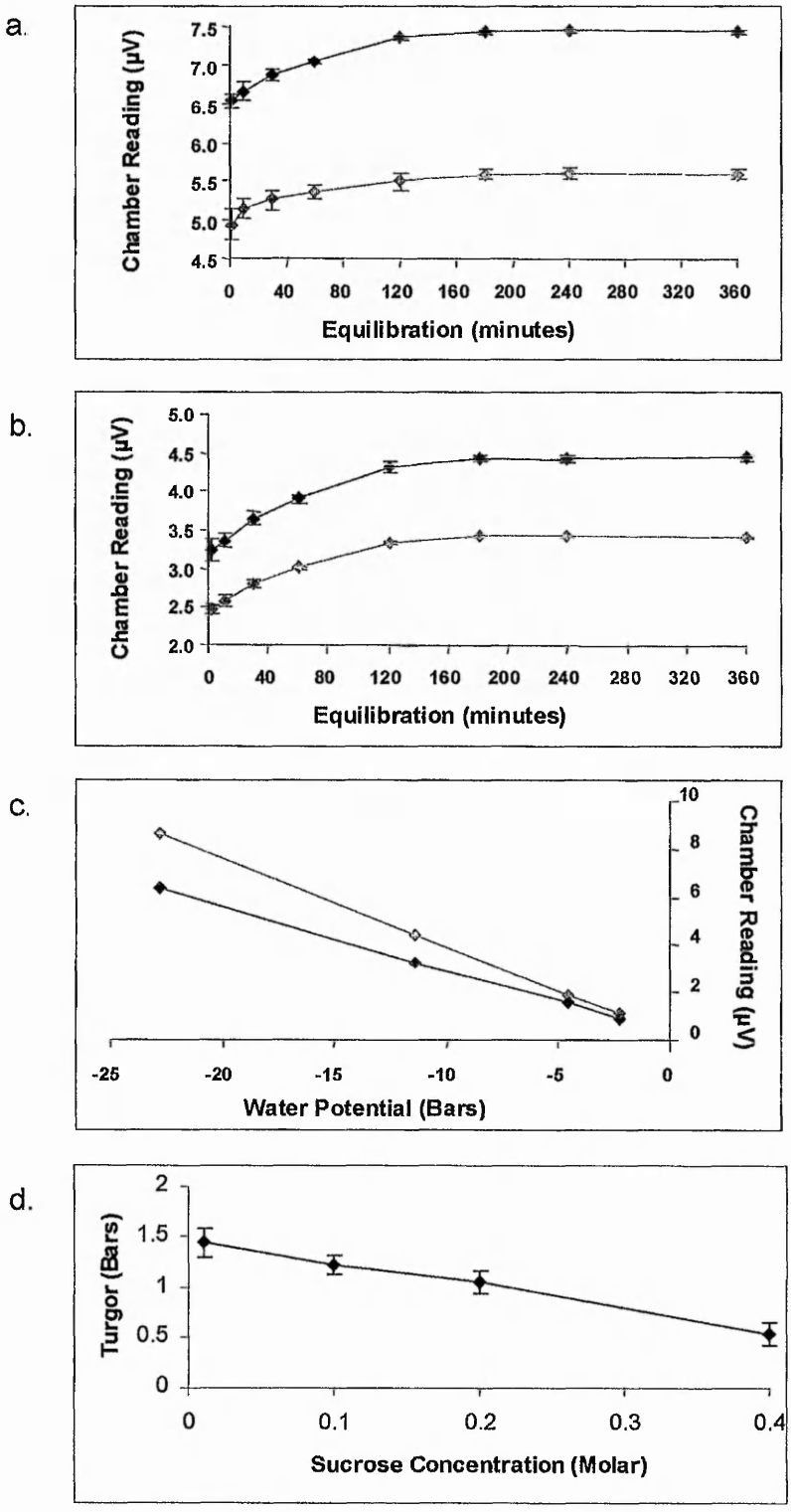


Figure 2.8 —◆— chamber 4539 —◇— chamber 4555 a. Equilibration of chambers using 0.25M NaCl. b. Equilibration of chambers using potato sections. c. Calibration curves for chambers. d. Water potential of tuber sections following incubation in sucrose solutions of varying molarity. Values are means ± S.E., where n = 5.

2.3.2.5 Water Potential, Osmotic Potential and Turgidity of Potato Tubers Through Storage: Water potential and osmotic potential declined (i.e. became more negative) in tubers of cultivar Pentland Dell from both Arthur Rickwood (Tr1) and Terrington (Tr 7) sites between harvest and 40 wks in store (Fig 2.9 a – d). These changes were significant at $P<0.001$. There was no significant change in water potential or osmotic potential through storage for cv Maris Piper (Tr 3 and 9; Fig 2.9 a – d). No significant difference was found in turgor pressure through storage for either Maris Piper or Pentland Dell (Fig 2.9 e – f). A significant difference in water potential and turgor was found between sites for cv Pentland Dell ($P<0.001$). Tubers grown at Terrington had a higher turgor pressure (Fig 2.9 f) and water potential (Fig 2.9 b) than those grown at Arthur Rickwood (Fig 2.9 a and e) in this cv. This was not the case for osmotic potential which showed no significant difference between sites (Fig 2.9 c – d). There was no significant difference between sites in water potential, osmotic potential or turgor in cv Maris Piper ($P>0.05$). Pentland Dell had a significantly lower water potential ($P<0.001$) (Fig 2.9 a and b) and turgor ($P<0.05$) (Fig 2.9 e and f). Cultivar Maris Piper did have a significantly higher osmotic potential than cv Pentland Dell ($P<0.01$) but this difference was only seen at 40 wks in store (Fig 2.9 c and d).

2.3.3 Cation Analysis of Tubers Through Storage

2.3.3.1 Calibration of Cations: Figure 2.10a shows the chromatogram produced following a run with a stock solution containing 1000µM cations. Table 2.8 shows the equations of the best fit lines (with the R^2 values) calculated for each cation which represents the conversion of peak area to µM concentration.

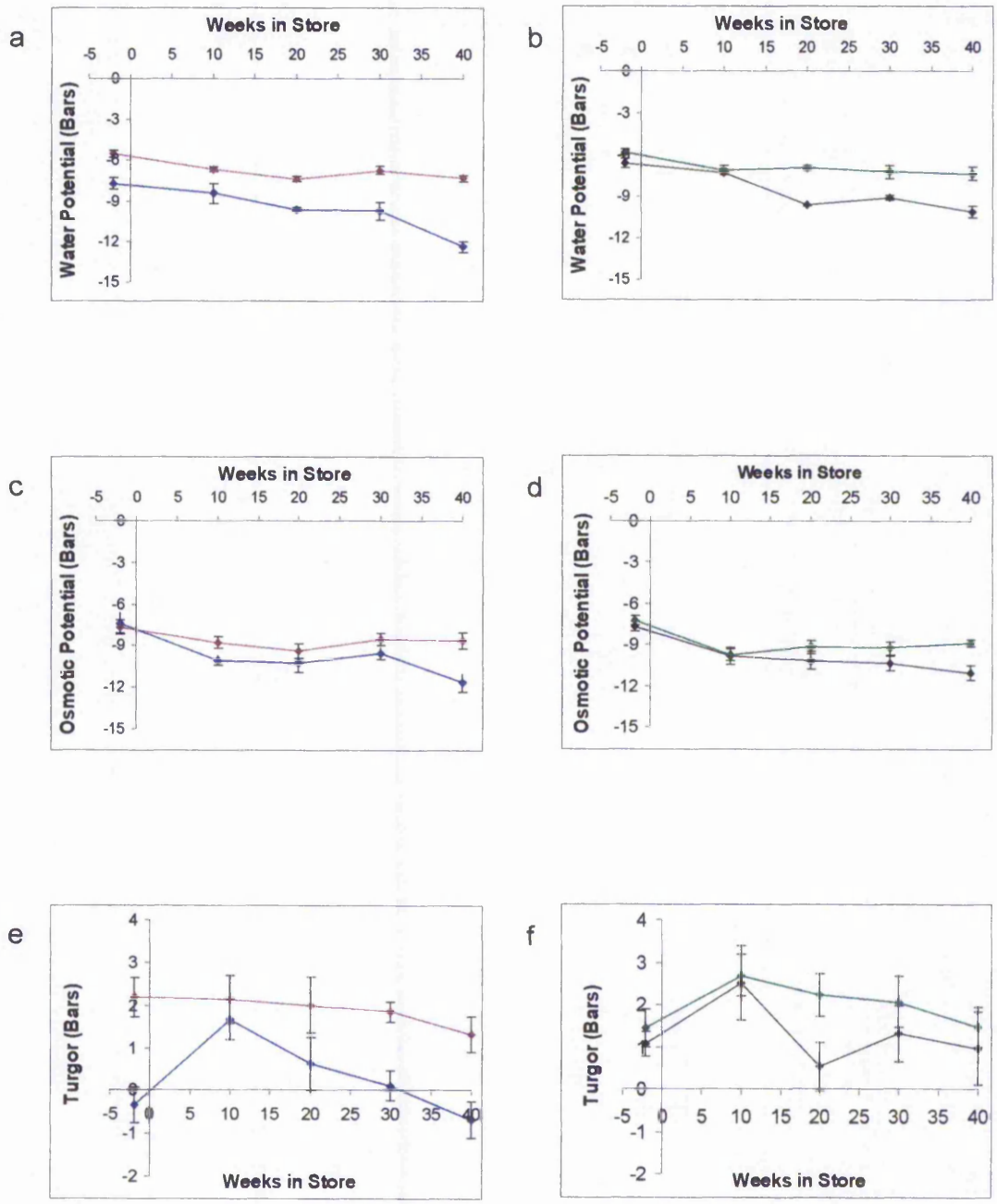
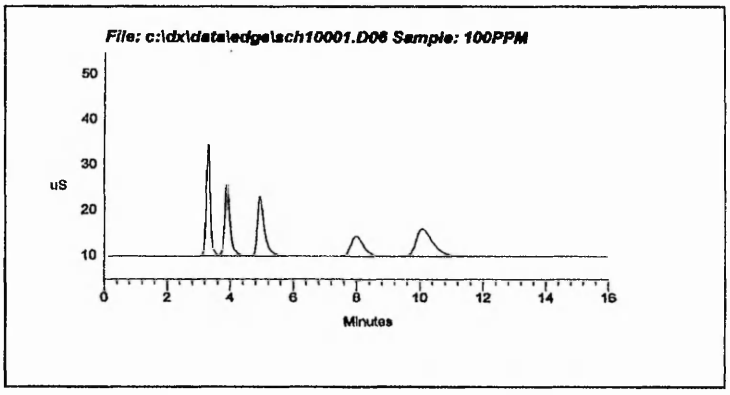
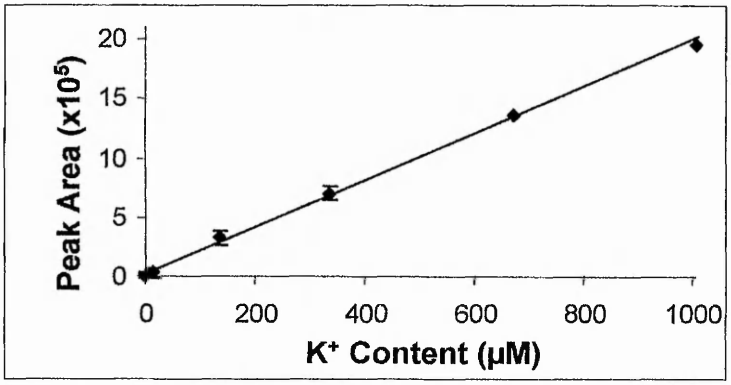


Figure 2.9. Changes in the water relations of tuber tissue through storage season 1997/8. Where —◆— Tr 1, —◆— Tr 3, —◆— Tr 7 and —◆— Tr 9 a. Water potential in tubers from Arthur Rickwood, b. Water potential in tubers from Terrington, c. Osmotic potential in tubers from Arthur Rickwood, d. Osmotic potential in tubers from Terrington, e. Turgor in tubers from Arthur Rickwood, f. Turgor in tubers from Terrington. Values are means ± S.E., where n = 5.

a.



b.



c.

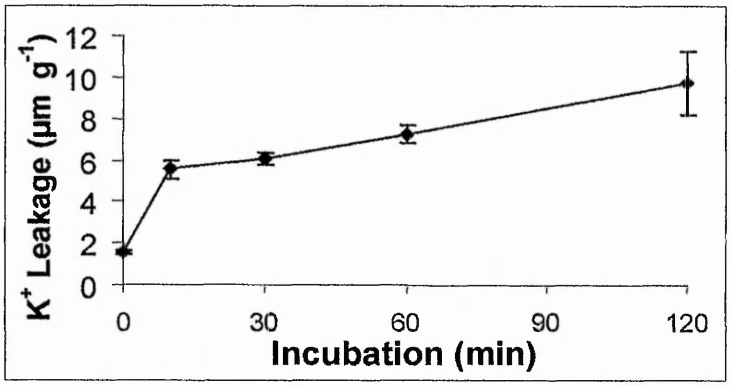


Figure 2.10. Preliminary work for the measurement of cation leakage. a. Example of a chromatogram produced from a 1000µM standard solution of cations (peaks represent from left to right sodium, ammonium, potassium, magnesium and calcium, µS represents a unit of conductivity). b. Calibration curve for potassium. c. Incubation time-course for potassium. Values are means ± S.E., where n = 5.

Table 2.8. Relationship between area under chromatogram (y) for cations and cation concentration in µM (x).

Cation	Equation	R^2
Sodium	x=(y-9191.816)/20981.67	0.9998
Ammonium	x=(y-440216.4)/3293.594	0.9594
Potassium	x=(y-43684.22)/1977.405	0.9939
Magnesium	x=(y+2222.56)/4240.479	0.9993
Calcium	x=(y+6008.4)/3944.967	0.9991

Figure 2.10 b shows the result of plotting the peak area against cation concentration (µM) for the cation potassium.

2.3.3.2 Cation Leakage from Potato Tuber Tissue Over Time: Cation leakage from tuber tissue increased at a constant rate between 10 and 120 min, although the S.E. value was noticeably higher at the latter time (Fig 2.10 c).

2.3.3.3 Cation Leakage Through Storage: Fig 2.11 show the results of cation leakage during storage season 1995/6 between 12 and 39 wks in store for cvs Pentland Dell and Record stored at 5 and 10°C.

Sodium leakage was significantly higher in tubers of cv Record than Pentland Dell throughout storage ($P<0.05$) (Fig 2.11 a and b). Sodium leakage remained stable up to 24 wks of storage for Pentland Dell and 27 wks in cv Record, at approximately 0.4 µm g^{-1} and 0.6 µm g^{-1} in Pentland Dell and Record, respectively. Following this there was a rise, which stabilised by the end of storage in both Pentland Dell and Record at 5 and 10 °C ($P<0.01$). No consistent difference was seen between tubers stored at 5 °C and 10 °C.

There was no significant difference between cvs or storage temperature for ammonium leakage (Fig 2.11 c and d). Leakage increased from approximately 0.15 µm g^{-1} to 0.45 µm g^{-1} between 12 and 24 wks in Pentland Dell and 15 to 27 wks for Record. This change in leakage during storage was significant ($P<0.001$). Following this there was no change in ammonium leakage towards the end of storage.

No consistent differences were seen in potassium leakage between Pentland Dell and Record (Fig 2.11 e and f). Leakage of potassium was the

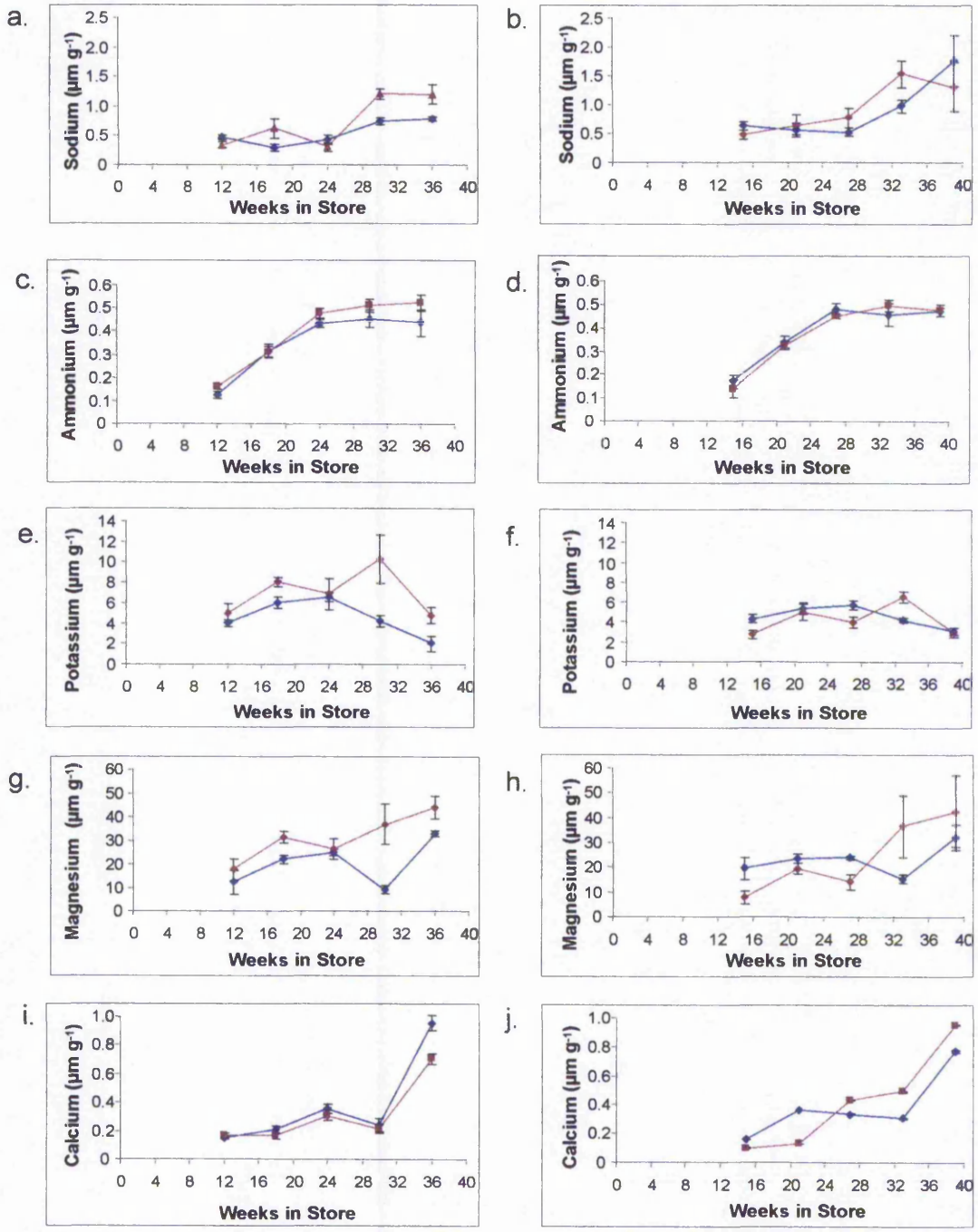


Fig 2.11. Cation leakage of tubers through the 1995/6 storage season. ◆ = stored at 5°C ■ = stored at 10°C. a. Sodium leakage in Pentland Dell, b. Sodium leakage in Record, c. Ammonium leakage in Pentland Dell, d. Ammonium leakage in Record, e. Potassium leakage in Pentland Dell, f. Potassium leakage in Record, g. Magnesium leakage in Pentland Dell, h. Magnesium leakage in Record, i. Calcium leakage in Pentland Dell, j. Calcium leakage in Record. Values are means ± S.E., where n = 5.

highest of all the cations with values ranging from approximately 2 to over 10 μm g^{-1}. Fig 2.11 e and f shows the changes in potassium content during storage. These changes were not significant ($P>0.05$) in tubers stored at 10 °C. However, tubers stored at 5 °C did show significant changes in potassium leakage throughout storage ($P<0.05$). A gentle rise in potassium leakage can be seen during early to mid-storage peaking after 24 wks in Pentland Dell and 27 wks in Record. Following this peak there was a decline in potassium leakage over the last 12 wks of storage in both cv.

There was an overall increase in magnesium leakage throughout storage in Pentland Dell and Record at 5 °C and 10 °C (Fig 2.11 g and h) which was significant at $P<0.01$. This was more pronounced in tubers stored at 10 °C although there was no significant difference between tubers stored at 5 and 10 °C ($P>0.05$). Cultivar had no significant effect on magnesium leakage ($P>0.05$).

Calcium leakage rose slightly over the first 30 wks of storage in Pentland Dell and 33 wks of storage in Record (Fig 2.11 i and j). Following this there was a dramatic rise in calcium leakage in both cvs at 5 and 10 °C during the last 6 wks of storage ($P<0.001$). No consistent affect of temperature or cv on calcium leakage was seen.

Fig 2.12 - 2.16 show cation leakage for the storage season 1997/8. With the exception of magnesium ion leakage in Pentland Dell and potassium ion leakage in general, there was a significant ($P<0.05$) change in the leakage of all cations throughout storage for all treatments during the 1997/8 storage season. For sodium ion leakage, there was a decline between 10 wks and the end of storage (Fig 2.12). A similar pattern was observed for calcium ion leakage (Fig 2.16). In comparison, ammonium ion leakage (Fig 2.13) increased throughout storage from 10 wks. Prior to this leakage was stable following harvest. With the exception of treatments 3 and 4, which demonstrate an increase in potassium ion leakage from 10 weeks in store ($P<0.01$), potassium leakage (Fig 2.14) did not significantly change throughout storage season 1997/8 ($P>0.05$). There was no significant change in magnesium ion leakage through storage for Pentland Dell ($P>0.05$). Fig 2.15

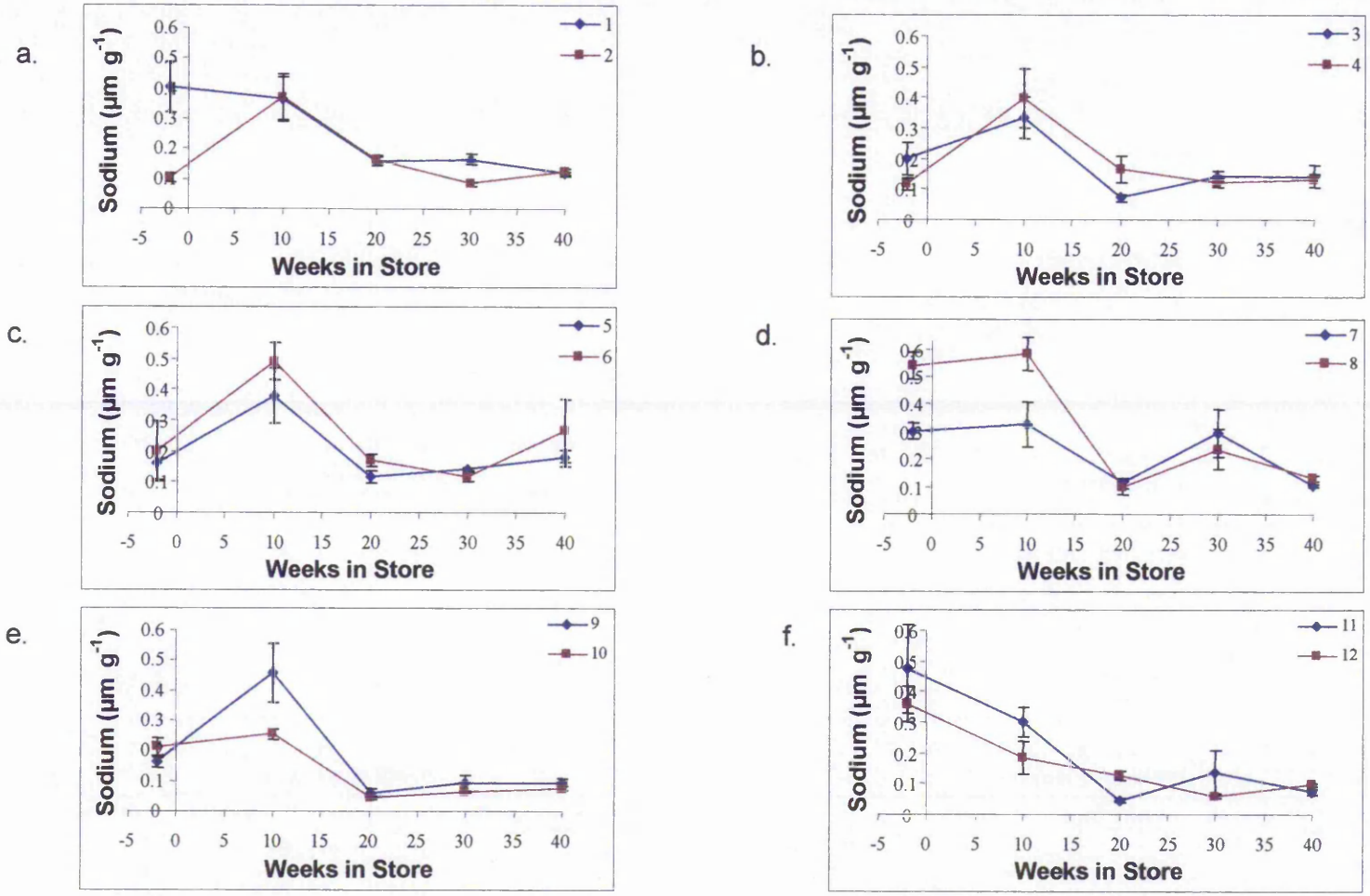


Fig 2.12. Sodium leakage during a 30 min incubation of tuber sections of treatments 1 - 12 through the 1997/8 storage season. Values are means ± S.E., where n = 5.

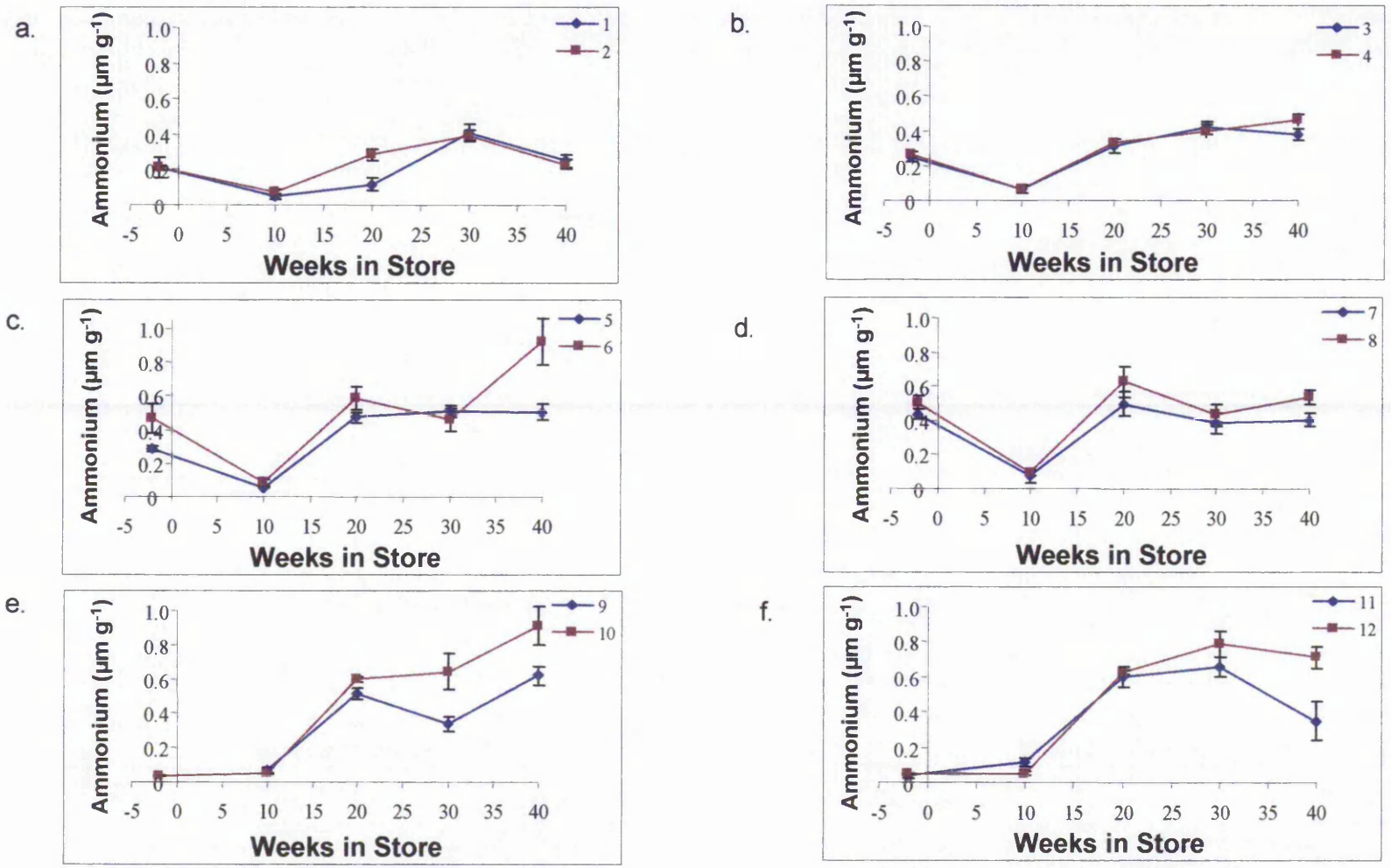


Fig 2.13. Ammonium leakage during a 30 min incubation of tuber sections of treatments 1 - 12 through the 1997/8 storage season. Values are means ± S.E., where n = 5.

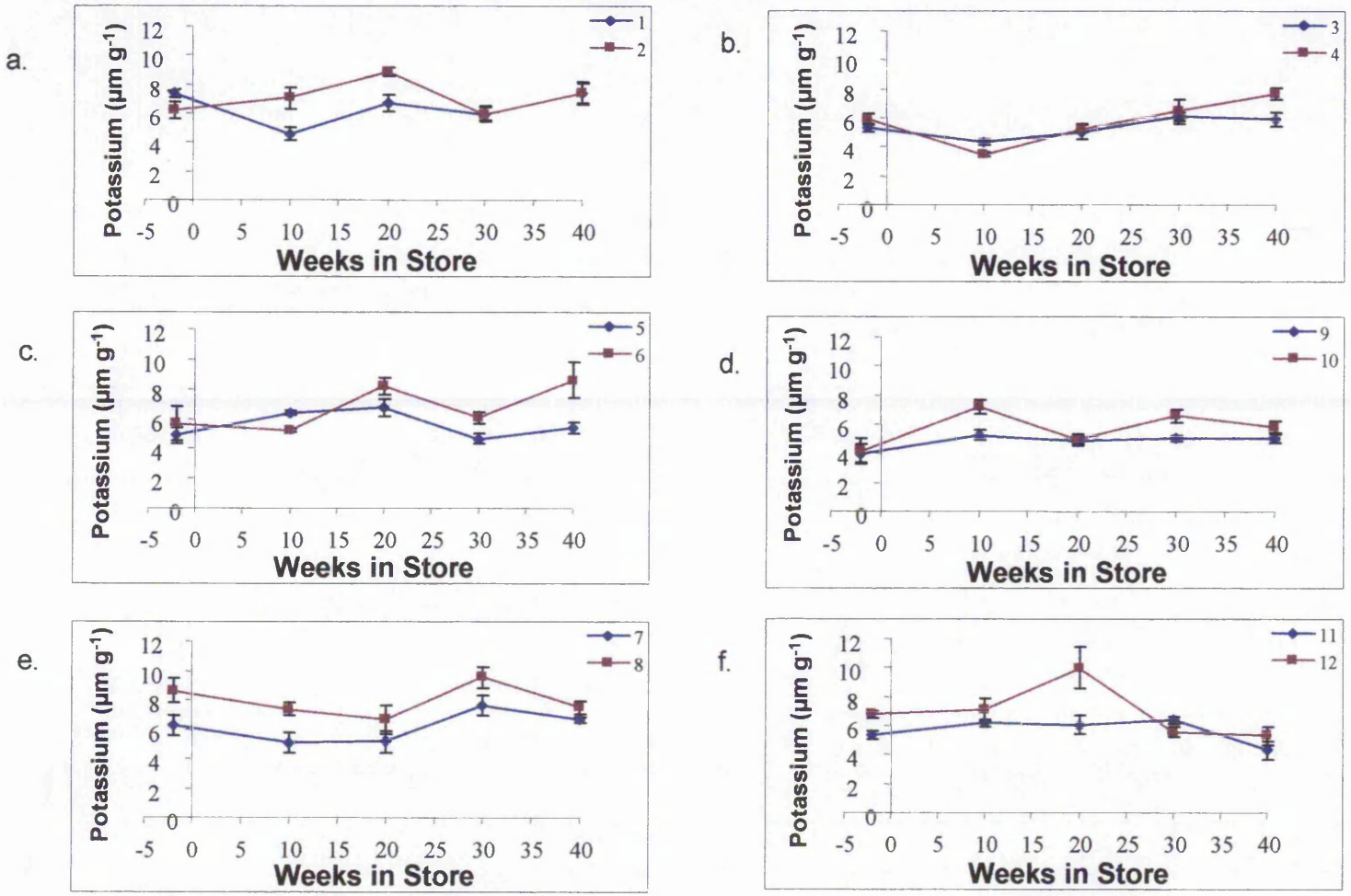


Fig 2.14. Potassium leakage during a 30 min incubation of tuber sections of treatments 1 - 12 through the 1997/8 storage season. Values are means ± S.E., where n = 5.

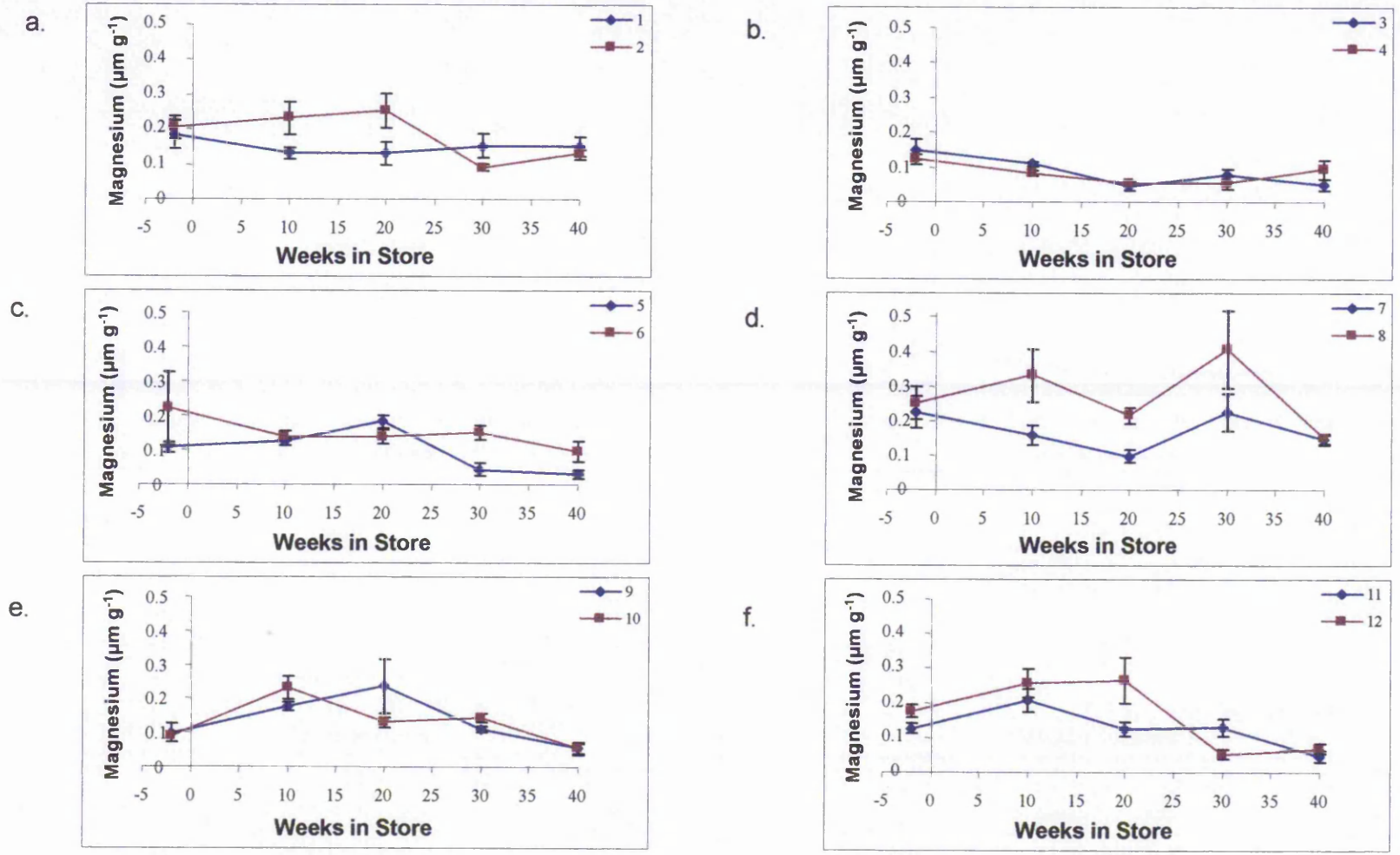


Fig 2.15. Magnesium leakage during a 30 min incubation of tuber sections of treatments 1 - 12 through the 1997/8 storage season. Values are means ± S.E., where n = 5.

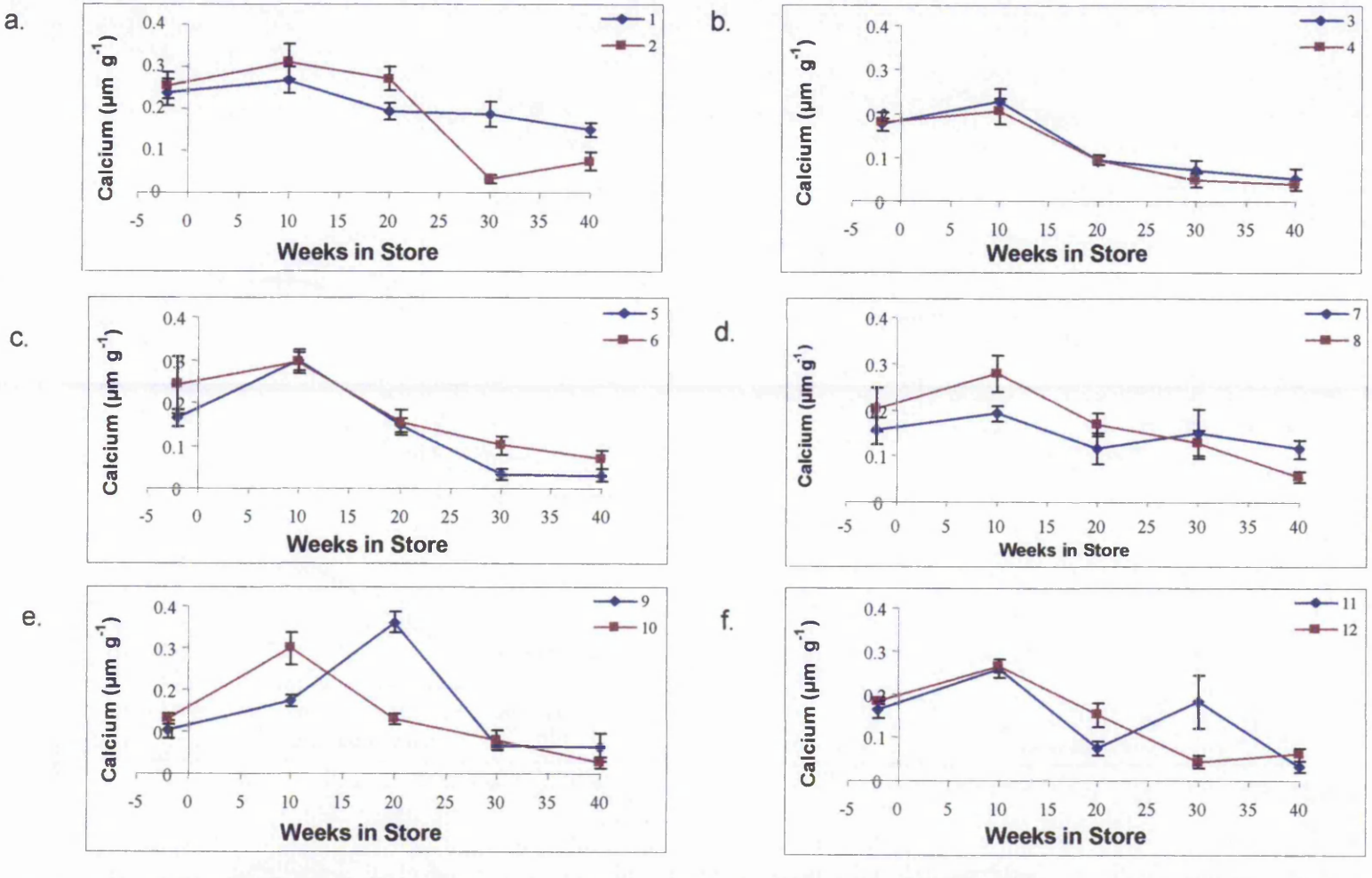


Fig 2.16. Calcium leakage during a 30 min incubation of tuber sections of treatments 1 - 12 through the 1997/8 storage season. Values are means ± S.E., where n = 5.

a and d. Maris Piper and Record did show significant changes through storage ($P<0.05$), which can be seen as a decline between 10 and 40 wks in store for tubers grown at Terrington.

Tuber cv had a significant effect on cation leakage ($P<0.05$). In general, Pentland Dell had the greatest leakage of cations, followed by cv Record and Maris Piper gave the lowest cation leakage. Whilst this relationship was true in most instances, leakage of ammonium ions was similar for Pentland Dell and Maris Piper, and lower than Record.

The physiological age of seed tubers did not have a significant effect on cation leakage.

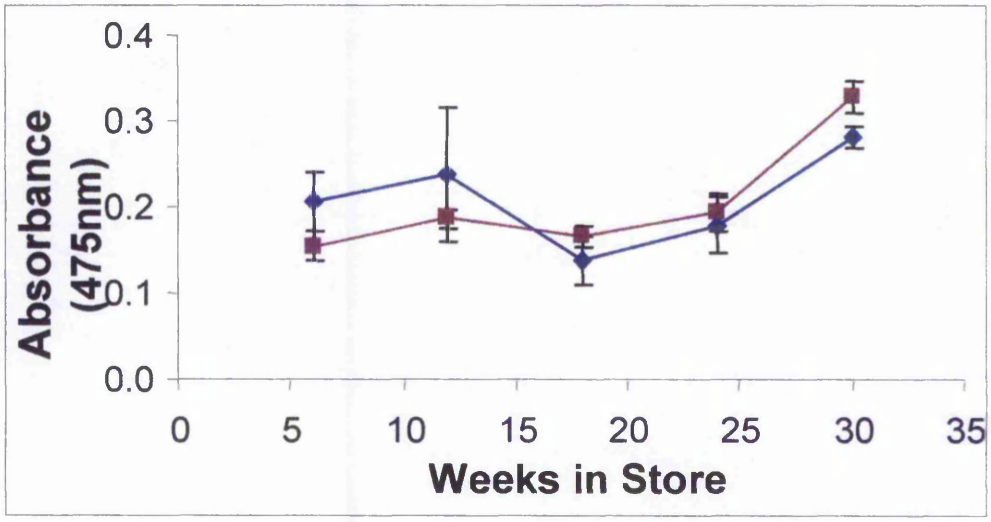
Site significantly influenced ammonium and magnesium ion leakage in Pentland Dell and Maris Piper ($P<0.05$). Tubers grown at Arthur Rickwood tended to have a lower rate of ion leakage than those grown at Terrington. No significant difference between sites was seen for sodium, calcium and potassium ion leakage or for cv Record ($P>0.05$).

2.3.4 Potential Colour Formation at (475 nm) Through Storage

During storage season 1995/6, potential colour formation at 475 nm of homogenised tuber tissue remained stable during early to mid storage (Fig 2.17) with an increase until the end of storage. This increase in potential colour formation was significant for cv Pentland Dell ($P<0.01$) but not found to be significant in cv Record using general linear model. However a t-test performed on the data found a significant difference between colour formation at 18 wks and that at 30 wks. No significant difference was found between the two cv or storage temperatures ($P>0.05$).

During the 1996/7 storage season, colour formation at 475 nm increased from harvest until 24 or 36 wks, after which it either declined or remained constant until the end of storage (Fig 2.18). The change in time over storage was significant ($P<0.001$). There was a significant difference between cvs (<0.001). Generally, cv Pentland Dell had the highest colour formation, followed by Record with Maris Piper having the least. No significant differences was seen between tubers grown from seed of different physiological ages ($P>0.05$), nor were any significant differences seen

a.



b.

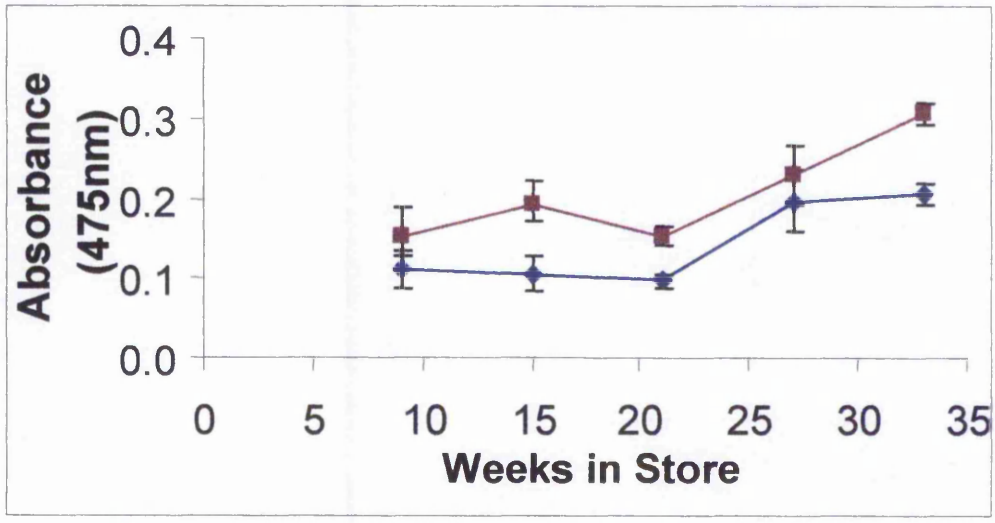


Figure 2.17. Colour formation (absorbance 475 nm) through storage season 1995/6. —◆— = 5°C —■— =10°C. a. Cultivar Pentland Dell, b. Cultivar Record. Values are means ± S.E., where n = 5.

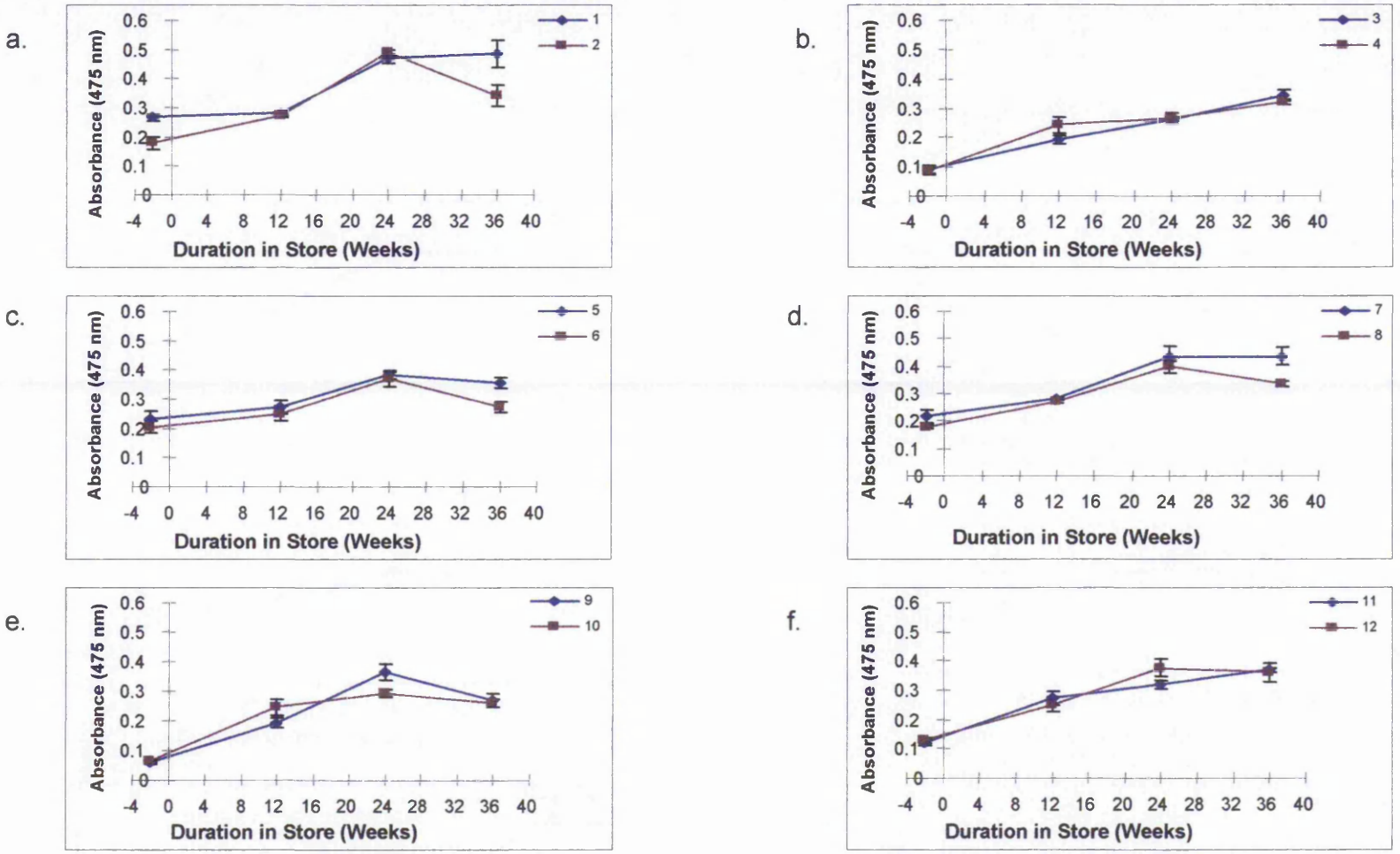


Figure 2.18. Potential colour formation (absorbance 475nm) through storage in tubers from treatments 1 - 12 during storage season 1996/7. Values are means ± S.E., where n = 5.

between tubers grown at Arthur Rickwood and those grown at Terrington ($P>0.05$).

