THE ACCUMULATION OF CHLOROPHYLLS AND GLYCOALKALOIDS IN STORED TUBERS

EVERARD JOHN EDWARDS

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

May 1997

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 authors individual contribution. The author has attended appropriate lectures, seminars and conferences in partial fulfilment of the requirements of the degree.

Signed:.....
(Candidate)

Signed:.....
(Director of Studies)

COPYRIGHT

ABSTRACT

Exposure to light causes potato tubers to green, due to the conversion of amyloplasts to chloroplasts (Anstis and Northcote, 1972), and accumulate toxic steroidal glycoalkaloids (Conner, 1937). The two major alkaloids, comprising 95% of the total (TGA) are α-solanine and α-chaconine (Olsson, 1989). The consumption of potatoes with high TGA concentrations can cause illness and even death (Morris and Lee, 1984).

This study reports the successful adaptation of leaf Chl analysis methods to potato tubers. Existing HPLC methods of TGA analysis have been critically examined and a number of problems have been addressed. This combined with the use of a new SPE sorbent has resulted in sample recoveries of 93 and 99% for α-solanine and α-chaconine respectively. The reliability and reproducibility of these methods allow them to be used routinely.

Exposure of potato tubers to low PPFD at various temperatures has shown that even at a PPFD of 12 μmol photons m^{-2} s^{-1} tubers will accumulate detectable Chl within 48 hr. It has also been demonstrated that storage at 5°C will delay the onset of greening and greatly reduce its severity. Long term storage of tubers reduced the potential for light-induced accumulation of TGA but did not have any significant effect on Chl synthesis. The extent of both greening and TGA accumulation were dependant on cultivar choice. Artificial neural networks were used to model the data produced by these experiments and were shown to closely follow the actual data. These could then be used to predict tuber response to specific storage and light exposure conditions.

Preliminary studies on the physiology of greening demonstrated that the accumulation of Chl and TGA were not biosynthetically connected. Also, carotenoid composition during greening was shown to change markedly, including the synthesis of carotenoids not present in unexposed tubers and results indicated that greened tubers are capable of fixing atmospheric CO_2.

ACKNOWLEDGEMENTS

The official thanks go to the Potato Marketing Board and The Nottingham Trent University for funding my laboratory exploits and supplying the necessary potatoes. Also to Andy for coaxing my writing skills into something reminiscent of readability and not despairing to much when I informed him of more disasters. Not least, thanks go to the technical staff within most sections of the Life Sciences department, especially Nigel and James who's HPLC repair skills have been so useful.

Personal thank you's must go to all those who have helped me put up with the 'joys' of doing a PhD; to Kerry for giving me an insight into this writing up lark, to Sarah for putting up with the worst of it and to Edge for encouraging me to get the thing finished!

Final thanks go to the Old Dears for the odd financial boost and occasional words of 'wisdom', everyone who isn't already mentioned and anyone else who'd feel aggrieved otherwise.

CONTENTS

PUBLICATIONS

At the time of submission the following papers have been, or are about to be, published.

Refereed Publications

E.J. Edwards & A.H. Cobb. (1996). An improved High Performance Liquid Chromatographic Method for the analysis of potato (*Solanum tuberosum*) glycoalkaloids. *The Journal of Agricultural and Food Chemistry.* **44**, 2705-2709.

E.J. Edwards & A.H. Cobb. (1997). The effect of temperature on glycoalkaloid and chlorophyll accumulation in potatoes (*Solanum tuberosum* L. cv. King Edward) stored at low photon flux density, including preliminary modelling using an artificial neural network. *The Journal of Agricultural and Food Chemistry.* **45**, 1032-1038.

E.J. Edwards & A.H. Cobb. (1997). Is there a link between greening and light enhanced glycoalkaloid accumulation in potato (*Solanum tuberosum* L.) tubers? *The Journal of the Science of Food and Agriculture.* In press.

Conference and Popular Press

E.J. Edwards & A.H. Cobb. (1996). The accumulation of chlorophylls and glycoalkaloids in stored tubers. *Abstracts of Conference Papers, Posters and Demonstrations of the 13th Triennial Conference of the European Association for Potato Research.* pp. 41-42.

E.J. Edwards & A.H. Cobb. (1996). Recent improvements in the analysis of glycoalkaloids by HPLC. *Abstracts of Conference Papers, Posters and Demonstrations of the 13th Triennial Conference of the European Association for Potato Research.* pp. 536-537.

E.J. Edwards & A.H. Cobb. (1996). When going green spells bad business. *Potato Review.* **6**, 32-35.

E.J. Edwards & A.H. Cobb. (1997). The accumulation of glycoalkaloids and chlorophylls in potato tubers exposed to light. Poster to be presented at Crop Protection and Food Quality conference in Sept. '97.

ABBREVIATIONS

A	Absorbance
A_{sf}	Peak asymmetry
ABA	Abscisic acid
AFPA	4-Amino-5-fluoropentanoic acid
ALA	5-aminolaevulinic acid
ANN	Artificial neural network
ANOVA	Analysis of variance
aq.	Aqueous
AU	Absorbance units
°C	Degrees centigrade
Chl	Chlorophyll
CIPC	3-chloro-*iso*-propylphenylcarbamate
cm	Centimetre
CPB	Colorado potato beetle
cv.	Cultivar
d	Days
e	Base of natural logarithm
et al.	Et allia
DMF	*N,N*-dimethylformamide
DNA	Deoxyribonucleic acid
fc	Foot-candles
Fig.	Figure
FR	Far red light
FWT	Fresh weight
g	Grammes
g	Gravities
GABA	γ-aminobutyric acid
gabaculine	3-amino-2,3-benzoic acid
Glu	Glucose
Glu-TR	Glu-tRNA reductase

GSA-AM	glutamate-semialdehyde aminotransferase
ha	Hectares
HCl	Hydrochloric acid
hr	Hours
HPLC	High performance liquid chromatography
IRGA	Infra red gas analysis
kg	Kilogrammes
kJ	Kilojoules
L	Linnaeus
LHC	Light harvesting complex
m	Metres
M	Molar
MES	2-[N-morpholino]ethanesulfonic acid
MF-NE	Monofunctional-nonendcapped
mg	Miligrammes
min	Minutes
mm	Millimetres
mol	Moles
MSE	Mean squared error
µ	Micrometres
µg	Microgrammes
µl	Microlitres
µm	Micrometres
µmoles	Micromoles
n	Number
nm	Nanometres
PAR	Photosynthetically active radiation
pH	Hydrogen ion concentration
PLH	Potato leafhopper
PPFD	Photosynthetically active photon flux density
PS	Photosystems
PSI	Photosystem I

PSII	Photosystem II
R	Red light
RH	Relative humidity
Rs	Separation of retention time
RuBisCO	Ribulose bisphosphate carboxylase/oxygenase
s	Seconds
SE	Standard error
SPE	Solid phase extraction
t	Time
t	Tonnes
TF-EC	Trifunctional-endcapped
TF-NE	Trifunctional-nonendcapped
TGA	Total glycoalkaloids
UV	Ultra violet light
v/v	Volume to volume
vis	Visible light
w	Peak width
w/w	Weight to weight

CHAPTER 1 - INTRODUCTION

1.1 - The History of Potato Use and Their Importance to Mankind

1.1.1 - Introduction and use of potatoes in Europe.

The genus *Solanum* L. contains about 2000 members spread throughout the world in tropical and subtropical regions. Less than 10% of these, about 150 species, are tuber bearing.

The potato was first classified by a European in 1596 as *Solanum tuberosum esculentum* (Bauhin, 1596) but was truncated to *S. tuberosum* by Linnaeus (1753). However, the modern potato is actually a series of hybrids between *S. tuberosum* and other species, including *S. antigenum* (Brouk, 1975), *S. demissum, S. stolonifera* and *S. acaule* (Howard, 1970). Breeding has increased resistance to diseases such as *Phytophthora* and potato viruses x and y.

It is likely that the potato was introduced by the Spanish from the Peruvian highlands in the Sixteenth century and indeed the English word potato is derived from the Spanish 'patata' which in turn was derived from the American native word 'batata' (Brouk, 1975). Cultivation of the potato probably first occurred in the highlands of what is now Peru, and could have been as early as 8000 BC (Martins Farias, 1976; Hawkes, 1985).

Spanish cultivation of the potato probably dates to about 1580 (Burton, 1989). There is also evidence that potatoes were being grown in Italy and England by the end of the 16th century. The potato was first used as a food crop in Europe by the poor, as consumption of tubers was met with resistance due to the conservative nature of the aristocracy. Indeed, it was not until the mid Eighteenth century that large scale potato production began, initially in Germany (Laufer, 1938). The potato did not become a major part of the English diet until the late Eighteenth century, when it was eaten and grown by Irish immigrants. The potato crop in Europe grew steadily in size until yields were devastated by *Phytophthora infestans* in the 1840's. During the latter part of the Nineteenth century the area under

potato cultivation increased, but it has declined during this century, although this has been partly offset by increases in yield (van Loon and Crosnier, 1982).

1.1.2 - Modern potato production and usage.

World production of potatoes is currently 270 million tonnes grown on 18 million ha (FAO, 1993) and is the worlds 4th largest crop, after rice, wheat and maize. Potatoes are used as both staple foodstuff and animal fodder throughout the world, with the Russian Federation, Poland, China and Ukraine growing almost half the world crop between them. It is particularly important as a food crop in poorer regions of Europe and the Americas.

In the 1993/94 growth season 152,000 ha were planted with potatoes in the UK. This represents a drop in area of over 50% since 1960. However, yield in the same period has risen from 22.6 t ha^{-1} to 43.5 t ha^{-1}, which equates to only a 4.5% drop in production (PMB, 1995a). Total production of potatoes in the UK during the 93/94 season was 6,384,000 t, valued at £1.5 billion, of which the domestic market (potatoes and products purchased for the home) used 3,618,000 t (PMB, 1995b). Almost 70% of this was purchased raw, 8% as crisps and 9% as frozen products. The remainder was canned or purchased dehydrated.

Possibly the most important trend in usage of potatoes in recent years has been the amount processed. This has increased steadily from 18% of total potato production in 1987/88 to almost 25% in 1993/94 (PMB, 1995b). This has implications both for growing and storage as potatoes used for processing have to be of very high quality and low temperature sweetening prevents storage at the lowest temperatures.

1.1.3 - The dietary value of the potato.

Potatoes are often thought of by the general public as lacking in nutrients and sometimes as fattening. However, as a source of energy the potato is fairly poor giving only about 3.35 kJ g^{-1} fresh weight cf. 15.23 kJ g^{-1} for rice, 13.89 kJ g^{-1} for wheat and 5.40 kJ g^{-1} for corn (Paul and Southgate,

1978; Nyman Siljestrom, Pederson, Bach Knudson, Asp, Johanson and Eggum, 1984). This is partly due to their high water content and partly to their very low fat content, 1 mg g^{-1}, which is 20% that of rice and 5% that of wheat. The proportion of total dietary energy intake which is formed by potatoes has been estimated as between 2.3% (in Italy) and 6.8% (in The Republic of Ireland) (Elton, 1978). However, about 20% of energy intake is in the form of lipids (Elton, 1978) and fried potato products such as chips and crisps can have between 20 and 25 kJ g^{-1}, many times that of fresh potatoes (Burton, 1989).

Potato tubers are an important source of protein and nitrogen. Indeed Burton (1966) calculated that 2 kg d^{-1} of boiled potatoes would supply all the nitrogen essential for an average adult. On a dry weight basis potatoes contain protein in amounts comparable to cereals. Potatoes possess sulphur containing amino acids such as methionine and cysteine at only about 70% of the concentrations present in wheat, but lysine content is nearly 3 times higher (Woolfe, 1987). However, potato protein is of particularly high quality, and in terms of biological value ha^{-1} is second only to soybean (Kaldy, 1972).

The area where potatoes play the most important role in human nutrition is possibly that most overlooked by the consumer, namely vitamin and mineral content, particularly vitamin C. Burton (1974) estimated that in the UK potatoes supplied 30% of the entire vitamin C intake. Kolasa (1993) calculated that 1 medium potato provided 15% of the US Recommended Daily Intake of vitamin B_6, a vitamin commonly consumed in inadequate amounts. The vitamin C content of fresh potatoes is approximately 200 µg g^{-1}. Unfortunately both this and the B vitamins are easily lost by overcooking and a potentially important supply of nutrients can be lost. Furthermore, potatoes are also a significant source of magnesium, copper, iron and iodine.

Potatoes also provide a supply of dietary fibre. Although containing only half the amount of fibre found in other common vegetables (Paul and Southgate, 1978), due to the large quantity of tubers consumed they are

probably the single most important source of unlignified fibre in the UK diet (Burton, 1989).

The importance of the potato crop and its rise to a global production comparable with cereals is due to its high nutritional value, providing a major source of protein, vitamins and minerals. The relatively high yield of the crop and its ability to tolerate many different climates have also helped to make the potato common on the dinner tables of virtually every part of the world.

1.2 - Storage of the Potato Crop

The British climate ensures that potatoes can only be harvested from late May for first earlies, until late October/early November for the maincrop. As lifting of the latter does not usually start until late August it is obvious that for potatoes to be available all year round some form of storage is needed. In the UK potatoes are usually stored in bulk in pallets. A large store of potatoes can have very different temperature and humidity characteristics throughout its mass. It is consequently necessary to ensure adequate ventilation to enable the full control of humidity and temperature needed to maintain a high quality product during long term tuber storage.

The temperature limits for tuber survival are between 2 and 30°C (Burton, 1989) but to minimise weight loss and pathogen development potatoes are stored at 3-6°C for pre-packing, 7°C for ware and 8-10°C for processing (PMB, 1992). Storage at lower temperatures causes sweetening and unacceptable fry colours when tubers are processed (Cunnington Mawson, Briddon and Storey, 1992). Also, humidity should be as high as possible, without causing condensation, in order to reduce evaporation from the tubers (Burton, 1989; PMB, 1992). However, these regimes are varied for particular cultivars and uses.

Before entering long term storage potatoes are 'cured'. This process involves holding the tubers at 12-15°C and 95% relative humidity for

approximately 2 weeks (PMB, 1992). These conditions allow suberisation and periderm formation at damaged areas of the tuber, i.e. wound healing. Curing is of immense importance as it maximises the subsequent storage period and the quality of the crop. Curing can also reduce skin spot (*Polyscytalum pustulans*) if humidity is reduced (Boyd, 1957). Lower humidity in conjunction with fungicides is also necessary if other fungal diseases are present.

1.2.1 - Sprouting.

The growth of sprouts is only a problem in potatoes to be stored for long periods as the inherent dormancy of harvested tubers put into store will prevent sprouting for some time. Sprouting occurs during dormancy break and is preceded by an increase in respiration (Isherwood and Burton, 1975). High respiratory rates cause weight loss due to the mobilisation of sugars and their subsequent breakdown, releasing CO_2. Further weight loss during sprouting is the result of increased evaporation of water. The epidermis of the potato sprout is about 100 times more permeable to water than the tuber periderm, therefore with a sprout surface area of just 1% of the tuber surface area, evaporative loss is doubled (Burton, 1955; Burton and Hannan, 1957).

Sprouting is traditionally controlled using low temperature storage combined with chemical suppressants such as chlorpropham (CIPC). However, the efficacy of other methods such as controlled atmosphere, reduced CO_2 or O_2 concentrations (Burton, 1958, 1968; Schouten, 1992; Khanbari and Thompson, 1994), and irradiation (Sparenberg and Ulmann, 1973) has also been examined. These have never been used to the extent of chemical suppressants because of limited effectiveness, relatively high cost and, in the case of irradiation, lack of public acceptance.

1.2.2 - Dehydration.

Evaporative water loss is the result of the tuber water potential moving towards equilibrium with that of the surrounding air. As such all tubers will be affected by dehydration and even the best measures can only

minimise this. Water moves along the cell walls through the tuber until reaching the periderm which presents the major barrier to evaporation. The extent to which the periderm affects water loss is shown by peeling the potato, this results in a 300-500% increase in evaporative losses (Burton, 1955). Inevitably with severe water loss there is a large drop in tuber weight, accompanied by wrinkling of the skin which is unacceptable to the consumer. Sprouting, wounding and temperature variation all affect water loss.

Evaporative water loss cannot be completely prevented, but by adjusting temperature and most importantly RH to minimise the difference between the water potentials of the tuber and the surrounding air, weight loss can be reduced to acceptable levels.

1.2.3 - Greening and glycoalkaloid formation.

When stored potatoes are exposed to light, a greening reaction will occur. This greening is the result of the initiation of chlorophyll production within the periderm and cortical parenchyma of the tuber (Larson, 1950). Concomitantly, a dramatic increase in the abundance of toxic glycoalkaloids is often observed within the tuber (Connor, 1937).

Exposure to light during growth, harvesting and handling of potatoes also causes greening and, even if followed by prolonged dark storage, the chlorophyll is not significantly degraded. Therefore, tuber chlorophylls exhibit great stability when compared with chlorophyll synthesised in leaves (Virgin and Sundqvist, 1992). Also glycoalkaloids do not degrade once formed (Baerug, 1962) and are not destroyed by cooking (Zobel and Schilling, 1964; Porter, 1972).

It therefore follows that any light exposure can have an adverse effect on the stored crop. Both the end user and processors will reject greened potatoes because of the association between greening and glycoalkaloids even though it is likely that the two phenomena are independent (Conner, 1937; Peterman and Morris, 1985).

1.3 - Glycoalkaloids

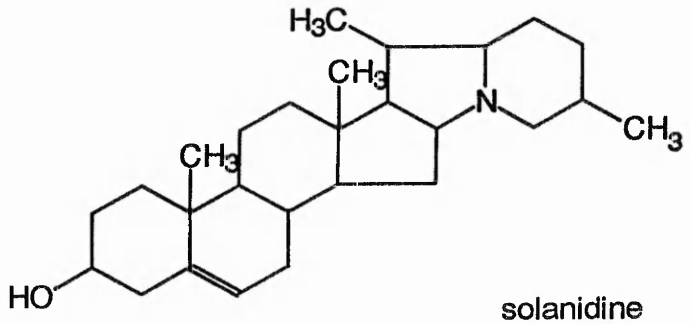
A glycoalkaloid is a molecule with a sugar moiety and an alkaloid. The glycoside can be composed of between 1 and 4 hexose sugars of varying types and is attached to the aglycone at C-3. Over 100 glycoalkaloids have been identified (Maga, 1994), mostly from the family *Solanaceae* (Schreiber, 1968), all possessing the C_{27} steroidal skeleton of cholestane.

Glycoalkaloids found to date in the potato plant are all based on solanidane or spirosolane type aglycones. The former includes the 2 major potato glycoalkaloids, α-solanine and α-chaconine, (Fig. 1.1), which together comprise approximately 95% of the total (Olsson, 1989). Other solanidane-based alkaloids identified include the mono and diglycosides of solanine and chaconine (Table 1.1) and demissidine. Spirosolane-based alkaloids include α- & β-solamarine (Shih and Kuc, 1974), both glycosides of tomatideno,l and the glycosides of solasodine (van Gelder and Schieffer, 1991).

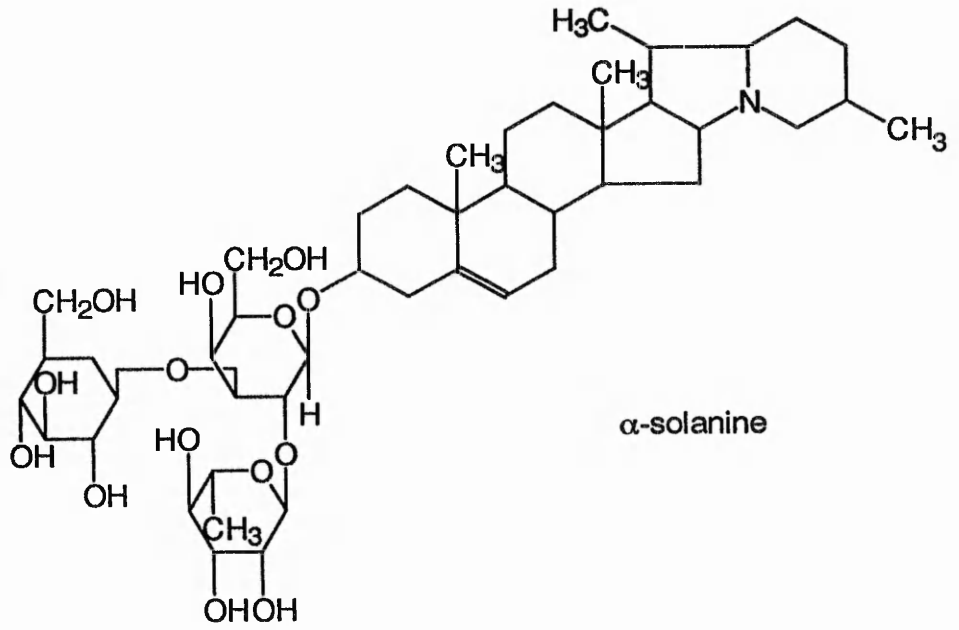
More recently Nash, Rothschild, Porter, Watson, Waigh and Waterman (1993) isolated a new family of alkaloids from potato tubers, which are derived from tropane.

Table 1.1 - Composition of glycosides of solanidine.

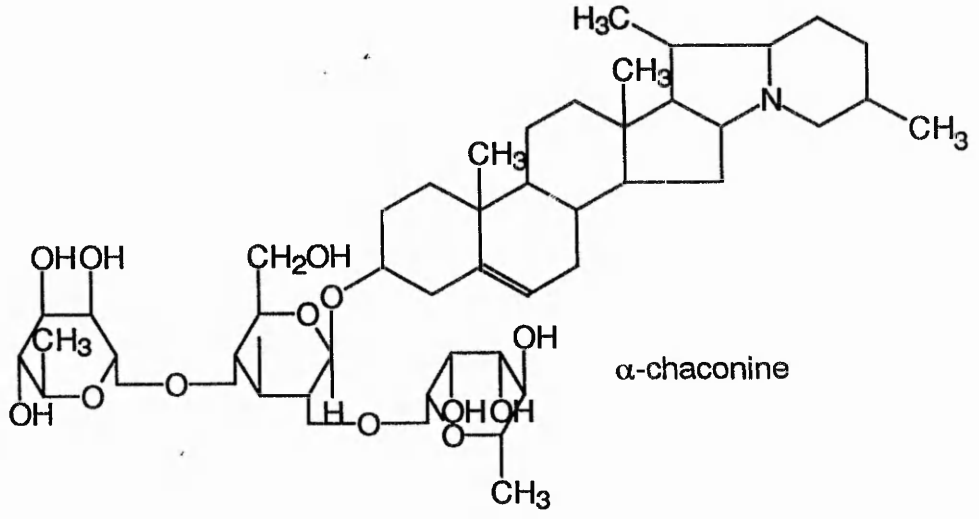
Molecule	Composition							
α-solanine	solanidine	+	galactose	+	glucose	+	rhamnose	
β-solanine	solanidine	+	galactose	+	glucose			
γ-solanine	solanidine	+	galactose					
α-chaconine	solanidine	+	glucose	+	rhamnose	+	rhamnose	
β-chaconine	solanidine	+	glucose	+	rhamnose			
γ-chaconine	solanidine	+	glucose					



solanidine



α-solanine



α-chaconine

Figure 1.1 - Structures of solanidine and its triglycerides.

Since biosynthesis of all steroids is via the acetate-mevalonate pathway (Fig. 1.2), it is assumed that solanidine is formed in the same manner. Some evidence for this has been forthcoming. Guseva and Paseschnichenko (1958) demonstrated that ^{14}C-labelled acetate is readily absorbed by potato shoots and that ^{14}C is incorporated into solanidine. However, in etiolated shoots ^{14}C was found in the sugar moiety of the glycoalkaloids. It was also observed that radio-labelled α-chaconine isolated in these studies possessed double the specific activity of α-solanine and that radio-labelled mevalonate was more readily incorporated into glycoalkaloids than acetate (Guseva, Borikhina and Paseschnichenko, 1960; Guseva, Paseschnichenko and Borikhina, 1961).

Following these studies, a large number of sterol intermediates have been shown to be involved in the synthesis of potato glycoalkaloids, including leucine, alanine and cholesterol (Heftmann, 1967; Jadhav, Salunkhe, Wyse and Dalvi, 1973). Indeed, inhibition of cholesterol synthesis prevents glycoalkaloid accumulation (Bergenstrahle, Borga, and Jonsson, 1996). However, the complete synthetic pathway from cholesterol to solanidine still remains to be elucidated. Of particular importance is the origin of the nitrogen atom in the F ring of solanidine. Kaneko, Tanaka and Mitsuhashi (1976) suggested that arginine is the source. This has been supported by a recent study that isolated 26-aminocholestanol, a novel intermediate between cholesterol and solanidine produced by a reaction involving arginine, from *S. abutiloides* roots (Ohmura, Nakamura, Tian, Yahara, Yoshimitsu and Nohara, 1995). Interestingly, this molecule was found as a triglyceride.

As only extremely low concentrations of solanidine are found in potatoes it is evident that the aglycone is rapidly glycosylated. This probably occurs via separate glucosyl- galactosyl- and rhamnosyl-transferase (Bergenstrahle, Tillberg and Jonsson, 1992a).

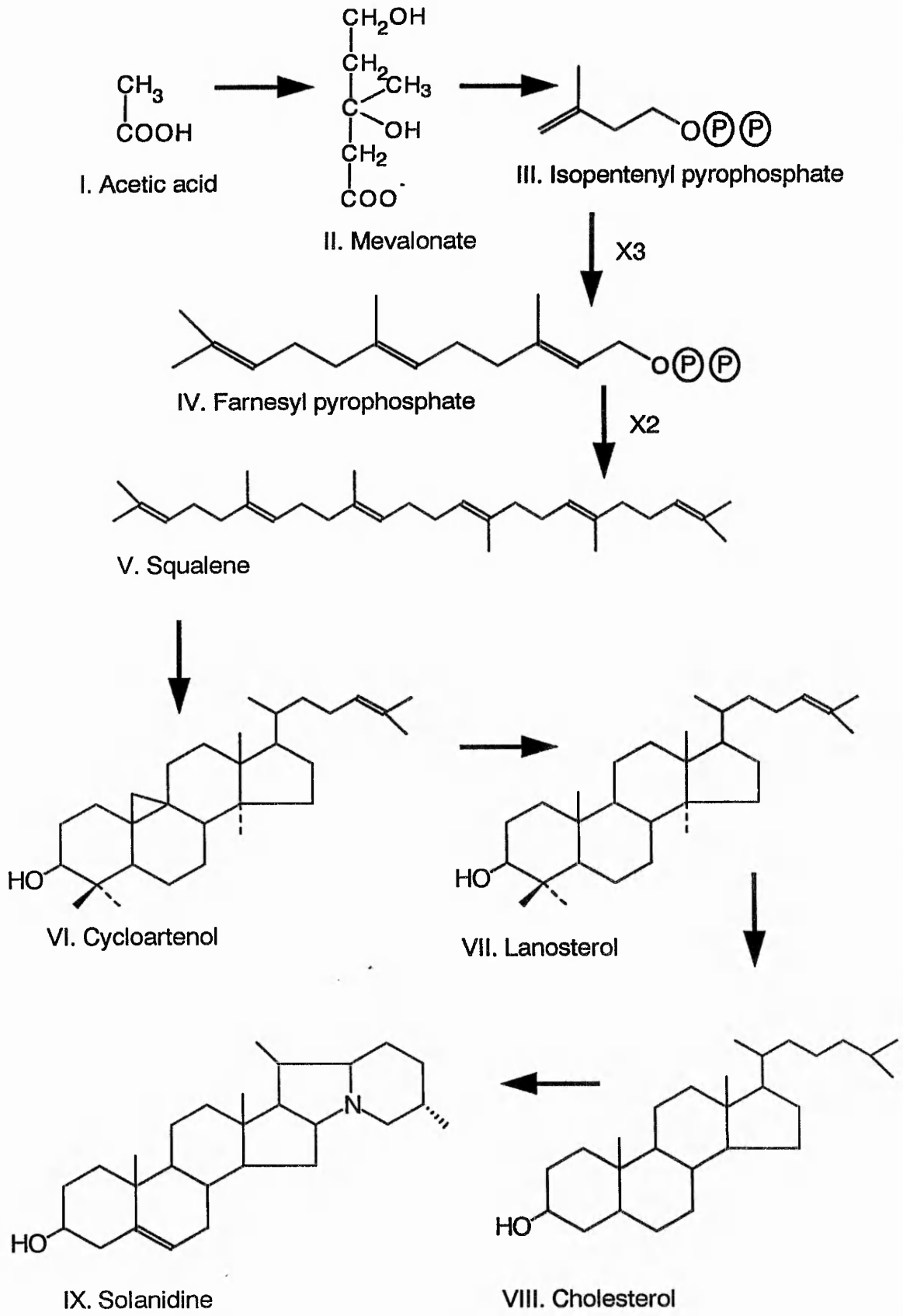


Figure 1.2 - Suggested pathway for the biosynthesis of solanidine (Maga, 1980).

1.3.1 - Causes of glycoalkaloid accumulation and their role in the potato.

Many factors have been suggested as affecting total glycoalkaloid (TGA) concentrations in potato tubers which range from pre- to post-harvest. Studies have been made of many environmental regimes, although rarely in a systematic manner.

Glycoalkaloids are found in all parts of the potato plant with the flowers possessing the highest concentrations, followed by the leaves, shoots, roots, stems and then tubers (Jadhav and Salunkhe, 1975; Kozukue, Kozukue and Mizuno, 1987; Friedman and Lao, 1992). Distribution of TGA within the cells of the potato is less well studied but it has been suggested that they are stored mainly in the soluble fraction of the cell (Roddick, 1976). However, it is uncertain whether the cytoplasm, vacuole or indeed both are involved. This is supported by Zimowski (1992) who found 2 sterol glucosyltransferases active on solanidine, one in the cytosol and one membrane-bound, but the cytosolic enzyme was by far the more active.

1.3.1.1 - Pre-harvest effects on glycoalkaloid accumulation: The initial, and possibly most significant, factor influencing TGA concentrations in tubers is the cultivar (cv.). A number of workers have investigated TGA in many different varieties (e.g. Wolf and Duggar, 1946; Uppal, 1987; Dale, Griffiths and Bain, 1992; Dale, Griffths Bain and Todd, 1993). Results have demonstrated that a wide range of TGA concentrations exist, although most varieties are within the range 30-100 $\mu g\ g^{-1}$ fresh weight (FWT). It is probable that the variation is related to the level of hybridisation with wild tuber-bearing species that has occurred in the breeding of a cultivar. Backcrossing has been found to cause both very high and very variable TGA concentrations in progeny (Deahl, Sinden and Young, 1993; Sanford, Deahl, Sinden and Kobayashi, 1995). Indeed, there have been a number of occasions where cvs. have not been released commercially or have had to be withdrawn, e.g. cv. Lenepe (Zitnak and Johnston, 1970) and cv. Magnum Bonum (Hellenas, Branzell, Johnsson and Slanina, 1995a).

Solanidine, unlike many other steroids has a nitrogen containing heterocyclic unit, and this has led to studies on tuber TGA concentrations from plants grown at different rates of nitrogen. Some confusion exists in the literature, with reports that increasing nitrogen application enhances TGA accumulation in tubers (Mondy and Munshi, 1990; Love, Herrman, Thompson-Johns and Baker, 1994), while others only found increases in the aerial parts of the plant (Ahmed and Muller, 1979).

Ahmed and Muller also found that TGA in all parts of the plant decreases towards maturity. High TGA in immature and small tubers has been well documented (Bomer and Mattis, 1924; Wolf and Duggar, 1946), although this is, at least in part, due to the higher surface area to volume ratio of smaller potatoes. The consequences of this are that early potato varieties often have comparatively high TGA concentrations (Hellenas, Branzell, Johnsson and Slanina, 1995b), which is of special importance as early potatoes are often eaten without peeling.

Insect damage to the aerial parts of the potato plant can also result in increased tuber TGA (Hlywka, Stephenson, Sears and Yada, 1994). Although this increase is probably due to injury, defoliation by Colorado beetle (*Leptinotarsa decemlineata*, Say) caused significantly higher tuber TGA than that by potato leafhopper (*Empoasca fabae*, Harris). Exposure of potatoes to sunlight in the field due to insufficient soil cover may also result in a significant rise of TGA accumulation (Hilton, 1951).

A number of studies have examined the effects of the weather conditions during the growing season on tuber TGA concentrations (Bomer and Matis, 1924; Hutchinson and Hilton, 1955; Sinden and Webb, 1972). However, results have not been unequivocal, but it does appear that stress in general to the parent plant can result in high tuber TGA concentrations (Friedman and McDonald, 1997).

1.3.1.2 - Post-harvest effects on glycoalkaloid accumulation: Several possible causes for increased TGA accumulation following harvest exist, ranging from mechanical injury to inadequate storage facilities.

Just as insect damage to the aerial parts of the potato plant cause an increase in TGA, so can mechanical damage to the tuber (Sinden and Webb, 1974). Injury due to careless harvesting and handling even when not visible to the naked eye causes TGA to accumulate in the tissues surrounding the wound (Ishizaka and Tomiyama, 1972). The enhanced glycoalkaloid production appears to be solely a defence response (Bergenstrahle, Tillberg and Jonsson, 1992b), although it has been suggested that it is a result of a general increase in membrane sterol synthesis (Hartmann and Benveniste, 1974). Initiation of TGA synthesis as a result of wounding begins within 24 hr of damage occurring (Shih, Kuc and Williams, 1973; Bergenstrahle *et al.*, 1992b), with the degree of TGA accumulation being governed by the severity of the injury (Olsson, 1986).

The most widely acknowledged cause of TGA increase in harvested tubers is, as stated above, exposure to light. This can occur at many times during the packing, storage and processing/retailing of a potato. A host of publications have examined this problem but much confusion still exists, and there are many contradictions in the literature. Studies are seldom comparable due to significant differences in sampling regimes or expression of results. Although it has been demonstrated unequivocally that prolonged exposure to broad spectrum light increases TGA accumulation.

A number of studies have made use of filters to investigate the effect of light quality on TGA concentration. The earliest of these showed blue light to have the greatest effect on accumulation (Conner, 1937), while red light had none. More recent studies using narrow bandwidth filters have contradicted this finding. Peterman and Morris (1985) found the spectral response curve of TGA accumulation to follow the shape expected by a phytochrome-mediated response but the peaks of activity were at 430 and 645 nm, this represents a shift towards the blue end of the spectrum of 30 and 45 nm. Different light sources have also been shown to effect TGA due to their differing spectral composition, with sodium lamps causing the highest accumulation of TGA followed by fluorescent lighting, with mercury lamps having a very minor effect (Percival, Dixon and Sword, 1994).

TGA concentrations in tubers increase in proportion to light intensity (Gull and Isenberg, 1958; Yamaguchi, Perdue and MacGillivray, 1960; Patil, Salunkhe and Singh , 1971) but the upper limit of this is uncertain, with Gull and Isenberg (1958) finding no increase at intensities above 50 foot candles (fc) while Patil *et al.* (1971) observed a response up to 100 fc with degradation of TGA at intensities above this. Percival and Dixon (1996) analysed TGA content of 6 cvs. exposed to between 0 and 1500 µmol PAR m^{-2} s^{-1}. The results were found to be markedly cv. specific but 5 of the 6 cvs. demonstrated no increase in TGA at light intensities above 1000 µmol PAR m^{-2} s^{-1} and 3 cvs. showed no increase when exposed to 750 µmol PAR m^{-2} s^{-1} or higher.

The influence of storage temperature on TGA concentration has received very little attention. There has been some suggestion that low temperatures increase TGA (Hilton, 1951). This is probably a result of low temperature stress to the tubers. Even less knowledge exists of the effect of temperature on light-enhanced TGA accumulation. Percival, Harrison and Dixon (1993) investigated the effect of 2 storage temperatures on subsequent light exposure accumulation, but found no effect. However, no attempt was made in that study to examine the interaction between temperature and the light exposure itself.

1.3.1.3 - Glycoalkaloids in disease and pest resistance: It has been demonstrated that potato glycoalkaloids are toxic to many forms of life including viruses (Thorne, Clarke and Skuce, 1985), fungi (Allen and Kuc, 1968), molluscs (Johnston and Pierce, 1994), insects (Wierenga and Hollingworth, 1992; Jonasson and Olsson, 1994) and mammals (Maga, 1980). It has, therefore, been assumed that the function of glycoalkaloids is in defence.

Experiments on the antifungal activity of glycoalkaloids have established that α-chaconine is more toxic than α-solanine and that there is a synergistic effect between them (Fewell and Roddick, 1993). This was shown to be effective over a wide range of ratios, even as low as 10-20% of

either molecule. The study also demonstrated that growth of *Rhizoctonia solani*, a potato pathogen causing dry rot, is inhibited far less by the 2 glycoalkaloids than non-pathogenic species such as the saprophytic *Ascobolus crenulatus*.

Shih *et al.* (1973) found that TGA accumulation was reduced when tuber slices were inoculated with *P. infestans*. This was accompanied by a large increase in rishitin, a sesquiterpenoid phytoalexin. Both compatible (causing little or no resistant reaction) and incompatible (producing a strong resistant reaction) races of *P. infestans* caused this effect but it was greater with the incompatible races.

Application of free sterols shortly before inoculation with incompatible races of *P. infestans* almost completely inhibits rishitin accumulation and leads to a slight increase in TGA (Mucharromah, Burton and Kuc, 1995). Exogenous arachidonic acid will elicit the rishitin accumulation/TGA suppression response (Bostock, Nuckles, Henfling and Kuc, 1983), however, application of free sterols with arachidonic acid has no effect on this response unless tuber slices are also inoculated with *P. infestans*.

These studies indicate that, while TGA appears to play a role in determining the pathogenicity of fungi towards tubers, they do not have a significant role in the resistance of potatoes to compatible fungal races.

A number of studies have also examined the role of TGA in insect resistance. Potato plants selected for resistance to potato leafhopper (PLH) were found to more than double foliar TGA concentrations within 7 generations (Sanford, Deahl, Sinden and Ladd, 1990). Sanford, Domek, Cantelo, Kobayashi and Sinden (1996) fed various *Solanum* glycoalkaloids to PLH adults. At the highest concentration used (0.27%) all the glycoalkaloids tested resulted in near complete mortality within 72 h, whilst at lower concentrations α-chaconine was much more toxic than α-solanine. Although at the lowest concentration (0.03%) both potato glycoalkaloids caused around 10% mortality.

Glycoalkaloids have also been shown to have a role in resistance to Colorado potato beetle (CPB), with high foliar concentrations of α-solanine

and α-chaconine causing up to 50% feeding inhibition (Sinden, Cantelo, Sanford and Deahl, 1991). The use of somatic hybrids to introduce glycoalkaloids from *S. chacoense* to *S. tuberosum* resulted in plants highly resistant to CPB (Cheng, Saunders and Sinden, 1995). However, this is likely to result in tubers unfit for human consumption as breeding for PLH resistance was found to significantly increase tuber TGA content (Sanford *et al.*, 1992).

Resistance of tubers to wireworm (*Agriotes obscurus*) is also, at least in part, due to TGA (Jonasson and Olsson, 1994) and it has been suggested that breeding tubers with high concentrations of TGA in the peripheral layers would give significant reductions in crop losses from this pest (Olsson and Jonasson, 1995).

1.3.2 - Glycoalkaloid poisonings and toxicity in man.

Glycoalkaloid poisonings are only rarely reported and until recent years have been largely ignored. As early as 1933 this danger was noted with the suggestion that many more mild poisoning cases were occurring but being mistaken for gastro-enteritis (Willimott, 1933). Willimott reported that a family of Cypriots had suffered symptoms including gastro-enteritis, abdominal pain, vomiting, depression and fever (temperatures of 40°C were observed). One person died as a result of the poisoning.

More recently, glycoalkaloid poisoning was reported in 78 schoolchildren who suffered similar symptoms to the above but with the addition of convulsions and hallucinations (Anon, 1979). The children were treated in a modern hospital and no deaths occurred.

In both of the above cases symptoms did not appear for approximately 12 hours after consumption of the glycoalkaloids, which is unusual for plant toxins (Oehme, 1978). Despite this it is thought that glycoalkaloids are more toxic to humans than to many other animals. Morris and Lee (1984) calculate from published reports of poisonings that the lethal dose is about 3-6 mg kg^{-1} body weight. This is comparable to strychnine and

arsenic, 5 mg kg^{-1} and 8 mg kg^{-1}, respectively. The acute toxic dose has been estimated as only 1.75 mg kg^{-1} body weight (van Gelder, 1990).

Glycoalkaloids exhibit 2 toxic effects, one disrupting phospholipid membranes (Roddick, 1974) and the other inhibiting acetylcholinesterase (Orgell, Vaidya and Dahm, 1958). The latter accounts for the depression of the central nervous system and neurological effects observed during poisoning. Roddick and Rijnenberg (1986) demonstrated that α-chaconine individually has a far greater effect on liposomes than α-solanine. They later found that the 2 molecules have a strong synergistic effect (Roddick and Rijnenberg, 1987). It has also been demonstrated that solanidine is much less toxic than its glycoside derivatives (Rayburn, Bantle and Friedman, 1994), so it is apparent that the carbohydrate moiety plays a most important role in glycoalkaloid toxicity.

A comprehensive study of glycoalkaloid mode of action on biomembranes has been carried out (Keukens, de Vrije, van den Boom, de Waard, Plasman, Thiel, Chupin, Jongen and de Kruijff, 1995) and a model produced (Fig. 1.3). It is thought that the aglycone forms a reversible complex with membrane sterols in a 1 to 1 ratio, followed by linking, due to hydrogen bonding, of the sugar moieties of the glycoalkaloids as their concentration in the membrane increases. The resulting stable matrix immobilises its constituent sterols which causes the sterols in the inner layer of the membrane to flip into the matrix. This causes the membrane to bud and eventually break apart. The toxicity is, therefore, dependant on the linking of the glycosides. This linkage is much more efficient between α-chaconine glycosides than α-solanine glycosides, explaining the greater toxicity of α-chaconine. However, a marked synergistic effect between the 2 molecules was observed, due to strong linkage between α-solanine and α-chaconine glycosides. Furthermore, any glycolipids present within the membrane interact with the glycoside, prolonging the duration of the sterol/aglycone complex thereby increasing the possibility of glycoside linkage and increasing toxicity.

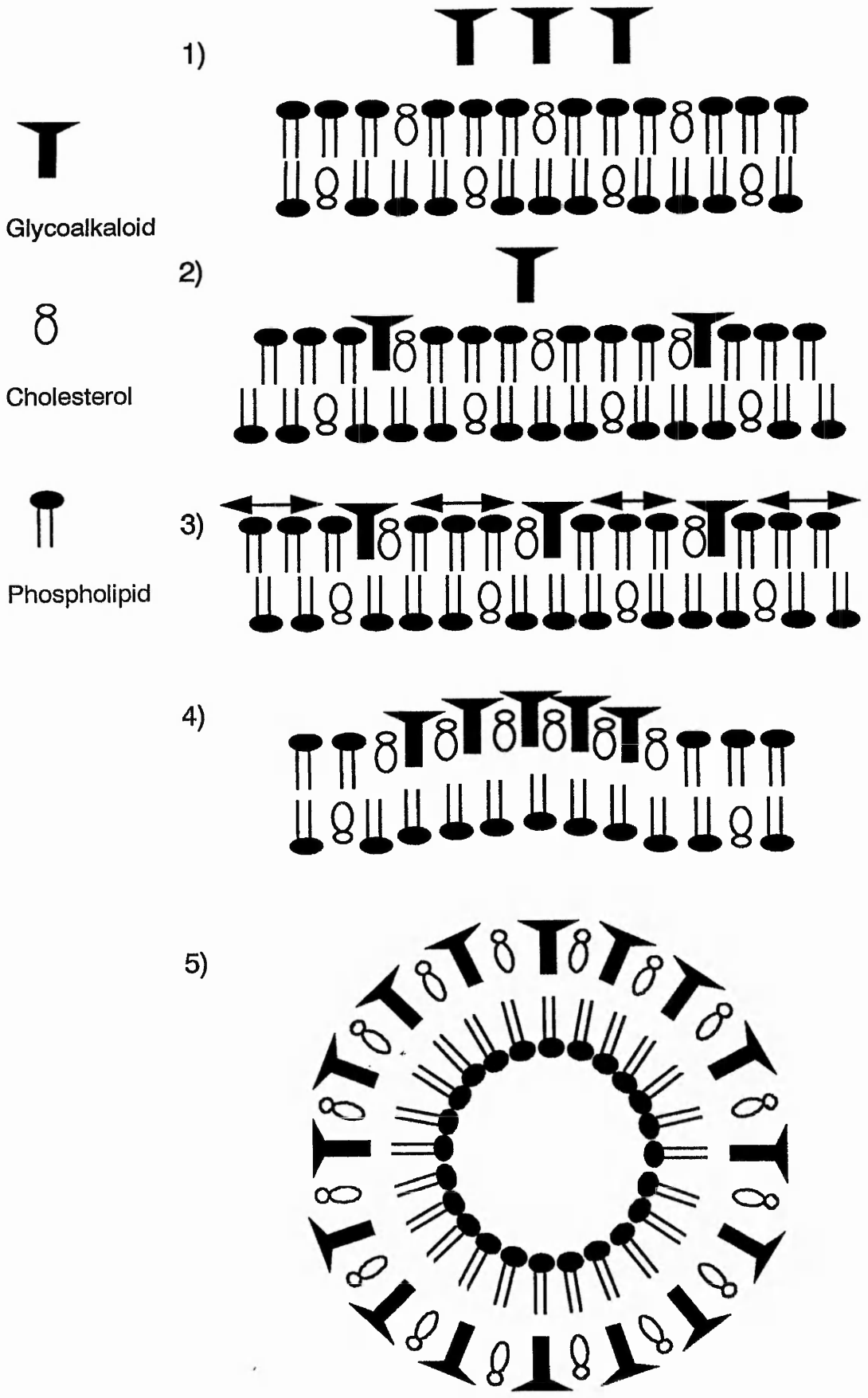


Figure 1.3 - Suggested model for glycoalkaloid disruption of biomembranes (after Keukens *et al.*, 1995).

The current interest in glycoalkaloids was started in the early 1970's when examination of data in the UK and USA led to the hypothesis that eating potatoes infected with *Phytophthora* was correlated with ancephaly and spina bifida (Renwick, 1972). However, this was not supported by feeding experiments in animals and attempts to repeat Renwick's work in other parts of the world were unsuccessful (Field and Kerr, 1973; Kinlen and Hewitt, 1973). Also, a limited clinical trial involving pregnant women avoiding consumption of potatoes showed fewer incidents of spina bifida than in the control group (Nevin and Merrett, 1975).

Studies in following years using specific potato glycoalkaloids showed abnormalities in rats (e.g. Swinyard and Chaube, 1973). A later study refuted this but observed that at high doses resorption of the foetus occurred (Chaube and Swinyard, 1976) and it was concluded that consumption of potatoes did not give any risk of developmental abnormalities (Harvey, Morris, McMillan and Marks, 1986). However, Keeler, Young and Brown (1976) found cranial abnormalities in 30% of hamster offspring when solasodine (a *Solanum* alkaloid very similar in structure to solanidine) was fed to the parent animals. This was repeated with other solanidane alkaloids (Gaffield and Keeler, 1996) and it was found that solanidine induced significant numbers of terata. Furthermore, Friedman, Rayburn and Bantle (1991, 1992) found developmental abnormalities and embryo toxicity when frog embryos were exposed to glycoalkaloids. Evidently the possibility of potato glycoalkaloid teratogenicity is still uncertain, however, it is unlikely that concentrations found in non-sprouting tubers could have any effect (van Gelder, 1989).

The publicity surrounding the possible teratogenicity of potatoes has led to a much greater awareness of glycoalkaloid toxicity and an increase in its study. Hopkins (1995) believes that toxicity testing of glycoalkaloids is still inadequate and advocates full toxicity trials equivalent to those needed for food additives. This is especially important as the current arbitrary limit for TGA of 200 $\mu g\ g^{-1}$ has little scientific basis and dates from the early 1920's (Bomer and Mattis, 1924).

1.4 - Photosynthetic Pigments in Potato Tubers

1.4.1 - The greening process.

When exposed to light, potato tuber cells initiate a greening process which involves the transformation of amyloplasts to chloroplasts (Anstis and Northcote, 1973). Although there is much data on the development of chloroplasts from etioplasts, very little is known about the transformation of amyloplasts. Both biochemical and ultrastructural changes are involved including the development of thylakoids and associated pigment-protein complexes, such as the photosystems (PS).

The chlorophyll molecule consists of 2 major units, a tetra-pyrrole ring with a central magnesium atom and a phytol group (Fig. 1.4). Chlorophyll b differs from chlorophyll a in the possession of a formyl group instead of a methyl group at position 3

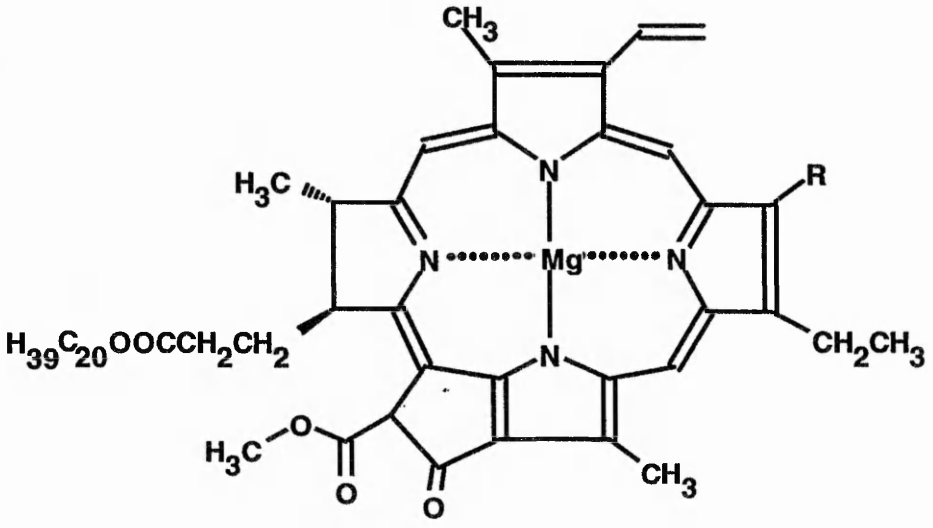


Figure 1.4 - The chemical structure of chlorophyll. R is a methyl group in chlorophyll a and a formyl group in chlorophyll b.