Colour formation at 475 nm also showed a slight general increase through storage season 1997/8 in cvs Pentland Dell and Maris Piper (Fig 2.19). The change in colour formation through storage was significant ($P<0.001$). Cultivar Record showed no significant change in colour formation over time. Cultivar had a significant effect upon colour formation ($P<0.001$), Maris Piper developed the least colour formation, Pentland Dell and Record showed similar results for colour formation during early storage but this increased to a greater extent in Pentland Dell after 20 wks in store. The site at which tubers were grown gave a significant difference in colour formation at 475 nm ($P<0.001$). As illustrated in Fig 2.19, tubers grown at Arthur Rickwood (Tr 1 – 6) declined significantly in colour formation between 20 and 30 weeks. This decline was not apparent in tubers grown at Terrington (Tr 7 – 12), in addition colour formation at 475 nm was generally higher in tubers grown at Terrington than those grown at Arthur Rickwood. Physiological age did not have any significant effect on potential colour formation ($P>0.05$).

2.3.5 Bruise Development During Storage

During storage season 1996/7 there was a significant change in bruise susceptibility, determined by bruise volume, as storage progressed ($P<0.001$), as illustrated in Figs 2.20 and 2.21 (percentage of tubers bruising) and in Figs 2.22 to 2.25 (change in categories of bruise volume over time). With the exception of values at harvest, where bruise susceptibility tended to be high, there was a general increase in the percentage of impacted tubers forming bruises from early to late storage in tubers grown at Arthur Rickwood (Fig 2.20). This was not always apparent in tubers grown at Terrington (Fig 2.21). A greater percentage of tubers impacted at 5 °C underwent bruise formation compared with those impacted at 10 °C (Figs 2.20 and 2.21). Additionally tubers impacted at 5 °C had a greater bruise volume than those impacted at 10 °C ($P<0.001$) e.g. Fig 2.22 a and b. This was true for all cvs. Cultivar Maris Piper showed a lower degree of bruising (e.g. Fig 2.24 e) than either Pentland Dell (e.g. Fig 2.24 a) or Record (e.g. 2.25 c) ($P<0.05$), these latter two

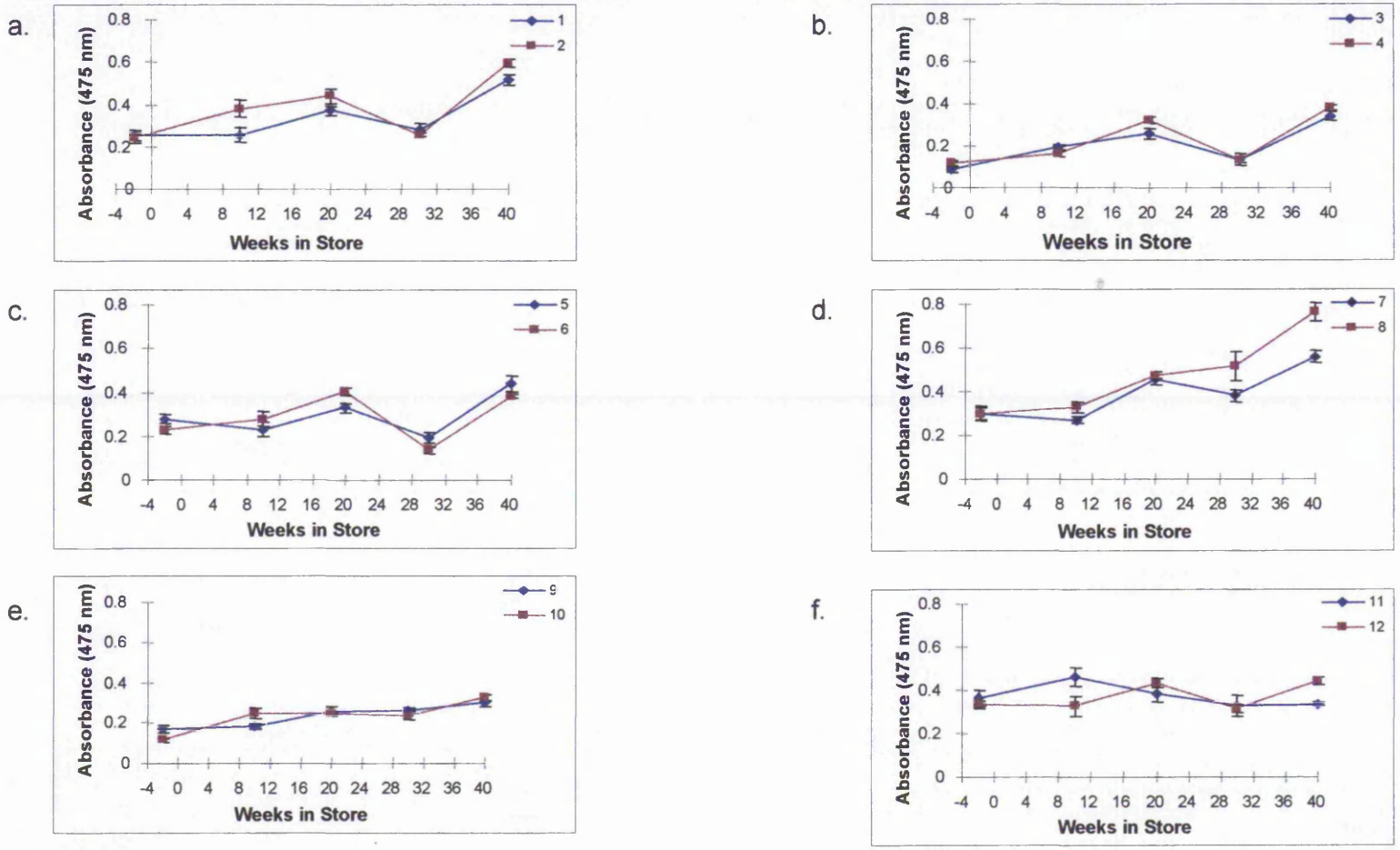


Figure 2.19. Potential colour formation (absorbance 475 nm) through storage in tubers from treatments 1 - 12 during storage season 1997/8. Values are means ± S.E. where n = 5.

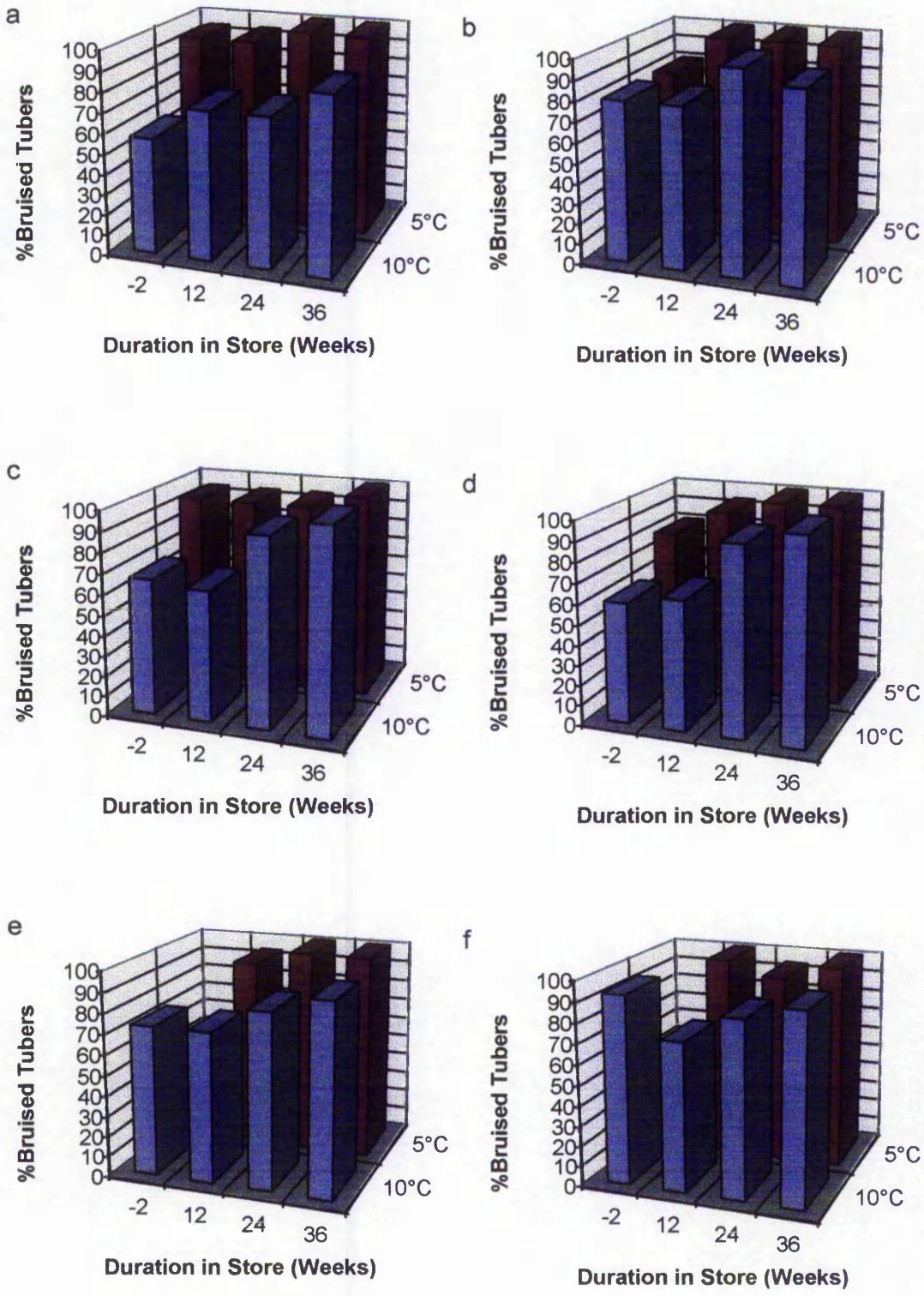


Figure 2.20. Percentage of tubers grown at Arthur Rickwood bruising through storage season 1996/7 following impact at 5 °C and 10 °C. a. Treatment 1, b. Treatment 2, c. Treatment 3, d. Treatment 4, e. Treatment 5 and f. Treatment 6.

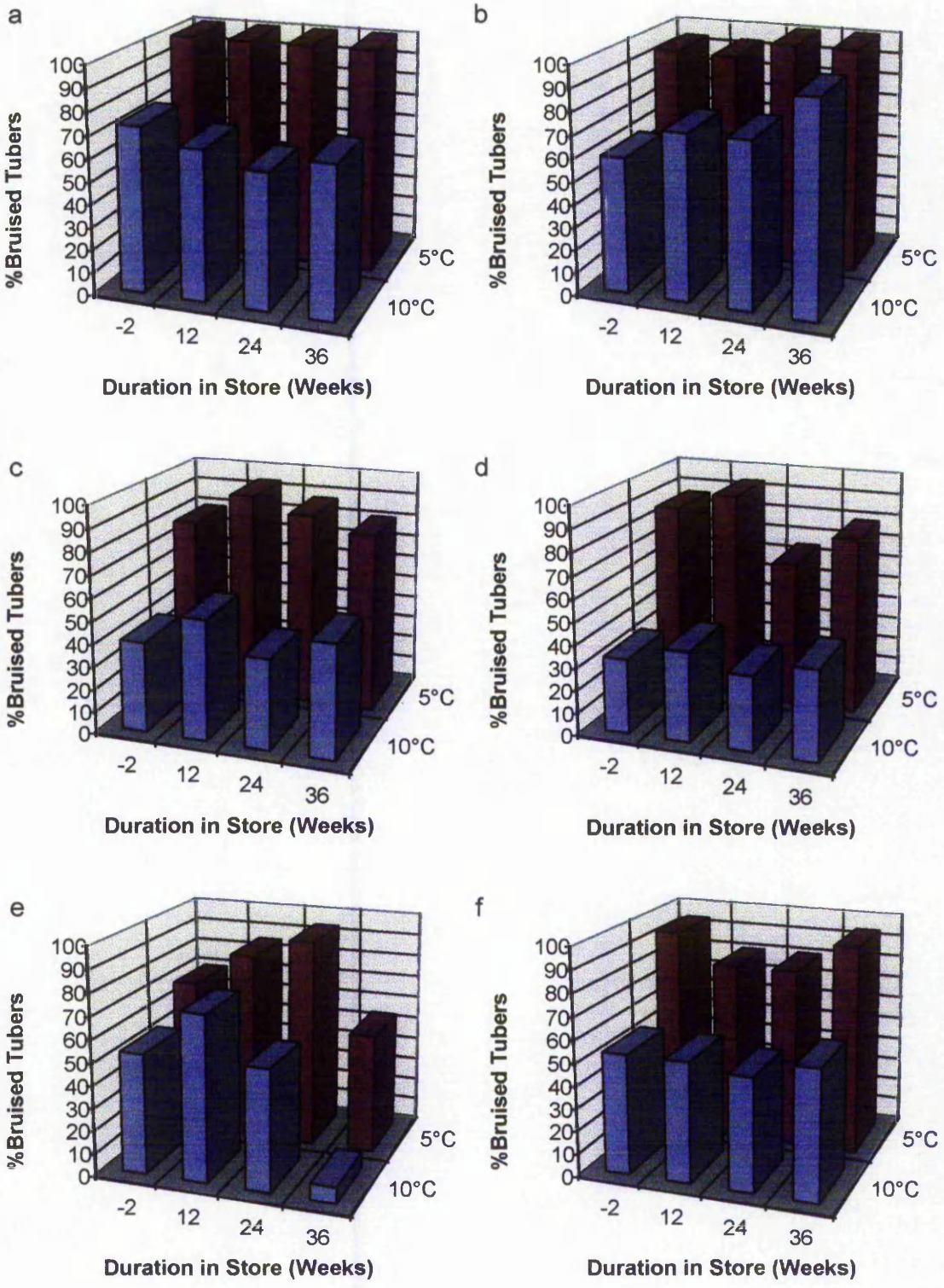


Figure 2.21 Percentage of tubers grown at Terrington bruising following impact at 5 °C and 10 °C through storage season 1996/7. a. Treatment 7, b. Treatment 8, c. Treatment 9, d. Treatment 10, e. Treatment 11 and f. Treatment 12.

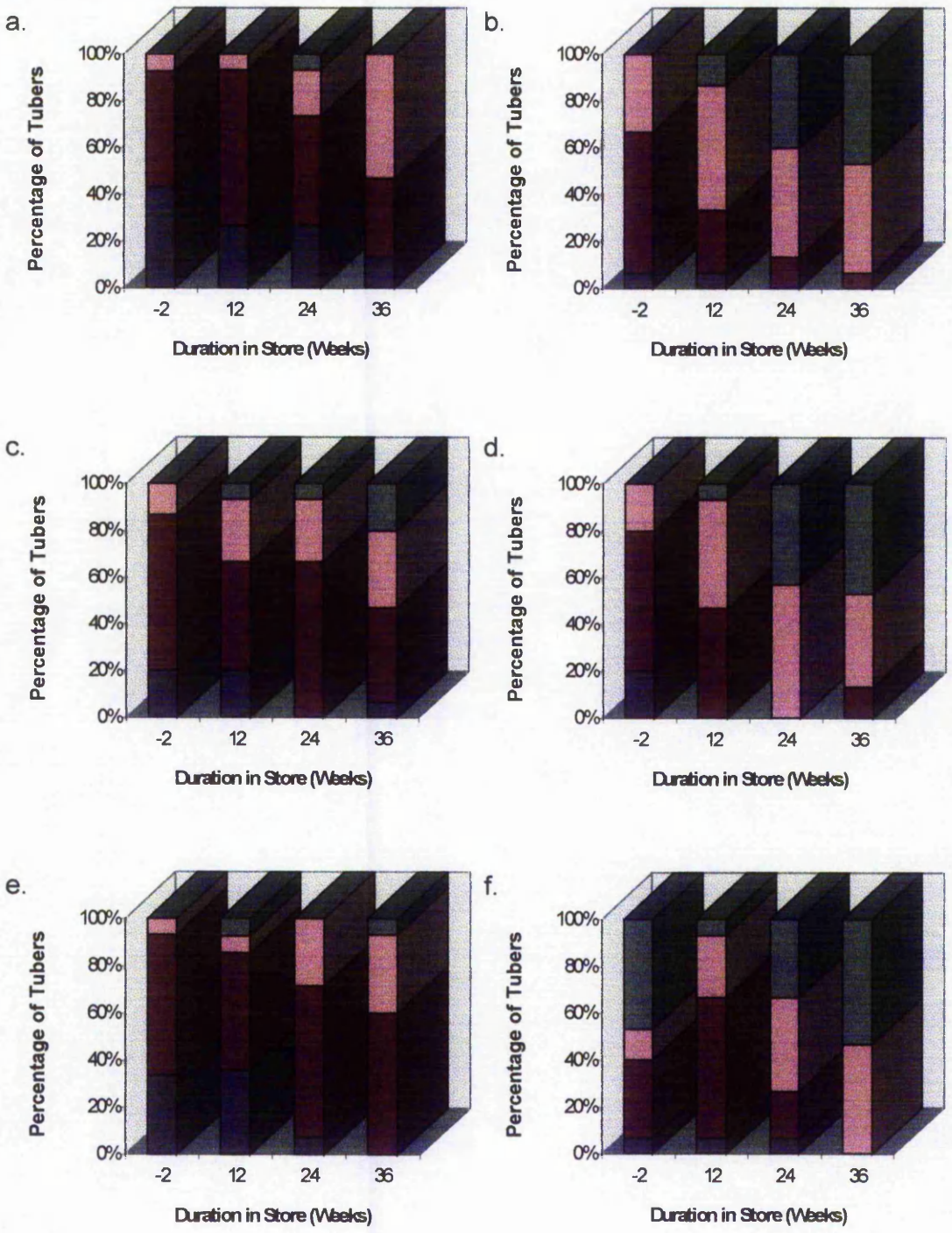


Figure 2.22 Percentage of impacted tubers showing ■ no visible bruise, ■ small bruise, ■ medium bruise and ■ large bruise through storage season 1996/7. a. Treatment 1, 10 °C impacted, b. Treatment 1, 5 °C impacted, c. Treatment 2, 10 °C impacted, d. Treatment 2, 5 °C impacted, e. Treatment 3, 10 °C impacted, f. Treatment 3, 5 °C impacted.

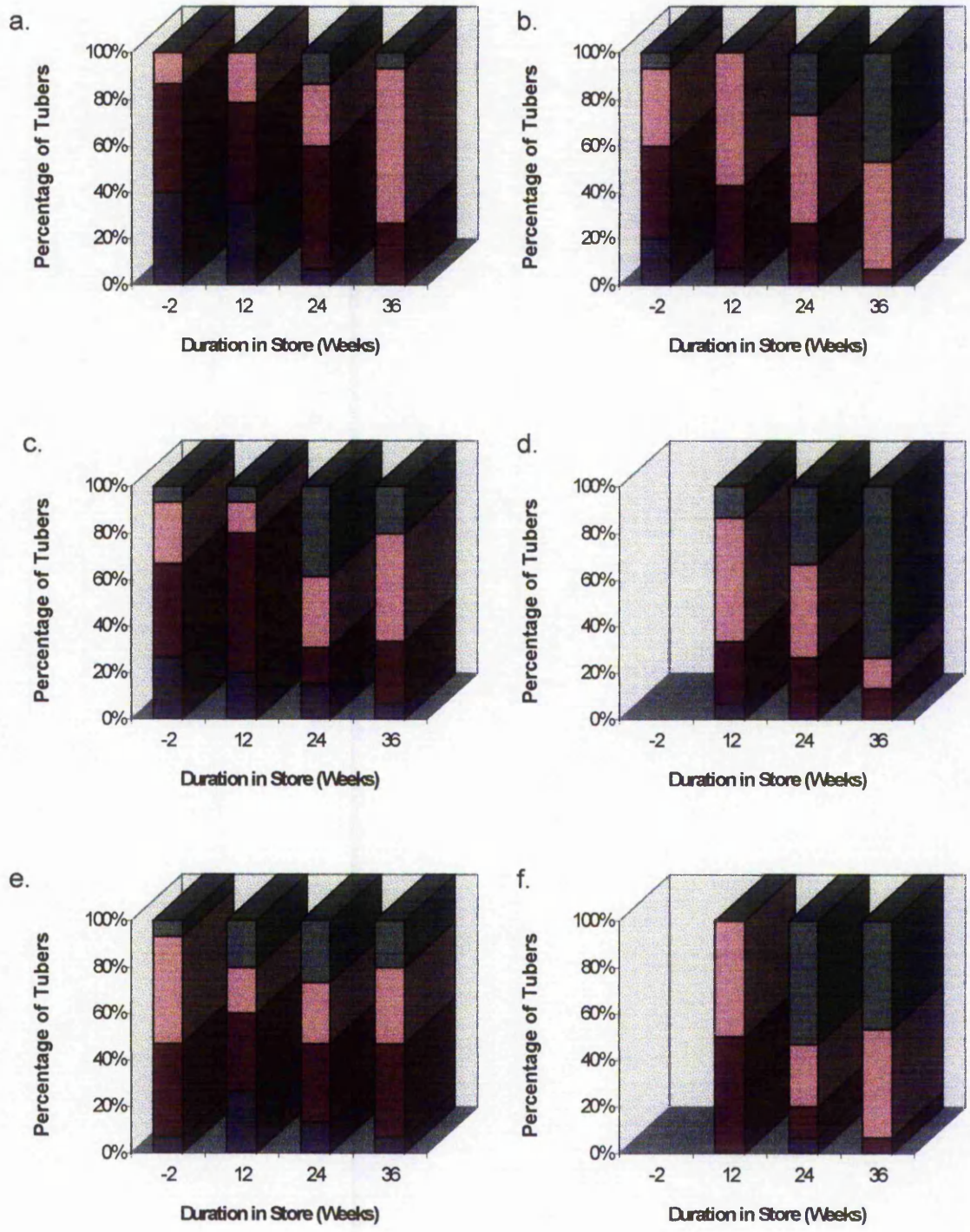


Figure 2.23 Percentage of impacted tubers showing ■ no visible bruise, ■ small bruise, ■ medium bruise and ■ large bruise through storage season 1996/7. a. Treatment 4, 10 °C impacted, b. Treatment 4, 5 °C impacted, c. Treatment 5, 10 °C impacted, d. Treatment 5, 5 °C impacted, e. Treatment 6, 10 °C impacted, f. Treatment 6, 5 °C impacted.

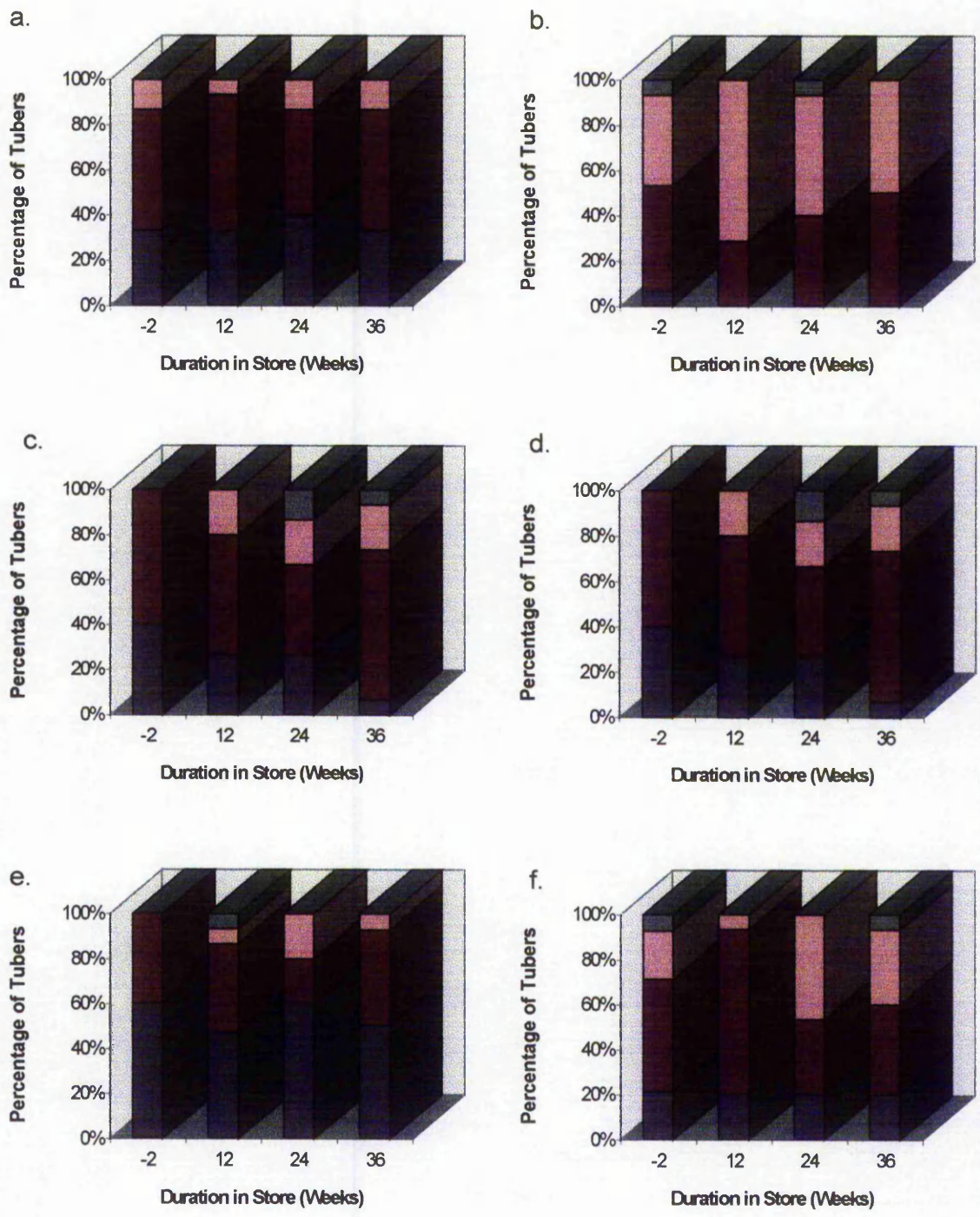


Figure 2.24 Percentage of impacted tubers showing ■ no visible bruise, ■ small bruise, ■ medium bruise and ■ large bruise through storage season 1996/7. a. Treatment 7, 10 °C impacted, b. Treatment 7, 5 °C impacted, c. Treatment 8, 10 °C impacted, d. Treatment 8, 5 °C impacted, e. Treatment 9, 10 °C impacted, f. Treatment 9, 5 °C impacted.

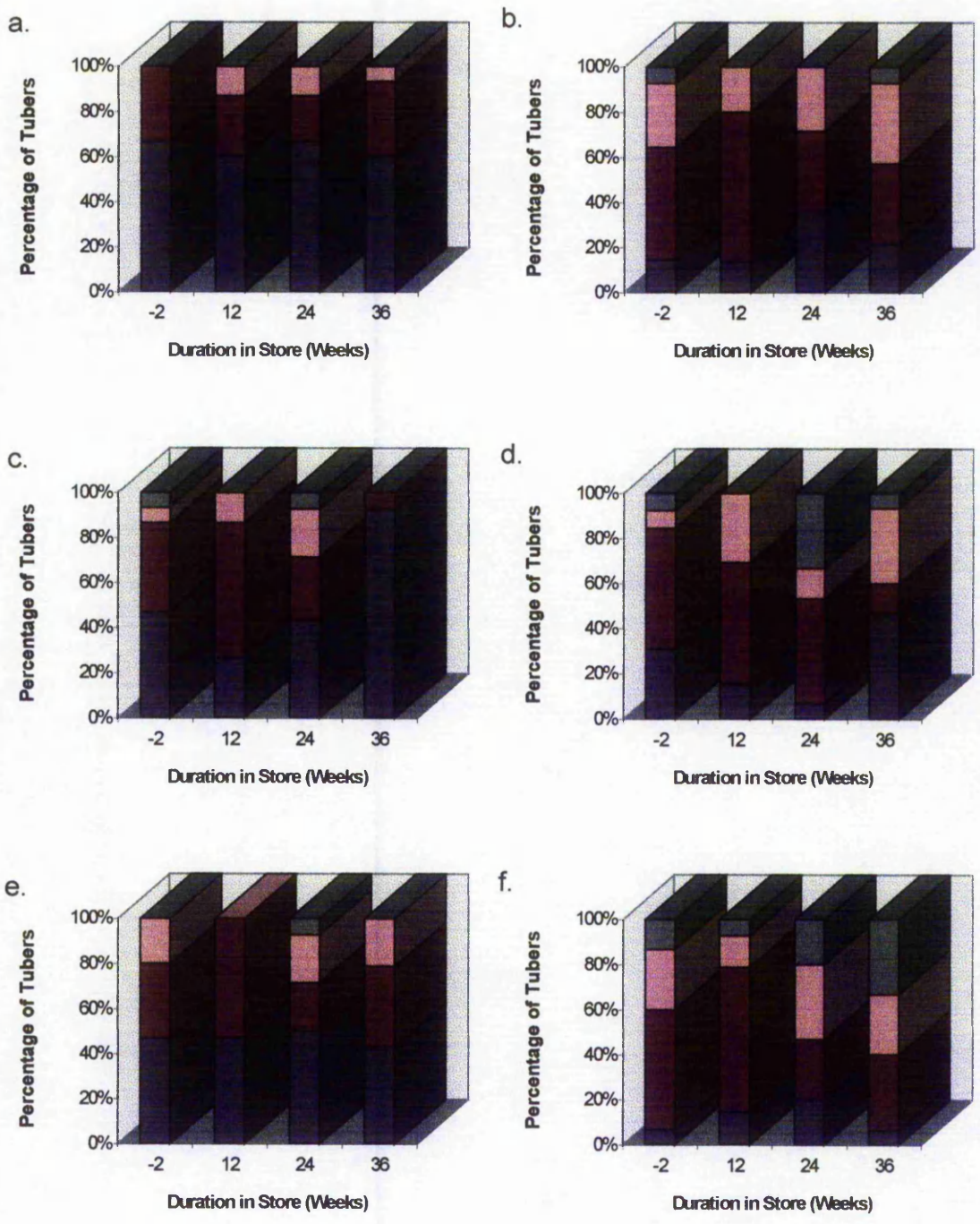


Figure 2.25 Percentage of impacted tubers showing ■ no visible bruise, ■ small bruise, ■ medium bruise and ■ large bruise through storage season 1996/7. a. Treatment 10, 10 °C impacted, b. Treatment 10, 5 °C impacted, c. Treatment 11, 10 °C impacted, d. Treatment 11, 5 °C impacted, e. Treatment 12, 10 °C impacted, f. Treatment 12, 5 °C impacted.

cvs showed no significant difference ($P>0.05$) in their bruise susceptibility by bruise volume. There was a significant difference in bruise volume between sites with tubers grown at Arthur Rickwood (e.g. Fig 2.23) bruising to a greater extent than those grown at Terrington (e.g. Fig 2.25) ($P<0.01$). No consistant or significant difference was observed between tubers grown from seed of differing physiological age in cv Pentland Dell, Maris Piper or Record ($P>0.05$) (Fig 2.22 – 2.25).

Figures 2.26 - 2.29 show the percentage of tubers bruising through storage during season 1997/8 following impact at 0.7 J (Figs 2.26 – 7) and 0.5 J (Figs 2.28 – 9). There was no significant change in bruise susceptibility, measured as bruise volume (Figs 2.30 – 2.37), through storage ($P>0.05$). This was true for tubers impacted at both 10 °C (Figs 2.30 – 2.33) and 5 °C (Figs 2.34 – 2.37) at low and high impact energy. A significant difference in bruise volume was seen between all 3 cvs ($P<0.001$). Generally, Record produced the greatest volume of bruise (e.g. Fig 2.30 e and f), closely followed by Pentland Dell (Fig 2.30 a and b), with Maris Piper developing the smallest bruises by volume (Fig 2.30 c and d). Physiological age had no significant affect on bruise susceptibility ($P>0.05$) e.g. Maris Piper from Arthur Rickwood impacted at 0.5 J of 0 degree days (Fig 2.32 c) and 500 degree days (Fig 2.32 d). No significant difference was seen between tubers grown at Arthur Rickwood (e.g. Fig 2.32) and those at Terrington (e.g. Fig 2.33) ($P>0.05$). Impact of tubers at 0.7 J (e.g. Figs 2.30 - 31) resulted in a significantly higher bruise volume than tubers impacted with 0.5 J (e.g. Figs 2.32 – 33) ($P<0.001$). Temperature had a significant affect on bruise volume. Tubers of Pentland Dell and Maris Piper when impacted at 5° C (e.g. Figs 2.35 a and b) bruised to a greater extent than those impacted at 10 °C. (e.g. Figs 2.31 a and b) ($P<0.001$). This was not the case with cv Record ($P>0.05$).

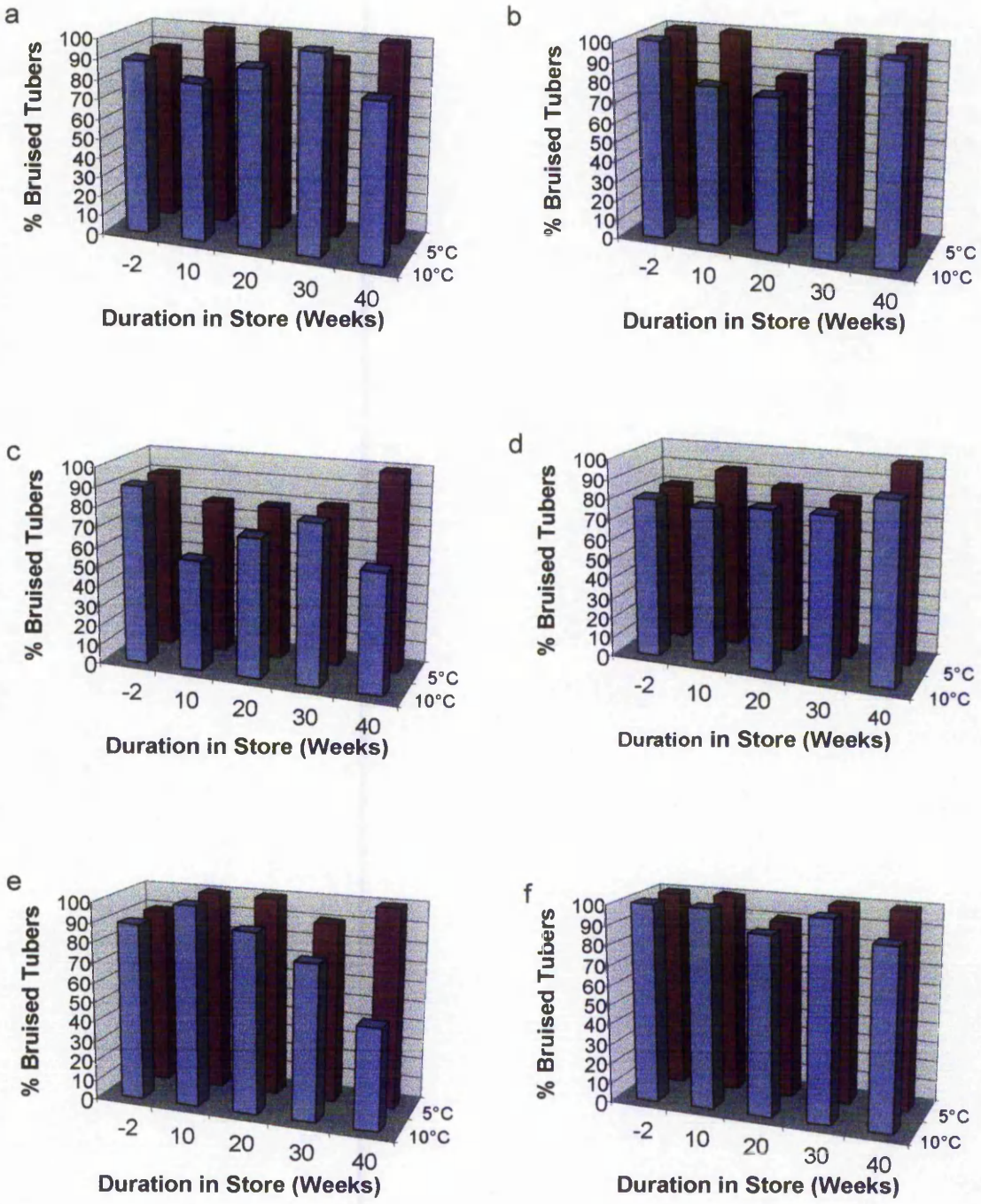


Figure 2.26. Percentage of tubers bruising through storage season 1997/8 following a 0.7J impact at 5°C and 10°C. a. Treatment 1, b. Treatment 2, c. Treatment 3, d. Treatment 4, e. Treatment 5 and f. Treatment 6.

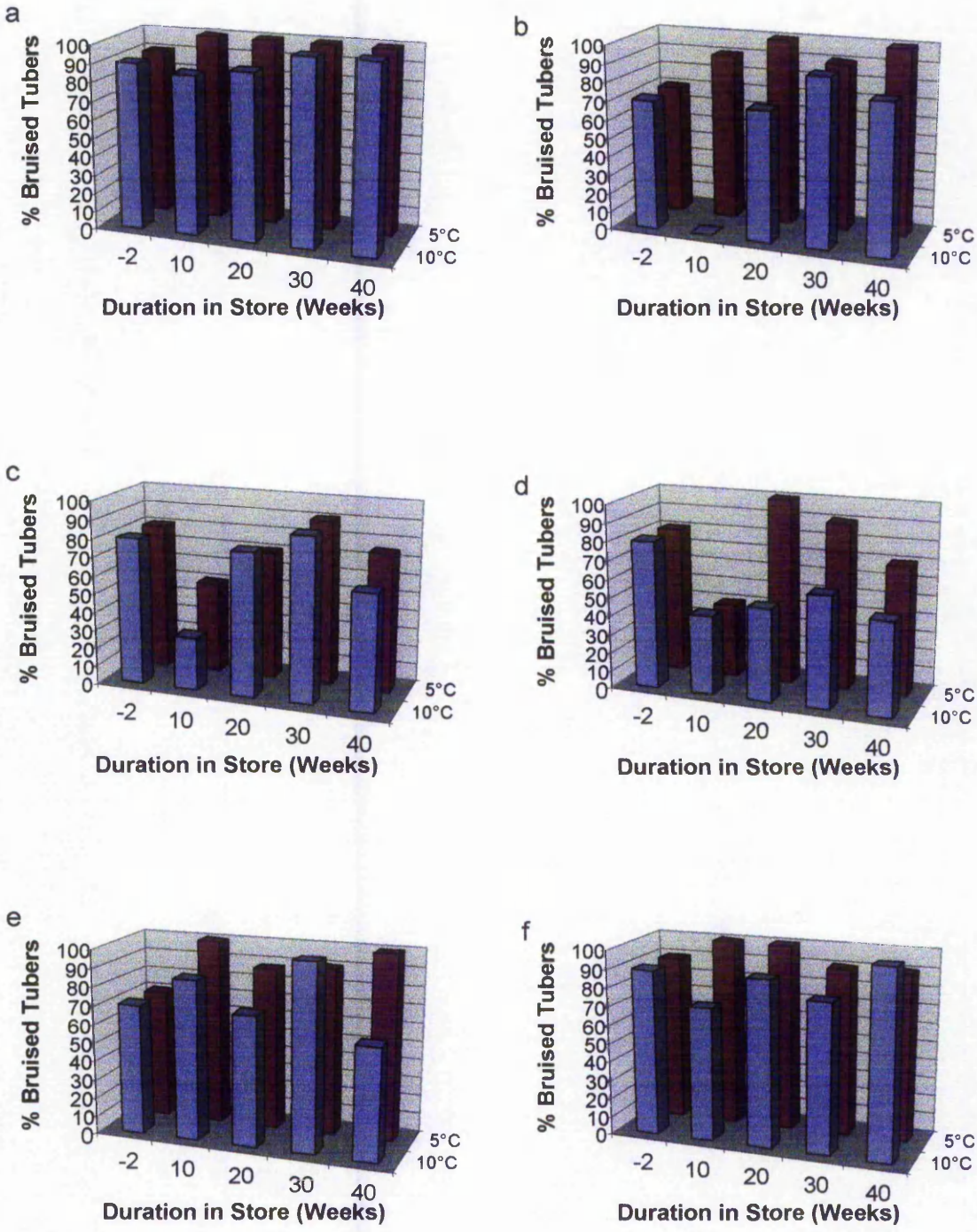


Figure 2.27 Percentage of tubers bruising following 0.7J impact at 5°C and 10°C through storage season 1997/8. a. Treatment 7, b. Treatment 8, c. Treatment 9, d. Treatment 10, e. Treatment 11 and f. Treatment 12.

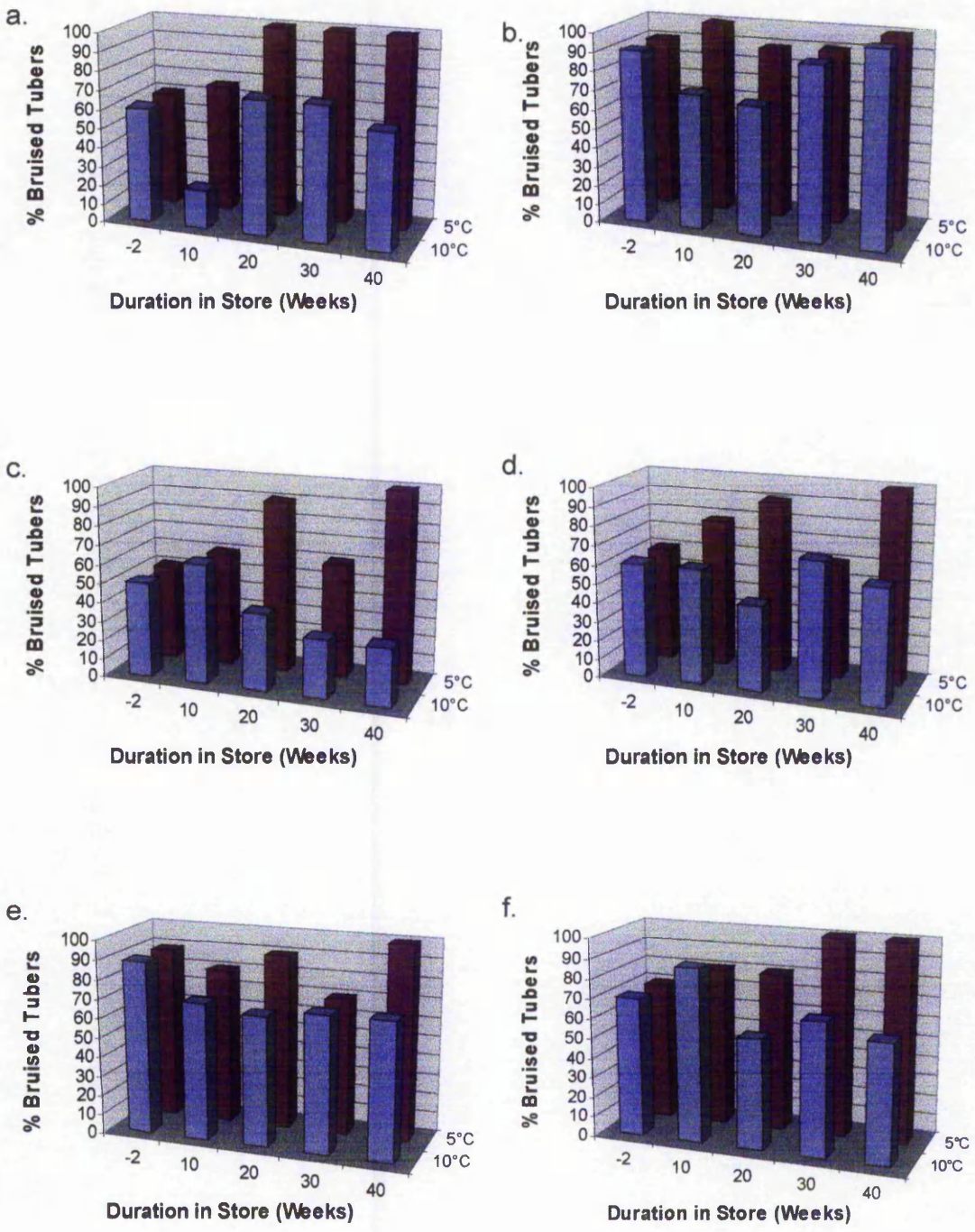


Figure 2.28. Percentage of tubers from Arthur Rickwood bruising through storage season 1997/8 following a 0.5J impact at 5°C and 10°C. a. Treatment 1, b. Treatment 2, c. Treatment 3, d. Treatment 4, e. Treatment 5 and f. Treatment 6.

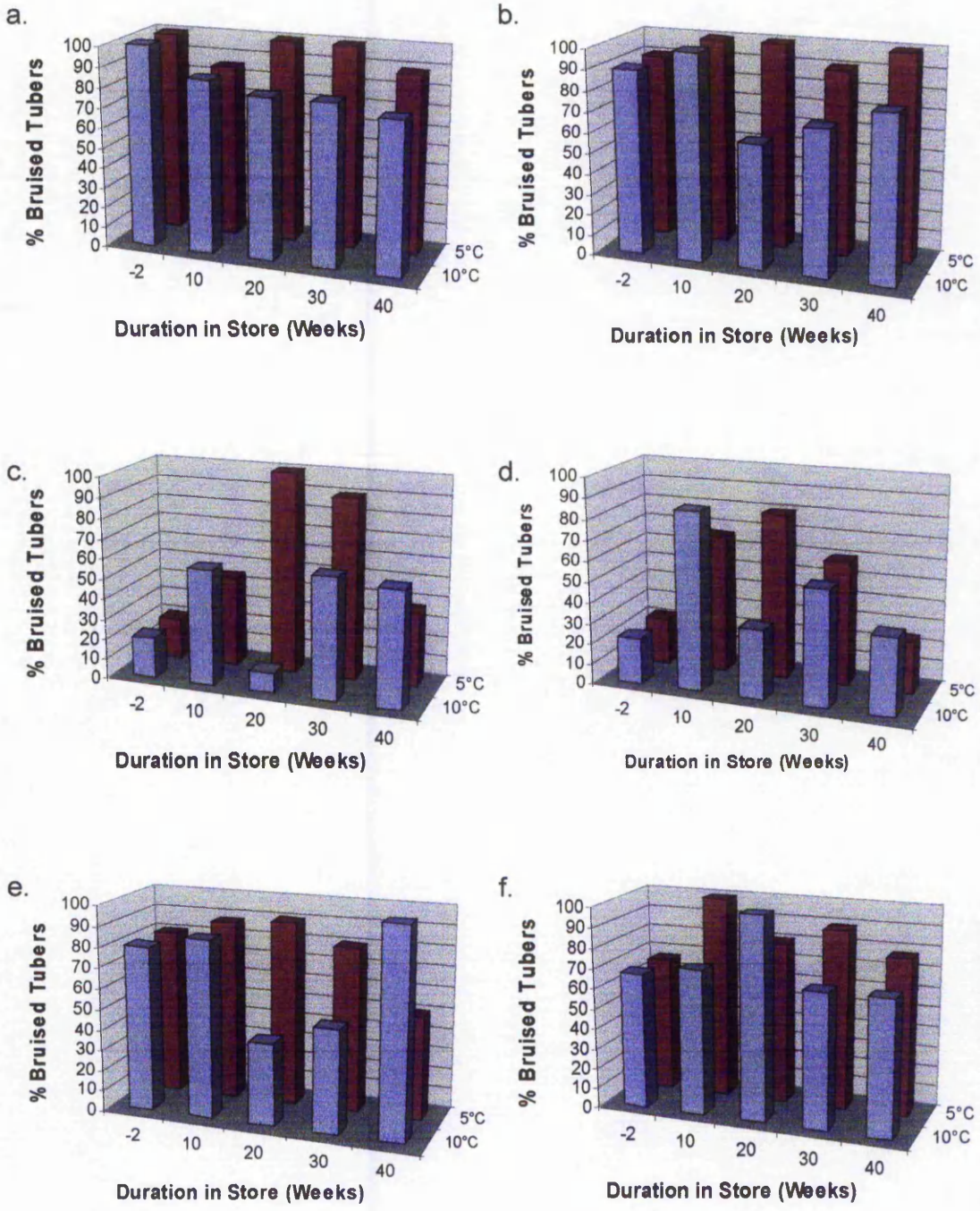


Figure 2.29 Percentage of tubers from Terrington bruising following 0.5J impact at 5°C and 10°C through storage season 1997/8. a. Treatment 7, b. Treatment 8, c. Treatment 9, d. Treatment 10, e. Treatment 11 and f. Treatment 12.