Synthesis of the phytol group is via the acetate-mevalonate pathway, from geranylgeranyl pyrophosphate (Fig. 1.5), while the tetra-pyrrole ring is formed from glutamic acid via 5-aminolevulinic acid. The final step in chlorophyll production is the conversion of protochlorophyllide (Pchl) to chlorophyll, which in higher plants is performed by a photochemical reaction (von Wettstein, Gough and Kannangara, 1995). The presence of Pchl in the roots of dark grown plants has been demonstrated both in monocots and dicots (McEwen and Lindsten, 1992). It is possible then that Pchl is present in potato tubers, enabling rapid production of Chl.

There is a lag period of about 20-24 h after illumination of the tuber before any chlorophyll or change in amyloplast structure can be detected (Anstis and Northcote, 1973; Zhu, Merkle-Lehman and Kung, 1984). This delay in transformation is common in plastids not specialised for photosynthesis (Rascio, Pasqua, Dalla Vacchia and Casadoro, 1990). Following the lag phase, both chlorophyll and ribulose bisphosphate carboxylase/oxygenase (RuBisCO) are detectable (Zhu *et al.*, 1984). Within the next 24 h activity of a PS I component, thought to be cytochrome f, has been observed (Muraja-Fras, Krsnik-Rasol and Wrischer, 1994). Other elements of the photosynthetic apparatus isolated at this time have included thylakoid membrane proteins and parts of the PS II reaction centre. Zhu *et al.* (1984) examined 3 week old chloro-amyloplasts for PS II activity, the positive result provided some evidence that the newly formed chloro-amyloplasts were capable of generating electrons for use in photosynthesis.

An increase in plastid DNA correlated with the greening transformation has also been observed in tuber tissues (Conover and Pryke, 1987), a process previously found in greening leaves (Boffey, 1985).

It is probable that control of the initiation of greening is by phytochrome, in common with other tissues. Indeed, if tubers are given a pulse of red (R) light before being exposed to white light there is approximately a 100% increase in chlorophyll formation after 72 h (Morris, Graham and Lee, 1979). This increase can be reversed by following the R light by a far-red (FR) pulse. However, this study demonstrates only that

phytochrome is likely to control the lag phase before chlorophyll synthesis begins which may explain why other workers have found blue light to be more effective than red when measuring chlorophyll formation after several days (Conner, 1937; Anstis and Northcote, 1973; Virgin and Sundqvist, 1992). Also, Koukkari and Hillman (1966) found no phytochrome in tuber tissue other than active eyes, but further examination with modern equipment would be needed to confirm this.

1.4.2 - The role of carotenoids in plants and their occurrence in tubers.

Potatoes contain a number of free carotenoids and their diesters, including lutein, lutein-5, 6-epoxide, β-carotene, neoxanthin, violaxanthin, and antheraxanthin (Tevini and Schonecker, 1986). These are present in ungreened tubers and cause the yellow appearance of potato flesh.

Carotenoids are also produced via the acetate-mevalonate pathway (Fig. 1.5), being based on the condensation of 2 geranylgeranyl pyrophosphate molecules to form the C_{40} phytoene (Packter, 1973). Xanthophylls are formed by the addition of an oxygen atom, as an hydroxy, oxo, epoxy or methoxy group, to the carotene hydrocarbons.

In leaves carotenoids have several functions, which include light harvesting, photoprotection, scavenging of activated oxygen, quenching of triplet state chlorophyll and dissipation of excess excitation energy (Pallett and Young, 1993).

While the majority of chloroplast carotenoids are associated with the light harvesting complexes and are thought to transfer absorbed light energy to chlorophyll very efficiently (Gust, Moore, Benasson, Mathis, Land, Chachaty, Moore, Liddell and Nemeth, 1985) possibly their most important role is that of photoprotection and energy dissipation.

Over the last 10 years understanding of the processes leading to loss of excess excitation energy, namely the xanthophyll cycle has been greatly enhanced (Demming-Adams and Adams, 1996). In excess light, violaxanthin is de-epoxidised via antheraxanthin to zeaxanthin which is involved in returning excited singlet chlorophyll to the ground state

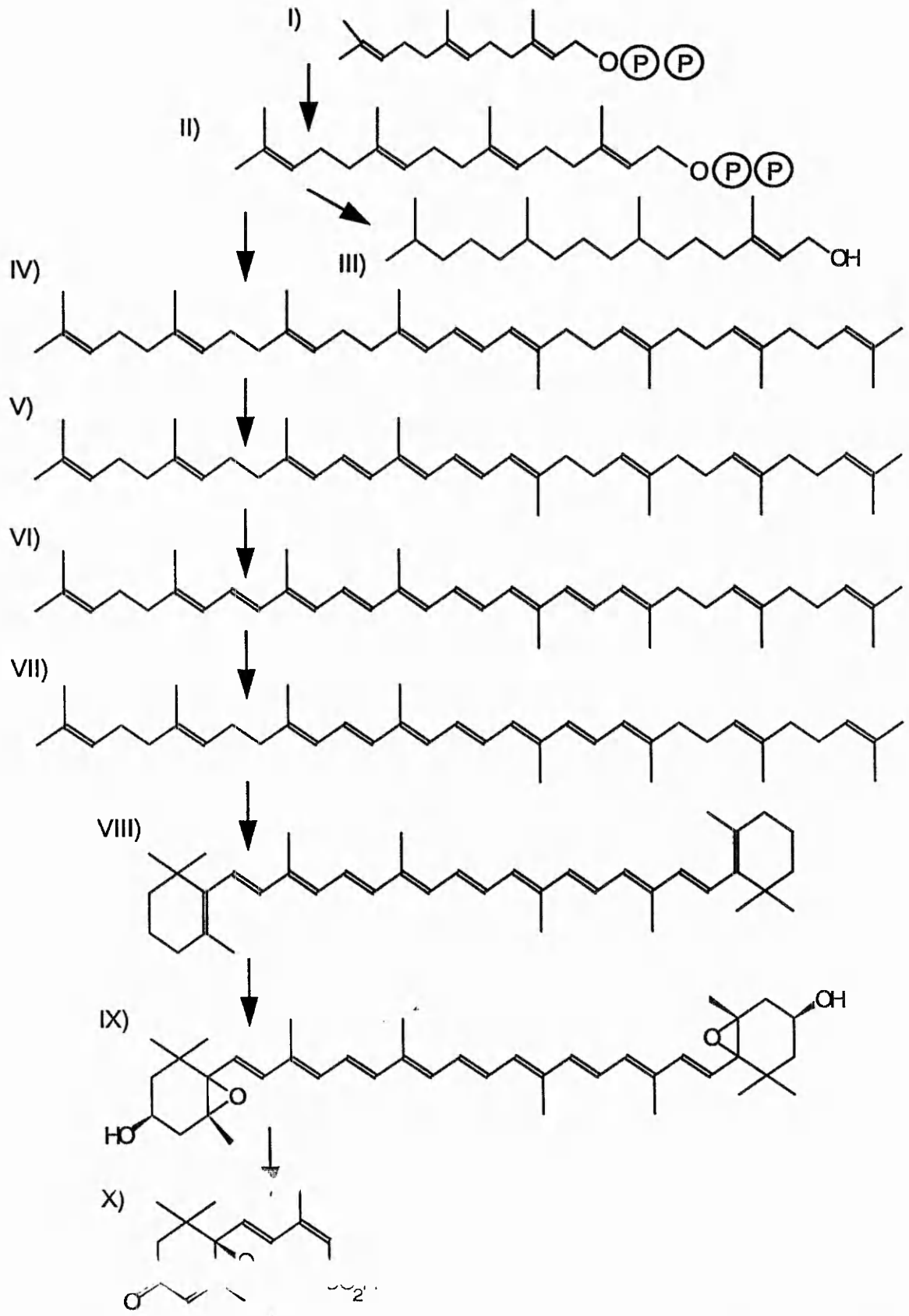


Figure 1.5 - Simplified carotenoid biosynthesis pathway. I) Farnesyl pyrophosphate, II) geranylgeranyl pyrophosphate, III) phytol, IV) phytoene, V) phytofluene, VI) ζ–carotene, VI) neurosporene, VII) lycopene, VIII) β-carotene, IX) violaxanthin and X) abscisic acid.

(Demming-Adams, 1990). During times of low photon flux density this process is reversed (Fig. 1.6).

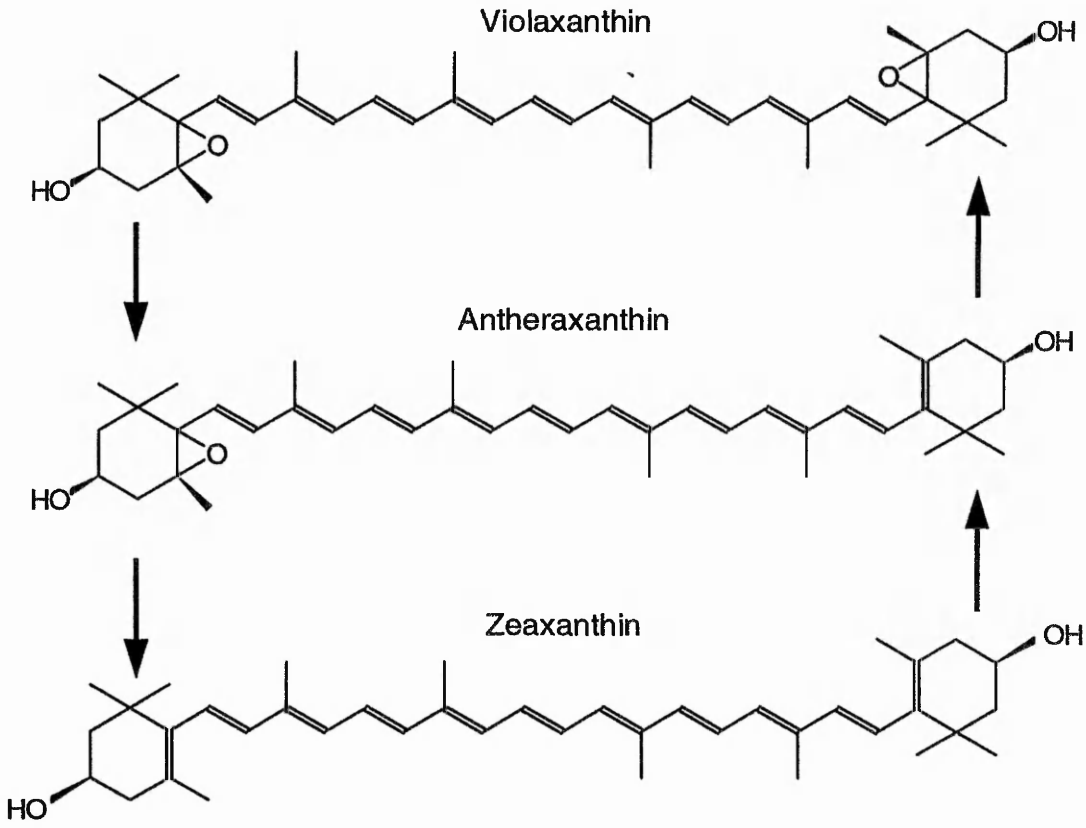


Figure 1.6 - Schematic of the xanthophyll cycle. Excess light causes the stepwise de-epoxidation of violaxanthin through antheraxanthin to zeaxanthin. During periods of low light this process is reversed.

Almost all environmental stress will lower a plant's photosynthetic rate, this requires that photoprotection be increased to avoid damage to the cell. Indeed a number of stresses have been shown to increase zeaxanthin accumulation (Morales, Abadia and Abadia, 1990; Demming-Adams and Adams, 1992).

The role of carotenoids in potato tubers is much less clear, with studies of tuber carotenoids being confined to measurement of changes in content. Storage of potatoes results in a decrease of free xanthophylls concomitant with an increase of xanthophyll diesters (Tevini, Schonecker,

Iwanzik, Riedman and Stute, 1984a). However, changes in total carotenoid concentrations during storage were found to increase or decrease depending on variety.

1.5 - Aims of the Investigation

The central aim of this study was to confirm or deny the proposed correlation between chlorophyll and glycoalkaloid syntheses in potato tubers (Dale *et al.*, 1993). This was to be achieved firstly, by optimisation of existing methods of glycoalkaloid and pigment analysis for potato tubers. Secondly, to establish the relationship between temperature and greening/TGA synthesis. Thirdly, to investigate the response of glycoalkaloid accumulation to dark storage in a number of varieties and finally, to establish if duration of storage has any effect on the potential of tubers to green once exposed to light.

CHAPTER 2 - OPTIMISATION OF PIGMENT AND GLYCOALKALOID ANALYSES

2.1 - Introduction

The presence of toxic glycoalkaloids in potatoes was first observed 170 years ago (Baup, 1826). However, the earliest methods of analysis were gravimetric and incapable of separating the different solanidine based glycoalkaloids. Consequently it was thought that only one glycoalkaloid, solanine, was present in potato tubers. Since this time there have been many developments and refinements in TGA analysis. Gravimetric methods were replaced by colorimetric procedures, which in turn have been replaced by sophisticated modern, mainly chromatographic, techniques (Maga, 1994). The use of these led to the discovery in the 1950's that solanine actually consisted of two different triglycerides, α-solanine and α-chaconine (Kuhn and Low, 1954).

Accurate pigment analysis predates this and spectrophotometric techniques based on the work of McKinney (1941) have been used for many years. However, potato tuber tissues are different in a number of ways to those of leaves and the measurement of chlorophyll from these poses unique problems, especially in the consideration of sampling regimes.

There are many variables that affect the concentrations of both photosynthetic pigments and glycoalkaloids in potatoes. The study of these requires reliable and reproducible methods of analysis to enable accurate comparison between experiments. This chapter describes the optimisation of methodology for pigment and glycoalkaloid analysis from potato tubers.

2.2 - Materials

Potato tubers (cv. King Edward) were purchased from a local supermarket and stored in darkness at room temperature for approximately 24 hr before use.

2.3 - Spectrophotometric Determination of Photosynthetic Pigments

2.3.1 - Introduction to tuber pigment analysis.

The major difficulty in accurately estimating tuber pigment concentrations is that even a severely greened tuber contains only a fraction of leaf Chl content. Initial Chl production is mainly in the outer layers of the tuber, although greening will eventually spread throughout the tuber. A short period of light exposure allows samples of the outer tuber cortex to be used rather than whole tubers, thus reducing dilution of the pigment in the sample with ungreened tissues.

The most commonly used procedures for spectrophotometric detection of photosythetic pigments use 80% acetone as an extraction medium and have been derived from the methods of McKinney (1941) and Arnon (1949). However, it has been reported that *N,N*-dimethylformamide (DMF) is a more efficient extraction medium for tissues with a low pigment concentration than 80% acetone (Moran and Porath, 1980).

2.3.2 - Methods.

Potato tubers were exposed to daylight for 14 d to ensure that TGA concentrations were enhanced. Mean daily maximum photosynthetically active photon flux density (PPFD) was 150 $\mu mol\ m^{-2}\ s^{-1}$.

A 6 mm diameter stainless steel cork borer was used to obtain 3 cores at random from each potato. The outer 10 mm, including the periderm, from each core was combined, frozen in liquid nitrogen and stored at -20°C.

Samples were taken from both the upper and lower surfaces of each tuber, then the central parts of each core were also combined and frozen. All subsequent procedures were performed at 4°C in the dark.

Samples were either extracted in 80% aq. acetone (v/v) with approximately 2 mg magnesium carbonate and 1 mg sodium bisulfite g^{-1} sample or 100% DMF. A DU-7 UV/Vis scanning spectrophotometer (Beckman Instruments, High Wycombe, UK) was used to measure the absorbance of acetone samples at 710, 663, 645 and 480 nm. DMF extract absorbance was measured at 710, 664 and 647 nm.

Chls *a* and *b* in acetone extracts were calculated according to the formulae of Lichtenthaler and Wellburn (1983) and total carotenoid concentration was estimated using the equations of Hendry and Price (1993). Chl concentrations in DMF extracts were calculated according to Inskeep and Bloom (1985).

Absorbance at 710 nm was subtracted from all other readings as an estimate of sample turbidity. Pigment concentration was given by:

Pigment=(Y.v/l)/weight

Where v=extraction volume and l=pathlength of cuvette.

For acetone extracts Y was given by:

Chl *a* (mg cm^{-3})=12.21 A_{663}-2.81 A_{645}

Chl *b* (mg cm^{-3})=20.13 A_{645}-5.03 A_{663}

Carotenoids (µmol g^{-1})= $((A_{480}+0.114.A_{663}-0.638.A_{645}).V.1000)/112.5.wt$

For DMF extracts Y was given by:

Chl *a* (mg cm^{-3})=12.70 $A_{664.5}$-2.79 A_{647}

Chl *b* (mg cm^{-3})=20.70 A_{647}-4.62 $A_{664.5}$

Mg cm^{-3} were converted to µmol g^{-1} using conversion factors of 1.119 for Chl a and 1.102 for Chl b (Hendry and Price, 1993).

2.3.3 - Results.

No detectable Chl was found in tuber tissues more than 10 mm below the skin, confirming that for light exposures of 14 days or less only the outer

10 mm of the tuber need be used for Chl samples. Indeed, it was observed that visual Chl occurred in only the outer 3-4 mm of the tuber.

Pigment extraction with DMF resulted in estimations of Chl content, especially Chl *a*, that were very variable compared with acetone extracts (Table 2.1). Therefore, 80% acetone was used for all subsequent spectrophotometric Chl analyses.

Table 2.1 - Chl *a* and *b* concentrations in acetone and DMF extracts. Mean Chl (μmol g^{-1} sample) ± SE, n=3

Extraction Medium	**Upper Surface**		**Lower Surface**	
	Chl *a*	**Chl *b***	**Chl *a***	**Chl *b***
80% Acetone	3.97±0.47	3.20±0.71	2.37±0.40	3.02±0.57
DMF	3.91±1.35	2.31±0.69	0.54±0.24	1.18±0.12

2.4 - Determination of Glycoalkaloids

2.4.1 - An introduction to existing methods of TGA analysis.

The potential toxicity of potatoes demonstrates the importance of accurate and reliable methods of analysis for tuber TGA. Modern commercial practice also necessitates that these methods be rapid and inexpensive. There are many methods for TGA analysis reported in the literature including mass-spectrometry (Chen, Derrick, Mellon and Price, 1994; Abell and Sporns, 1996), isotachyphoresis (Kvasnicka, Price, Ng and Fenwick, 1994), thin layer chromatographic scanning (Ferreira, Moyna, Soule and Vazquez, 1993), various colorimetric methods, gas chromatography (Lawson, Erb and Millar, 1992), counter-current chromatography (Fukuhara and Kubo, 1991), high-pressure liquid chromatography (HPLC) and enzyme immuno-assays. Each method has relative advantages and disadvantages, such as lack of sensitivity, a need for derivatisation, expensive chemicals or excessively long preparatory steps. Currently the most realistic methods utilised are based on HPLC and

immunoassays. The latter offers the possibility of a sensitive, simple, rapid and relatively cheap detection method. Indeed, Kamps-Holtzapple and Stanker (1996) have recently generated *E. coli* strains that produce recombinant antibodies to solanidine glycoalkaloids, thus negating the need for further use of animals or animal cells. This, combined with the relative ease of bacterial fermentation, could lead to a widely acceptable and low cost antibody-based test. However, polyclonal antibodies reported in the literature show a lack of specificity or immunological response (Morgan, McNeney, Matthew, Coxon and Chan, 1983; Plhak and Sporns, 1992; Plhak and Sporns, 1994) and the monoclonals that have been raised to date, although specific to solanidine-based compounds, cannot distinguish between individual glycoalkaloids, making them unsuitable for some studies (Plhak and Sporns, 1994; Stanker, Kamps-Holtzapple and Friedman, 1994).

There are a great many published HPLC methods due to the proven ability of HPLC to separate and quantify potato glycoalkaloids. In addition, there is a wide availability of HPLC equipment and these methods have the advantage of speed and ease of use. The majority of reported HPLC methods use reverse-phase (RP) C_{18} or NH_2 sorbents with a mobile phase consisting of acetonitrile and a biological buffer, commonly phosphate.

Detection of glycoalkaloids presents a problem as the molecules do not possess a chromaphore, which necessitates the use of UV. The reduction in the sensitivity of an assay due to this is compounded by the UV maxima of both α-solanine and α-chaconine being at 202 nm, close to the limit of many detectors and also a wavelength at which both water and dissolved oxygen absorb strongly.

Extraction techniques and reported recoveries vary greatly. Indeed, with over 400 papers published in the last 25 years (Plhak and Sporns, 1994) there is much contradiction and confusion in the literature on TGA analysis.

2.4.2 - Extraction.

A 10 mm diameter cork borer was used to take samples from fresh tubers. These were cut in half and either (a) used fresh and ground in a pestle and mortar in 15 cm^3 extraction medium (0.02M heptane sulfonic acid in 1% aq. acetic acid (v/v) with 1 mg cm^{-3} sodium bisulphite) or (b) frozen in 10 cm^3 0.1% aq. polyvinyl-pyrrolidone (w/v) to -70°C, lyophilised and ground to a powder with a glass rod. Five hundred mg of the powder was mixed with 15 cm^3 of extraction medium.

The extract was homogenised with an Ultra Turrax TP18/10 with a 10N probe (Fisons, Loughborough, UK) for 30 s then centrifuged at 5,310 *g* in a MSE Chilspin (Fisons) at 4°C for 15 min. Samples were kept on ice throughout the procedure. All solutions were prepared with ultra-pure water from an Elga Maxima (Elga Ltd., High Wycombe, UK) and all chemicals were AnalaR grade unless otherwise stated.

2.4.3 - Purification and Concentration.

Ten cm^3 of the supernatant was applied to a solid phase extraction (SPE) column. A comparison was made between 4 commercially available SPE sorbents; Sep-Pak C_{18} (Waters, Watford, UK), Isolute mono-functional non-endcapped C_{18} (MF-NE), Isolute trifunctional non-endcapped C_{18} (TF-NE) and Isolute trifunctional endcapped C_{18} (TF-EC) (all from Jones Chromatography, Hengoed, UK). Four weights of the TF-EC sorbent (200, 500, 1000 and 2000 mg) were tested to determine optimal recoveries of spiked samples.

The columns were activated with 5-10 cm^3 methanol, according to sorbent volume, and equilibrated with 10 cm^3 of extraction medium. Potato extract was applied to the column and interfering constituents were removed with a protocol adapted from Friedman and Levin (1992). This consisted of a series of washes; 5 cm^3 water, 5 cm^3 0.05M ammonium bicarbonate, 5 cm^3 methanol-ammonium bicarbonate (50:50 v/v) and 5 cm^3 water. All washings were collected and tested for the presence of glycoalkaloids. The

glycoalkaloids were finally eluted with 4-8 cm^3 methanol-0.1M hydrochloric acid (80:20 v/v). The eluent was neutralised with 15 µl 2M NaOH per cm^3 and dried under vacuum with a Jouan RC 10.22 centrifugal evaporator (Tring, UK). The residue was resuspended with 1 cm^3 methanol-0.5M HCl (60:40 v/v) and 50 µl samples analysed by HPLC.

2.4.4 - Detection and quantification.

HPLC of TGA was performed using a System Gold 126 pump, 166 variable wavelength UV detector and 507 autosampler (all Beckman Instruments, High Wycombe, UK) controlled with a Viglen SL personal computer (Alperton, UK) using System Gold chromatography software (Beckman Instruments).

Eight RP columns, 6 C_{18} and 2 C_8 were evaluated (Table 2.2) using a method adapted from that of Jonker, Koops and Hoogendoorn (1992). The mobile phase was acetonitrile-0.01M Tris HCl buffer (40:60 v/v) adjusted to pH 7.8 with HCl. This was filtered through a 0.22 µm filter and degassed for 20 min under reduced pressure. The HPLC flow rate was 1.5 cm^3 min^{-1} and the detector was set at 202 nm.

Absorbance spectra of the individual glycoalkaloids dissolved in mobile phase was measured using a Lambda 12 UV/Vis scanning spectrophotometer (Perkin Elmer, Beaconsfield, UK). The absorbance maxima of α-solanine and α-chaconine were 202 and 201.5 nm, respectively (Fig 2.1).

The method was calibrated using α-solanine and α-chaconine standards (purity >99% Fluka, Gillingham, UK) dissolved in methanol-0.5M HCl (60:40 v/v). Standards were prepared at 6 concentrations by serial dilution 250, 100, 10, 5, 2.5 and 1.25 µg cm^{-3}. These were run 3 times and a calibration curve calculated for each glycoalkaloid using the mean integrated peak area of the 3 runs.

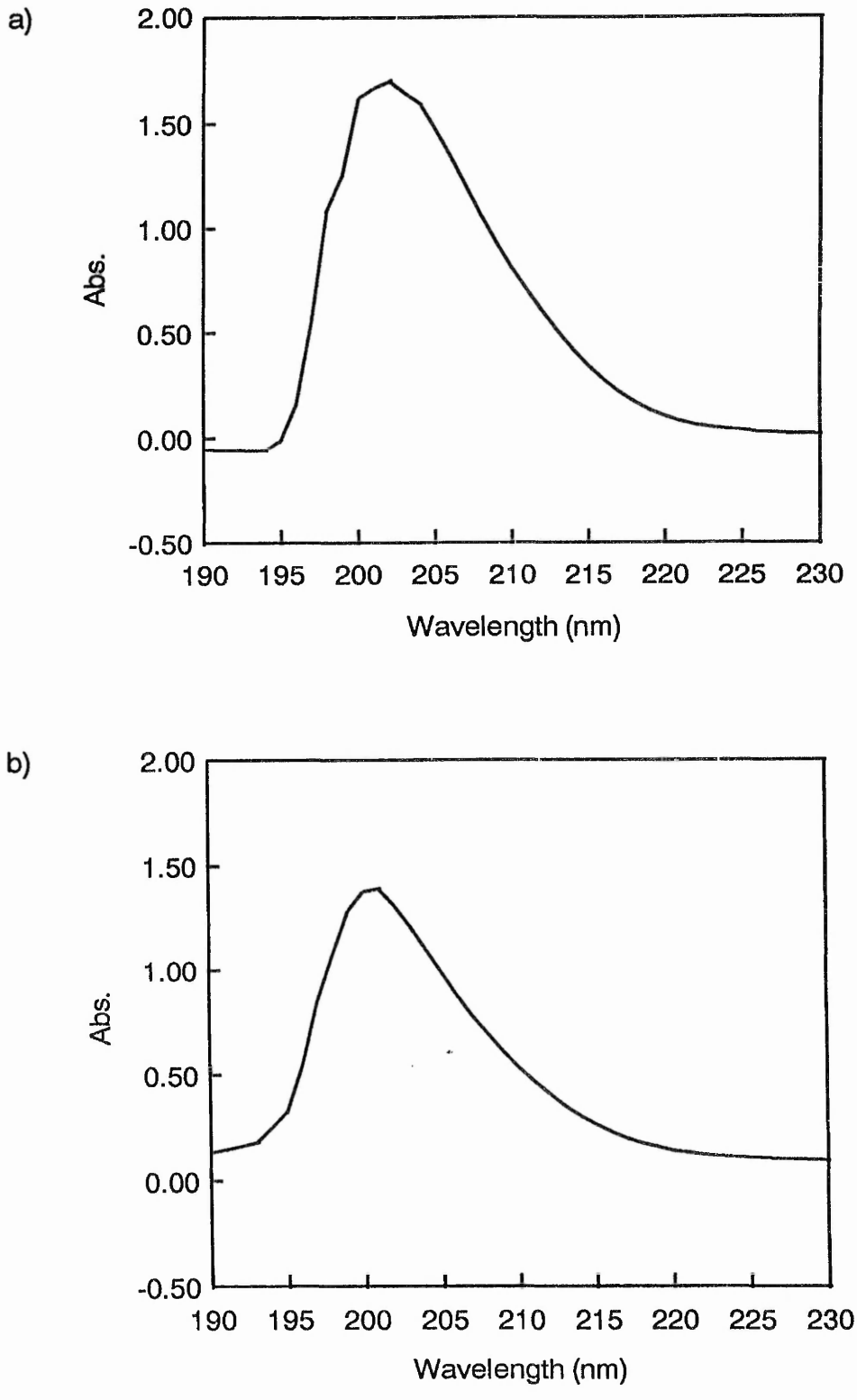


Figure 2.1 - UV spectra of a) 250 μg cm^{-3} α-solanine and b) 125 μg cm^{-3} α-chaconine.

Table 2.2 - HPLC columns used.

Sorbent	Particle Size	C_{18} / C_8	Manufacturer	Notes
μBondapak	5μ	C_{18}	Fisons, UK	
Spherisorb	5μ	C_{18}	Fisons, UK	
Techsphere 80	5μ	C_{18}	HPLC Technology, Macclesfield, UK	Low carbon
Techsphere 80	5μ	C_8	HPLC Technology, UK	Low carbon
Techsphere BDS	5μ	C_{18}	HPLC Technology, UK	
Ultrasphere	5μ	C_{18}	Beckman Instruments, UK	
Zorbax	5μ	C_{18}	Fisons, UK	
Zorbax	5μ	C_8	Fisons, UK	

The detector response was found to be non-linear, especially at low concentrations. The best fit was given by exponential and power equations for α-solanine and α-chaconine respectively. The calculated calibration equations were:

$$\alpha\text{-solanine (mg cm}^{-3}) = 1.5624 - 1.5612.e^{(-x/230.7153)}$$

$$\alpha\text{-chaconine (mg cm}^{-3}) = .0005 + 0.0090.x^{0.8808}$$

Where x=peak area. Actual and predicted concentrations using these equations were identical to 3 decimal places.

Recovery was estimated using internal standards of 0.25 mg per sample with 4 spiked and 4 control samples taken from the same tuber.

2.4.5 - Results

The use of freeze-dried powder gave recoveries with no significant difference to those from fresh tissue (data not shown). The highest recovery of 0.5 mg cm^{-3} standards was achieved using Isolute TF-EC columns (Table 2.3) with virtually 100% recovery, whereas Sep-Pak columns gave only 25-

30%. None of the other SPE columns used gave recoveries as high as those obtained with the TF-EC.

Table 2.3 - SPE sorbent type recoveries. Mean % recovery of standards ± SE, n=4.

Sorbent	Mean Recovery %	
	Solanine	Chaconine
Sep-Pak	26±1.3	30±1.4
Mono-functional non-endcapped	31±1.5	35±1.6
Trifunctional non-encapped	85±2.5	86±2.4
Trifunctional endcapped	99±1.8	101±3.2

Initially, 200 mg columns were used but it was found that breakthrough of sample occurred for potato samples spiked with standards. It was therefore necessary to increase the amount of sorbent in the columns. One g sorbent weight was found to give the best recovery (Table 2.4). It is probable that the 2 g columns gave a lower recovery due to irreversible adsorption of TGA.

Table 2.4 - SPE sorbent volume recoveries. Mean % recovery of standards from spiked samples ± S.E., n=4.

Sorbent Weight mg	Mean Recovery %	
	Solanine	Chaconine
200	54±1.9	59±1.6
500	68±1.6	64±1.1
1000	93±1.3	99±3.1
2000	71±3.3	59±5.5

Glycoalkaloids were not detected in any of the washes used to elute unwanted components from the SPE columns.

The degree of separation of α-solanine and α-chaconine by HPLC was dependant on both column choice and the composition of the mobile phase. Two sorbents, μBondupak and Techsphere BDS, gave no separation of standards. Zorbax C_{18} produced uneven peaks with excessively long retention times. Only 3 columns gave greater than 90% separation with 0.5 mg cm^{-3} standards; Techsphere 80 C_{18} and the 2 C_8 sorbents (Table 2.5). Asymmetry of the eluted peaks varied greatly between the columns, with the C_8 sorbents giving the least tailing. Techsphere 80 also was subject to low band broadening and had short retention times so was used for all subsequent work (Fig. 2.2). The scheme below (Fig 2.3) was therefore adopted and used for all further studies.

Table 2.5 - Separation, peak shape and retention of α-solanine (S) and α-chaconine (C) by representative C_8 and C_{18} HPLC sorbents. Separation calculated as $Rs=2(t_2-t_1)/(w_1-w_2)$, where Rs=separation, t=retention time and w=peak width at baseline; peak asymmetry calculated as $A_{sf}=b/a$, where A_{sf}=asymmetry, b=rear baseline segment at 10% peak height and a=front baseline segment at 10% peak height. Zorbax C_8 results with mobile phase of 55:45 tris:acetonitrile.

Sorbent	Separation	Tailing		Peak Width (min)		Retention Time (min)	
		S	C	S	C	S	C
Techsphere 80 C_{18}	0.93	3.0	3.5	1.0	1.3	7.4	8.5
Zorbax C_8	0.96	2.0	2.4	2.0	2.4	12.1	14.2

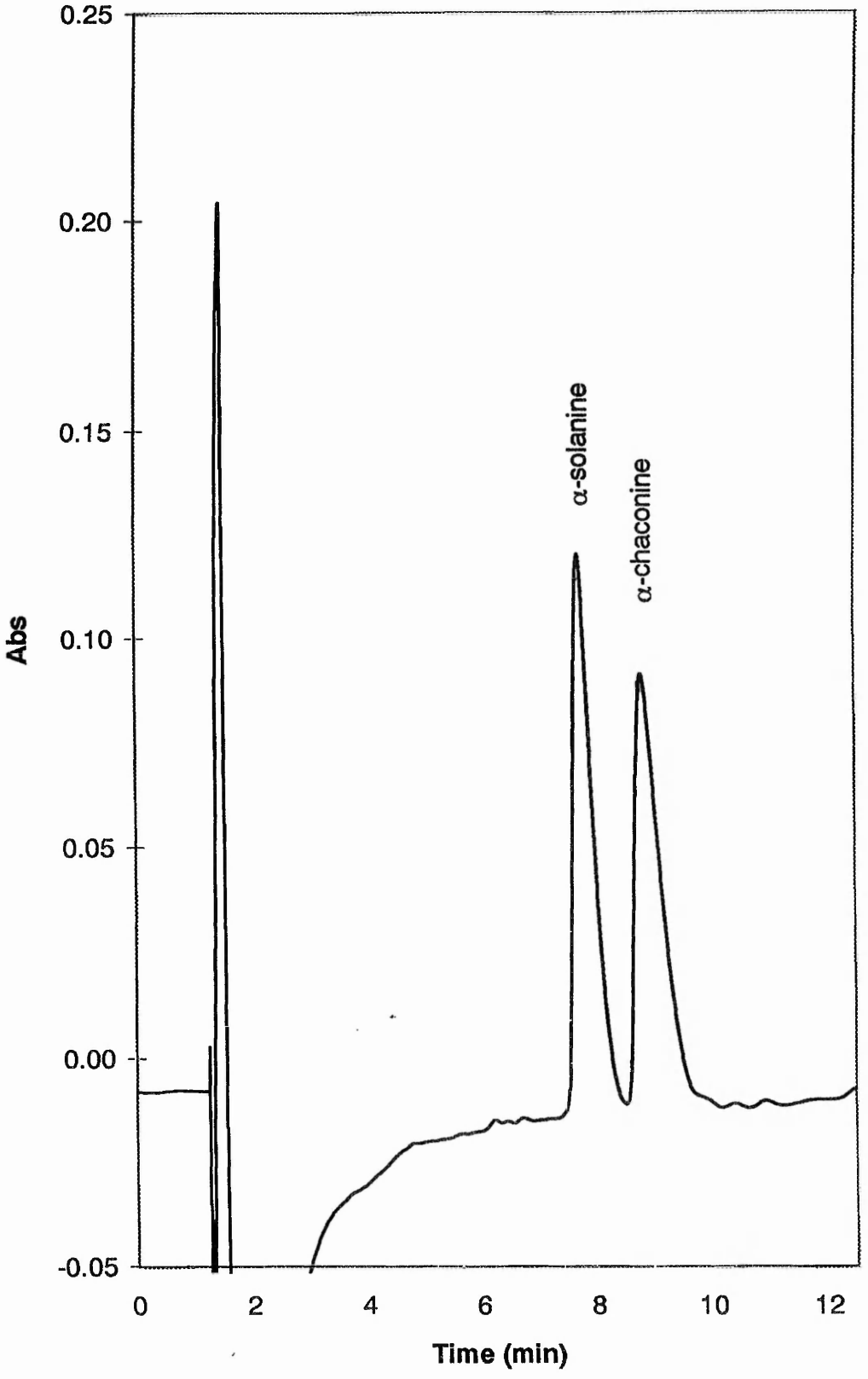


Figure 2.2 - Chromatogram of 0.25 mg cm^{-3} standards.

EXTRACTION

Sample taken from potato tuber with 10 mm cork borer and freeze dried

↓

0.5 g freeze dried powder homogenised with 15 cm^3 extraction medium using an Ultra Turrax

↓

Extract centrifuged at 5,310 g

↓

PURIFICATION AND CONCENTRATION

10 cm^3 supernatant applied to Solid Phase Extraction column pre-conditioned with 6 cm^3 methanol and 10 cm^3 extraction medium

↓

SPE column washed with 5 cm^3 water, 5 cm^3 ammonium bicarbonate, 5 cm^3 ammonium bicarbonate:methanol (50:50 v/v) and 5 cm^3 water

↓

Glycoalkaloids eluted with 6 cm^3 methanol:0.1M HCl (80:20 v/v)

↓

Eluate neutralised and evaporated to dryness then resuspended in 1 cm^3 methanol:0.5M HCl (60:40 v/v)

↓

SEPARATION AND ANALYSIS

50 µl injected onto HPLC

↓

HPLC mobile phase acetonitrile:0.01M Tris HCl (40:60 v/v) at 1.5 cm^3 min^{-1} flow rate, detector set at 202 nm

Figure 2.3 - Flow diagram of method described in the text.

2.5 - Discussion of Methodology

Careful consideration of the sampling procedure for tuber Chl is important as greening occurs most strongly in the outer layers of the tuber. Similarly almost all TGA found in freshly harvested, non-greened, potatoes are in the outer layers (Kozukue , Kozukue and Mizuno, 1987) and small and immature tubers have greater TGA concentrations (Hellenas, Branzell, Johnsson and Slalina, 1995). The results obtained by any sampling method are consequently affected by the ratio of the surface area of peel to volume of sample, with the effect being greatest when using whole tubers.

To minimise this, cores were taken from the potato, giving a much less variable surface area:volume ratio. Samples for Chl analysis need consist only of the outer 1 cm of the potato unless very extreme Chl accumulation is being studied, as this reduces the need for further dilution of the sample and contains all the accumulated Chl. However, as a small amount of TGA is found even in the middle of a potato, cores for TGA analysis must be taken through the centre of the tuber.

Initial attempts at Chl extraction were hampered by rapid browning of the sample during homogenisation, which was overcome by adding sodium bisulfite to the extraction medium as an anti-oxidant. The nature of the tuber tissue and its high polysaccharide content necessitated the use of relatively large amounts of extraction medium to prevent the sample becoming too viscous, this led to some samples being very dilute and requiring a very sensitive spectrophotometer to accurately measure absorbance.

Since tuber tissues are approximately 80% water by weight, the freeze-drying of samples provides a 5 fold concentration of TGA. It also allows samples to be stored for longer periods of time prior to analysis. In this study, no differences were found in TGA content between fresh and lyophilised potato samples. Furthermore, it has been shown that freeze-drying increases reproducibility between samples (Dao and Friedman, 1996). Considering these advantages, freeze dried tissues were used throughout.

There is a wide range of extraction solvents employed in published methods. Most are based on a weak solution of acetic acid with the addition of other solvents or salts. Methanol (Jonker *et al.*, 1992) or tetrahydrofuran (THF) (Bushway, Bureau and King, 1986) are commonly added. However, as glycoalkaloids are probably stored within the aqueous phase of the potato cell and readily soluble in dilute acid, the use of these solvents is unnecessary. Furthermore, they require removal before any SPE step as many organic solvents prevent full adsorption of TGA onto C_{18} SPE sorbents. The additional steps required for the removal of such solvents further contributes to a reduced recovery. The use of heptane sulfonic acid as an ion-pair reagent (Carmen, Kuan, Francis and Kirschenheuter, 1986) enhances complete adsorption of TGA and was therefore used in these investigations. Sodium bisulfite was used to reduce oxidation of the extract (Hellenas, 1986).

A number of published methods involve an extraction step using methanol (e.g. Kvasnicka *et al.*, 1994). However, the present study found potato glycoalkaloids to be virtually insoluble in cold methanol and so recovery from these methods can be, at best uncertain, even at the low concentrations present in most potato tubers.

Virtually every recent paper describing a clean-up method for TGA advocates the use of Sep-Pak C_{18} SPE cartridges. However, extremely variable results were observed between batches and 3 other cartridge types were tested for reproducibility of recoveries. The Isolute sorbent equivalent to Sep-Pak C_{18} (MF-NE) gave recoveries similar to Sep-Pak, but the TF-EC sorbent gave the highest recovery and good reproducibility. Unfortunately, the low selectivity of endcapped C_{18} sorbents necessitated a large sorbent weight due to the high number of compounds in the extract capable of binding to the column. No significant batch to batch variation was observed with these cartridges and they were therefore adopted for all subsequent work.

This investigation found that on drying samples, dilute HCl gave extremely variable recoveries, although Friedman and Levin (1992) did not

detect any hydrolysis of TGA when evaporating to dryness in the presence of acid. It was later demonstrated that 50% of α-chaconine is hydrolysed in 0.2N HCl at 50°C and that the presence of methanol increases the rate of hydrolysis (Friedman, McDonald and Haddon, 1993; Friedman and McDonald, 1995). Neutralising the SPE eluate prevented this hydrolysis and the excess NaOH had no detectable adverse effect on the glycoalkaloid extract. The surplus alkali did, however, make it necessary to resuspend the samples in a stronger acid than was used for the SPE elution.

The composition of the mobile phase used for HPLC was important in ensuring full separation of α-solanine and α-chaconine. A number of published methods have used acid phosphate buffers (Hellenas, 1986). However, full separation could not be attained with the HPLC columns reported above. Jonker *et al.* (1992) proposed the use of Tris-HCl at near neutral pH. Good separation was achieved on the Techsphere 80 column using this mobile phase.

Separation and retention of the analates was increased by raising the buffer pH. However, this resulted in reduced sensitivity of the system due to the glycoalkaloids being less soluble. No TGA was detected above pH 8 due to precipitation upon injection onto the column. The optimum pH was found to be 7.6 -7.8. Reducing the amount of acetonitrile in the mobile phase also improved separation. Using 40% acetonitrile, 60% buffer gave maximum separation, although glycoalkaloids are insoluble at lower acetonitrile ratios.

Detection of potato glycoalkaloids requires the use of UV as there is no chromaphore within the solanidine molecule. This limits sensitivity of detection, requiring the use of relatively high sample volumes. Spectra of α-solanine and α-chaconine in the adopted mobile phase show maxima at 202 and 203 nm respectively. Therefore 202 nm was used for quantification of TGA. This also means that mobile phases containing THF are unsuitable as it has an UV cut-off of 212 nm.

2.6 - Conclusions

Findings presented in this chapter demonstrate the successful adaptation of leaf Chl analysis methods to potato tubers, providing a standard assay to be used in future experiments.

A number of problems of HPLC analysis of potato glycoalkaloids are addressed and the use of a new SPE sorbent is described resulting in sample recoveries of 93 and 99% for α-solanine and α-chaconine, respectively. The method is reliable and reproducible enabling it to be used routinely for determination of TGA.

CHAPTER 3 - THE EFFECT OF TEMPERATURE ON PIGMENT AND GLYCOALKALOID ACCUMULATION IN TUBERS STORED AT LOW PHOTON FLUX DENSITY

3.1 - Introduction

Despite the need for optimal conditions to enhance the storage lifetime of the potato crop, the role of temperature and its effects on the synthesis of photosynthetic pigments and glycoalkaloids in potato tubers is largely unreported in the literature. Several publications have documented the effect of temperature on tuber chlorophyll formation, but disagree on the optimum temperature for storage. Ramaswarmy and Nair (1974) stated that maximum greening occurred at 0°C, agreeing with an earlier investigation by Larson (1950). However, Harkett (1975) found that 15-20°C caused maximum Chl accumulation. It has also been reported that low temperatures lead to a loss of carotenoids in tubers stored in the dark (Bhushan and Thomas, 1990).

TGA accumulation is thought to increase with storage at low temperatures (Hilton, 1951; Zitnak, 1955), although Nair, Behere and Ramaswarmy (1981) found no change in TGA content of tubers stored at 0-28°C.

Although some studies of the response of potatoes to light have examined chlorophylls and TGA in conjunction (Peterman and Morris, 1985; Kaaber, 1993; Griffiths, Dale and Bain, 1994), the results are often difficult to compare due to absolute concentrations of chlorophyll not being determined and the use of different sampling regimes for TGA analysis. Furthermore, while a number of publications have considered the effect of light quality and quantity on these responses (Gull and Isenberg, 1960; Baerug, 1962; Peterman and Morris, 1985; Percival, Dixon and Sword ,1994), only 1 recent paper has examined the role of temperature (Percival, Harrison and Dixon, 1993). However, the latter study did not investigate the effects of

temperature during chlorophyll and TGA synthesis, but only the temperature during storage prior to light exposure. This chapter examines the tuber response to light at a range of storage temperatures, and suggests that there is no link between TGA and chlorophyll production.

3.2 - Materials and Methods

3.2.1 - Plant material

Potato tubers (cv. King Edward) were purchased from a local supermarket and stored in darkness at room temperature for approximately 24 h before use.

3.2.2 - Experimental design

Potatoes were half-buried, longitudinally, in potting compost (Seed and Potting Compost, J. Arthur Bowers, Lincoln, UK) in trays (600x320x80 mm). Each tray contained 17 potatoes of which 5 were covered with a smaller opaque tray (225x175x50 mm) to act as dark controls (Fig. 3.1). Three trays of potatoes were stored in each of 4 incubators (Mercia Scientific, UK) set to 5, 10, 20 and 25°C (Plate 3.1). The photo-period was 16 hrs light 8 hrs dark with a photosynthetic photon flux density (PPFD) of 12 µmole photons m^{-2} s^{-1}. At 0, 2, 5 and 8 d of storage 1 tray of potatoes per treatment was analysed for pigment and glycoalkaloid content.

3.2.3 - Pigment and TGA extraction and quantification

The procedures described in Chapter 2 were used without modification. The regime used for sampling individual tubers is shown in figure 3.2.

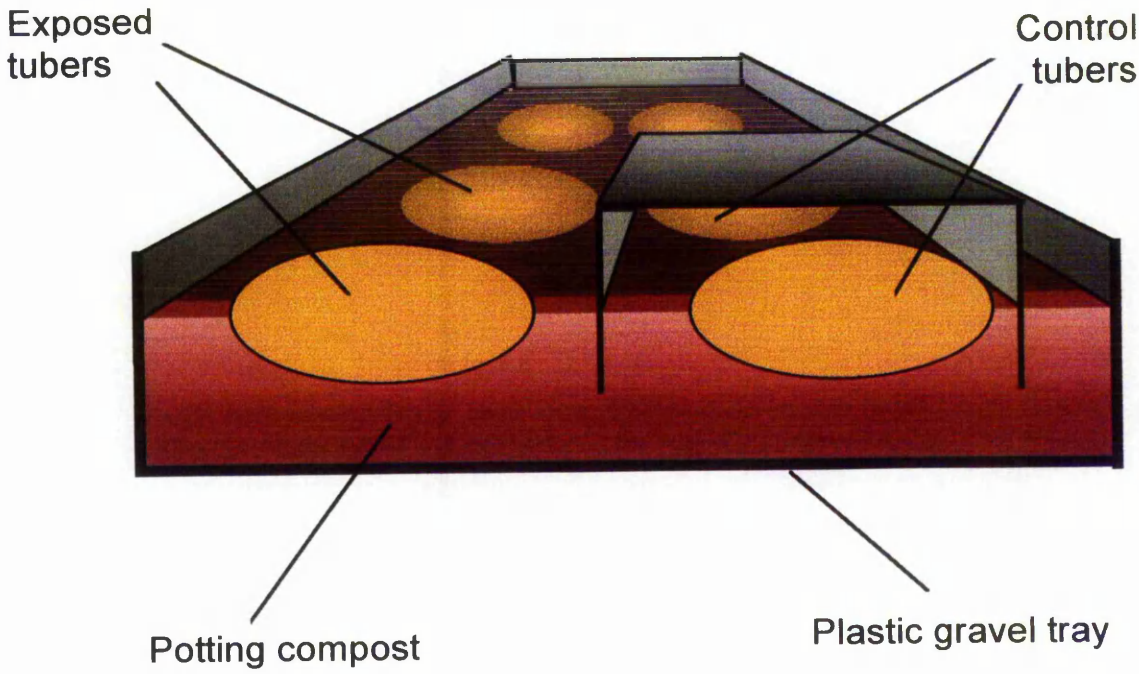


Figure 3.1 - Experimental design.

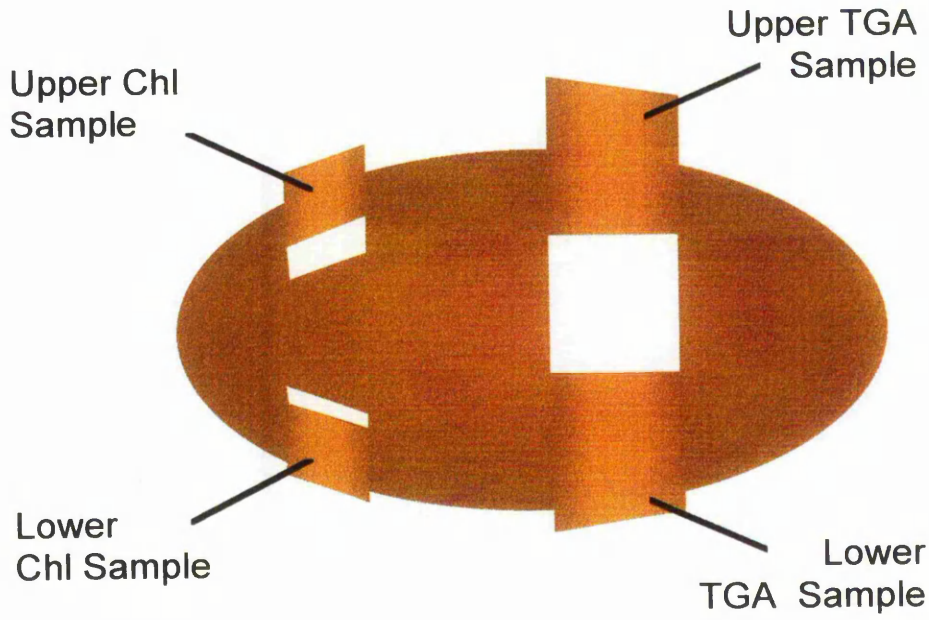


Figure 3.2 - Tuber sampling regime.

Plate 3.1 - Incubator containing tubers at 20°C.

3.2.4 - Statistical analysis

Initial analysis of the data set was with regression and two-way analysis of variance (ANOVA). Students t test and Kolmogorov-Smirnov test (a non-parametric test similar to the t test but applicable to non normally distributed data) were used to provide further analysis in depth.

3.3 - Results

Initial analysis by ANOVA indicated the possibility of significant differences between Chl concentrations in unexposed control samples (data not shown). However, the 'detected' Chl in these samples appeared to be due to a lack of spectrophotometer sensitivity and further analysis using Students t and Kolmogorov-Smirnov tests demonstrated that no control samples contained any significant Chl. Therefore, it was concluded that tubers maintained in darkness did not accumulate detectable Chl nor exhibit any significant changes in TGA concentrations irrespective of storage temperature. However, total carotenoid concentrations in unexposed control samples did exhibit significant fluctuations (Fig 3.3). The tubers stored at 20 and 25°C showed increased carotenoid content after 2 d of storage, whereas carotenoid concentrations in samples stored at 5 and 10°C did not significantly alter. After 8 d of storage all control tubers had an increased carotenoid content, although after 5 d there was no significant difference between any of the samples and the control tubers analysed at the start of the experiment. All results given below were significant to the 5% level or greater, unless otherwise stated. Complete pigment and glycoalkaloid results are tabulated in Appendix 1.

3.3.1 - Photosynthetic pigments in tissues exposed to light.

Temperature was observed to have a marked effect on Chl accumulation (Fig 3.4). Chl was detected after 2 d of light exposure

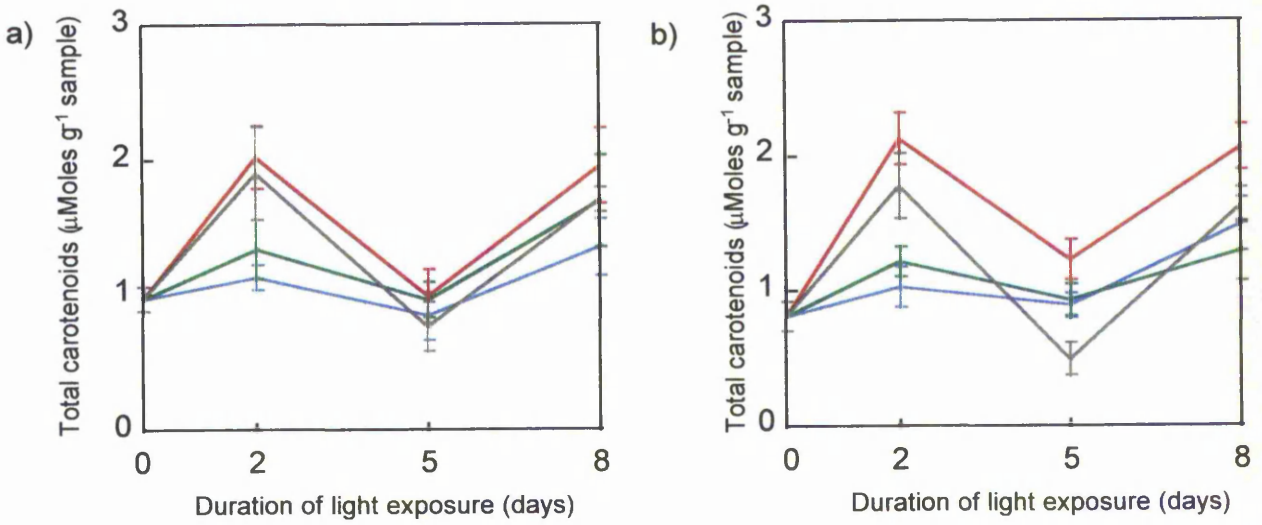


Figure 3.3 - Accumulation of total carotenoids in control tubers stored in darkness at 5-25°C. Samples stored at —— 5°C, —— 10°C, —— 20°C, —— 25°C. Each point is the mean of 5 samples ± standard error. a) upper surface of tuber and b) lower surface of tuber.

in all exposed samples (significant in all samples except those stored at 5°C). Tubers stored at 20°C accumulated higher concentrations of Chl than tubers stored at the other temperatures throughout the experiment (Fig. 3.4a). After 5 d of light exposure tubers stored at 20°C contained 4.55 µmoles Chl g^{-1} sample, more than double the total Chl concentration in tubers stored at other temperatures. However, after 8 d of exposure there was no significant difference in total Chl concentrations between tubers stored at 20 or 25°C (5.84 and 5.54 µmoles g^{-1} sample, respectively).

Samples stored at 10°C also exhibited a large increase in Chl content, although only reaching approximately 65% of the concentrations found in the samples stored at higher temperatures. The samples stored at 5°C surprisingly had a significantly higher total Chl content after 5 d light exposure than those stored at 10°C. Indeed, this result was not significantly lower than that of the tubers stored at 25°C.

An examination of the Chl *a* to *b* ratio (Fig 3.4d) and the concentrations of the individual Chls (Fig. 3.4b & c), revealed that the majority of Chl in all samples was Chl *a* and that the Chl *a* to *b* ratio rose between 2 and 8 d of exposure irrespective of storage temperature.

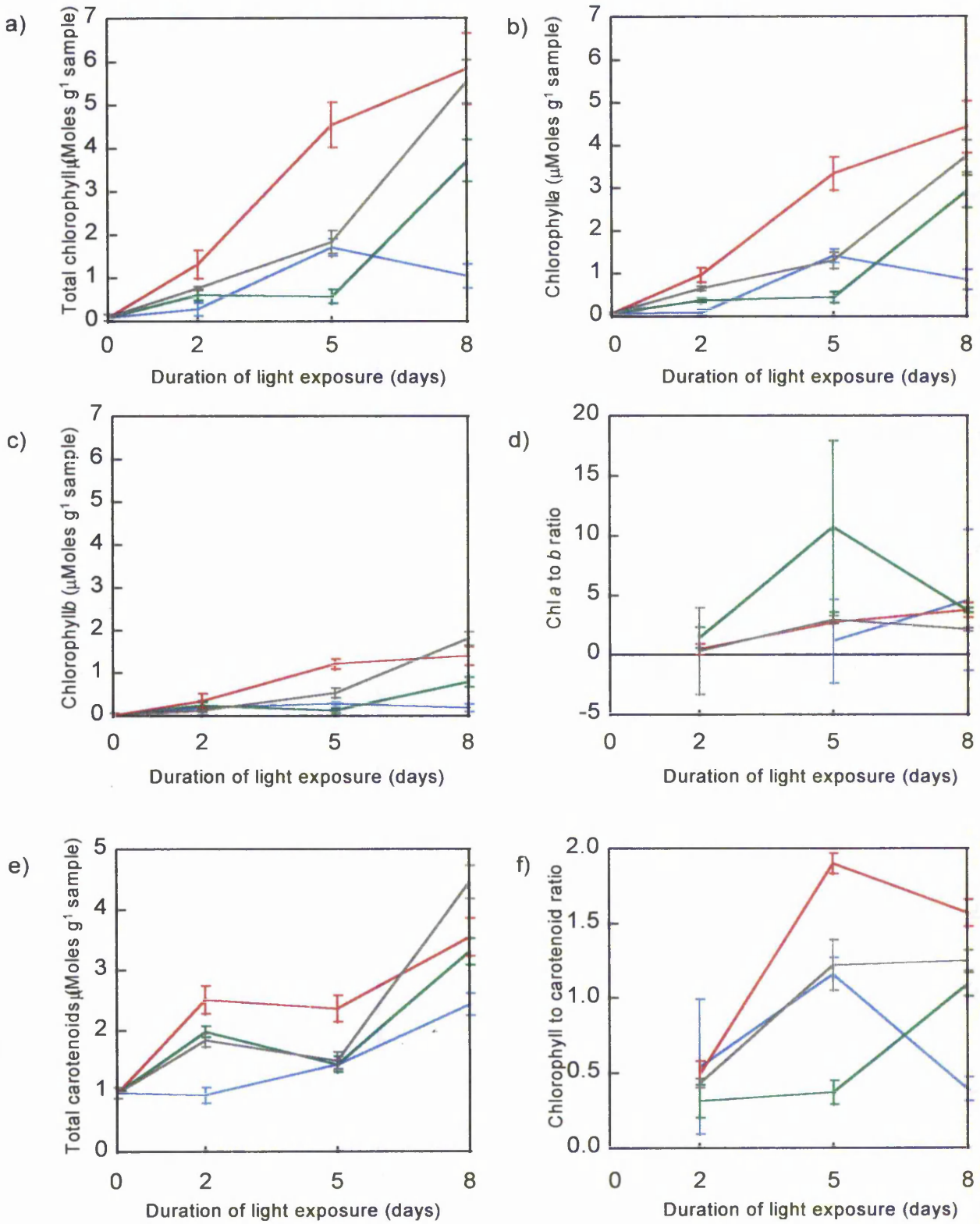


Figure 3.4 - Accumulation of photosynthetic pigments in upper surface of tubers exposed to 12 µmole photons m^{-2} s^{-1} for 8 days at 5-25°C. Samples stored at —— 5°C, —— 10°C, —— 20°C, —— 25°C. Each point is the mean of 12 samples ± standard error. a) Total chlorophyll, b) Chl *a*, c) chlorophyll *b*, d) Chl *a* to Chl *b* ratio, e) total carotenoids and f) Chl to carotenoid ratio.

However, while Chl *a* concentrations closely followed total Chl concentrations in all samples, the Chl *b* content of tubers stored at 25°C increased proportionally more than Chl *a* and was significantly higher than that in the tubers stored at 20°C. This was mirrored by a drop in Chl *a* to *b* ratio in these samples from almost 3 to 2.11 between 5 and 8 d of light exposure. Chl *a* to *b* ratios in tubers stored at 10 and 20°C were similar after 8 d at 3.7, this was slightly lower than in tubers stored at 5°C but the SE of the latter was very high.

Tubers stored at the 3 higher temperatures all showed a significant increase in carotenoids after 2 d of light exposure (Fig. 3.4e). This was greatest in the samples stored at 20°C. The carotenoid concentration in the tubers stored at 5°C rose steadily between 2 and 8 d of light exposure, while the tubers stored at the higher temperatures did not show any further increase until after 5 d of exposure. The tubers stored at 25°C had a carotenoid content after 8 d of 4.45 μmoles g^{-1} sample, which, unlike the total Chl concentrations, was significantly higher than any of the other samples.

Tubers stored at all temperatures exhibited an increase in Chl to carotenoid ratio between d 2 and 5 of exposure due to the significant increase in Chl content. However, only the tubers stored at 10°C showed any further increase in Chl to carotenoid ratio, and the samples stored at 5°C had a greatly reduced ratio after 8 d exposure.

3.3.2 - Pigment concentrations in unexposed tissues.

The lower, unexposed surface of tubers exposed to light also showed some greening, despite receiving no direct light themselves (Fig 3.5). The differences in Chl accumulation with temperature were much less distinct than in the upper tissues and the highest total Chl concentrations were found in the tubers stored at 10 and 25°C (Fig 3.5a). Even in these samples, total Chl content only reached about 25% of the highest Chl content of the upper tissues.

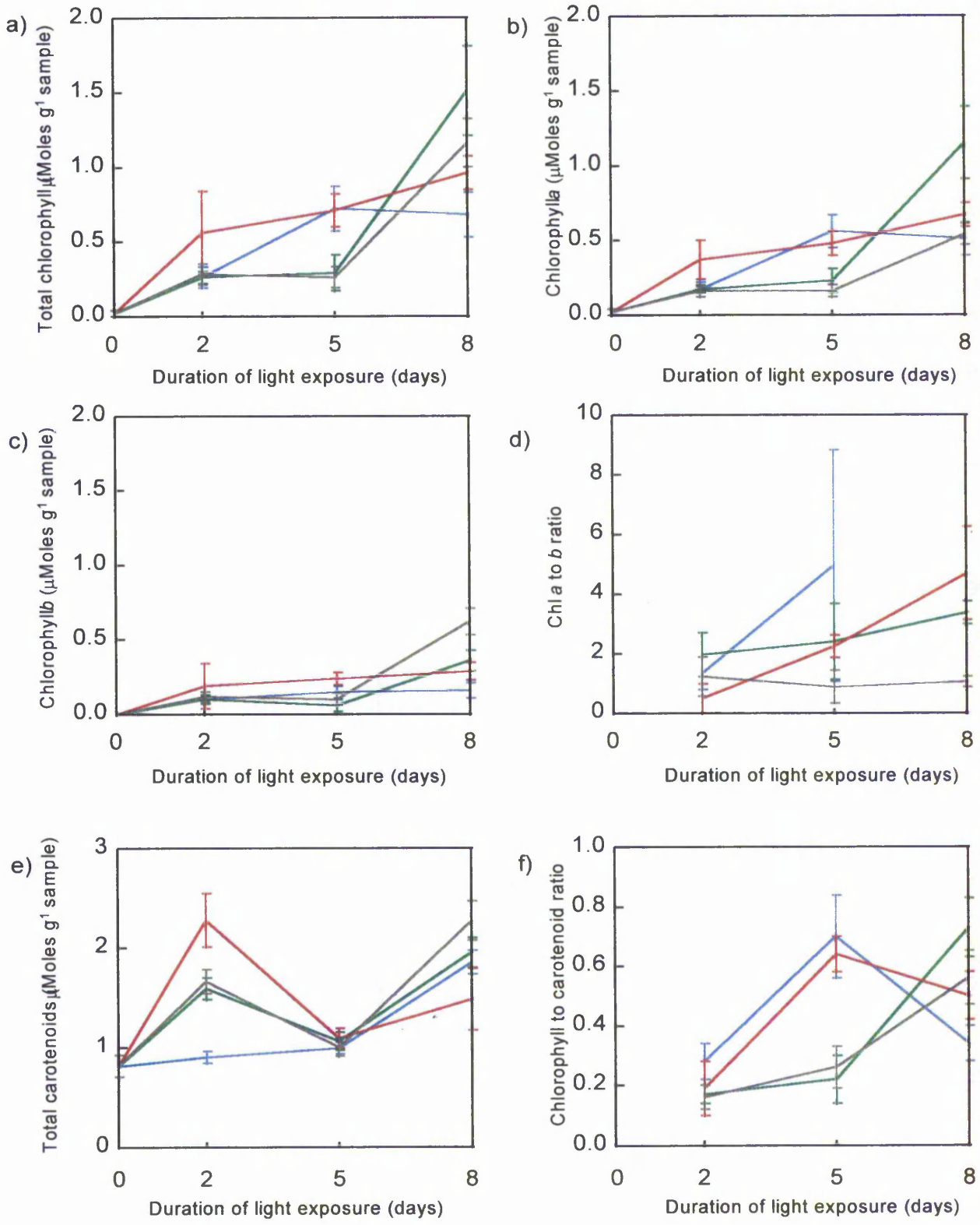


Figure 3.5 - Accumulation of photosynthetic pigments in lower surface of tubers exposed to 12 µmole photons m^{-2} s^{-1} for 8 days at 5-25°C. Samples stored at —— 5°C, —— 10°C, —— 20°C, —— 25°C. Each point is the mean of 12 samples ± standard error. a) Total chlorophyll, b) Chl *a*, c) chlorophyll *b*, d) Chl *a* to Chl *b* ratio, e) total carotenoids and f) Chl to carotenoid ratio.

Chl *a* again constituted the major part of total Chl irrespective of storage temperature (Fig 3.5b). Indeed, significant concentrations of Chl *b* were only detected after 5-8 d of light exposure (Fig 3.5c). Total carotenoid concentrations did not show any significant difference from control samples (Fig 3.5e).

3.3.3 - Glycoalkaloid results.

Examination of the glycoalkaloid data using ANOVA indicated some significant variations between or within treatments. Further analysis using Students t test demonstrated a small number of significant differences among the TGA data for the lower surface of exposed potatoes. However, these showed a random pattern and were not supported by Kolmogorov-Smirnov tests. There were no other significant differences exhibited by the glycoalkaloid data even to the 10% level (Table 3.1).

Table 3.1 - TGA concentrations and α-solanine to α-chaconine ratios (S:C) in the upper halves of tubes exposed to a PPFD of 12 μmoles m^{-2} s^{-1} or darkness (control) for 8 d at 4 different temperatures (μg g^{-1} sample). Values are means ± SE, where N=5 for control and 12 for exposed samples.

		5°C	10°C	20°C	25°C
TGA	Control	72.5±12.7	69.7±21.7	69.6±13.2	46.5±8.3
	Exposed	46.9±6.8	89.7±22.0	48.3±8.3	93.2±30.4
S:C	Control	0.30±0.07	0.36±0.03	0.44±0.05	0.40±0.09
	Exposed	0.31±0.05	0.41±0.02	0.32±0.05	0.40±0.07

Regression analysis of both the entire data set and the exposed upper surface data alone showed no relationship between chlorophyll and TGA concentrations (R^2 of 0.044 and 0.100, respectively).

3.4 - Discussion

Exposure of potato tubers to a low PPFD caused detectable greening within 48 hr irrespective of the storage temperature used. However, Chl accumulation in tubers stored at 5°C was not statistically significant at this time. After 8 d of light exposure the tubers stored at 5°C had a Chl content significantly lower than tubers stored at higher temperatures and there was an indication of loss of Chl *a*.

Sub-optimal temperatures can generate several effects in greening leaves, including a considerable reduction in Chl content (Nie and Baker, 1991). Thylakoid biogenesis is also affected and there is a reduction in plastid-encoded gene products and soluble proteins (Robertson, Baker and Leech, 1993). A further factor in the response of greening tissues to low temperatures is PPFD. Low growth temperature reduces the quantum yield of photosystem II (PSII) which can result in photo-oxidative damage when the PPFD is high (Baker, Long and Ort, 1988; Oberhuber and Edwards, 1993). However, the use of a low PPFD in this study would reduce the possibility of photo-oxidative damage. Therefore, it can be assumed that the observed effects are solely due to low temperature storage and not light quantity.

The data presented in this chapter indicate that the optimal temperature for Chl accumulation in tubers is approximately 20°C. Therefore, storage at both 5 and 10°C can be considered sub-optimal. Tubers stored at both these temperatures exhibited a delay in the greening response and a significantly reduced Chl content after 8 d of light exposure.

As potatoes are an underground storage organ of a montane plant, high storage temperature would be likely to cause stress to the tubers. Indeed, in tubers exposed to light at 25°C the initial rate of greening was reduced and when Chl accumulation increased there was a decrease in the Chl *a* to *b* ratio, indicative of stress (Hendry, Houghton and Brown, 1987).

In leaves, high carotenoid concentrations have also been associated with environmental stresses (Pallett and Young, 1993), which may account

for the observation that after 8 d of light exposure the carotenoid content of tubers is directly related to temperature, with the highest temperatures resulting in the highest carotenoid concentrations. Accumulation of carotenoids as part of the new photosynthetic apparatus of the developing chloroplasts alone does not explain this, as the highest degree of greening does not equate to the highest carotenoid concentration. Furthermore, low temperature storage did not inhibit carotenoid accumulation to the same extent as it did Chl accumulation. This latter phenomenon has previously been observed in maize leaves (Haldiman, 1996). High concentrations of carotenoids in photosynthetic tissues exposed to low temperatures could be particularly important due to their role in the dissipation of excess light energy (Deming-Adams and Adams, 1996).

There was also an increase in carotenoid content in all tubers following the start of the experiment. This was short lived with concentrations falling back to their original levels after 5 d, followed by an increase in the exposed tubers. This increase could possibly be a response to the temperature change which the tubers in the experiment were subjected to or a stress before purchase, e.g. storage under light or at high temperature. These responses indicate that with more study carotenoid concentrations could possibly be used as a monitor of tuber stress.

All these factors are important when considering the storage of potatoes during retail and in the home. It is apparent that the display lifetime of stock is dependant on storage temperature with maximum greening being caused by approximately the temperatures currently maintained in typical supermarkets and the home i.e. 20°C. These data would suggest that potatoes exposed to light be stored at low temperatures, ideally 5°C, which would extend their shelf-life. It is unlikely that the duration of any display, or home storage would be long enough to cause any reduction in quality associated with low temperature storage (Ewing, Senesac, and Sieczka, 1981).

Accumulation of photosynthetic pigments also occurred in the unexposed periderm of potatoes that were exposed to light. It is unlikely that

there was any transmission of light through the potato, as tuber tissue is fairly opaque and there was no Chl accumulation in the central cortex of the tuber. Dark synthesis of Chl involving a light-independent protochlorophyllide oxidoreductase has been reported in dark grown seedlings of a number of *Pinus* spp. (Ou and Adamson, 1995) and in barley (*Hordeum vulgare*) seedlings transferred to darkness after initial growth in light (Adamson, Griffiths, Packer and Sutherland, 1985; Walmsley, 1991). Chl is not transported within plant tissues, but synthesised within the chloroplast (von Wettstein, 1995), therefore it maybe that a similar enzyme is present in potato tubers.

It is possible that the receptor for initiation of Chl synthesis in the exposed areas of the periderm, probably phytochrome (Morris *et al.*, 1979), also initiates a signal transduction through the tuber which interacts with a second receptor in the periderm promoting light-independent Chl synthesis. Leaf tissues placed in darkness undergo rapid Chl breakdown (Bennett, 1981), but Chl formed in potato tubers is remarkably stable (Virgin and Sundqvist, 1992), therefore even a very low activity of light-independent protochlorophyllide oxidoreductase could lead to significant accumulation of Chl.

It is evident that light exposure will cause greening of potatoes during storage throughout the 5-25°C temperature range. However, these results do not indicate any light-enhanced glycoalkaloid accumulation at any of the temperatures used. This is surprising as light-induced promotion of TGA biosynthesis is well known (Conner, 1937). However, it is possible that TGA concentrations are not affected by the very low flux densities used in this study, as light quantity is known to be important in the alteration of TGA pools (Hilton and Gamborg, 1957; Percival and Dixon, 1996). This could suggest that light-enhanced TGA synthesis is controlled by a photo receptor other than phytochrome. Alternatively, it may be that cv. King Edward does not strongly accumulate TGA in response to light, as light-enhanced TGA synthesis is known to be cultivar specific (Percival, Dixon and Sword, 1996).

The α-solanine to α-chaconine ratios appear to be stable and were not significantly affected by any treatment. This provides some evidence that the high degree of variation and lack of statistical significance of the TGA data is natural and not due to experimental error. It also suggests that tuber glycoalkaloids are stable and not rapidly metabolised, even when increasing metabolic activity is evident.

Ramaswarmy, Behere and Nair (1976) suggested that glycoalkaloids are formed within the chloroplast as a direct product of photosynthesis. This study contradicts this, certainly at low PPFD. The lack of TGA accumulation despite significant Chl production suggests that the 2 light responses have independent receptors and/or signal transduction pathways and that photosynthetic pigment and TGA accumulation are not biochemically related, as has been frequently suggested (Dale *et al.*, 1993).

3.5 - Conclusions

The principal findings reported above are:

1. Even at extremely low PPFD, potatoes will accumulate significant concentrations of chlorophyll within 48 hr.
2. Storage of potatoes at 5°C, while exposed to light, delays the onset of, and reduces the extent of, greening and possibly leads to breakdown of accumulated chlorophyll after several days storage.
3. Tuber carotenoid concentrations when stored in the presence of light are a function of the storage temperature and could serve as an indicator of tuber stress.
4. The α-solanine to α-chaconine ratios indicate that the glycoalkaloids present are stable. Under the conditions used in this study there was no increase in tuber TGA concentrations and therefore TGA and Chl accumulation are not linked.

CHAPTER 4 - EFFECT OF STORAGE DURATION ON THE POTENTIAL OF TUBERS TO ACCUMULATE GLYCOALKALOIDS AND PHOTOSYNTHETIC PIGMENTS IN RESPONSE TO EXPOSURE TO LIGHT

4.1 - Introduction

Potato tuber greening and glycoalkaloid accumulation in response to light has been studied for over 50 years (Jadhav and Salunkhe, 1975). However, it is rare that the metabolic state of the tubers or their storage conditions and duration before use is noted. The physiological age of a tuber affects many aspects of its metabolism and its response to external conditions (Harris, 1992). Therefore it is likely to affect tuber response to light.

Few publications to date have reported any investigation of the role of tuber maturity in determining the greening response. Buck and Akeley (1967) found that potatoes harvested very early or very late greened less than those harvested in between. They also observed that after 4 months of storage light exposure caused slightly less greening in tubers than in those that had been stored for 2 months. However, these results used optical density as a measure of greening and differences observed were not significant. More recently Griffiths *et al.* (1994) examined a number of cvs. at harvest and after 3 months storage. The results indicated no clear trend between cvs., although again quantitative analysis of chlorophyll concentrations was not made.

A number of studies have examined the role of storage duration in glycoalkaloid production, often with inconclusive or contradictory results (Friedman and McDonald, 1997). A possible explanation for this was offered by Olsson and Roslund (1995) who found a cycling of TGA during 9 months of storage.

However, the role of storage in determining the response of tubers to subsequent light exposure has not previously been examined and is studied in this Chapter using a limited selection of potato cvs.

4.2 - Materials and Methods

4.2.1 - Plant material

Potato tubers (cvs. Brodick, King Edward, Pentland Dell and Record) were provided by the Potato Marketing Board Experimental Station, Sutton Bridge. Tubers of cv. Brodick were placed into storage on 7/11/94, following curing for 14 days at 15°C. Tubers of cvs. King Edward, Pentland Dell and Record were placed in storage on 11/11/94, following similar curing. The potatoes were stored at 7°C and 95% RH for up to 30 weeks. Sprouting was inhibited by 4 applications of 3-chloro-*iso*-propylphenylcarbamate (CIPC) as a thermal fog. The initial application was on 9/11/94 for Brodick and 14/11/94 for the other cvs. Further applications, for all cvs., took place on 9/12/94, 20/1/95 and 13/4/95.

After 0, 10, 20 and 30 weeks of storage tubers of each cv. were sampled, brought to The Nottingham Trent University and stored in the dark at room temperature for 24 hr before use.

4.2.2 - Experimental design

Sample tubers were set out in trays as described in Chapter 3 and exposed to daylight in a glasshouse (Plate 4.1). Daylight was supplemented during winter months with sodium halide lamps. One tray of potatoes per cv. was analysed for pigment and glycoalkaloid content after 0, 3, 6 and 10 days of light exposure. Light quantity was measured at 4 wavelengths/bandwidths; PAR, 730 nm, 660 nm and 470 nm using a SKR 1850 4-channel light sensor with a SDL 2580 datalogger (both Skye Instruments, Llandrindod, Wales). Air temperature of the glasshouse compartment was monitored using a shielded T-type thermocouple

Plate 4.1 - Greenhouse exposure of potatoes to daylight.

(Thermocouple Instruments Ltd., Cardiff, UK) connected to a MFI 100 switching box and MFI 1010 datalogger (Central Instruments Ltd., Birmingham, UK).

4.2.3 - Pigment and glycoalkaloid extraction and quantification

The procedure for pigment determination described in Chapter 2 was used without modification. Glycoalkaloids were extracted and analysed as described in Chapter 2 with the following modifications. The addition of a 7955 column heater/chiller (Jones Chromatography, Hengoed, UK) allowed the column to be maintained at 40°C. The mobile phase was consequently adjusted to 55% aq. Tris buffer, 45% acetonitrile.

4.2.4 - Statistical analysis

Analysis of the results was as described in Chapter 3, using Students t and Kolmogorov-Smirnov tests.

4.3 - Results

4.3.1 - Environmental measurements.

Accumulated PAR was similar, about 60-70 mol photons m^{-2} after 10 days of light exposure, for all exposures except that of the tubers stored for 20 weeks, which was approximately double this (Fig. 4.1a). However, the spectral composition was more variable. Accumulated light at the blue, R and FR bandwidths was very similar for the exposures following 0 and 10 weeks of storage (Figs. 4.1b, c &d). The 2 later exposures were higher at all 3 bandwidths with the R component of the exposure after 20 weeks of storage particularly high.

This pattern was repeated by the mean daily PFD (Fig 4.2). Although, the exposure of tubers stored for 20 weeks was considerably more variable from day to day than the other exposures.

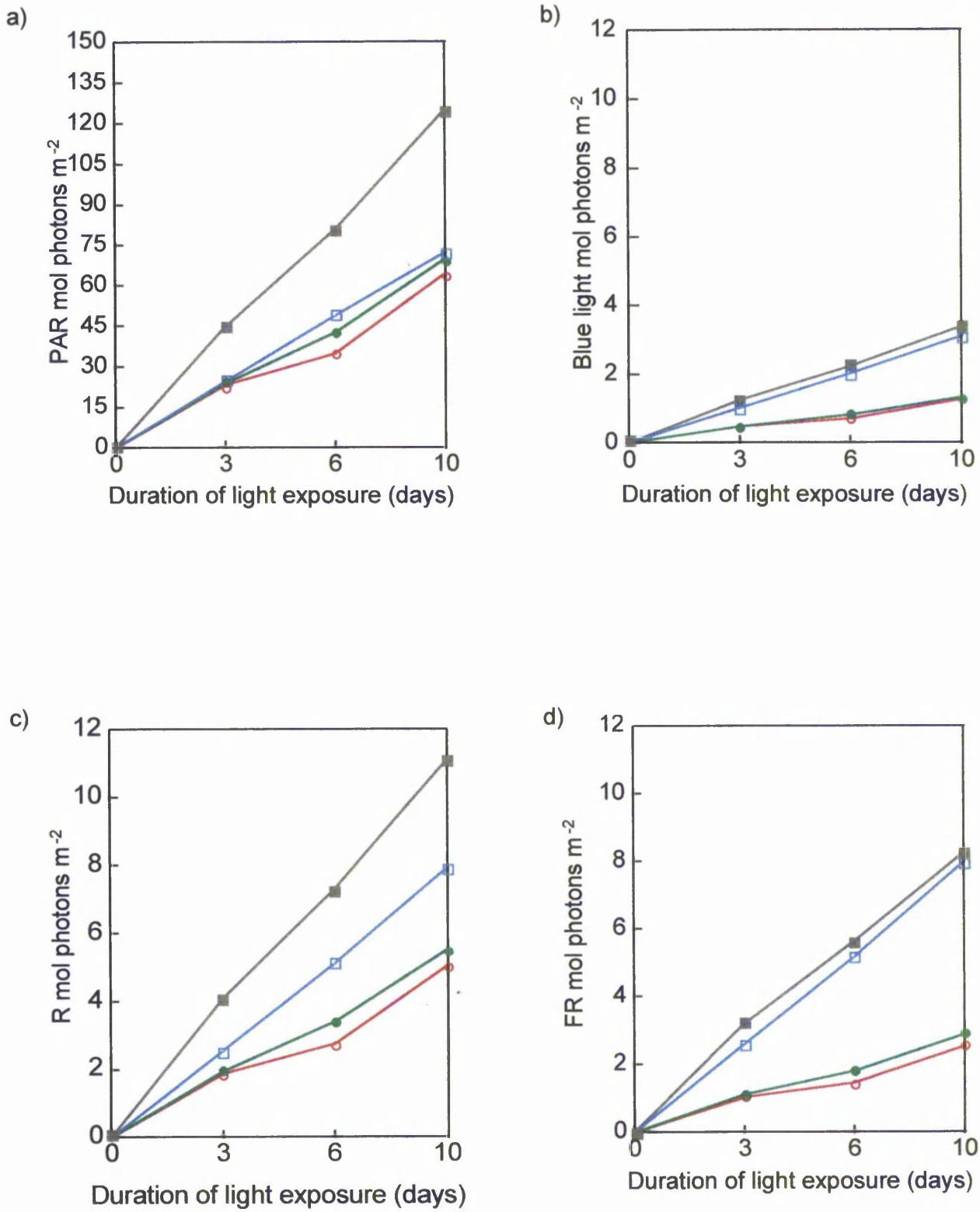


Figure 4.1 - Accumulated photon flux density during the exposure of stored tubers to light. Tubers were stored for —○— 0, —●— 10, —■— 20 and —□— 30 weeks. a) Photosynthetically active radiation, b) blue light, c) red light and d) far-red light.

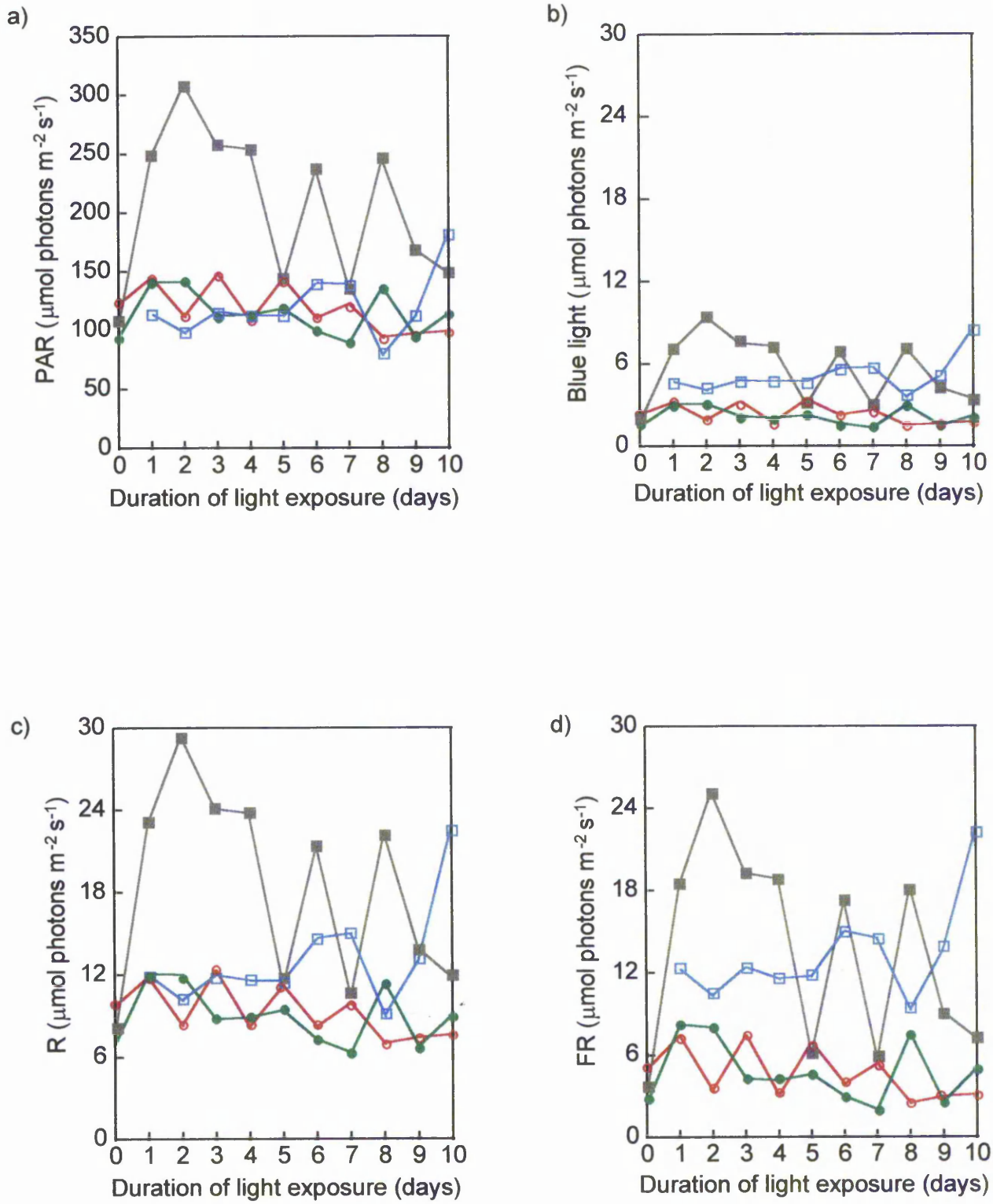


Figure 4.2 - Mean daily photon flux density during the exposure of stored tubers to light. Tubers were stored for —○— 0, —●— 10, —■— 20 and —□— 30 weeks. a) Photosynthetically active radiation, b) blue light, c) red light and d) far-red light.

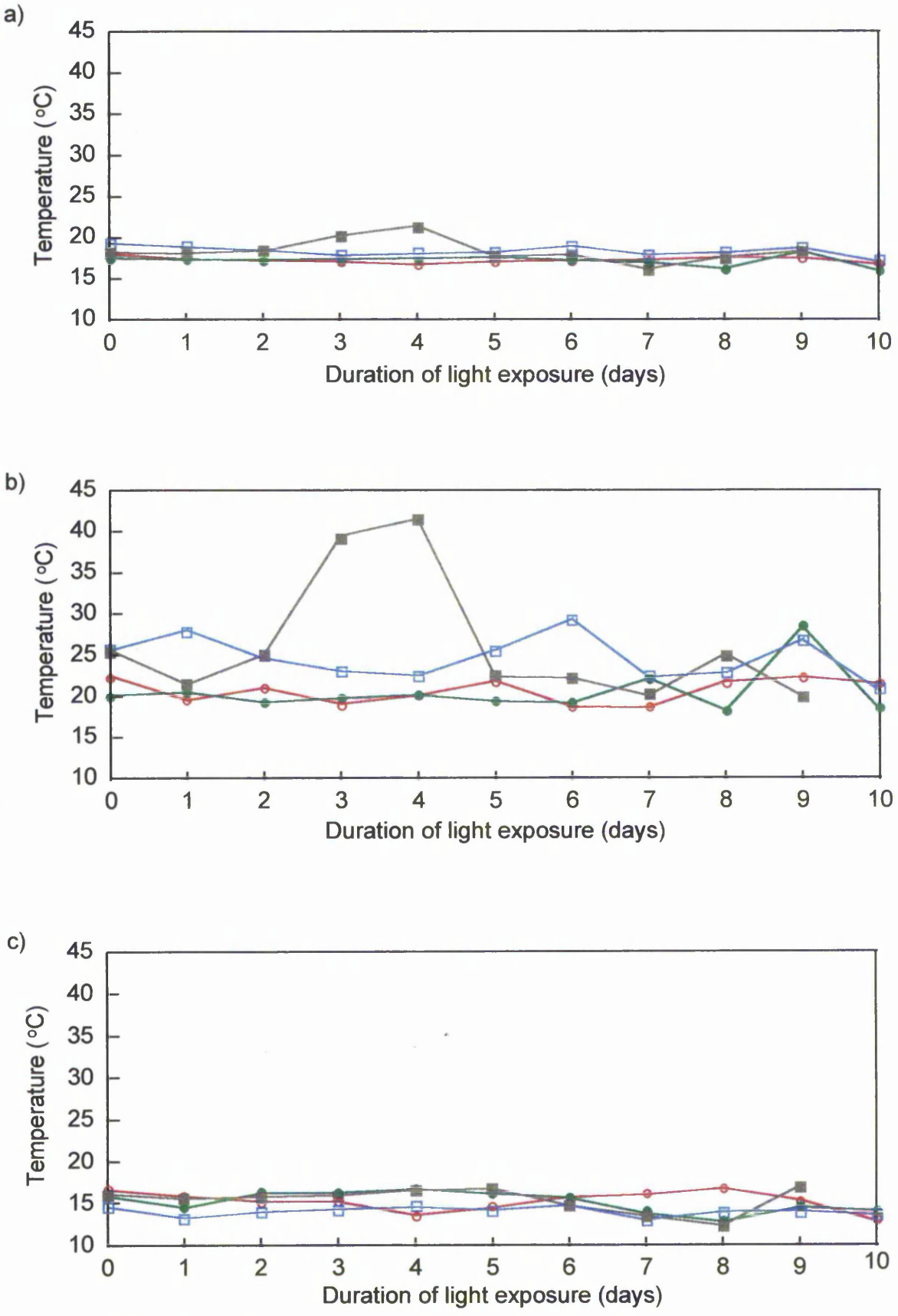


Figure 4.3 - Daily mean, maximum and minimum temperatures during the exposure of stored tubers to light. Tubers were stored for 0, 10, 20 and 30 weeks. a) Mean daily temperature, b) maximum daily temperature and c) minimum daily temperature.

Glasshouse temperatures during all 4 exposures were very similar (Fig. 4.3). Mean daily temperatures were about 18°C, with maxima of 20-25°C and minima of 14-17°C.

4.3.2 - Pigment concentrations.

Storage for 20 weeks in darkness led to a significant increase in total carotenoids, over initial concentrations, in 3 cvs. (Fig. 4.4); Brodick, Record (both significant to the 1% level) and Pentland Dell (5% level). However, after a further 10 weeks of storage only Brodick had concentrations significantly different from the freshly harvested and cured samples.

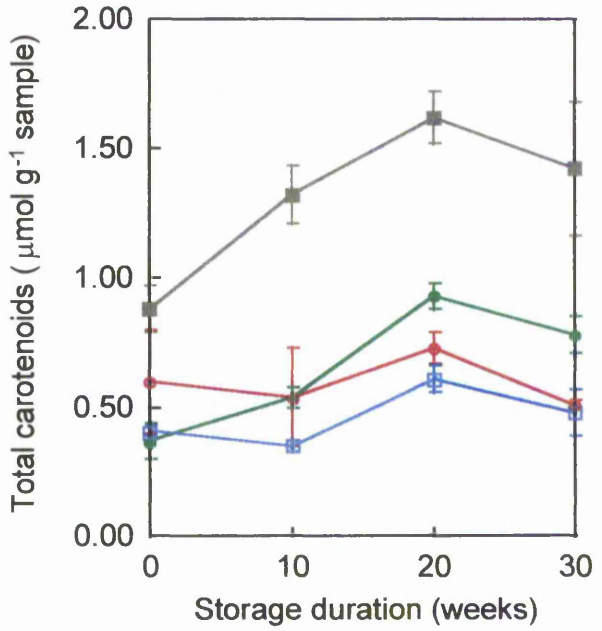
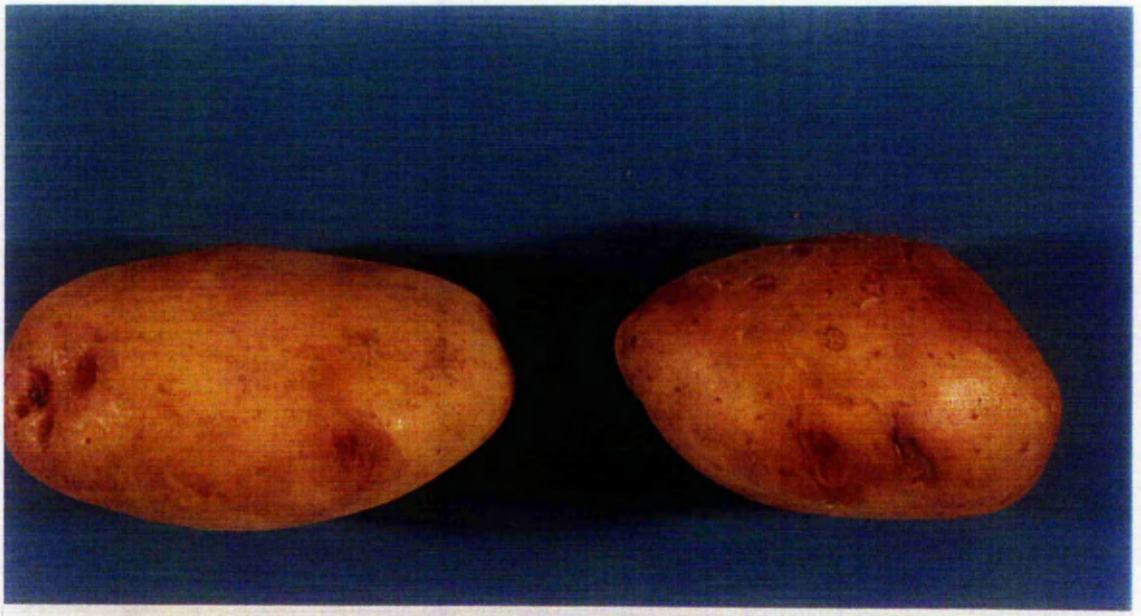


Figure 4.4 - Fluctuation in total carotenoid content of tubers during storage under conditions described. Each point is the mean of 5 samples ± standard error. —○— King Edward, —●— Brodick, —■— Record and —□— Pentland Dell.

Irrespective of storage duration and cv., greening was visible to the naked eye after 3 days of exposure to light in the upper surface of exposed potatoes. After 10 days marked greening was observed and greened sprouts were also visible (Plates 4.2 - 4.5).



DAY 0 CONTROLS

Plate 4.2 - King Edward potatoes before exposure to light.

DAY 3 CONTROL EXPOSED

Plate 4.3 - King Edward potatoes after 3 days of exposure to light.

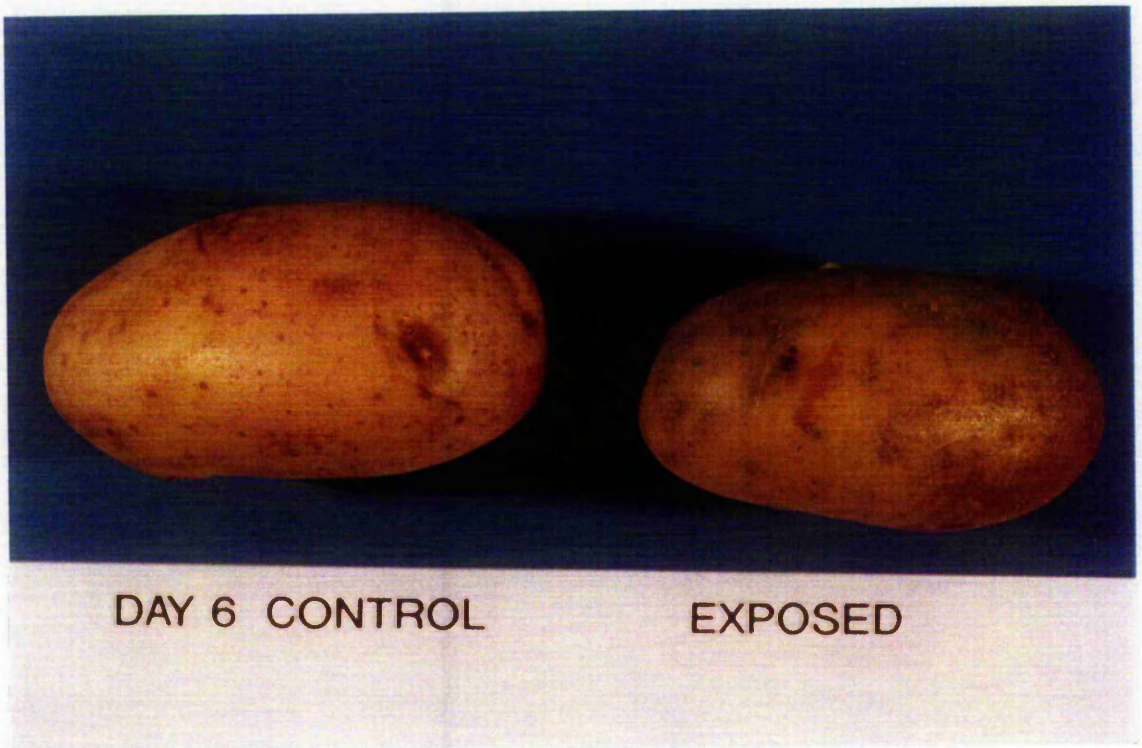


Plate 4.4 - King Edward potatoes after 6 days of exposure to light.