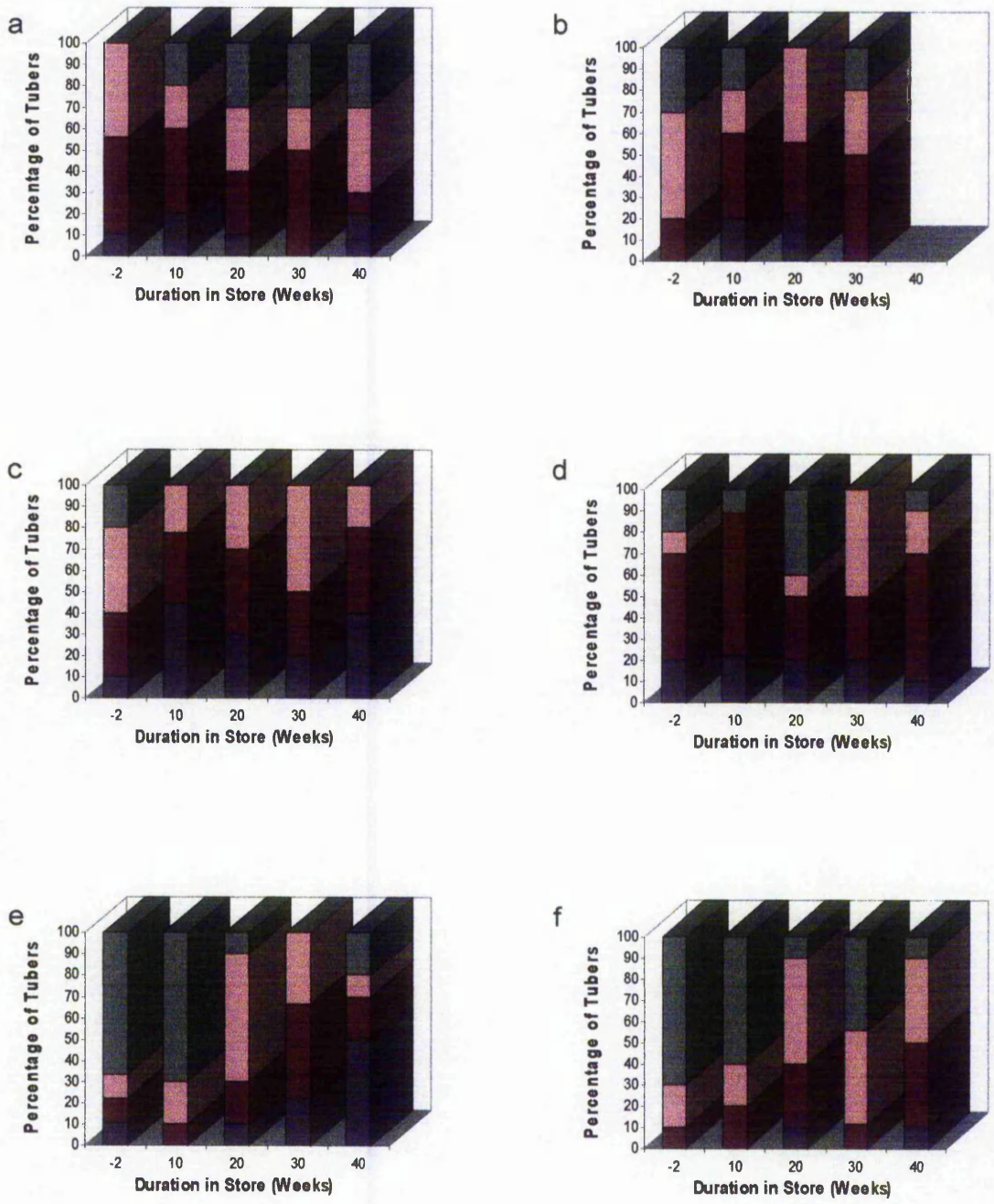


Figure 2.30 Percentage of 10°C stored tubers showing ■ no visible bruise, ■ small bruise, ■ medium bruise and ■ large bruise following impact at 0.7J through storage season 1997/8. a. Treatment 1, b. Treatment 2, c. Treatment 3, d. Treatment 4, e. Treatment 5, f. Treatment 6.

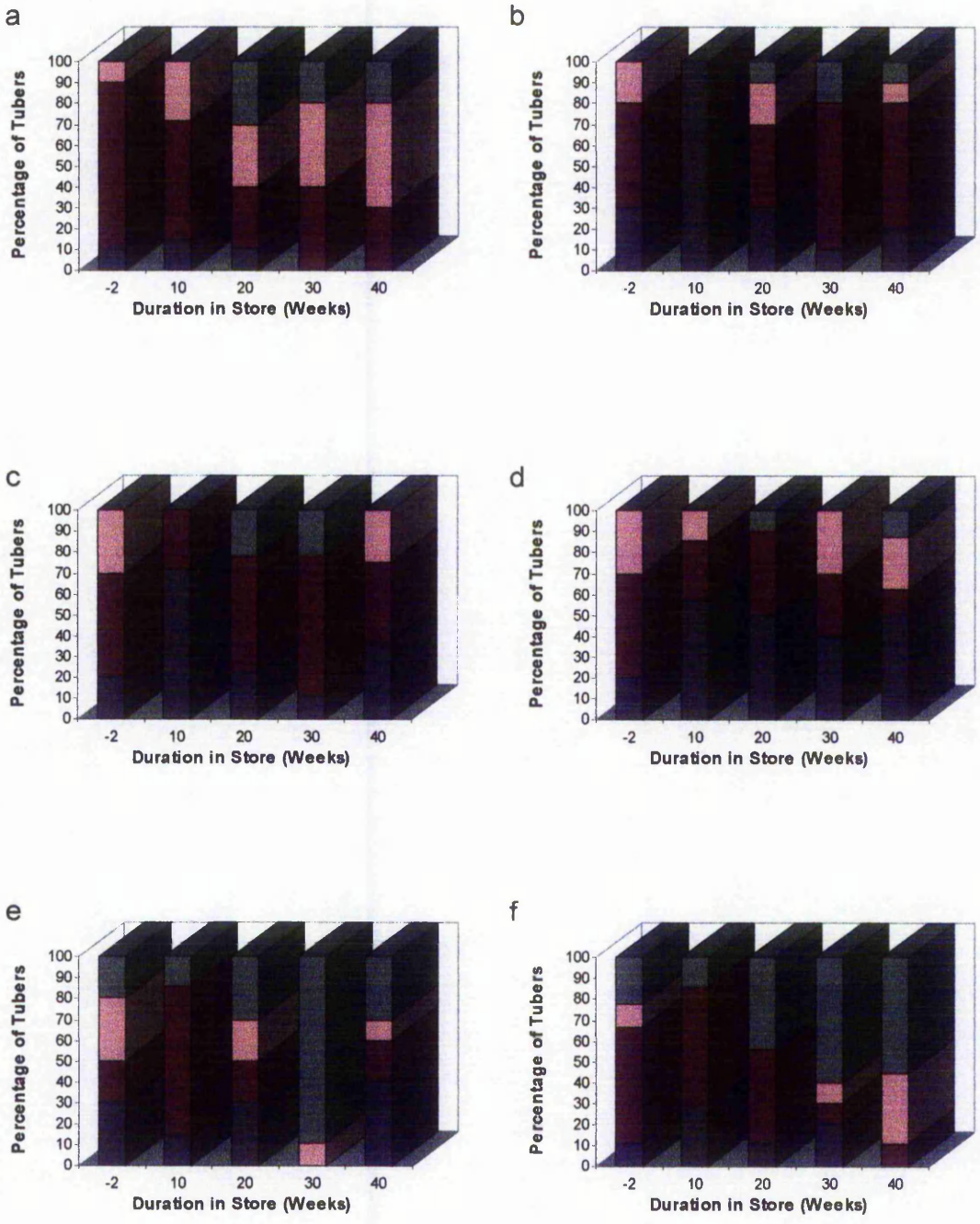


Figure 2.31 Percentage of 10°C stored tubers showing ■ no visible bruise, ■ small bruise, ■ medium bruise and ■ large bruise following impact at 0.7J through storage season 1997/8. a. Treatment 7, b. Treatment 8, c. Treatment 9, d. Treatment 10, e. Treatment 11, f. Treatment 12.

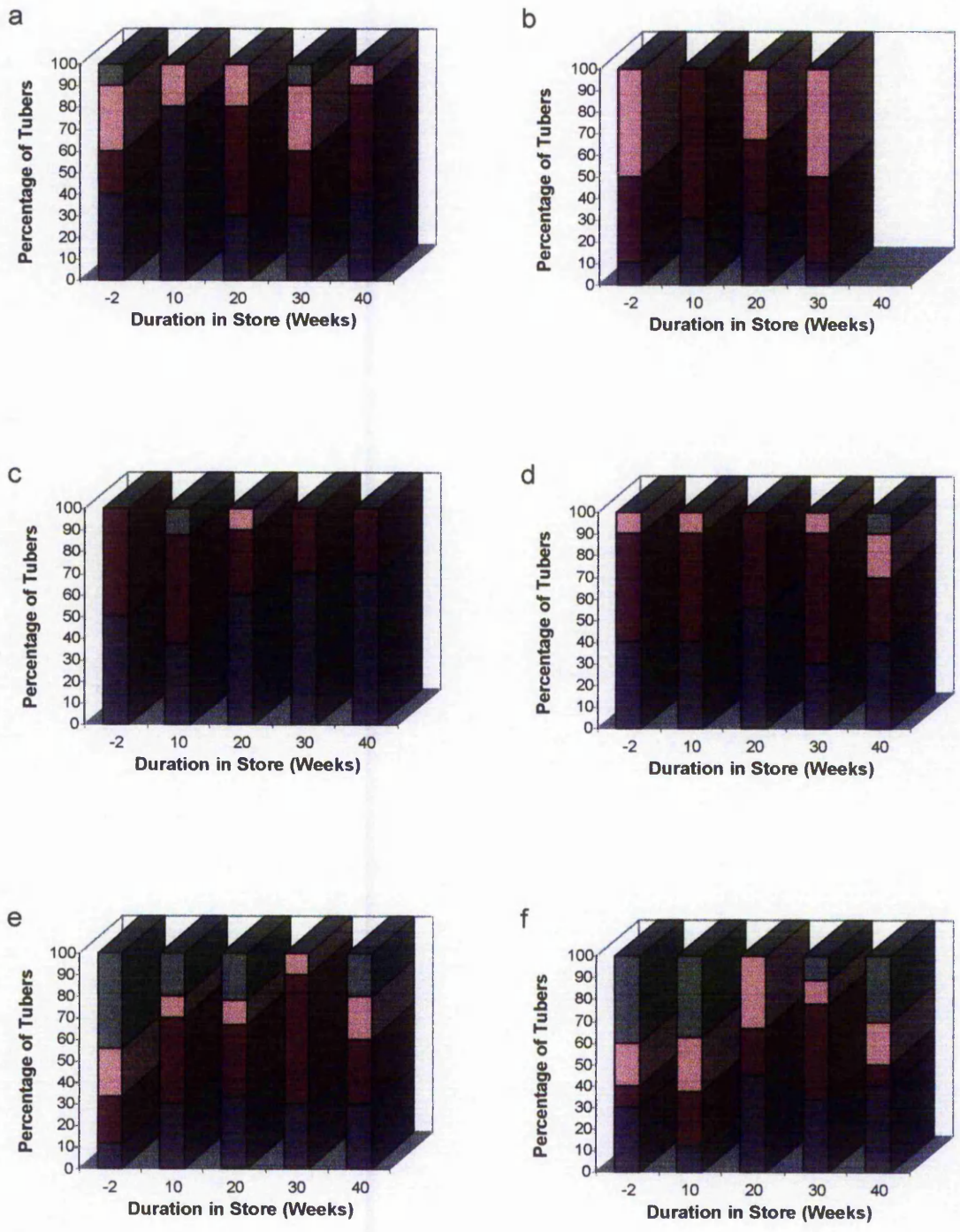


Figure 2.32 Percentage of 10°C stored tubers showing ■ no visible bruise, ■ small bruise, ■ medium bruise and ■ large bruise following impact at 0.5J through storage season 1997/8. a. Treatment 1, b. Treatment 2, c. Treatment 3, d. Treatment 4, e. Treatment 5, f. Treatment 6.

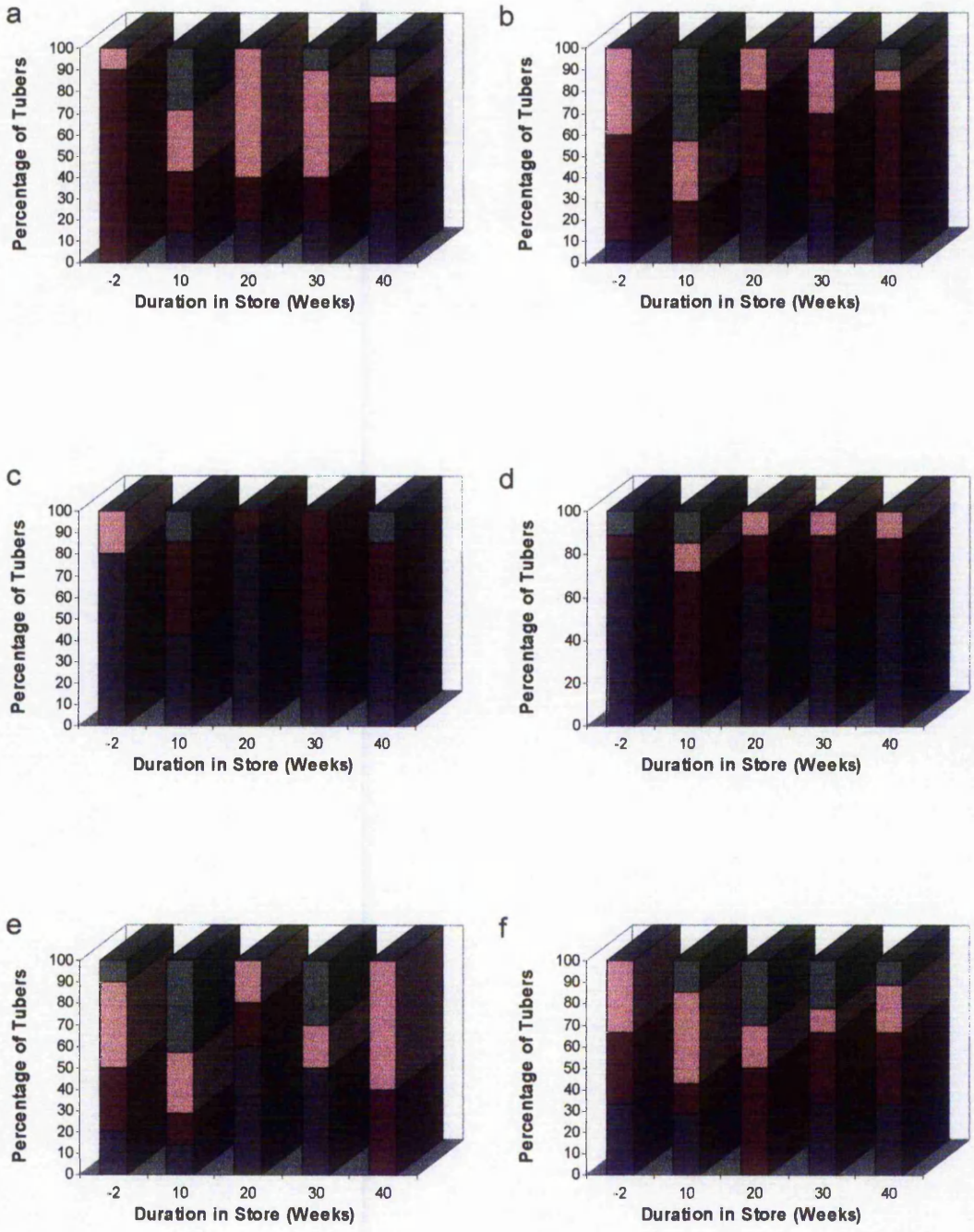


Figure 2.33 Percentage of 10°C stored tubers showing ■ no visible bruise, ■ small bruise, ■ medium bruise and ■ large bruise following impact at 0.5J through storage season 1997/8. a. Treatment 7, b. Treatment 8, c. Treatment 9, d. Treatment 10, e. Treatment 11, f. Treatment 12.

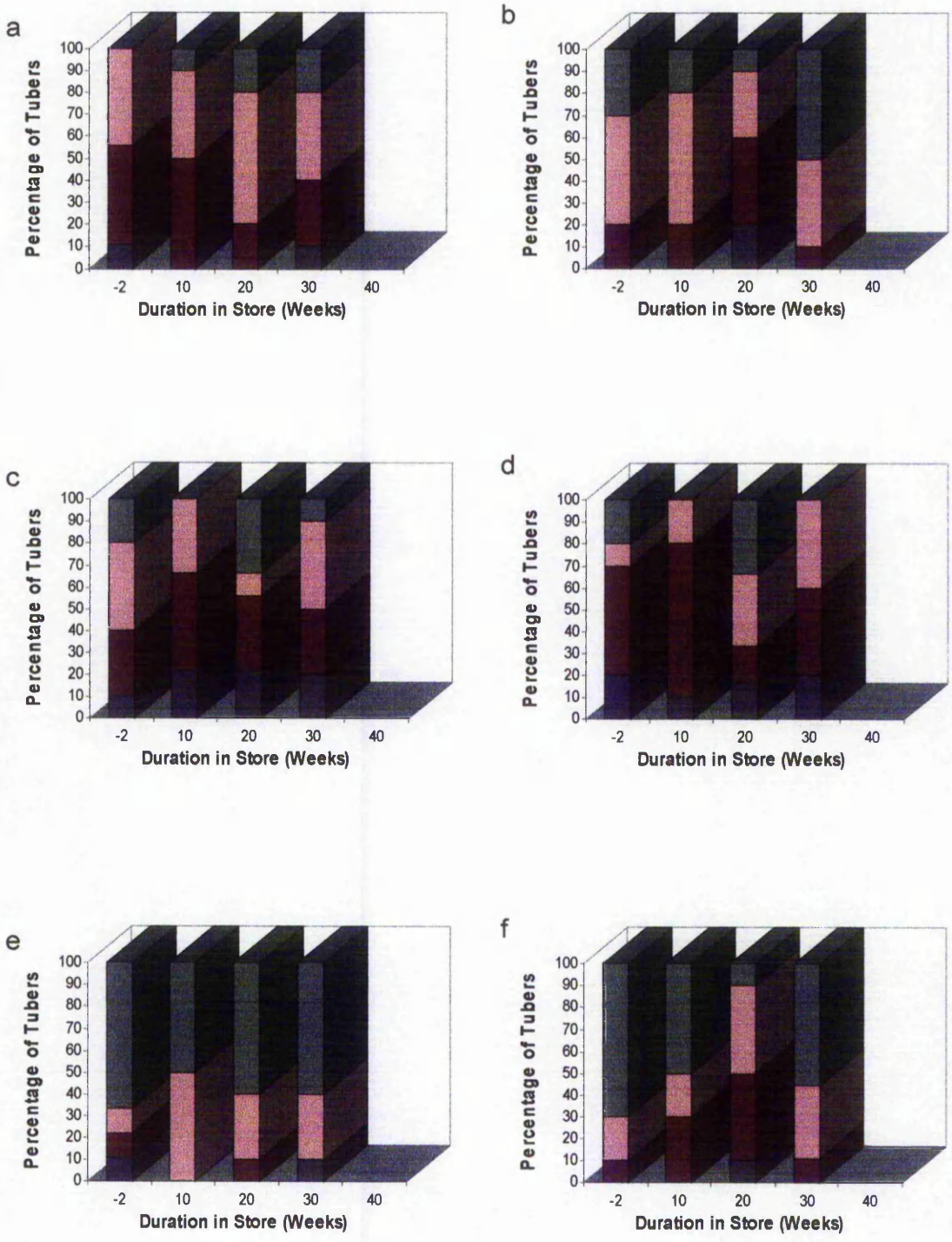


Figure 2.34 Percentage of 5°C stored tubers showing ■ no visible bruise, ■ small bruise, ■ medium bruise and ■ large bruise following impact at 0.7J through storage season 1997/8. a. Treatment 1, b. Treatment 2, c. Treatment 3, d. Treatment 4, e. Treatment 5, f. Treatment 6.

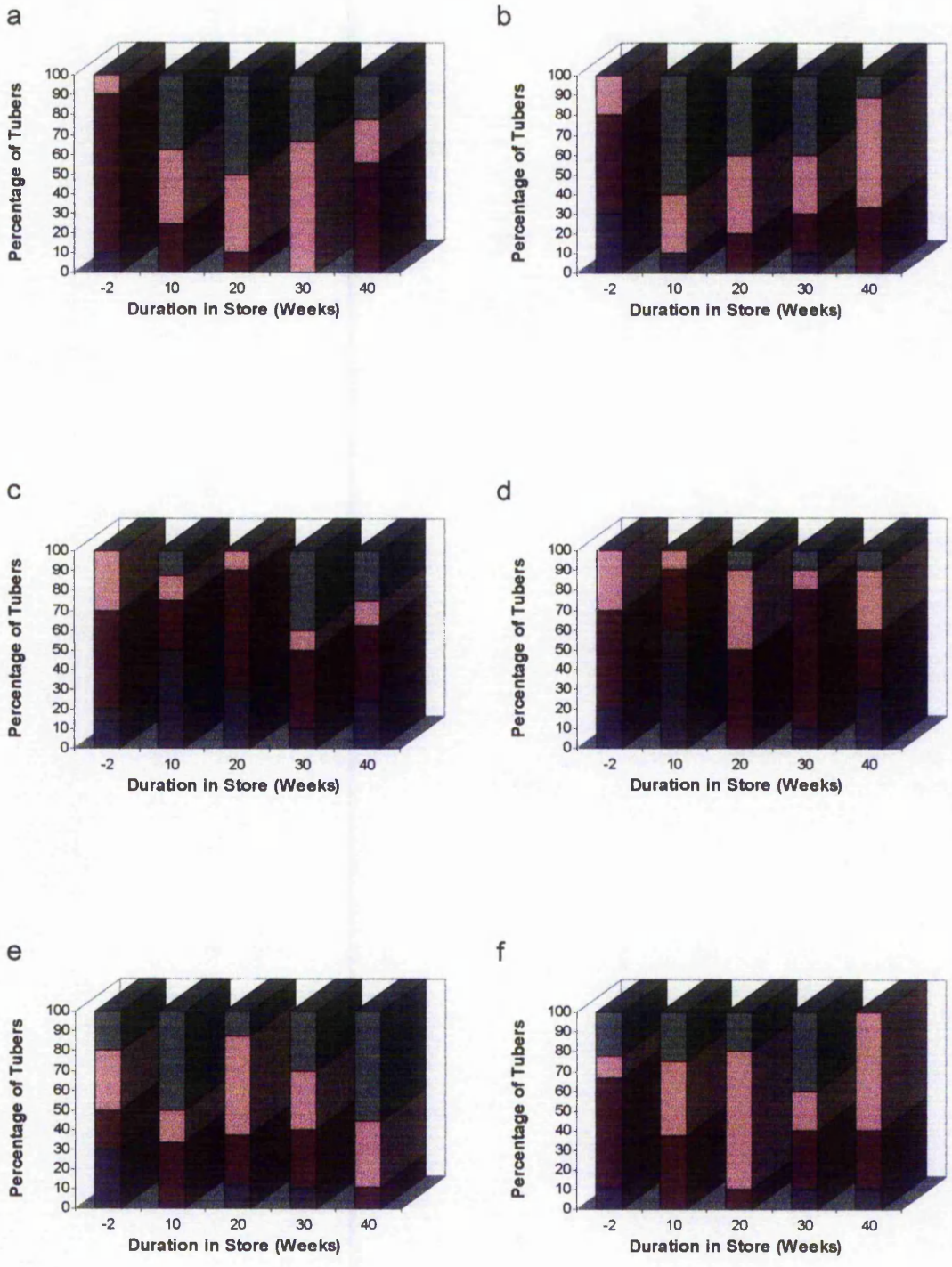


Figure 2.35 Percentage of 5°C stored tubers showing ■ no visible bruise, ■ small bruise, ■ medium bruise and ■ large bruise following impact at 0.7J through storage season 1997/8. a. Treatment 7, b. Treatment 8, c. Treatment 9, d. Treatment 10, e. Treatment 11, f. Treatment 12.

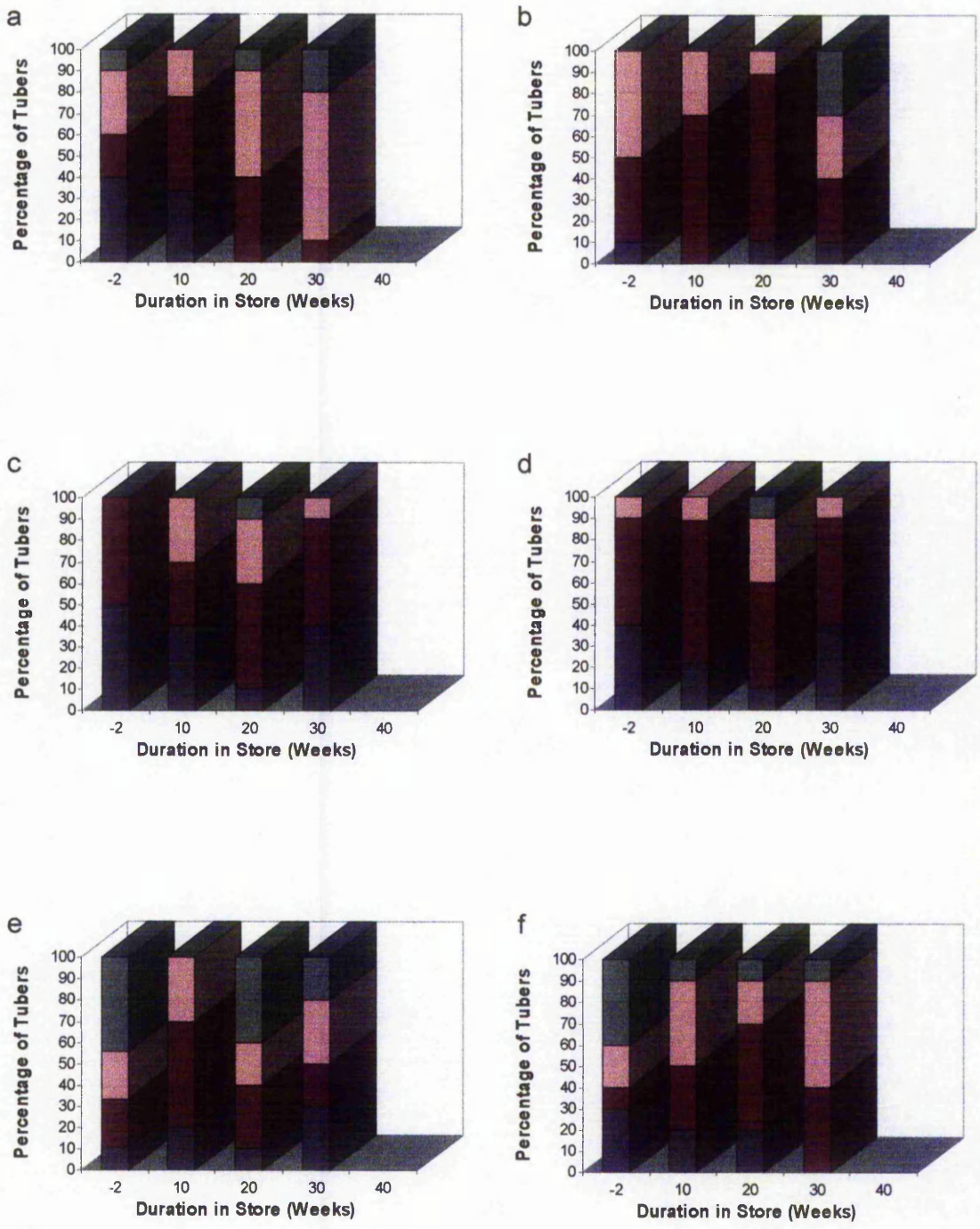


Figure 2.36 Percentage of 5°C stored tubers showing ■ no visible bruise, ■ small bruise, ■ medium bruise and ■ large bruise following impact at 0.5J through storage season 1997/8. a. Treatment 1, b. Treatment 2, c. Treatment 3, d. Treatment 4, e. Treatment 5, f. Treatment 6.

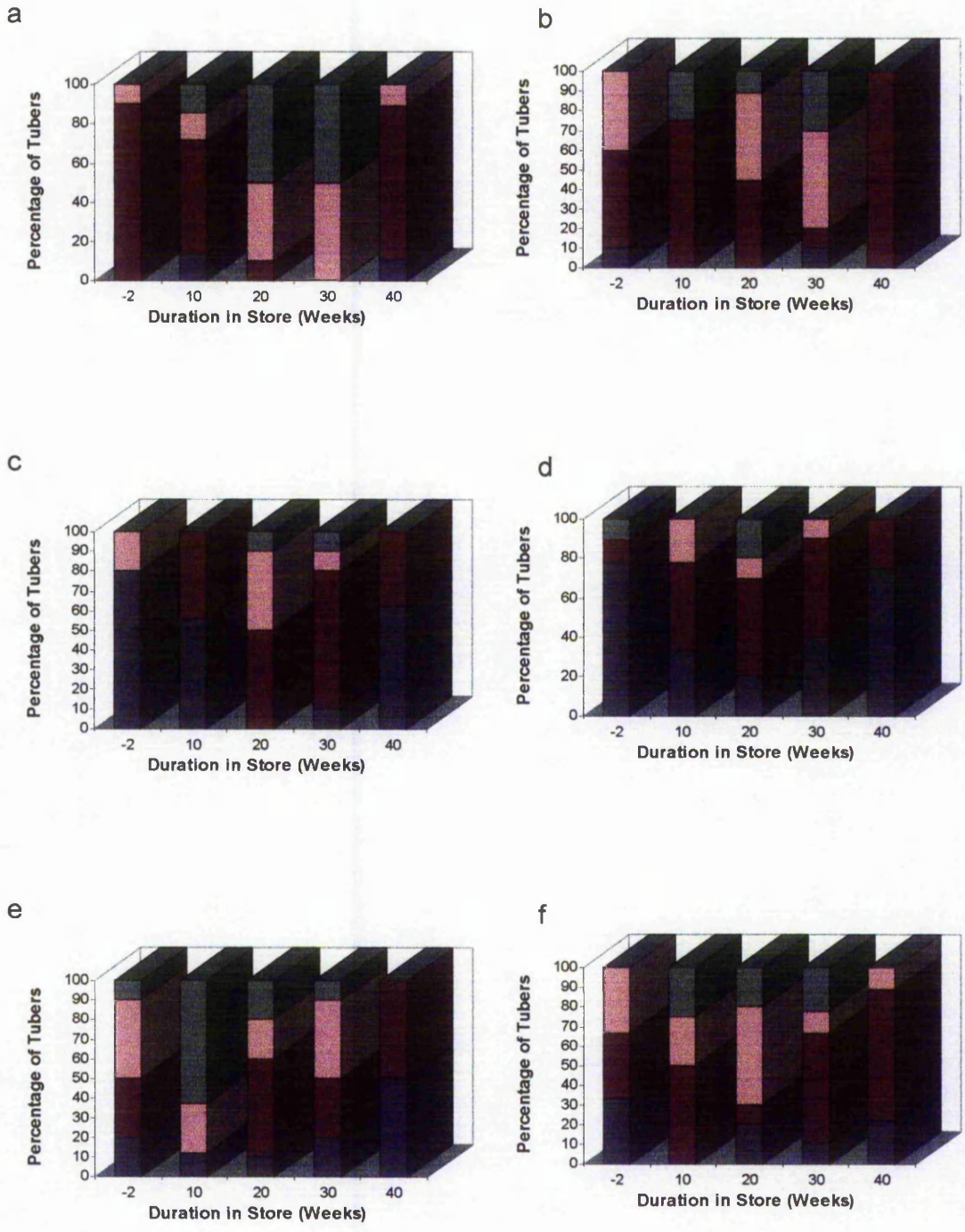


Figure 2.37 Percentage of 5°C stored tubers showing ■ no visible bruise, ■ small bruise, ■ medium bruise and ■ large bruise following impact at 0.5J through storage season 1997/8. a. Treatment 7, b. Treatment 8, c. Treatment 9, d. Treatment 10, e. Treatment 11, f. Treatment 12.

2.3.6 Data Generated at the Sutton Bridge Experimental Unit

2.3.6.1 Sugar Content of Stored Potato Tubers: Dry matter content during storage season 1996/7 remained constant at 20-25% of the fresh weight. This pattern was the same for all cultivars, at both sites and physiological ages (Fig 2.38). Figures 2.39 to 2.40 show the glucose and fructose contents respectively of tubers from the 1996/7 storage season. An almost identical pattern is followed for both sugars, increasing for all 12 treatments from harvest to 12 weeks, followed by a levelling off or decline between 12 and 36 weeks in cv Maris Piper and Record. In cv Record from both Arthur Rickwood and Terrington, fructose and glucose concentrations levelled off or declined between 12 and 24 weeks, followed by a large increase before the end of storage. Sucrose concentration gave a similar increase in cv Pentland Dell between 24 and 36 weeks with a slight increase also between 12 and 24 weeks in storage, which was not seen in the other cvs. Sucrose concentration declined in all treatments between harvest and 12 weeks (Fig 2.41). This decline continued in cv Maris Piper and Record between 12 and 24 weeks, and until the end of storage in Maris Piper, whereas in cv Record the concentration of sucrose remained stable or even increased.

2.3.6.2 Dry Matter Content of Stored Potato Tubers: During the 1997/8 storage season dry matter content again remained relatively stable between 20 and 25 % for all 12 treatments (Fig 2.42).

Figures 2.43 and 2.44 show glucose and fructose content respectively, which rose steadily between 0 and 20 weeks. Glucose concentration subsequently remained stable throughout storage, with the exception of Pentland Dell, which rose from 20 weeks onwards in tubers from Arthur Rickwood and from 30 weeks in tubers grown at Terrington. The same pattern is seen for fructose concentration. Sucrose concentration either declined or remained stable between harvest and 10 weeks in store (Fig 2.45). This decline continued until 30 weeks in store in tubers of cv Maris Piper which then remained constant. For cv Record there was an increase in sucrose concentration between 30 and 40 weeks in store. For cv Pentland Dell there was an increase from 20 weeks in store with a decline between 30 and 40 weeks in treatment 2.

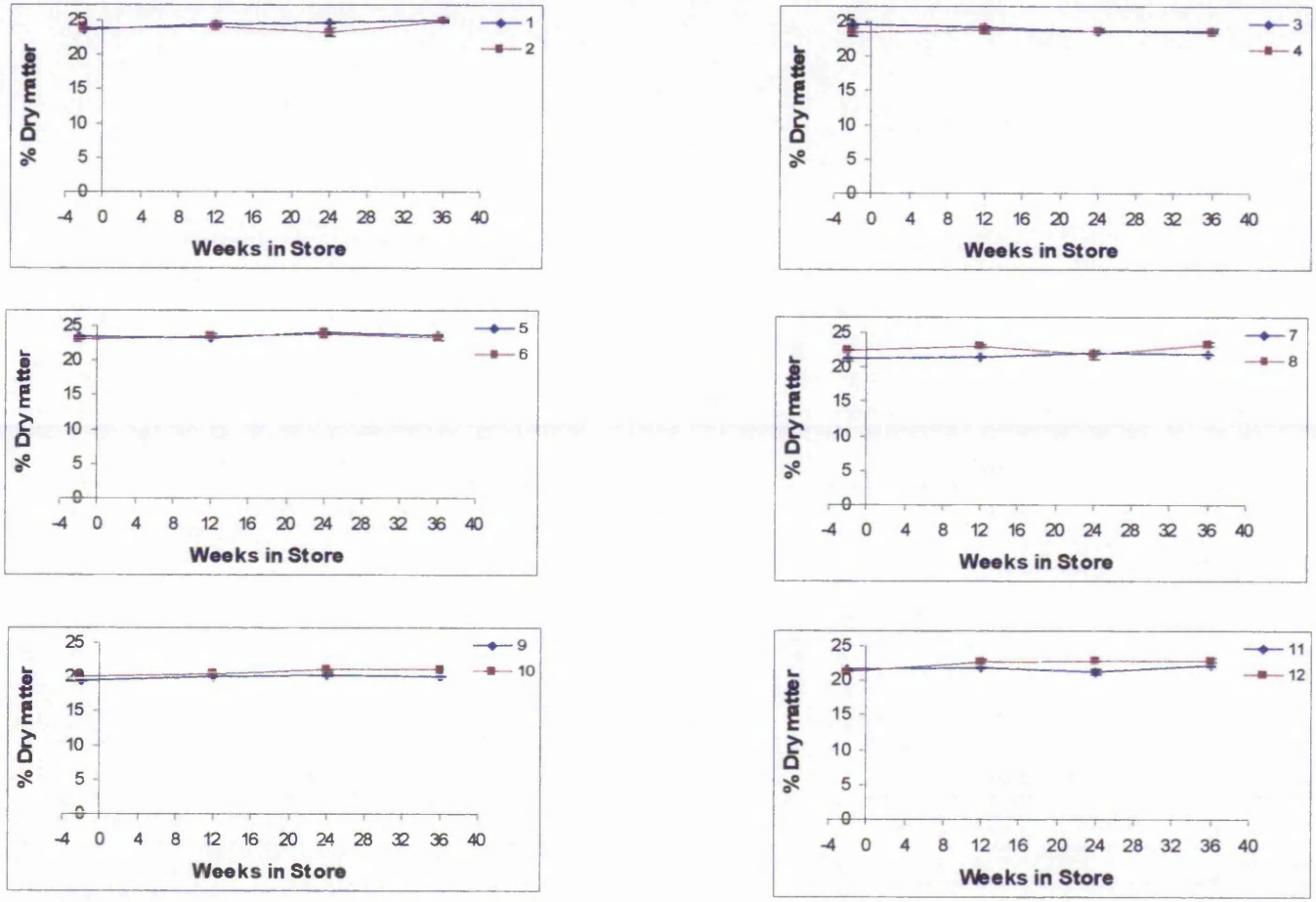


Fig 2.38 Dry matter content of potato tubers as percentage of fresh weight from treatments 1 - 12 during storage season 1996/7. Values are means ± S.E., where n = 3.

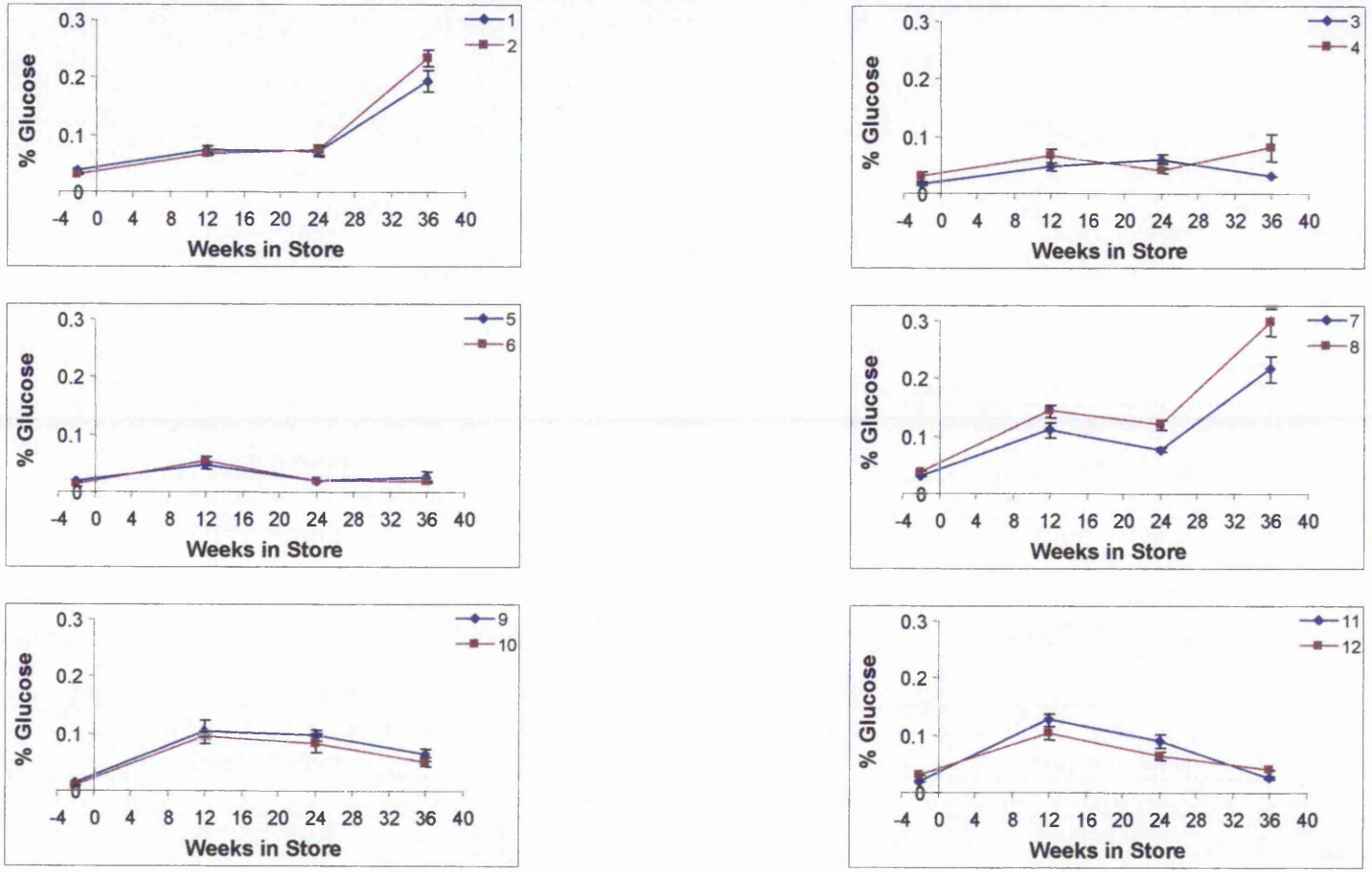


Fig 2.39 Glucose concentration of potato tubers as percentage of fresh weight from treatments 1 - 12 during storage season 1996/7. Values are means ± S.E., where n = 3.

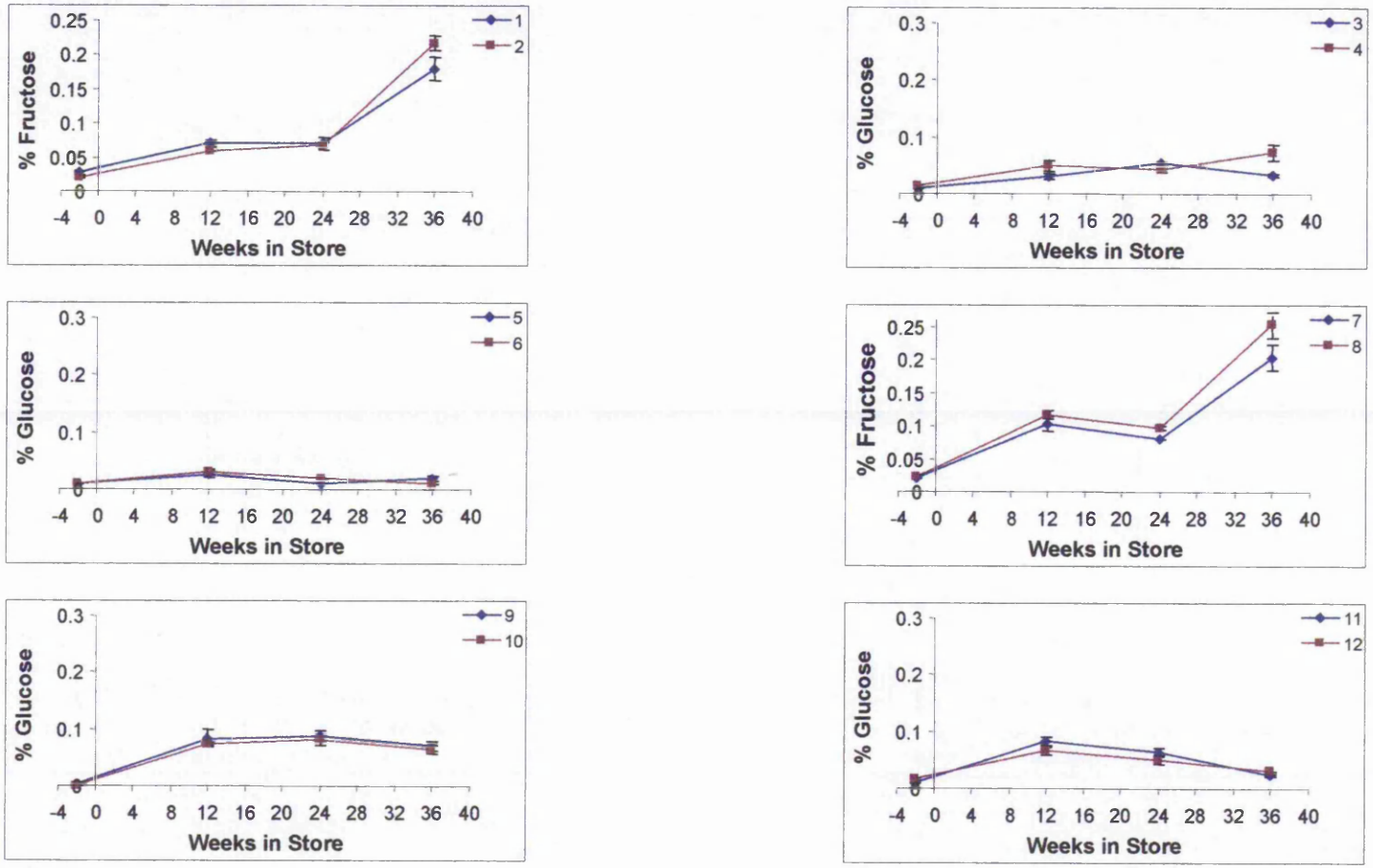


Fig 2.40 Fructose concentration of potato tubers as percentage of fresh weight from treatments 1 - 12 during storage season 1996/7. Values are means ± S.E., where n = 3.