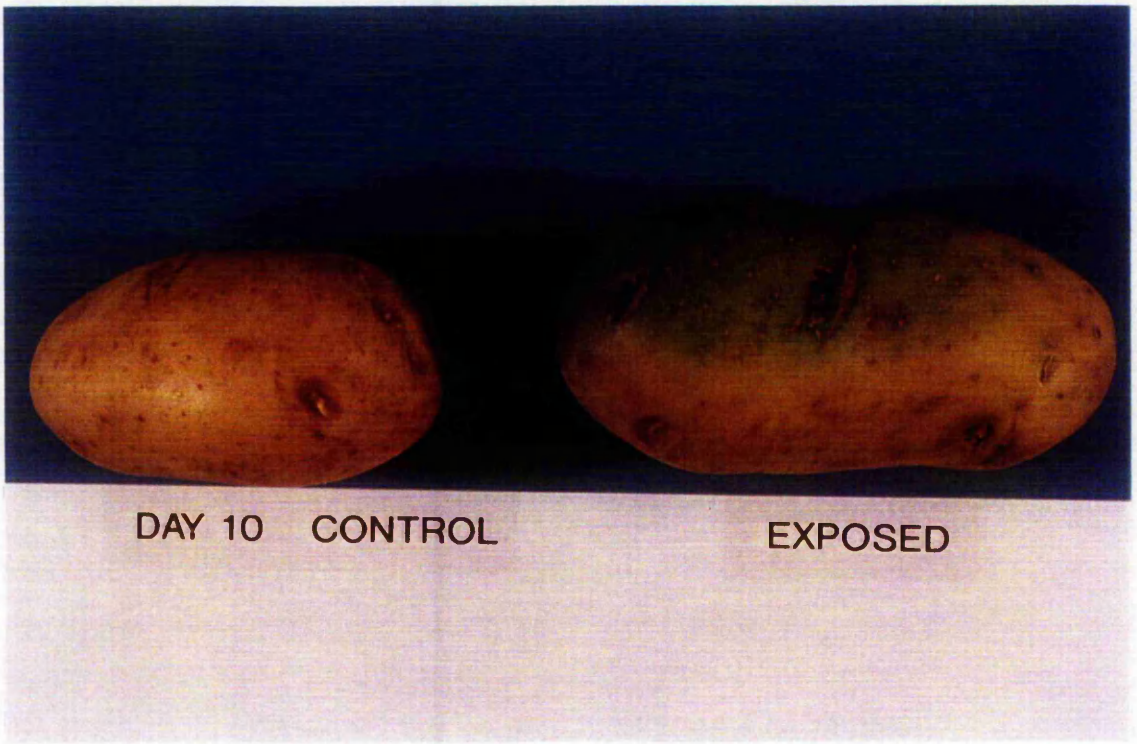


Plate 4.5 - King Edward potatoes after 10 days of exposure to light.

Chl in control tubers did not differ significantly from zero time irrespective of cv. or storage duration. Total carotenoid content in control tubers did not significantly vary from initial concentrations during exposure of tubers to light in any of the four exposures.

4.3.2.1 - Pigment concentrations in the upper surface of tubers: Greening was confirmed by pigment analysis; both Chls and carotenoids increased in the periderm of the tubers exposed to light, regardless of cv. or storage duration (Fig 4.5). Full pigment results are given in Appendix 2. However, there were clear differences in the extent of this response between cvs. Brodick and Record accumulated the greatest concentrations of total Chl, reaching maxima of 12-15 μmol g^{-1} sample and 10-12 μmol g^{-1} respectively. Greening was less in King Edward and Pentland Dell tubers with maximum concentrations of 5-6 μmol g^{-1} and 4-5 μmol g^{-1}.

Storage duration did not have a marked effect on total Chl accumulated after 10 days of light exposure. Total Chl content did vary between exposures but there was no overall pattern between cvs. and most differences were not significant (10% level). However, there did appear to be an effect of storage duration on initial rates of Chl accumulation. Brodick, Record and King Edward tubers that had been stored for 10 weeks all accumulated significantly (1% level) less total Chl after 3 days light exposure than freshly harvested tubers. Similarly, Brodick and Record tubers stored for 20 weeks accumulated less Chl than those stored for 10 weeks after 3 days exposure.

Chl *a* to *b* ratios were variable and again the cvs. did not exhibit any similar responses (Fig 4.6). However, as the duration of light exposure increased Chl *a* to *b* ratios tended towards a value of 4 irrespective of initial values. This was especially noticeable in the cvs. that accumulated higher total Chl concentrations, i.e. Brodick and Record.

Total carotenoid concentrations followed a similar pattern to total Chl concentrations (Fig. 4.7). Brodick and Record accumulated significantly (1% level) higher carotenoid contents than Pentland Dell or King Edward. There

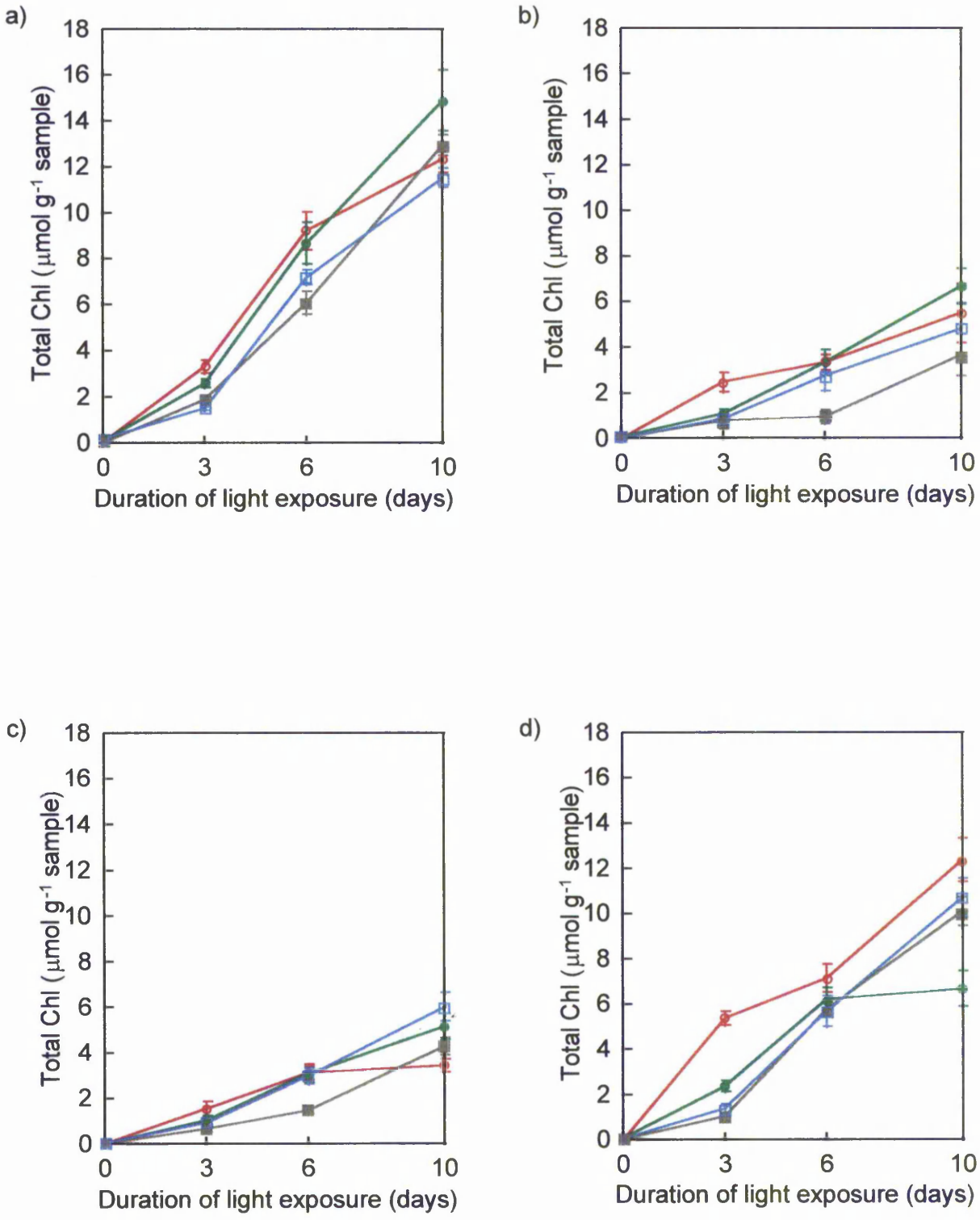


Figure 4.5 - Accumulation of total Chl in the upper, illuminated, surface of tubers exposed to light after various storage durations. Each point is the mean of 12 samples ± standard error. Tubers were stored for —○— 0, —●— 10, —■— 20 and —□— 30 weeks. a) Brodick, b) King Edward, c) Pentland Dell and d) Record.

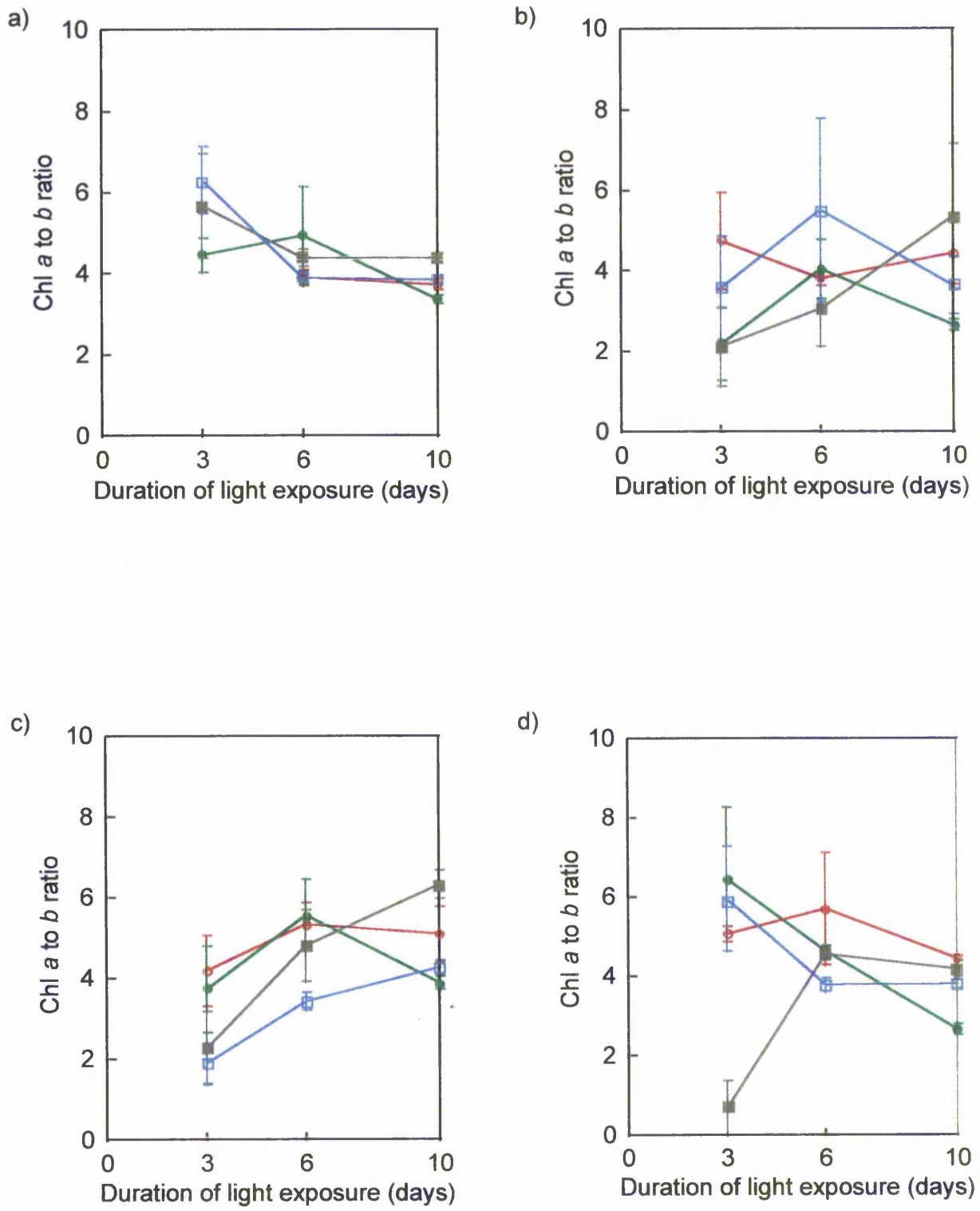


Figure 4.6 - Chl *a* to *b* ratios in the upper, illuminated, surface of tubers exposed to light after various storage durations. Each point is the mean of 12 samples ± standard error. Tubers were stored for —○— 0, —●— 10, —■— 20 and —□— 30 weeks. a) Brodick, b) King Edward, c) Pentland Dell and d) Record.

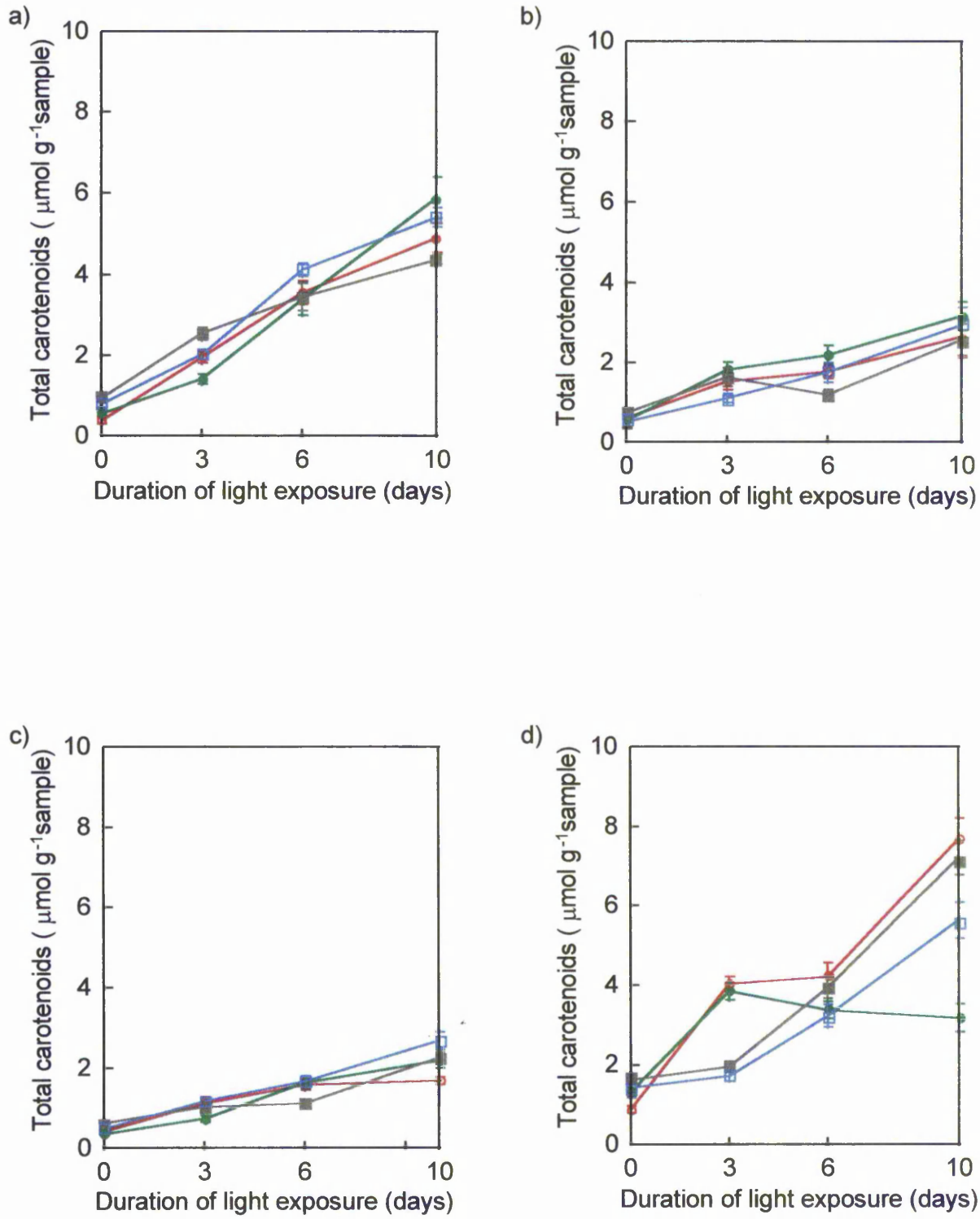


Figure 4.7 - Accumulation of total carotenoids in the upper, illuminated, surface of tubers exposed to light after various storage durations. Each point is the mean of 12 samples ± standard error. Tubers were stored for —○— 0, —●— 10, —■— 20 and —□— 30 weeks. a) Brodick, b) King Edward, c) Pentland Dell and d) Record.

were no significant (10% level) effects of storage duration on carotenoid accumulation.

Total Chl to total carotenoid ratios rose significantly (5% level) in all cvs. irrespective of storage duration between 3 and 6 days of light exposure (Fig. 4.8). However, significant (10% level) increases did not always occur between 6 and 10 days of light exposure. In all 4 varieties the highest Chl to carotenoid ratios were achieved during the exposure following 10 weeks of storage and the lowest following 20 weeks. In Brodick and King Edward final Chl to carotenoid ratios were significantly (1% and 5% levels, respectively) higher after 0 and 10 weeks of storage than after 20 or 30 weeks of storage. Whereas in Pentland Dell and Record this difference was only significant between the exposures following 10 and 20 weeks of storage.

4.3.2.2 - Pigment concentrations in the lower surface of tubers: Significant (1% level) accumulation of total Chl occurred in the unexposed surface of tubers exposed to light in all 4 cvs. (Fig 4.9). However, the maximal Chl concentrations were much lower than in the upper surfaces, approximately 2 μmol g^{-1} in Brodick and Record, 1.4 μmol g^{-1} in King Edward and only 0.25 μmol g^{-1} in Pentland Dell.

Greening of the lower surface of King Edward tubers was to a similar extent as Brodick and Record even though the upper surface accumulated much less than these cvs.. Lower surface greening in Pentland Dell was very low, irrespective of storage duration, with very low final concentrations.

Increasing storage duration resulted in a decrease in final Chl concentrations in the lower surfaces of all 4 cvs., but this was not linear with time. Pentland Dell tubers stored for 30 weeks did not accumulate significant (1% level) Chl at all.

Total Chl composition was variable, but in general Chl *a* to *b* ratios increased during light exposure (Fig. 4.10). Ratios achieved after 10 days exposure were lower than those found in the upper surfaces, especially in Pentland Dell.

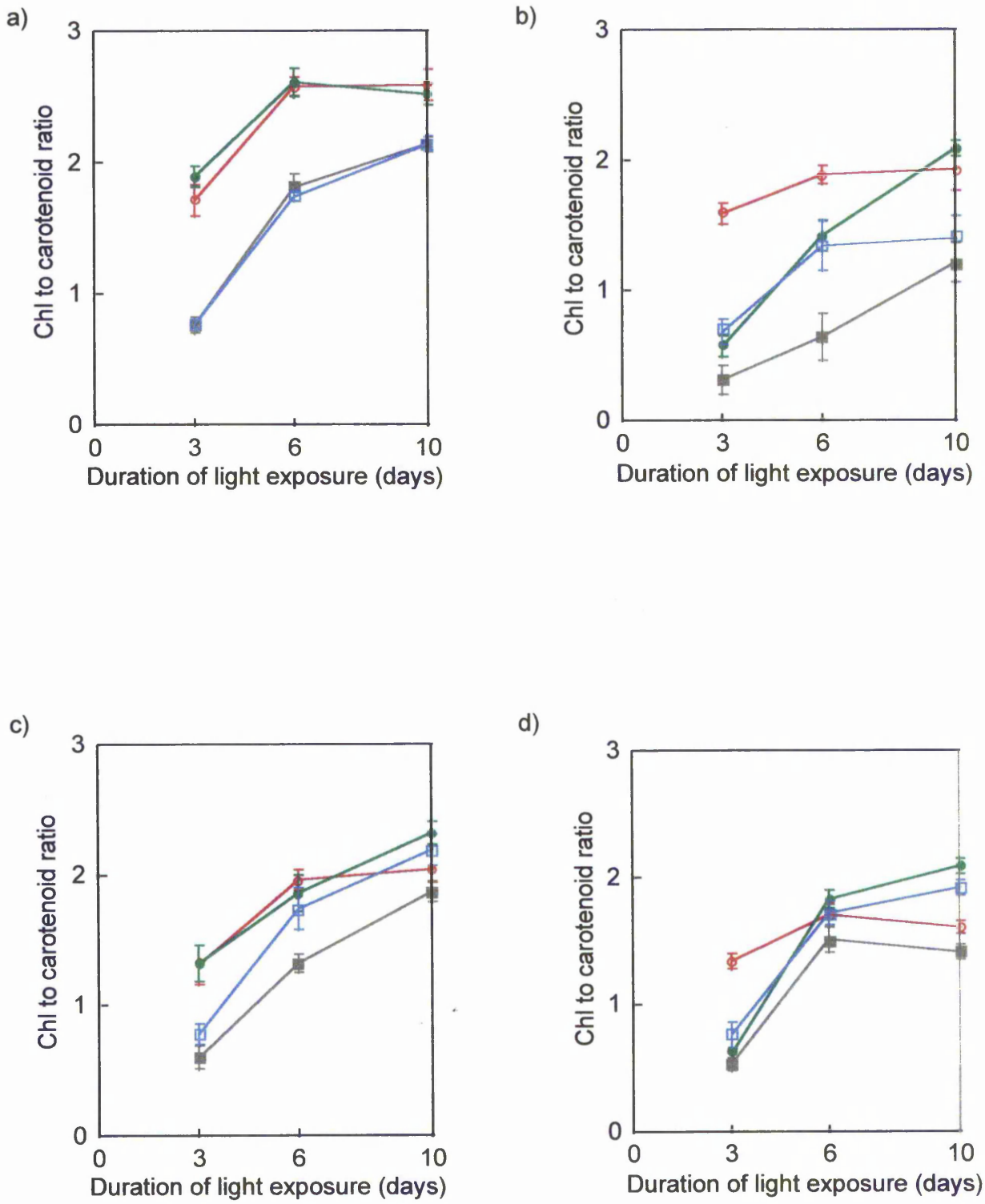


Figure 4.8 - Chl to total carotenoid ratios in the upper, illuminated, surface of tubers exposed to light after various storage durations. Each point is the mean of 12 samples ± standard error. Tubers were stored for —○— 0, —●— 10, —■— 20 and —□— 30 weeks. a) Brodick, b) King Edward, c) Pentland Dell and d) Record.

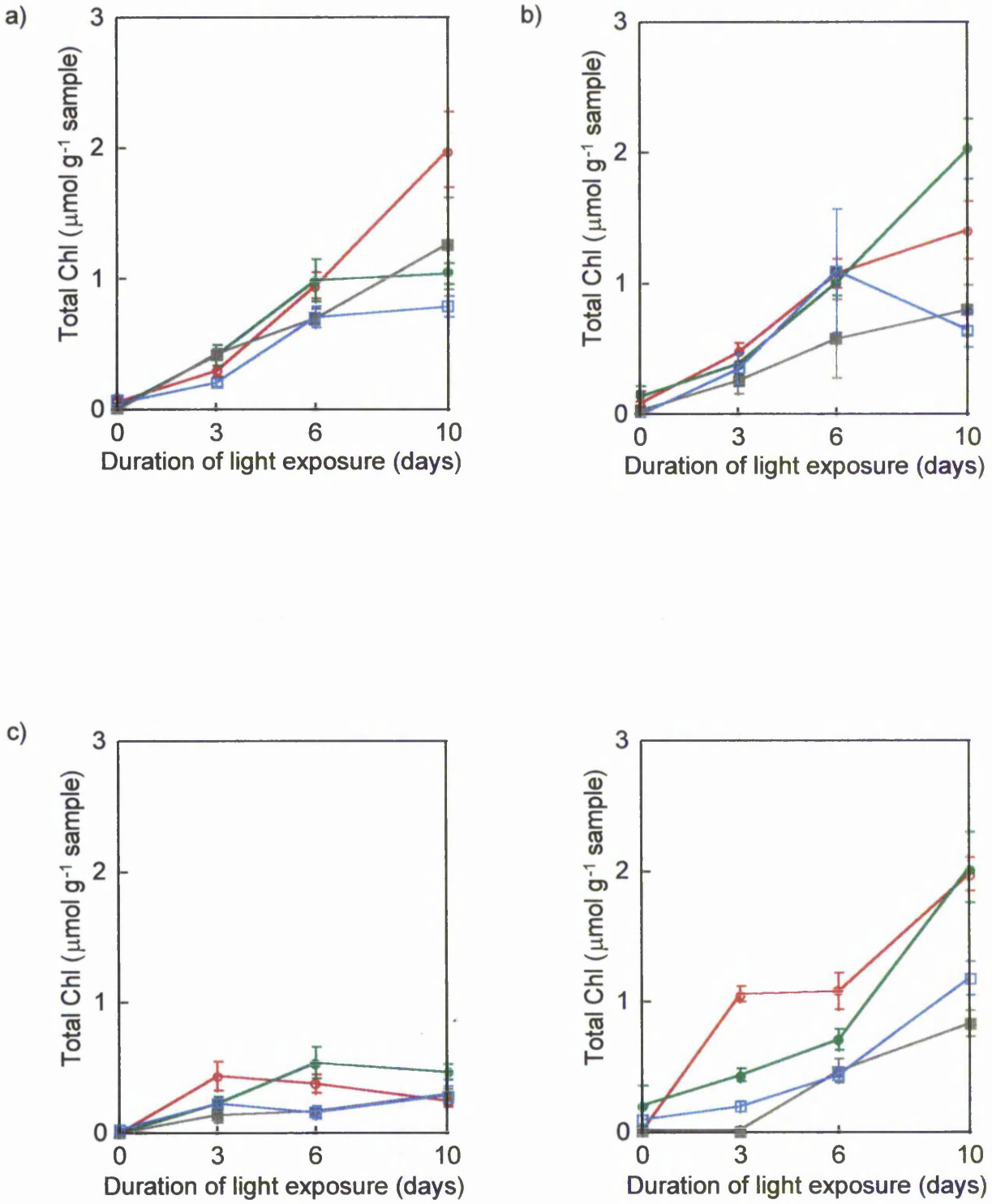


Figure 4.9 - Accumulation of total Chl in the lower, non-illuminated, surface of tubers exposed to light after various storage durations. Each point is the mean of 12 samples ± standard error. Tubers were stored for —○— 0, —●— 10, —■— 20 and —□— 30 weeks. a) Brodick, b) King Edward, c) Pentland Dell and d) Record.

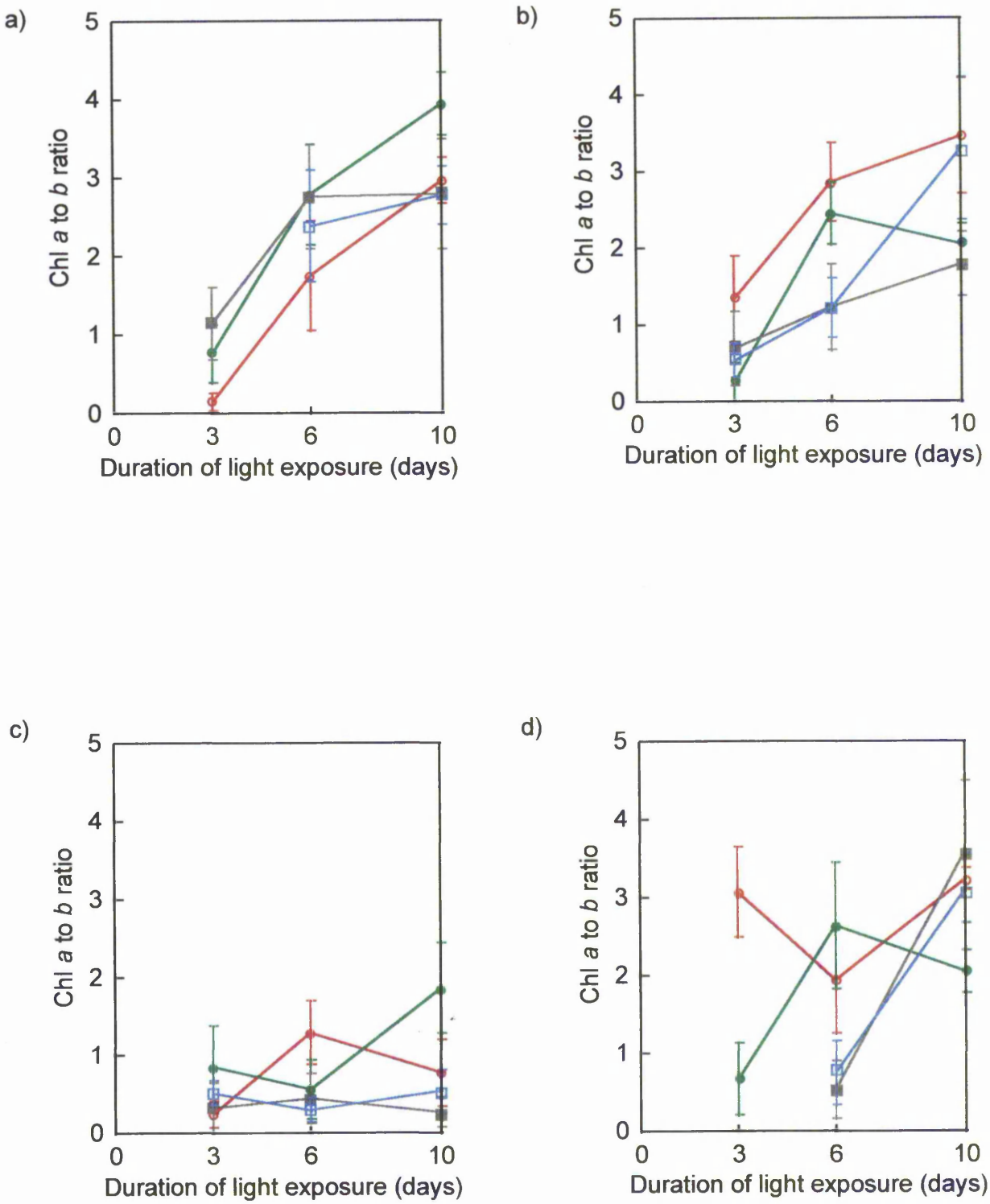


Figure 4.10 - Chl *a* to *b* ratios in the lower, non-illuminated, surface of tubers exposed to light after various storage durations. Each point is the mean of 12 samples ± standard error. Tubers were stored for —○— 0, —●— 10, —■— 20 and —□— 30 weeks. a) Brodick, b) King Edward, c) Pentland Dell and d) Record.

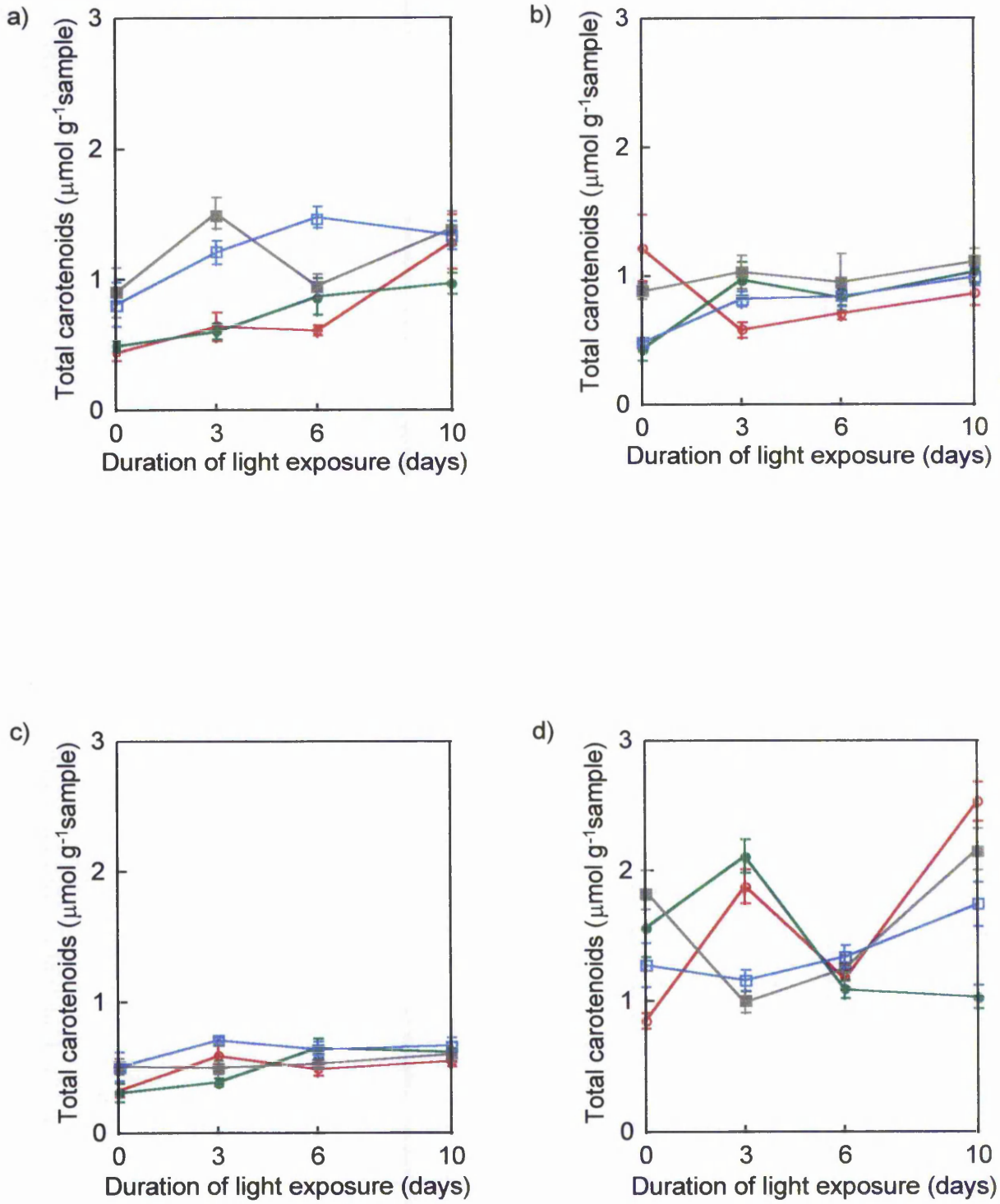


Figure 4.11 - Accumulation of total carotenoids in the lower, non-illuminated, surface of tubers exposed to light after various storage durations. Each point is the mean of 12 samples ± standard error. Tubers were stored for —○— 0, —●— 10, —■— 20 and —□— 30 weeks. a) Brodick, b) King Edward, c) Pentland Dell and d) Record.

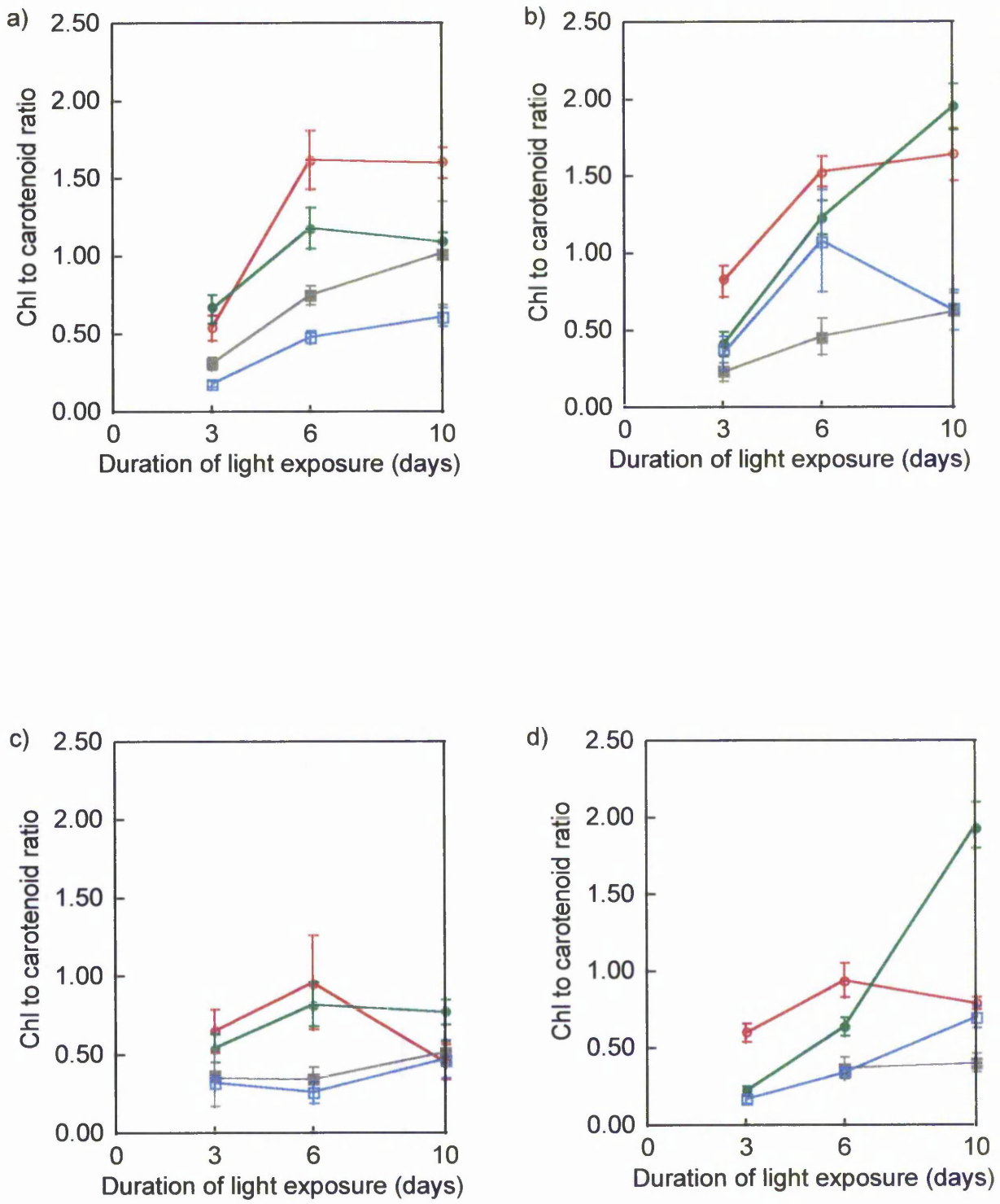


Figure 4.12 - Chl to total carotenoid ratios in the lower, non-illuminated, surface of tubers exposed to light after various storage durations. Each point is the mean of 12 samples ± standard error. Tubers were stored for 0, 10, 20 and 30 weeks. a) Brodick, b) King Edward, c) Pentland Dell and d) Record.

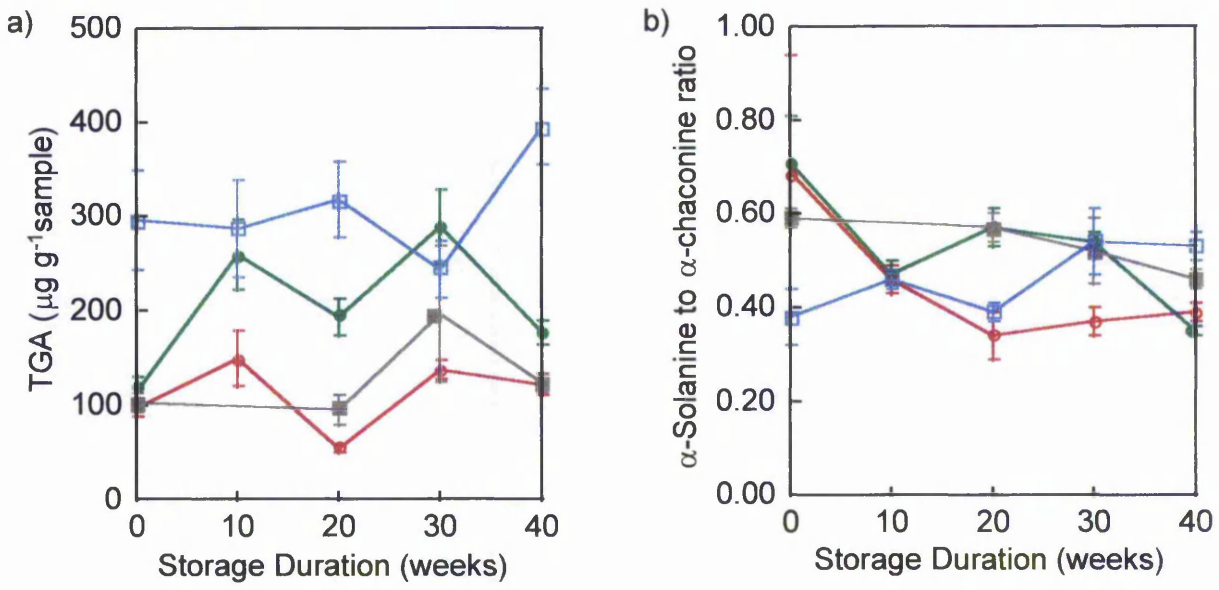


Figure 4.13 - Fluctuation in a) total glycoalkaloid content and b) α-solanine to α-chaconine ratios of tubers during storage in the dark under conditions described. Each point is the mean of 10 samples (2 samples from each of 5 tubers) ± standard error. —○— King Edward, —●— Brodick, —■— Record and —□— Pentland Dell.

Accumulation of carotenoids was slight or non-existent in all 4 cvs. (Fig. 4.11), therefore, Chl to carotenoid ratios were largely dependant on total Chl content (Fig 4.12).

4.3.3 - Glycoalkaloid concentrations.

Full glycoalkaloid data are given in Appendix 2. A marked effect of cv. on both TGA content and accumulation was observed throughout the study.

4.3.3.1 - Glycoalkaloid content and composition of stored tubers: Pentland Dell tubers consistently had the highest TGA concentrations (Fig. 4.13a) throughout the 30 weeks of storage, followed by Brodick, with the TGA content of Record and King Edward remaining lower throughout. There was some evidence of TGA cycling during storage in all the varieties except Pentland Dell, however, this was only significant (5% level) in Brodick.

Notably, there was no significant difference in the TGA content of non-stored tubers and those stored for 30 weeks in any cultivar.

After harvest/curing King Edward and Brodick tubers had high initial α-solanine to α-chaconine ratios of approximately 0.7 (Fig. 4.13b). These had dropped significantly (5% level) after storage for 30 weeks in both cvs. to slightly under 0.4. Record exhibited a slow but non-significant decrease in α-solanine to α-chaconine ratios from 0.6 initially to 0.46 after storage. Conversely non-stored Pentland Dell tubers had a very low α-solanine to α-chaconine ratio below 0.4, which rose to almost 0.6 during the storage period.

4.3.3.2 - Glycoalkaloid changes in the upper surface of tubers exposed to light: Exposure of King Edward tubers to light did not cause an increase in TGA in any of the experiments. However, exposure of tubers of the other cvs. did result in increased TGA concentrations (Fig. 4.14). There was a marked cv. effect on the lag period before TGA accumulation started, and consequently on the degree of TGA accumulation. TGA concentrations in tubers of Brodick had risen significantly after 3 days of light exposure, whereas Pentland Dell tubers did not demonstrate a significant increase until 6 days of exposure and Record until 10 days.

Storage duration also had a significant effect on the extent of TGA accumulation. In all three varieties that exhibited light-induced TGA accumulation this was significantly (1% level) reduced by storage for 20 or 30 weeks. Indeed, Brodick tubers stored for 30 weeks did not significantly (10% level) accumulate any TGA in 10 days of light exposure, despite accumulating higher TGA concentrations than any other cv. after 10 weeks storage, 1.22 mg g^{-1} sample.

No significant effects on α-solanine to α-chaconine ratios were observed, although, Brodick and Pentland Dell tubers did appear to show a slight rise in α-solanine to α-chaconine ratios during light exposure (Fig. 4.15).

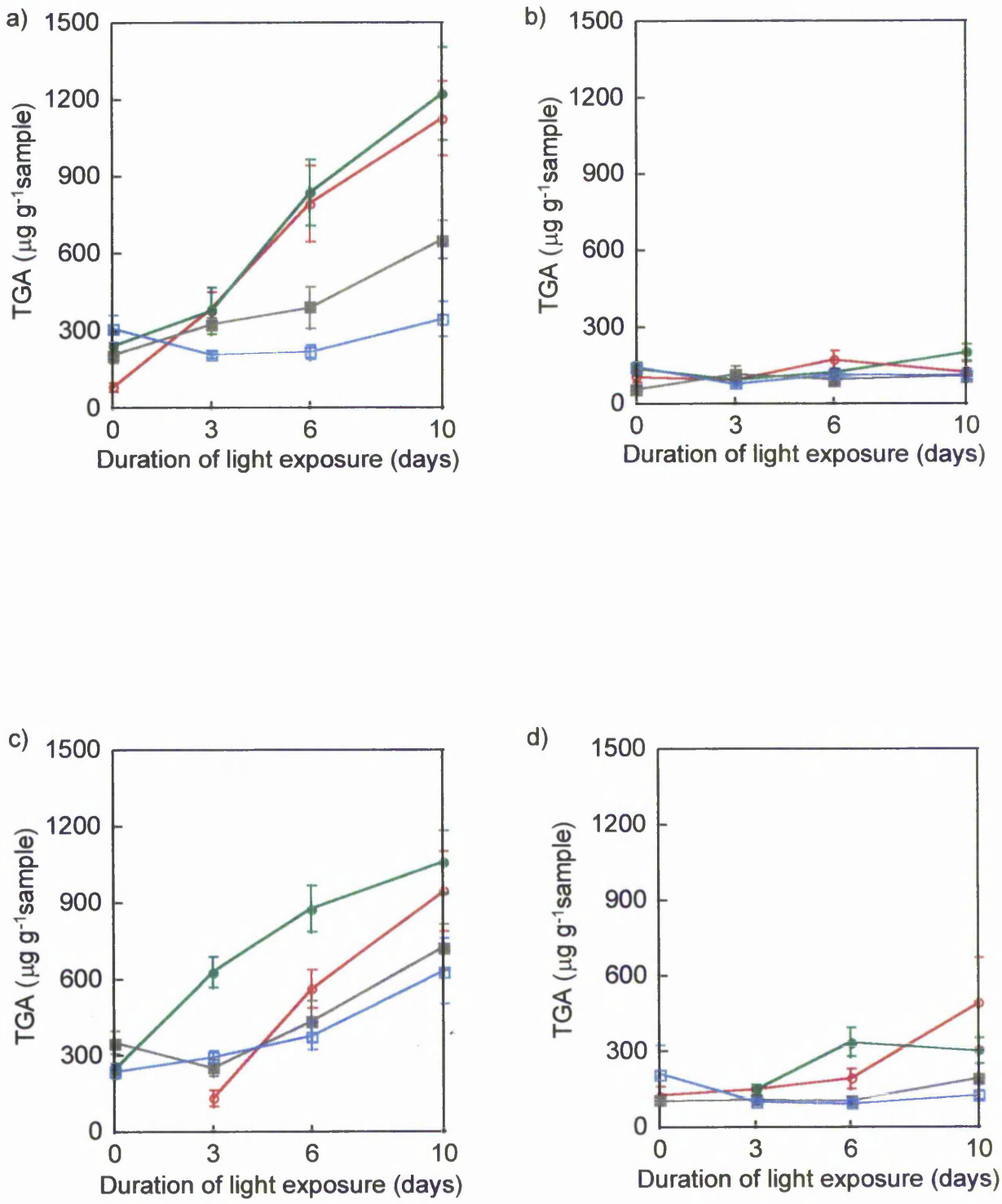


Figure 4.14 - Accumulation of total glycoalkaloids in the upper, illuminated, surface of tubers exposed to light after various storage durations. Each point is the mean of 12 samples ± standard error. Tubers were stored for —○— 0, —●— 10, —■— 20 and —□— 30 weeks. a) Brodick, b) King Edward, c) Pentland Dell and d) Record.

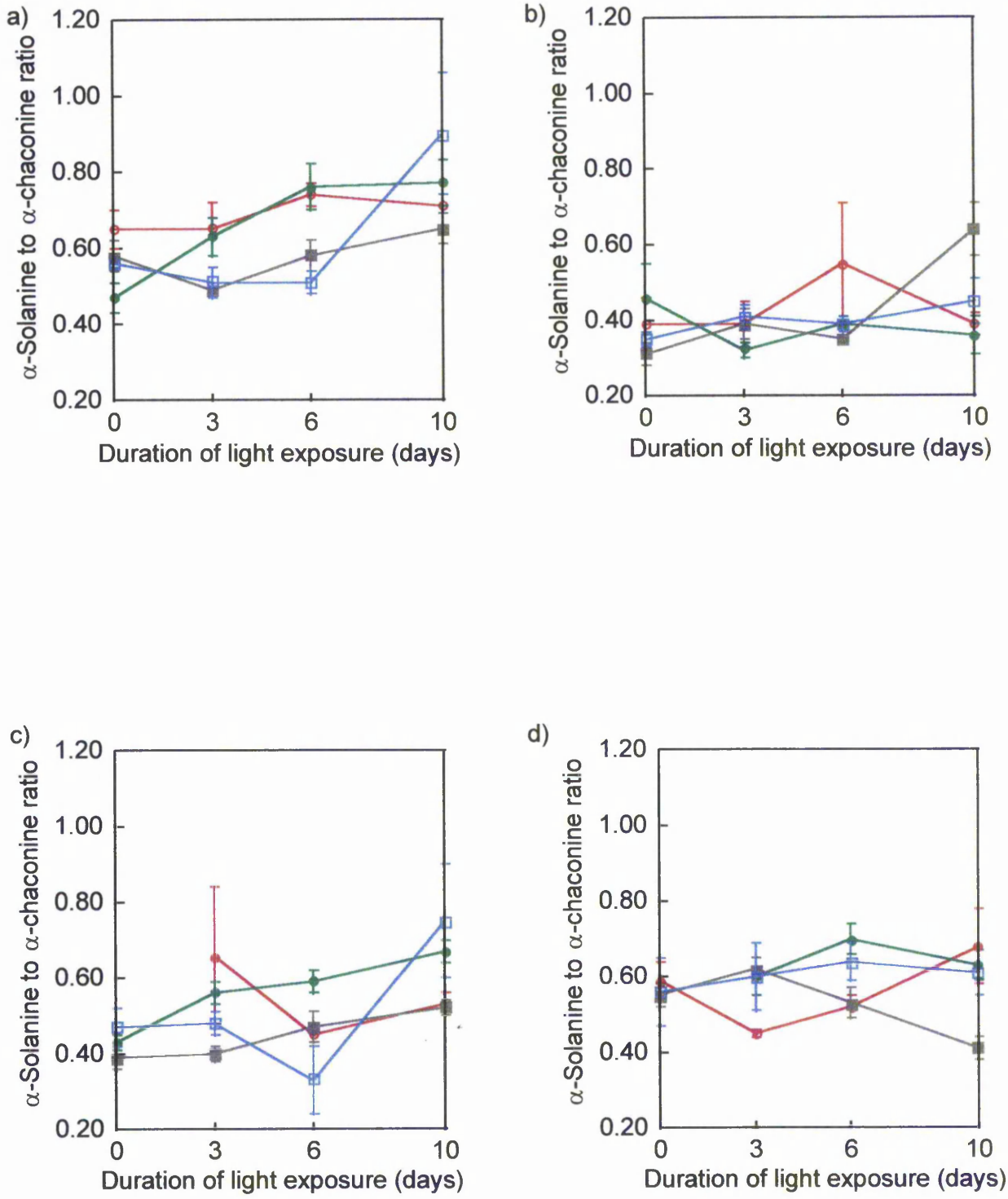


Figure 4.15 - α-Solanine to α-chaconine ratios in the upper, illuminated, surface of tubers exposed to light after various storage durations. Each point is the mean of 12 samples ± standard error. Tubers were stored for 0, 10, 20 and 30 weeks. a) Brodick, b) King Edward, c) Pentland Dell and d) Record.

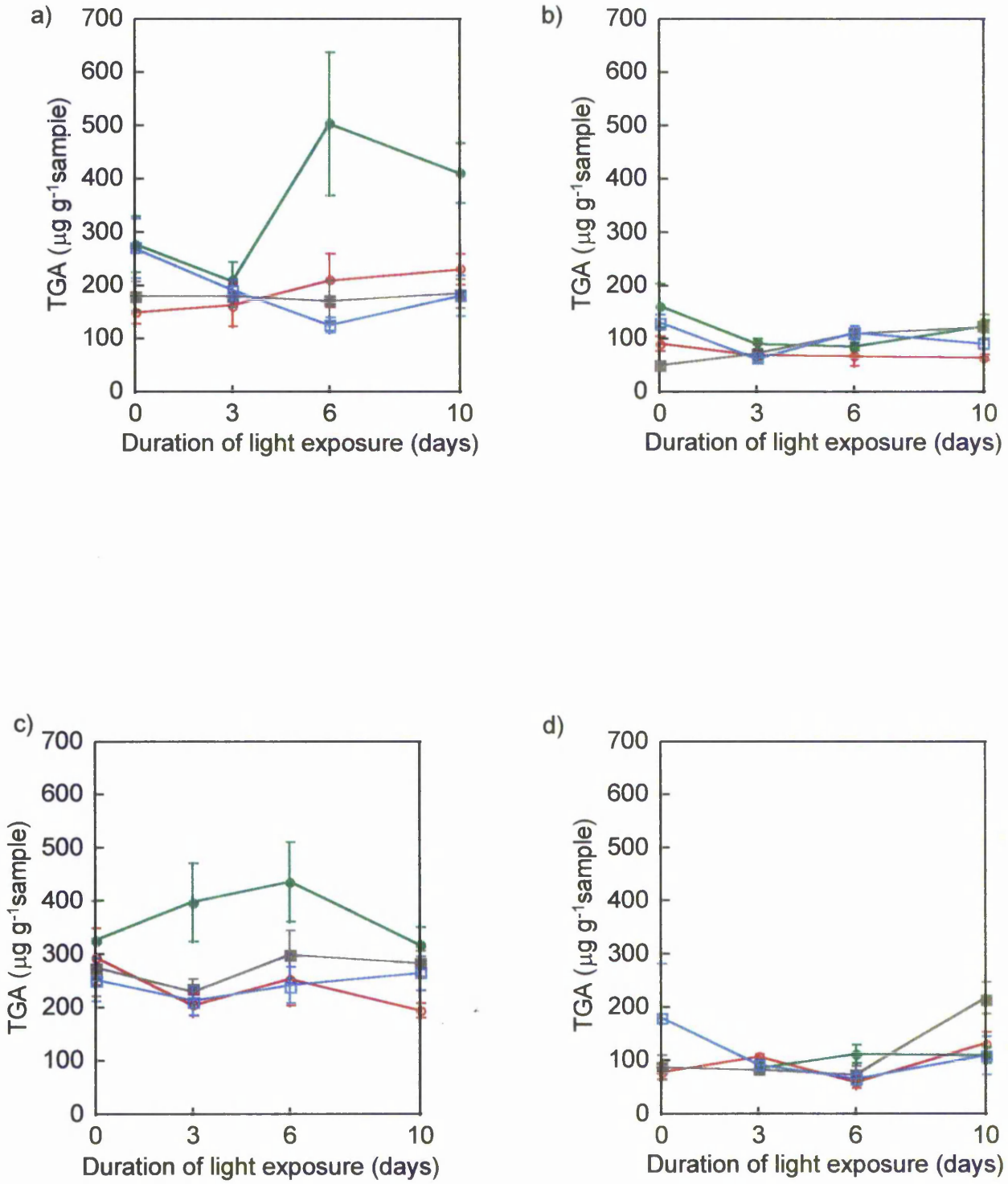


Figure 4.16 - Accumulation of total glycoalkaloids in the lower, non-illuminated, surface of tubers exposed to light after various storage durations. Each point is the mean of 12 samples ± standard error. Tubers were stored for —○— 0, —●— 10, —■— 20 and —□— 30 weeks. a) Brodick, b) King Edward, c) Pentland Dell and d) Record.

Table 4.1 - Summary of total Chl and TGA results for the upper, exposed, surface of tubers after 10 days of light exposure following 0, 10, 20 and 30 weeks of dark storage. Figures are % of maximum for that cv.

Cultivar	Total Chl				TGA			
	0	10	20	30	0	10	20	30
Brodick	82.3	100.0	83.9	77.4	92.2	100.0	53.3	28.3
King Edward	82.5	100.0	54.7	72.3	61.8	100.0	53.9	55.7
Pentland Dell	57.1	85.5	71.0	100.0	89.1	100.0	68.3	59.6
Record	100.0	53.9	81.4	86.5	100.0	61.3	38.8	25.4

4.3.3.3 - Glycoalkaloid concentrations in the lower surface of tubers exposed to light: There was no significant change in TGA content of the lower half of King Edward and Record tuber samples when the upper surface of the tuber was exposed to light (Fig. 4.16). Only the exposure after 10 weeks storage caused any accumulation in Brodick and Pentland Dell. This increase was not continuous with time and much smaller than that in the upper, exposed, surface.

None of the 4 cvs. demonstrated any significant alteration to α-solanine to α-chaconine ratios in the unexposed tissues (Fig. 4.17), although variation in these samples was very high.

4.4 - Discussion

The greening response was observed in all tubers that were exposed to light, irrespective of cv. or storage duration. There were significant varietal differences in the extent of this response, as has previously been observed (Patil, Salunkhe and Singh, 1971; Griffiths *et al.*, 1994). Notably the 2 cvs. with the highest Chl accumulation in response to light, Brodick and Record, are both varieties used for processing and, therefore, selected for their high dry matter content. While this may account for some of the difference in Chl content when measured on a fresh weight basis it is unlikely to explain the large difference in Chl concentrations between the four cvs.

A further factor which could be involved is periderm thickness. Both Brodick and Record have a deep outer layer around the tuber, which is much thinner in King Edward and Pentland Dell. Visually greening is less evident in the former 2 cvs. in spite of greater Chl content. Therefore, it is likely that the thicker cork layer will reduce the amount of PAR reaching the cortical tissues immediately below it, which could affect greening in either of 2 ways; high PPFD will inhibit Chl accumulation in potato tubers (Patil *et al.*, 1971, Percival and Dixon, 1996) therefore thinner periderm layers could lead to

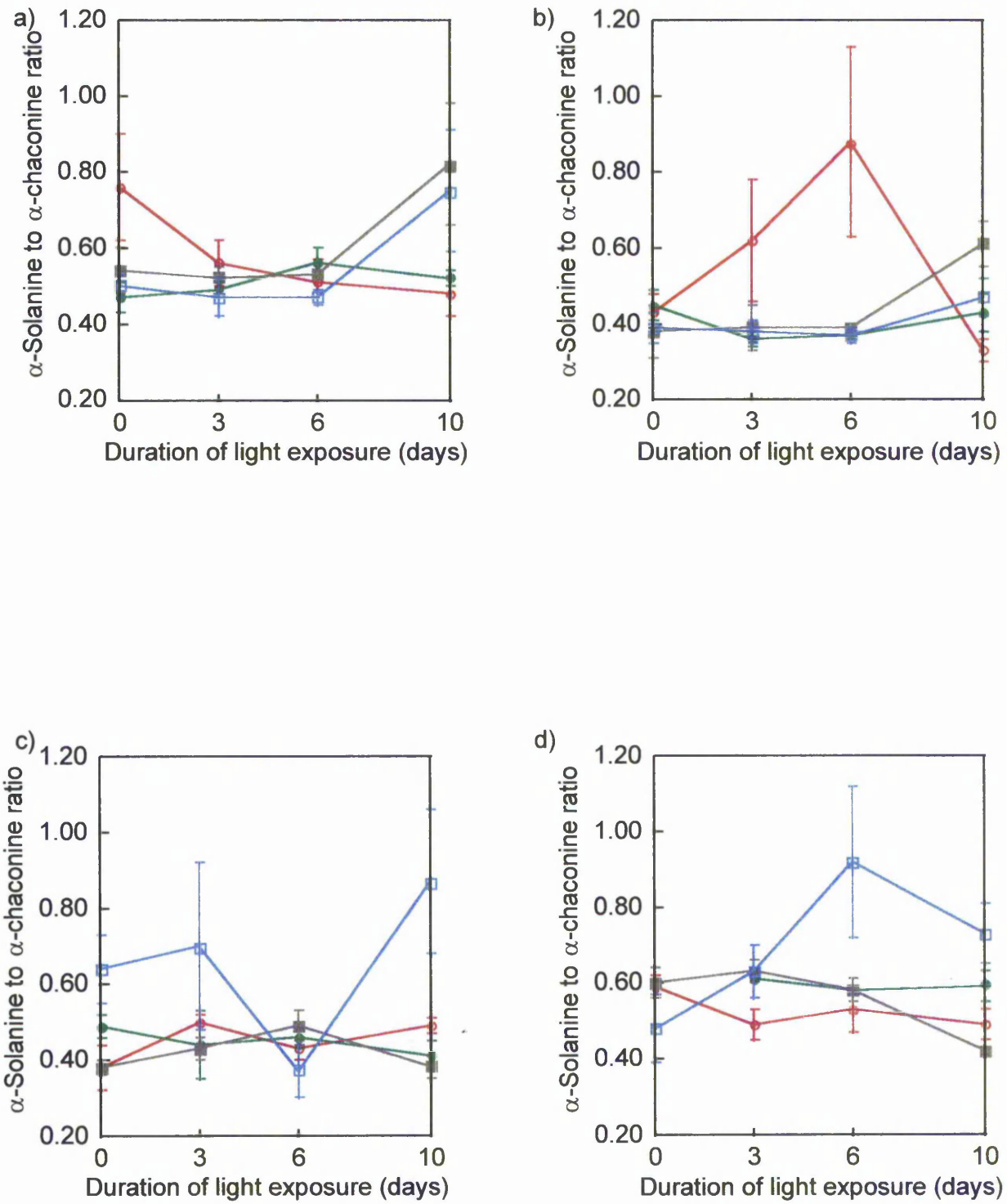


Figure 4.17 - α-Solanine to α-chaconine ratios in the lower, non-illuminated, surface of tubers exposed to light after various storage durations. Each point is the mean of 12 samples ± standard error. Tubers were stored for —○— 0, —●— 10, —■— 20 and —□— 30 weeks. a) Brodick, b) King Edward, c) Pentland Dell and d) Record.

reduced greening, or secondly higher Chl production could be an adaptation to the lower amount of PAR received, much as in some shade-tolerant plants (Boardman, Bjorkman, Anderson, and Goodchild, 1975).

There was also a considerable varietal difference in the light-induced accumulation of TGA. As tuber TGA content was measured on a dry weight basis there can be no affect of tuber water content on these results. Furthermore, TGA concentrations could not be related to any obvious physical factors and it is likely that TGA accumulation is entirely a physiologically governed response.

Whereas the lag phase before the onset of Chl synthesis is similar irrespective of cv., and thought to be about 24 h (Morris, Graham and Lee, 1979) there was a marked difference between cvs. in the period before detectable accumulation of TGA occurred. This significantly affected the TGA content of tubers after 10 days of light exposure. Brodick, which accumulated more TGA than the other 3 cvs., had the shortest lag phase, with a detectable increase in TGA by the first sampling date, after 3 days.

This variation in lag phase between Chl and TGA accumulation and the difference between maximum accumulation of TGA and Chl (Table 4.1) indicates that the light receptor and signal transduction pathways for induction of synthesis of the 2 molecules are separate. Indeed, Peterman and Morris (1985) demonstrated that, although similar, the action spectrums of the 2 responses are distinct.

Unlike Chl, both TGA and carotenoids are present within the potato tuber at all times and changes in the concentrations of these could be indicative of tuber stress. Significant changes in the content of both TGA and carotenoids was observed during storage. However, there was no continuous accumulation of either. Olsson and Rosalund (1995) suggested that TGA are cycled during long term storage, with sharp rises in TGA followed by slow declines. The results from this study indicate some degree of cycling, but are not conclusive and a shorter period between samples would be needed to unequivicably demonstrate this. The slow rise in carotenoids during 20 weeks of storage could be indicative of the stress

which low temperature storage causes to potato tubers. The decrease in carotenoids after 20 weeks of storage could be due to the general deterioration in tuber quality after this time, although, if carotenoids were accumulated as a general response to tuber stress this would not be expected.

Although storage duration had no significant effect on the Chl content after 10 days of light exposure it did affect the Chl concentrations after 3 days of exposure. This was probably due to an increased lag phase before the onset of greening, which in turn could have been due to tuber dormancy.

In contrast, storage duration had no effect on the lag phase before TGA synthesis was increased, but did markedly affect the subsequent rate of TGA accumulation. Storage for more than 10 weeks caused a significant reduction in the potential of tubers to accumulate TGA when exposed to light. The extent of this effect increased with further storage and was also cv. specific. Brodick tubers stored for 0 and 10 weeks had near identical TGA accumulation, whereas those stored for 20 weeks stored only 50% of this and those stored for 30 weeks had no significant TGA accumulation. This was repeated in Pentland Dell tubers but tubers stored for 20 and 30 weeks still accumulated approximately 60% of those stored for 0 and 10 weeks.

This effect was not due to environmental conditions during light exposure, as there was no correlation with either mean daily PAR or temperature and the amount of TGA accumulated. It is likely that the reduced accumulation is related to the tubers metabolic state. It has been previously reported that the physiological age of a tuber can effect TGA content and accumulation (Bomer and Matis, 1924; Griffiths *et al.*, 1994). This could be important in determining the fitness of long term stored tubers for human consumption. As old tubers with slight greening would be unlikely to contain enhanced TGA concentrations and, therefore, would still be safe to eat. Although more research would be required before greening in aged tubers could be discarded as a health risk.

It has previously been observed that light exposure can effect α-solanine to α-chaconine ratios (Percival, Dixon and Sword, 1996). However, in this study there was no evidence of change in these apart from a slight, and non-significant, rise in Brodick tubers stored for 10 weeks then exposed to light. The lack of any change in α-solanine to α-chaconine ratio in stored tubers not exposed to light suggests that the turnover of glycoalkaloids in stored tubers is low. It is possible that the availability of free sugars determines this ratio but as there is no significant change in α-solanine to α-chaconine ratios in tubers exposed to light and accumulating TGA irrespective of storage duration or cv., it is perhaps more likely that α-solanine to α-chaconine ratios are under genetic control.

Although storage duration did not significantly affect either total Chl or total carotenoids accumulated after 10 days of light exposure it did appear have a significant effect on Chl to carotenoid ratios. A low ratio is indicative of photo-oxidative stress as the quenching abilities of carotenoids become more important (Deming-Adams and Adams, 1996). In all 4 cvs. the lowest Chl to carotenoid ratios were found in the tubers that had been stored for 20 weeks. This exposure had by far the highest accumulated PAR and the highest mean daily PPFD. It is likely that the relatively high PPFD was enough to stress the newly forming photosynthetic apparatus of the greening tubers, leading to the need for higher carotenoid concentrations relative to Chl. In all 4 cvs. the exposure following 30 weeks of storage led to lower Chl to carotenoid ratios than was found in the potatoes stored for 10 weeks, however, only in Brodick and King Edward were Chl to carotenoid ratios higher in the tubers stored for 0 weeks than those stored for 30 weeks. Therefore, it is unclear if storage duration, and consequently tuber age, has any role in affecting Chl to carotenoid ratios, or if these are purely a result of stress induced by a relatively high PPFD.

The lower, unexposed, surfaces of tubers exposed to light all accumulated significant concentrations of Chl, although in Pentland Dell samples these were extremely low. The extent of greening in the other 3 cvs. was similar, reaching about 2 μmol g^{-1} sample, suggesting that the level

of greening in light exposed tissues has little effect on greening in non-exposed tissues.

As greening in these tissues is likely to be a response to a signal from light exposed tissues it may be that this a simple on/off response, not affected by signal concentrations. Since greening in exposed tissues is a phytochrome-mediated response, it also appears likely that greening in the non-exposed tissues could result from a signal transduction pathway originating from phytochrome.

Carotenoid concentrations in non-exposed tissues did not alter significantly either with storage duration or length of exposure. This is surprising as carotenoids are an essential part of the photosynthetic apparatus (Goodwin, 1980). It may be that carotenoids already present in tuber plastids were utilised in the formation of the photosystems and were adequate for the essential carotenoid requirement. Further incorporation of carotenoids would not be necessary as the tissues were not subjected to light exposure.

Significant accumulation of TGA also did not occur in non-exposed tissues. If TGA accumulation was a result of greater sugar availability due to photosynthesis occurring this could be expected. It is also unlikely that diffusion from tissues with high TGA concentrations occurs as glycoalkaloids are likely to be compartmentalised due to their disruptive effects on all steroid containing phospholipid membranes (Keukens *et al.*, 1995).

The marked difference in the light-induced response of Chl and TGA accumulation to storage is further indication that there is no biosynthetic link between the two chemical classes.

4.5 - Conclusions

The principal findings reported above are:

1. Increasing storage duration reduced the potential for TGA accumulation, but did not effect Chl accumulation. This was, in part, dependant on cv.

2. Although storage did have some effect on carotenoid and TGA concentrations in tubers that had not been exposed to light these were relatively minor and had no effect on suitability of the tubers for human consumption.
3. Greening of non-exposed tissues occurred regardless of cv. but this had no effect on TGA concentrations in these tissues.
4. TGA concentrations in control tubers are no indication of the potential for TGA accumulation when they are exposed to light.

CHAPTER 5 - ARTIFICIAL NEURAL NETWORK MODELLING OF GREENING AND GLYCOALKALOID ACCUMULATION

5.1 - Introduction

Artificial neural networks (ANN) are a relatively new computing technique, originally based on the way that the human brain processes information. They are a useful tool in understanding and interpreting very variable data (Dayhoff, 1990). Consequently, ANN's have been used for a diverse range of modelling and predictive tasks, including taxonomic identification (Simpson, Williams, Ellis and Culverhouse, 1992), predictive modelling of growth (Yee, Prior and Florence, 1993), predictions of cardiac complications after surgery (Lette, Colletti, Cerino, McNamara, Eybalin, Levasseur and Nattel, 1994), forecasting of share prices (Davalo and Niam, 1990) and the effects of microclimate on ozone injury (Balls, Palmer-Brown and Sanders, 1996). They have even been used to provide an automatic potato cultivar classification technique, which was found to be more accurate than traditional multivariate statistical classification method (Jensen, Tygesen, Kesmir, Skovaard and Sondergaard, 1997).

The most commonly used ANN's are back-error propagation networks, which typically consist of 3 sets of nodes (Fig. 5.1). The first set is the input layer, i.e. the data that may have caused the results; time, exposure, etc. The second is a layer of hidden nodes, these are the 'neurons' and each one is connected to every input and every output node. The hidden nodes apply a mathematical transformation to the data which is then passed to the third set of nodes. This output layer consists of the results from the network.

The ANN is trained by being shown 'real' data, e.g. the results of an experiment. This data is presented in a series of patterns, each consisting of a number of inputs and outputs. For example, an ANN used to model the effects of climate on vegetation would be given patterns consisting of

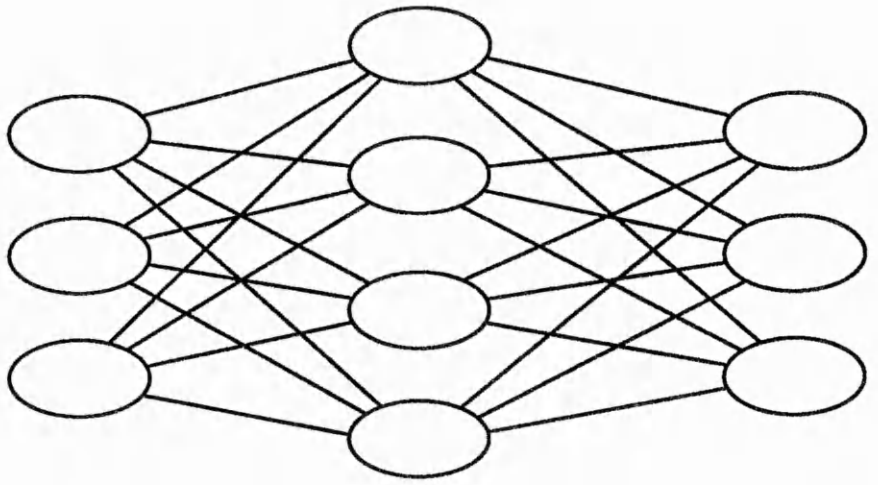


Figure 5.1 - Conceptual representation of ANN structure. Each node in the input and output layers represents 1 data point and each node in the hidden layer represents 1 neuron.

environmental data as inputs and measurements of the vegetation effects as outputs. The ANN applies individual weightings to each of the connections between nodes and compares its calculated results with the actual results that have been shown to it. These weightings are then altered in order to minimise the error. When the difference between the input and output is at its minimum training of the network is stopped and the ANN can then be used to predict results from inputs that are previously 'unseen'.

This Chapter describes the use of one such ANN to model the data obtained from the experiments described in Chapters 3 and 4.

5.2 - Materials and Methods

5.2.1 - Data origin

The entire data set from the experiments in Chapters 3 and 4 were used for network training. The outputs were: Chl *a*, Chl *b*, total Chl, Chl *a* to *b* ratio, total carotenoids, Chl to carotenoid ratio, α-solanine, α-chaconine,

TGA, α-solanine to α-chaconine ratio. Results for upper and lower surfaces of each tuber were given as separate outputs of the same sample. Inputs used were: mean temperature during exposure, mean daily maximum temperature, mean daily minimum temperature, mean daily PAR, FR, R and blue light, mean daily maximum PAR, FR, R and blue light, total PAR, FR, R and blue light (calculated from light period and PAR measurements), duration of exposure, cv., duration of storage prior to exposure and whether the tuber had been exposed to light or not.

5.2.2 - Neural network modelling

The data was used to train back propagation networks using the Neuroshell 2 software package (Ward Systems Group, Frederick, MD, USA) on a 386 DX personal computer (Tiny Computers, Salfords, Surrey, UK).

The network architecture was varied in several ways to produce the most effective network. These included the number of hidden layers, the number of hidden nodes, the interconnections between layers of nodes and the mathematical transformation used in each layer.

Optimal training of the network was estimated as follows. The network was trained with only 80% of the data, samples were presented individually in a random order until the network had 'seen' the entire training data set. The network was then tested with the remaining 20% of the original data and the error between the network predicted results and the actual results of this data was logged. This process was repeated until no further reduction in error could be achieved, which was determined to have occurred if 20,000 patterns had been presented to the network since the error of test results had been reduced. The figure of 20,000 patterns is estimated by the software as the optimal number to prevent overlearning of the data without reducing learning ability. The network was then saved and could be used as a model of greening and glycoalkaloid accumulation.

The data was presented to the networks in a number of different forms. The data from the experiments in Chapter 3 was used alone or in combination with the data from Chapter 4. Also the data from different cvs. was given to

networks separately. Finally, networks were trained using a single output, e.g. Chl *a*, Chl *b*, total carotenoids, etc. alone.

After training was complete the entire original data set was then presented to the network and the mean squared error (MSE) between actual and predicted results was used to determine the architecture that was most effective at modelling the actual data. The R^2 value (correlation) between actual and predicted results was used to compare the effectiveness of the network's ability to model the individual outputs.

The final network(s) used could then be given a range of inputs to examine their effect on greening in more detail than could be allowed from the original data.

5.3 - Results

A total of 7 networks were found to effectively model the data. These were: network 1: pigment data from Chapter 3, n=205,

network 2: pigment & glycoalkaloid data for cv. Brodick, n=195,

network 3: pigment & glycoalkaloid data for cv. Pentland Dell, n=178,

network 4: pigment & glycoalkaloid data for cv. Record, n=168,

network 5: pigment & glycoalkaloid data for cv. King Edward (data from Chapter 4 only), n=183,

network 6: pigment data for cv. King Edward (data from Chapters 3 and 4), n=426 and

network 7: all pigment & glycoalkaloid data from Chapter 4, n=724.

The architecture found to model the actual data with least error contained two blocks of hidden nodes in parallel, each with a different transformation function, both containing 15 nodes (Fig. 5.2). There was no connection between these two blocks, but both were connected with all inputs and outputs. Block 1 applied a gaussian transformation function and block 2 either a tan h (network 1) or a complimentary gaussian transformation

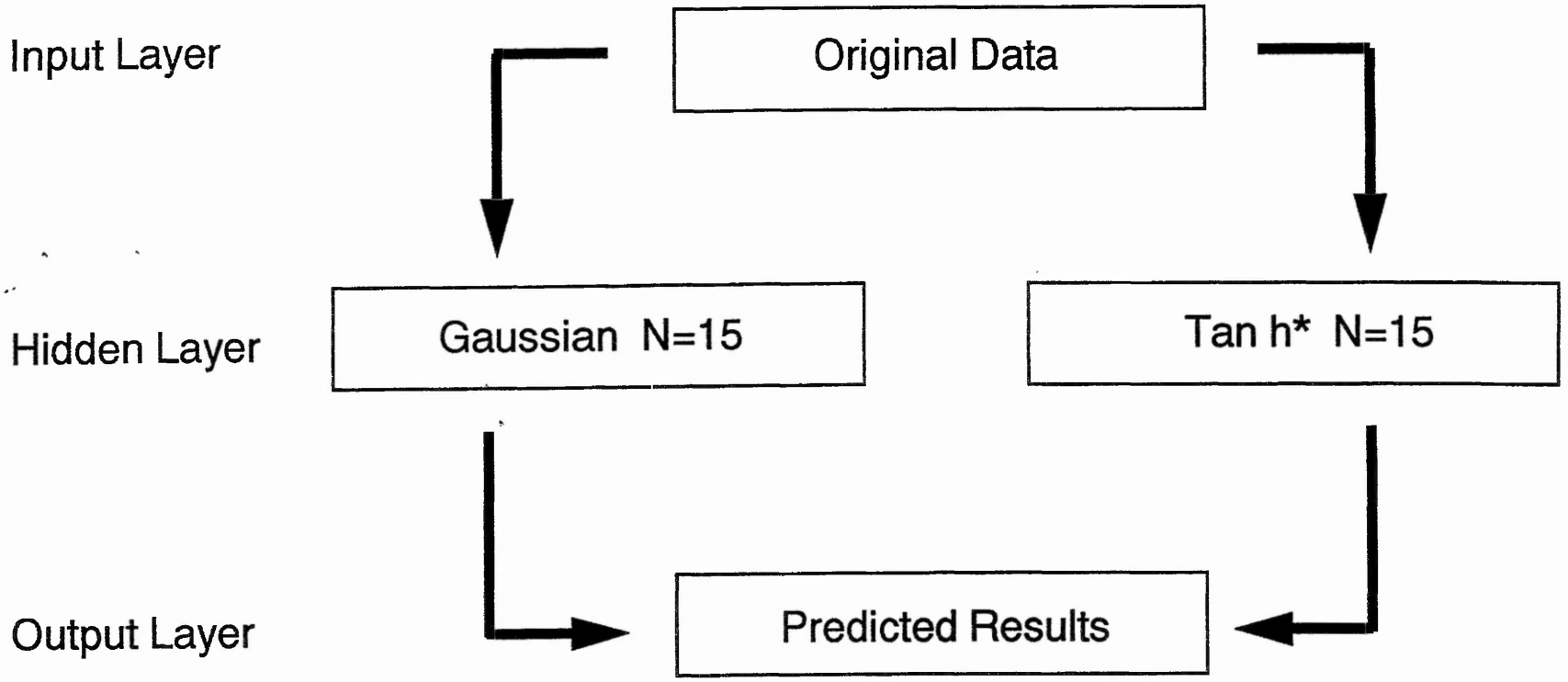


Figure 5.2 - Neural network architecture used. The text in the hidden layer shows the mathematical transformation used and number of nodes within the block. The arrows represent the connections between layers. *Tan h used in Network 1, gaussian compiement used in networks 2-7.

(inverse gaussian, all other networks). This architecture was found to be 22% more efficient at modelling the data than a standard 3 layer back propagation architecture (network 1).

Three approaches were used for training networks; using individual networks for individual cvs., using individual networks for the 2 sets of experimental data or using one network to model all the data. The network trained using all of the available data was found to be less successful than the other approaches and was therefore discontinued. Networks trained using only one output were similar in accuracy of predictions as networks trained using all outputs for the samples from the upper surface of tubers, but the latter networks were 90% more effective at modelling the data from the lower surface samples.

The most efficient networks were determined by examining MSE values for the network results when compared to the original data (Tables 5.1 & 5.2). MSE is a measurement of absolute error, i.e. the lower the value the lower the error and the better the model, this allowed direct comparison between the remaining networks. MSE values for network 7, which incorporated all the data from Chapter 4, have been shown both for the network as a whole and for the individual cvs.

The data in Table 5.1 demonstrate that error in modelling by networks 2-5 is similar to network 7 for the results from the upper surface of tubers. However, the MSE for the glycoalkaloid results is better than network 7 for Record and King Edward. It is clear from Table 5.2 that while network 7 predicts the glycoalkaloid results from the lower surface of tubers with a similar error to networks 2-5, it is inferior at modelling the pigment data from these samples.

The accuracy of these networks in modelling the data was estimated by determining the correlation between the predicted and the actual results, giving an R^2 value (Tables 5.3 & 5.4). All 7 networks were effective at predicting pigment concentrations in the upper surface of tubers, but whilst the estimates of total Chl to total carotenoid ratios were good, only networks 1,3 and 4 had any accuracy in predicting Chl *a* to *b* ratios. Network 7 did not

Table 5.1 - MSE for network outputs versus actual data of upper, exposed, surface results.

Network	Chl a	Chl b	T. Chl	Chl a: b	T. Car	Chl : Car	α-solanine	α-chaconine	TGA	α-solanine : α-chaconine
1	0.67	0.14	1.24	1.41	0.36	0.25	-	-	-	-
2	2.14	0.14	3.17	149.61	0.68	0.75	22,576	22,434	84,011	0.03
3	0.61	0.06	0.95	3.45	0.14	0.12	16,545	24,271	80,646	0.09
4	1.79	0.11	2.58	7.69	0.94	0.04	4,275	4,564	17,082	0.04
5	2.10	0.20	3.45	102.35	0.65	0.15	683	2,437	5,084	0.30
6	1.34	0.16	2.26	48.25	0.53	0.19	-	-	-	-
7	1.81	0.14	2.79	69.49	0.70	0.10	12,076	14,717	50,872	0.12
7 - Brodick	2.12	0.13	3.03	141.95	0.70	0.07	22,675	22,114	84,484	0.04
7 - P. Dell	0.64	0.06	0.91	8.74	0.19	0.10	16,072	23,710	76,669	0.11
7 - Record	2.12	0.14	3.45	12.48	1.19	0.06	7,148	8,784	31,303	0.05
7 - K. Edward	2.32	0.22	3.75	103.75	0.74	0.15	1,418	3,533	7,930	0.29

Table 5.2 - MSE for network outputs versus actual data of lower, unexposed, surface results.

Network	Chl a	Chl b	T. Chl	Chl a: b	T. Car	Chl : Car	α-solanine	α-chaconine	TGA	α-solanine : α-chaconine
1	0.09	0.03	0.19	1.65	0.19	0.06	-	-	-	-
2	0.16	0.02	0.20	4.09	13.99	0.15	5,008	9,808	28,554	0.66
3	0.02	0.01	0.03	7.66	0.03	0.15	3,365	6,698	18,032	0.06
4	0.04	329.37	325.05	n/a	0.17	0.03	824	1,824	4,857	0.07
5	0.30	0.02	0.37	13.77	0.12	1.05	383	1,044	2,696	0.12
6	0.18	0.03	0.27	7.67	0.18	0.52	-	-	-	-
7	0.14	85.76	85.46	n/a	4.15	0.38	2,360	5,097	13,266	0.26
7 - Brodick	0.17	13.43	16.01	n/a	14.50	0.17	4,447	9,247	24,913	0.67
7 - P. Dell	0.03	9.03	7.14	610.47	0.14	0.17	3,137	6,458	17,168	0.08
7 - Record	0.05	340.98	335.47	n/a	0.41	0.05	1,027	2,514	6,223	0.11
7 - K. Edward	0.31	3.18	6.17	164.74	0.45	1.12	604	1,722	3,525	0.15

Table 5.3- R^2 for network outputs versus actual data of upper, exposed, surface results.

Network	Chl a	Chl b	T. Chl	Chl a: b	T. Car	Chl : Car	α-solanine	α-chaconine	TGA	α-solanine : α-chaconine
1	0.73	0.63	0.73	0.52	0.71	0.51	-	-	-	-
2	0.89	0.90	0.90	0.05	0.84	0.93	0.50	0.57	0.56	0.24
3	0.81	0.68	0.80	0.60	0.80	0.86	0.39	0.48	0.43	0.04
4	0.85	0.87	0.87	0.44	0.79	0.93	0.23	0.30	0.27	0.10
5	0.57	0.58	0.58	0.11	0.54	0.79	0.12	0.15	0.16	0.05
6	0.62	0.61	0.63	0.09	0.61	0.67	-	-	-	-
7	0.84	0.83	0.84	0.09	0.79	0.89	0.53	0.63	0.59	0.04
7 - Brodick	0.89	0.91	0.90	0.10	0.84	0.94	0.50	0.58	0.56	0.00
7 - P. Dell	0.80	0.65	0.81	0.00	0.72	0.87	0.41	0.49	0.46	0.00
7 - Record	0.82	0.84	0.82	0.10	0.73	0.90	0.00	0.00	0.00	0.00
7 - K. Edward	0.54	0.54	0.54	0.09	0.48	0.79	0.00	0.00	0.00	0.00

Table 5.4 - R^2 for network outputs versus actual data of upper, exposed, surface results.

Network	Chl a	Chl b	T. Chl	Chl a: b	T. Car	Chl : Car	α-solanine	α-chaconine	TGA	α-solanine : α-chaconine
1	0.43	0.46	0.43	0.32	0.51	0.42	-	-	-	-
2	0.52	0.53	0.58	0.35	0.00	0.64	0.07	0.18	0.11	0.06
3	0.54	0.12	0.47	0.16	0.10	0.36	0.01	0.14	0.08	0.04
4	0.81	0.09	0.10	0.08	0.53	0.75	0.06	0.06	0.06	0.01
5	0.35	0.61	0.46	0.21	0.26	0.26	0.36	0.13	0.22	0.24
6	0.37	0.50	0.44	0.14	0.49	0.29	-	-	-	-
7	0.48	0.00	0.00	0.01	0.00	0.35	0.30	0.42	0.39	0.00
7 - Brodick	0.49	0.00	0.00	0.00	0.00	0.61	0.17	0.22	0.22	0.05
7 - P. Dell	0.20	0.00	0.00	0.00	0.00	0.26	0.08	0.17	0.12	0.00
7 - Record	0.72	0.06	0.07	0.01	0.00	0.63	0.00	0.00	0.00	0.00
7 - K. Edward	0.34	0.00	0.00	0.00	0.00	0.22	0.00	0.00	0.00	0.01

successfully predict pigment concentrations in the lower surface of tubers, but all the other networks had positive correlations between predicted and actual results. Figs. 5.3 - 5.6 demonstrate the accuracy of these predictions, even where R^2 values are only 0.5.

The data from Chapter 3 was modelled satisfactorily either on its own, or in conjunction with the cv. King Edward data from Chapter 4. Network 6 was therefore used to predict pigment content of samples stored at temperatures from 5-25°C at 1°C intervals (Figure 5.7). In general the network predictions were accurate and allowed much more detail to be observed then the actual data. However, the network predictions lost accuracy when the duration of light exposure was close to zero.

5.4 - Discussion

The data generated by the experiments in Chapters 3 and 4 exhibited considerable variation, typical of analytical studies using potato tubers. As ANN's are capable of producing accurate models even when data is scattered or highly variable, this approach was used to model the data.

The initial aim of this chapter was to combine all the data described in the previous chapters into one model. It was hoped that this could then be used to predict the response of tubers to fixed storage and environmental conditions. However, it is clear that modelling was only successful for all the outputs when the cvs. were modelled using separate networks. The complexity of the data and its inputs is shown by the necessity of a complex neural network architecture, as a simple 3 layer back-propagation network (as shown in Fig. 5.1) was incapable of accurately modelling the results. The software used to produce these networks was unable to accept distinct, unrelated, categories i.e. cv. within a single input. To produce network 7 each cv. was given a random integer as input. The software interpreted these as a continuum and therefore assigned a significance that was not present to the higher integers thus affecting some outputs, most notably the

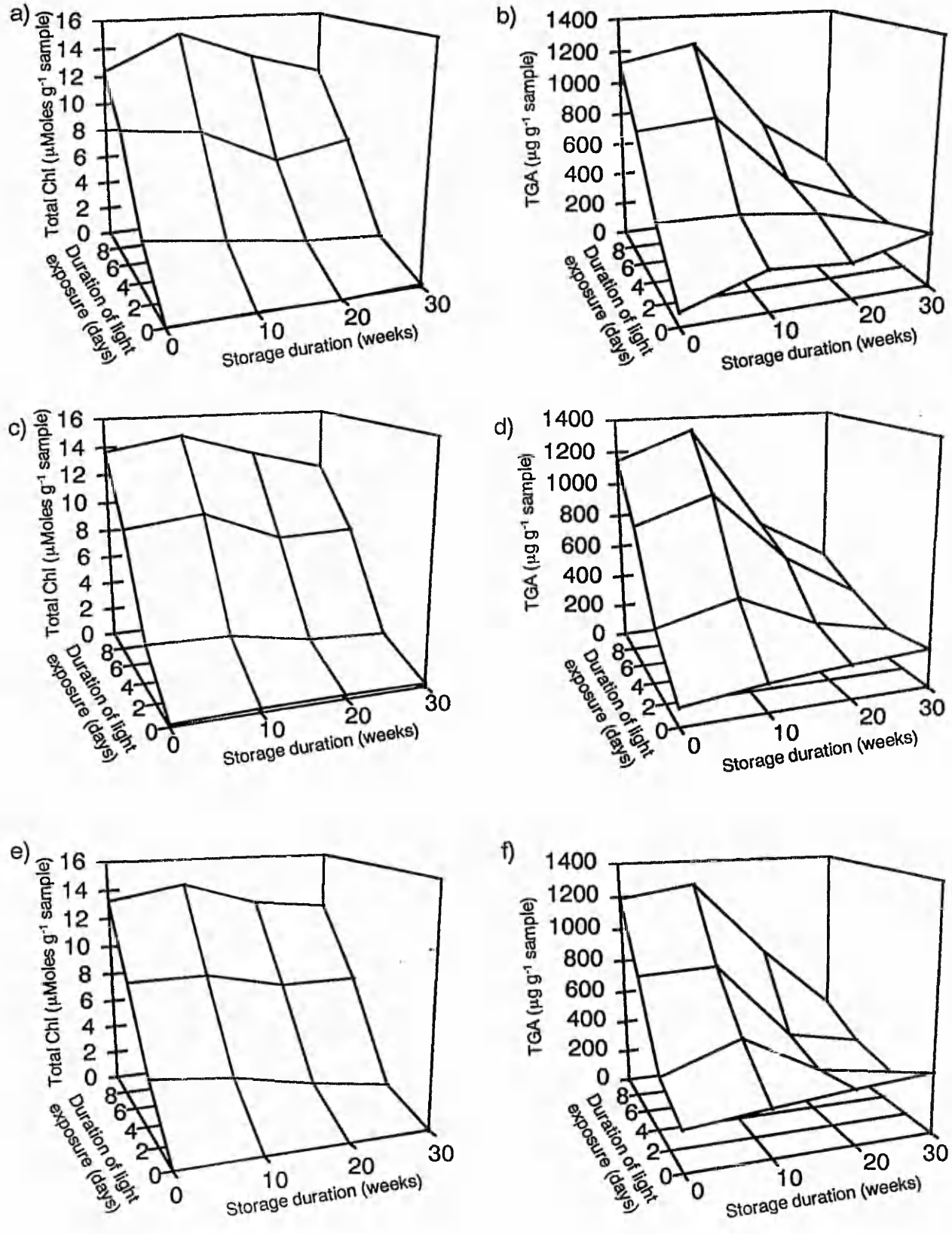


Figure 5.3 - Comparison of actual and network predicted total Chl and TGA concentraions in the upper surface of Brodick tubers exposed to light. a) Actual total Chl results, b) actual TGA results, c) & d) results predicted by network 2, e) & f) results predicted by network 7.

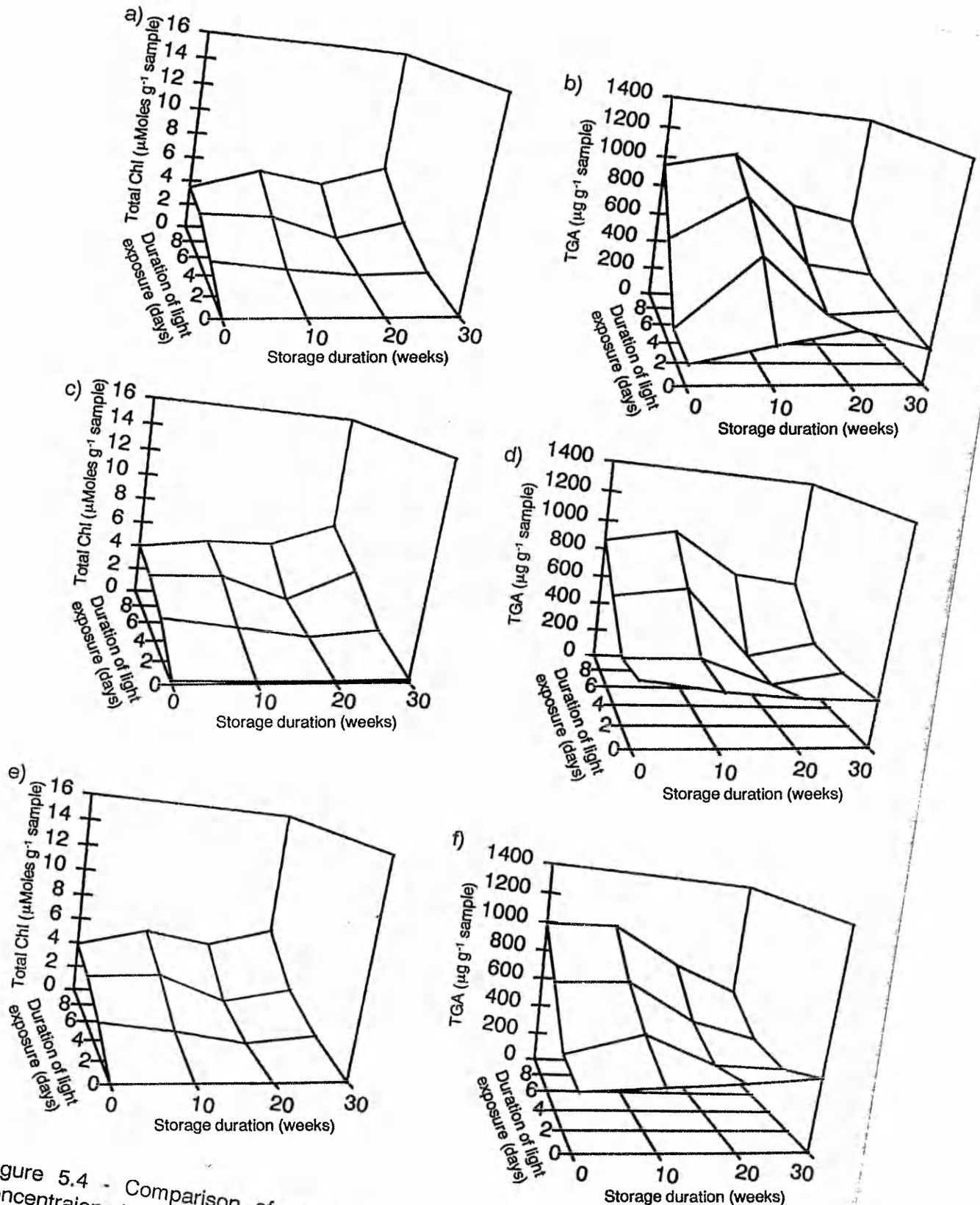


Figure 5.4 - Comparison of actual and network predicted total Chl and TGA concentraions in the upper surface of Pentland Dell tubers a) Actual total Chl results, b) actual TGA results, c) & d) results predicted by network 3, e) & f) results predicted by network 7.

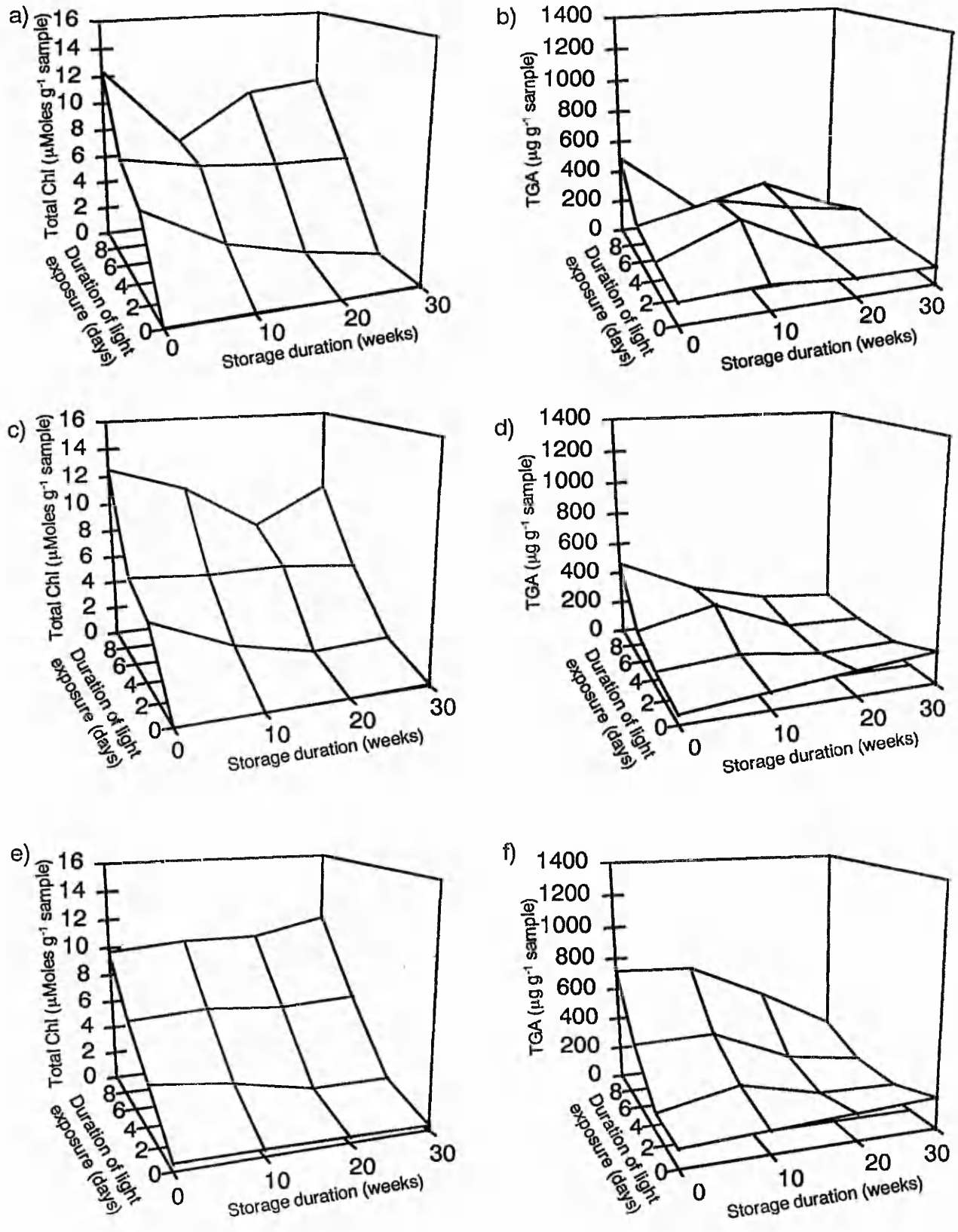


Figure 5.5 - Comparison of actual and network predicted total Chl and TGA concentraions in the upper surface of Record tubers a) Actual total Chl results, b) actual TGA results, c) & d) results predicted by network 4, e) & f) results predicted by network 7.