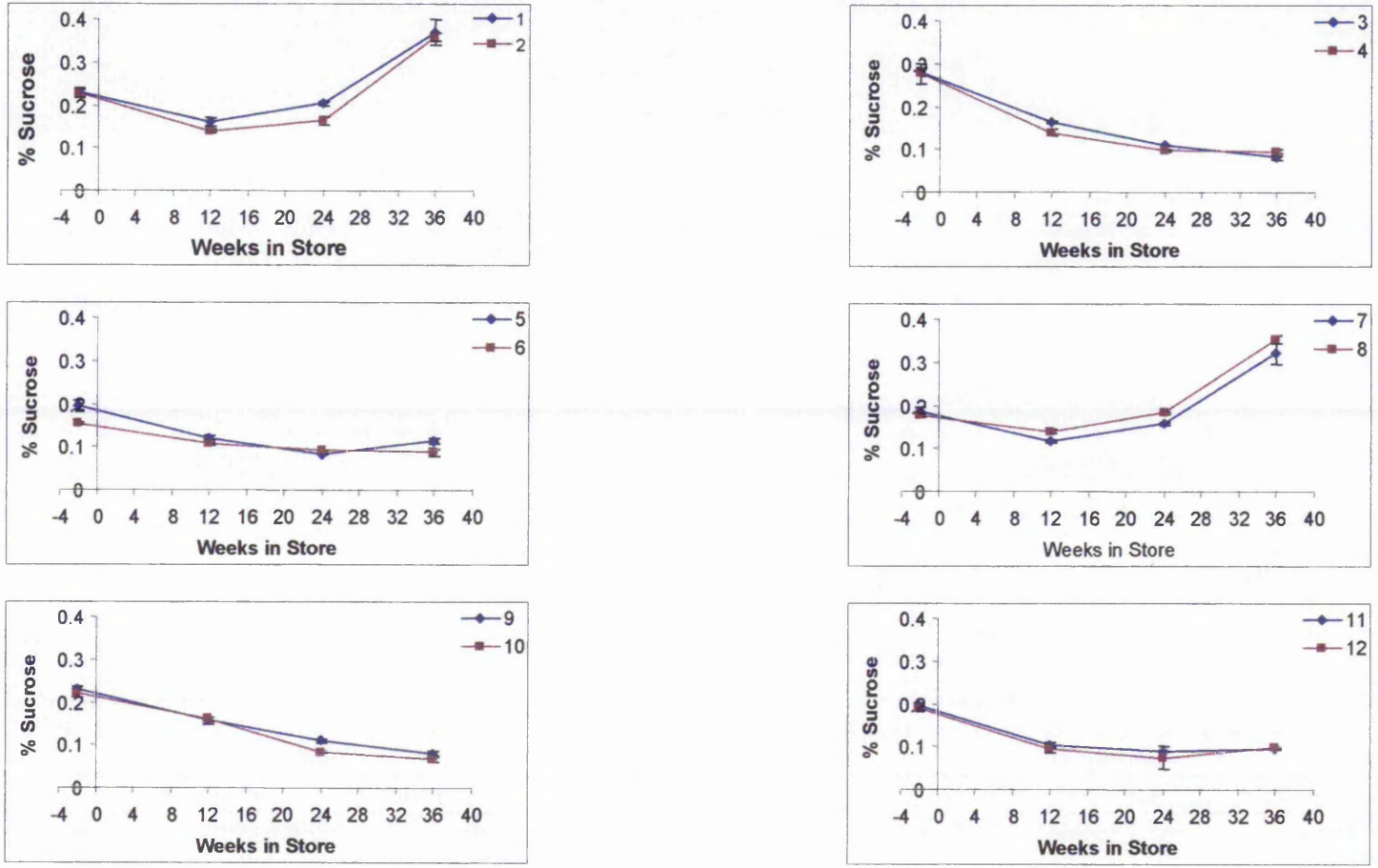


Fig 2.41 Sucrose concentration of potato tubers as percentage of fresh weight from treatments 1 - 12 during storage season 1996/7. Values are means ± S.E., where n = 3.

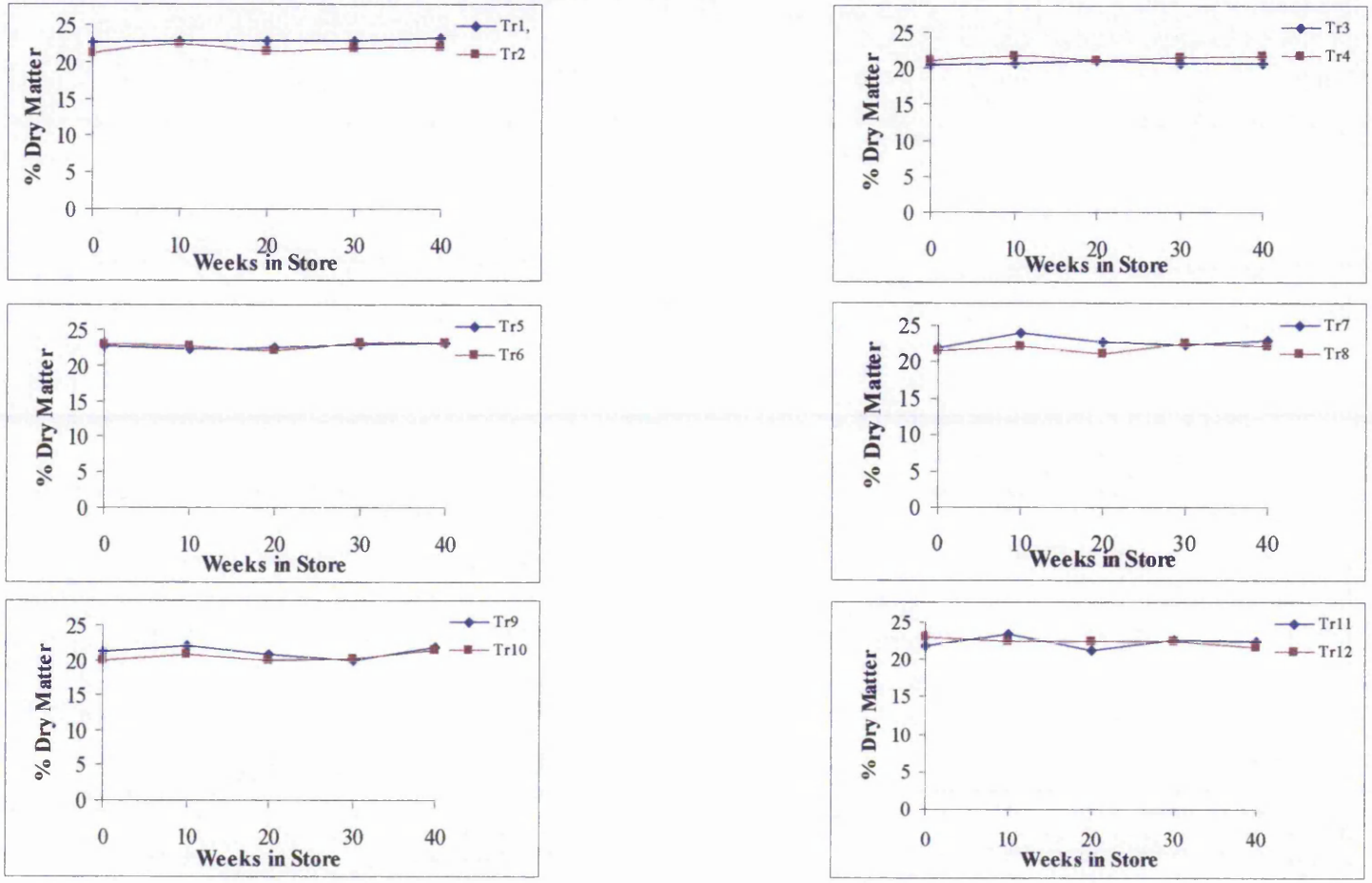


Fig 2.42 Dry matter content of potato tubers as percentage of fresh weight from treatments 1 - 12 during storage season 1997/8. Values are means ± S.E., where n = 3.

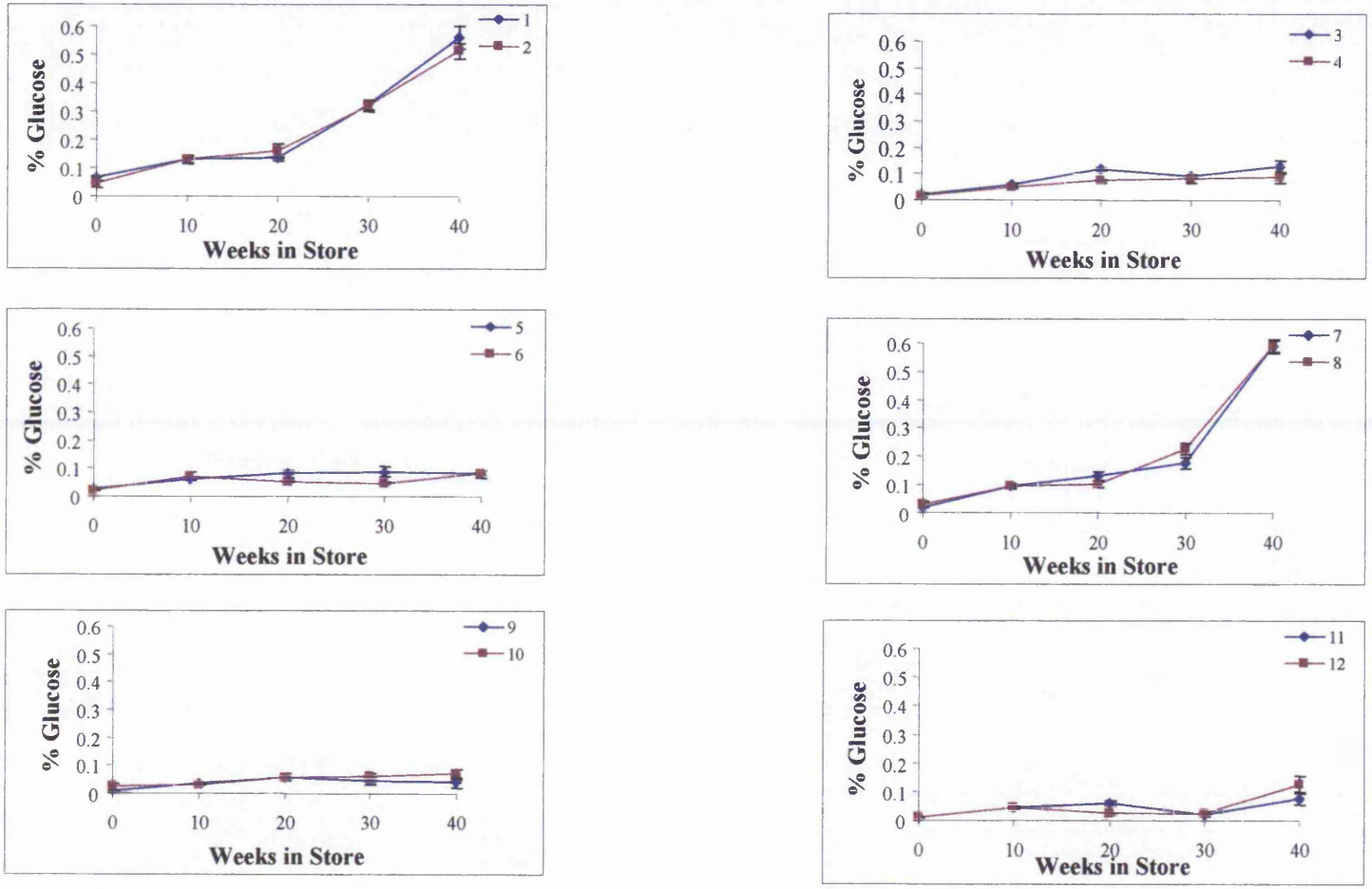


Fig 2.43 Glucose concentration of potato tubers as percentage of fresh weight from treatments 1 - 12 during storage season 1997/8. Values are means ± S.E., where n = 3.

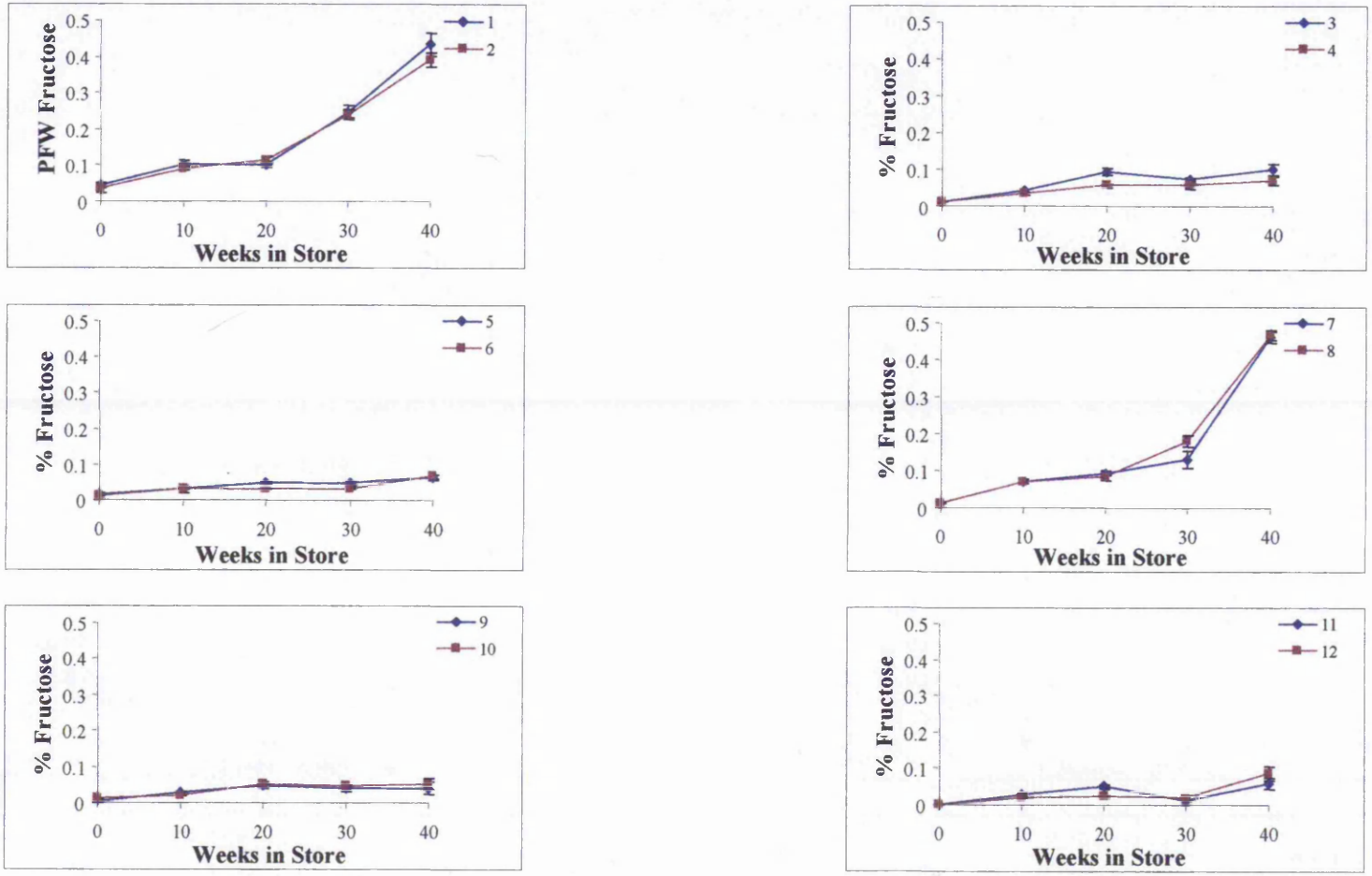


Fig 2.44 Fructose concentration of potato tubers as percentage of fresh weight from treatments 1 - 12 during storage season 1997/8. Values are means ± S.E., where n = 3.

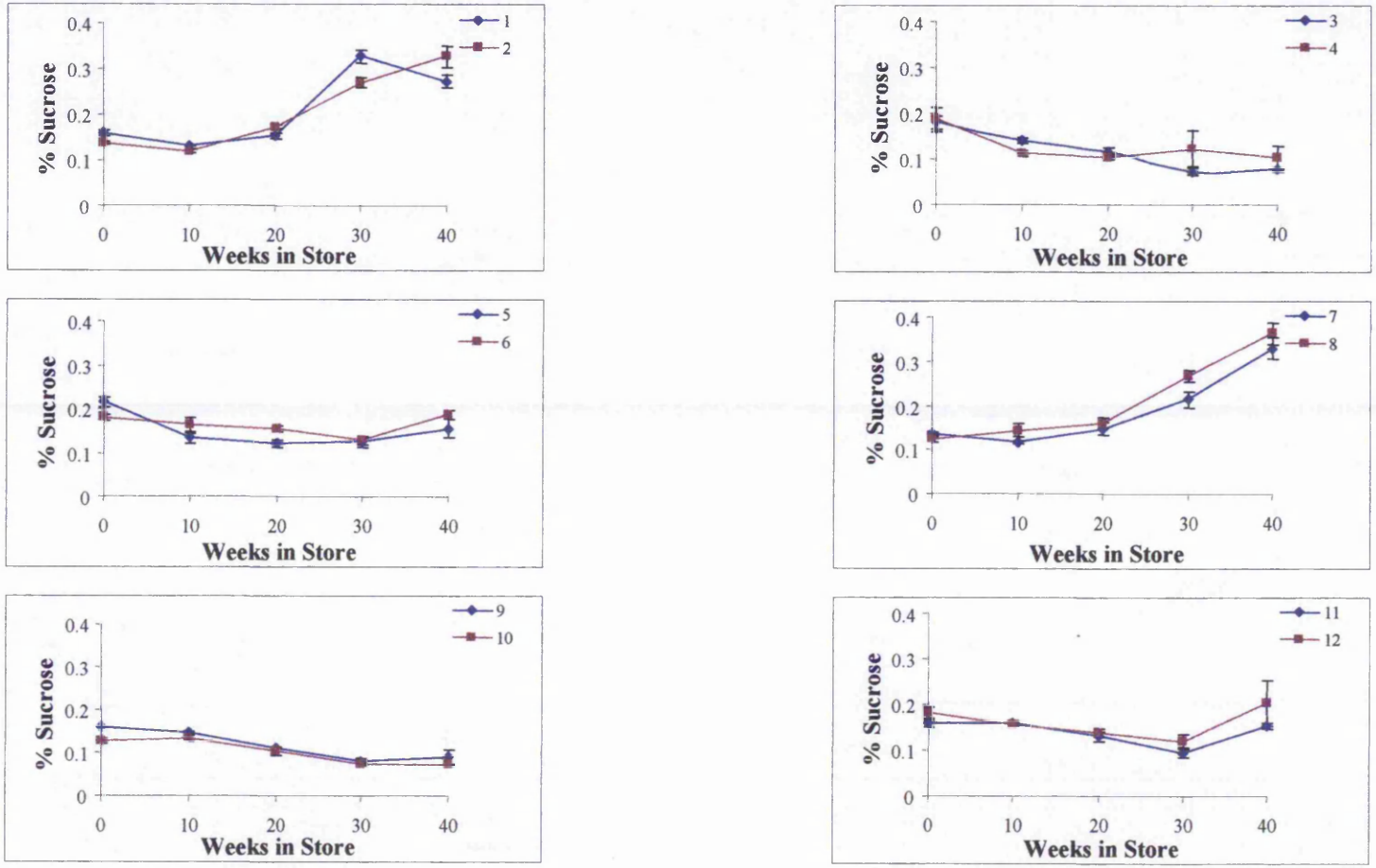


Fig 2.45 Sucrose concentration of potato tubers as percentage of fresh weight from treatments 1 - 12 during storage season 1997/8. Values are means ± S.E., where n = 3.

2.4 Discussion

The large number of variables measured during this study in some instances hindered the quality of the work. Factors such as physiological age and site might have been dropped from the investigation however contractual commitments meant these parameters had to be measured. The nature of the project as an investigation through 3 storage seasons also meant that once methods had been determined initially, there was little scope for alterations once the project was underway.

2.4.1 Respiration

Respiration of tuber populations gives a good indication of the metabolic state of the tubers through storage and was therefore an important measurement to be taken. A decline in respiration was apparent in all 12 treatments following harvest during the storage seasons 1996/7 and 1997/8. This indicates that the tubers were more metabolically active at harvest, something that has previously been noted in a variety of different cvs and storage temperatures (Amir, et al. 1977; Schippers, 1977; Dizengremel, 1985; Williams & Cobb, 1993; Wiltshire & Cobb, 1996). This increased metabolism may be important for good periderm formation which occurs once the mature tubers are lifted. Respiration, reached a minimum between 12 and 24 wks during all 3 storage seasons 1995/6, 1996/7 and 1997/8. This was followed by a rise towards the end of storage. This again is a typical pattern observed previously where tuber respiration reaches a basal rate several weeks after being placed into storage (i.e. dormancy), which is maintained until sprout initiation late in storage when respiration increases to fuel the increased demand for energy to enable sprout growth (Schippers, 1977; Li, 1985; Williams & Cobb, 1993; Wiltshire & Cobb, 1996).

One possible source of error in this investigation is the measurement of respiration on a fresh weight basis. Loss of water and dry matter of the tuber over storage (both of which are a result of respiration) would influence the values of respiration calculated. Figures 2.38 and 2.42 suggest that dry matter content of tubers through storage remained constant and results obtained from the analysis of relative water content (Fig 2.7) and turgor (Fig 2.9 e and f) found that little water loss occurred in store. However, handling

tubers through storage, it was evident that tubers became softer as storage progressed. A decline in water content alongside that of dry matter would mean that measurements of dry matter content would remain constant despite loss of dry matter.

Due to the design of the respiration investigations, statistical analyses could not be performed upon the data and hence determining the significance of results was not possible. While 10 measurements of respiration were made on each set of 5 tubers, measurement of respiration upon more than one set of tubers would be necessary in order to statistically analyse the data. This was not possible due to the limited number of tubers available and also the time involved in measuring respiration in the large number of tuber treatments. However, as similar pattern of respiration was seen in all treatments throughout storage, it was presumed that these results are typical respiration rates occurring within the tuber populations. Unfortunately, comparisons between sites, cv and physiological age could not be made with any confidence due to the lack of statistical back up. However no obvious differences were observed between seed tubers of different physiological age. A site difference was apparent in storage season 1996/7 with tubers grown at Terrington having a higher rate of respiration than those grown at Arthur Rickwood at harvest and during early storage. This difference was not observed during storage season 1997/8. Respiration in tubers of cv Maris Piper was lower than that of Pentland Dell or Record for those tubers that were measured.

During storage season 1995/6 there was a slightly higher rate of respiration in tubers stored at 10 ºC compared those stored at 5 ºC. Temperature is known to influence metabolic processes in the potato (Rastovski, 1987) and an increase in respiration corresponding to change in storage temperature is well documented. The effects of low temperature on reducing respiration are utilised for the post-harvest storage of crops (Taiz & Zeiger, 1991) including the potato.

Brooks (1996) suggested that an increased susceptibility to bruising is accompanied by a proportional rise in respiration. However, comparing the rate of respiration through storage to the percentage bruised tubers and the

severity of bruising, it can be seen that respiration rate and therefore tuber metabolism did not influence bruising susceptibility, in this investigation.

2.4.2 Water Relations

During storage, potato tubers respire and transpire resulting in a number of physiological changes, particularly, water loss (Scanlon, Pang & Biliaderis, 1996). The water status of the tuber is believed to play an important role in determining bruise susceptibility within a potato variety (Hughes, 1980; McGarry et al. 1996). Smittle et al. (1974) reported that there was a negative correlation between tuber turgidity and blackspot bruise development, whereas a positive relationship existed with shatter bruise. Thus, turgid tubers following harvest are more susceptible upon impact to the cracking and splitting damage associated with shatter bruise due to the high stresses within the tissue (Hughes, 1980; Garcia, Ruiz-Altisent & Barreiro, 1995). Yet after subsequent storage during which tubers lose water, the turgidity of tubers declines with an increase in the susceptibility to blackspot bruise (Kunkel & Gardener, 1959; Smittle et al., 1974; Burton, 1977; Hughes, 1980; Brook, 1996; McGarry et al., 1996).

Where precautions have been taken to reduce tuber water loss, a reduction in blackspot bruise was noted. In Washington State (USA), where blackspot bruise is a field rather than a storage problem, recommendations to harvest when fields were moist rather than under conditions favourable for dehydration (at 85 °F, when relative humidity is approximately 30 %) resulted in a reduction in blackspot bruising (Kunkel and Gardner, 1965). Smittle, et al., (1974) dehydrated tubers by leaving them at 70 °F (~22 °C) and 20 % RH for 0, 3, 6 or 9 wks (which resulted in a 2.7 %, 5.6 % and 11.2 % weight loss). Following impact, blackspot susceptibility was low in hydrated tubers but increased with dehydration followed by a slight decrease. While these results may give an indication of the effect of turgidity on blackspot susceptibility, direct measurements of turgidity and water relations were not taken. Over the 9 wks in store tubers may have been subject to other influences and will have undergone biochemical and physiological changes, which could have affected blackspot susceptibility.

Much of the previous work has not measured turgor, but has estimated it for example using measurements of specific gravity (Kunkel & Gardner, 1965), curvature of potato plugs under static load (Smittle et al., 1974) or tissue stiffness (McNabnay, Dean, Bajema & Hyde, 1999).

During this project several methods for measuring turgor were investigated. The first was the compensation method in which tuber sections were incubated in sucrose solutions and weight change accurately measured to determine water and osmotic potentials from which turgor could be calculated. It was suggested by Linn and Pitt (1986) that inclusion of a 0.02 M phosphate buffer with the incubation solutions minimised the degradation of tuber tissue. This buffering has subsequently been used by other authors undertaking the compensation method (Scanlon et al., 1996). However, during this investigation, a comparison of tuber weight change following incubation in buffered and control sucrose solutions found that whilst tuber weight was maintained between 36 and 48 hrs of incubation in buffered sucrose solution compared with controls, tuber sections incubated in buffered solutions underwent a significantly greater weight change than controls throughout the incubation period ($P<0.05$). Non-buffered sucrose solutions were therefore used for the compensation investigations to prevent any distortion of results. In this investigation, producing an accurate measurement of tuber turgor using the compensation method was not possible due to difficulties in obtaining precise values for osmotic potential. A similar method using buffered mannitol solutions was used by Linn and Pit (1986) who also had difficulties in that they could not identify incipient plasmolysis using measurements of sample volume change. As an alternative they used measurements of tissue stiffness to determine at what point incipient plasmolysis occurred. A further disadvantage in using this method was the requirement for a large volume of tissue in order to incubate at a wide range of solution water potentials and consequently a large numbers of tubers were required. The drawbacks of this method made it unsuitable for determination of potato tuber turgidity in this investigation.

Relative water content (RWC) measures the water content within the tissue in comparison to that of fully turgid tissue and hence it is not a direct measurement of turgidity but can be used as an indication. A decline in

turgidity (and therefore RWC) was expected during storage. Therefore, the maintenance of RWC, which was seen at the end of storage season 1995/6, was surprising (Fig 2.7). Consequently, there was a possibility that the method for measuring RWC was not sensitive enough to detect the changes in water status within the tuber although the high relative humidity (95 %) in which tubers were stored may have prevented a significant loss of water from the tuber through storage.

Due to the area of tissue being examined, the pressure probe was not appropriate for measurements of tuber turgidity. It was therefore decided that the thermocouple psychrometer should be employed to measure water potential and osmotic potential of tuber sections in order to determine turgidity.

Preliminary work found that vapour equilibrium within the C52 sample chambers took 3 hrs incubation for potato tuber and saturated filter paper discs (Fig 2.8 a and b). According to the type of substance being measured for water potential using the psychrometer, the time taken to reach vapour equilibration varies. Brown & Oosterhuis (1992) estimated equilibration times of approximately 2 hrs for solutions and 4 hrs for leaves, although alternative reports detail longer incubation periods than this for leaves. Barrs (1965) recommended 24 hrs. The internal workings of thermocouple psychrometers differ slightly (Brown & Oosterhuis, 1992) and therefore it is essential to calibrate each chamber. The equations produced for the two C52 sample chambers converting chamber readings to water potential both had an R^2 of 0.9999 enabling confidence in the conversions. Finally, tuber tissue was incubated in a range of sucrose solutions between 0.01 M and 0.4 M prior to measurements of the tissue turgor using the method outlined in 2.2.3.4. This allowed the method to be tested ensuring differences in turgidity were borne out in the results given by the psychrometer. A significant decrease in the turgor of the tissue ($P<0.05$) was brought about by an increase in the molar concentration of the sucrose solution. Consequently, it was decided that this method was accurate enough to determine changes in tuber turgidity. Water potential and osmotic potential both had to be measured using the psychrometer in order to calculate turgor pressure. All 3 results give useful information about different aspects of tissue water status and therefore have

been included in the assessment of tuber water relations through storage season 1997/98.

There was no significant change in turgor pressure during storage for either Maris Piper or Pentland Dell in tubers grown at Arthur Rickwood or Terrington although there a slight decline was recorded between 10 and 40 wks in store. These results were very surprising as it has previously been reported that there is a decline in tuber turgor through storage (Hughes, 1980). It is possible that evaporatory losses were kept to a minimum due to the high relative humidity maintained throughout storage at SBEU (95%). However, the loss of turgor pressure over a period of 40 wks in store would seem inevitable as water loss occurs through respiration (Fig 2.3 – 2.5). Indeed, through handling tubers during sampling it was evident that a loss of water was taking place with tubers becoming noticeably softer as storage progressed.

It might be expected that the decline in turgor would be greatest following harvest, as tubers are likely to lose far more water once they have been removed from the ground, particularly if respiration at this time is high. Consequently, the absence of a significant decline between harvest and 10 wks in store is very surprising. One possibility for this is that prior to curing tuber skin set will not be complete and consequently water loss through evaporation will be greatly increased following harvest. The time taken for harvested tubers to be transported from the field to the laboratory and the subsequent time taken for all the analyses to be completed may have allowed a decline in turgor of the outer cells of the potato tuber, which was not seen later following storage when skin set would have been completed. In addition, following harvest tubers were maintained at 15 °C (harvest temperature) rather than 10 °C (storage temperature) at which tubers were maintained after sampling through storage. This increase in temperature will have allowed even greater evaporatory and respiratory losses.

Tubers of cv Pentland Dell grown at Terrington had a higher turgor pressure than those grown at Arthur Rickwood, a difference which was significant at $P<0.001$ (Fig 2.9 e and f). Again, this is surprising as tubers grown at ADAS Arthur Rickwood were irrigated during the growing season, whereas tubers grown at Terrington were not. This difference may represent

differences in rainfall patterns during the period immediately prior to harvest. Alternatively, it may represent differences in the soil water holding capacity and potassium content. A deficiency in potassium has been found to increase blackspot susceptibility and it has been proposed that this is through an effect on the water economy of the plants (Kunkel & Gardener, 1959; Smittle et al., 1974). Consequently it was suggested that blackspot bruise reduction by potassium application was a result of increased tuber water content (Smittle, et al., 1974). However, both sites under went fertiliser applications of 250kg ha^{-1} 3 times each year, reducing the likelihood of potassium deficiency being a significant factor.

No significant difference was observed in osmotic potential between tubers grown at the two sites and consequently the higher turgor pressure found in tubers grown at Terrington was also seen in the results for water potential. It may be worth noting that an increase in turgor causes an increase in water potential. It was observed by Hughes (1974) that while extremities of turgor affect a tubers susceptibility to bruising, it does not always explain the differences in bruising and hence other factors must be involved.

No significant change in water potential or osmotic potential was found in cv Maris Piper during storage (Fig 2.9). In contrast, tubers of cv. Pentland Dell declined in water and osmotic potential, indicating an increase in the dissolved solutes as storage progressed. Comparing this data with the results of sugar content of the tubers during storage, it can be seen that the increase in glucose, sucrose and fructose content was far greater in Pentland Dell than Maris Piper, which indicates that an increase in content of these three sugars through storage in cv Pentland Dell is partly responsible for the decline in osmotic potential (and therefore water potential) through storage.

According to Burton (1966) during the first wks of storage, the hexose content of potatoes increases, which does not necessarily correspond to a decline in sucrose concentration. It can be seen from Figures 2.39-41 and 2.43 - 45 that this was so during this investigation However, following this Burton describes a slow decline in both hexose sugars and sucrose until the end of storage. This investigation found that while this held true for cvs Maris Piper and Record during storage season 1996/7, it was not the case for cv

Pentland Dell which underwent a dramatic increase in glucose, fructose and sucrose at the end of storage. Storage season 1997/8 also saw this rise in sucrose, although there was no decline in glucose or fructose. The increase in glucose fructose and sucrose at the end of storage was also evident in the results for osmotic potential which declined between 30 and 40 weeks in store in cv Pentland Dell.

One point that can be noted from Fig 2.9 is the relatively large standard errors associated with the turgor measurements. The inherent variation within the tubers is unlikely to be the only reason for this. Psychrometric measurements are very sensitive in the range of plant water potential, often leading to variability within data (Simonneau & Habib Inra, 1991). One source of this with the psychrometric method is the evaporative losses, which occur following excision of tissue up until the time the tissue is sealed within the chamber. While every attempt was made to transfer the tuber tissue as quickly as possible such that the tissue was only exposed to the air briefly, it is inevitable that slight time differences will have occurred. In addition, any slight chamber temperature differences between samples will have caused a deviation in the results. In this investigation, tissue was incubated at 25 °C and the temperature of incubators was regularly checked using external thermometers. Furthermore, the chambers were housed within a polystyrene box upon which a lid was sealed immediately prior to removal from the incubators to further reduce temperature changes. However, again, any very slight variations will have resulted in a small amount of error. The standard errors for water potential and osmotic potential were relatively small, in comparison to those for turgor. Calculations of turgor were made using the formula $\Psi = P + \pi$ and thus encompass both measurements of water potential and osmotic potential, including any associated error. A final source of error in the measurement of tuber turgidity using the psychrometric method is that incurred by the mixing of apoplast and symplast during tissue homogenisation for the measurement of osmotic potential. In measuring turgor we are only interested in the internal hydrostatic pressure of the cells made up of the symplast, the apoplast consists of the intercellular spaces surrounding the cells, the mixing of the two will result in some error. However, in potato tuber tissue the

intercellular space accounts for only about 1 % of the tissue (Linn and Pitt, 1986) resulting in only a small degree of error.

The aim of the work on water relations was to be able to correlate a decline in tuber turgidity with an increase in bruise susceptibility through storage. Unfortunately this has not been possible as turgor was not shown to significantly decline during storage. Confidence in the results obtained using the psychrometric method was low, it is likely that the psychrometric method was not suitable for determining accurate quantitative measurements of tuber turgidity. Measuring tuber specific gravity whilst taking into account changes in dry matter content, might have provided a better, although indirect, indication of how tuber turgidity was changing during storage.

2.4.3 Cation Leakage

Changes in the extent of cation leakage through storage from tuber tissue imparts information on membrane integrity and leakage of intact tuber cells. It is unfortunate that no data is available for storage season 1996/7. However, from an examination of the results from the 1997/8 storage season, information on changes in cation leakage following harvest can be obtained. Thus, both sodium (Fig 2.12) and calcium ion leakage (Fig 2.16) at harvest were high, and that this was, in general maintained or even increased up until 10 wks in store. There was a subsequent decline until the mid to late storage, following which the leakage of these two cations was generally maintained. Measurements of sodium and calcium ion leakage from storage season 1995/6, however differ, with a significant increase in leakage from mid to late storage continuing until the end of storage (Fig 2.11). This increase in calcium and sodium ion leakage later in storage indicated disruption of tuber cell membranes (Turnbull and Cobb, 1992). Sodium is presumed to be stored in the vacuole and is also involved in the structural functions of the cytoplasmic and cellular membranes. Increased leakage of sodium ions indicated increased permeability or disruption of the tonoplast allowing sodium to be lost from cells. The vacuole is thought to be where the PPO substrates are located (McGarry et al., 1996). Consequently, any increase in tonoplast permeability or disruption during storage could enable leakage of the PPO substrates and hence increased melanin formation.

Calcium is located primarily in the cell walls and is also involved in the maintenance of certain cytoplasmic organelle membranes. An increase in the leakage of calcium ions indicates a decline in the integrity of intracellular membranes as storage progressed, during season 1995/6. Conversely, there is no increase in the leakage of either sodium or calcium ions towards the end of storage in season 1997/8 and in some instances a slight decline can be observed. This would indicate that the condition of cellular membranes is maintained during this season. The high levels of leakage at harvest and following the first 10 wks in store, is more difficult to explain. If the high level of leakage, was for some reason associated with increased metabolism immediately following harvest and during early storage, a similar increase would be expected during the latter stages of storage and this is not seen. One difference between harvested tubers and tubers removed from storage for sampling was temperature. Harvest temperature in 1997/8 was 15 °C, whereas the tubers were stored at 10 °C. It might be possible that this difference in temperature had the effect of increasing sodium and calcium ion leakage at harvest, perhaps due to an increase in metabolism seen in respiration data. Glinka (1974) investigating the flux of thiourea in carrot tissue noted that temperature affected the rate of movement. However, it should be remembered that tissue samples were incubated at 25 °C for cation analysis. A greater insight into tuber membrane quality might have been achieved if the temperature for incubation had been that of the tubers rather than the standard 25 °C. The temperature difference of tubers at harvest cannot account for the continued high levels of sodium and calcium leakage at 10 wks in storage (Fig 2.12 and 2.16).

Leakage of magnesium ions did appear to rise during storage season 1995/6 (Fig 2.11), however this result was not significant. During storage season 1997/8 there was no significant change in magnesium ion leakage for cv Pentland Dell (Fig 2.15). For cvs Maris Piper and Record there was a significant decline in magnesium ion leakage through storage ($P<0.05$) (Fig 2.15). This cation is found in amyloplasts and mitochondria (Turnbull and Cobb, 1992), and the results suggest that no significant decline in amyloplast or mitochondrial membrane integrity had occurred throughout storage.

The results for potassium ion leakage are unclear. During storage season 1995/6, a slight rise in leakage up until mid storage was followed by a decline (Fig 2.11). This pattern was significant ($P<0.05$) only in tubers stored at 5 °C. During storage season 1997/8 there is no clear pattern of potassium ion leakage and any changes seen were insignificant ($P>0.05$) (Fig 2.14). Potassium is found within the soluble fraction of the plant cell as a free ion which acts as an enzyme cofactor for a number of enzymatic reactions, in particular those involved in phosphorylation. The importance of this cation in bruising is well known (Brook, 1996; Burton, 1966) with deficient soils often resulting in blackspot susceptible tubers possibly through it's role maintaining cellular turgidity. It is thought however that there is a threshold limit above which altering the potassium ion content of the tubers has no effect on blackspot bruise. Bruising results indicate that there was no clear relationship between susceptibility and changes in potassium leakage through storage. It is surprising that the leakage of potassium does not correlate better with that of sodium which is also found within the vacuole. This might indicate that increased sodium leakage is not a result of tonoplast degradation but the degradation of cytoplasmic and cellular membranes.

A significant increase in the concentration of ammonium ion leakage was seen during storage seasons 1995/6 (Fig 2.11) and 1997/8 (Fig 2.13) ($P<0.001$). Nitrogen is a constitutive structural component of plant tissue, in particular forming proteins within the tuber. It can be seen from the 1997/8 results that the increase in leakage begins following 10 wks in storage and that prior to this the rate of ammonium leakage either declines or remains constant. The alteration in pattern of ammonium leakage from harvest to storage may be the result of the temperature difference between freshly harvested and stored tubers, or alternatively the change in physiology between harvest and storage. An increase in ammonium leakage through storage may indicate deamination of proteins is occurring.

Whilst differences in cation leakage through storage may indicate changes in the quality of different membranes, any leakage that takes place from the cell must pass through the cell membrane in order to be measured in the bathing solution. Hence leakage of all cations was dependent upon changes in the quality of this membrane.

Studies of electrolyte leakage through storage in potato tubers by Knowles and Knowles (1989) found a decline in leakage between 2 and 7 months in storage, following which there was an increase up to 24 months. Their results indicated deterioration in membrane quality (leading to an increase in electrolyte leakage) through storage as a result of free radical-mediated peroxidation of unsaturated fatty acids within the lipid bilayer. It was also noted that changes in electrolyte leakage through storage may be a consequence of changes in the total concentration of electrolytes in the tissue and not simply changes in membrane quality. Changes in the double bond index of tubers were inversely correlated with change in membrane quality measured by leakage. These results suggested that fatty acyl composition of the lipid bilayer of the membrane is a determinant of membrane permeability in stored tubers. The results from this investigation indicate a decline in membrane quality during storage season 1995/6 but not 1997/8. As bruise determination was not done in 1995/6 and bruising did not decline during 1997/8, it cannot be determined where changes in membrane permeability affect blackspot bruise.

2.4.4 Dry Matter Content

Dry matter content remained stable throughout storage season 1996/7 (Fig 2.38) and storage season 1997/8 (Fig 2.42), which indicated that the tuber tissue was not being significantly degraded. This is contradictory to the literature in which starch breakdown, known to be essential for respiration and metabolism, takes place throughout storage resulting in a decline in a reduction in dry matter content (Burton, 1966; Wiltshire & Cobb, 1996). It is likely that a decline in fresh weight during storage, masked any decline in dry matter content in this study. A better method of determining loss of dry matter through storage would have been to have weighed tuber samples prior to storage for their fresh weight value. Dry matter content could then be calculated through storage as the percentage of this original fresh weight value rather than the fresh weight value at following storage.

2.4.5 Potential Melanin Formation

The measurement of colour (dopachrome) formation at 475 nm provides an indication of the extent to which enzyme activity and substrate content are combining within the intracellular environment to affect melanin formation. Summarising the results found during the 3 storage seasons, there was an increase in colour formation as storage progressed. The times during storage at which these increases were greatest varied between the 3 years with tubers from storage season 1995/6 increasing from mid to late storage (Fig 2.17), compared with tubers from the subsequent storage season increasing during early and mid storage and the degree of potential bruise formation then remaining constant until the end of the storage period (Fig 2.18). In the majority of treatments, the increases in colour formation over time were significant, although there were a few cases when this did not hold true, in particular for cv Record. Cultivar Maris Piper tended to have the lowest degree of colour formation throughout storage, indicating a low biochemical potential to produce melanin, with Record and Pentland Dell forming significantly more dopachrome in this investigation. No significant difference was seen between tubers grown from seed tubers of different physiological age ($P>0.05$). During storage season 1997/8 the site at which tubers were grown did have a significant affect on colour formation (Fig 2.19). This difference probably resulted entirely from a decline in abs 475 nm at 30 wks in store in tubers grown at Arthur Rickwood which was not seen in tubers grown at Terrington.

Gubb, et al., (1989), employed freeze dried potato tissue followed by wetting, sonication and incubation for 3 hrs at 30 °C, centrifugation and measurement of absorbance at 500 nm as one form of measurement of blackspot susceptibility. In addition, they measured colour difference spectrophotometrically following incubation of fresh and frozen tissue at 30 ºC for 3 hrs compared with prior to incubation and a visual assessment of fresh and frozen slices were made. This method would allow determination of susceptibility at a later date, although freezing may affect enzyme activity.

The increase in colour formation at 475 nm during storage indicated an increase in the biochemical propensity of the tubers to produce melanin. Comparing the results of these studies with those of bruise susceptibility and

percentage bruise formation indicates that the biochemical ability to produce melanin is not the main influencing factor involved in bruise susceptibility.

2.4.6 Bruise Susceptibility

Tubers impacted during storage season 1996/7 showed a significant change in the volume of bruise during storage ($P<0.001$) (Figs 2.22 – 2.25). Tubers grown at Arthur Rickwood showed an increase in percentage bruise formation and severity of bruise through storage (e.g. Figs 2.22-3). This was not apparent in tubers from Terrington (e.g. Figs 2.24-5), for which there was no clear pattern. While there is no obvious explanation for this difference, the sites on which the tubers were grown are different. Tubers at Arthur Rickwood were grown on a free draining peaty loam pH 6.8, which is irrigated during the summer, whereas the soil at Terrington is a silty clay, pH 8.2 which is not irrigated. Variations in composition of the soil may play some role in affecting bruise susceptibility and the Terrington site had a greater soil phosphorus, potassium and magnesium content than Arthur Rickwood (ADAS, 1996; 1997 &1998).

There was no significant change in bruise volume during the subsequent storage season, with no consistent pattern unfolding as storage progressed. This result is very surprising. Previous work has indicated a rise in tuber susceptibility to blackspot bruise throughout storage (Burton, 1989), although McGarry et al. (1996) states that evidence is in fact conflicting, with a number of studies recording a decline in tubers bruising through storage and variations in tuber response to storage differing between cultivars. In addition, they note the possibility that following impact there can sometimes be a delay in bruise formation, such that analysis of tubers 48 hrs after bruising may not be sufficient to pick up damage in all cases. However, preliminary work done for this investigation found 48 hrs sufficient for bruise development.

One disadvantage of the method used to inflict impact on the tubers was that it allowed the possibility of multiple impacts as the falling bolt rebounds from the tuber and impacts for a second time. The rebound will vary considerably according to the turgidity of the tuber such that flaccid tubers will absorb more of the initial impact and reduce any rebound which

may occur. In contrast, the pendulum method of impact provides more control such that a single impact can more easily be administered. A further problem with impacting methods is that the size and shape of the tuber affects its bruise susceptibility. Dean et al. (1993) discussed the importance of standardising the physical properties of tubers if this method is to be used. Angle of curvature at the point of impact affects the amount of force delivered at that single point. In this investigation, every effort was made to ensure that tubers with basal ends displaying a similar degree of curvature, as well as size, were used for the bruising investigations, however variability is to some extent inevitable and may have been a source of error. It may have been worth measuring curvature at the point of impact in bruising investigations to enable the amount of error to be established. Unfortunately this would have been time consuming, in particular as this would have to be done using a non-destructive method prior to impact. One advantage of the falling bolt method is that results were quantitative, some work detailed in the literature only use qualitative methods such as Sawyer & Collins (1960), who noted the average severity of blackspot.

Dean et al., (1993) compared abrasive peeling, impact bruising followed by bruise assessment and tissue homogenisation as methods for estimating blackspot bruising. Comparison of the three methods using different genotypes and sampling through storage demonstrated that all three methods could distinguish between tuber genotypes and tubers from differing sample dates. They found that the best correlation of methods was between abrasive peeling and lyophilisation, as might be expected, since both methods are determining the biochemical capabilities of the tuber to produce melanin. Dean et al. (1993) concluded that the impacting method lacked sensitivity compared with the other two methods tested, however this method may show more varied results in accordance with the variation within tubers. Impacting methods take a larger number of factors into consideration such as turgor and dry matter content, not simply the biochemical constitution of the potatoes and therefore should be more accurate in their determination of bruise susceptibility. All methods undertaken by Dean et al. (1993) found that *S. hjertingii*, a wild species known for it's lack of browning, exhibited lower levels of enzymic browning than *S. tuberosum* cv. Maris Piper.

During this study blackspot susceptibility was found to vary considerably between tuber varieties, cv Maris Piper was found to have a significantly lower degree of bruising than either Pentland Dell or Record both in storage season 1996/7 and 1997/8. While no significant difference was seen between tubers of Pentland Dell and Record during 1996/7 Record did bruise to a greater extent than Pentland Dell in the subsequent year. The reduced susceptibility to blackspot bruise seen in Maris Piper can also be seen in the results for potential colour formation indicating that this difference between Maris Piper and the other cvs used in this investigation may be partly due to differences in the biochemical ability to produce melanin. Maris Piper also had a reduced cation leakage compared with other cvs ($P<0.05$) suggesting that difference in tuber membrane quality may also have been important in reducing blackspot bruise susceptibility in this cv.

Temperature at the time of impact was found to affect blackspot bruise susceptibility with an increase in susceptibility when tubers were impacted at a cooler temperature. Tubers impacted at 5 °C had a significantly higher degree of bruising than those impacted at 10 °C ($P<0.001$). This has been noted by a number of other workers and is believed to hold true between 1 and 30 °C (Burton, 1970).

2.5 Conclusions

The results from this investigation show clearly that tubers grown, harvested and stored in different years can have very different physiological properties. Thus investigations into tuber physiology should ideally be performed over several years to ensure a clear picture of what happens to tubers as storage progresses and how their external environment effects tuber properties

Table 2.9 and 2.10 summarise the main trends in tuber physiology measured during the 3 storage seasons. It can be seen from Table 2.9 that bruising susceptibility varied between storage season 1996/7 and 1997/8 both in colour formation at 475 nm and in bruise volume. During storage season 1996/7 colour formation increased during early and mid storage but remained stable at the end of storage whilst bruise volume increased from the beginning to the end of storage. Storage season 1996/7 was an average

year in terms of storage but tubers from this season were highly susceptible to bruising compared with storage season 1995/6 or 1997/8 (SBEU, personal communication, 1998). During the subsequent storage season colour formation at 475 nm increased throughout storage however bruise volume showed no consistent pattern as storage progressed. Dry matter content results for storage seasons 1996/7 and 1997/8 remained stable but are misleading in that they only indicate the dry matter content in relation to fresh weight, which is itself likely to have declined through storage.

Respiration rates showed the same pattern during all three storage seasons, declining following harvest, maintaining a reduced respiration during mid storage followed by an increase towards the end of storage.

It can be seen from Table 2.10 that Maris Piper was less susceptible to bruise formation than either Pentland Dell or Record. This correlates with a lower respiration rate, cation leakage and biochemical potential to produce melanin and also a higher turgor, osmotic potential in Maris Piper than cv Pentland Dell. Respiration rate, membrane quality, biochemical potential for melanin formation, and turgor have all been found previously to influence blackspot susceptibility and hence no conclusions can be drawn as to which of these factors, if any, are responsible for the reduced bruise susceptibility in Maris Piper. In particular as none of these factors consistently correlated with bruise susceptibility it can only be concluded that no one factor measured is solely responsible for blackspot susceptibility.

There were no significant differences in dry matter content between cvs which indicates that this does not play an important role in bruise susceptibility. Tuber physiological age did not have any significant affects on bruise susceptibility or any of the physiological and biochemical factors measured.

Table 2.9. Summary of general changes in tuber physiology during storage, → no change, ↑ increase ↓ decrease,

		Storage season								
		1995/6			1996/7			1997/8		
		Early	Mid	Late	Early	Mid	Late	Early	Mid	Late
Respiration			→	↑	↓	→	↑	↓	→	↑
Water Relations	Ψ							↓	↓	↓
	π							↓	↓	↓
	P							→	→	→
Cation Leakage	Sodium		→	↑				→	↓	↓
	Ammonium		↑	→				→	↑	↑
	Potassium		→	→				→	→	→
	Magnesium		↑	↑				→	→	→
	Calcium		→	↑				→	↓	↓
Abs (475nm)		→	↑	↑	↑	↑	→	↑	↑	↑
Bruise Vol					↑	↑	↑	→	→	→
Dry Matter					→	→	→	→	→	→
Sugar Content	Glucose				↑	→		↑	→	
	Fructose				↑	→		↑	→	
	Sucrose				↓	↓				
Industry perspective*	storage	Good			Average			Good		
	bruising	Low			High			Low		

*Sutton Bridge Experimental Unit (1998), personal communication

Table 2.10. Summary of the influences on tuber physiology. MP = Maris Piper, PD = Pentland Dell and R = Record, T = Terrington and AR = Arthur Rickwood, x = no influence.

		CV	Storage temp	Site	Phys Age
Respiration		MP < PD = R	5 °C < 10 °C	T > AR (yr 2)	x
Water Relations	Ψ	MP > PD			
	π	MP > PD			
	P	MP > PD			
Cation Leakage	Sodium	MP < R < PD	x	x	x
	Ammonium	MP = PD < R	x	T > AR	x
	Potassium	MP < R < PD	x	x	x
	Magnesium	MP < R < PD	x	T > AR	x
	Calcium	MP < R < PD	x	x	x
Abs (475 nm)		MP < PD = R	x		x
Bruise Vol		MP < PD ≤R		AR > T (Yr 2)	x
Dry Matter		x		x	x
Sugar Content	Glucose	MP = R < PD		x	x
	Fructose	MP = R < PD		x	x
	Sucrose			x	x

3.0 Potato Tuber Physiology and Biochemistry Following Impact: A Time-course Study

3.1 Introduction

According to Kahl & Wielgat (1976), plants respond defensively to wounding in three ways;

1. using dead cells at the wound site as a barrier,
2. accumulating protective compounds at the wound surface and
3. undergoing cell division and modifying cell walls e.g. with lignin and suberin

If bruising in potatoes was categorised as above it would fit category 2, i.e. that the tuber synthesises melanin at the site of impact as a protective compound. However, the actual role of melanin has not been identified and in the potato melanin does not form at the surface of the tuber but beneath the periderm within the vascular ring (Burton, 1989). This may indicate that a potato tuber responds differently to bruising than it does to wounding.

Burton (1970) suggested that impact results in a distortion of the tissue leading to a gradual intracellular breakdown and bruising. Breakdown of the intracellular structure of the potato would both involve and result in physiological and biochemical changes, as part of an activation of secondary metabolism (Belknap et al., 1990).

Much of the work done to date on damage response has concentrated on wounding, usually by cutting (Johnson & Schaal, 1957; Kahl & Wielgat, 1976; Galliard, 1978). This investigation aims to provide information on various aspects of tuber physiology and biochemistry following damage caused by impact.

3.1.1 Changes in Tuber Physiology Following Impact

The tuber responds to impact in a number of ways and in particular respiration has been found to increase in bruised and wounded tubers compared with non-damaged tuber tissue (Kahl & Wielgat, 1976; Dizengremel, 1985; Brook, 1996). This response indicates an increase in the metabolic rate of tuber cells (Burton, 1970).

Another aspect of tuber physiology that may be affected by impact is turgidity. It is likely that the cellular damage resulting from impact will cause

some loss of turgidity. Damage to the cells close to the periderm may result in direct moisture loss to the surroundings. Previous investigations have found that tubers that underwent pressure bruising during storage had an elevated loss of water despite no apparent cracking or breaking of the periderm (Lulai et al., 1996). However as no direct measurements of turgidity following impact have been reported, measuring turgidity will provide interesting information on how the tuber tissue water relations are affected by impact. Loss of water from the tuber cells will in itself affect both the physiology and the biochemistry in the area of damage.

3.1.2 Biochemical Changes Following Impact

A number of adaptations to tuber biochemistry have been previously noted as occurring upon impact (Burton, 1970). One effect is a general increase in the content of PPO substrates found in the potato, chlorogenic acid, tyrosine and caffeic acid (Burton,1970; Stevens & Davelaar, 1996). This rise was found not only in the damaged tissue but also in the area surrounding it (Burton, 1970), indicating that the increase was not a result of substrates being transported into the damaged area from the surrounding cells. One possibility is that substrate synthesis is increased during this time. Alternatively metabolism of the substrates may be blocked (Burton, 1970). Evidence supporting the synthesis of new substrates can be found in investigations into the formation of polysomes following damage. An immediate increase in ribonucleic acid (RNA) content can be found after damage followed by an increase in deoxyribonucleic acid (DNA) (Burton, 1970). Ishizuka, & Imaseki, (1990) found an increase in ribosomes following wounding indicating that damaged cells are producing new proteins. In addition, there is an increase in the synthesis of ribosomal RNA within the nucleolus for up to 48 hrs after tuber damage (Kahl & Wielgat, 1976).

Although the exact role of PPO has not been determined, it is probably involved in defence against pathogens and pests (Thygesen, Dry & Robinson, 1995). PPO activity within the tissue has not generally been found to increase upon impact and it is not thought that the concentration of PPO within the cells is limiting to melanin formation (Burton, 1970; Belknap, 1990). However, results have not always been consistent and there have been

some reports of increased PPO upon wounding (Cheung & Henderson, 1972; Thomas & Delincee, 1981). Much of this work was performed on tissue damaged by cutting injury. It would therefore be valuable to confirm whether or not there is any alteration in PPO activity following impact.

Other biochemical alterations found to occur following mechanical damage of potatoes include stimulation of glycoalkaloid biosynthesis (Mondy, Leja & Gosselin, 1987). This is also likely to be a defence response to protect tubers from attack by pathogens. There has been conflicting evidence concerning changes in the concentration of the antioxidant ascorbic acid following bruising. Mondy, et al. (1987) found ascorbic acid to be significantly lower in bruised tubers compared with controls over 12 wks of storage. However, Burton (1990) has reported a transient increase in ascorbic acid content at the site of impact. Johnson & Schaal (1957) found that ascorbic acid transiently increased in tuber tissue slices, although in the damaged cells of cut surfaces ascorbic acid was oxidised.

Increases in enzyme activities associated with wounding are found following impact, including as phenylalanine ammonia lyase, ubiquitin and peroxidase. This results from an increase in gene expression (Belknap, et al., 1990; Burton, 1990).

3.1.2.1 Measurements of PPO Activity: There are two ways in which PPO activity can be measured. The first is to measure the change in colour formation spectrophotometrically of a potato extract and the second is to measure change in rate of oxygen consumption (which is essential for the formation of polyphenols) of the potato extract.

When using a spectrophotometric method to determine PPO activity, the formation of quinone polymers is measured at 475 nm. This method is only suitable for relative measurements of PPO activity because the assay is only linear for a short period of time. Subsequently, the enzyme may be inactivated to some extent during catalytic activity (Mayer & Harel, 1979).

The rate of PPO activity is in part dependent upon environmental conditions such as pH (which is optimally between 5.0 and 7.0) and substrate (Mayer & Harel, 1979), and it is important to establish the exact assay conditions in order for the reaction to be running at it's V_{max}.

One problem with spectrophotometric assays is that due to the low affinity of O_2 for PPO, well aerated substrate solutions are necessary for V_{max} to be established (Mayer & Harel, 1979; Vaughn & Duke, 1984). Whilst this is less of a problem where measurements are made using oxygen electrodes, this method can be more time consuming and therefore a spectrophotometric method was decided upon for use in this study.

3.1.3 Aims

The aims of this investigation were to measure a number of physiological and biochemical parameters at regular intervals during the 24 hrs following impact to determine how the tuber responded. This investigation was undertaken firstly during early storage and secondly during late storage to determine how this affected the tuber response to impact.

Measurements undertaken include tuber respiration, cation leakage (to provide information on intracellular membrane damage occurring within the tissue), tuber turgidity, PPO activity and bruise formation over the 24 hrs following impact. In addition, ascorbate content and LAH activity were measured Brierley (1998) unpublished.

3.2 Materials and Methods

3.2.1 Plant Material

Tubers of cv. Pentland Dell and Maris Piper grown at ADAS Arthur Rickwood from seed tubers of 500 day degrees were used for both time-course investigations. These tubers were from the 1997/8 storage season. The tubers were harvested, cured and stored as in Chapter 2.2.1.3. Tubers were then transported for analysis after 15 wks and 32 wks in storage as in Chapter 2.2.1.1. Tubers were stored in incubators at 5 °C and 10 °C to equilibriate. In tubers of cv Pentland Dell this incubation period was 18 hrs and for cv Maris Piper 42hrs.

3.2.2 Tuber Impact

Tubers were impacted with 0.7 J energy using a falling bolt as in Chapter 2.2.6.1 at either 5 °C or 10 °C and returned to incubators of corresponding temperature until analyses were carried out. Investigations into cation leakage, bruise formation and PPO activity were carried out prior to impact and 0, 1, 2, 6 and 24 hrs after impact. All analyses were performed using 5 tubers for each combination of tuber cv and temperature at each analysis time throughout the two investigations, with the exception of percentage bruise formation where 10 tubers were used, and turgor determinations in which 4 tubers were measured.

3.2.3 Respiration of Tubers Following Impact

Respiration measurements on control tubers of cv Pentland Dell and Maris Piper stored at 10 °C were carried out as outlined in Chapter 2.2.2.2 with the following exceptions;

1) readings were taken every 3 mins (rather than every 2 mins),

2) measurements were carried out at 10 °C (rather than room temperature) and

3) respiration was measured prior to impact for 1 hr as a control, following a 30 min equilibration.

Following this tubers were removed from their gas tight chamber, impacted as in Chapter 2.2.6.1, and replaced. Measurements of respiration were resumed for a further 24 hrs allowing for a 30min re-equilibration time.

This investigation was repeated 3 times and an average and standard error was calculated. Due to the amount of data produced results were plotted only every 30 mins and error bars could not be displayed.

3.2.4 Cation Leakage Following Impact

The Dionex was calibrated as in Chapter 2.2.4.2. Measurements of cation leakage were performed on tubers of cvs Pentland Dell and Maris Piper at 5 and 10 °C (Chapter 3.2.1). Measurements were taken 1 hr prior to impact and following impact as in Chapter 3.2.2. Tuber tissue was prepared and cation leakage measured as in Chapter 2.2.4.3.

3.2.5 Turgor Following Impact

Tuber turgidity was measured on control tubers 6 hrs prior to impact and tubers 0, 6 and 24 hrs after impact following incubation at 10 °C, according (Chapter 3.2.2). Turgidity was measured as in Chapter 2.2.3.4.

3.2.6 Tuber Bruise Formation Following Impact

Tubers of cv Pentland Dell and Maris Piper incubated at 5 and 10 °C were used in this investigation. Following impact as in Chapter 3.2.2 tubers were analysed for percentage bruise formation 1 hr prior to and 0, 1, 2, 6, and 24 hrs after impact. Tuber periderm was removed from the site of impact and tubers were assessed for bruising damage. The percentage of tuber bruising was calculated.

3.2.7 PPO Activity Following Impact

3.2.7.1 Measuring PPO Activity: The assay for PPO activity was based on that of Chen (1991). Tuber tissue was homogenised in 10 ml 100 mM MES pH 6.0. The extract was filtered through 2 layers of muslin and centrifuged for 5 minutes at 10,000 g in a MSE Micro Centaur (Jennings & Co., East Bridgford, UK). 2.5ml of supernatant was desalted using a Sephadex G25 column to give the tuber enzyme extract.

The reaction mixture for this assay was 2 ml 15 mM chlorogenic acid in 100 mM MES at pH 6.0 and 450 µl of MES, pH 6.0, to which 50 µl enzyme extract was added (12 mM concentration of chlorogenic acid in final mixture).

PPO activity was measured spectrophotometrically at 395 nm and 25 °C using a Perkin Elmer Lambda 12 spectrophotometer (Perkin-Elmer Limited, Beaconsfield, England). Protein determination of the enzyme extract was measured using the Coomassie Blue assay of Bradford (1976). PPO activity was expressed as absorbance units mg^{-1} protein sec^{-1}.

3.2.7.2 Optimising Assay for PPO Activity with Regards to Substrate: The assay outlined in Chapter 3.2.7.1 was carried out using 15 mM chlorogenic acid, 15 mM caffeic acid and 4 mM tyrosine. This was repeated 3 times.

3.2.7.3 Optimising pH of Assay Conditions for PPO Activity: Activity of PPO was measured using the method outlined in Chapter 3.2.7.1 varying the pH of the buffer solution between pH 4.0 and 7.0 at intervals of 0.5 units. For each pH, a measure of activity was made on 5 tubers individually. The investigation was repeated.

3.2.7.4 Measurement of PPO Activity Following Impact: PPO activity was measured using the method outlined in Chapter 3.2.7.1 following impact at 10 °C as in Chapter 3.2.2 on tubers of cv Pentland Dell and Maris Piper following 15 wks in storage.

3.2.8 Statistical Analyses for Impact Investigations

Statistical analyses were performed on data as in Chapter 2.2.7 as appropriate. No statistical analyses were performed on the results of percentage bruise formation. T tests were performed on respiration data comparing the 5 measurements made prior to impact with,

a. the 5 measurements made following equilibration after impact and
b. the 5 measurements made at the end of the 24 hrs after impact.

In addition the general linear model was used to test for a decline in respiration over the 24 hrs following impact. In all cases values have been presented as a mean of the sample and where possible standard error has been calculated.

3.2.9 Additional Measurements

In addition to the above, measurements of lipid acyl hydrolase (LAH) activity and ascorbate were made on the sub-samples of tubers from the timecourse investigation 15 wks into storage by Brierley (1998, unpublished).

3.3 Results

N.B. Line graphs have been used to illustrate results in this chapter. Sample times prior to and following impact that are linked by a line are not intended to indicate a continuous or gradual change in parameter over that time.

3.3.1 Tuber Respiration Following Impact

Respiration rates of Pentland Dell and Maris Piper tubers increased significantly upon impact after both at 15 and 32 wks in storage ($P<0.001$), see Fig 3.1. There was a subsequent decline in respiration in the 24 hrs following impact ($P<0.001$). The respiration rate did return to that prior to impact in the 24 hrs after impact in Maris Piper at 15 wks ($P>0.05$). This contrasts with the results for Maris Piper at 32 wks and Pentland Dell in which the rate of respiration did not return to that prior to impact over the following 24 hrs ($P>0.05$).

Storage had a significant effect on the rate of respiration of control tubers, which was significantly higher after 32 wks in storage compared with 15 wks of storage in both Pentland Dell and Maris Piper ($P<0.001$).

3.3.2 Cation Leakage Following Impact

Table 3.1 shows instances in which cation leakage increased immediately following impact. Leakage of sodium and ammonium ions from tuber tissue did not increase significantly immediately following impact in Pentland Dell or Maris Piper at 5 or 10 °C, either 15 or 32 wks into storage ($P>0.05$). See Figs 3.2 and 3.3 for sodium and ammonium leakage, respectively.

There was a significant increase in potassium (see Fig 3.4), magnesium (see Fig 3.5) and calcium ion leakage (see Fig 3.6) immediately after impact for tubers of Pentland Dell impacted after 15 wks in store at both 5 and 10 °C ($P<0.05$). In addition, calcium ion leakage in Maris Piper stored for 15 wks and impacted at 5 ºC and potassium and calcium ion leakage in Pentland Dell stored for 32 wks and impacted at 5 ºC did increase significantly immediately following impact ($P<0.05$).

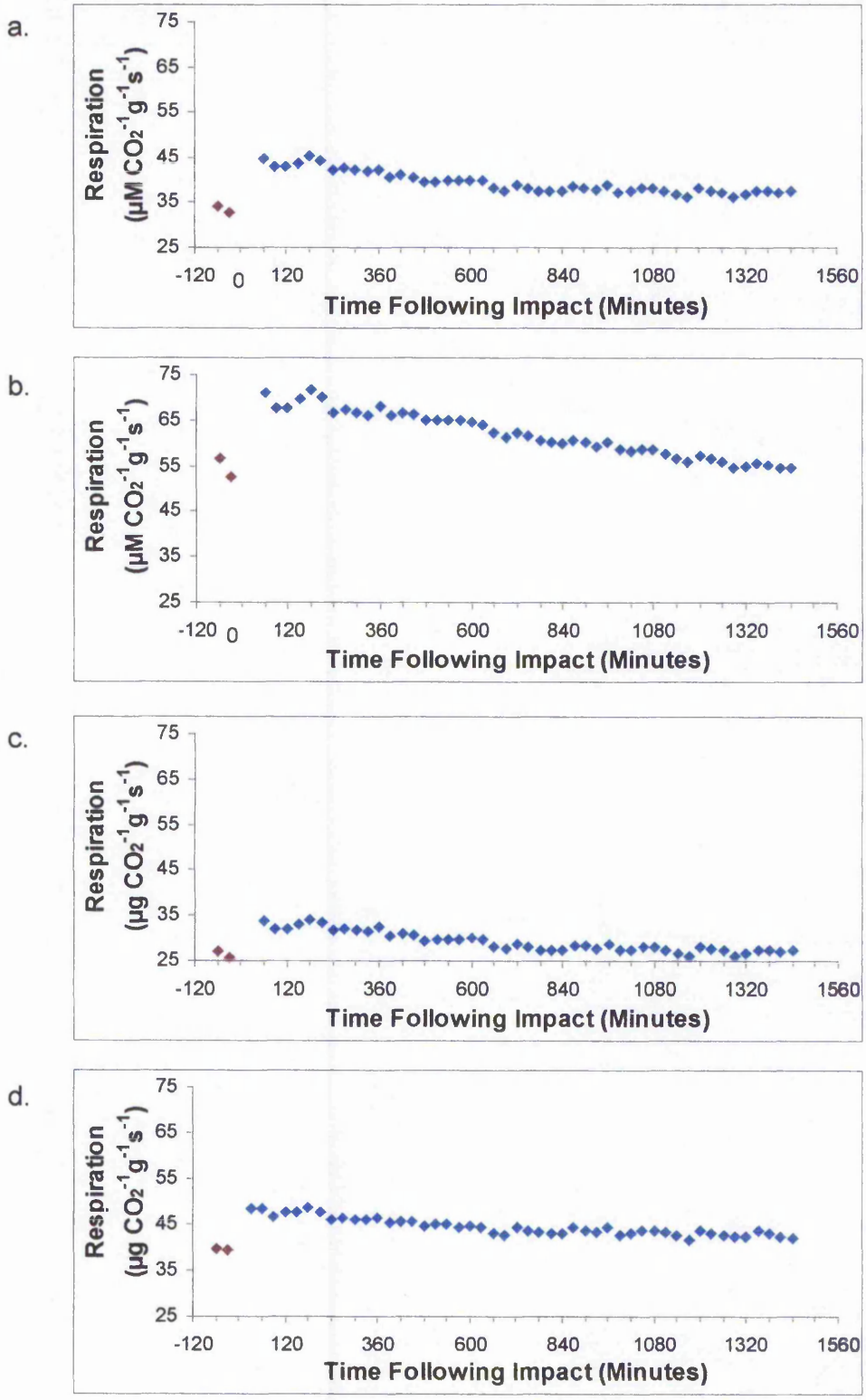


Figure 3.1. Respiration rate of tubers in the 24 hrs following impact at time 0. ◆ = respiration prior to impact, ◆ = respiration following impact. a. Pentland Dell following 15 wks in store. b. Pentland Dell following 32 wks in store. c. Maris Piper following 15 wks in store. d. Maris Piper following 32 wks in store.

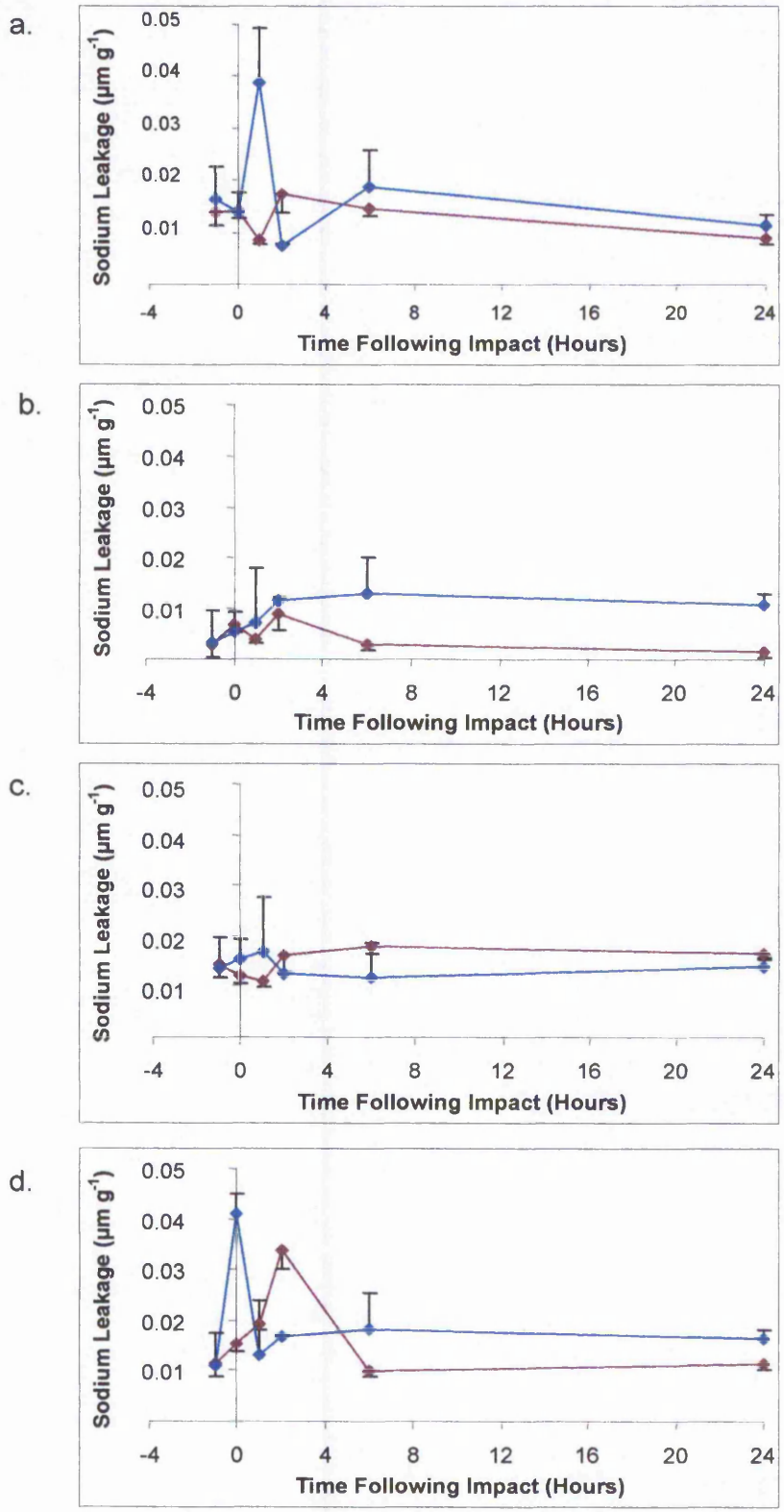


Figure 3.2. Sodium ion leakage during a 30 min incubation of tuber tissue impacted at time 0. —◆— = impacted at 10°C —◆— = impacted at 5°C. a. Pentland Dell following 15 weeks in store. b. Maris Piper following 15 weeks in store. c. Pentland Dell following 32 weeks in store. d. Maris Piper following 32 weeks in store. Error bars = ± standard error where n = 5.

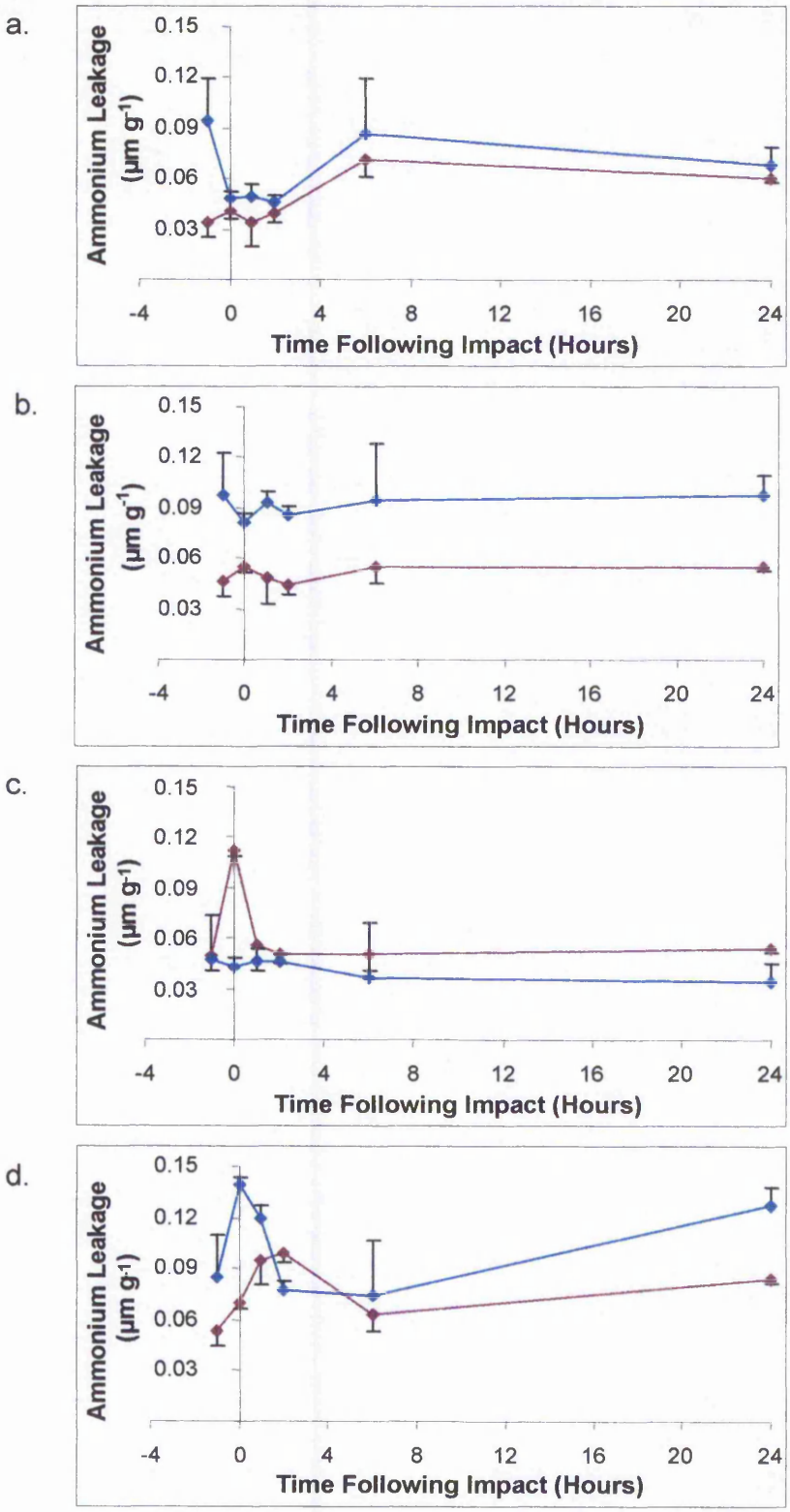


Figure 3.3. Ammonium ion leakage during a 30 min incubation of tuber tissue impacted at time 0. —◆— = impacted at 10°C —◆— = impacted at 5°C. a. Pentland Dell following 15 wks in store. b. Maris Piper following 15 wks in store. c. Pentland Dell following 32 wks in store. d. Maris Piper following 32 wks in store. Error bars = ± standard error where n = 5.

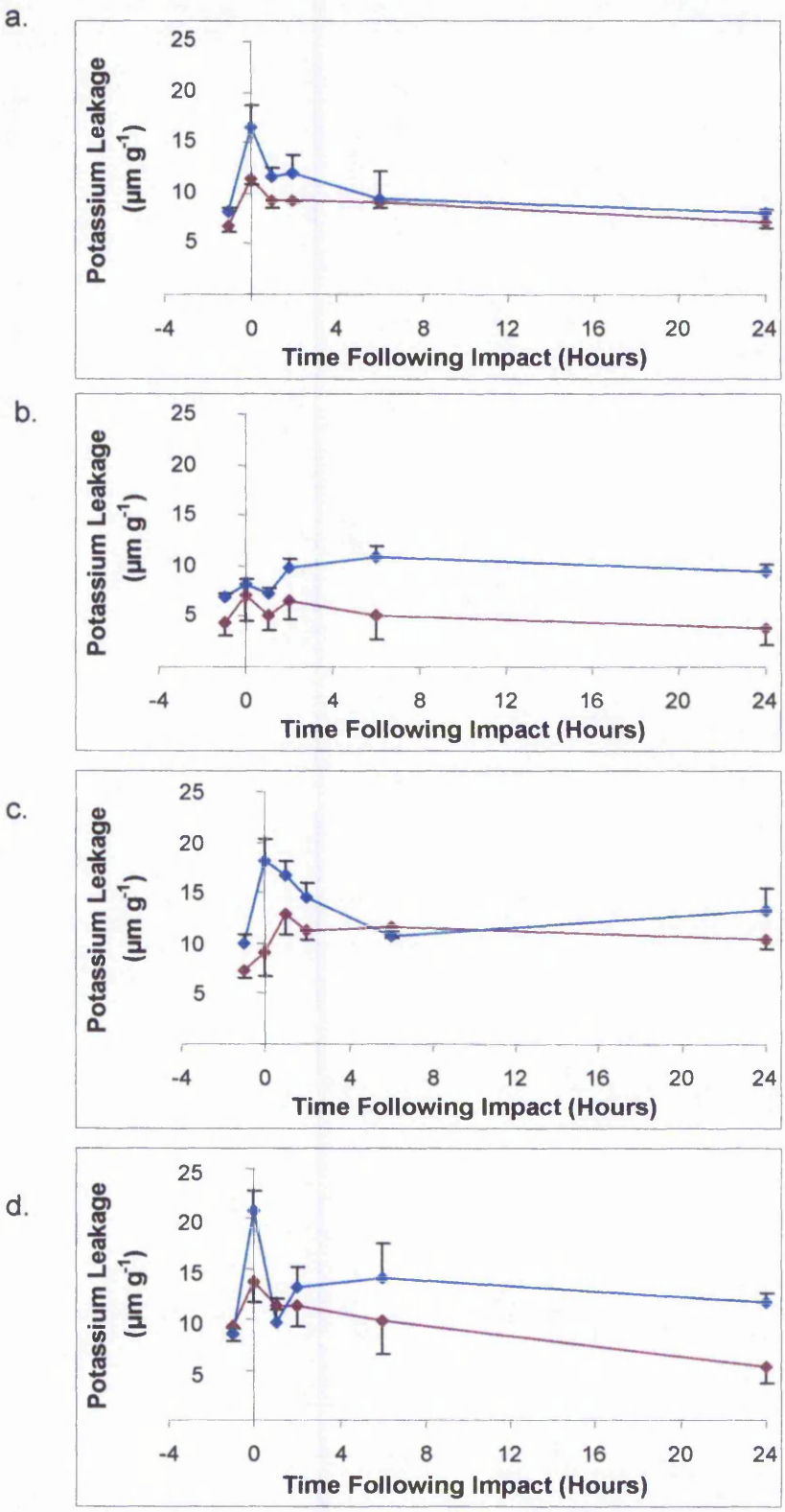


Figure 3.4. Potassium ion leakage during a 30 min incubation of tuber tissue impacted at time 0. —◆— = impacted at 10°C —◆— = impacted at 5°C. a. Pentland Dell following 15 wks in store. b. Maris Piper following 15 wks in store. c. Pentland Dell following 32 wks in store. d. Maris Piper following 32 wks in store. Error bars = ± standard error where n = 5.

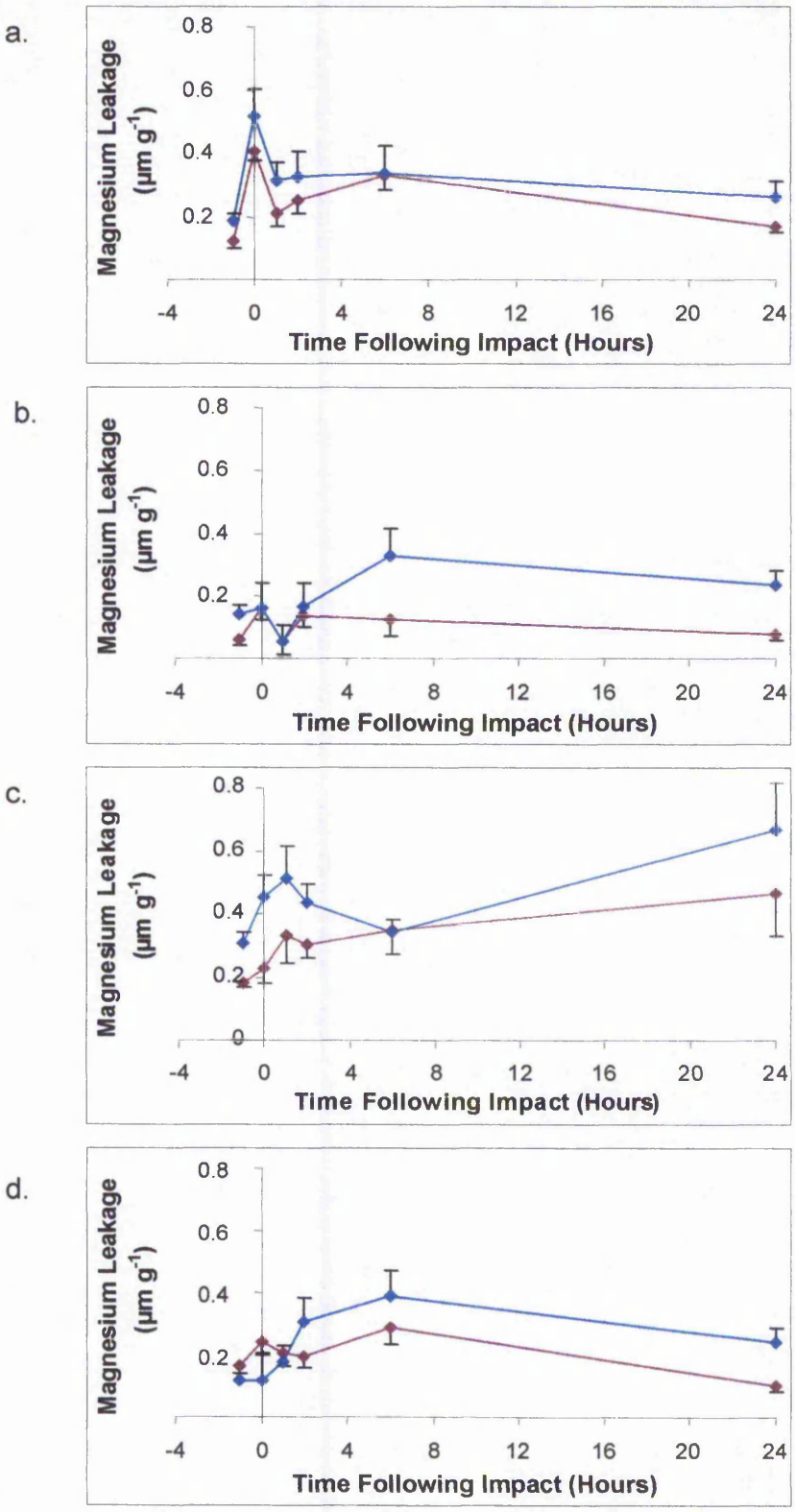


Figure 3.5. Magnesium ion leakage during a 30 min incubation of tuber tissue impacted at time 0. —◆— = impacted at 10°C —◆— = impacted at 5°C. a. Pentland Dell following 15 wks in store. b. Maris Piper following 15 wks in store. c. Pentland Dell following 32 wks in store. d. Maris Piper following 32 wks in store. Error bars = ± standard error where n = 5.

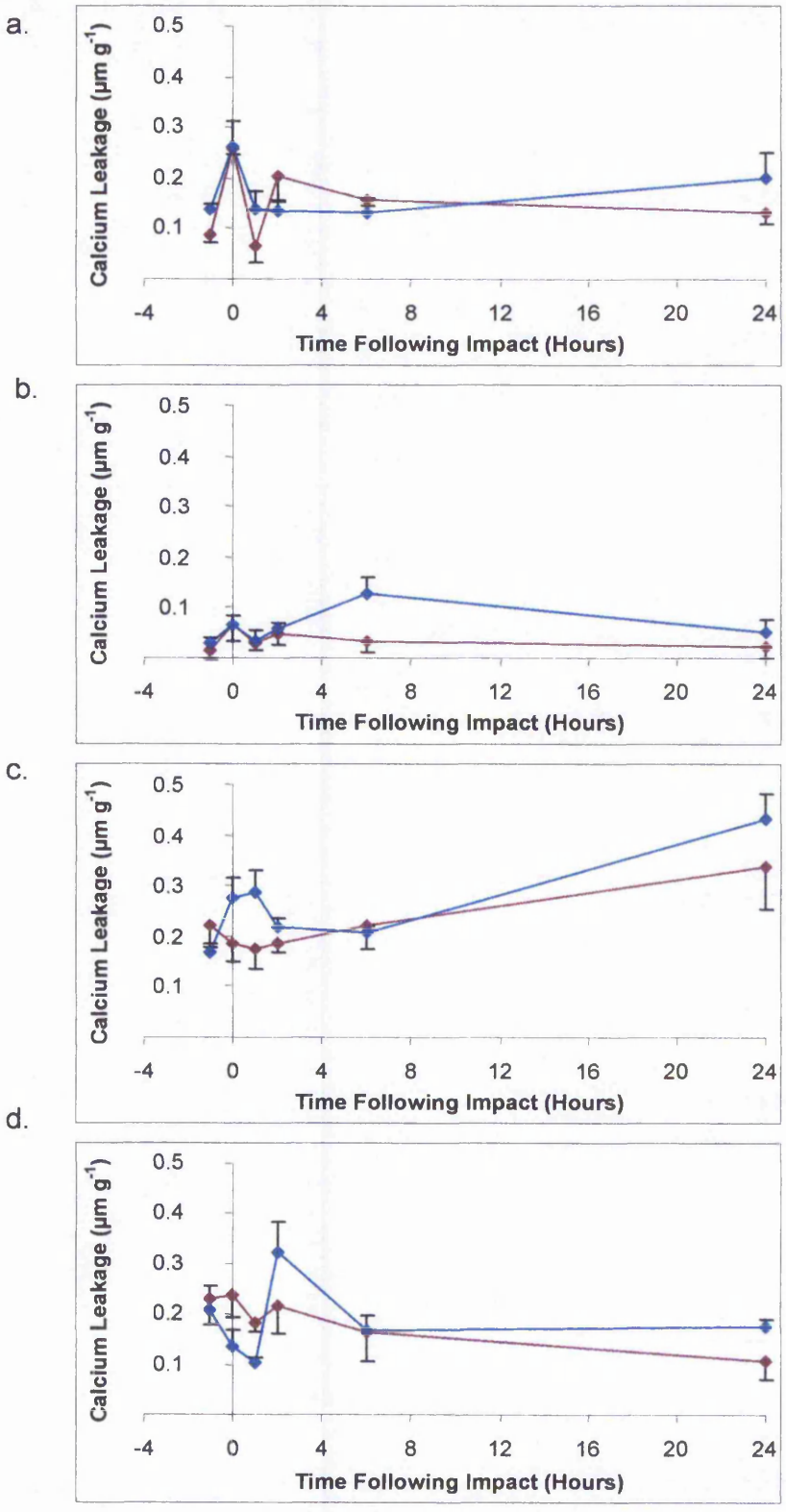


Figure 3.6. Calcium ion leakage during a 30 min incubation of tuber tissue impacted at time 0. —◆— = impacted at 10°C —◆— = impacted at 5°C. a. Pentland Dell following 15 wks in store. b. Maris Piper following 15 wks in store. c. Pentland Dell following 32 wks in store. d. Maris Piper following 32 wks in store. Error bars = ± standard error where n = 5.

Table 3.1 Instances where cation leakage was significantly greater immediately after impact compared with that prior to impact ($P<0.05$)

Storage (weeks)	cv	Temp °C	Na	NH_4	K	Mg	Ca
15	P.Dell	10			√	√	√
15	P.Dell	5			√	√	√
15	M.Piper	10					
15	M.Piper	5					√
32	P.Dell	10					
32	P.Dell	5			√		√
32	M.Piper	10					
32	M.Piper	5					

Table 3.2 indicates changes in the leakage of cations in the 24 hrs following impact. Again no significant changes were seen in the leakage of sodium or ammonium ions. There was a significant change in potassium and magnesium ion leakage in the 24 hrs after impact for tubers of Pentland Dell impacted at 10 °C after 15 wks in store ($P<0.05$). Figs 3.4 and 3.5 demonstrate that potassium and magnesium ion leakage declined in these tubers following the rise immediately after impact. Tubers of Maris Piper stored for 32 wks and impacted at 10 °C also showed a significant decline in potassium ion leakage in the 24 hrs following impact. Figure 3.4 shows a decline in leakage from impact up until 24 hrs after impact. In addition, calcium ion leakage (Fig 3.6) increased significantly at 24 hrs following impact in Pentland Dell stored for 32 wks impacted at 5 and 10 °C and in Maris Piper stored for 32 wks before impact at 10 °C in which there was a decline in cation leakage ($P<0.05$).

Table 3.2 Instances where there was a significant change in cation leakage during the 24 hrs after impact (P<0.05)

Storage (weeks)	cv	Temp °C	Na	NH_4	K	Mg	Ca
15	P.Dell	10			√	√	
15	P.Dell	5			√		
15	M.Piper	10					
15	M.Piper	5					
32	P.Dell	10					√
32	P.Dell	5					√
32	M.Piper	10			√		√
32	M.Piper	5					

There was a significantly greater leakage of potassium and magnesium ions in cv Pentland Dell in tubers impacted at 5 °C than tubers impacted at 10 °C (P<0.001).

In cv Maris Piper stored at 5 °C there was a significant difference in the sodium, potassium and calcium ion leakage between tubers stored for 15 and 32 wks. Figs 3.2, 3.4, and 3.6 indicate that there was a greater increase in ion leakage in tubers following impact later in storage.

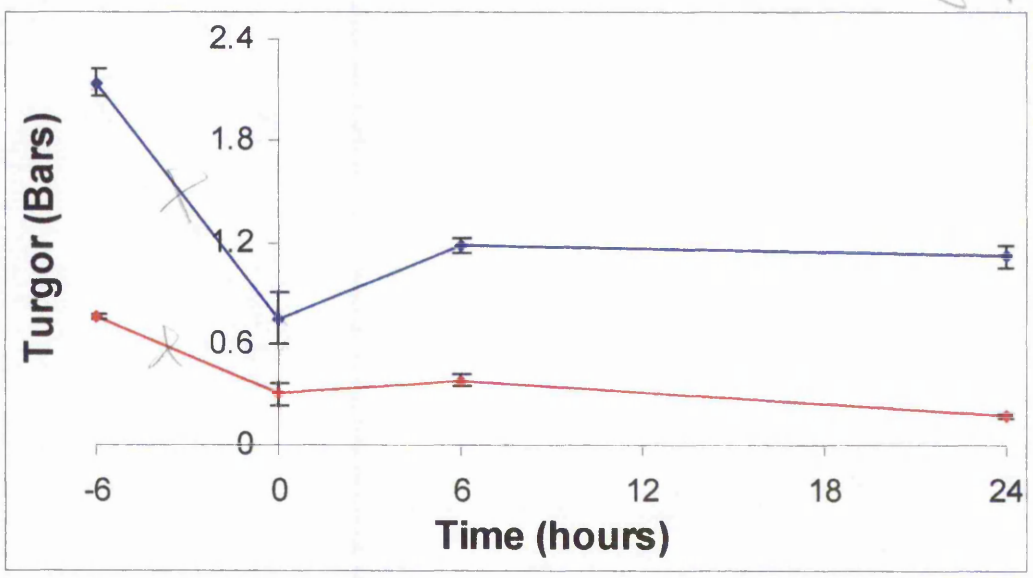
3.3.3 Tuber Turgor Following Impact

Turgor declined significantly upon impact in tubers of Pentland Dell and Maris Piper following 15 weeks in store (P<0.01), see Fig 3.7a. There was no significant change in turgor in the 24 hrs following impact (P>0.05).

Tubers of Maris Piper and Pentland Dell stored for 32 wks did not show any significant change in turgor either immediately after impact or in the following 24 hrs (P>0.05), see Fig 3.7b.

Tuber turgidity was lower in Pentland Dell than Maris Piper at 15 and 32 wks in storage (P<0.001).

a.



b.

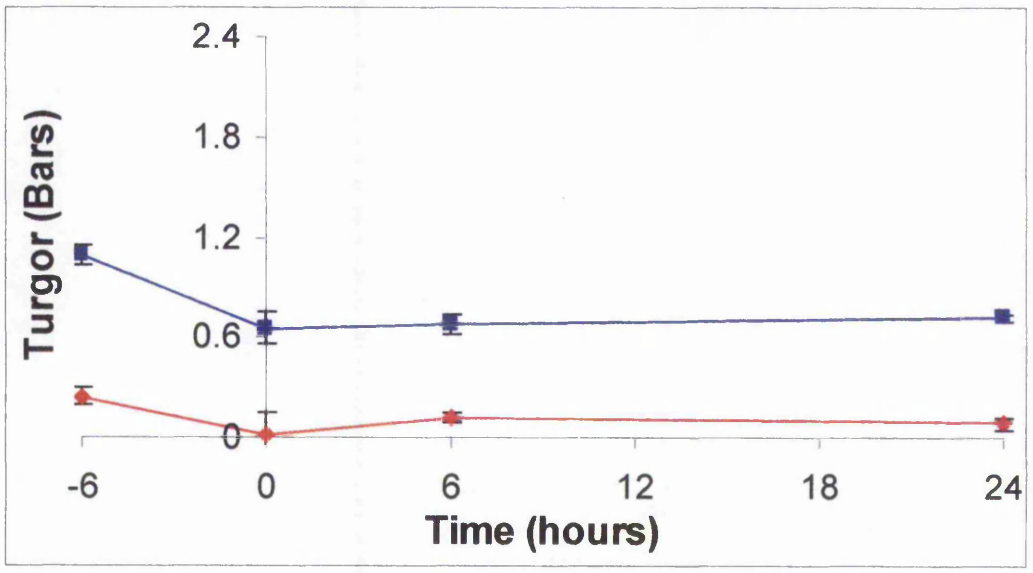


Figure 3.7. Tuber turgor from 6 hours prior to impact to 24 hours after impact in Pentland Dell —◆— and Maris Piper —■— impacted at 10°C. a. Tubers impacted following 15 wks in storage. b. Tubers impacted following 32 wks in storage. Error bars = ± standard error, where n = 4.

3.3.4 Percentage of Bruised Tubers Following Impact

Figure 3.8 shows that no tubers had formed a bruise immediately after impact. The number of tubers showing discolouration increased rapidly between 0 and 2 hrs after impact with percentage bruising reaching a maximum 6 hrs after impact. The percentage of bruised tubers 24 hrs after impact was higher after 32 wks in store compared with 15 wks in store for both Maris Piper and Pentland Dell impacted at 5 and 10 °C. Also, maximum bruising is higher for tubers impacted at 5 °C, compared with those at 10 °C.

3.3.5 Investigation into PPO Activity Following Impact

3.3.5.1 Comparison of PPO Substrates: Figure 3.9a shows that using the substrate chlorogenic acid for assaying PPO activity resulted in a greater activity than either caffeic acid or tyrosine. Indeed negligible activity could be obtained with tyrosine as substrate. A lower concentration of tyrosine was used compared with the 2 other substrates due to low solubility. Very similar results were obtained in repeat investigations.