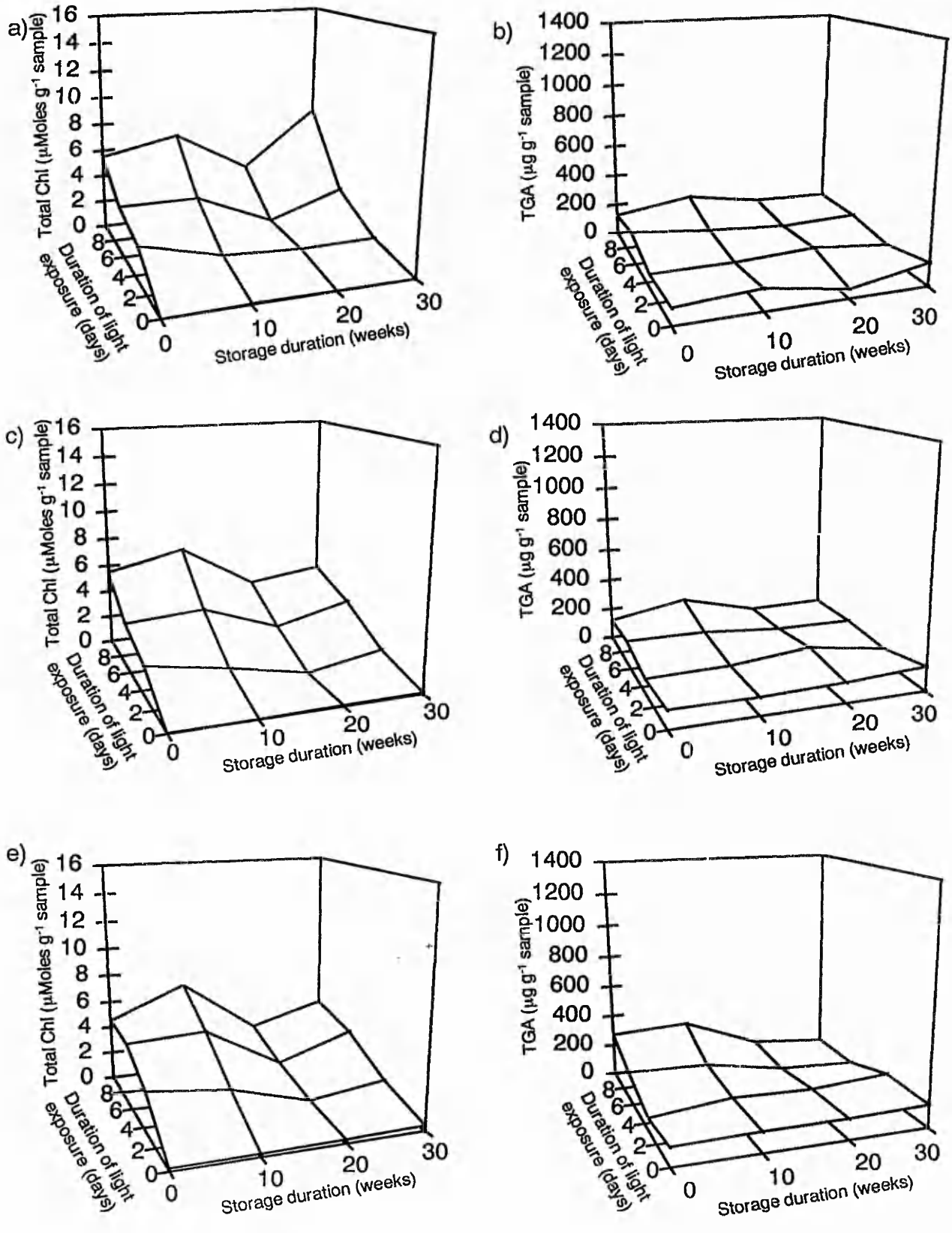


Figure 5.6 - Comparison of actual and network predicted total Chl and TGA concentraions in the upper surface of King Edward tubers a) Actual total Chl results, b) actual TGA results, c) & d) results predicted by network 5, e) & f) results predicted by network 7.

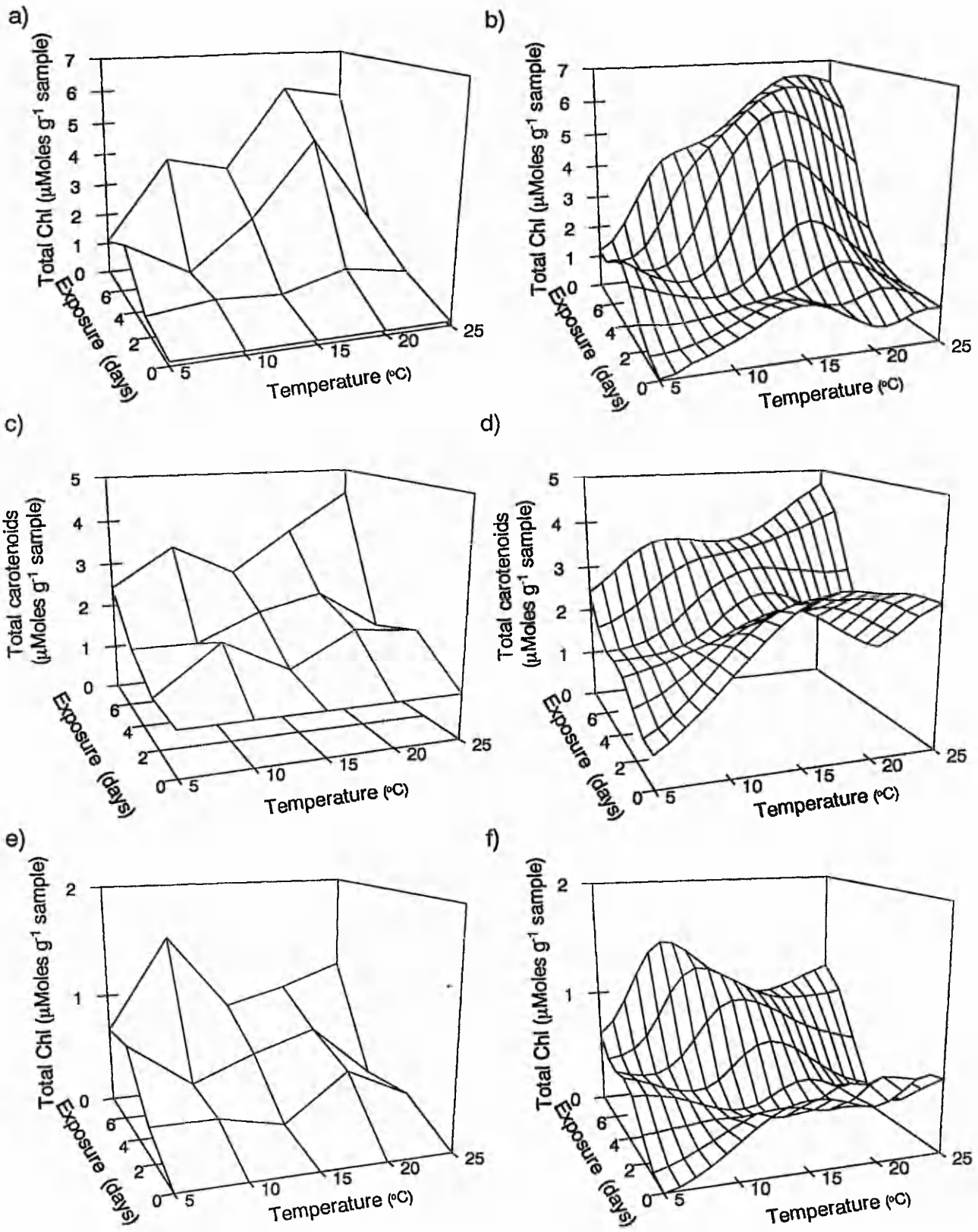


Figure 5.7 - Comparison of actual data and neural network predictions for King Edward tubers, network 1. a) Actual total Chl in upper surface of exposed tubers, b) network predicted total Chl in upper surface of exposed tubers, c) actual total carotenoids in upper surface of exposed tubers, d) network predicted total carotenoids in upper surface of exposed tubers, e) actual total Chl in lower surface of exposed tubers and f) network predicted total Chl in lower surface of exposed tubers.

glycoalkaloid results. This problem could be circumvented by using specifically written software or by using a range of cv. characteristics instead of the cv. itself as input. An advantage to the latter approach is that any characteristics which may be connected with the tuber response to light e.g. periderm thickness, may then be used to predict the response of cvs. previously unseen by the network.

All 7 networks produced were able to closely predict pigment concentrations in exposed tissues, however, the much more variable glycoalkaloid data appeared to be more difficult for the networks to model. There is some difficulty in measuring the accuracy and validating an ANN model. As with any statistical model this can only be done by testing the models predictions and then incorporating any new results. Since it was not possible to physically test these ANN's accuracy could only be judged by how closely they modelled the data which was shown to them. For this reason neural network architecture and the form in which the data was presented to the ANN was decided purely by the error between network predicted results and the actual data. Unfortunately this gave no indication of how well any ANN was actually modelling the data. In order to determine if the networks were a useful model, the correlation between the actual data and the predicted results was calculated. The original data contained between 5 and 12 results for one set of conditions, whereas the ANN could only predict one result for a single set of inputs the correlation, therefore, the correlation included a measure of the variation inherent in the original data. Indeed, many of the R^2 values seemed to depend largely on the variability of the data set. While this effect can be seen as an advantage it also makes interpretation of R^2 values difficult, as a moderate R^2 value may not actually indicate an inferior model to a high R^2 value, but just more variable data.

Some evidence for this can be seen as pigment results were predicted more accurately than the glycoalkaloid results and the exposed surface results were, similarly, predicted more accurately than the unexposed surface results. Sample variation was much higher in the glycoalkaloid and lower surface data and it can be seen from Figs. 5.3 - 5.6 that the accuracy of the

model is not entirely dependant on R^2. As an output with an R^2 of 0.5 e.g. Brodick TGA results may model the original data almost as well as an output with an R^2 value of 0.9 e.g. Brodick total Chl results.

Although predicted results followed the actual data closely there were some discrepancies. When the duration of exposure was close to 0 the network predictions deviated more markedly from the actual data and network 1 tended to over-estimate the pigment concentration at 8 days of light exposure. This was possibly due to using a gaussian transformation in the hidden layer, which reduces the importance of the extremes of the data and, while improving the modelling of variable data, may be reducing the accuracy of the predictions at the extremes of the data set.

While the data set used to produce these networks is fairly large (n=929) much more data is required before a neural network could be considered useful for estimating the response of tubers to storage and light exposure. However, these networks are an effective preliminary model and demonstrate the possibilities of more widely encompassing models incorporating more data from a range of different exposures and storage regimes.

5.5 - Conclusions

1. ANN's can accurately model the complex data produced by the experiments described in Chapters 3 and 4.
2. Using 'unseen' inputs could aid future work and experimental design.
3. The ANN's described in this Chapter could provide the basis of a large ANN model which could be used to predict the behaviour of tubers both in store and in retail outlets.
4. There is difficulty in measuring the accuracy of an ANN model and validation of any such would be needed before the model could be of use commercially.

CHAPTER 6 - PHYSIOLOGICAL STUDIES OF GREENING AND GLYCOALKALOID ACCUMULATION

6.1 - Introduction

This chapter groups together 3 studies of tuber physiology, namely changes in photosynthetic pigment composition during greening, the effect of greening on tuber CO_2 exchange and the effect of inhibitors of Chl and phytochrome biosynthesis on TGA accumulation. These are preliminary studies on aspects of tuber greening that are poorly covered in the literature and provide a basis for further study.

6.2 - The Determination of Carotenoid Composition During Greening

Carotenoids possess light-harvesting and photo-protective roles in photosynthetic tissues, but their role in non-photosynthetic tissues is less well understood. High concentrations of carotenoids are found in flowers and fruits, where they aid pollination and seed dispersal by attracting insects and herbivores. However, the role of carotenoids in roots has yet to be elucidated. Indeed, the bright orange colour, given by β-carotene, in carrots (*Daucus carota* L.) is thought to be a product of plant breeding by mankind (Kirk and Tilney-Basset, 1978). A photoprotective role is clearly unnecessary, but it is probable that the anti-oxidant properties of carotenoids are still employed in root tissues. Furthermore, as abscisic acid (ABA) synthesised in roots is formed exclusively via xanthophyll cleavage (Parry and Horgan, 1991) it is possible that the tuber xanthophyll pool may also be used for ABA production.

ABA has two important roles within potato roots, firstly it is involved in whole plant water regulation (Davies and Zhang, 1991) and secondly in control of tuber dormancy and growth (Sorce, Piaggesi, Ceccarelli and

Lorenzi, 1996). ABA accumulates in water stressed roots and moves to shoots via the xylem stream, where it is involved in regulation of shoot development and stomatal conductance. Rapid production of ABA would require a xanthophyll pool, which in tubers is available as lutein and violaxanthin are major carotenoids in white fleshed potatoes (Iwanzik, Tevini, Stute and Hilbert, 1983). However, the fluctuation of the tuber carotenoid pool in relation to ABA synthesis has not been studied.

The lipid fraction of tuber amyloplast membranes has been shown to contain up to 1% carotenoids by weight (Fishwick and Wright, 1980). This helps to maintain membrane integrity as carotenoids have the ability to quench free radicals, thus preventing lipid peroxidation. The antioxidant properties of β-carotene have been studied (Krinsky and Denecke, 1982), and it was found that antioxidant behaviour only occurred at low oxygen concentrations. However, the *in vivo* oxygen concentration of most cells is around 2%. A number of other carotenes and xanthophylls have also been found to have similar antioxidant properties. Relatively high carotenoid concentrations have also been found in the mitochondrial and microsomal fractions of potato tuber cells (Costes, Burghoffer, Carrayol, Ducet and Diano, 1976).

The distribution of carotenoids in the tuber is uneven, with the pith containing less total carotenoids than the cortex (Tevini, Iwanzik and Schonecker, 1984). Lutein and violaxanthin were the most prevalent pigments irrespective of location within the tuber.

A number of studies have examined, in varying detail, the carotenoid composition of fresh or dark-stored potato tubers, but none have examined the changes in tuber carotenoid content during the greening of potatoes. This section describes the initial adaptation of a carotenoid analysis method to potato tubers and a preliminary study of the changes in tuber carotenoid composition during greening.

6.2.1 - Materials and methods

6.2.1.1 - Plant material: Potato tubers (cv. King Edward) were purchased from a local supermarket and stored in darkness at room temperature for approximately 24 h before use. Tubers were either greened in a glasshouse or kept in darkness. Samples were taken at 0, 4, 7, 10, and 14 days and analysed for photosynthetic pigment content as described below.

Mature sugar beet (*Beta vulgaris* L.) leaves were kindly provided by J. Dixon (The Nottingham Trent University) and were grown under standard greenhouse conditions.

6.2.1.2 - HPLC and TLC determination of carotenoids composition: The outer 5 mm, including the periderm, was peeled from the tuber and homogenised in 80% aq. acetone (v/v) with 20 mg sodium carbonate g^{-1} sample. Pigments were transferred to diethyl ether using a separating funnel. The ether was dried off by vacuum and the sample resuspended in HPLC-grade acetone. Fifty µl of the sample was injected onto the HPLC system. Sugar beet leaves (approximately 25 g) were treated identically.

HPLC of carotenoids was performed using a System Gold 126 pump, 507 autosampler, 406 analogue to digital converter (all Beckman Instruments, High Wycombe, UK) and a LC-85 spectrophotometric variable wavelength detector (Perkin Elmer, Beaconsfield, UK) fitted with a Beckman 250x4.5 mm Ultrasphere 5 µm reversed phase C_{18} column (Beckman Instruments) controlled by a Viglin SL personal computer (Alperton, UK) using System Gold chromatography software (Beckman Instruments).

A gradient mixture of (A) methanol to water (75:25 v/v) and (B) ethyl acetate (Cano, 1991) was used. The initial composition of the mobile phase was 100% A followed by gradient elution to a semi-final composition after 20 min of 30% A 70% B. A further gradient of 10 min to a final composition of 100% B was followed by a 10 min re-equilibration with 100% A. The flow rate was 1.7 cm^3 min^{-1}. Initial samples were run 4 times with the detector wavelength set to 415, 430, 450 or 470 nm.

Extracts were also applied to a Silica 60 thin layer chromatography plate (20x20 cm) (Fisons, Loughborough, UK). The plate was run for 1.25 h in a chamber containing acetone and 40-60^{o} petroleum ether (40:60 v/v). Resulting pigment bands were scraped off, resuspended in HPLC-grade acetone and centrifuged at 13,000 rpm in a MSE Micro Centaur Microfuge (RW Jennings, East Bridgford, UK). An aliquot of each band was applied to the HPLC column as above and a second aliquot scanned from 700 to 300 nm on a 550 S scanning spectrophotometer (Perkin Elmer, Beaconsfield, UK).

6.2.2 - Results

Initial HPLC analysis of tissue from greened tubers produced 14 peaks (Table 6.1, Figs. 6.1 - 6.4). The detector response at 415 and 470 nm was much lower than at 430 or 450 nm, with peak 13 being undetectable at the former wavelengths. Detector response at 430 nm was slightly higher for the early peaks than 450 nm, however, it was lower for peak 10. Furthermore, separation of peaks 10 and 11 was better at 450 nm (Fig. 6.3). A detector wavelength of 450 nm was used for all further HPLC analysis of carotenoids.

Comparison of maxima obtained by UV-vis spectroscopy of TLC bands with published absorbance maximas and the HPLC retention times of the resuspended TLC bands enabled peaks 7, 10, 12, 13 and 14 to be positively identified as lutein, Chl *b*, Chl *a*, phaeophytin and β-carotene, respectively. Other peaks were tentatively identified using published data (Cano, 1991) as *cis*-violaxanthin, *cis*-neoxanthin, violaxanthin and neolutein A & B (Table 6.2). The C_{10} epimeric isomer of Chl *b* was also detected. No attempt at quantification was made.

Five carotenoids were found in the control samples (Table 6.3). However, only lutein and β-carotene were found in all samples. Violaxanthin and *cis*-violaxanthin were found in most control samples, with neolutein A being found in just one. After 4 days of light exposure Chl's and a small

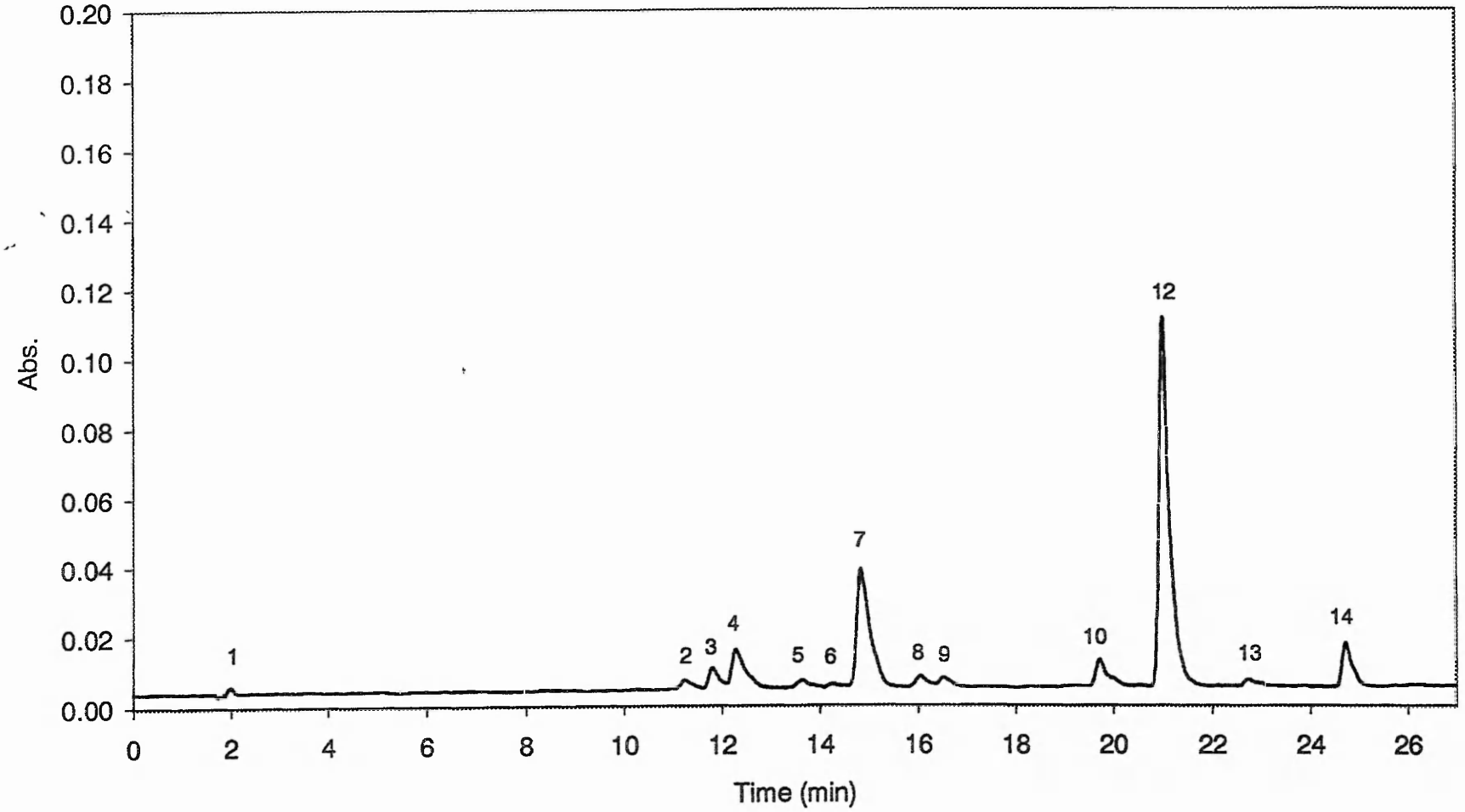


Figure 6.1 - HPLC chromatogram of pigment extract from greened tuber. Absorbance measured at 415 nm.

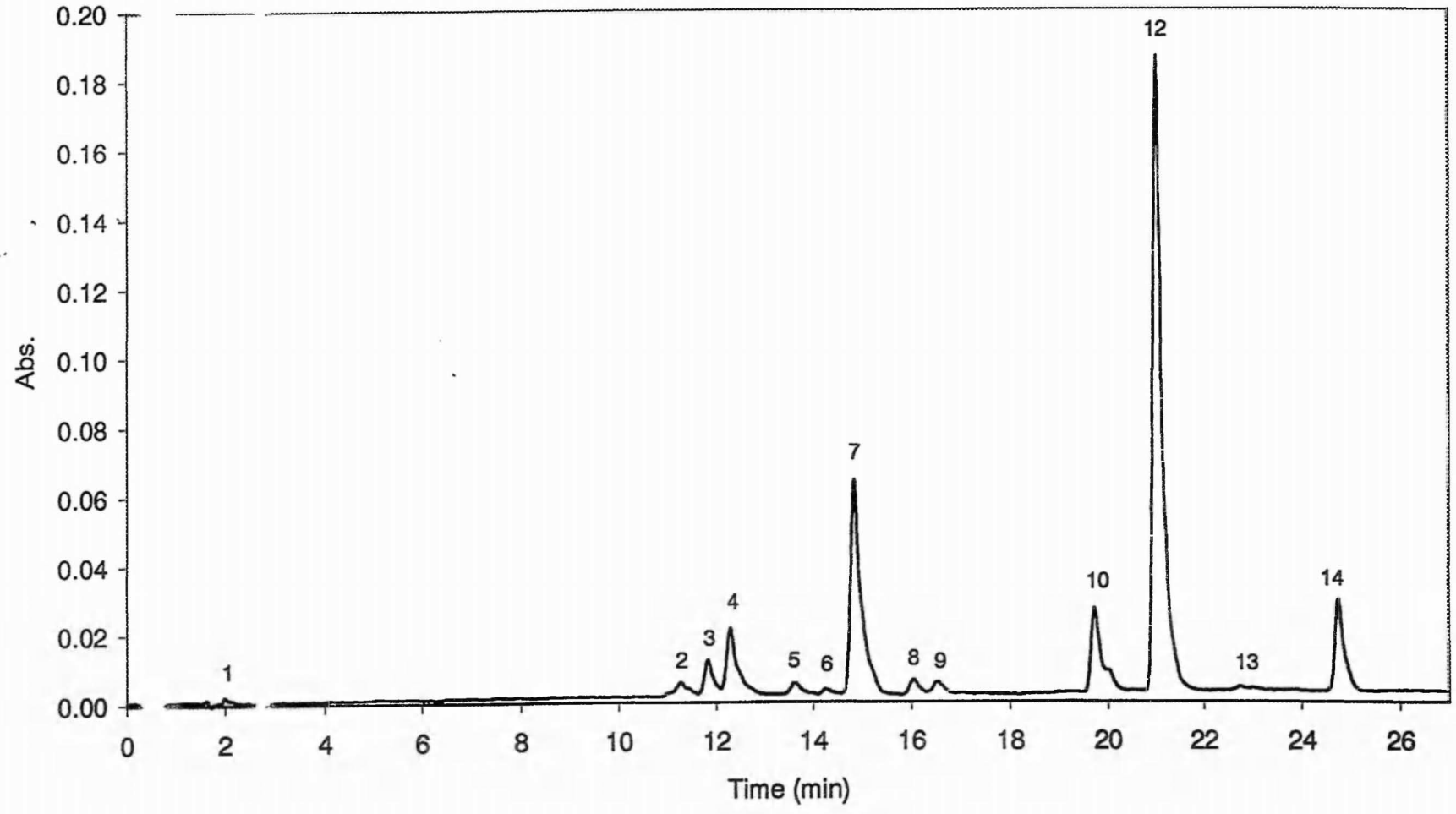


Figure 6.2 - HPLC chromatogram of pigment extract from greened tuber. Absorbance measured at 430 nm.

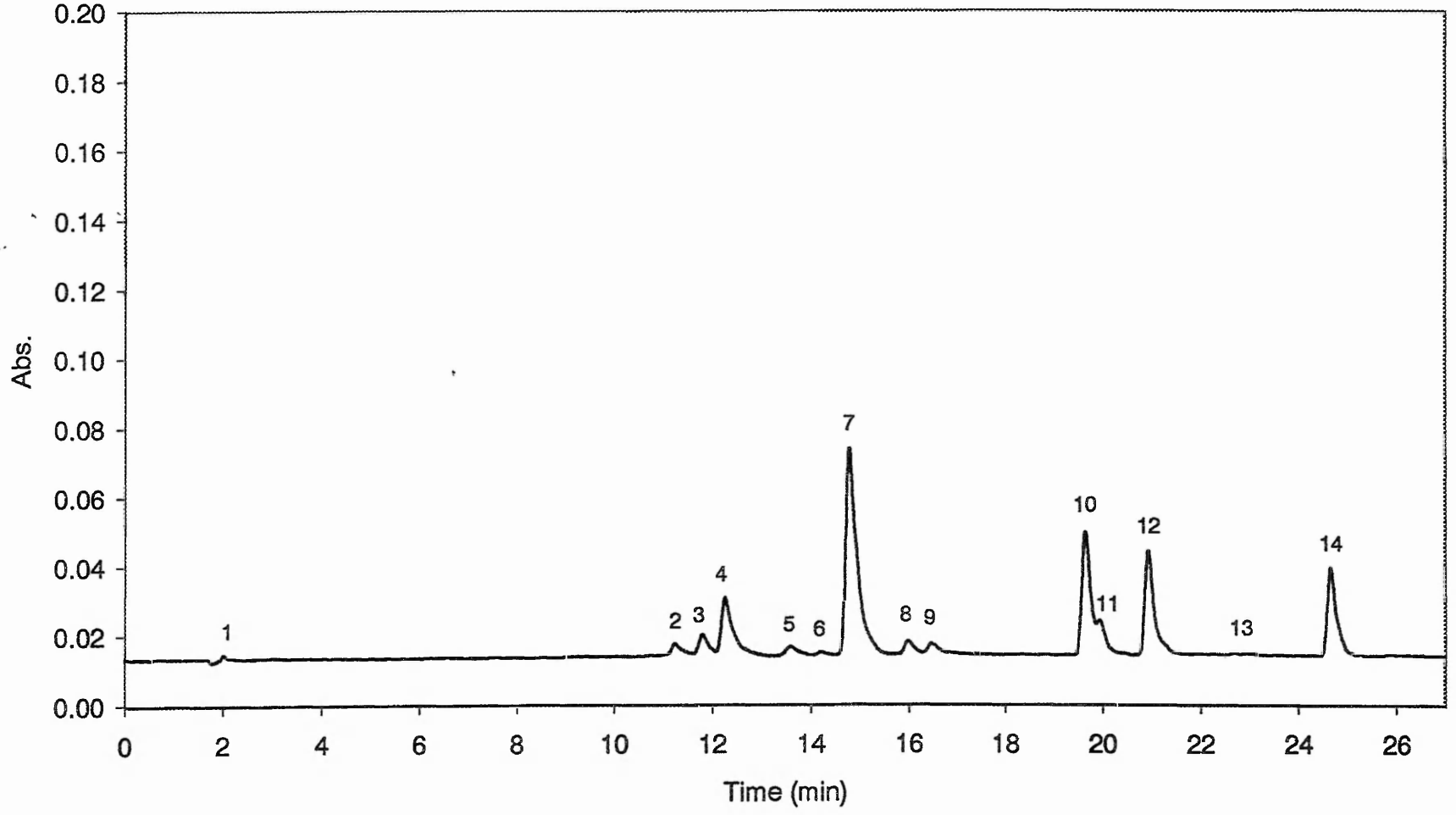


Figure 6.3 - HPLC chromatogram of pigment extract from greened tuber. Absorbance measured at 450 nm.

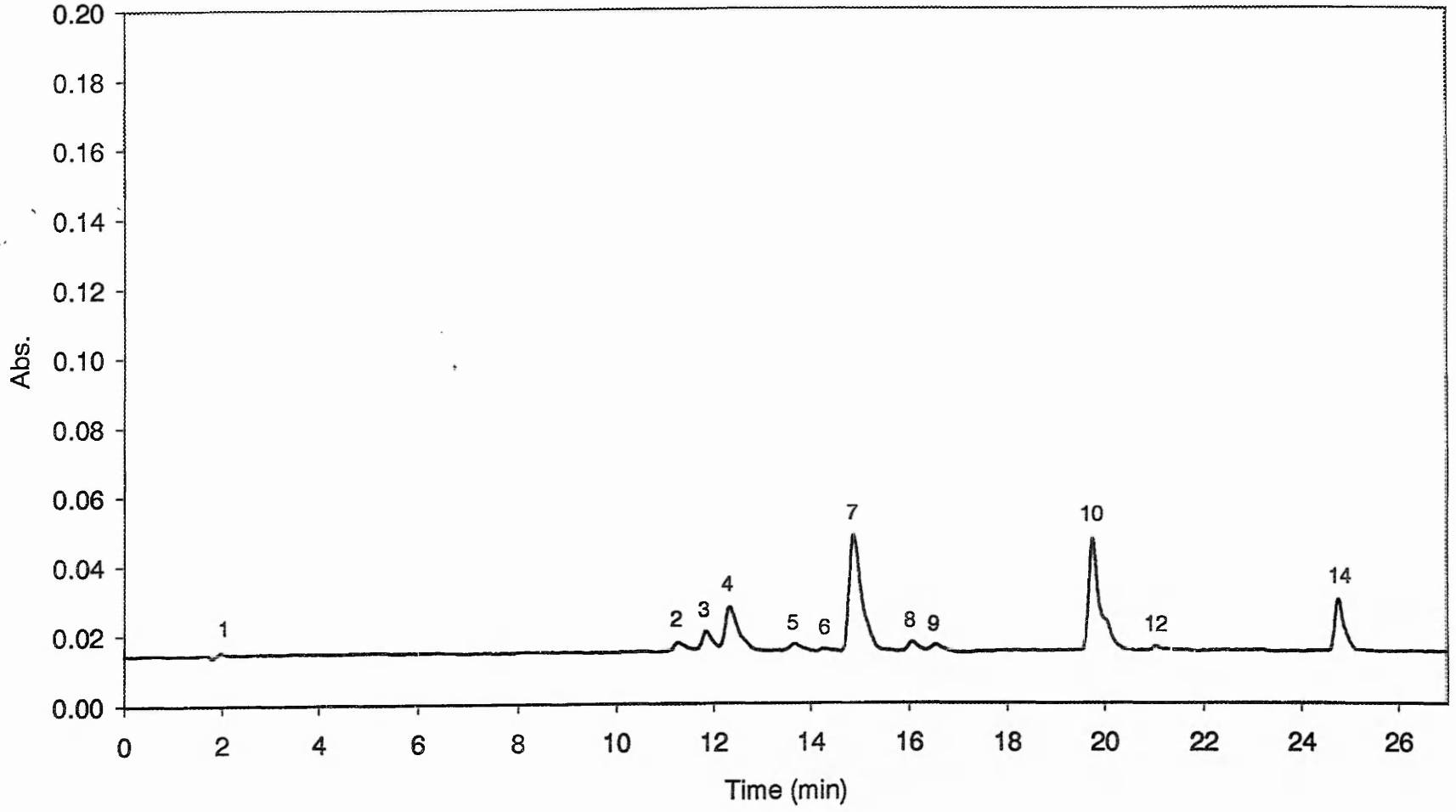


Figure 6.4 - HPLC chromatogram of pigment extract from greened tuber. Absorbance measured at 470 nm.

Table 6.1 - Height and area of chromatogram peaks at various detector wavelengths.

Peak No.	Retention Time (min)	Peak Height ($AUx10^{-4}$)				Peak Area (relative units)			
		415	430	450	470	415	430	450	470
1	1.9	-	-	-	-	-	-	-	-
2	11.2	27	39	34	22	86	96	90	55
3	11.8	60	102	61	51	144	220	134	117
4	12.3	109	192	166	123	338	496	442	369
5	13.6	17	31	24	18	34	71	61	43
6	14.3	1	14	5	2	2	25	5	2
7	14.8	335	620	599	333	954	1552	1587	971
8	16.1	28	43	36	28	65	99	77	69
9	16.5	21	34	27	20	48	86	59	55
10	19.7	75	242	354	320	185	512	733	754
11	19.9	20	61	67	83	19	105	189	105
12	21.0	1061	1834	298	10	2580	4063	638	15
13	22.7	15	8	3	-	26	14	3	-
14	24.7	125	264	253	146	289	572	549	320

peak no. 5 were present in the chromatogram. The C_{10} isomers of both Chl *a* and *b* were observed after 7 days of light exposure and after a further 7 days all 9 carotenoids were detectable.

Although quantification of the results was not possible comparison of the peak areas with that of lutein, the largest peak present in all samples, showed that all the Chl's and β-carotene increased with light exposure at a greater rate than lutein, whereas the other carotenoids present did not vary with respect to lutein content.

Comparison of chromatograms from greened tuber tissue and sugar beet leaf tissues (Fig. 6.5) demonstrated that the distribution of pigments was very similar. However, the concentration of pigments in the leaf extract

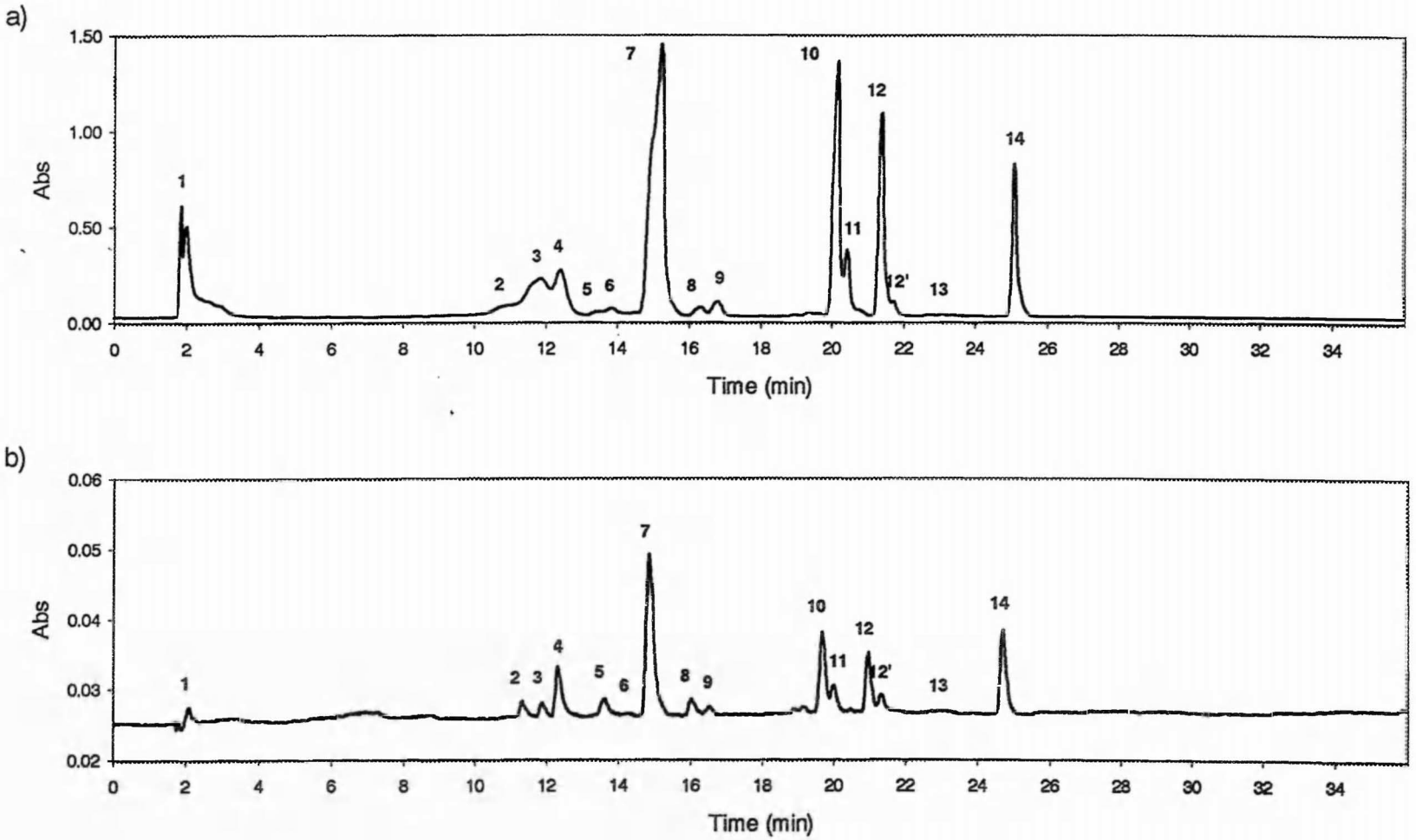


Figure 6.5 - HPLC chromatograms of a) sugar beet leaf and b) potato tuber exposed to daylight for 14 days.

Table 6.2 - Identification of chromatogram peaks.

Peak No.	Identity	
1	solvent front.	
2	*cis*-violaxanthin	tentatively identified from published data (Cano, 1996)
3	*cis*-neoxanthin	tentatively identified from published data
4	violaxanthin	tentatively identified from published data
5	?	
6	lutein epoxide	tentatively identified from published data
7	*all-trans*-lutein	identified by TLC and spectra
8	neolutein B	tentatively identified from published data
9	neolutein A	tentatively identified from published data
10	chlorophyll *b*	identified by TLC and spectra
11	chlorophyll *b'*	tentatively identified from published data
12	chlorophyll *a*	identified by TLC and spectra
13	pheophytin	identified by TLC and spectra
14	β-carotene	identified by TLC and spectra

was more than 30 times greater and the early xanthophyll peaks were poorly separated, indicating the possibility of other xanthophylls being present. Also, whereas in tuber tissues the peak area of neolutein A was larger than that of neolutein B, this situation was reversed in the leaf tissue. Peak no. 5 was much smaller in the leaf tissue chromatogram relative to violaxanthin compared with the tuber extract and appeared to consist of 2 peaks.

6.2.3 - Discussion

The HPLC method detected a range of carotenoids and chlorophylls and demonstrated that tuber greening involves the biosynthesis of carotenoids not previously detectable in unexposed tubers.

Table 6.3 - Presence of various pigments in greened and control tubers.

	Control					Exposed			
	0	4	7	10	14	4	7	10	14
cis-violaxanthin	✓			✓	✓	✓			✓
cis-neoxanthin									✓
violaxanthin	✓		✓		✓	✓		✓	✓
Peak no. 5						✓			✓
lutein epoxide								✓	✓
all-trans-lutein	✓	✓	✓	✓	✓	✓	✓	✓	✓
neolutein B								✓	✓
neolutein A				✓				✓	✓
chlorophyll *b*						✓	✓	✓	✓
chlorophyll *b*'							✓	✓	✓
chlorophyll *a*						✓	✓	✓	✓
chlorophyll *a*'							✓	✓	✓
pheophytin								✓	✓
β-carotene	✓	✓	✓	✓	✓	✓	✓	✓	✓

As the absorption maxima of different carotenoids can vary quite considerably an ideal HPLC analysis method would involve the use of a photo-diode array detector, which as well as enabling the detection of very small amounts of pigment would give valuable information towards identification of the peaks in the chromatogram. However, the prohibitive cost of such equipment makes the development of a carotenoid analysis method using a single detection wavelength valuable. The method of Cano (1991) effectively separated a number of carotenoids and Chls from Kiwi fruit extracts. Initial attempts using the above HPLC system and potato extracts gave inadequate peak separation. Also, as the carotenoid composition of Kiwi fruit and potatoes was likely to be different, it was possible that 430 nm was not the ideal wavelength for detection of potato tuber carotenoids.

Lengthening the gradient duration to 30 min resulted in greatly improved peak separation, apart from that of the Chl isomers. It is important that the wavelength used should allow accurate detection of as many carotenoids as is reasonably possible. As many carotenoids have absorption maxima between 420 and 480 nm, 4 wavelengths between these were used. It is clear from the chromatograms (Figs. 6.1 & 6.4) that detection at 415 or 470 nm was not ideal. However, detection at 430 or 450 nm both gave chromatograms with 13 well separated peaks. Detection at 450 nm gave the best separation of the C_{10} Chl isomers and allowed integration of these peaks.

Three types of pigment were detected by this method. The first to be eluted were the oxygenated carotenoids, the xanthophylls, of which a total of 8 were detected in greened tubers. Secondly were the chlorophylls and their breakdown products and thirdly the hydrocarbon carotenes, of which only β-carotene was found in tubers.

Positive identification of some of these peaks was made by running an aliquot of the sample on a TLC plate, resuspending the resulting bands and obtaining both the spectra and HPLC retention time for these. Order of elution was proportional to TLC R_f values because reversed phase sorbents were used for both forms of chromatography. Comparison of spectra with published data for absorbance maxima (Davies, 1976; Cano, 1991) allowed 5 peaks to be identified. Recovery of the other pigments from the TLC plate was not sufficient to allow accurate spectra to be obtained. The 8 unidentified peaks were tentatively identified by comparing the elution order and retention times with the chromatograms of Cano (1991). Identification of these was uncertain. Determination of the stereochemistry of detected carotenoids could be made by running a second aliquot of a sample at a near UV wavelength, as *cis* isomers all possess an absorbance maximum at approx. 310-330 nm, whereas, *trans* isomers do not absorb strongly at these wavelengths.

The position of peak no. 5 would indicate that it is auroxanthin. However, it is widely separated from any other peaks in the chromatogram

and could therefore be any one of a number of similar xanthophylls. Zeaxanthin has been shown to be present in significant amounts in potato tubers (Granado, Olmedilla, Blanco, Rojas-Hidalgo, 1992), but was unidentified in this study. Indeed, as the order of xanthophyll elution is partially governed by number of oxygen atoms in a carotenoid, the position of peak no. 5 is also reconcilable with the peak identity being zeaxanthin or antheroxanthin. Furthermore, auroxanthin is more commonly found in senescent tissues (Biswal, 1995).

This peak was, relative to the other xanthophylls, more abundant in the greened tuber tissue than in sugar beet leaf tissue. If the true identity of this xanthophyll was zeaxanthin or antheroxanthin it could be indicative of the stress that light exposure causes potato tubers. The xanthophyll cycle involves the de-epoxidation of violaxanthin to antheroxanthin and zeaxanthin in order dissipate excess energy. This leads to the accumulation of antheroxanthin and zeaxanthin in photosynthetic tissues in plants subjected to environmental stress (Demming-Adams and Adams, 1996). It is likely that the concentrations of these compounds in the leaf would be, relatively, less than in the tuber as the sugar beet plant had not been subjected to stress before analysis.

Quantification at this stage of method development was not possible due to the lack of pure pigment standards. Furthermore, recovery of pigments after the acetone to ether transfer and after the vacuum drying was not monitored. However, changes in the relative concentrations of pigments would be both possible and useful if careful sampling of tuber tissue was carried out. This preliminary study was used to determine only the presence or absence of a pigment.

Analysis of tubers that had not been exposed to light produced large lutein and β-carotene peaks in all samples, and it is likely that these pigments give white tubers their colouration (Gross, 1991). Violaxanthin and *cis*-violaxanthin were also found in the majority of control samples and also impart colouration to tuber flesh.

Tubers exposed to light exhibited a swift and ongoing change in pigment composition. The rapid onset of Chl synthesis in tubers after exposure to light is well documented (Jadhav and Salunkhe, 1975). Indeed, after 4 days of exposure there were clear Chl *a* and *b* peaks and a small peak 5. After 7 days the C_{10} isomers of both Chl *a* and *b* were also detected. Further light exposure led to the production of 5 more xanthophylls and phaeophytin. The latter pigment being an electron acceptor in PS II and a breakdown product of Chl in tissues exposed to light. However, it was not until after 14 days of exposure to light that the number of carotenoids present in greened tubers was similar to those in sugar beet leaves. Although there is a rapid onset of greening after exposure to light it appears that the photosynthetic apparatus is not complete for some time.

6.2.4 - Conclusions

This preliminary study has described an analysis method for tuber carotenoids which has potential, with further development, as an useful means of investigating both qualitative and quantitative changes in the composition of these pigments within tubers. Initial data has indicated that major changes do occur when tubers are exposed to light and has raised a number of further questions. The method could also be used to investigate carotenoid changes in response to other storage conditions and could allow carotenoid quantity and composition to be used as tuber stress indicators.

6.3 -The Analysis of Tuber CO_2 Output During Greening

Although much is now known about the transformation of amyloplasts to chloroplasts during potato tuber greening, the occurrence of photosynthesis in the new chloroplasts has yet to be demonstrated. Furthermore, the extent of any carbon fixation and its effect on tuber CO_2 exchange and respiration is also unknown.

There is some biochemical evidence for active photosynthesis in greened tubers, Zhu, Merkle-Lehman and Kung (1984) showed that electron transport and O_2 evolution occurred in isolated chloroplasts. They also demonstrated that RuBisCO was being synthesised, an observation supported by the work of Muraja-Fras, Krsnik-Rasol and Wrischer (1994) who also detected proteins which probably formed part of the light-harvesting complex II (LHC II) and the photosystem II (PSII) reaction centre.

The use of Infra-Red Gas Analysis (IRGA) allows the carbon exchange of whole tubers to be monitored during the greening process. Unlike other atmospheric gases, CO_2 absorbs infra-red (IR) light strongly, which allows accurate measurements of small CO_2 concentrations. However, water vapour also absorbs IR which necessitates the removal of all water vapour from the analysis sample. Modern IRGA equipment takes advantage of this to detect not only CO_2 but also the water content of sample air, thus allowing the analysis of both water and CO_2 exchange from the sample plant material.

This study describes the use of IRGA to investigate gaseous exchange from greening tubers, and the effect of this on water loss by the tubers.

6.3.1 - Materials and Methods

6.3.1.1 - Plant material: Potato tubers (cv. King Edward) were purchased from a local supermarket and stored in darkness for 24 h before use.