3.3.5.2 The Effect of pH on PPO Activity: Figure 3.9b shows the results from the initial investigation into the effect of pH on PPO activity. pH 6.0 gave optimal PPO activity with chlorogenic acid as substrate. This was confirmed when the investigation was repeated.

3.3.5.3 PPO Activity Following Impact: It is evident from Fig 3.10 that there was no significant change in PPO activity following impact or in the subsequent 24 hrs ($P>0.05$) for tubers impacted at 15 wks in storage.

Cultivar Pentland Dell had significantly higher PPO activity than Maris Piper, both prior to impact and in the following 24 hr period.

3.3.6 Data Generated By Brierley (1998, unpublished)

Tuber ascorbate content declined in the 2 hrs following impact before stabilising and finally increasing to approximately the content before impact by 24 hrs (Fig 3.11 a). Fig 3.11b indicates a similar pattern occurs with LAH activity with a possible increase in activity immediately following impact,

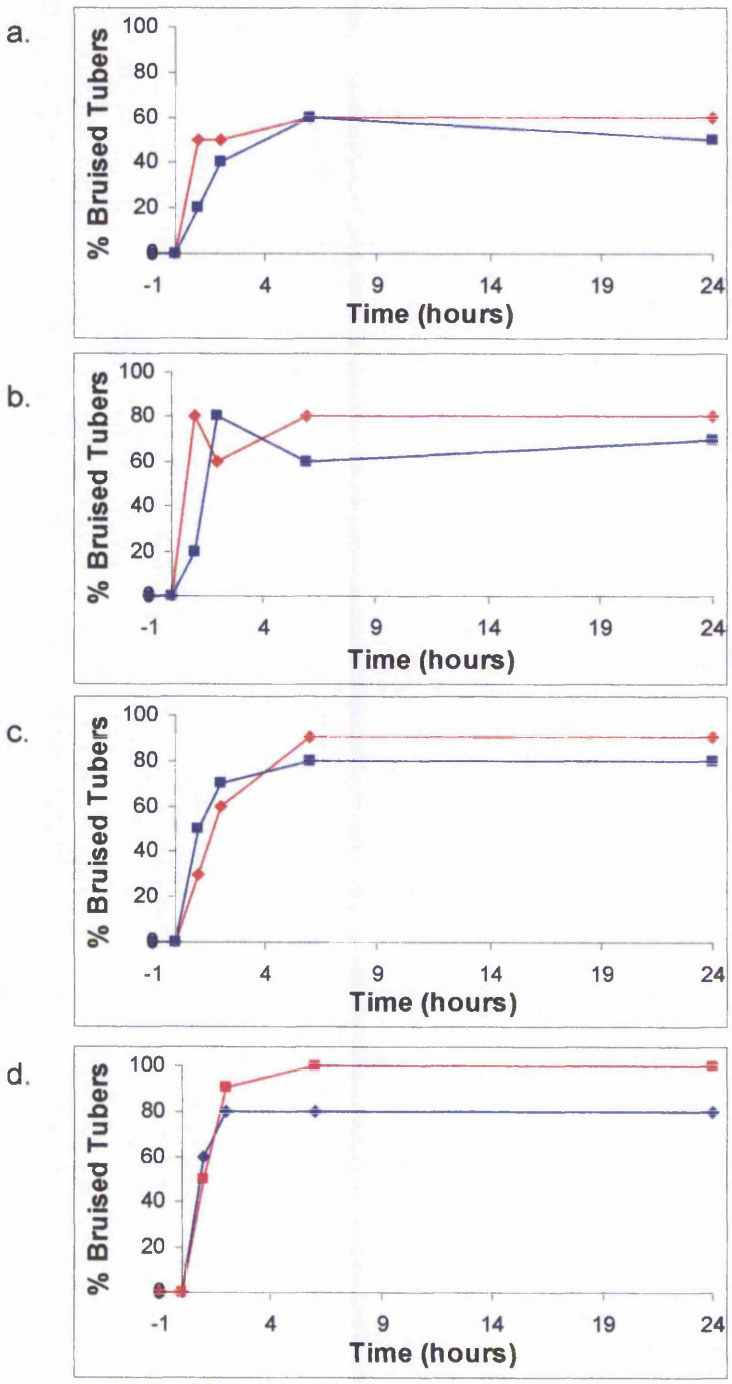


Figure 3.8. The percentage of tubers bruising with time after impact in Pentland Dell —◆— and Maris Piper —■— a. Tubers impacted at 10°C after 15 wks in storage. b. Tubers impacted at 5°C after 15 wks in storage. c. Tubers impacted at 10°C after 32 wks in storage. d. Tubers impacted at 5°C after 32 wks in storage.

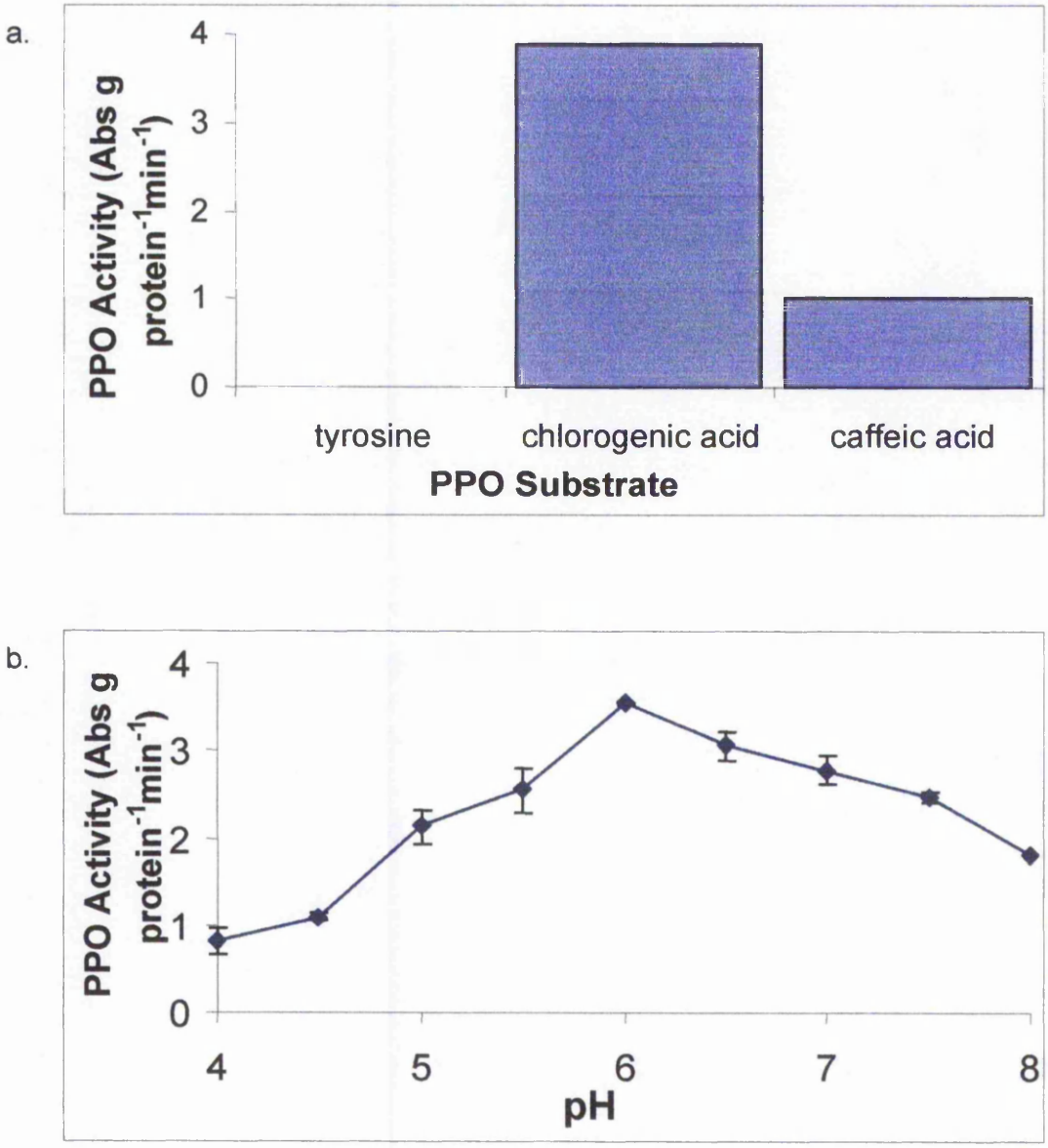
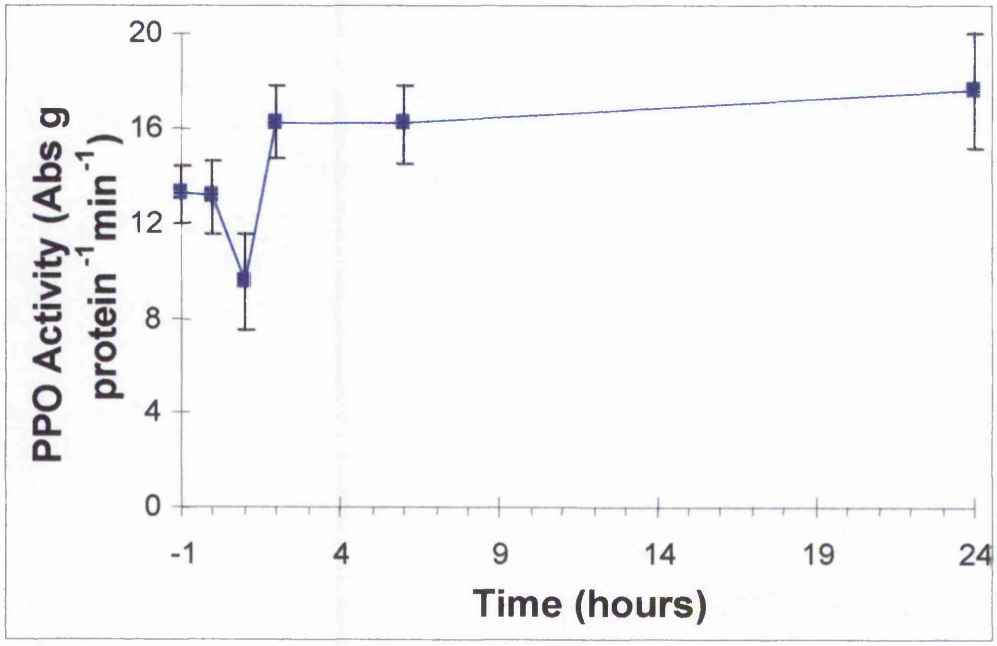


Figure 3.9. a. A comparison of PPO activity obtained using substrates 4 mM tyrosine, 15mM chlorogenic acid and 15 mM caffeic acid. b. The effect of pH on PPO activity with chlorogenic acid as substrate. Error bars = ± standard error where n = 5.

a.



b.

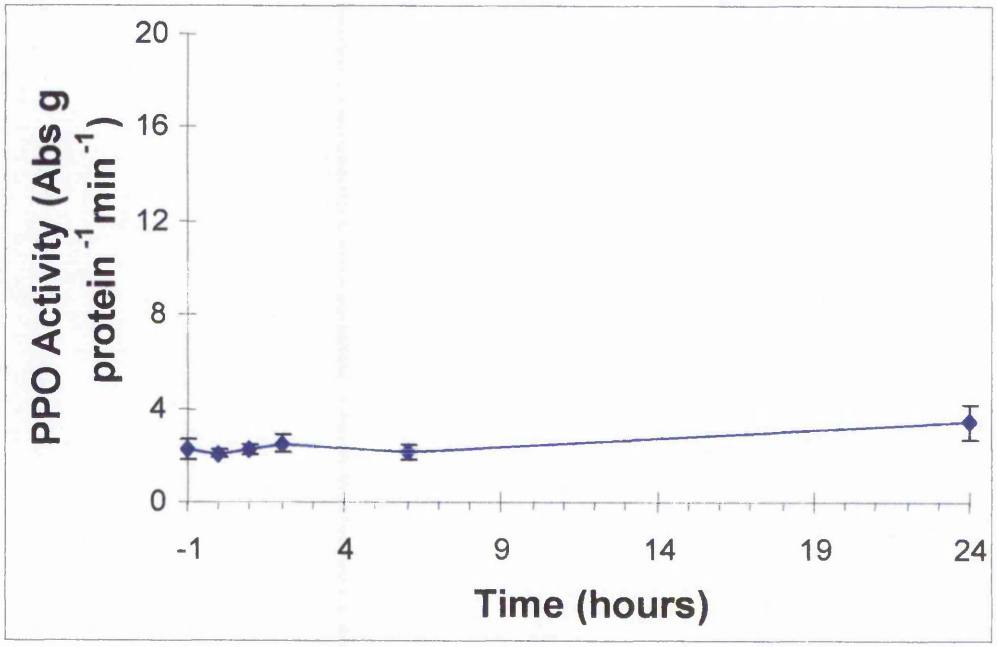
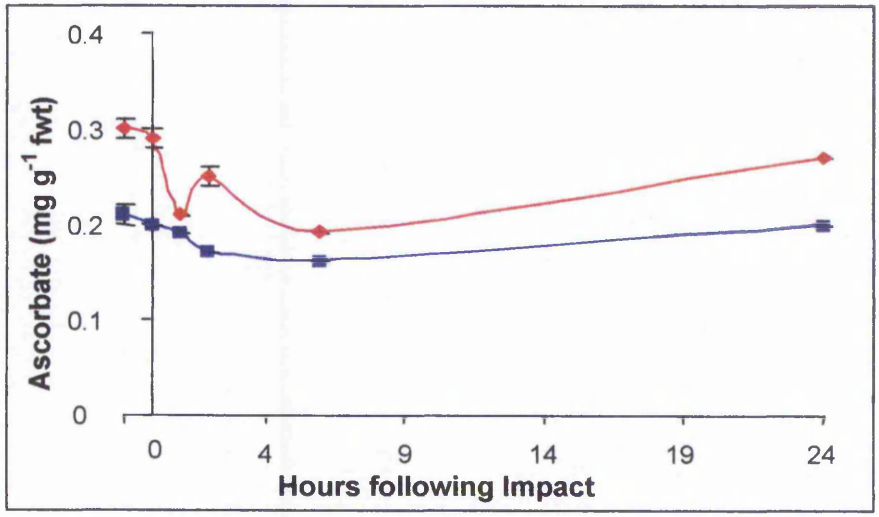


Figure 3.10. PPO activity prior to and in the 24 hrs following impact at 10°C. a. Pentland Dell. b. Maris Piper. Error bars = ± standard error, where n = 5.

a.



b.

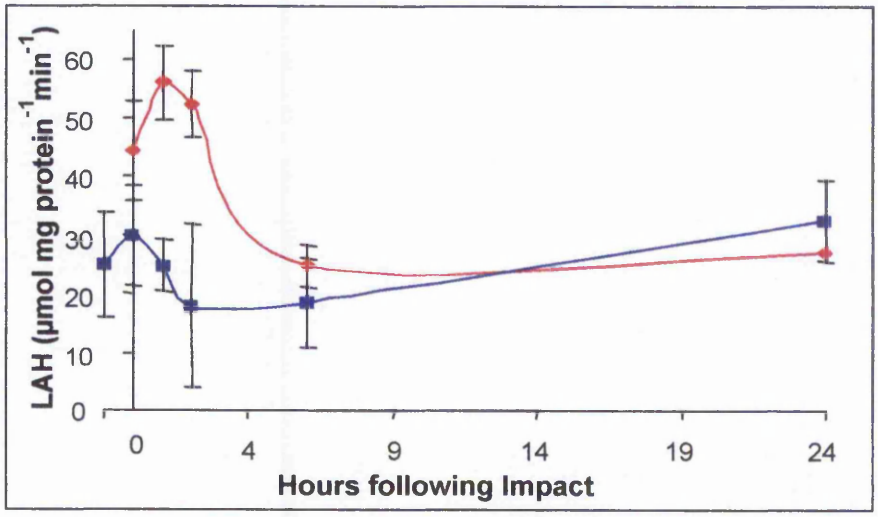


Figure 3.11. a. Ascorbate content and b. LAH activity prior to and following impact in tubers of —◆— Pentland Dell and —■— Maris Piper.

followed by a decline over the subsequent 2 hrs before increasing by 24 hrs after impact. The variability in this data was high resulting in large error bars.

Ascorbate content and LAH activity were both lower in cv Maris Piper than in Pentland Dell.

3.4 Discussion

Due to the large number of experiments carried out on the tubers during the 24 hr investigations it was necessary to carry out the work on the 2 cvs on 2 consecutive days. This is unlikely to have had a significant affect on the results but it is important to note this when comparisons between cvs are being made.

3.4.1 Respiration

In these investigations there was an immediate increase in respiration upon impact in tubers of Maris Piper and Pentland Dell during both time-course investigations early and late in storage. However, because the tubers had to be removed from the sealed respiration chamber in order for impact to take place this increase could only be confirmed after a half hour re-equilibration period. Following the rise in respiration, there was a subsequent decline, which began approximately 3 hrs after impact. Only in cv Maris Piper following 15 wks in storage did the respiration rate return to that of control tubers prior to impact during the 24 hr timecourse, in all other instances respiration was still declining 24 hrs after impact. It is interesting to note that Maris Piper following 15 wks in storage also had the lowest percentage bruise formation of the tubers impacted at 10 °C.

It has previously been established that following wounding there is an increase in the metabolic rate of the damaged cells which involves an increase in the rate of respiration (Burton, 1970; Kahl & Wielgat, 1976; Theologis & Laties, 1981; Dizengremel, 1985; Brook, 1996). Burton (1970) states that this increase in respiration occurs both in damaged cells and those of the surrounding tissue. However, Theologis and Laties (1981) found that wound-induced respiration increased between 3 and 5 times, but only in the damaged tissue and the adjacent 1 mm. The extent to which respiration increased following impact in this investigation was therefore unexpected. While some increase was predicted, it was suspected that this would have been masked by the large proportion of the tissue that had not been damaged by impact. The results suggest that the rate of respiration may increase not only in the damaged cells, but also in the surrounding the tissue. It should be noted that temperature would have a significant effect on

respiration rate. The respiratory measurements performed by Theologis and Laties (1981) were at 25 °C, where-as in this investigation measurements were made at 10 °C. The greater rate of respiration at 25 °C may have masked to some extent any increase in respiration that took place outside 1 mm from the wounded tissue in the investigation by Theologis and Laties (1981).

It would have been more accurate to measure respiration of the damaged tissue only rather than that of the whole tuber in order to establish the full extent of the rise in respiration. In order to do this, a method such as the use of oxygen electrodes could have been employed, but this would have meant destruction of the tissue, which in itself would have affected the results. In addition measuring respiration using oxygen electrodes is time consuming and somewhat artificial unlike measurements using infra red gas analysis.

Increased respiration following impact provides the damaged tissue with enough energy to fuel an adequate wound healing response, including the initiation of defence compounds. This response is similar to that found in tubers which have been wounded or infected by parasites in which the production of secondary metabolites helps to defend the tuber against further damage (Dizengremel, 1985). Schippers (1977) reports that in an early investigation by Lutman cutting injury resulted in increased respiration but that this relationship was not found with bruising. However, the measurements of respiration may have been taken too late to pick up any respiration burst, which had occurred. The majority of research points to an immediate increase in respiration following damage either by infection, bruising or wounding (Ilker et al., 1977; Dizengremel, 1985; Brook, 1996). This rise in the rate of respiration provides ATP to fuel the resultant damage responses. Evidence suggests that this rate will fall again over time.

3.4.2 Cation Leakage

Tables 3.1 and 3.2 clearly demonstrate that there was no change in sodium or ammonium ion leakage either immediately following impact or in the subsequent 24 hrs. These cations are primarily located in the vacuole and structural components of the cells, respectively. This would indicate that

there was no significant decline in tonoplast quality and that structural breakdown either did not occur or did not result in the release of ammonium ions.

It was clear that increased cation leakage was more common in cv Pentland Dell than in Maris Piper, particularly during time-course one. Fifteen weeks into storage, potassium, magnesium and calcium ion leakage increased in Pentland Dell tubers when impacted both at 5 and 10 °C in comparison with Maris Piper that only showed an increase in calcium ion leakage when impacted at 5 °C. An increase in calcium ion leakage indicates a possible decline in structural composition of the cell walls as well as some of the organelle membranes and an increase in magnesium ion leakage, found in the amyloplasts and mitochondria, points to a decline specifically in the quality of the amyloplast and mitochondrial membranes. Leakage of potassium ions, which are located in the soluble fraction of the tuber cell, might indicate a decline in the quality of the cell membrane following impact. These results indicate that the quality of membranes within the cell was negatively affected by impact in Pentland Dell.

Cation leakage in tubers impacted following 32 wks in store did not increase to the same extent as earlier in storage immediately after impact. Only potassium and calcium ion leakage increased in Pentland Dell, impacted at 5 °C. This would indicate that membranes were not damaged to the same extent when impacted later in storage, in particular at higher impact temperature. One possible reason for this is that loss of membrane quality during mid to late storage means that impact does not have such a significant effect on membrane quality of cation leakage. Alternatively, a reduction in turgor following a longer time in store might result in less physical damage being inflicted upon the tuber on impact. The increased leakage of potassium and magnesium ions resulting from impact at a lower temperature as seen in Pentland Dell indicates that a greater amount of internal membrane damage is taking place compared with tubers impacted at 10 °C. The effect of temperature seen on potassium and magnesium leakage in Pentland Dell in which leakage increased with a lower impact temperature suggest that damage to membranes was increased, perhaps due to changes in membrane fluidity.

In addition to the immediate increase in cation leakage in some tubers following impact, there were also some significant changes in the 24 hrs following impact. In some instances this was the decline that took place when leakage returned to it's pre-impact rate for example with potassium ion leakage in tubers of Pentland Dell following 15 wks in storage. However, in the case of calcium ion leakage in tubers from 32 wks in store with the exception of Maris Piper at 10 °C these changes include significant increases later in storage which may indicate that membranes are slowly being degraded following impact damage, possibly due to the autocatalytic nature of the membrane damage such that damage to cells releases enzymes, for example LAH from the vacuole, which would result in membrane degradation and subsequently yield the products of membrane lipid degradation, fatty acids which stimulate the action of the digestive enzyme LAH. The results from investigations into LAH activity performed on tubers during this timecourse investigation suggest that in the 24 hours following impact, activity declines in the first couple of hrs and then increases for at least the first 24 hours. This was particularly evident in cv Maris Piper. Unfortunately these results showed a large degree of variability and therefore precise conclusions cannot be drawn however it is possible to speculate that the process of membrane lipid degradation in the 24 hrs after impact is bringing about some increase in the LAH activity.

Theologis & Laties (1981) found there was a loss of lipids from membranes of tuber cells following slicing, including linoleic and linolenic acid. These workers also found that lipid losses took place up to 10 mm from the sliced surface. An investigation into the effects of slicing on tubers found that tissue with cutting damage had lower endogenous membrane lipid within seconds of wounding (Galliard, 1978), a result of active degradation of the intracellular membrane structure which in turn affects function. Interestingly, lipid has been found to be the source of energy for respiration in tissue damaged by cutting whereas carbohydrate is normal for intact tubers. However, Dizengremel (1985) reported that energy for the respiration burst following damage is provided by starch breakdown. It would be interesting to investigate the effect of impact upon lipid breakdown and in particular how

this is affected by low temperature and whether these lipid compounds are also used to fuel impact induced respiration.

3.4.3 Water Relations

Results from investigations into turgor following impact found that turgor declined following impact after 15 wks in storage. This was not surprising, as it has been shown previously that moisture loss in tubers increases following pressure-bruising damage (Lulai et al., 1996). This indicated that cellular damage was resulting in water loss and therefore loss of turgor. Conversely, tubers impacted following 32 wks in store did not show a significant decline in bruising. This might indicate that less damage is occurring to the cells upon impact during late storage, which would agree with results of cation leakage. Control tubers at this time were found to be significantly lower in turgidity than tubers earlier in storage; consequently loss of turgor following impact may have been to a lesser degree. The higher pressure within the cells during early storage resulting in a greater loss of water following impact than in the less turgid tubers later in storage.

Measurement of water potential required for turgor determination were time-consuming, taking 3 hrs for tubers to equilibrate, therefore measurements on control tubers had to be made 6 hrs prior to impact rather than 1 hr as with other investigations. While there is a possibility that in the 6 hrs prior to impact the tubers lost turgor pressure, the tubers were being incubated at 10 °C in the dark and it is therefore assumed that during this period there was no significant change in turgidity. The time taken for measurements of water potential and osmotic potential making up turgor is one of the limitations with this method. However results in Chapter 2.3.2 indicated that this would be the most suitable method for investigating tuber turgidity. Loss in turgor following impact will have a great influence over the physiology of the cell with a reduction in intracellular pressure that will influence transport processes, etc.

3.4.4 Blackspot Susceptibility

The percentage of bruised tubers increased in all cases in the 24 hrs after impact in both time-course 1 and 2, such that there was no bruising at

the time of impact or immediately after. Bruises began to develop by 1 hr after impact increasing to a maximum between 2 and 6 hrs after impact. It might be thought that this response was very rapid, since some reports suggest it takes up 3 – 4 days for a bruise to develop. It should be remembered that this result is only a percentage of the tubers forming a bruise. While the maximum number of tubers had formed a bruise 6 hrs after impact that is not to say that these bruises were not still developing 6 hrs after impact. Belknap et al. (1990) note that discolouration as a result of impact occurs approximately 4 hrs after impact and full bruise development took 24 hrs. No statistical analyses were performed on the results of this investigation, but it evident from Fig 3.8 that the increase in bruising is a result of impact which is as expected. Again, it was found that tubers impacted at 5 °C resulted in a greater percentage of bruised tubers than those impacted at 10 °C. It has been suggested that this might be a result of the rheological properties of tubers at 5 °C compared with those impacted at 10°C (Gray & Hughes, 1978).

3.4.5 PPO Activity

It is now possible to prevent the formation of blackspot bruise by inhibiting the expression of the key enzyme PPO (Bachem, Speckmann, Linde, Verheggen, Hunt, Steffens & Zabeau, 1994) in tubers of transgenic potato plants. Although this procedure prevents melanin formation upon impact, it does not reduce the damage incurred by the tuber tissue and has no effect on the disruption to tissue texture. The physiological effects noted during this investigation upon impact would be unaffected by the transformation with the exception of percentage bruise formation. It would be interesting to see how transformed tubers stored and additionally how they responded over time to various types of tissue damage, attack and wounding in comparison with wild type tubers. Useful information on the role of PPO in tuber damage might be gained. The importance of the physical damage caused by impact in the absence of melanin formation is in any case significant. Damaged tissue absorbs more oil during frying and will therefore still be a problem to the potato industry (Baritelle, et al., 2000).

Comparison of PPO substrates for use in assaying PPO activity found that although tyrosine is the primary phenol correlated with blackspot in bruising (Brook, 1996), no *in vitro* activity was found with this substrate under these assay conditions. Possibly another trigger compound is required to allow PPO and tyrosine to interact. Unfortunately, the low solubility of tyrosine meant that a direct comparison with other substrates could not be made. Figure 3.9 a shows that chlorogenic acid resulted in the most PPO activity. It was also found that activity was optimal when the pH was 6.0 (Fig 3.9 b), consequently this was the substrate and pH used for the investigations into PPO activity following impact.

The results of this investigation agree with the majority of earlier investigations, that the activity of PPO in tuber tissue was not found to increase upon impact and is not thought to be a limiting factor in the formation of melanin (Burton, 1970). This was true for cvs Maris Piper and Pentland Dell after impact at 10 °C 15 wks into storage. Belknap et al. (1990) did not find a change in PPO activity in the 72 hrs following impact indicating that activities present in control tissue are sufficient to respond to impact damage. However, there have been some contradictory reports for example that of Cheung & Henderson (1972) who found an increase in PPO activity in bruised tubers of the cv Pontiac and Netted Gem, no statistical backup was provided for these results. Criticisms can be made of this investigation in that control and bruised tissue were taken from different areas of the same tubers. PPO activity varies between the stem and bud end of tubers which would have affected the results in this investigation and may have resulted in differences in PPO activity between the 2 sample points rather than there being a difference in control and impacted tissue.

An increase in a number of other enzymes has however been noted in damaged potato tuber tissue. Galliard (1978) suggests an increase in the activity of peroxidase and Belknap et al. (1990) found that activity of the enzyme phenylalanine ammonia lyase (PAL) increased 200 fold following impact and that of ubiquitin and the heat shock protein HSP70 also increased over the subsequent 48 hrs. The reason behind the increase in activity of particular enzymes following impact and stress is unknown and may be a part of a general activation of defence mechanisms (Hughes, 1974).

Belknap et al. (1990) report that enzymes involved in the metabolism and synthesis of tyrosine and phenylalanine were activated thus increasing PPO substrate levels. However with the exception of PAL, the activities of these regulatory enzymes were not effected. Other effects of impact that have been found include an increase in ribosomes following damage (Kahl, 1971; Edgell, Brierley & Cobb, 1998) detected by electron microscopy. Bruised tubers have also been found to have more phenols, ascorbic acid and TGA than controls (Mondy et al., 1987). It has been found that in the few days after wounding there is an increase in the concentrations of chlorogenic acid, tyrosine and caffeic acid (Burton, 1970). Results from Brierley (1998, unpublished) indicate that this was not the case with ascorbate following impact of tubers in these timecourse investigations and that in fact ascorbate content of tubers dropped immediately following impact and that this level was approximately regained over the following remainder of the 24 hr studies (Fig 3.11a). It is possible however that ascorbate levels continued to rise after the 24 hrs of this investigation.

3.5 Conclusions

Table 3.3 summarises the immediate effects impact has upon tuber physiological and biochemical properties measured in this investigation. Impact of tubers resulted in bruise formation, which was evident in both Pentland Dell and Maris Piper 1 hr after impact both early and late in storage. In addition to this the rate of respiration rose immediately upon impact in all samples of tuber that were measured.

A decline in turgor was seen immediately following impact in Pentland Dell and Maris Piper tubers. However this decline was only seen in tubers impacted early in storage. Tubers impacted after 32 wks in store did not show a significant decline in turgor.

There was no significant change in PPO activity in either Pentland Dell or Maris Piper, nor did sodium or ammonium ion leakage change immediately following impact. This was not the case with potassium, magnesium or calcium ion leakage which increase significantly upon impact in cv Pentland Dell, in particular during early storage. This was seen to a lesser extent in tubers impacted late in storage and in cv Maris Piper which only underwent a significant increase in calcium ion leakage in tubers impacted at 5 °C following 15 wks in store.

Table 3.3. The changes in tuber physiology and biochemistry which occurred within 1 hr of tuber impact in cvs Maris Piper and Pentland Dell at 5°C and 10°C following storage for either 15 or 32 weeks. ↑ = increase, - = no change, ↓ = decrease and x not sampled.

Cultivar	Storage (wks)	Temp (°C)	Resp	Cation Leakage					Turgor	Bruise	PPO Activity
				Na	NH_4	K	Mg	Ca			
P.Dell	15	5	↑	-	-	↑	↑	↑	x	↑	x
P.Dell	15	10	↑	-	-	↑	↑	↑	↓	↑	-
P.Dell	32	5	↑	-	-	↑	-	↑	x	↑	x
P.Dell	32	10	↑	-	-	-	-	-	-	↑	-
M.Piper	15	5	↑	-	-	-	-	↑	x	↑	x
M.Piper	15	10	↑	-	-	-	-	-	↓	↑	x
M.Piper	32	5	↑	-	-	-	-	-	x	↑	x
M.Piper	32	10	↑	-	-	-	-	-	-	↑	x

4.0 Microscopy of Stored and Impacted Tuber Tissue

4.1 Introduction

4.1.1 Determining The Ultrastructure of Bruised Potato Tubers

A background of what occurs biochemically and physiologically within impacted potato tubers has previously been outlined. However, less information is available on the cellular damage that takes place upon and following impact.

It has been suggested that prior to impact, PPO and its substrates are kept apart by intracellular compartmentation, the former being associated with the plastids and the latter located within the vacuole (McGarry, et al. 1996). It is understood that damage to intracellular membranes following impact results in PPO and its substrates being brought together, thus initiating melanin formation in the presence of oxygen. Hughes (1980) suggested that the presence of starch grains in tuber cells may be responsible for rupturing cell membranes on impact and therefore tuber starch content is an important component of bruise susceptibility.

Microscopy of plant tissue is an important technique for enabling visualisation of the internal structure of the cells. Exactly what takes place within tuber cells upon impact remains largely unknown, with only a few ultrastructural studies to date. Reeve, Hautala & Weaver (1968) investigated some of the ultrastructural characteristics of bruising using light microscopy techniques and observed the darkening of the cytoplasm in bruised tuber tissue. However, no obvious structural damage was noted. McIlroy (1980) claimed that melanin formation was the result of granules that formed from existing peroxisomes. Further studies by Barkhausen (1978) and Ishizuka & Imaseki (1990) have provided more information of what occurs within potato tubers following impact and wounding damage. However a clear ultrastructural sequence of events following impact is not available.

Additionally, changes in tuber ultrastructure through storage may influence bruise formation. Turnbull and Cobb (1992) reported an increase in the abundance of mitochondria within the cytoplasm during late storage, indicative of an increase in metabolism of the tubers, which may in turn affect bruise susceptibility. It is possible that the changes in turgidity of tubers

through storage may be visualised ultrastructurally along with any consequences of impact. Hughes (1980) reported that flaccid tubers with high starch content were most susceptible to blackspot bruising, in particular if tyrosine content and PPO activity were high. In contrast, very flaccid tubers, which have for example sprouted, were less prone to blackspot (Gray & Hughes, 1978). However, in order to sprout, the tuber is likely to have mobilised some of its starch reserves, again this might be visualised as a decline in starch through storage. More information on the ultrastructural effects of storage on bruising may give a greater insight into how storage plays a role in influencing bruising in stored potato tubers.

4.1.2 Techniques Involved in Microscopy

Light microscopy gives a clear overall view of the whole plant cell, magnifying up to 1000 times. However, it is of limited use for determining finer intracellular detail. In this case, electron microscopy is employed with a resolving power of approximately 1000 times that of the light microscope enabling magnification of up to 1 million times.

As with a light microscope, the electron microscope has 4 basic components (Fig 4.1), the source of illumination, which in an electron microscope is the electron gun. Condenser lenses, then focus the illumination onto the specimen. Beneath the specimen an objective lens forms the primary image of the specimen, which is subsequently magnified by the magnifying lenses, providing an image on a screen, which can be viewed and recorded.

More recently the confocal microscope has been developed for the visualisation of 3D structures in the presence of dyes and fluorescence. Incubating plant tissue with fluorescent stains enables cellular structures to be visualised by the confocal microscope. Selective staining procedures can provide images, which show only the intracellular detail of interest. By recording images at intervals throughout a section of tissue, a 3D image can be produced making this a useful tool for ultrastructural studies.

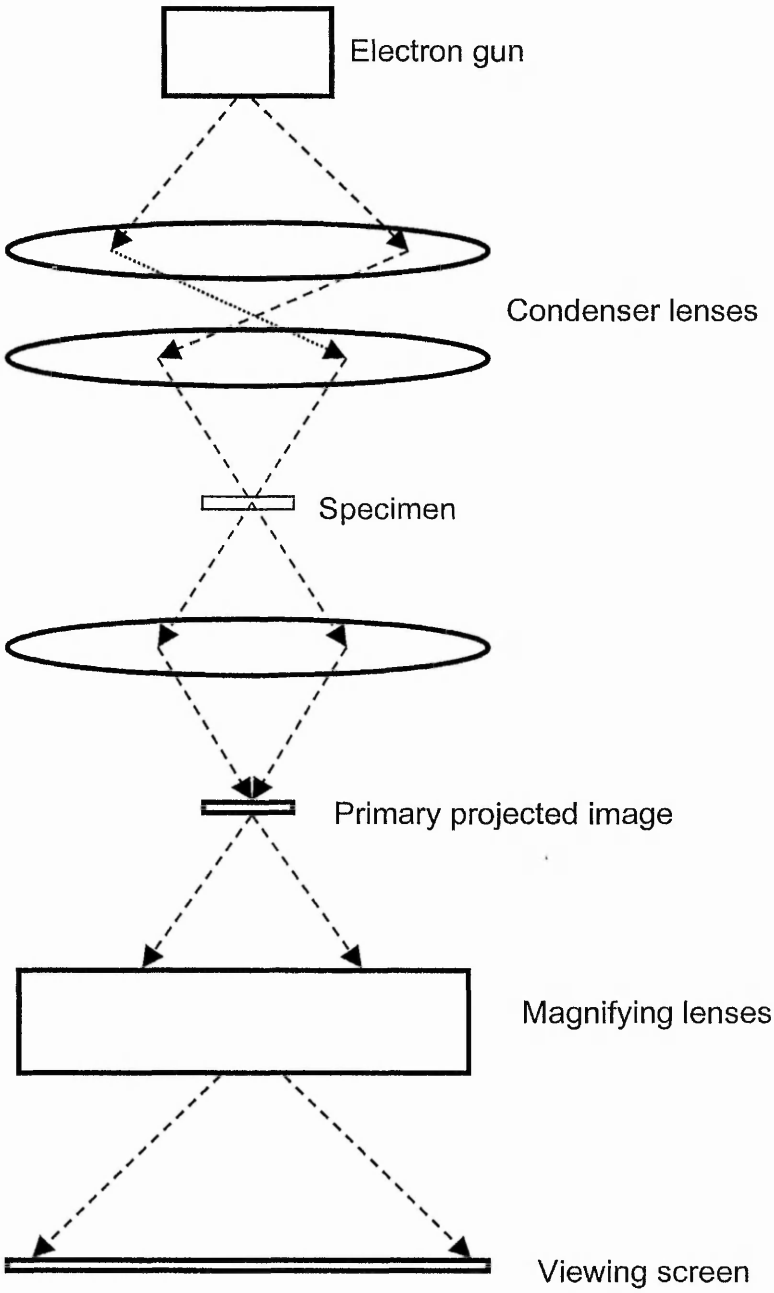


Figure 4.1. Simplified diagram of an electron microscope

4.1.2.1 Specimen Preparation for Electron Microscopy: Before observation by electron microscopy, biological tissue has to be prepared. There are several stages to this process, fixation which kills and preserves the tissue, in-block staining which can be performed to reveal ultrastructural characteristics of the specimen, dehydration to allow penetration of the embedding medium and embedding in which resin infiltrates the tissue and polymerises to enable ultra-thin sections of the sample to be cut. Following this samples can be mounted and stained to further improve the visibility of the ultrastructural detail. Table 4.1 gives details of methods used for preparation of plant tissue including that of potato in investigations by other workers.

4.1.2.2 Fixation: Fixation of the biological specimen must occur as quickly as possible after the tissue has been dissected so that as few changes as possible occur within the tissue. Fixatives preserve tissue by forming cross-links between reactive groups. Non-coagulant fixatives are used to fix biological samples and a common example are the aldehydes e.g. Glutaraldahyde and the additive fixatives e.g. osmium tetroxide and the ruthenium tetroxides.

Glutaraldahyde ($C_5H_8O_2$) is the most effective of the aldehyde fixatives. It quickly stabilises cell structures by cross-linking proteins thereby increasing the density of the tissue (Fig 4.2a). It also increases permeability to embedding media. However, it is unable to stabilise lipids, which will then be extracted during dehydration. Glutaraldahyde is therefore not suitable as the sole method for fixation of plant cell membranes due to its inability to fix phospholipid components of membranes (Carde, 1978).

Osmium tetroxide (OsO_4) is a strong oxidant (Meek, 1976; Roland, 1978). It combines chemically with almost all cellular constituents, attaching firmly to structures and imparting physical density (see Fig 4.2b), stabilising and delineating them (Meek, 1976). It is used as a fixative (of proteins, lipids and lipoproteins), but is also an electron-dense stain and the best known substance for electron contrast. It provides good fixation of

Table 4.1. Methods Used in Preparation of Plant Tissue for use with Electron Microscopy

Plant Tissue	Fixation	Post-Fixation	Dehydration	Embedding	Post-fixative staining	Reference
radish & bean root, oat coleoptiles, tobacco callus, root & stem microbodies	3% glutaraldahyde (v/v), pH 6.8	2% OsO_4 (v/v)	Acetone → propylene oxide	Araldite-Epon	uranyl acetate, lead citrate	Frederick & Newcomb, 1969
potato tuber	6% glutaraldahyde (v/v)	2% OsO_4 (v/v)	acetone	Spurr's	uranyl actetate, lead citrate	Thomson et al.,1995
potato tuber	2% glutaraldahyde (v/v)	1% OsO_4 (v/v)→ 0.5% uranyl acetate (v/v)	ethanol	Spurr's	lead citrate	Turnbull & Cobb, 1992
potato tuber	4% glutaraldahyde (v/v), pH 7.4	2% OsO_4 (v/v)→ 0.5% uranyl acetate (v/v)	ethanol	Epon	uranyl acetate, lead citrate	Ohad et al., 1971
potato tuber, plant storage organs	glutaraldahyde	OsO_4 or chromate/ OsO_4		"Epoxy"	uranyl acetate, lead citrate	Barkhausen, 1978
plant cell membranes	2.5% glutaraldahyde (v/v), pH7.2	1% OsO_4 (v/v)	ethanol	"Epoxy"	uranyl acetate, tannic acid	Carde, 1987 (review)
potato tuber	4% glutaraldahyde (v/v), pH 7.4	1% OsO_4 (v/v)	ethanol	low-viscosity resin	lead citrate, iodine	Sowokinos, et al.,1987

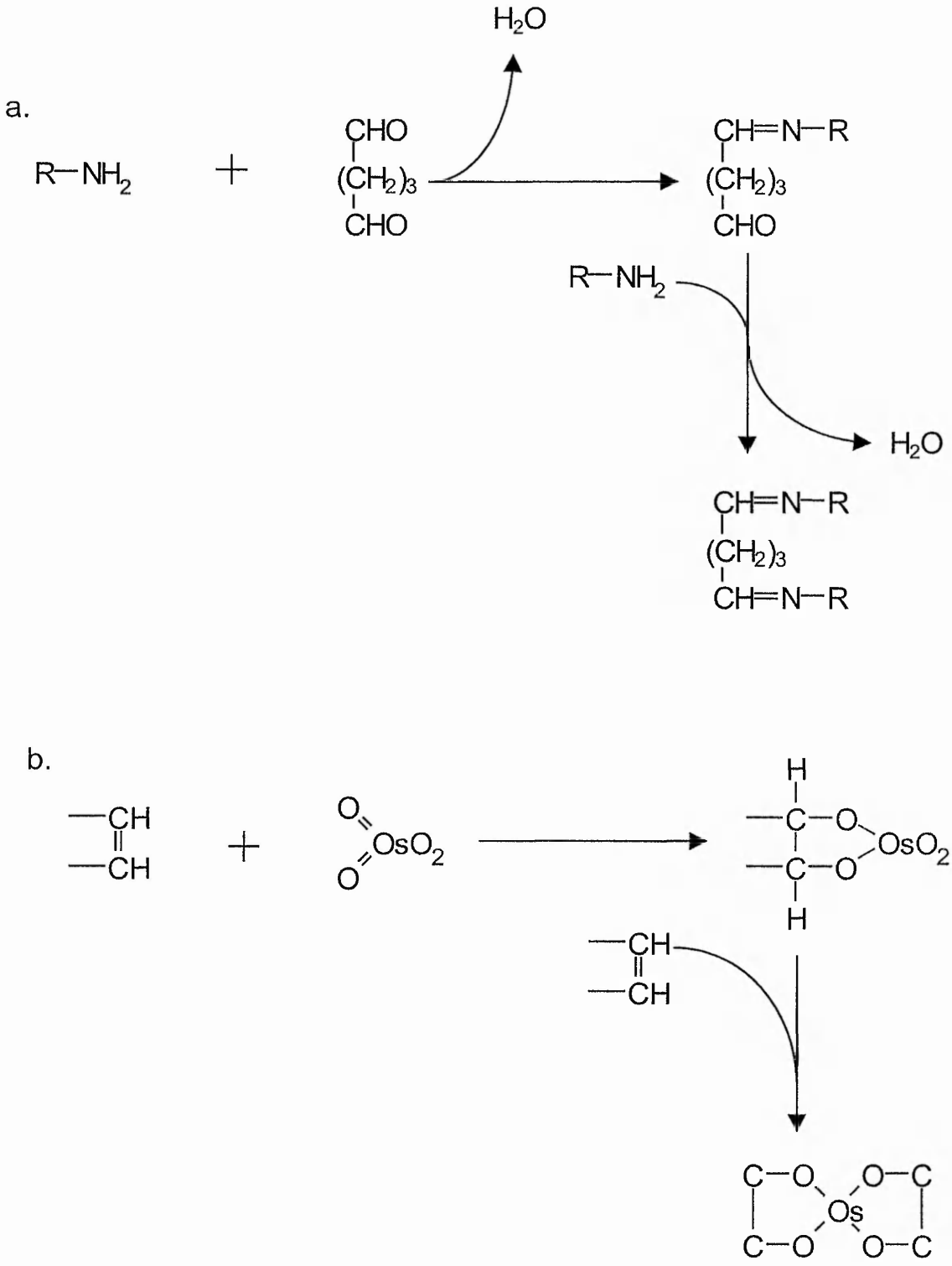


Figure 4.2. a. Cross linking of protein by glutaraldehyde and b. Cross linking with double bonds of unsaturated lipid or protein by osmium tetroxide (From Hall, Flowers and Roberts, 1981).

membranes, reacting strongly with phospholipids due to their numerous unsaturated fatty acids (Roland, 1978). Osmium tetroxide is poor at preserving carbohydrates and nucleic acids.

The most commonly used method of fixation for electron microscopy of biological samples is a combination of glutaraldahyde, used as a pre-fixative and osmium tetroxide (Meek 1976; Hunter, 1993). See Table 4.1. The glutaraldahyde acts quickly, stabilising cell structures. Slower penetration by osmium tetroxide is then used to stabilise lipids and other pre-fixed cellular constituents as well as staining the tissue.

An alternative primary fixative is potassium permanganate ($KMnO_4$) that was used in early studies on plant material and is now less common (Meek, 1976). This penetrates deep into the specimen and preserves phospholipids giving sharp delineation of cell membranes. However, it has unfavourable effects on soluble proteins and nucleic acids within the cell (Carde, 1987) and consequently does not fix ribosomes (Hall, Flowers & Roberts, 1981). The use of potassium ferrocyanide or potassium ferricyanide with osmium tetroxide has also been used as a post-fixative following fixation with glutaraldahyde. This method gives improved fixation of the nuclear envelope, endoplasmic reticulum and plastid membranes including thylakoids according to the mix used (Carde, 1987).

Other fixatives include ruthenium tetroxide (RuO_4), acrolein, (C_3H_40), formaldahyde (HCHO) and uranium salts. These are all less commonly used for electron microscopy of biological samples. In addition to chemical fixation it is also possible to freeze tissue quickly using liquid nitrogen resulting in very few chemical changes within the tissue (Hall et al., 1981). Before tissue can be observed under the electron microscope it must be dehydrated which is done by freeze drying. While the principles behind this methodology are simple, in practice freezing samples is inconvenient resulting in fragile samples, which can undergo freezing damage (Hall et al., 1981). Freeze etching employs the above technique and following freezing the specimen is fractured, revealing the structural composition within the tissue, the tissue is then freeze dried and the surface is coated with a thin layer of heavy metal and carbon vapour. Following this the tissue is thawed

and the replica can be collected and viewed under the electron microscope (Hall et al., 1981). While this method is a good alternative to chemical fixation it does simply provide a morphological representation of the tissue and it is less widely used.

4.1.2.3 In-Block Staining: In-block staining is done to further increase the contrast of the specimen. Heavy-metal ions bind to structures within the specimen impeding the passage of electrons. Uranyl acetate is often used as a stain and this also benefits the preservation of various cellular components e.g. DNA and membranes (Roland, 1978). Iron, bismuth, and potassium permanganate solutions have also been used (Meek, 1976).

4.1.2.4 Dehydration: Removal of water from the tissue is a vital component of tissue preparation since water absorbs electrons and will hence reduce the quality of the sample image. Dehydration takes place in a series of steps (Roland, 1978) with ethanol, methanol, isopropanol or acetone. Ethanol is the preferred dehydrating solution (Carde, 1987) as this does not harden the specimen as others (e.g. acetone) do.

4.1.2.5 Embedding: Early electron microscopists used butyl methacrylate as an embedding medium. This is no longer chosen because the tissue shrinks as the resin polymerises, producing artefacts. The replacement epoxy resins such as Araldite, Epon and Spurrs hardly shrink at all on polymerisation and are now the most commonly used. In plant tissue, epoxy resins provide good preservation of cell structure and enable easy sectioning.

Following infiltration by the embedding medium, the specimen is placed into a mould that is then filled with the resin and heated to give a polymerised block, which can then be sectioned.