6.3.1.2 - Experimental design: A LCA4 IRGA (ADC, Hertfordshire, UK) was used to analyse the gaseous exchange of tubers. The LCA4 gas circuit is shown in Fig. 6.6. Two sets of 5 tubers were weighed and placed in 2 gas tight perspex chambers aranged in an open system (Fig. 6.7, Plate 6.1). PVC/PTFE tubing was used throughout and a flow rate of approximately 268 μmoles air s^{-1} was maintained. Ambient air was drawn from outside the laboratory via an inlet approximately 1.5 m above the ground, fitted with a downward facing funnel to prevent the entry of any rain into the system. A 2 m tube allowed the air to warm to room temperature before entering the reference inlet port of the WA161 gas switching box (also ADC). The reference outlet port on the WA161 was connected to the IRGA reference port. Each sample chamber was connected to sample inlet ports on the WA161 and the sample outlet port on the IRGA. The sample outlet port on the WA161 was connected to the sample inlet port on the IRGA.

One chamber was covered in several layers of black polythene to prevent any light reaching the tubers, over which was placed a layer of white polythene to reduce heat absorption. Both chambers were illuminated using a high pressure sodium lamp. PPFD was measured using a SKR 1850 4-channel light sensor with a SDL 2580 datalogger (both Skye instruments, Llandrindod, Wales). A fan was used to dissipate excessive heat. Air temperature around the chambers was kept to between 20 and 24°C.

The WA161 switching box was set to sample each chamber every 20 min. At each occasion the IRGA recorded reference and sample CO_2, reference and sample H_2O vapour pressure, atmospheric pressure, atmospheric temperature and time. The chambers were exposed to continuous light for 14 d, and measurements taken for the same period.

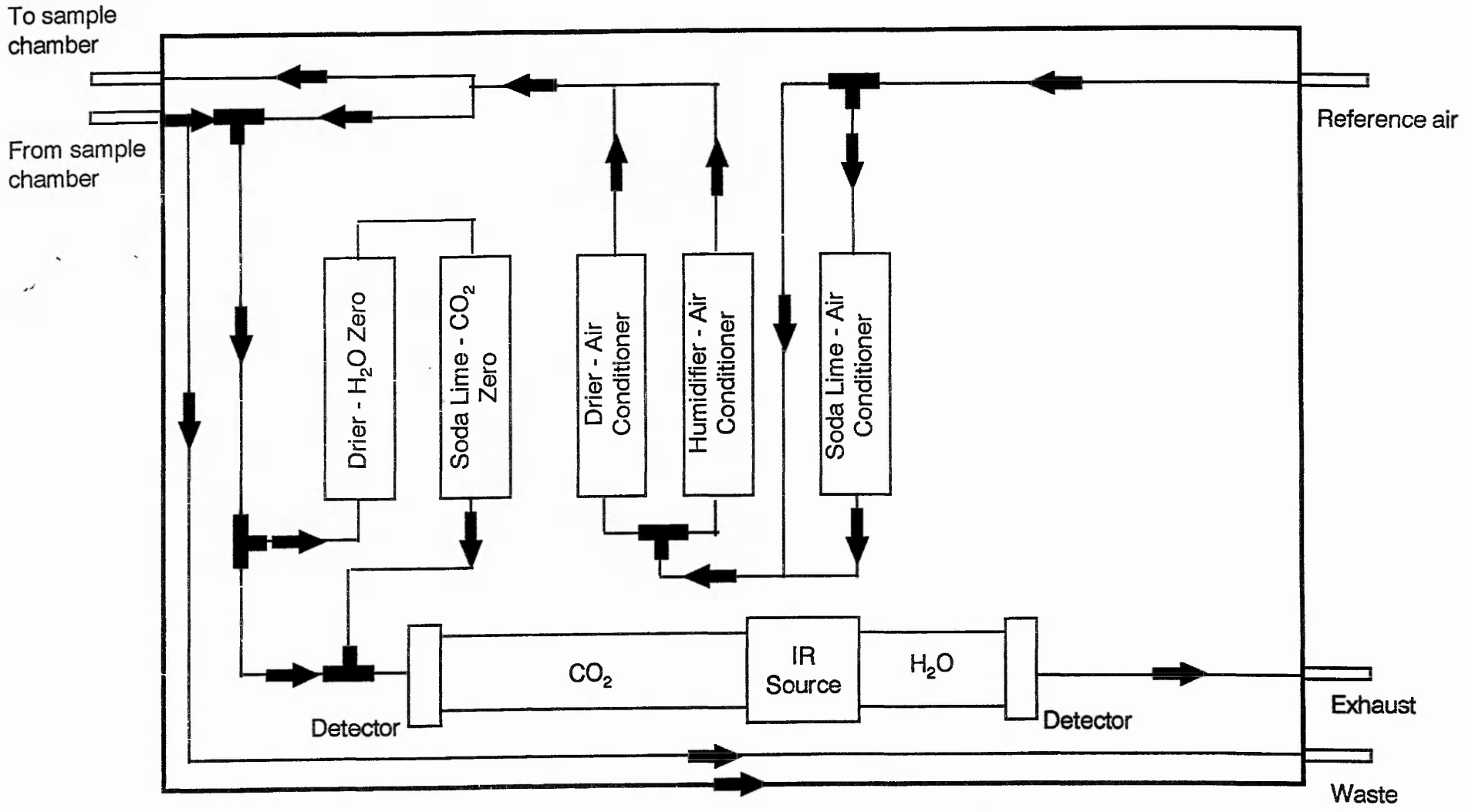


Figure 6.6 - Simplified diagram of LCA4 gas flow circuit. ➡ direction of air flow. ┻ switching valve.

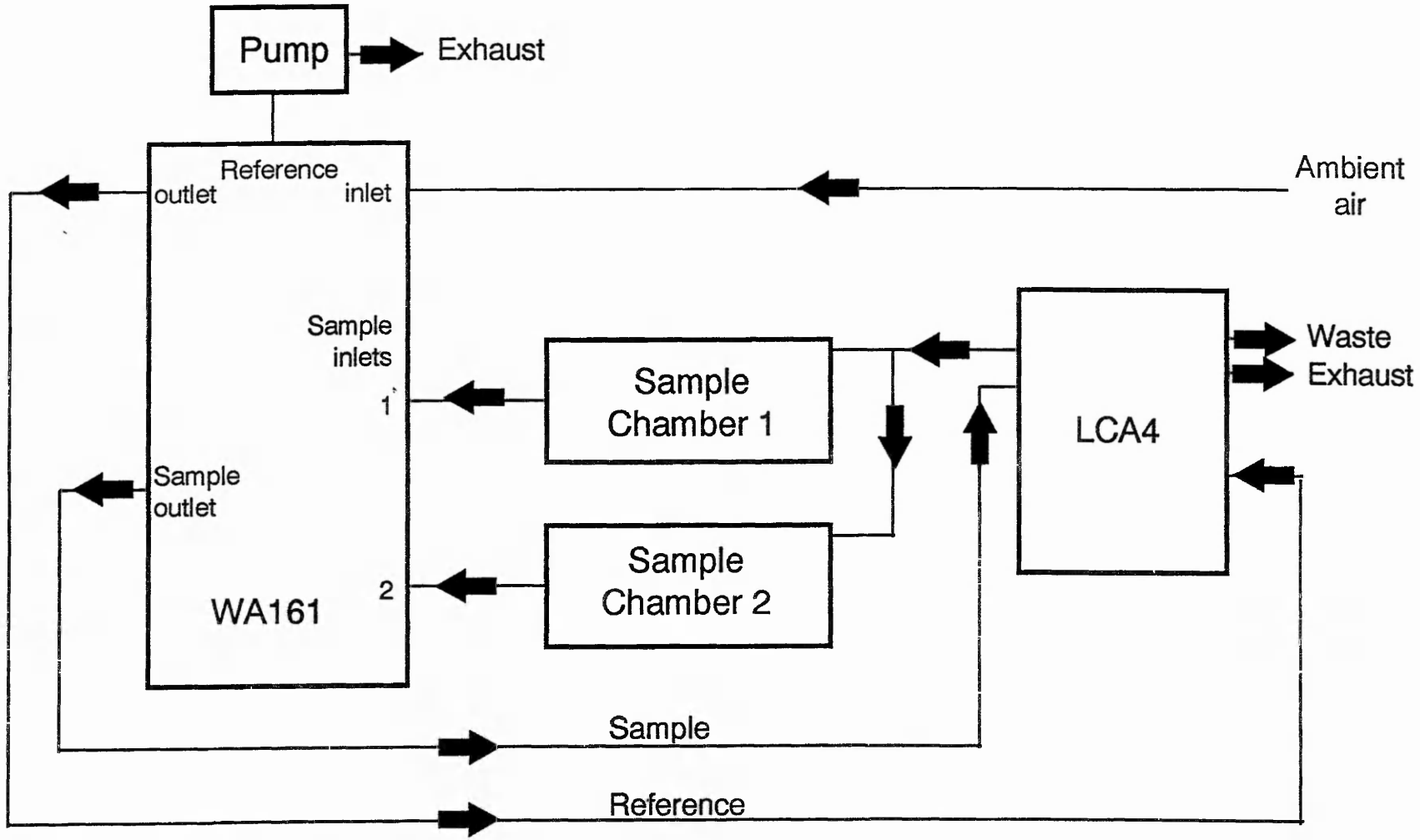


Figure 6.7 - Experimental design for the measurement of CO_2 and H_2O exchange by tubers in dark (chamber 1) and light (chamber 2). ➡ direction of flow.

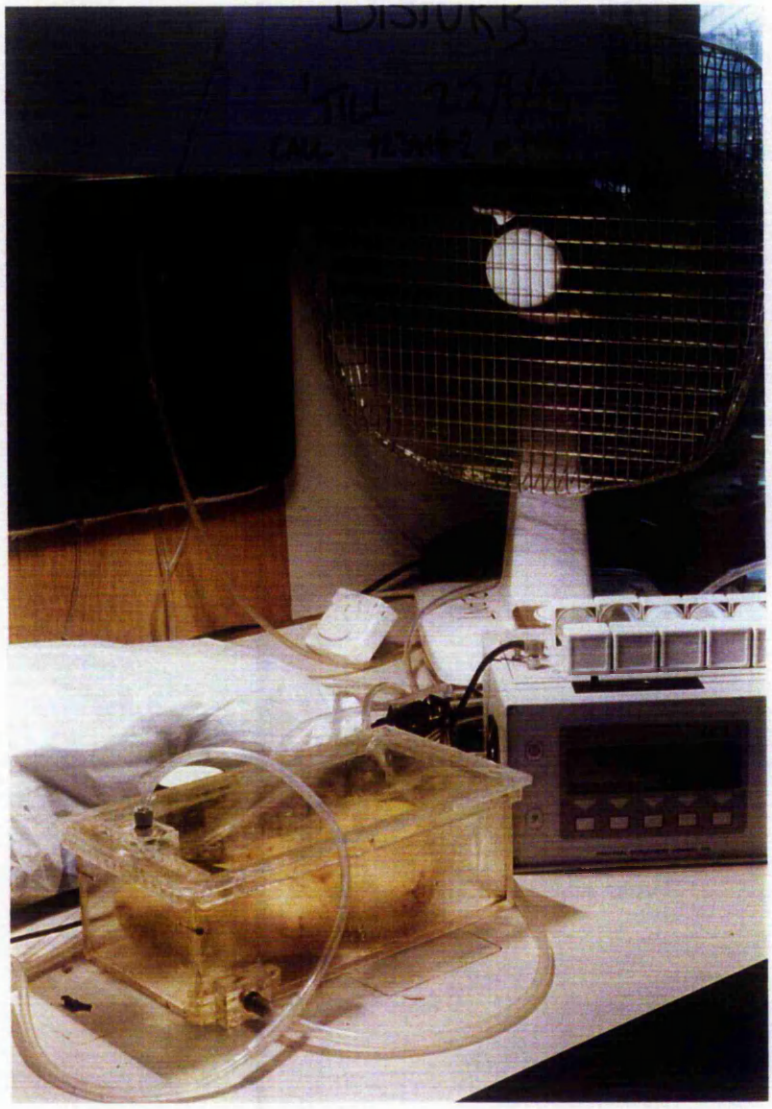


Plate 6.1 - Photograph of the IRGA apparatus.

6.3.2 - Results

During the experiment PPFD varied from 300 to 400 µmoles photons m^{-2} s^{-1}. After 14 days the tubers that had been exposed to light exhibited considerable greening, whereas control tubers possessed no obvious green colouration (Plate 6.2). Control tubers showed a slight loss of fresh weight of 56 g from an original weight of 790 g (7.1%). Exposed tubers lost much more weight, from 919 to 764 g (17%).

6.3.2.1 - CO_2 exchange: CO_2 exchange was calculated per g of initial sample weight. Readings were very variable for the first 4 days of light exposure (Fig. 6.8). However, after this time the CO_2 evolved by tubers exposed to light decreased. This continued throughout the rest of the experiment, although the rate of decrease was small after 12 days. The CO_2 evolved by the control tubers, though variable remained steady during this period. After 14 days CO_2 evolution by control tubers was more than 10 times that of exposed tubers.

6.3.2.2 - H_2O evolution: H_2O vapour pressure was calculated per g of initial sample weight. After 5 days of very variable readings H_2O loss from tubers was fairly steady, decreasing slightly through the experiment (Fig. 6.9). There was no marked difference in H_2O loss between exposed or control samples.

6.3.3 - Discussion

There was considerable variation in the readings for both H_2O and CO_2 for the first 4-5 days of the experiment. This coincided with a number of problems with condensation within the system. It is likely that the variable results during this time were due to the condensation as high relative humidity would overload the IRGA air conditioners and affect the readings. This was combated by adding Drierite, self-indicating anhydrous calcium

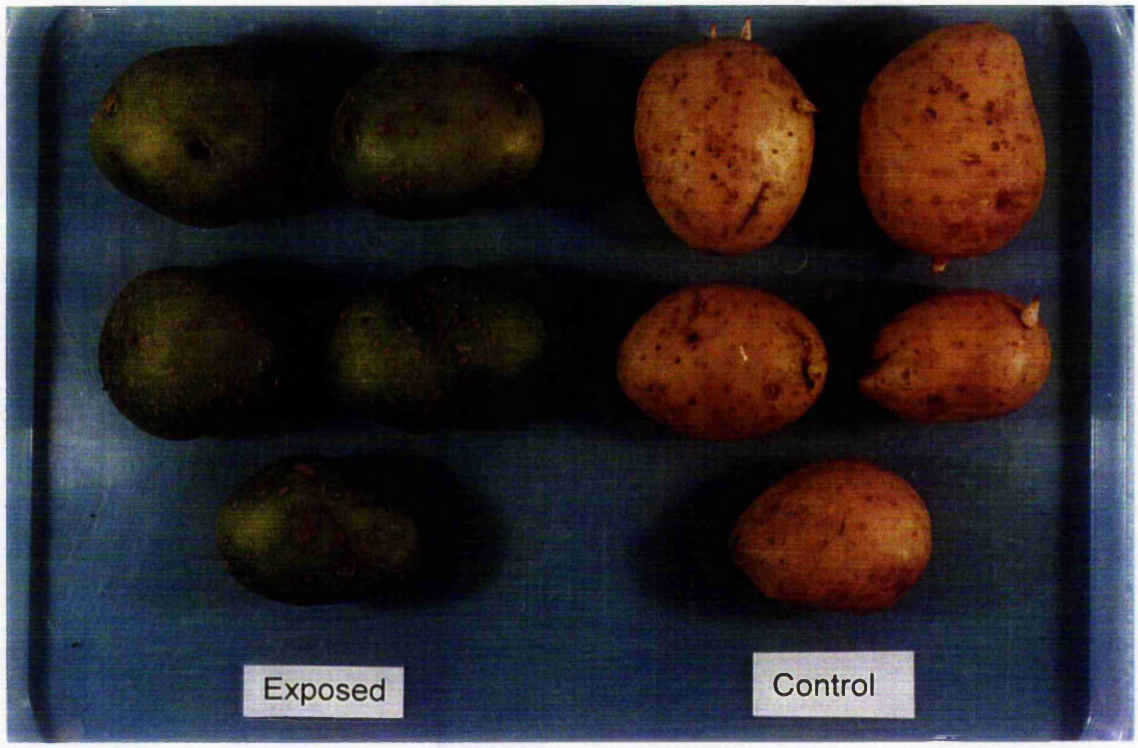


Plate 6.2 - Potato tubers (cv. King Edward) after 14 days exposure to light or darkness.

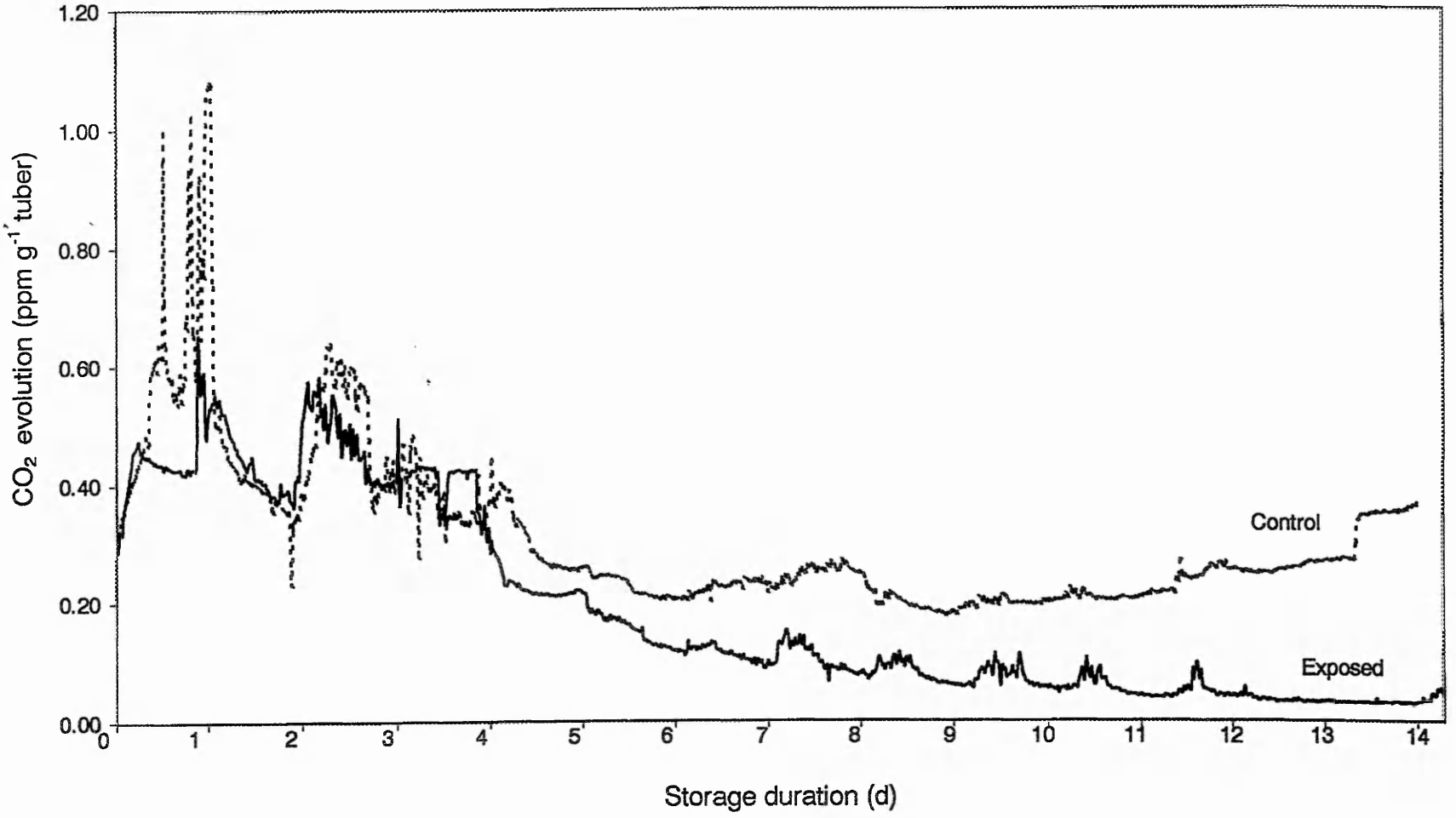


Figure 6.8 - CO_2 evolution from control and exposed tubers during 14 days storage.

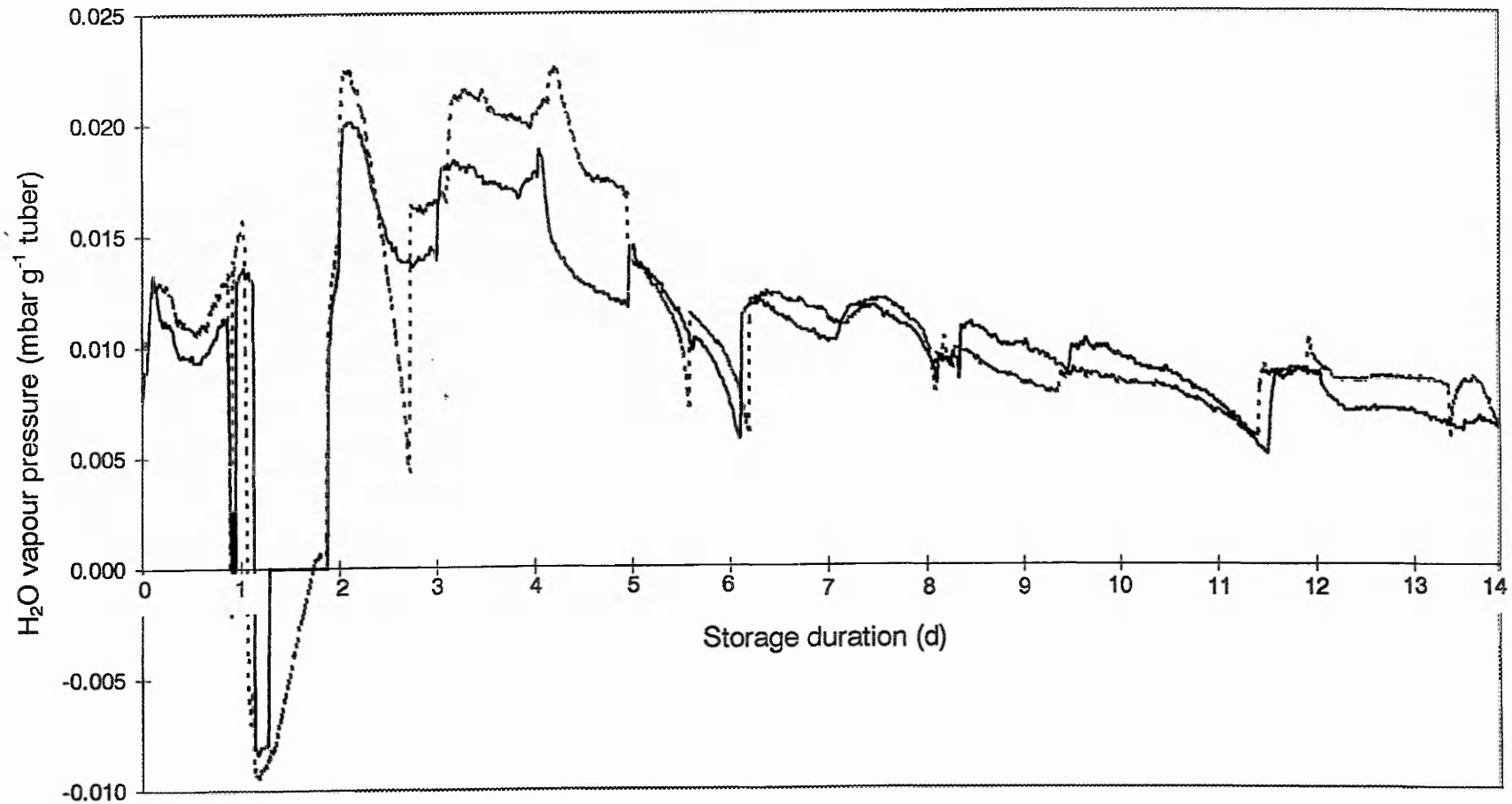


Figure 6.9 - H_2O evolution from control and exposed tubers stored for 14 days.

sulfate (also ADC) to the reference line, but took some time to have its full effect.

There is a clear difference in CO_2 exchange between greening and control tubers. If this was attributable to a difference in respiration between the 2 sets of tubers it could be expected that water loss from the 2 samples would also be different. However, this did not occur. There are two possible explanations for this, either respiration of both samples was equal, or evaporative water loss from the tubers was much greater than respiratory water loss, therefore, making respiratory water loss undetectable. The most probable reason for the low CO_2 evolution in the greening tubers is that carbon fixation is occurring, although net carbon gain was not observed.

Although it has previously been shown that greened tubers contain RuBisCO and possess electron transport activity, this result is the most direct evidence of actual carbon fixation. Interestingly, the exposed tubers lost a much greater proportion of their weight during the storage time than the dark-stored tubers. As water loss has been shown to be equal from the 2 samples it is difficult to ascertain how this weight loss occurred. If photosynthesis was occurring then there must be splitting of water. As water is not entering the tuber tissues this water must be coming from the tuber itself. Therefore, there may be considerable loss of water from the tuber and the O_2 given off is not detectable by the IRGA. If the increased weight loss from the exposed tubers was due to increased metabolic breakdown of starch it would be expected that water loss to the atmosphere would be higher than that from the control sample. However, as previously observed this did not occur.

If carbon fixation was occurring, an increase in dry weight, or a reduction in dry weight loss could be expected. Unfortunately, dry weight was not measured in this study. Further study, with measurement of O_2 and tuber dry weight, may provide some insight into this.

6.3.4 - Conclusions

This preliminary experiment has provided some evidence towards determining in the photosynthetic apparatus in greened tubers is fully functioning. However, it has also raised a number of questions that cannot be answered without further experimentation.

6.4 - Inhibition of Chlorophyll Synthesis and its Effect on TGA Accumulation

6.4.1 - Introduction

As greening is often used by the public as a rough measure of the health risk posed by a potato it is important to fully understand the factors leading to both Chl and TGA accumulation and the relationship between them. It has often been suggested, most recently by Dale *et al.* (1993), that the phenomena of greening and light-enhanced TGA accumulation are connected. However, no direct link has been experimentally proven.

4-Amino-5-fluoropentanoic acid (AFPA) (Fig. 6.10a) is a potent mechanism-based inhibitor of glutamate aminotransferases (Silverman and Invergo, 1986) and has previously been reported to inhibit Chl biosynthesis in plants (Gardner, Gorton and Brown, 1988). AFPA binds irreversibly to glutamate-semialdehyde aminotransferase (GSA-AM) (Silverman and Levy, 1980) and prevents the formation of 5-aminolaevulinic acid (ALA), the precursor of porphobilinogen, which in turn forms the pyrrole components of the Chl molecule. ALA formation is the rate-limiting step in Chl biosynthesis (Kumar, *et al.*, 1996) and so is an ideal point at which to inhibit the synthesis of Chl. The more commonly used Chl biosynthesis inhibitor 3-amino-2,3-dihydrobenzoic acid (gabaculine) (Fig. 6.10b) also inhibits this enzyme.

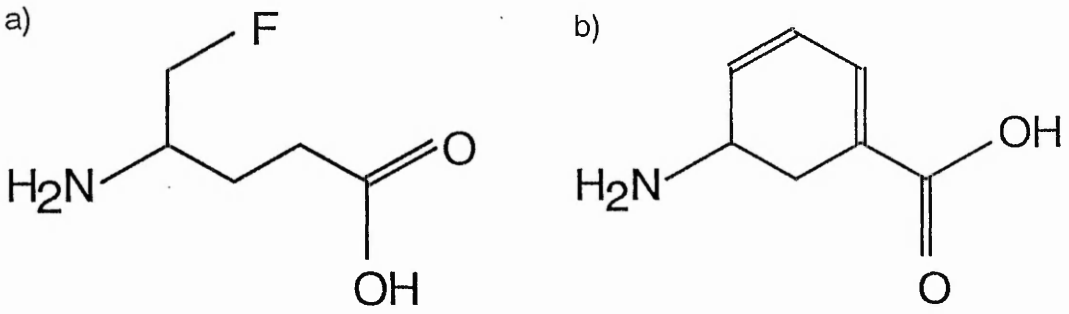


Figure 6.10 - Chemical structures of a) AFPA and b) gabaculine

This study has focused on two commercially important UK cultivars Pentland Dell, primarily used for fries, and Record, a crisping variety. AFPA

and gabaculine were applied to whole tubers which were then exposed to daylight, in order to establish whether the greening process could be separated from light-enhanced TGA accumulation and to indicate the presence or absence of any direct biosynthetic link between the two.

6.4.2 - Materials and methods

6.4.2.1 - Plant material: Potato (*Solanum tuberosum* L.) tubers (cv. Record) were provided by the UK Potato Marketing Board Experimental Station, Sutton Bridge, near King's Lynn, UK for experiment 1. These had been stored under optimal conditions in a commercial store for 5 months prior to use. Tubers for experiment 2 (cvs. Pentland Dell and Record) were freshly harvested from a commercially grown crop. All tubers were stored in darkness at room temperature for 24 h before use.

6.4.2.2 - AFPA synthesis: AFPA was synthesised by R.E. Saint (Department of Chemistry and Physics, The Nottingham Trent University) essentially as described by Silverman and Levy (1980) (Fig 6.11), but with some modifications. Reduction of (*S*)-(+)-5-carbethoxy-2-pyrrolidone was accomplished using sodium borohydride (Valasinas and Frydman, 1992). The resulting alcohol was brominated by treatment with N-bromosuccinimide and tryphenylphosphine for 12 h (Smith and Fuchs, 1995). Further steps in the synthesis were carried out according to the original protocol.

6.4.2.3 - Experiment 1: Potato tubers (cv. Record) were dipped in either distilled water, 100 mM 2-[N-morpholino]ethanesulfonic acid (MES), adjusted to pH 5.5 or gabaculine in 100 mM MES also pH 5.5 (both Sigma-Aldrich Co. Ltd, Poole, UK) for 5 min. Air dried tubers were half-buried, longitudinally, in peat-based potting compost (Seed and Potting compost, J. Arthur Bowers, Lincoln, UK) in trays (600x320x80 mm). Smaller opaque trays (225x175x50 mm) were used to cover half of the total number of tubers as dark controls.

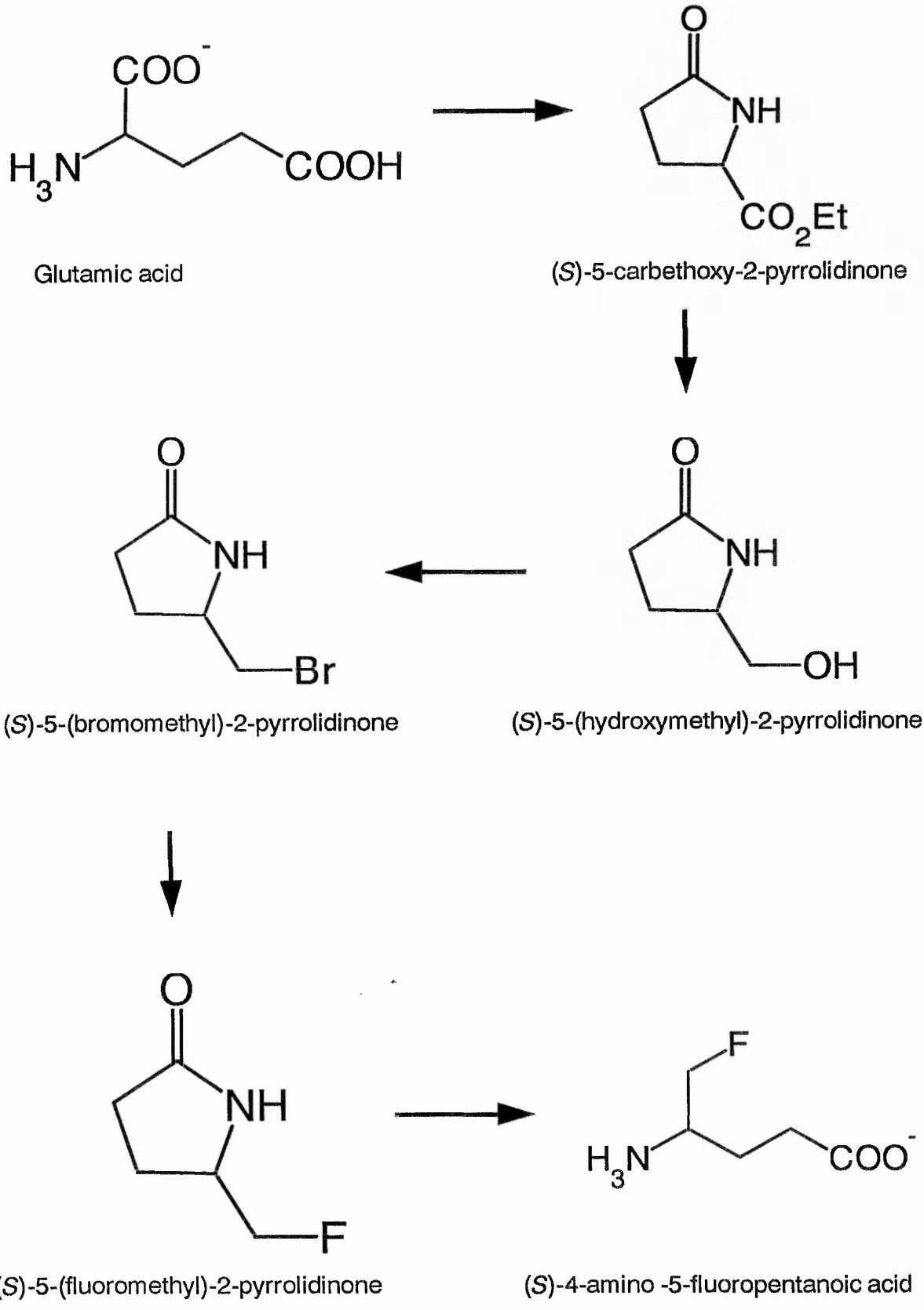


Figure 6.11 - Flow diagram of AFPA synthesis.

The trays were placed in a greenhouse and exposed to daylight at 18°C, with a photoperiod of 16 h. Light quantity was measured using a SKR 1850 4-channel light sensor with a SDL 2580 datalogger (both Skye instruments, Llandrindod, Wales). After 0, 3, 6 and 10 days of exposure, 5 control and 5 exposed tubers from each treatment were analysed for chlorophyll, carotenoid and glycoalkaloid content as described below, with the exception of the water-treated tubers which were sampled after 10 days only.

6.4.2.4 - Experiment 2

Potato tubers (cvs. Pentland Dell and Record) were dipped in either 100 mM MES, pH 5.5 or 5 mM AFPA in 100 mM MES also pH 5.5 for 20 min. Tubers were placed in trays and exposed to daylight as described above. The photoperiod was 12 h light, 12 h darkness. After 0, 3, 6 and 10 days exposure, 10 tubers of each cultivar, 5 of each treatment, were analysed as described below.

6.4.2.5 - Pigment and TGA extraction and quantification: Pigment analysis was carried out according to the procedure described in Chapter 2. The glycoalkaloid analysis procedure was modified as described in Chapter 4.

6.4.2.6 - Statistical analysis: Analysis of the results was as described in Chapter 3, using Students t and Kolmogorov-Smirnov tests.

6.4.3 - Results

Tubers maintained in darkness did not accumulate detectable Chl in either experiment and did not exhibit any significant changes in glycoalkaloid concentrations, irrespective of cultivar or inhibitor treatment. Full Chl and glycoalkaloid results are presented in Appendix 3.

6.4.3.1 - Experiment 1: There were no significant differences in the accumulation of photosynthetic pigments after light exposure for 10 days

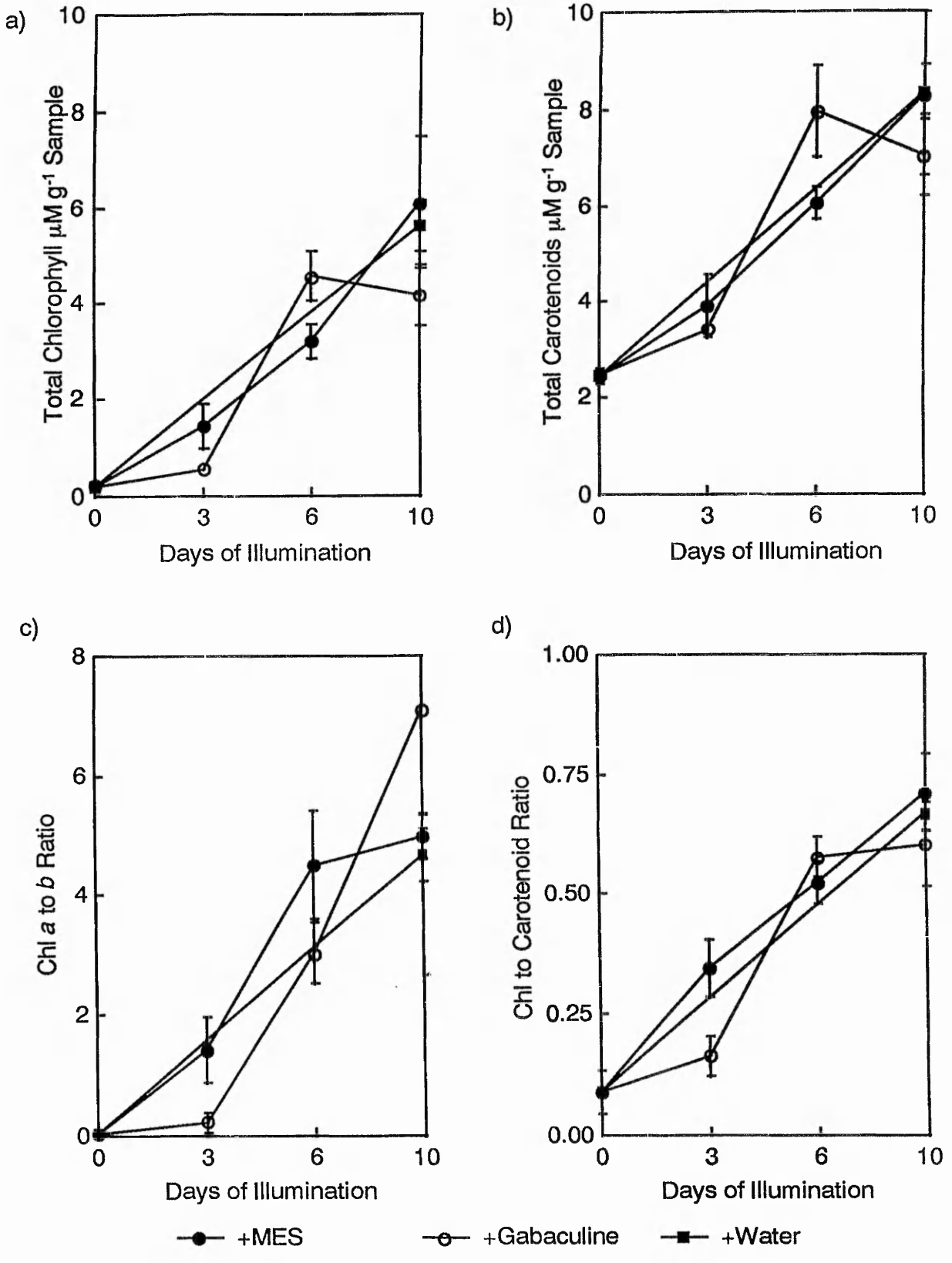


Figure 6.12 - Effect of gabaculine and MES on the accumulation of Chls and carotenoids in tubers (cv. Record) exposed to daylight at 18°C a) total Chl accumulation, b) total carotenoid accumulation, c) Chl *a* to *b* ratio and d) Chl to carotenoid ratio. Each point represents the mean of 5 tubers with SE.

between tubers treated with water or MES buffer (Fig. 6.12). After 3 days of exposure to light tubers treated with MES had accumulated Chl and showed an accompanying increase in total carotenoid concentrations. However, tubers treated with gabaculine showed no significant rise in Chl content and exhibited a marginal inhibition of carotenoid accumulation (Fig. 6.12a and b). After a further 3 days of light exposure the gabaculine-treated tubers had accumulated significantly ($p \leq 0.01$) greater concentrations of both Chl and carotenoids than tubers treated with MES alone, reaching a maximum of 4.57 and 7.96 μmoles g^{-1} sample, respectively. After 10 days the tubers treated with gabaculine had a slightly lower pigment content than the control tubers treated with MES buffer, which continued to increase both Chl and carotenoids, to 6.11 and 8.31 μmoles g^{-1} sample, respectively.

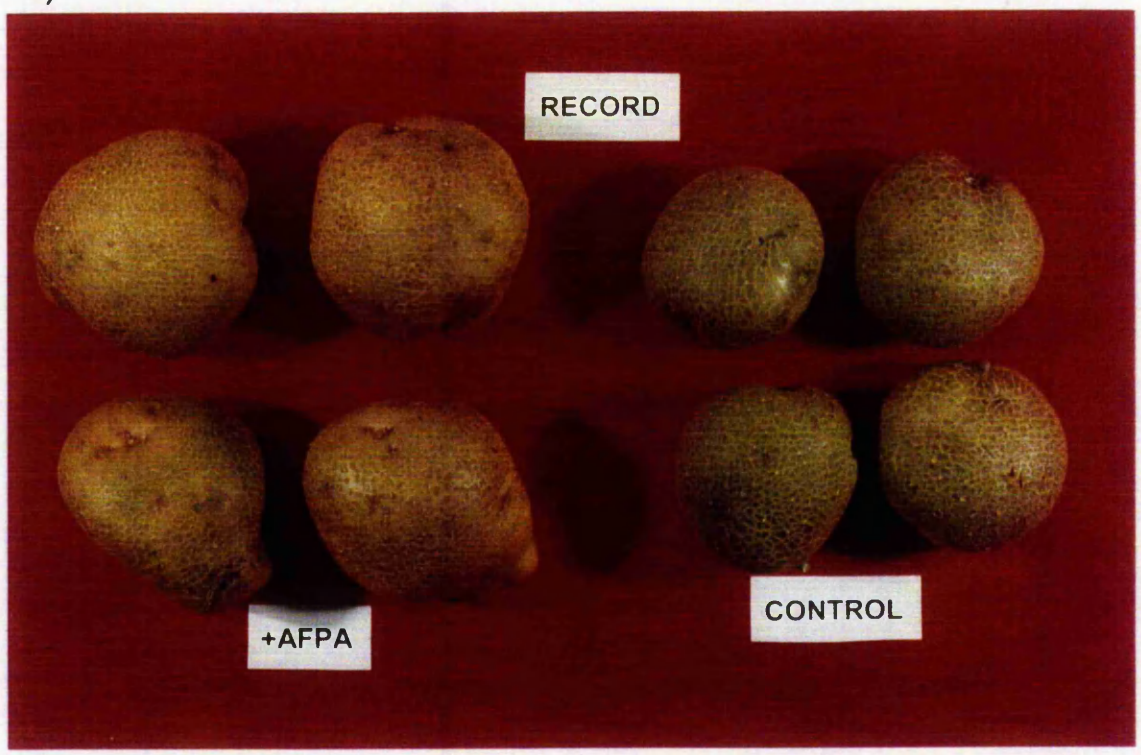
The Chl *a* to *b* ratio (Fig. 6.12c) in tubers treated with MES buffer initially increased but after 3-6 days of light exposure steadied at approximately 4.5, whereas the Chl *a* to *b* ratio in tubers treated with gabaculine only started to rise after 6 days of exposure and increased sharply to 7 after 10 days exposure.

The Chl to carotenoid ratio (Fig. 6.12d) mirrored the accumulation of total Chl, but no final reduction in gabaculine-treated tubers was observed.

Initial TGA content was 130.6±40.4 μg g^{-1} sample. There was no light-enhanced accumulation of TGA until tubers had been exposed to light for 10 days, when tubers contained 182.7±20.8 μg TGA g^{-1} sample and this was not significantly affected by treatment with gabaculine.

6.4.3.2 - Experiment 2: Exposure to daylight caused tubers of both cultivars treated with MES to green (Plates 6.3 & 6.4). Treatment with AFPA caused a significant ($p \leq 0.01$) reduction of total chlorophyll in both cultivars (Fig. 6.13a & b). Although, cv. Record accumulated twice as much Chl as cv. Pentland Dell after 10 days of light exposure, the maximum inhibition observed was similar, at about 70% (Table I). However, inhibition of Chl was highest after 10 days in cv. Pentland Dell, whereas maximum inhibition in cv. Record was after 6 days.

a)



b)

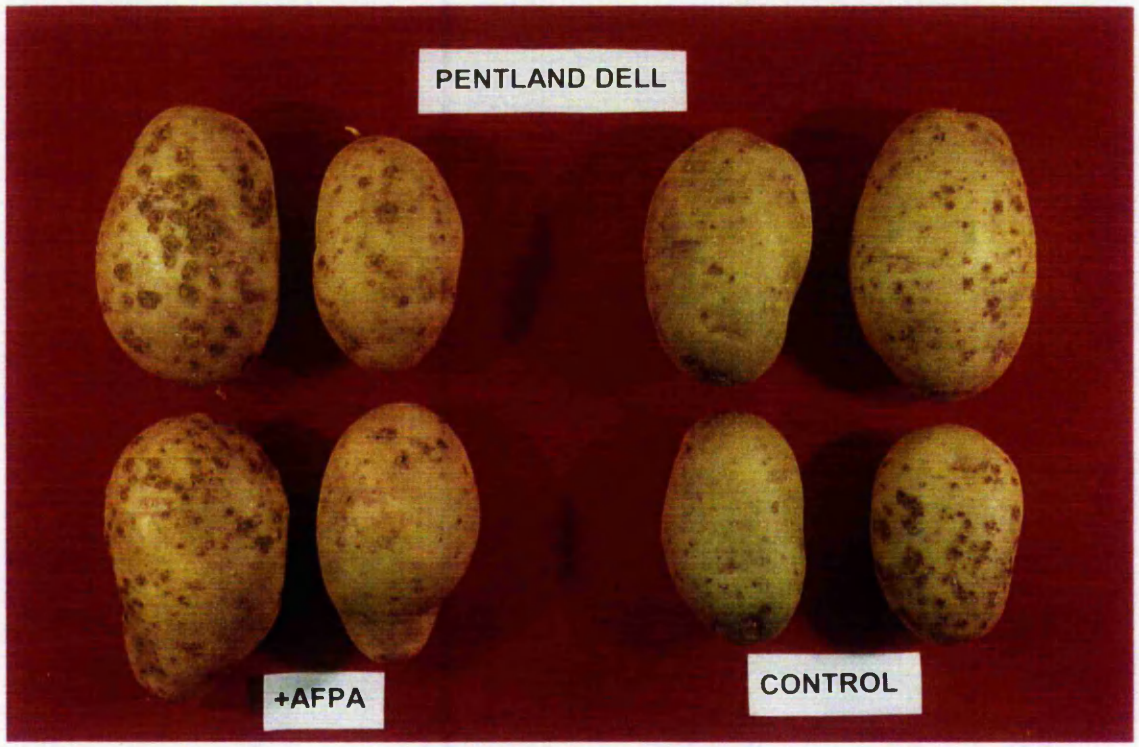
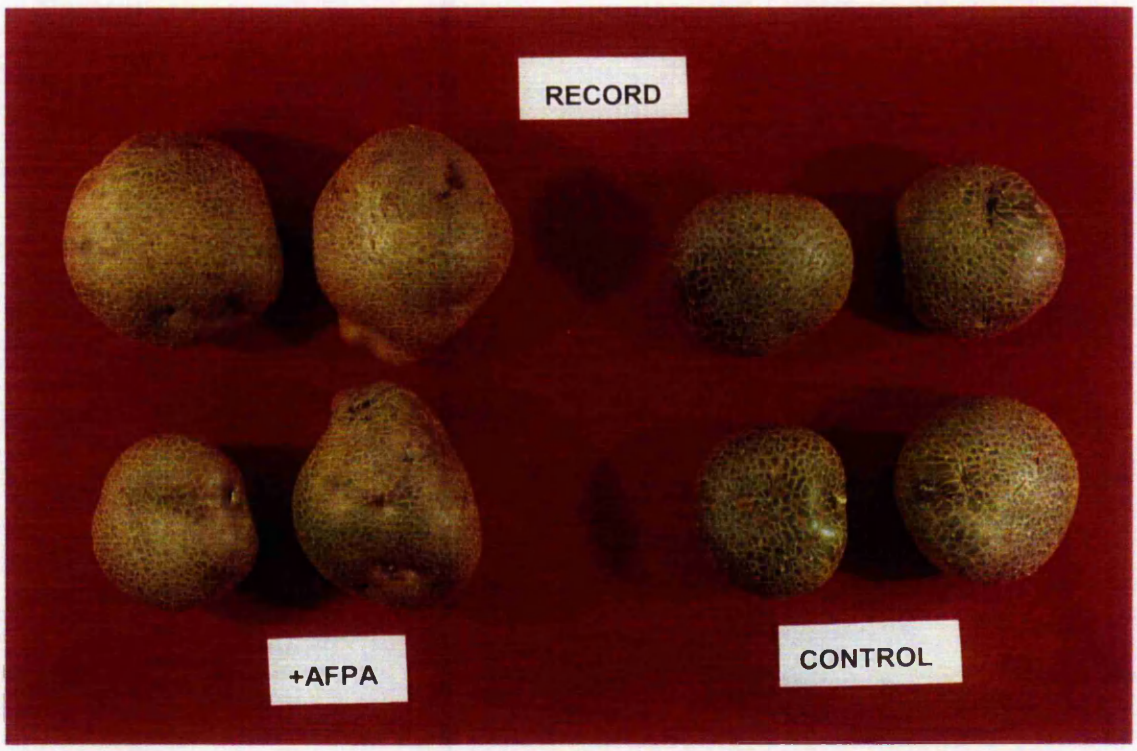


Plate 6.3 - Potato tubers dipped in either AFPA or MES, then exposed to daylight for 6 days a) cv. Record and b) cv. Pentland Dell.

a)



b)

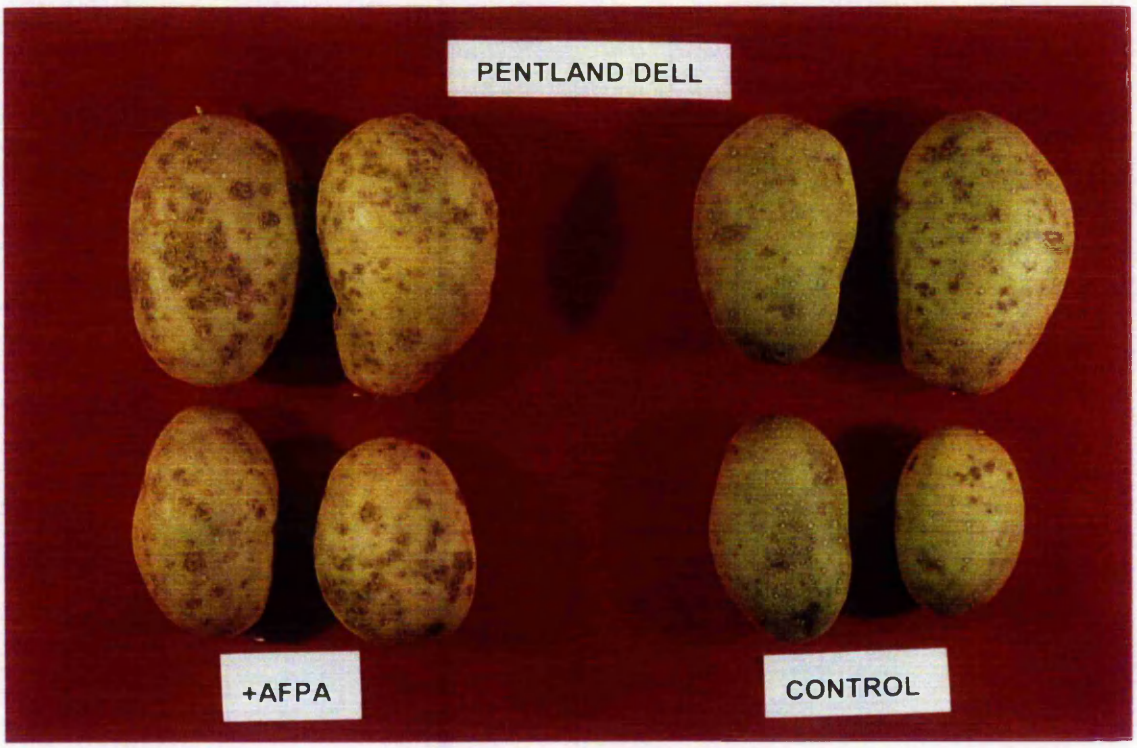


Plate 6.4 - Potato tubers dipped in either AFPA or MES, then exposed to daylight for 10 days a) cv. Record and b) cv. Pentland Dell.