4.1.2.6 Sectioning and Mounting Tissue In order for the sample to be observed under the transmission electron microscope it must be cut into sections, typically between 60-150 nm thick. In order to do this, the block is cut using the glass or diamond blade of a microtome. Approximate section thickness is estimated using its colour (see Table 4.2).

Table 4.2. Approximations of section thickness judged by colour of section and possible uses

Colour of Section	Thickness of Section	Uses
Grey	< 60 nm	High resolution work
Silver	60 - 90 nm	Generally used for TEM
Gold	90 - 150 nm	Lower power TEM
Purple	150-190 nm	
Blue	190-240 nm	

Sections are mounted on copper grids coated in formvar or collodion, which provide support.

4.1.2.7 Post-Fixative Staining of Thin Sections: Staining is used to further enhance the contrast of the mounted specimen. This is typically a two stage procedure, firstly with uranyl acetate and then lead citrate (Table 4.1).

4.1.3 Specimen Preparation for Confocal Microscopy

Confocal microscopy is still a relatively new technique. The staining procedures used are the same as those for traditional florescence work. Table 4.3 gives some details of fluorescent stains used for biological material in general.

Very little information is available on compounds suitable for staining cellular constituents. Acridine orange was suggested (Leica-personal communication) to be a good general stain for plant cells due to a differing affinity to various cell constituents and being widely used as a fluorescent dye in histochemistry. Cell walls that are non-lignified stain red and vacuoles may stain red or green according to the acidity of their contents (Lillie, 1977).

Table 4.3 Application and Absorption of fluorochromes commonly used for staining biological material (Molecular Probes, 2000, Sheppard & Shotten, 1997)

Fluorochrome	Absorption max.	Absorption min.	Application
Acridine orange	490	590, 640	General, RNA, DNA (nuclear)
7-Amino-actinomycin D	555	655	DNA stain
BOPIDY phospholipid dyes			cell membranes golgi apparatus
Calcein Lucigenin Rhodamine	495	520	cell viability mitochondria endoplasmic reticulum
Texas Red	592	610	Immunoconjugates

4.1.4 Aims of this Study

There have been few ultrastructural studies of tuber bruising and previous work tends to have concentrated on impact or wounding upon recently harvested tubers without considering how storage might affect the process of melanin formation. In this investigation the ultrastructure of the potato tuber cortical cells of cv Pentland Dell, a variety widely used in the U.K for French fries, has been examined following impact. The aim has been to establish an ultrastructural sequence of events following impact and to characterise the differences in bruising ultrastructure of tubers between harvest and following late storage.

4.2 Materials and Methods

4.2.1 Light and Electron Microscopy Studies

4.2.1.1 Plant Material: Potato tubers of cv. Pentland Dell from ADAS Arthur Rickwood, UK, grown from seed tubers of 0 degree days were harvested, stored at 10 °C, and transported as in Chapters 2.2.1.1 and 2.2.1.2 for ultrastructural investigations during storage season 1995/6 and 1996/7, respectively.

4.2.1.2 Preliminary Investigation 1995/6: After 12 and 24 wks of storage, control and impacted tubers (impacted as in chapter 2.2.6.1) were incubated at 25 °C and sampled after 0, 1, 2, 3 and 6 days for ultrastructural analysis (See Chapter 4.2.1.3).

4.2.1.3 Sample Preparation for Microscopy: For each sample longitudinal cores, 10 mm in diameter, were taken from 5 tubers, the periderm was removed and the basal 5 mm were diced into 1 mm cubes. These cubes were fixed using 2 % aq. (v/v) glutaraldahyde (containing 50mM sodium cacodylate buffer and 200mM sucrose, pH 7.2), washed 3 times in Palades buffer, (pH 7.2) for 15 mins followed by post-fixation in 1 % aq. (v/v) osmium tetroxide (in Palades buffer). Samples were washed 3 times in deionised water for 15 mins before in block staining with 0.5 % aq. (w/v) uranyl acetate for 1 hr. Samples were then dehydrated in an ethanol series (30 %, 70 % and 95 % ethanol each for 5 mins, followed by 2 washes of 100 % ethanol for 10 mins) and incubated in low viscosity Spurr's resin (25 % in ethanol for 30 mins, 50 % in ethanol for 10 hrs, 100 % for 2 hrs) followed by embedding in 100 % Spurr's Resin at 70 °C for 9 hrs. 90 nm thick sections were mounted on 100 mesh size copper grids (coated in 0.5 % formvar resin in chloroform). Mounted sections were stained with saturated solution of aqueous uranyl acetate followed by Reynold's lead citrate (Reynolds, 1963) (both of which had been filtered through 0.2 μm acrodisc filters) before examination with a JEOL 2010 transmission electron microscope (TEM). For each sample of 5 tubers, 3 sections were examined by electron microscopy

for each tuber. Mitochondrial, ribosome and vesicle density were individually assessed and allocated a value between 0 and 4 in which:

0 - none visible

1 - few

2 - moderate

3 - abundant

4 - cytoplasm densely packed

4.2.1.4 Ultrastructure of Bruising Through Storage 1996/7: Tubers were sampled at harvest and 12, 24 and 36 wks in store. Impacted tubers (see Chapter 2.2.6.1) and controls were incubated at 25 °C for 48 hrs prior to analysis. Control tubers at 10 °C were also prepared for ultrastructural analysis (see Chapter 4.2.1.3).

4.2.2 Confocal Microscopy of Bruising in Potato Tubers

Supermarket bought tubers of cv Pentland Dell were incubated at 10 °C and impacted as in Chapter 2.2.6.1. These and control tubers were incubated at 25 °C for 48 hrs. Longitudinal cores, 10 mm in diameter, were taken from 5 impacted and 5 control tubers, the periderm was removed and the basal 5 mm was thinly sliced into approximately 0.2 mm slices. Slices were incubated for 2 mins in a 35 mM solution of acridine orange containing 200 mM sucrose at room temperature. Sections were viewed using a Leica TCS NT confocal microscope equipped with a FITC lens. The samples were excited at 500 nm and emitted wavelengths were detected at 525 nm (green) and 650 nm (red). Five sections were viewed from each tuber and images were recorded digitally.

4.2.3 Statistical Analyses

General linear model was carried out on investigations into changes in mitochondrial number.

4.3 Results

4.3.1 Ultrastructure of a Typical Cell at Harvest

Typical ultrastructural changes were established during storage season 1996/7 when tubers from a full storage season were analysed. At harvest, a typical tuber cell observed by TEM had an intact tonoplast with cell and amyloplast membranes close alongside the cell wall and starch grains respectively (Plate 4.1a). Intracellular organelles were few in number (see Plate 4.1b). Table 4.4 details the changes in organelle numbers and cellular details throughout storage.

Table 4.4. Organelle and vesicle number and membrane integrity (scaled) seen in stored potato tubers between harvest and 36 wks in store. Numbers represent an average taken from 45 cells from a population of 5 tubers. Key: pm - cell membrane, cw – cell wall.

	mitochondria	ribosomes	membrane quality	vesicles
Harvest	1	1	intact, pm close to cw	1
12 weeks	2	1	intact, pm close to cw	1 (along cw)
24 weeks	3	2	intact, pm pulling away from cell wall in areas	2 (along pm and cw
36 weeks	2	3	breakdown, 10 % cells	4

4.3.2 Tuber Ultrastructure During Storage

As storage progressed to 24 wks (Table 4.4), cell and amyloplast membranes became distinct and less closely appressed from the cell wall and the starch grains, respectively (Plate 4.2a). The number of mitochondria increased from the few seen at harvest to becoming abundant following 24 weeks of storage (see Plate 4.2b). The density of ribosomes within the cell was found to increase. Unfortunately statistical analyses were not

Key to Plates

ae = amyloplast membrane
c =cytoplasm
ca = cavity
cw = cell wall
d = dictyosome
m = mitochondria
ml = melanin
n = nucleus
ns = nucleolus
p = peroxisome
pm = plasma membrane
pp = precipitated protein
r = ribosomes
rc = ribosome rich cytoplasm
rer = rough endoplasmic reticulum
sg = starch grain
t = tonoplast
v = vacuole
vs =vesicle

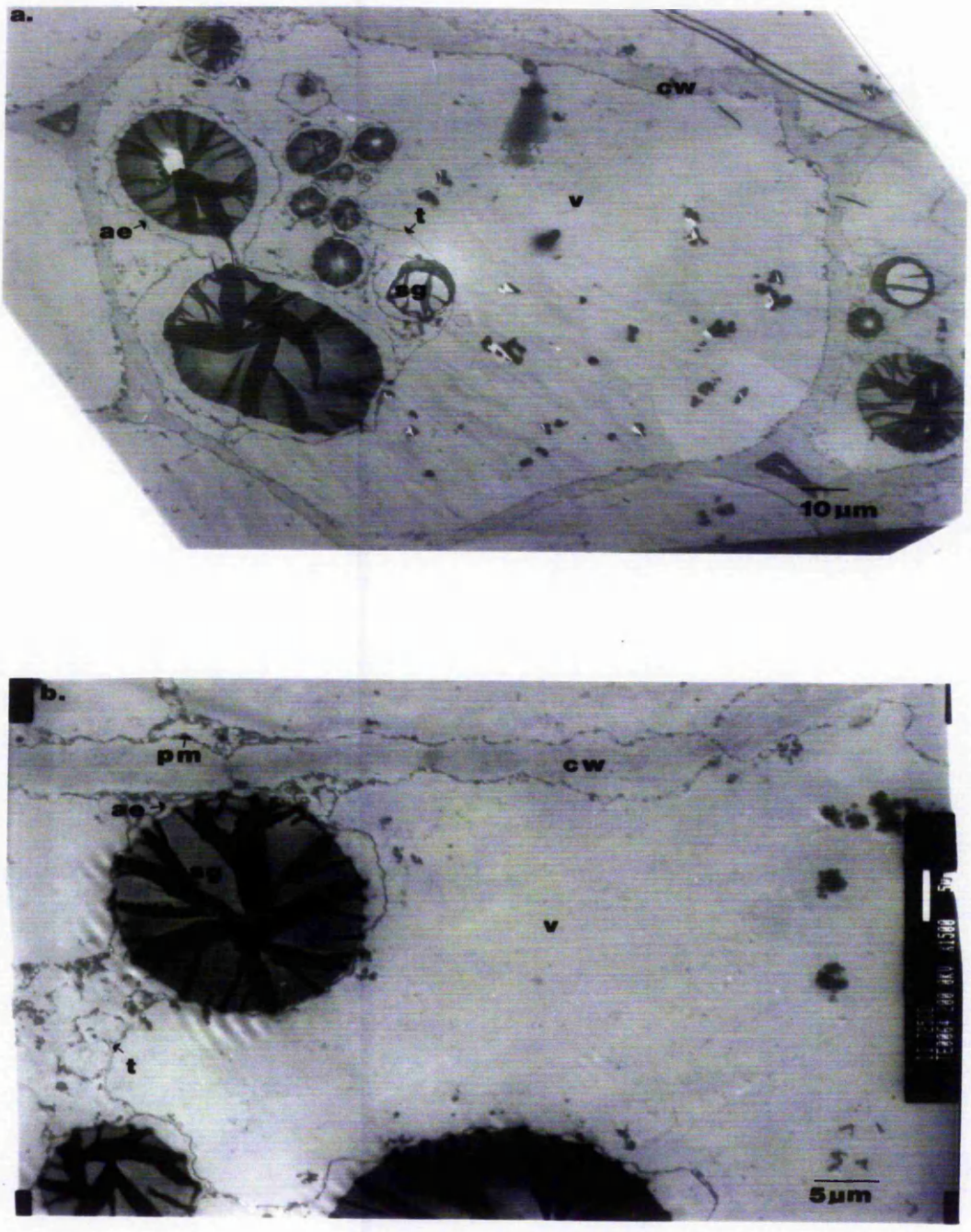


Plate 4.1. A typical tuber cell from harvest, R value = 1. a, Entire cell with an intact tonoplast (x 700). b, Intracellular detail, amyloplast envelope and plasma membrane are close alongside starch grain and cell wall respectively. Organelles are few in number (x1500).

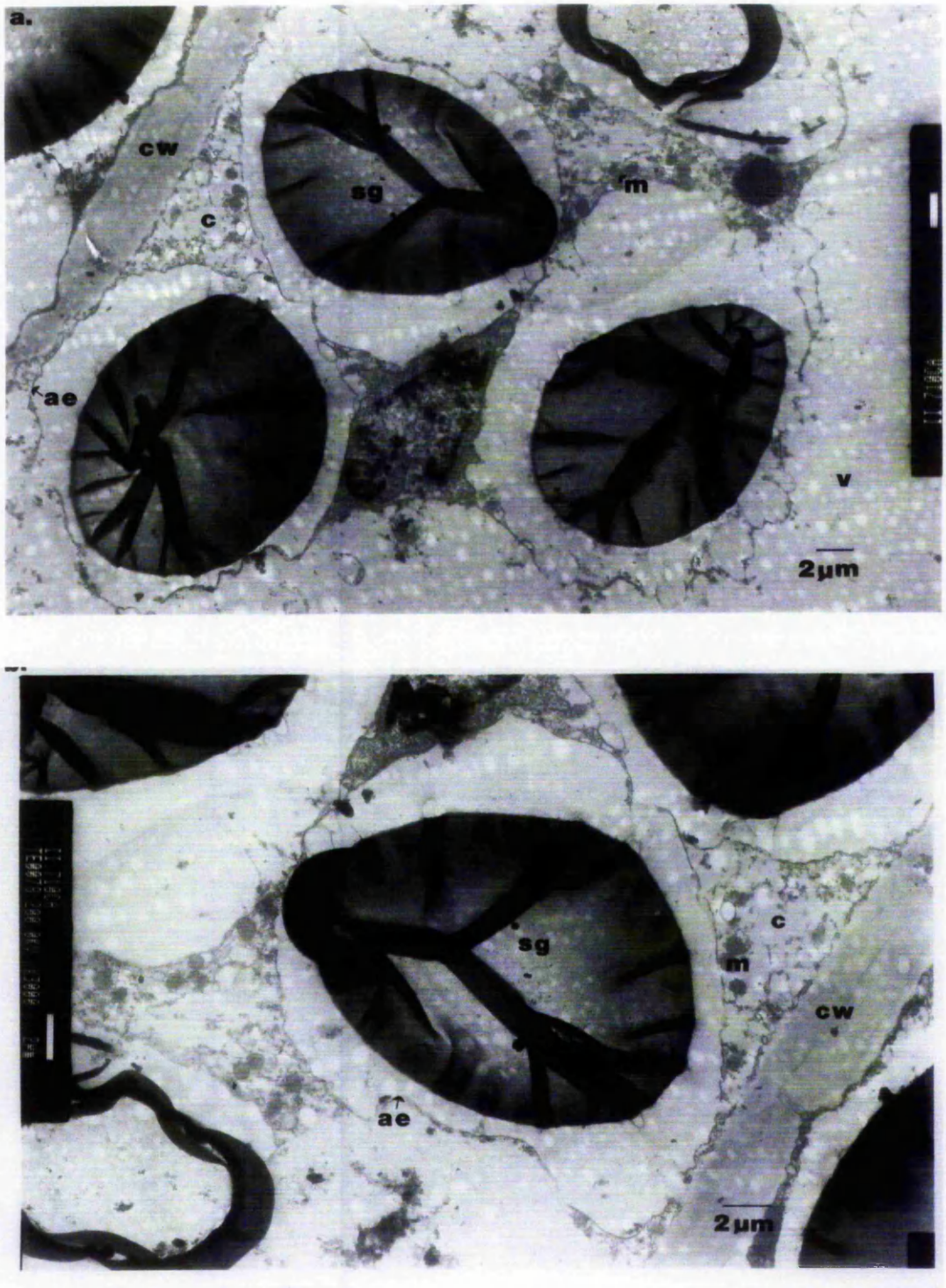


Plate 4.2. Tuber cell following 24 wks in store, R value = 2. a, Cell and amyloplast membranes pulling away from the cell wall and starch grains respectively (x 2000). b, High power micrograph showing an increased number of organelles (x 3000).

performed on this data due to its qualitative nature. This was noted after 18 wks in store in storage season 1995/6. Following 36 wks in store, the cytoplasm darkened in approximately 30% of cells observed (Plate 4.3a). However no further significant change in organelle numbers were seen.

In approximately 10% of sections examined there was a loss of membrane integrity due to disruption of the tonoplast in tubers taken from late storage (36 wks). See Plate 4.3b.

4.3.3 Ultrastructural stages in blackspot bruise formation

Results from the preliminary investigation in which tubers were impacted after 24 wks in store indicated that there were four ultrastructural stages in melanin formation (Plate 4.4a). After 1-2 days some intracellular breakdown was observed, in particular in the region surrounding the starch grains (Plate 4.4b). The numbers of mitochondria and ribosomes (both as polysomes and rough endoplasmic reticulum increased (Table 4.5) from moderate numbers prior to impact to organelles becoming abundant 1-2 days after impact. (Plate 4.5a). Three days after impact the cytoplasm adjacent to the cell wall and surrounding the starch grains became increasingly granular compared with controls (Plate 4.7a). However organelles were still visible and the density of ribosomes within the cytoplasm had increased (Plate 4.5b). After 6 days the ribosomes and organelles were in general no longer visible and the cytoplasm consisted of a dark amorphous layer (Plate 4.6a). Individual membranes were not visible where this dark discolouration was present, although the discolouration was delimited by what must once have been the cell membrane and amyloplast envelopes (Plate 4.6b). This can be compared with controls in which no dark discolouration was seen within the cells (Plate 4.7b).

Tuber cortical parenchyma cells showed no obvious cell wall damage.

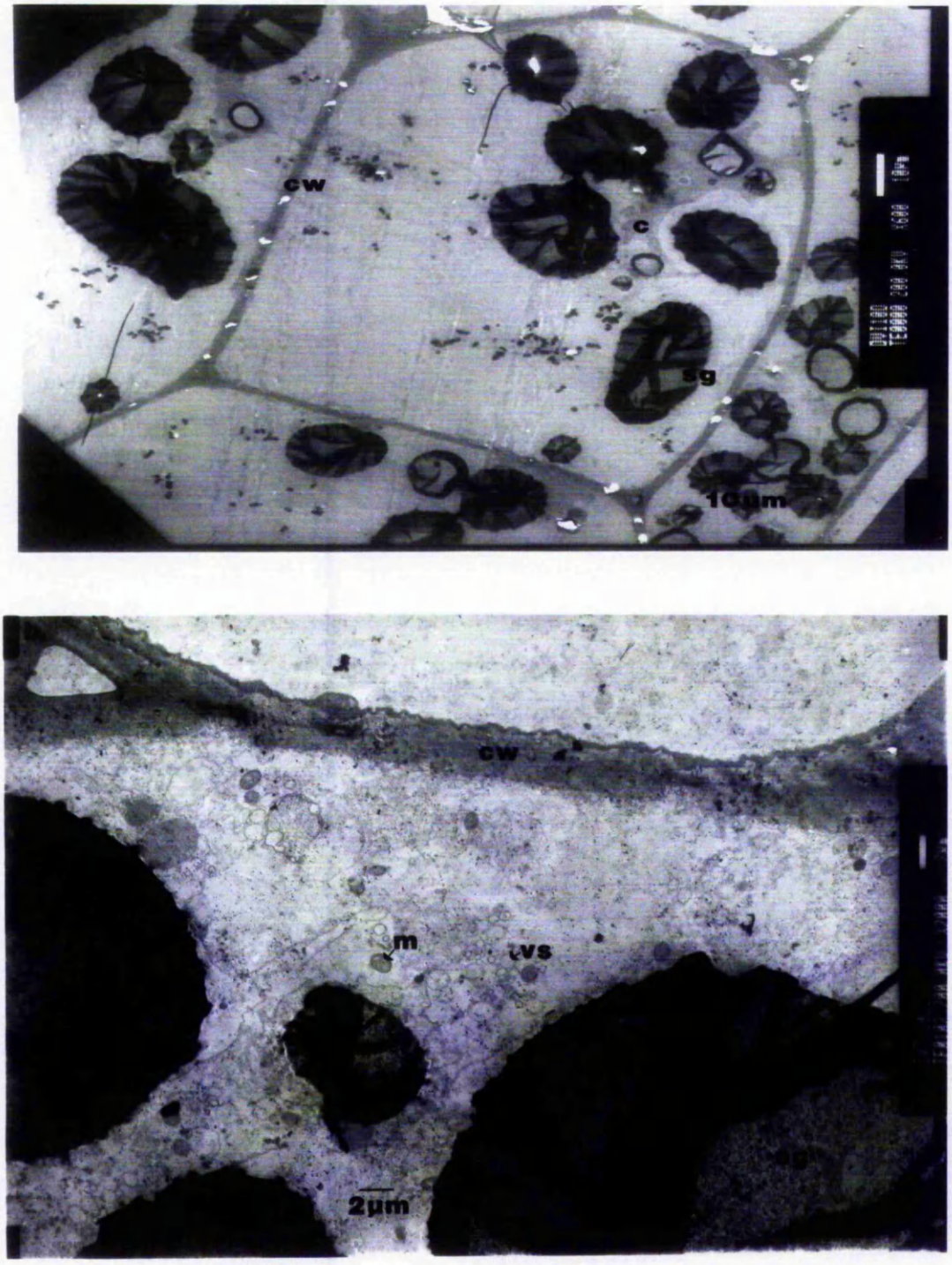


Plate 4.3. Tuber cells following 36 wks storage, R value = 3. a, The cell cytoplasm has darkened (x 600). b, The intracellular structure of the cell has become disrupted (x 2000).

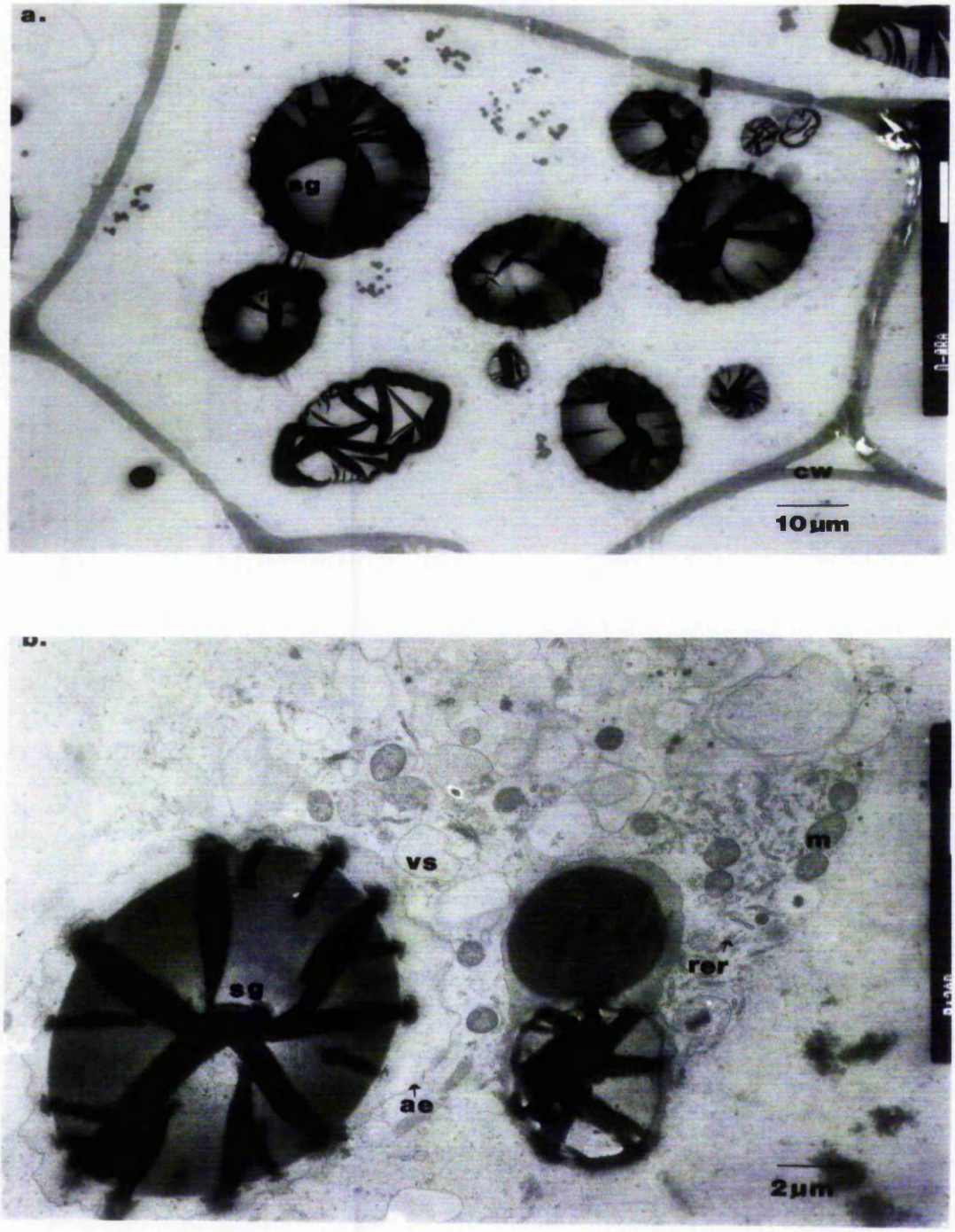


Plate 4.4. Stages in melanin formation. a, Control tuber cell prior to impact, R value = 2 (x 800). b, Partial collapse of intracellular structure of following impact, R value = 3 (x 4000).

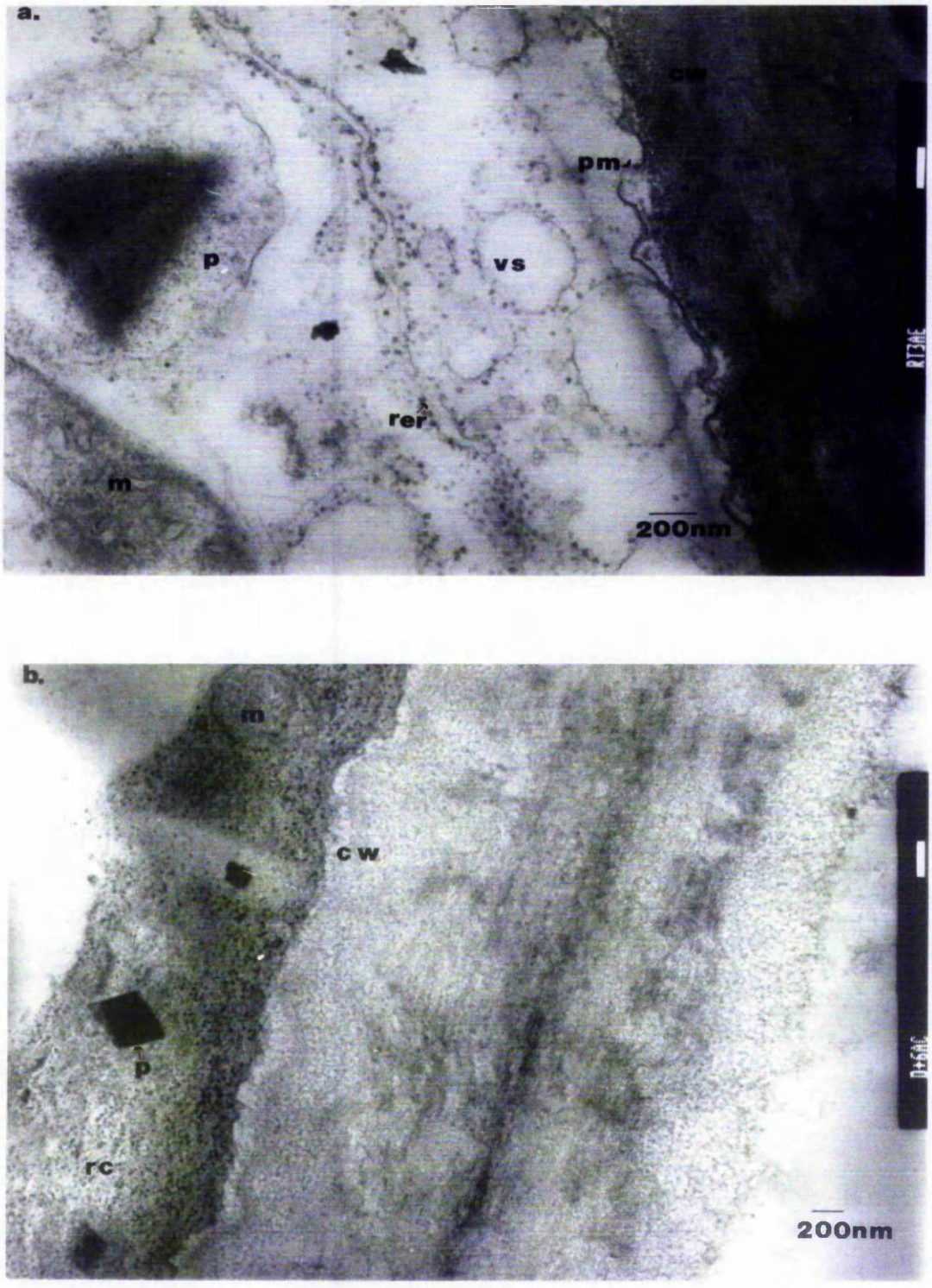


Plate 4.5. Stages in melanin formation. a, Increased mitochondrial and ribosomal abundance, R value = 3 (x 25 000). b, Darkening of the cytoplasm adjacent to the cell membrane and surrounding the starch grains, R value = 4 (x 20 000).

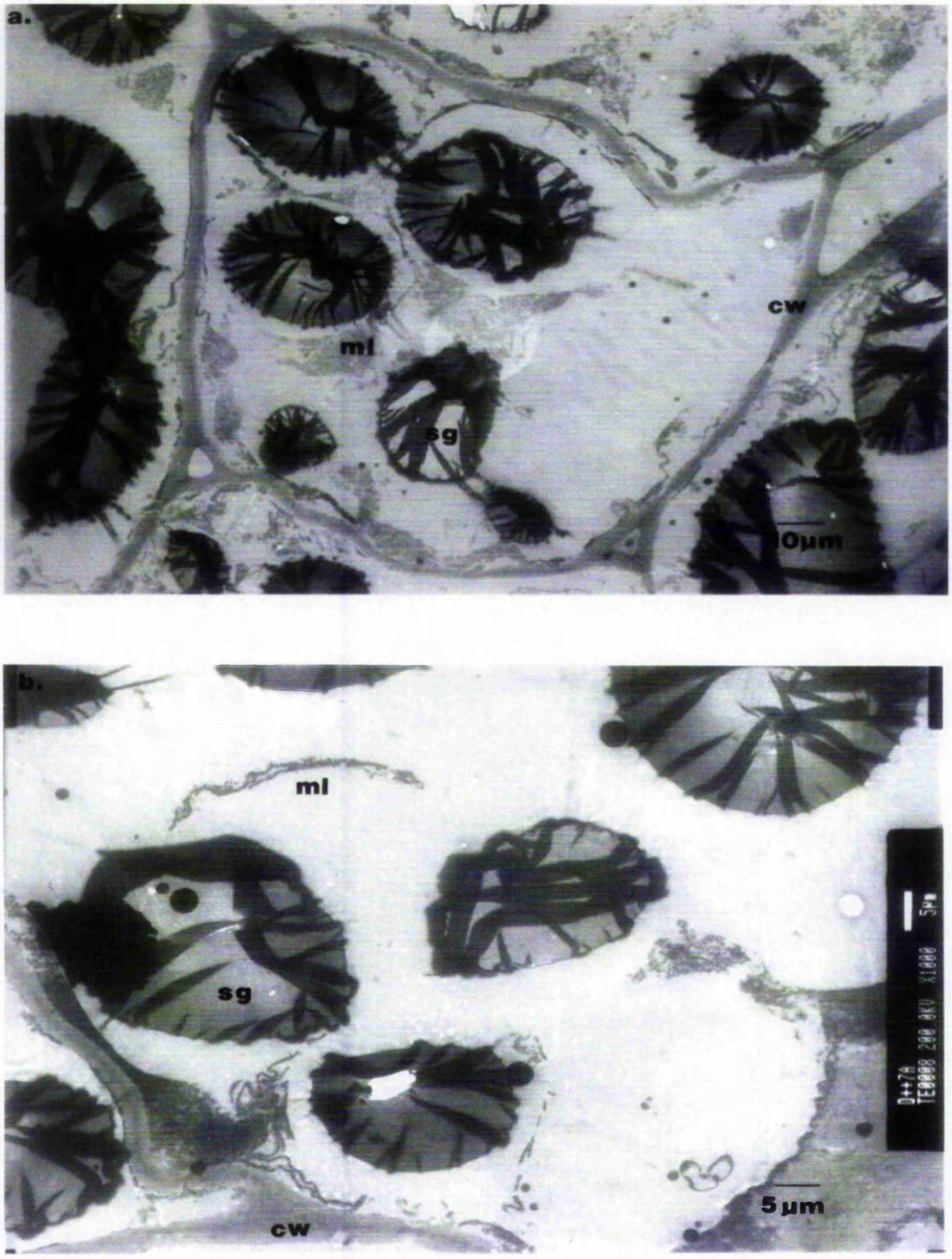


Plate 4.6. Final stage in the formation of melanin, R value. a, Melanin formation in bruised tuber cell (x 600). b, Tuber cell showing melanin formation delineated by membranes (x 1000).

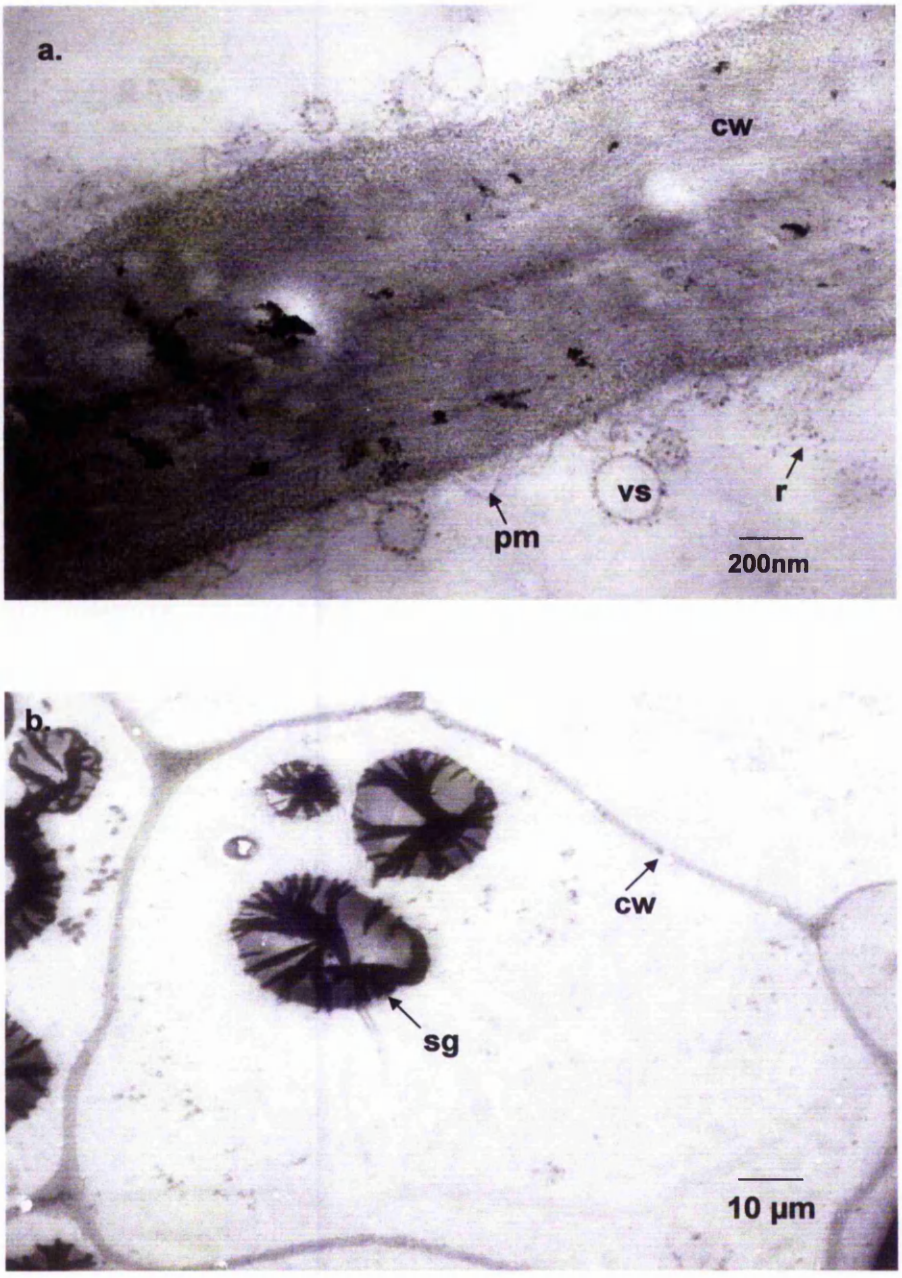


Plate 4.7. Stages in melanin formation. a, Control section from tuber incubated for three days at 25°C showing an area alongside the cell wall, R value = 2 (x 20 000). b, Control section showing an entire cell following incubation for 6 days at 25°C (x 800).

Table 4.5. Organelle numbers per cell (scaled) and cytoplasmic discolouration in potato tubers following impact after 24 wks in store. Numbers represent means taken from 45 cells from a population of 5 tubers.

	mitochondria	ribosomes	% cells with discolouration
Control	2	2	0
1 day	3	3	0
2 days	3	3	65%
3 days	3	4	92%
6 days	0	0	96%

4.3.4 Bruising ultrastructure through storage

During the 1995/6 storage season preliminary impact studies 12 and 24 wks into storage indicated that tubers during early storage were less susceptible to blackspot bruising when impacted, compared with tubers impacted after 24 wks in storage. Impact with 0.7 J energy after 12 wks in storage did not result in any visible bruise formation and ultrastructural studies revealed no melanin. However, an increase in abundance of ribosomes and mitochondria was observed 1-2 days after impact along the periphery of the cell and surrounding the starch grains (Plate 4.8a).

This was also seen in tubers as an early stage in bruise formation after impact following 24 wks in storage. However, these tubers did develop blackspot, which tended to be restricted to the vascular region of the tuber. This was observed as a dark amorphous layer as noted in 4.3.3.

Occasionally dictyosomes were observed in impacted tissue throughout the storage period (Plate 4.8b). Whilst these were never observed in control tissue or in samples looked at for changes in ultrastructure through storage, the infrequency of their occurrence in impacted tubers meant that this was not found to be significant ($P>0.05$).

With occasional exceptions melanin was only present in cell sections containing starch grains, in cells sections that had not cut through any starch grains, no melanin was visible (Plate 4.8c).

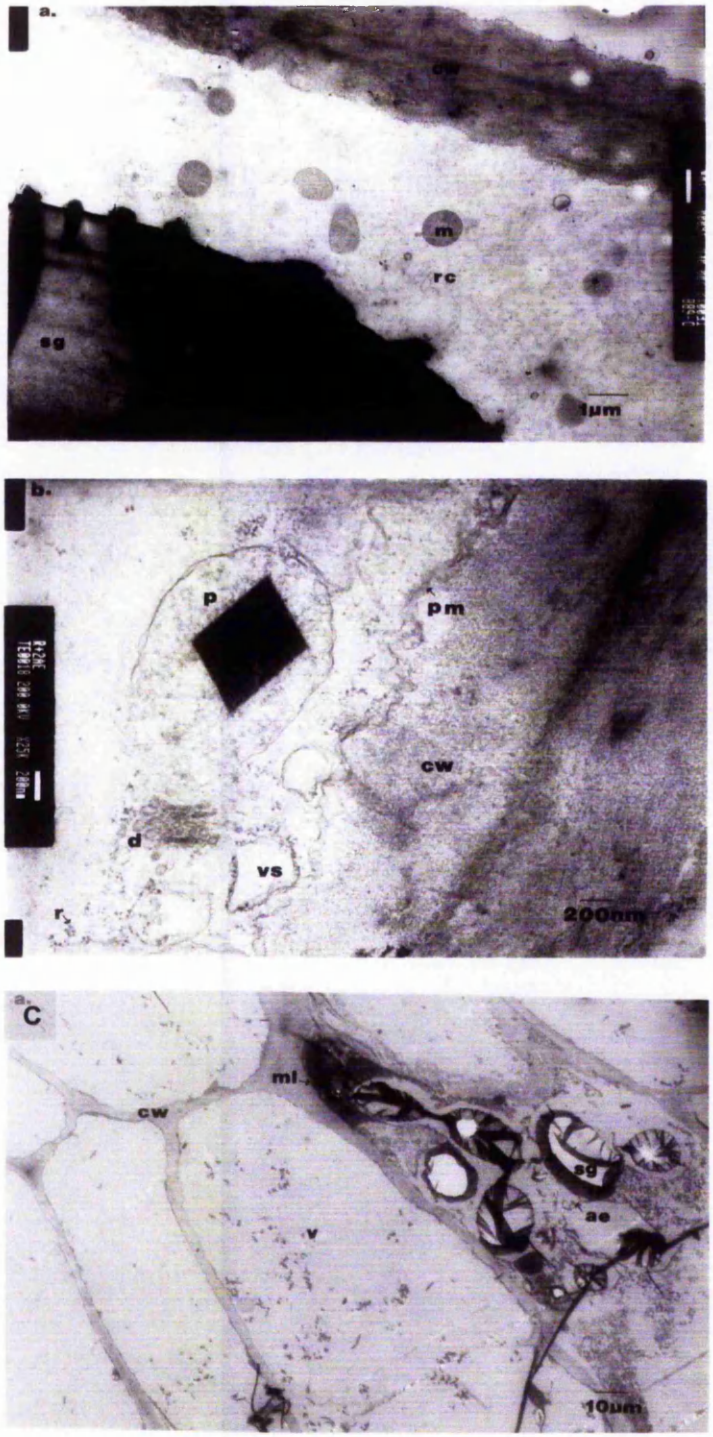


Plate 4.8. Tuber cells following impact. a, Tuber after 12 weeks in store (tuber did not bruise), increased abundance of ribosomes and mitochondria, R value = 3 (x 6000). b, Dictyosome observed in cell 24 weeks into storage, R value = 2 (x 25 000). c, Section through an impacted tuber demonstrating how melanin forms around amyloplasts and not in the absence of amyloplasts (x 400).

Table 4.6. Organelle numbers per cell (scaled) in stored potato tubers 2 days after impact. Numbers represent means taken from 45 cells from a population of 5 tubers.

	mitochondria	ribosomes	% cells with discolouration
Harvest	4	2	36
12 wks	3	3	13
24 wks	2	2	72
36 wks	1	1	89

4.3.4.1 Observations on Shatter Bruise Verses Blackspot Bruise Development: Ultrastructural studies revealed that tubers from storage season 1996/7 developed microscopic shatter bruise upon impact at harvest rather than blackspot. Impact at harvest resulted in cracks in the periderm and fissures throughout the tissue in occasional tubers, which were discarded. Ultrastructural analysis of impacted tissue, which had not obviously shattered, revealed several differences in the bruising ultrastructure when compared with tubers 12-36 wks in store exhibiting typical blackspot bruise. Complete or partial destruction of the tonoplast was seen in tuber cells from harvest (Plate 4.9a) that had been replaced by a large number of vesicles. Deposits, stained black, were present in the majority of these vesicles and are presumed to be precipitated protein (Plate 4.9b). Vesicles seen in tubers affected by blackspot only rarely contained these deposits. Whilst numerous mitochondria were seen in shatter bruised tissue, there were less ribosomes than is typically seen with blackspot (Plate 4.9b). In addition, the nuclei of tubers from tissue exhibiting microscopic shatter were highly invaginated and surrounded by numerous membrane bound vesicles (Plate 4.10a), compared with both those from tubers exhibiting blackspot (Plate 4.10b) and unimpacted control tubers at harvest (Plate 4.10c).

In occasional tubers which had undergone severe damage following impact at harvest cells contained cavities within them and intracellular discolouration (Plate 4.11a) was very extensive. This can be compared with

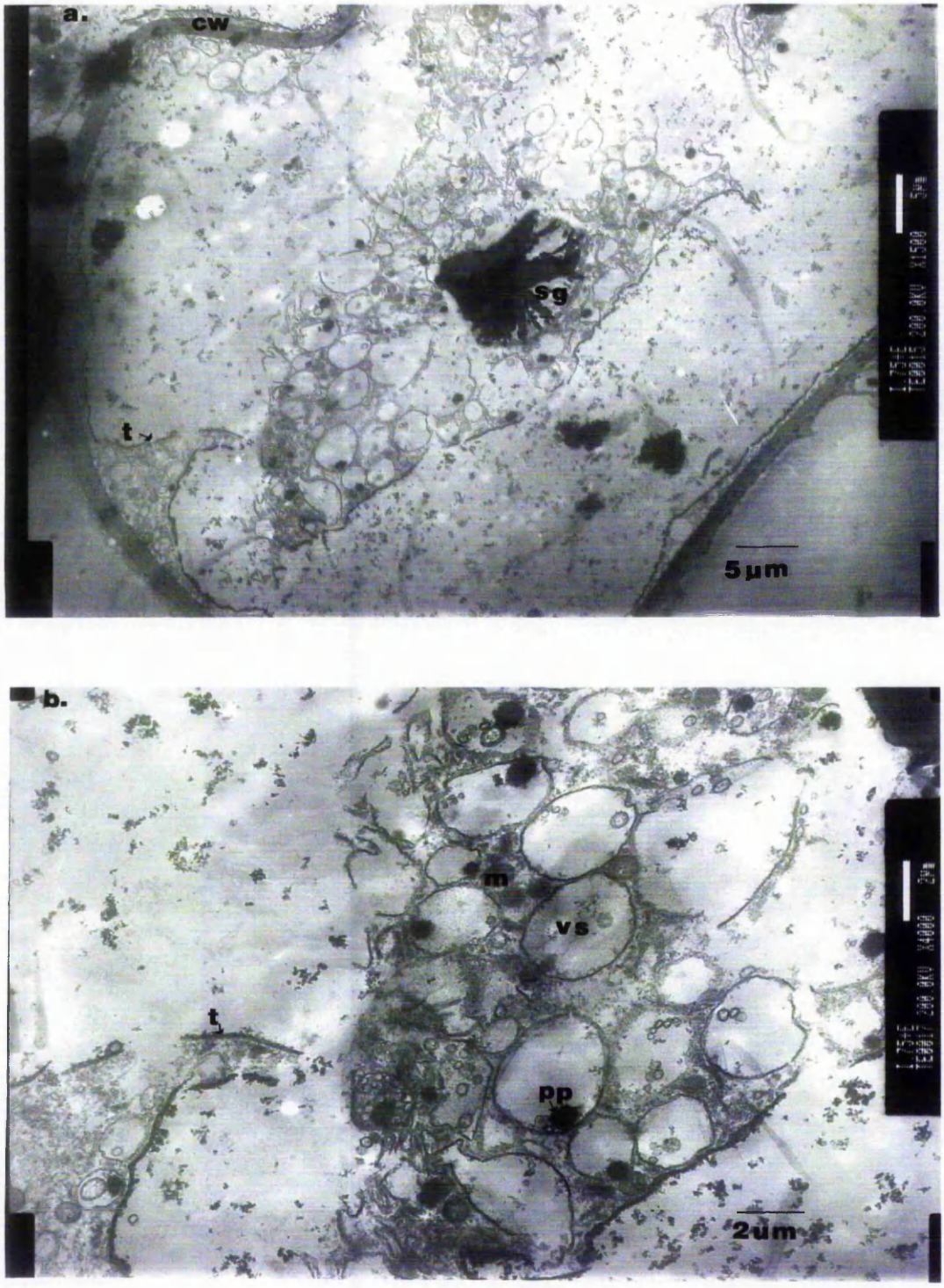


Plate 4.9. Shatter bruised cell following impact at harvest R value = 2. a, Entire cell showing the disrupted tonoplast, (x 1500). b, high power micrograph showing protein precipitation within numerous vesicles and many mitochondria (x 4000).

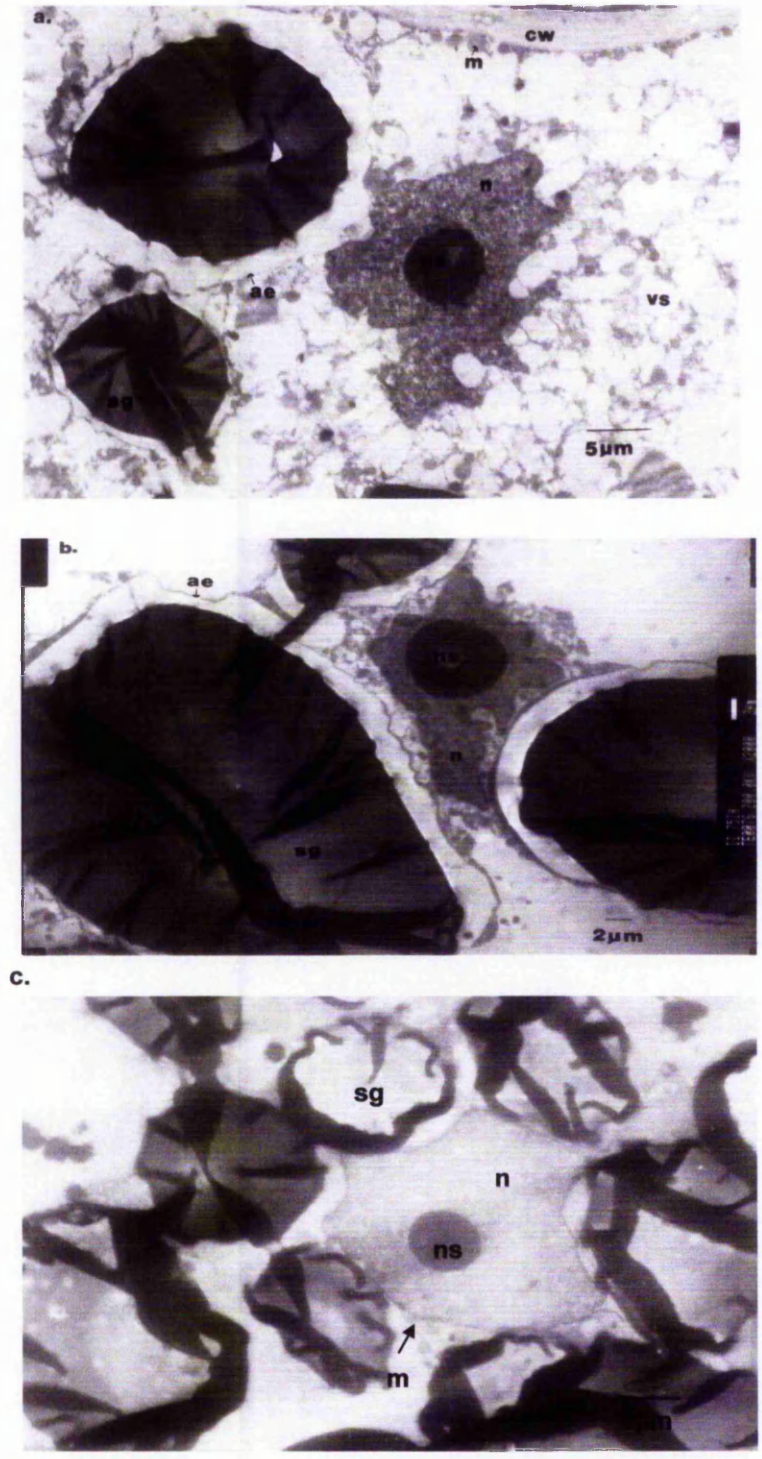


Plate 4.10. Nuclei of impacted and control tuber cells. a, Cell showing microscopic shatter following impact at harvest. Nuclear envelope is invaginated and surrounded by vesicles R value = 2 (x 2000). b, Tuber cell following impact after 12 weeks in store, surrounded by few vesicles, R value = 2 (x 2000). c, Nucleus of control cell at harvest, R value = 2 (x 2000).

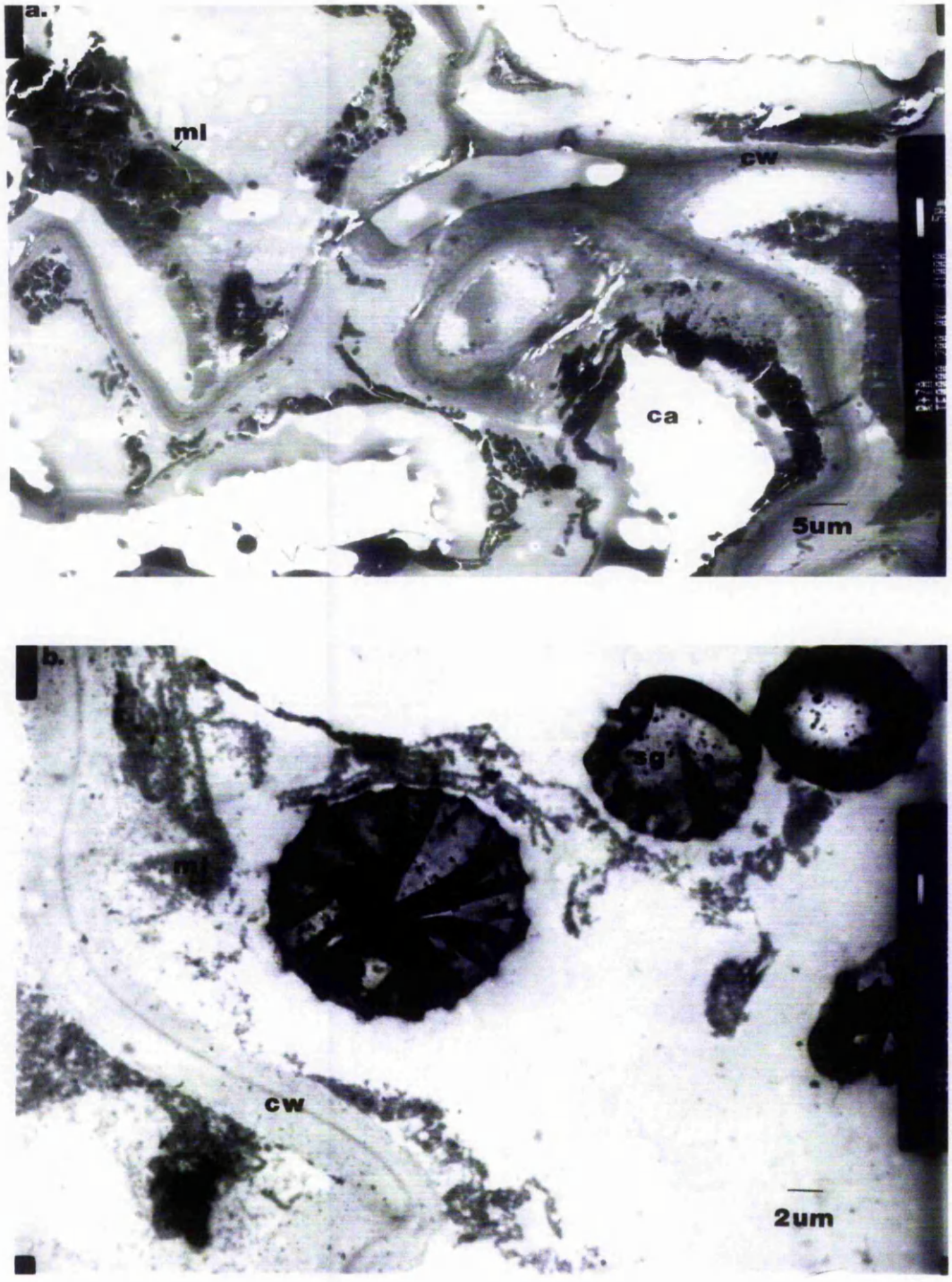


Plate 4.11. Tuber cells following impact, R value = 0. a, Microscopic shatter following impact at harvest has resulted in cavities within the cell and a large area of melanin formation (x 1000). b, Blackspot bruised cell showing melanin formation along the cell wall and surrounding the amyloplasts. No cavities had formed (x 2000).

tubers impacted after 12 wks in storage that had undergone little discolouration (Plate 4.11b).

Table 4.7. Comparison of tuber cell ultrastructure in potatoes impacted at harvest and those impacted following storage. Results indicate the typical cell

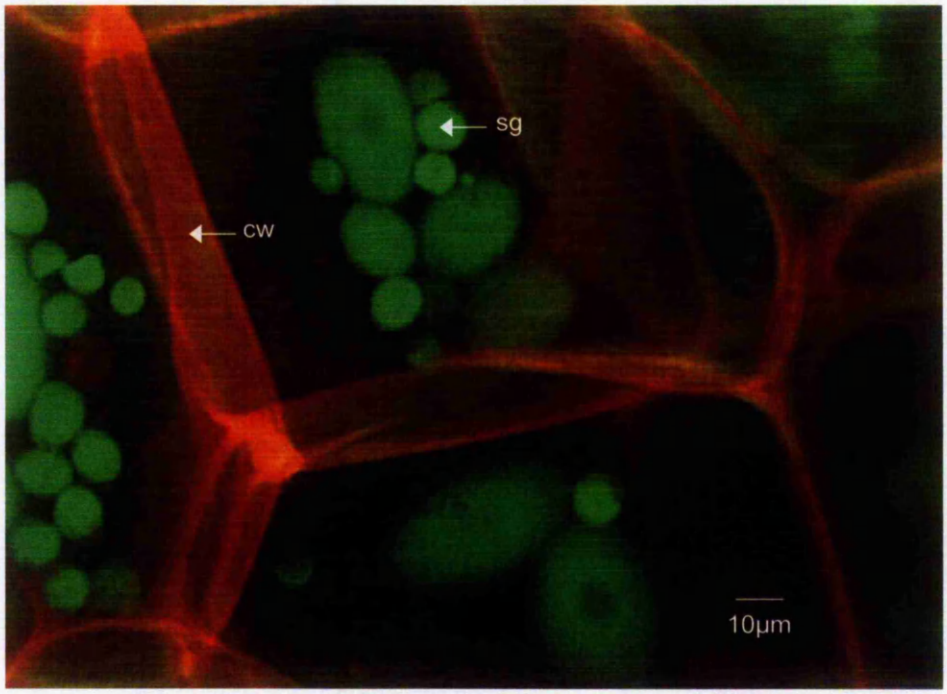
Character	Impact at Harvest	Impact following storage
Mitochondrial abundance	Increased	Increased
Ribosomes abundance	No change	Increased
Vacuole integrity	Shattered	Intact
Vesicles appearance	Many containing deposits	Few rarely containing deposits
Nuclear envelope	Invaginated	Not invagingated
Melanin formation	Present	Present later in storage

4.3.5 Confocal Microscopy of Bruised Potato Tissue

Plate 4.12a shows an image produced from 3 sections 10 nm apart which have been overlaid of a control tuber cell that has not undergone impact. It can be seen that tuber cell walls fluoresce red whilst tuber starch grains fluoresce green following staining with acridine orange. No other intracellular components were visible in control tuber cells. Plate 4.12b is also an image produced from 3 sections 10 nm apart, which have been overlaid, in this case the tuber cells were those from an impacted tuber. It can be seen that in addition to the cell wall and starch grains seen fluorescing in control tissue there was also some red fluorescing discolouration within the plant cells. The location of this discolouration is difficult to pinpoint accurately due to the depth of the image.

Plate 4.13a shows a control tuber section in which emitted fluorescence was only detected at 650 nm and therefore is only picking up red light. Both cell walls and starch grains fluoresced red. It can be seen that the cell walls were straight compared with those of impacted cells (Plate 4.13b), which appeared distorted and even broken.

a.



b.

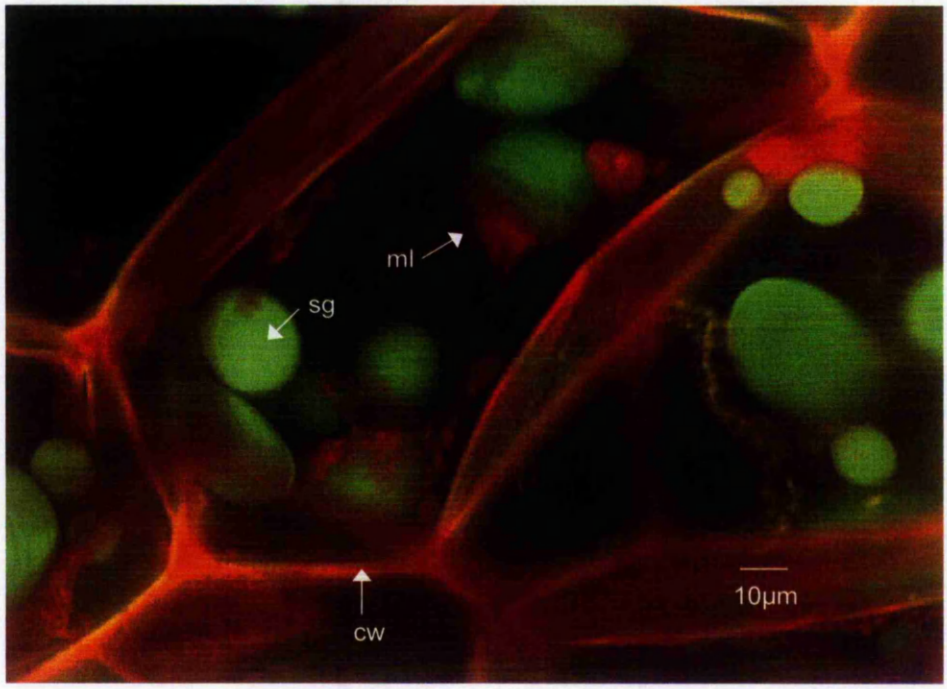


Plate 4.12. Confocal images of potato tuber cells produced by overlaying 3 images at 10nm intervals a. Control cells showing cell wall and starch grains b. Impacted tuber cells showing cell walls, starch grains and red discolouration.

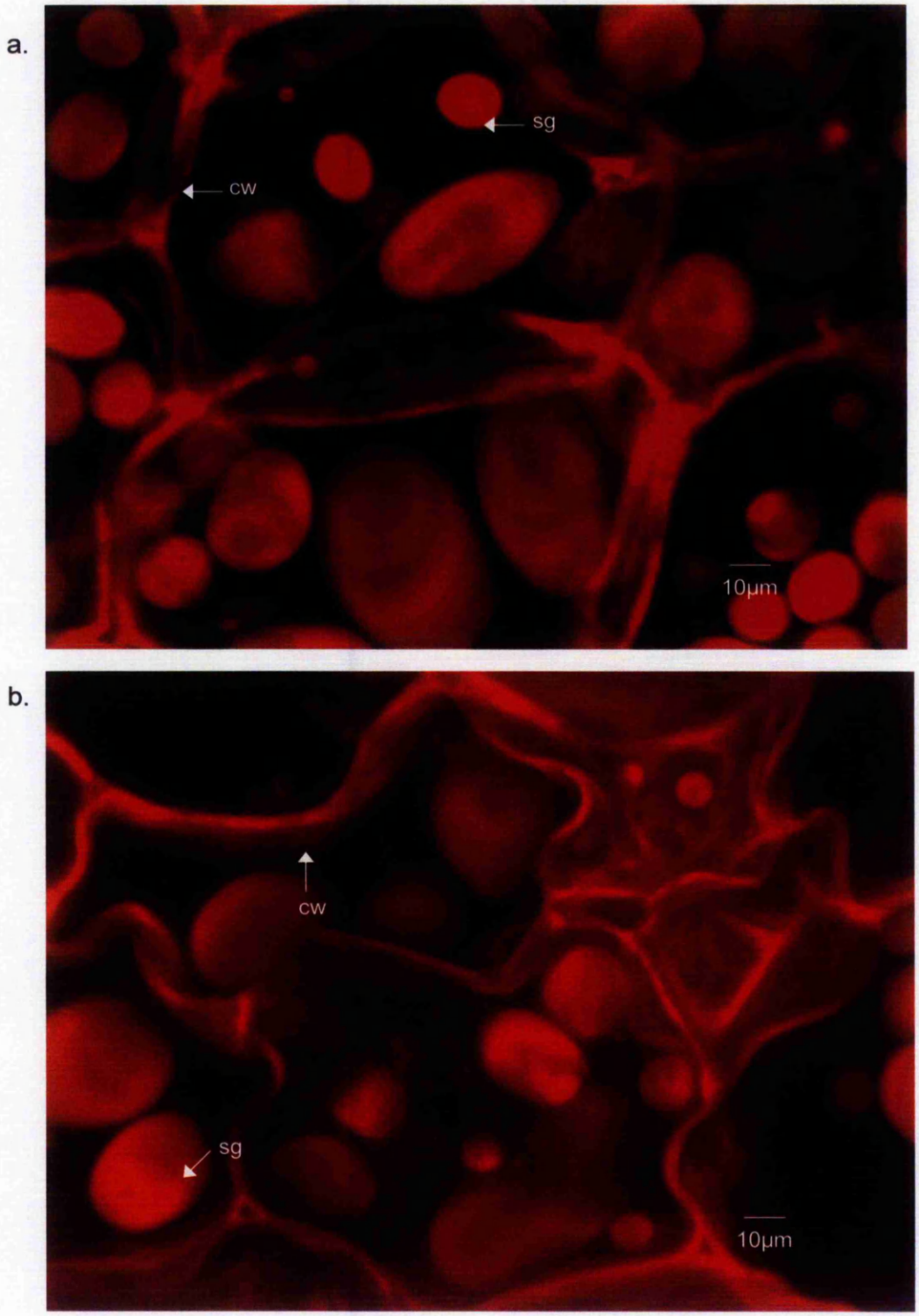
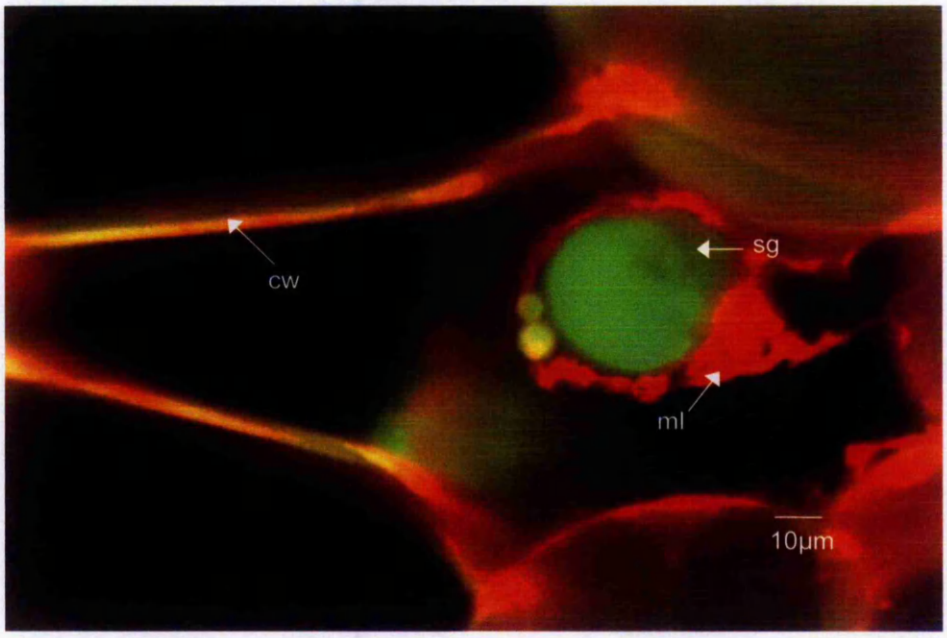


Plate 4.13. Confocal images of potato tuber cells produced by detecting only light emitted at 650nm a. Control cells showing cell wall and starch grains b. Impacted tuber cells showing convoluted cell walls and starch grains.

Plate 4.14a and b are both images of impacted tissue. Plate 4.14a demonstrates that the discolouration formed around the starch grain. Cytoplasmic strands are visible which implies that the discolouration formed from within the cytoplasm. This image also indicated that in the absence of starch grains little discolouration formed. In contrast Plate 4.14b shows a tuber section containing numerous starch grains and subsequently an increase in the red discolouration is seen, not always around starch grains but along the walls of the tuber cells.

a.



b.

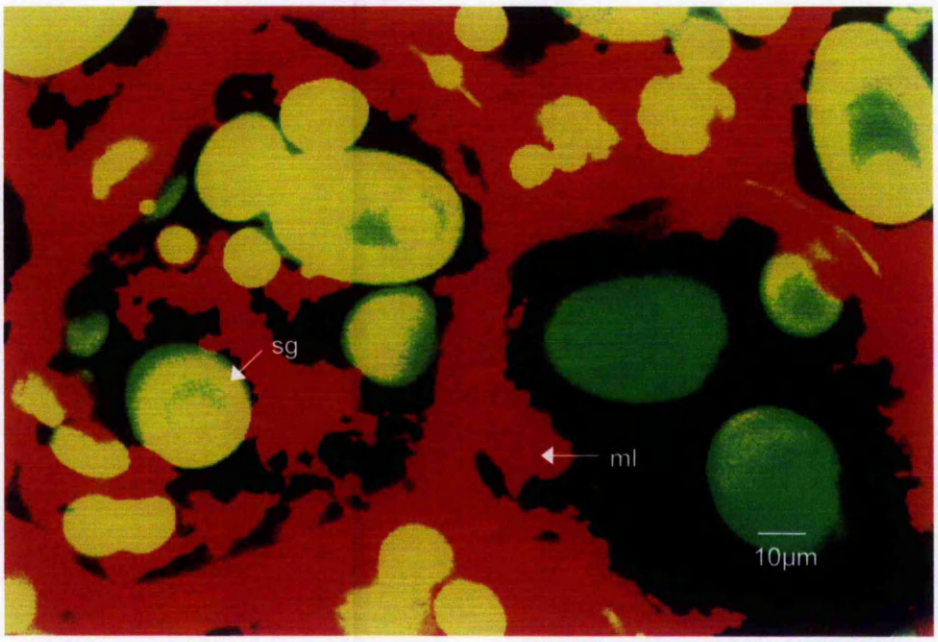


Plate 4.14. Confocal images of cells from impacted potato tubers a. Melanin formation surrounding a starch grain b. Cells containing large deposits of melanin

4.4 Discussion

Dissection of tuber tissue into 1mm cubes prior to fixation was necessary to allow penetration of osmium tetroxide into the centre of the section. However, assuming an average cell diameter of 200μm, there would only be approximately 25 cells per cube. Cutting damage would affect the outer layer of cells and the adjacent layer, which are adjoined by plasmodesmata may also be affected leaving only one central cell that is unaffected by cutting damage. This might suggest that some of the damage seen in examined sections is a result of cutting damage rather than as a result of bruising. However, the use of controls indicated that the damage seen was a result of impact as control tissue did not display the same characteristics.

A further criticism of the methodology is that the area of tissue sectioned was taken over a 5 mm depth, variation in cell size (and therefore response to impact) will occur over this distance as the cells increase in size from the basal end of the tuber. This will increase the variation seen within results.

4.4.1 Tuber Ultrastructure During Storage

Studies using electron microscopy, suggest that the tissue was losing water during storage as there was some loss of membrane integrity (Chapter 4.3.2). In addition, the cell membrane became distinct from the cell wall as the tissue became more flaccid. This supports the work of Turnbull and Cobb (1992) in which studies of tubers through storage showed a breakdown of the tonoplast during mid storage.

An increase in ribosomes and mitochondria within the tissue after 18 weeks in storage (storage season, 1995/6) indicated that changes are taking place in tubers. These may be associated with a general increase in the metabolic rate as the tubers come out of their dormant phase and begin sprout growth development (Lisinska & Leszczynski, 1989). Espen, Morgutti & Cocucci, (1999) report that changes in the protein composition occurs within tubers during storage, such changes might be indicated by an increase in ribosome numbers prior to protein formation. The cytoplasmic darkening seen in approximately 10% of cells late in storage may indicate a

variety of things. Firstly there may have a been a change in the cytoplasmic constitution, perhaps in preparation for sprout initiation. Alternatively, a reduction in membrane integrity may have allowed components of the cells normally compartmented within cellular organelles to leach into the cytoplasm and react together.

4.4.2 The formation of Melanin in Blackspot Bruised Tissue

Melanin formation took place over several days and four distinct stages could be identified (chapter 4.3.4). Following impact intracellular breakdown was noted around the starch grains and an increase in the number of mitochondria and ribosomes was observed. This was followed by a darkening of the cytoplasm adjacent to the cell wall and surrounding the starch grains, which became increasingly darker, embedding all of the organelles within it. This was presumed to be melanin.

This is in agreement with the work of Reeve et al. (1968), working with the cultivars Russet Burbank and White Rose, observed using light microscopy that the cytoplasm became dark and granular when tissues were bruised. No obvious structural damage was noted and melanin was identified as forming on intracellular surfaces of protoplasts and inner cell walls. A later study by Barkhausen (1978), which examined wound healing in plant storage tissues, including bruising in potato tubers, found that the abundance of rough endoplasmic reticulum and ribosomes increased dramatically after wounding. The increase in ribosomes began 4 hours after wounding, the time taken for the association of ribosomal subunits within the cells. Ishizuka & Imaseki (1990) found that wounding resulted in increased ribosome and polysome formation for up to 48 hrs after damage following an increase in the synthesis of rRNA within the nucleolus. Ribosomal RNA is transported from the nucleus into the cytoplasm within protective proteins to prevent damage by nucleases (Kahl & Weilgat, 1976) It was found that this was often associated with mitochondrial division. This suggests a common requirement for increased protein synthesis involved in wound healing in general and is not specific to bruising. Wound-induced synthesis of proteins, such as ubiquitin (Garbarino, Rockhold & Belknap, 1992) and phenylalanine

ammonia lyase associated with bruising and cell damage, have also been reported by Belknap and Rickey, (1990) and Belknap et al. (1990).

In these early investigations a breakdown of the intracellular structure was not noted as a feature of bruise formation. McIlroy (1980) suggested that while the cytoplasm of bruised tissue became brown compared with that of undamaged tissue, the main contributor to the dark colouration of the bruised tissue was melanin granules 0.5-1.5 μm in diameter, which, he claimed, were derived from peroxisomes. During this investigation no such structures were seen. Moreover, where tissue was obviously damaged, such that the cytoplasm became granular and dark (with what is presumed to be melanin), intact crystals within the peroxisomes could be seen embedded in the melanin. This suggests that these did not form the melanin granules identified by McIlroy. It may be that the structures visible to McIlroy were some form of protein which had precipitated during intracellular breakdown, as was noted in this investigation with shatter damaged tissue.

Previous investigations tend to have been carried out upon tubers immediately following harvest, whereas in this study the effect of impact was looked at through storage to establish how time in store affects blackspot.

These observations are consistent with the hypothesis that impact results in a loss of intracellular compartmentation or membrane damage and a mixing of enzyme and substrates for blackspot colour development, with oxidative metabolism resulting in melanin formation. Enhanced mitochondrial and ribosomal abundance is evidence of increased metabolism and wound healing in general. The enzyme LAH is thought to be located within the vacuole (Galliard, 1978) and the disruption caused by impact or cutting damage allows the enzyme to become released into the cytosol with a resultant increase in lipid hydrolysis and therefore membrane degradation (Galliard, 1978).

The formation of melanin surrounding starch grains and along the cell membrane supports the theory that the enzyme PPO is associated with plastid membranes and may indicate some association with the cell membrane. In particular the absence of melanin formation where starch grains are absent from cell sections suggests that starch grains are vital for this response to occur, this might be due to the association of PPO with the

amyloplast membranes or alternatively the physical presence of these large organelles may have assisted in the mechanical damage caused to intracellular structure when the tuber underwent impact.

The presence of occasional dictyosomes in bruised tissue (Chapter 4.3.4) and the associated dictyosome vesicles have also been observed previously by Thomson et al. (1995) after tissue had undergone either cutting or bruising wounds. These workers were looking at the development of suberin lamellae development following damage and frequently observed dictyosomes in the damaged tuber tissue, usually alongside the cell membrane to which many of the dictyosome vesicles fused. Dictyosomes may be responsible for the increased number of vesicles often found alongside the cell membrane in bruised tuber tissue, the vesicles perhaps contain chemicals important for wound healing.

4.4.3 Bruise Ultrastructure Through Storage

It has previously been proposed that during prolonged storage, tubers lose water and hence their turgidity declines (McGarry et al., 1996). Turnbull and Cobb (1992, 1995) reported loss of intracellular membrane integrity and an increase in number of vesicles during mid to late storage of Pentland Dell tubers. The plasmalemma began during mid storage and late in storage dissociated from the cell wall. They also noted an increase in the space between starch grain and amyloplast envelope. All of these changes are indicative of a decrease in turgidity throughout storage and the increased distance between amyloplast envelope and starch grain may also indicate a decline in starch content.

During this study, impacting Pentland Dell with 0.7 J at harvest resulted in a different types of bruising to that seen when tubers were impacted following storage (Chapter 4.3.4.1). At harvest impacted tubers developed what might be described as microscopic shatter bruise (despite any obviously shatter bruised tubers being discarded) compared with tubers impacted following storage, which developed blackspot. It has long been established that there is a negative correlation between tuber turgidity and blackspot bruise development, whereas a positive relationship exists with

shatter bruise (Smittle et al., 1974). Thus, turgid tubers following harvest are more susceptible to cracking and splitting due to the high stresses occurring on impact (Hughes, 1980a; Garcia et al., 1995). Flaccid tubers with a high starch content were most susceptible to blackspot bruising (Hughes, 1980).

Observations of cells from tubers impacted at harvest revealed a complete disruption of the tonoplast, unlike blackspot, with the formation of numerous membrane bound vesicles containing black staining amorphous material which is assumed to be various precipitated proteins. These vesicles were surrounded by large numbers of mitochondria, membrane fragments and appeared to be very active areas of the cell. However, few ribosomes were present in these shattered cells. This damage occurred throughout the cell and was not restricted to surrounding the starch grains and alongside the cell wall as is the case with blackspot bruise. This is demonstrated in severely damaged shatter bruised tissue in which the cells contained numerous cavities and a large amount of melanin (Chapter 4.3.4.1). Sectioning this tissue was difficult as it tended to disintegrate during microtoming. This was not found in tubers impacted following storage. Additionally, the nuclear envelope became highly invaginated following impact at harvest in comparison both with controls and tubers demonstrating blackspot damage following impact later in storage. These nuclei were surrounded by numerous vesicles and mitochondria indicating that the response to damage was still taking place 48 hours after impact when the tissue was sampled.

Whilst it is probable that the ultrastructural differences between impacted tubers at harvest and throughout storage are due to the type of bruise resulting from impact, it is possible that curing and storage could have brought about these differences. It would therefore be necessary to carry out a direct comparison of blackspot versus shatter bruise on tubers of identical background before conclusions can be drawn.

4.4.4 Confocal Microscopy Studies

Results from the confocal microscopy study were limited in the detail that they revealed of tuber tissue. However, particular areas of interest within the cell were revealed i.e. cell wall and starch grains. In addition to

this what is presumed to be melanin also fluoresced in impacted cells and these images were in agreement with those of the TEM studies with melanin forming in particular along the edges of the cells and surrounding the starch grains, with little melanin formation occurring in intracellular areas where amyloplasts were not present. This study also revealed distortion of the cell walls in damaged tissue (Plate 4.13) that appeared convuluted compared with controls.

This technique allows images to be overlaid such that 3D images can be put together. It was hoped that a full 3D image of a damaged and control cell might be produced, however this was not possible as the content of the cells was so great that it became impossible to distinguish intracellular structures within the cell.

5.0 Final Discussion

5.1 A criticism of techniques employed

Due to the nature of this project, a number of constraints were put in place at the outset of the work which meant that once methods had been determined initially, there was little scope for alterations at a later date. This also meant that a large number of variables had to be measured. Correlating biochemical and physiological properties of stored potato tubers with blackspot bruise formation required a large number of investigations to be performed regularly through storage over a number of storage seasons. Due to problems encountered with various investigations during the three storage seasons, a complete set of results was not possible.

Lack of data on certain characteristics for specific storage seasons was particularly a problem where the data for the remaining two storage seasons were inconsistent. In particular, the loss of cation leakage data for storage season 1996/7 was a problem. This data would have provided a good deal of information changes to the cellular compartmentation as it was during this season that bruise susceptibility increased through storage, at least in tubers grown at Arthur Rickwood Experimental Station. Storage season 1997/8 was the only season for which data was available on cation leakage and bruise formation. As bruise formation did not increase through storage in this season, correlations between blackspot susceptibility and damage to specific intracellular membranes was difficult. Consequently, for leakage of sodium and calcium ions which differed between storage season 1995/6 and 1997/8, conclusions cannot be drawn as to whether this leakage may have resulted in differences in bruise susceptibility.

This work was based on the assumption that bruise susceptibility would increase through storage. However measurements of bruise severity and percentage bruise formation did not always support this contention during storage seasons 1996/7 and 1997/8. No direct measurements of bruise susceptibility were taken during storage season 1995/6. Consequently, while the results obtained from storage season 1995/6 gave useful information on how the physiology and biochemistry of the tubers changed throughout storage, this could not be correlated with bruise susceptibility.

Measurements of percentage bruise formation through storage highlighted the degree of variation within tubers, such that whilst one tuber may form a sizable bruise another may not bruise at all within the same sample population. Variability in tuber susceptibility to bruising may have been a result of slight differences in curvature at the basal end of the tuber. While tubers used in these investigations were selected for a standardised size and shape, slight variations in the degree of curvature at the basal end of the tuber was inevitable and would have resulted in variations in the amount of energy hitting any one point and therefore variations in bruise formation. A method of bruising which could measure the degree of curvature and consequently the amount of tissue impacted, and therefore the exact amount of force to reach the potato, would have given more accurate results. This type of measurement would be possible using the pendulum method of impact, however this would have been too costly to build. Unfortunately, time limitations would not allow analysis of tubers for degree of curvature prior to impact and therefore a degree of error had to be accepted and taken into consideration when analysing results.

Ultrastructural investigations into bruising indicated that there was microscopic shattering of the intracellular structure within tubers impacted at harvest which did not generally occur later in storage. Consequently, when analysing results through storage for any biochemical or physiological factors it should be remembered that the biochemistry and physiology of potato tuber bruising may differ at harvest compared with later in storage. In some instances, the general pattern of increase in bruise susceptibility through storage was disturbed by increased bruise susceptibility at harvest which might be explained by the existence of microscopic shatter.

While a lot of useful information was gathered during microscopy of tubers, statistical analyses were not possible due to its qualitative nature. While this caused some problems in the comparison of data with results of a quantitative nature, confidence in the results was high due to the large number of sections examined from a total of 5 tubers per sample group.

A similar problem occurred in the measurement of respiration through storage during the 3 storage seasons in which time limitations did not allow for replicated investigations to be carried out and consequently no statistical

analyses could be performed. Again, the consistency of results between cultivars, seasons and other treatments allow confidence to be placed in the overall pattern of respiration rate measured through storage although direct measurement between tuber treatments was not possible. For impact time-course investigations in which more time was available for respiration measurements, replication was carried out and therefore statistical analyses could be performed on this data.

A number of problems were met in trying to develop an accurate and quantitative method for determining turgor and water relations of the tuber tissue. Consequently, measurements of turgidity through storage were only made during storage season 1997/8 and the results that were produced were surprising in that they did not show any significant decline in turgor during storage. An increase in turgor between tubers investigated at harvest and at 10 wks in store highlighted an inconsistency with the sampling procedure. It was desirable to take measurements on tubers as soon as possible after harvest and this was carried out at the harvest temperature to maintain some tuber stability. Consequently tubers were being incubated 5 °C warmer than they were during storage. While the investigations at harvest gave useful information in the biochemical and physiological state of the tubers at this time, it would have been an advantage to also sample tubers following the 2 wk curing period i.e. at 0 weeks in store. This would have provided data that was directly comparable with that of tubers during storage. The measurements of turgor provided by the psychrometric method indicate that this method may not have been accurate enough to determine the changes in tuber turgor evident during storage.

Despite the problems which occurred during these studies, a considerable body of data on the physiology and biochemistry of potato bruising in relation to storage was collected over the three storage seasons which can provide some information on factors influencing potato bruising.

5.2 Interpretation of main findings

The overall aim of this project was to investigate biochemical and physiological factors in relation to potato tuber blackspot bruising.

The first approach was to monitor tuber biochemical and physiological properties through storage alongside measurements of bruise susceptibility in order to identify key determinants in bruise susceptibility.

It has been previously demonstrated that tuber susceptibility to blackspot increases throughout storage (Smittle et al., 1974). This was not always found to be the case in these studies. The results that were gathered from tubers grown at ADAS, Arthur Rickwood showed an increase in bruise susceptibility throughout storage season 1996/7. However, this pattern was not evident in samples taken from ADAS, Terrington. No such increase in bruise susceptibility was seen during storage season 1997/8. The inconsistency in the pattern of bruise susceptibility through storage made correlation with tuber physiological and biochemical properties difficult.

It has been suggested that tuber respiration rate is positively correlated with bruise susceptibility (Brooks, 1996). During this investigation tuber respiration rate through storage followed a consistent pattern with a decline immediately following harvest and subsequently tubers maintained a low rate of respiration until mid to late storage when the rate increased. .This pattern did not correlate with bruise susceptibility and consequently it can be concluded that the tuber respiration rate, and indeed the metabolic rate, did not have a significant effect on bruise susceptibility during storage.

Providing energy for the continuous respiration through storage would require the breakdown of storage reserves. However no decline in dry matter was seen through storage, probably due to a parallel decline in water content. Consequently the investigations into tuber dry matter content did not provide a true measure of the change in dry matter content during storage and therefore cannot tell us whether this has a significant effect on bruise susceptibility.

Similarly no conclusions could be drawn as to the effect of turgor on bruise susceptibility due to a low degree of confidence in the results.

Results from investigations into potential melanin formation were particularly useful in that they highlighted that the biochemistry involved in tuber bruising was not the critical determinant in bruise susceptibility. This data takes into account PPO activity, substrate concentration, ascorbate activity and the influence of any other intracellular constituents that might

affect bruising in potato tubers. Results from these studies indicated that the potential for melanin formation to occur was increasing during storage. However, a parallel increase was not seen in the results from bruise susceptibility measurements. It is likely that there is a threshold limit for each of the important biochemical characteristics in relation to bruising such that if a tuber is below this limit for any one characteristic bruise susceptibility is reduced. This can be demonstrated by tubers engineered to be deficient in PPO. Bruising cannot occur as PPO is a vital component in the bruising reaction (Bachem et al., 1994). Above this threshold concentration, the biochemical components are not limiting and therefore bruising is not affected by the increased potential for producing melanin seen as storage progressed. To calculate the biochemical potential for melanin formation, tubers are homogenised allowing the constituents of the bruising reaction to be brought together. This increase during storage implies that without any tuber and cellular structure bruise severity following impact would have increased during storage; it is the intracellular compartmentation within the tubers that is preventing this increase in potential melanin formation from being observed.

Investigations into the effect of loss of tuber compartmentation on bruise susceptibility during storage were carried out firstly using measurement of cation leakage. It was expected that these measurements would act as a guide to how the quality of the membranes were changing through storage. During storage season 1995/6 there was an increase in the leakage of sodium and calcium ions from tuber samples during late storage, which may imply degradation or disruption of the tonoplast as storage progressed. This may have enabled the leakage of PPO substrates e.g. tyrosine from the vacuole enabling them to react with PPO resulting in some darkening of the cytoplasm as was seen in microscopy studies of tubers from late storage (Plate 4.3a). However no measurements of bruise susceptibility were made during this storage season. No increase in leakage of sodium ions through storage was during storage season 1997/8, however nor was there any increase in bruise susceptibility during this season. The lack of data for cation leakage during storage season 1996/7 means that information on cation leakage and bruise susceptibility was only available for one storage

season and consequently no conclusions can be drawn on the change in membrane quality through storage and subsequent effects on blackspot susceptibility.

In addition to measurements of cation leakage, investigations into tuber compartmentation were also carried out using electron microscopy allowing visualisation of the tuber intracellular structure as storage progressed. Results suggested a loss of water was taking place from the cells as storage progressed with the cell membrane pulling away from the cell wall, with some loss of membrane integrity by late storage. This evidence indicates that a decline in intracellular compartmentation is taking place during late storage resulting in a darkening of the cytoplasm seen in approximately 10% of cells. Again no correlations could be made between these results and those of bruise susceptibility.

The effect of impact on tuber ultrastructure was also investigated through storage and compared with bruise susceptibility. Following impact at harvest a number of differences in tuber ultrastructure could be seen compared with tubers impacted following storage. For example, tonoplast disruption and the formation of numerous membrane-bound vesicles was seen following impact at harvest but was not noted in any tubers from 12, 24 or 36 wks in store. It is likely that tubers at harvest underwent microscopic shatter following impact. This correlates with the unexpectedly high degree of bruising at harvest seen in the bruise susceptibility studies.

The second approach used to identify key influences in bruise susceptibility was measurement of tuber physiological and biochemical characteristics prior to and in the subsequent hours following impact. Timecourse investigations were carried out following 15 and 32 weeks in storage. It was evident that the length of time in store affected the tuber response to impact. Tubers impacted following 15 wks in store demonstrated a greater loss of turgor and increased cation leakage compared with those impacted 32 wks into storage. During this time there was an increase in bruise susceptibility such that a greater disruption to the intracellular compartmentation found early in storage resulted in a lower degree of bruise susceptibility.

Turgor declined immediately following impact. This implies that the tissue had been seriously disrupted by the damage, which was supported by the evidence of potassium, magnesium and calcium ion leakage which increased immediately upon impact in cv Pentland Dell following 15 weeks in store.

It is likely that this increase in cation leakage and decrease in turgor seen immediately after impact in tubers from early storage was the result of severe membrane disruption that was followed by repair as membranes reformed. Later in storage, the impact may have resulted in more permanent membrane damage being sustained, which ultimately resulted in a greater degree of bruise formation. Where cation leakage increased upon impact this also returned to pre-impact levels often within only a few hrs after impact perhaps indicating that damaged membranes had been repaired.

In agreement with previous workers (Li, 1985; Brook, 1996) there was an increase in the rate of respiration was seen following impact, indicating that an increase in metabolism is required following damage. This is likely to be to fuel repair of the tissue. This rise in respiration rate had almost returned to pre-impact rates in the 24 hrs following impact. This indicates either that once wound responses have been initiated and are underway, additional energy is not required to keep them up or alternatively that the damage response is completed within this time.

Measurement of PPO activity found that there was no significant change in activity following impact. This indicates that the amount of enzyme activity present within the cell is enough to allow bruise formation and an increase in PPO activity is not necessary.

Ultrastructural time-course studies were carried out separately from physiological and biochemical time-course studies. One aim of this work was to establish an ultrastructural sequence of events following impact. This was done using electron microscopy.

Microscopy studies on tubers following impact (Chapter 4.3.3) revealed that the tuber initially responds with an increase in numbers of mitochondria and ribosomes which increased significantly in the first 24 hrs after impact and were clearly visible on examination of sections of damaged tissue. This can be associated with the increase in respiration following impact seen during the time-course investigations. Barkhausen (1978) also found an

increase in ribosome numbers 4 hrs after wounding and an associated division of mitochondria. These results indicate that there has been an up-regulation in tuber metabolism following impact. This may be required to fuel an increase in protein synthesis. Certain proteins have been found to be up-regulated following damage for example the enzymes PAL and ubiquitin, as reported by Belknap et al. (1990) and the cell wall protein, extensin gene (Croy et al., 1998)

Subsequently, there was a darkening of the cytoplasm which became more granular in appearance between 48 and 72 hrs after impact. This darkening increased until organelles within the cytoplasm were no longer visible and the cytoplasm was black. This darkening was presumed to be the result of melanin formation. It is evident from these results that it took several days for melanin formation to be completed. From the time-course investigation percentage bruise formation was measured over the 24 hrs following impact and consequently melanin formation may not have been complete. This was of little importance as the melanin development was easily detectable by this time.

During these studies a significant difference between cvs was observed in their susceptibility to blackspot bruise formation and various physiological and biochemical characteristics. In particular Maris Piper generally had a lower blackspot susceptibility than either Pentland Dell or Record and also tended to have the lowest biochemical potential for producing melanin according to results from potential colour formation. In addition, Table 3.3 shows that little change in cation leakage occurred following impact in Maris Piper compared with cv Pentland Dell. This would imply that intracellular membranes undergo less damage following impact in Maris Piper. Tubers of Maris Piper differ from those of Pentland Dell and Record physiologically in that they have a significantly lower rate of respiration and lower leakage of cations but higher turgor, osmotic potential and water potential. Turgor has been shown previously to have a positive relationship with blackspot susceptibility (Smittle et al., 1974) and this may be an important factor in reducing susceptibility of Maris Piper to blackspot bruise.

Seed tubers can be physiologically aged by increasing the storage temperature for a period of time. This effectively results in a more mature tuber. Consequently, sprouting and subsequently tuber initiation occur earlier in seed tubers that have been aged and this in turn influences the yield of the crop. Tubers produced from aged seed should therefore be older themselves than equivalent tubers produced from seed that has not been aged and might consequently have different characteristics that could affect blackspot susceptibility. It was shown in this study that differences in the physiological age of seed tubers did not result in significant differences in the resultant crop of any of the physiological or biochemical factors measured in this investigation.

The site at which tubers were grown, while differing in soil type and irrigation practices, had no consistent effect on blackspot susceptibility.

Increased susceptibility to bruising at lower temperatures has been reported previously and was evident in these investigations (Brierley, Edgell, Wilthshire & Cobb, 1998). The effect of temperature upon bruise susceptibiity is particularly important from a practical point of view for farmers harvesting, and transporting tubers. Handling the tubers at cooler temperatures is likely to cause a greater degree of damage.

In these studies tubers impacted at 5 °C showed a greater percentage of bruised tubers and average bruise volume than tubers impacted at 10 °C. The reason for this was not demonstrated by any of the investigations into physiology following impact at 5 and 10 °C. While tuber respiration is known to be greater at higher temperatures, the general pattern of respiration did not fit that of bruise susceptibility. Alternatively, temperature influences membrane fluidity. It was noted by Sowokinos (1990) that leakiness of tuber membranes was greater in tubers under cold stress and hence the temperature affect on membranes may influence leakage of substrates from the vacuole in addition to the damage caused upon impact. Espen, Morguttim, Abruzzese, Begrini, Rivetta, Quattrini, Cocucci and Coccuci, (1999) investigated the changes in MDA (malondealdehyde) concentration and potassium release in potato tubers stored at 3 and 23 °C for up to 120 days. At the higher temperature they found no change in either MDA levels

or potassium leakage where as at 3 °C there was an increase both in the concentration of MDA indicating an increase in lipid peroxidation and potassium leakage indicating a decline in membrane integrity. In addition to these findings an increase in reducing sugars and sucrose was found at 3 °C which was not seen at 23 °C indicating that the tubers were undergoing cold acclimation.

These studies have enabled us to conclude that differences in tuber temperature and compartmentation are of primary importance in determining the variations seen in bruise susceptibility.

5.3 Suggestions for further study

Where data sets are incomplete it would be useful to collect measurements on additional storage seasons. For example a better indication of the effect of storage on cation leakage and bruise susceptibility might be gained by repeating this study for additional years. Similarly changes to methods for example for measuring dry matter content and turgor (as outlined in Chapter 2.4) and then measuring these alongside bruise susceptibility would provide the information which was not successfully acquired during this study.

A useful addition to this study would have been the analysis of membrane lipid composition. It is well known that a reduction in temperature decreases membrane fluidity. This is partly due to the degree of saturation of the fatty acyl chains of lipid molecules within the membranes such that in a saturated state the lipid is less fluid. The degree of saturation is influenced by temperature and fatty acyl chains will transform from being rigid to fluid once raised above a transition temperature. The structure of the lipid and length of the hydrocarbon chain influences the transition temperature. Consequently, variations in the lipid content of tuber membranes may affect the fluidity of the membranes that in turn will affect the degree to which the membrane can respond to impact damage. A rigid membrane is more likely to be damaged upon impact than a fluid one. Variations in membrane lipid composition through storage or between cultivars may be one influencing factor in bruise susceptibility. Changes in membrane lipid composition during cold storage have been recorded with an increase in unsaturated fatty acids (Spychalla &

Desborough, 1990). It would also be useful to determine the transition temperature of tuber membranes, which could be done by measuring the saturation levels of lipids from membrane extracts at a series of temperatures. Identification and quantification of lipids is now a relatively simple process which can be done using ideally gas chromatography or alternatively high performance liquid chromatography. However, membrane isolation is difficult, in particular attempting to isolate a specific type of membrane e.g. the tonoplast may not be practical.

An alternative way of looking into membrane quality during storage would be to measure MDA levels in tubers over storage. This would indicate the amount of lipid peroxidation that was occurring within the membranes and would therefore indicate whether tuber membranes were being damaged in this way during the storage process. Measurements taken through storage following impact would also demonstrate how the extent of damage caused by impact might change as storage progressed.

It would also be interesting to carry out a more detailed study using confocal microscopy, using alternative stains to select for membranes which, together with acridine orange, would give a more complete picture of the cell ultrastructure. Visualisation of the intracellular structure in this way might indicate the role of the starch grains in damaging the internal structure upon impact.

Characterisation of the tuber periderm by microscopy would also be a useful area for extra study. The structure and depth of this layer of the tuber may have a significant affect on bruise susceptibility. Differences in periderm structure would influence the absorption of impact energy by this layer and consequently would affect the amount of energy that reaches the tuber cortex ultimately the vascular system where bruise formation takes place.

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