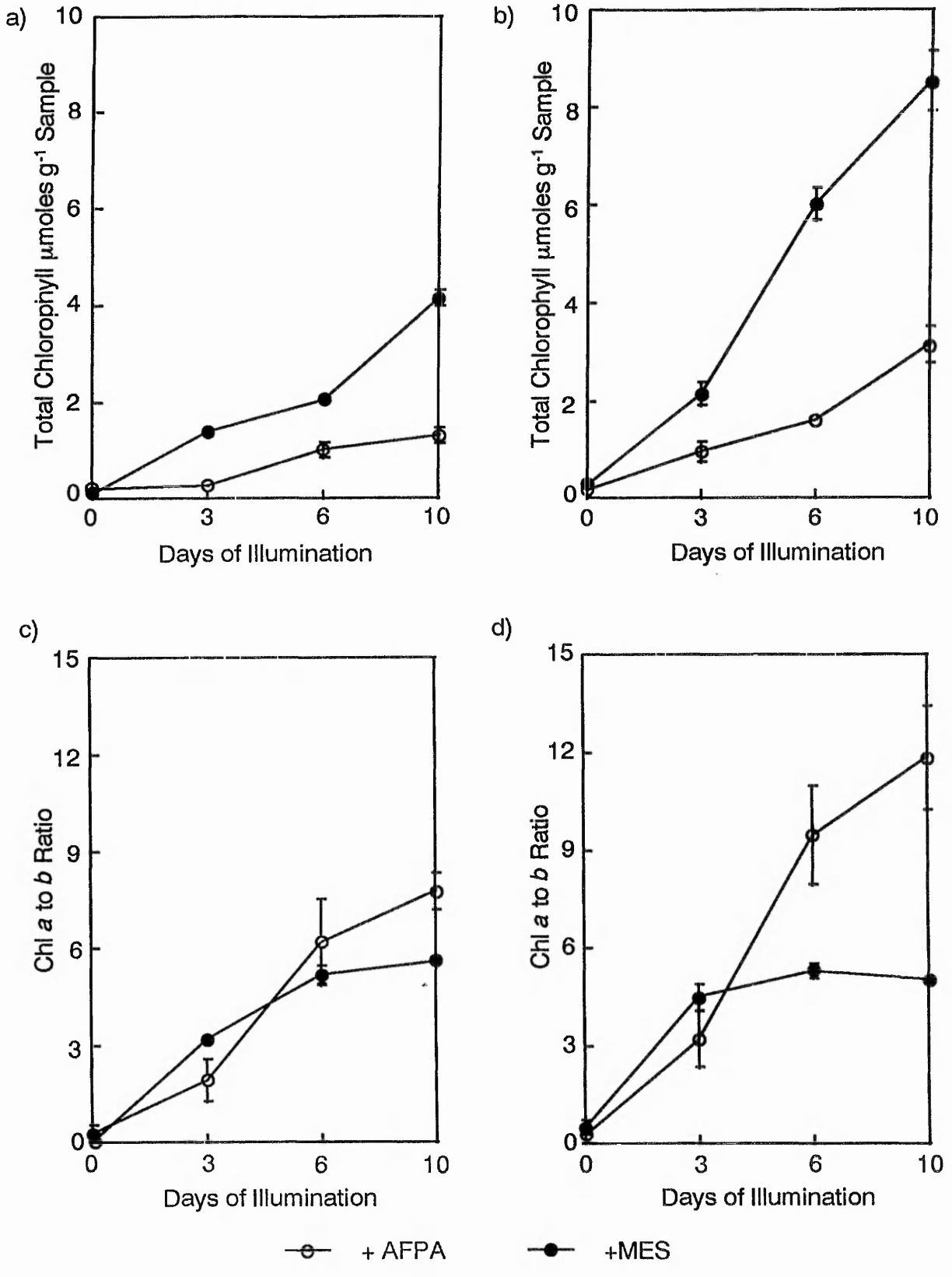


Figure 6.13 - Effect of AFPA on the accumulation of Chl in tubers exposed to daylight at 18°C a) total Chl accumulation in cv. Pentland Dell, b) total Chl accumulation in cv. Record, c) Chl *a* to *b* ratio in cv. Pentland Dell and d) Chl *a* to *b* ratio in cv. Record. Each point represents the mean of 5 tubers with SE.

Table 6.4 - Inhibition of Chl synthesis in potato tubers by 5 mM AFPA.

Illumination (days)	Pentland Dell			Record		
	Chl *a*	Chl *b*	Total Chl	Chl *a*	Chl *b*	Total Chl
3	57.7%	17.1%	48.1%	51.4%	81.1%	56.2%
6	49.4%	56.6%	50.6%	70.9%	85.6%	73.2%
10	66.2%	80.2%	68.4%	58.9%	82.4%	62.9%

Tubers treated with MES exhibited a slow rise in Chl *a* to *b* ratio to 5-5.5, irrespective of cultivar (Fig. 6.13c & d). Chl *a* to *b* ratio in tubers treated with AFPA continued to increase throughout the experiment, reaching 11.8 in Record and 7.8 in Pentland Dell. This was due to a greater inhibition of Chl *b* than Chl *a*, reaching over 80% in both cultivars.

Total carotenoid concentrations were slightly inhibited by AFPA treatment (Fig. 6.14a & b), to a maximum of 20% inhibition. The lack of inhibition of carotenoid accumulation led to low Chl to carotenoid ratios in tubers treated with AFPA (Fig. 6.14c & d).

After 10 days of exposure to light cv. Pentland Dell had 5 times the initial TGA concentration, with a significant increase occurring by day 6 (Fig. 6.15a). However, TGA content in cv. Record remained stable for the 10 day period (Fig. 6.15b). Treatment with AFPA did not significantly affect TGA accumulation in either cultivar. The ratio of α-solanine to α-chaconine increased with time in cv. Pentland Dell, but was not significantly affected by light exposure in cv. Record (Fig. 6.15c & d).

6.4.4 - Discussion

This study has used specific Chl inhibitors during light exposure of tubers to investigate the possibility of a biosynthetic link between greening and light-enhanced Chl accumulation in potato tubers. The storage of tubers in darkness prevented any alteration in either Chl or TGA content. Therefore, any changes occurring in light-exposed tubers were solely

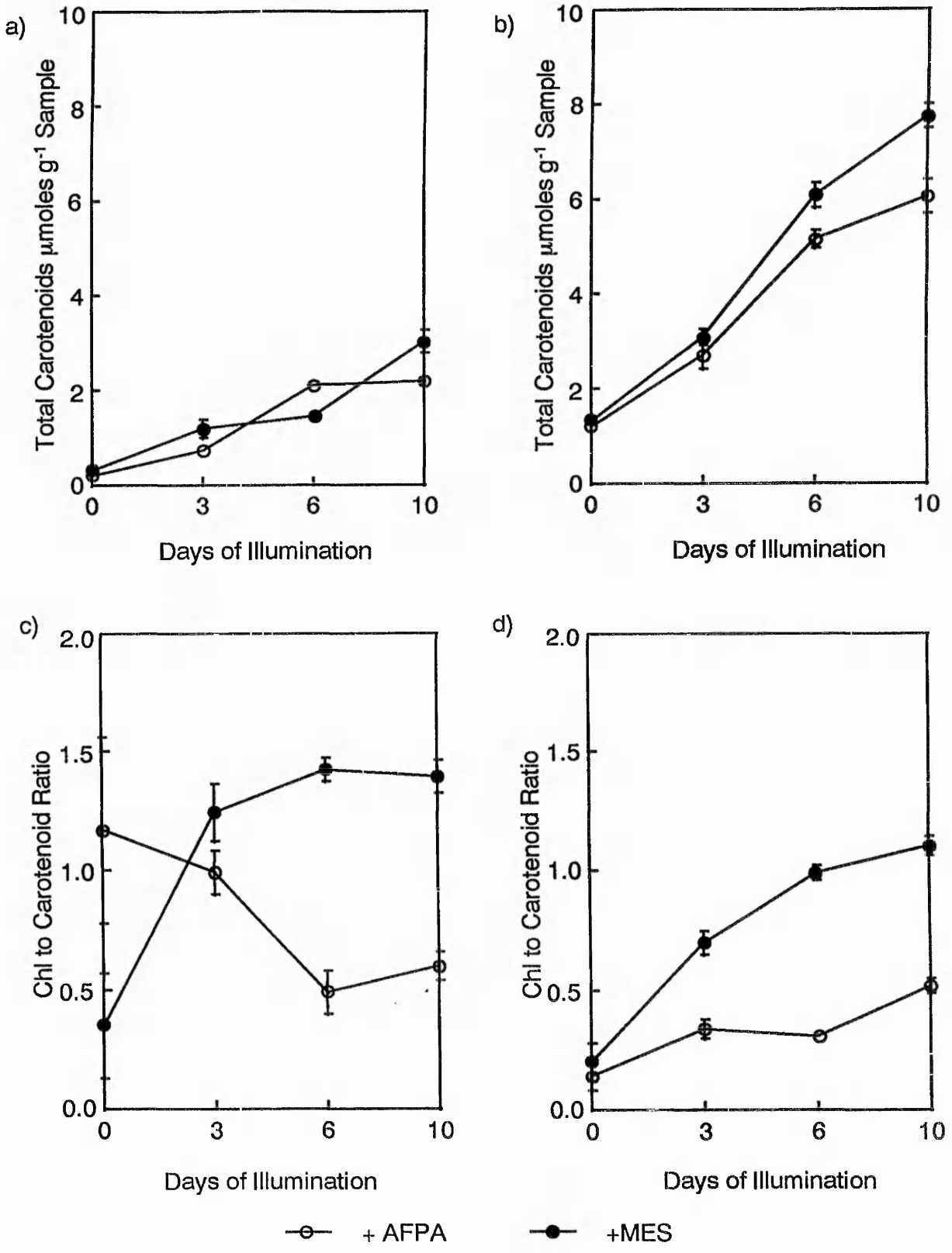


Figure 6.14 - Effect of AFPA on the accumulation of carotenoids and the Chl to carotenoid ratio in tubers exposed to daylight at 18°C a) total carotenoid accumulation in cv. Pentland Dell, b) total carotenoid accumulation in cv. Record, c) Chl to carotenoid ratio in cv. Pentland Dell and d) Chl to carotenoid ratio in cv. Record. Each point represents the mean of 5 tubers with SE.

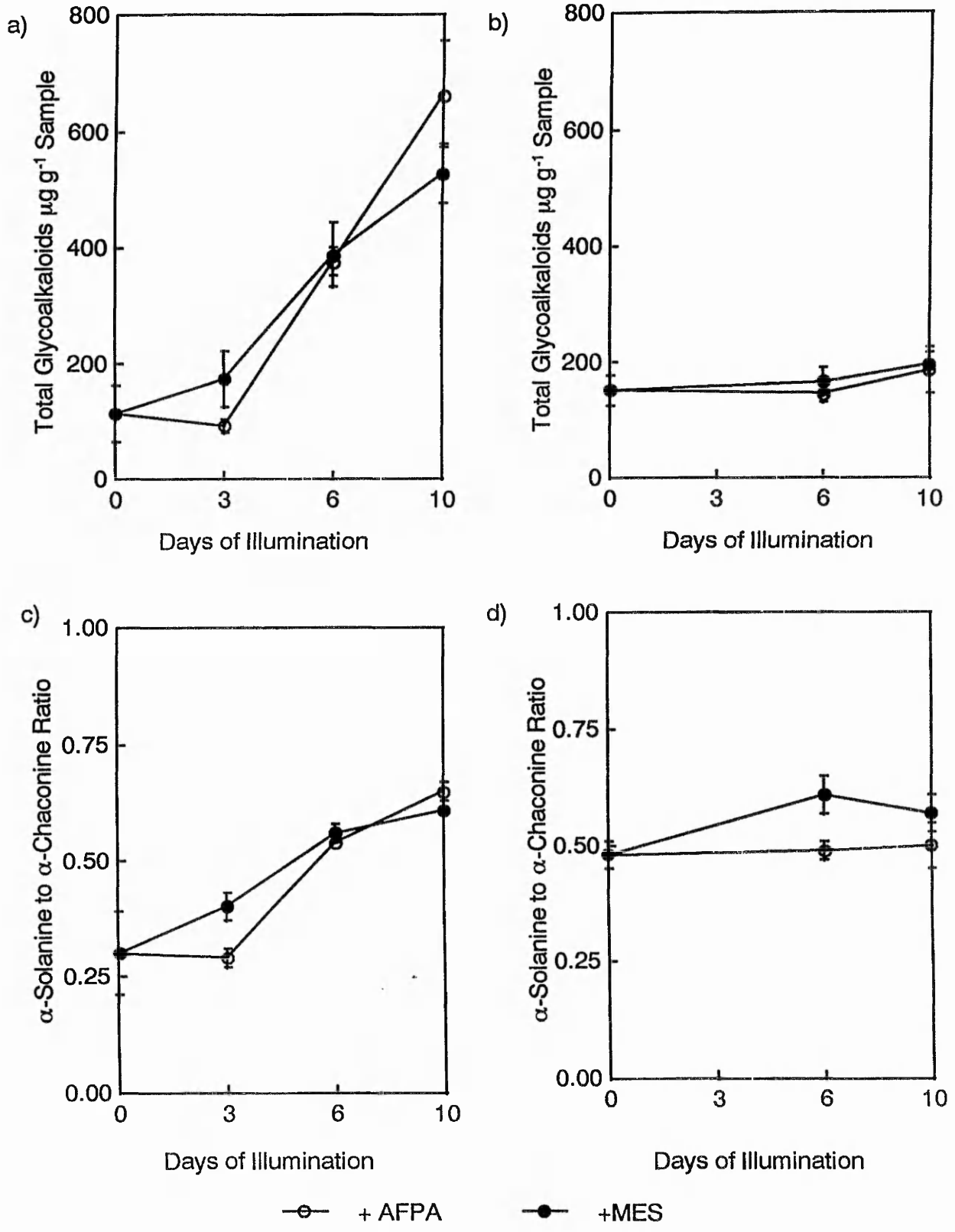


Figure 6.15 - Effect of AFPA on the accumulation of TGA and α-solanine to α-chaconine ratio in tubers exposed to daylight at 18°C a) TGA accumulation in cv. Pentland Dell, b) TGA accumulation in cv. Record, c) α-solanine to α-chaconine ratio in cv. Pentland Dell and d) α-solanine to α-chaconine ratio in cv. Record. Each point represents the mean of 5 tubers with SE.

attributable to light exposure and inhibitor treatment, rather than any metabolic changes associated with storage or other greenhouse conditions.

Exposure to light caused visible greening of tubers treated with MES after 24 h in both cultivars. Although tubers of cv. Record accumulated Chl at twice the rate of cv. Pentland Dell, this difference could not be correlated with light-enhanced TGA accumulation. The TGA content of cv. Record, whilst increasing marginally, was not significantly affected by 10 days of light exposure. Conversely, cv. Pentland Dell had significantly accumulated TGA after 6 days of light exposure. The variation in lag-phase before the onset of light-enhanced TGA accumulation and the lack of any correlation between this and Chl accumulation indicates the absence of any biosynthetic link between the two phenomena. Furthermore, the strong cultivar differences suggest the need for a close examination of light-induced responses in other cultivars, as the results from one cultivar clearly cannot be extrapolated to another.

Both inhibitors were formulated to a pH of approximately 5.5 with MES buffer alone with no use of adjuvants. However, to ensure that MES itself was inert, a second set of controls was included in Experiment 1 which were dipped in distilled water. There were no significant differences in any parameter between tubers treated with MES or water in both light and dark conditions. Consequently, it was assumed that MES did not affect tuber physiology and that any effect was due to the inhibitor alone. Tubers treated with MES were consequently used as controls in both experiments.

The inhibition of Chl synthesis by gabaculine was only significant ($p \leq 0.01$) for the first 3 days after application. After a further 3 days of light exposure in the presence of gabaculine, Chl concentrations were significantly higher than tubers treated with MES. Both enzymes in the C_5 pathway, Glu-tRNA reductase (Glu-TR) and GS-AM, are regulated by light (Ilag, Kumar and Soll, 1994). Furthermore, transcription of the genes which encode them (*HEMA* and *GSA*, respectively) is not thought to be co-regulated (Ilag *et al.*, 1994). Therefore, although GS-AM was inhibited by gabaculine, Glu-TR activity would have increased in response to light

exposure, possibly leading to the accumulation of Glu-tRNA which would allow the rapid synthesis of ALA when the tuber overcame GS-AM inhibition.

Feedback inhibition by metabolites and precursors of the Chl biosynthetic pathway is also thought to control ALA synthesis (Beale and Weinstein, 1990) and would arrest over-production. However, further study is needed to elucidate the processes occurring in the tuber during the inhibition of Chl synthesis by gabaculine.

Although tubers treated with AFPA exhibited a lasting inhibition of GS-AM, tubers of cv. Record appeared to be overcoming this after 10 days of light exposure (Fig 6.13b). However, inhibition of Chl accumulation in Pentland Dell was still increasing after 10 days (Fig 6.13a). As AFPA is an irreversible, mechanism-based inhibitor of aminotransferases, inhibition occurs on a one molecule/one active site basis (Silverman and Invergo, 1986). Consequently, inhibition of ALA synthesis will depend on the number of active sites, the abundance of enzyme and the number of molecules of inhibitor. It is likely that as ALA synthesis is the rate limiting step in Chl synthesis, the activity of the C_5 pathway enzymes in cv. Record may be greater than in cv. Pentland Dell, allowing the former to overcome inhibition before the latter. This hypothesis is supported by the observation that cv. Record greens at a faster rate than cv. Pentland Dell.

Treatment of tubers with both AFPA and gabaculine resulted in 80-85% inhibition of Chl *b*, even in cv. Record when Chl *a* inhibition was reduced. Chl *a* is an essential component of both photosystems if light-harvesting is to occur, whereas Chl *b*-less mutants can be viable, although deficient in the light-harvesting complex II (Batschauer *et al.*, 1986). Therefore, when there is a strong inhibition of Chl formation reducing the loss of Chl *a* to Chl *b* would maximise light harvesting. It is thought that an oxygenase enzyme converts Chl *a* to Chl *b* (von Wettstein, Gough and Kannangara, 1995) and since it is extremely unlikely that AFPA or gabaculine could inhibit this enzyme, an active control of this enzyme must be in operation.

The majority of Chl *a/b* binding proteins are rapidly degraded proteolytically in the absence of Chl (Bennett, 1981). Therefore, inhibition of Chl must reduce the abundance of these proteins and also affect their binding of carotenoids. In both experiments carotenoid accumulation was slightly inhibited by the application of GS-AM inhibitors. Carotenoids are synthesised via the acetate-mevalonate pathway as are steroids and therefore synthesis is not directly affected by AFPA or gabaculine. However, the reduction in carotenoid accumulation is only significant after a number of days of light exposure and is indicated by a rise in Chl to carotenoid ratios between 6 and 10 days in both cultivars (Fig 6.14b & c). Furthermore, phytol might be expected to accumulate in treated tubers, thus total carotenoid accumulation may not be strongly inhibited. Chl to carotenoid ratios in tubers treated with MES exhibited a rapid increase with the onset of Chl production, which stabilised as Chl and carotenoids were accumulated at a similar rate, the exact value being cultivar-dependant and determined by the initial carotenoid concentration.

Tubers of cv. Record treated with gabaculine and AFPA had TGA concentrations almost identical to those treated with MES. However, as accumulation was so slight in this cultivar, significant differences were difficult to detect. TGA concentrations of cv. Pentland Dell tubers treated with AFPA were not significantly different from those of MES treated controls. Indeed, after 10 days the tubers treated with AFPA had a slightly greater TGA content than the MES controls. This result demonstrates that light-enhanced TGA accumulation is not a secondary product of photosynthetic carbon metabolism and that there is no correlation between tuber greening and glycoalkaloid content.

The α-solanine to α-chaconine ratios are important since α-chaconine is significantly more toxic than α-solanine (Fewell and Roddick, 1993). However, there is also a synergistic effect when the two are combined and toxicity is dependant on the ratio of the glycoalkaloids. Initially, almost 75% of the glycoalkaloids in cv. Pentland Dell was α-chaconine. This rose steadily throughout the experiment until nearly 75% of TGA was α-solanine

(Fig 4c). As peak toxicity of glycoalkaloid combinations is approximately 60% to 40% (α-chaconine to α-solanine) this alteration of the α-solanine to α-chaconine ratio actually decreases the potential toxicity of the TGA. Therefore, it appears that despite the proven toxicity of TGA to herbivores and fungi (Fewell and Roddick, 1993; Olsson and Jonasson, 1995) the ratio between the individual glycoalkaloids is governed by factors other than maximal toxicity.

It is clear that with so many factors involved in the accumulation of Chl and TGA, combined with the lack of any obvious biosynthetic relationship between the two phenomena, there is a potential danger in the assumption that non-greened potatoes are safe for human consumption but greened tubers are not. The data presented here demonstrates that slightly greened tubers can be high in TGA and that highly greened tubers can have a low TGA content.

It has been suggested that GSA-AM would be an ideal enzyme to act as a target for a novel herbicide as there is no homologous enzyme in mammals (Kumar, Schaub, Soll and Ujwal, 1996). The data from this study would support this view and suggest the potential usefulness of AFPA in the prevention of tuber greening during retail. However, AFPA and its analogues were originally developed to inhibit γ-aminobutyric acid (GABA) aminotransferase in epileptic patients, and they also inhibit glutamic acid decarboxylase and have proved to be fairly toxic (Silverman and Nanavati, 1990). The similarity of GABA and glutamate-semialdehyde may make it difficult to find a non-toxic inhibitor of GSA-AM, and therefore, Glu-TR may provide a better target enzyme for herbicide development.

6.4.5 - Conclusions

This study demonstrates that inhibition of Chl synthesis with a GS-AM inactivator does not affect glycoalkaloid synthesis or accumulation. Therefore, although there is no direct biosynthetic link between Chl accumulation and light enhanced glycoalkaloid accumulation, AFPA may be a useful tool for the further study of any interactions between the two.

CHAPTER 7 - GENERAL DISCUSSION

7.1 - A General Criticism of Methodology Employed in This Investigation

It is essential in any study of the physiology of potato tuber greening and TGA formation that accurate methods exist for the quantification of photosynthetic pigments and potato glycoalkaloids. Furthermore, experimental design must take account of the distribution of these molecules within the tuber and the high levels of variation that occur between potatoes, even of the same cv.

Whilst accurate methods for the analysis of photosynthetic pigments in leaves have existed for many years this is not the case with glycoalkaloids. Detection of glycoalkaloids poses some difficulties due to their lack of a chromophore. Early gravimetric analysis methods were wholly inadequate for physiological research as they require very large initial samples and very efficient extraction techniques. These and many other methods preclude the separation of α-solanine and α-chaconine, which also reduces their usefulness to the researcher as several publications have suggested that α-solanine to α-chaconine ratios play a significant physiological and toxicological role (Friedman and McDonald, 1997).

The difficulty in separating α-solanine and α-chaconine is due to the similarity between the molecules and is amply demonstrated by the existence of α-chaconine not being recognised until 1954. Modern chromatographic techniques were required to successfully do this, but the actual detection and quantification is still difficult. Recent monoclonal antibodies show excellent affinity for solanidine based glycoalkaloids but cannot discriminate between the two common potato alkaloids or their hydrolysis products (Stanker, *et al.*, 1994). For these reasons HPLC techniques similar to the one described in Chapter 2 are commonly used. However, although UV absorbance has been successfully used for detection the limit of this is relatively high, requiring extensive concentration and

purification of samples. Furthermore, virtually all solvents compatible with HPLC techniques absorb UV wavelengths, creating a higher background absorbance and further reducing the methods sensitivity. HPLC-mass-spectrometry may well provide a more accurate method of detection, reducing the need for sample concentration and allowing detection of glycoalkaloids in much smaller samples. Unfortunately, use of these techniques is restricted by the high cost of equipment and its maintenance.

Further problems with the HPLC assay used throughout these studies is the susceptibility of peak separation to external conditions, such as laboratory temperature, and the need for very accurate mixing and pH control of the mobile phase. Also the method requires an acidic column, which is not commonly available in many analytical laboratories.

HPLC was also used to analyse carotenoid composition. As carotenoids all absorb visible wavelengths the difficulties with this technique were fewer than with the TGA analysis. The main problem was choice of analysis wavelength, as the method needed to detect as many carotenoids as possible. Using a single-wavelength detector limits the sensitivity of the method towards those carotenoids that have absorption maxima furthest from this wavelength. However, the analysis can then be used more widely as expensive photo-diode array detectors are not needed.

Over 90% of potato tuber glycoalkaloids are located within the first 3-5 mm of tissue under the periderm (Kozukue *et al.*, 1987). Because of this any sampling of tubers must allow for the surface area to volume ratio effects that occur. A problem with a number of previous studies is that whole or half tubers are used (e.g. Sanford *et al.*, 1995). Unless the sample tubers used in these studies are identical in size and shape TGA concentrations will largely appear to be a result of tuber size, with small tubers having the highest TGA content. Several authors (e.g. Wolf and Dugger, 1946; Sinden and Webb, 1972) have suggested that small tubers are high in glycoalkaloids, and yet did not show that their experiments were not affected by differing surface area to volume ratios.

In the studies described in this thesis a cylinder of tuber tissue with a fixed area of periderm was used. A sampling regime of this type allows much better comparison between different tubers, and perhaps more importantly different cvs. Such a sample will still be affected by tuber size to some extent as a larger tuber would result in a longer cylinder of tissue and therefore dilute the TGA in the outer tissue. However, as glycoalkaloids are found throughout the tuber any sample must include tissue from the whole tuber, not the outer tissues alone. A second effect of this sampling regime was to negate any toxicological relevance the results may have had, as whole tubers, not cylindrical sections, are used for potato products. Therefore, TGA concentrations in cooked and processed potatoes are affected by tuber size.

As TGA concentrations in tuber samples are affected by injury to the tuber tissue (Salunkhe, Wu, and Jadhav, 1972) and light exposure samples need to be stored in a manner that will prevent any changes in TGA content. Rapid freezing and freeze-drying effectively does this (Dao and Friedman, 1996), and was used throughout the experiments reported here. However, although preliminary work indicated that freeze-drying did not affect TGA concentrations within samples no extensive testing of this has been carried out, and it is possible that storage of the freeze-dried tissue in adverse conditions or for an extensive time could reduce sample TGA content.

The high level of variation between tubers is a problem that must be surmounted in many areas of potato research. Experiments need to be designed in order to minimise variation and with a sufficient sample size to provide statistically significant results despite this. Preliminary experiments indicated that a sample size of 12 would be sufficient to provide a standard error of 10% or less. However, whilst pigment results were almost always within this the glycoalkaloid results proved to be both more variable and subject to greater experimental error. Consequently, the standard errors of these latter results were sometimes considerably higher than this which reduced the significance of some data. Furthermore, the smaller sample sizes used for controls also proved to have a high standard error, probably

due to inherent variation. However, relative to exposed samples the absolute error was normally very low.

The significance of cv. differences was clearly demonstrated in Chapter 4. Therefore, choice of cv. is also important in order to achieve relevant and representative results. The four cvs. used in Chapter 4 covered a range of potato uses and of tuber responses. However, the use of King Edward in Chapter 3 affected the usefulness of the data as this variety was subsequently found not to accumulate TGA within a relevant timescale. Substitution of King Edward for Pentland Dell in this experiment could have provided better information on the effect of exposure temperature on TGA synthesis.

ANN's have proven to be an useful tool in a number of areas of computer modelling and prediction. Their 'intelligent' method of data analysis, based on the mammalian brain, is uniquely suited to complex and variable biological data. Despite this few applications for ANN's have been attempted within the biological field. The simple, generic, ANN's used in this study proved to be remarkably effective at predicting the very variable information that was presented to them. However, there were some apparent limitations; extreme data was less well modelled and no indication of variability could be given, the latter sometimes being of note in itself.

The preliminary studies described in Chapter 6 aimed to provide a basis for further study in areas of greening physiology that had not previously been investigated. The use of IRGA to measure the CO_2 exchange of potatoes is useful in that it does not affect the tubers thus variability in the results is reduced as the same tubers can be used throughout an experiment. Two methods of investigation can be used; the first is to expose tubers to light and take regular, e.g. daily, readings which can them be plotted to show any change in CO_2 emission, the second is to expose the tubers to light within sealed chambers and take continuous readings throughout the experiment. The former method presented difficulties in that the chambers used took a considerable time to equilibrate, in which time tuber respiration had been affected due to atmospheric

temperature and CO_2 content was different around the IRGA equipment to the exposure site. Therefore, the second method was used, which reduced the number of samples that could be used to one control and one exposed. This prevented any statistics being used on the data and also meant that any air leaks in the system affected all samples. Although IRGA is a non-destructive way of measuring CO_2 exchange it cannot measure photosynthesis directly in the tuber. Consequently unless a positive photosynthetic rate was achieved, i.e. CO_2 from the sample chambers was less than ambient, there is no direct evidence of CO_2 fixation. However, a large reduction in CO_2 emission by metabolically active tubers would be hard to explain any other way.

The use of AFPA to inhibit tuber Chl synthesis without directly inhibiting tuber TGA synthesis was effective. However, the formulation and application of the inhibitor was crude and study of the concentrations of the molecule entering the tuber and its effects there would be necessary in order to ascertain if complete Chl synthesis inhibition does not affect TGA synthesis.

7.2 - Interpretation of Results in Relation to the Literature

Potato greening and glycoalkaloid accumulation has serious effects on food safety. Consequently the study of the physiology of these responses is important and of interest to the scientist, the potato producer and the potato consumer.

These studies have examined a number of effects on the greening process, as well as that process itself.

7.2.1 - Light

It has long been known that light, including most forms of artificial lighting, causes potatoes to green and to accumulate TGA (Conner, 1937; Gull and Isenberg, 1960). Throughout these experiments all tubers exposed

to light synthesised Chl. However, the response of the TGA pool was cv. dependant. Percival, Dixon and Sword (1996) observed that initial TGA content prior to light exposure was not related to rate of accumulation nor final TGA content after exposure. Similarly, in this study Brodick accumulated higher TGA concentrations than any other cv. despite Pentland Dell having the highest initial TGA content. There was also a cv. effect on the lag phase before the onset of detectable TGA accumulation, with King Edward tubers not initiating TGA formation even after 10 days of light exposure. It is possible that this effect explains some anomalous results in the literature where tubers have not accumulated TGA (Percival, Harrison and Dixon, 1993).

King Edward potatoes were exposed to a PPFD of 12 μmol m^{-2} s^{-1} in the experiment described in Chapter 3 and approximately 100-150 μmol m^{-2} s^{-1} in the later experiments but accumulated Chl concentrations were very similar after 8-10 days of exposure. Furthermore, the higher light intensity did not increase TGA accumulation. This contradicts the findings of earlier studies which observed that increasing light intensity leads to an increase in Chl content (Liljemark and Widoff, 1960; Patil, Salunkhe and Singh, 1971). However, Gull and Isenberg (1958) found no effect of light intensities above 50 fc and more recently Percival and Dixon (1996) observed that while tubers exposed to 500 μmol m^{-2} s^{-1} accumulated higher TGA concentrations than those at 250 μmol m^{-2} s^{-1}, a PPFD of 1000 μmol m^{-2} s^{-1} or higher actually inhibited TGA accumulation. Thus it appears that the effect of light intensity on Chl and TGA accumulation is not a simple one and that at low light intensities PPFD does not play an important role in determining the extent of Chl and TGA accumulation whereas a moderate PPFD leads to an increase in these molecules.

Initiation of light-induced Chl synthesis (Morris *et al.*, 1979) and possibly TGA accumulation (Peterman and Morris, 1985) in potato tubers is controlled by phytochrome. AFPA has been shown to inhibit phytochrome biosynthesis (Gardner *et al.*, 1988) yet TGA increases were unaffected by AFPA application. This indicates that the TGA pool is influenced by a

separate light receptor, however, as Chl inhibition was not total and phytochrome is inhibited to a lesser extent than Chl by AFPA phytochrome may not have been significantly affected.

7.2.2 - Storage

Several publications have examined the effect of storage on tuber glycoalkaloid concentrations (Wolf and Duggar, 1946; Fitzpatrick, Herb, Osman and McDermott, 1977; Griffiths *et al.*, 1994) but conclusions have been varied, with authors concluding that storage had no effect, caused an increase in TGA or that any effect was cv. specific. Olsson and Roslund (1995) observed a cycling of TGA through storage, similar to the observations in Chapter 4. Not all of these results were statistically significant, but a cv. specific cycling of TGA could explain the many contradictions in published data.

Griffiths *et al.* (1994) investigated the effect of 48 hr of light exposure on Chl and TGA content of tubers stored for 0 or 3 months. Their results were inconclusive and very much dependant on cv. choice. The observations presented here indicated that while the extent of greening varied somewhat after different storage durations there was no significant effect of storage, but that 20 months storage or longer reduced TGA accumulation. This period is longer than has previously been examined and storage for 10 weeks did not have any significant effect on light-induced TGA synthesis, which may explain the difference between these and previously published results. Storage for up to 30 weeks is necessary within the industry so the effects of long term storage are important.

7.2.3 - Temperature

Another aspect of potato greening about which there is confusion in the literature is the effect of temperature. Authors have observed higher Chl concentrations in tubers exposed at low temperatures (Ramaswamy and Nair, 1974), higher Chl concentrations in tubers exposed at 15-20 °C (Harkett, 1975), faster greening after storage at 5 °C (Yamaguchi, Hughes

and Howard, 1960), higher TGA concentrations in tubers stored at low temperatures (Jadhav and Salunkhe, 1975), higher TGA concentrations in tubers stored at high temperatures (Linnemann, van Es and Hartmans, 1985) and that temperature has little effect on TGA formation (Percival *et al.*, 1993).

It is difficult to reconcile all of these results but careful storage of potatoes at low PPFD to prevent photo-oxidative effects in the experiment described in Chapter 3 produced little greening in tubers stored at 5 °C and higher Chl concentrations at 20 °C which agrees with the majority of published studies and the response of leaves to low temperature (Nie and Baker., 1991). Glycoalkaloids are produced as a result of stress to the tuber, therefore, it might be expected that a combination of stressful conditions would result in the highest TGA concentrations. The King Edward potatoes used in this study did not exhibit any TGA accumulation despite the high temperature stress caused by exposure to light at 25 °C which was evidenced by the high carotenoid concentrations. King Edward appear to be very slow TGA accumulators, but the increased stress of very high or low temperatures might be expected to increase this. Percival *et al.* (1993) also found little effect of temperature on light-induced TGA formation. It seems likely then that although temperature during storage may have an effect on the TGA content of tubers not exposed to light it has little effect on light-induced TGA synthesis.

7.2.4 - The physiology of greening and glycoalkaloid accumulation

The reason for potato tuber greening in response to light is almost certainly photosynthesis. It seems odd then that only one publication has investigated whether greened tubers are capable of fixing CO_2. Zhu *et al.* (1984) found that electron transport was occurring in isolated tuber chloroplasts. The existence of O_2 evolution combined with the study described in Chapter 6, which indicated that CO_2 fixation was occurring confirms that greening does result in active photosynthesis. This has a

number of implications for tuber physiology, which may in turn help explain light-induced TGA accumulation.

The possibility of a light-independent protochlorophyllide reductase being present in potato tubers is also interesting. If such an enzyme exists in tubers this is the first reported incidence in the roots of an angiosperm. Furthermore, it is also evidence for the existence of a signal transduction pathway within the tuber. The lack of TGA accumulation in unexposed tissues of exposed tubers indicates not only that glycoalkaloids do not diffuse through a tuber from the point of origin but also that there is a fundamental difference in the pathways leading to TGA and Chl synthesis.

Throughout these experiments carotenoid concentrations were measured as well as Chl. In leaves high carotenoid concentrations can be indicative of environmental stress (Demming-Adams and Adams, 1992). In these studies carotenoids in control tubers have shown some potential as stress indicators. In greened tissues Chl to carotenoid ratios may also prove useful. In Chapter 4 it was shown that although neither Chl nor carotenoid concentrations appeared to be significantly affected by storage Chl to carotenoid ratios were, although this was clearer in some cvs. than others.

The ratio of α-solanine to α-chaconine is also important as toxicity of TGA is directly related to its composition (Roddick and Rijnenberg, 1987; Rayburn, Friedman and Bantle, 1994). While no clear effects of storage or light exposure was seen on these there were significant cv. differences. However, Percival *et al.* (1996) have observed an increase in α-solanine to α-chaconine ratios in certain cvs. As a low ratio has greater toxicity it would be expected that if potatoes were producing high TGA concentrations purely as a defence reaction such ratios would be low therefore control of α-solanine to α-chaconine ratios is controlled to maximise toxicity. The large variation between cvs. in α-solanine to α-chaconine ratios also emphasises the difficulties in setting safe concentrations of TGA for consumption.

In conclusion it is clear that there is no direct link between light-induced Chl and TGA accumulation, indeed, it is probable that the two

processes have a different light receptor, and that cv. has a significant role in all aspects of the physiology of greening and glycoalkaloid formation.

7.3 - Further Studies

Despite the many studies of potato glycoalkaloids that have been published in recent years there is still an imperative need for further research. Many early studies generated conflicting data that is only now starting to be explained. Friedman and McDonald (1997) outline a number of areas of research that have either been inadequately studied or not studied at all. They also list a range of studies that are necessary to provide information allowing growers and consumers to chose and handle the safest cvs. These include determining the susceptibility of all commercial cvs. to glycoalkaloid accumulation as a result of greening and mechanical damage, evaluation of food-compatible inhibitors that could inactivate TGA toxicity or synthesis and fully investigating the role of tuber size and maturity on tuber TGA content.

The ANN's described in Chapter 5 could be of great assistance in this work. All the data from experiments such as these could be used to train specifically written ANN software, producing a model that could be used for recommendations for all aspects of potato storage and processing. As any attempt to study all commercial cvs. is by necessity a huge undertaking the use of ANN's could also take account of any lack of standardisation in analysis and exposure techniques between different research groups, particularly in underdeveloped areas of the world. It is also possible that the ANN's so developed could be used to predict the response of new potato cvs. and to identify previously unrecognised factors in the response of tuber TGA pools to the storage environment. If a measure of sample error was given to the ANN it could also be used to predict the variability of the tuber response, allowing any recommendations on tuber storage to be adjusted accordingly.

Some very basic aspects of tuber glycoalkaloid physiology are still unknown. An example of this is the sub-cellular localisation of glycoalkaloids. It has been suggested that glycoalkaloids are stored in an aqueous compartment of the cell, but the evidence for this has not been definitive and no rigorous study has investigated the problem. Two simple approaches to this could be effective; fractionation, which is time consuming, would cause analysis difficulties and would be unlikely to prove the exact location of the glycoalkaloids, or immuno-gold electron microscopy. The latter technique could take advantage of established methods for transmission electron microscopy of potato tuber cells (Turnbull and Cobb, 1992) and the very specific monoclonal antibodies already produced (Plhak and Sporns, 1994; Stanker *et al.*, 1994) to give a very accurate appraisal of intracellular distribution. The most likely problem to be encountered with this method would be the low concentrations of TGA within the cells. However, if periderm and/or highly greened cells were initially used it is likely that the sensitivity of the technique could be improved enough to investigate non-greened cells. A further advantage of this method is that it could easily be used to follow the development of chloroplasts within the tuber and any association between these and light-induced TGA accumulation.

The preliminary experiment described in Chapter 6 indicates that greening tubers are capable of CO_2 fixation and carbohydrate synthesis. Further experimentation using radioisotope studies utilising $^{14}CO_2$ would enable this to be determined. If greening tubers are capable of CO_2 fixation then the effect on tuber water content could be significant as a stored tuber cannot replace water hydrolysed as part of photosynthesis. It is also likely that free sugars within the tuber would increase, which may in turn affect starch content and even have an effect on tuber TGA concentrations.

Also described in Chapter 6 is a simple HPLC method for carotenoid analysis. Use of this method with potatoes stored under sub-optimal conditions, such as high temperature, would enable the composition of tuber carotenoids and the changes observed in tuber total carotenoid content to be determined. These studies could in turn lead to a simple

spectrophotometric test for tuber stress based on an understanding of tuber carotenoid response to external stresses.

Although much of the synthesis of solanidine based glycoalkaloids is identical to that of other steroids the steps beyond cholesterol have not been elucidated. Of particular interest is the insertion of the nitrogen atom, recently Ohmura *et al.* (1995) have suggested a mechanism for this. However, further research is needed to fully understand this process. Inhibition of one of the enzymes catalysing these steps, by addition of a novel molecule or by genetic manipulation, could prevent the accumulation of TGA.

Another enzyme system that could be blocked to improve the safety of potatoes is the transferases that convert solanidine to the two glycoalkaloids. Stapleton *et al.* (1991) have partially purified the glucosyltransferase and continuance of these studies could lead to a detailed understanding of these enzymes. Changes in the activities of the glyco-transferases, given sufficient activity, investigated using relatively simple activity assays, during greening and wound healing may give an insight into how TGA accumulation occurs and is regulated. Such assays could also be used to find sites of high activity within the cell or tissue.

The exact role of glycoalkaloids within the tuber is still not fully elucidated. Although it is likely that the primary use of glycoalkaloids in tubers is for defence there is still some doubt as to the degree to which this is important against actual potato pests. Furthermore, secondary glycoalkaloid roles which have been suggested, such as nitrogen storage, have not been sufficiently investigated. Exposure to light results in a tuber TGA content far higher than is found naturally, again the purpose of this is not clear. Studies described here have shown that the biosynthesis of Chl and TGA are separate, so high TGA concentrations is not simply a secondary product of tuber greening. An understanding of glycoalkaloid synthesis and the sites, both of this, and TGA storage would be valuable in answering these questions.

The commonly quoted industry standard of 200 μg TGA g^{-1} FWT as the limit for safe consumption of potatoes is not based on any scientific study but has merely been continually suggested until accepted. Furthermore, this standard has no legal enforcement in the UK. Considering the high toxicity of TGA and the considerable position which potatoes have in the UK diet it is clear that this situation is not sufficient for public health. Indeed, these inadequacies have been noted several times in previous publications (Hopkins, 1995; Friedman and McDonald, 1997).

There are many factors involved in establishing a safe level for potato TGA content. These include; average consumption of potatoes, maximum consumption of potatoes, particularly within the poorer areas of society, the form in which potatoes are prepared and eaten, i.e. with/without skins, cooking method and time, and the absorption of glycoalkaloids by the human intestine. These also need to be adjusted for the possibility of poor storage within the home, adverse weather conditions leading to high TGA concentrations within a crop etc. Given the toxicity estimates of Morris and Lee (1984) an average adult would only have to consume half a kg of potatoes at the accepted safe level of TGA content to experience toxic effects. TGA content in Brodick tubers triples after 7 days of light exposure, therefore, if glycoalkaloid concentrations were enhanced due to inadequate storage etc. this amount would be considerably smaller. It is apparent then that the risk posed by consumption of potatoes with a high TGA content is a very real one and is compounded by a lack of any significant risk assessment having been implemented.

REFERENCES

ABELL, D.C. AND SPORNS, P. (1996). Rapid quantitation of potato glycoalkaloids by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Journal of Agricultural and Food Chemistry.* **44**, 2292-2296.

ADAMSON, H., GRIFFITHS, T., PACKER, N. AND SUTHERLAND, M. (1985). Light-independent accumulation of chlorophyll *a* and *b* and protochlorophyllide in green barley (*Hordeum vulgare*). *Physiologia Plantarum.* **64**, 345-352.

AHMED, S.S. AND MULLER, K. (1979). Seasonal changes and the effect of nitrogen- and potash-fertilization on the solanine and a-chaconine content in various parts of the potato plant. *Z. Pflanzenernaehr. Bodenkd.* **142**, 275-279.

ALLEN, E.H. AND KUC, J. (1968). α-Solanine and α-chaconine as fungitoxic compounds in extracts of Irish potato tubers. *Phytopathology.* **58**, 776-781.

ANON. (1979). Solanine poisoning. *British Medical Journal.* **8**, 1458-1459.

ANSTIS, B.J.P. AND NORTHCOTE, D.H. (1973). Development of chloroplasts from amyloplasts in potato tuber discs. *New Phytologist.* **72**, 449-463.

ARNON, D.I. (1949). Copper enzymes in isolated chloroplasts. *Plant Physiology.* **24**, 1-17.

BAERUG, R. (1962). Influence of different rates and intensities of light on solanine content and cooking quality of potato tubers. *European Potato Journal.* **5**, 242-251.

BAKER, N.R.; LONG, S.P. AND ORT, D.R. (1988). Photosynthesis and temperature, with particular reference to effects on quantum yield. In *Plants and Temperature.* Long, S.P. and Woodward, F.I. (Eds.) The Company of Biologists Ltd., Cambridge, UK. pp. 347-375.

BALLS, G.R., PALMER-BROWN, D. AND SANDERS, G.E. (1996). Investigating microclimatic influences on ozone injury in clover (*Trifolium subterraneum*) using artificial neural networks. *New Phytologist.* **132**, 271-280.

BATSCHAUER, A., MOSINGER, E., KRUEZ, K., DORR, I. AND APEL, K. (1986). The implication of a plastid-derived factor in the transcriptional control of nuclear genes encoding the light-harvesting chlorophyll *a/b* protein. *European Journal of Biochemistry.* **154**, 625-634.

BAUP, M. (1826). Extrait d' une lettre sur plusieurs nouvelles substances. *Ann. Chim. Phys.* **31**, 108-?

BAUHIN, G. (1596). *Phytopinax.* Basle.

BEALE, S.I. AND WEINATEIN, J.D. (1990). Tetrapyrrole metabolism in photosynthetic organisms. In: *Biosynthesis of Heme and Chlorophylls.* Dailey, H.A. (ed). McGraw-Hill, New York, pp 287-391.

BENNETT, J. (1981). Biosynthesis of the light harvesting chlorophyll *a/b* protein. Polypeptide turnover in darkness. *European Journal of Biochemistry.* **118**, 61-70.

BERGENSTRAHLE, A., TILLBERG, E. AND JONSSON, L. (1992a). Characterization of UDP-glucose: solanidine glucosyltransferase and UDP-galactose: solanidine galactosyltransferase from potato tubers. *Plant Science.* **84**, 35-44.

BERGENSTRAHLE, A., TILLBERG, E. AND JONSSON, L. (1992b). Regulation of Glycoalkaloid accumulation in potato tuber discs. *Journal of Plant Physiology.* **140**, 269-275.

BERGENSTRAHLE, A., BORGA, P. AND JONSSON, L.M. (1996). Sterol composition and synthesis in potato tuber discs in relation to glycoalkaloid synthesis. *Phytochemistry.* **41**, 155-161.

BHUSHAN, B. AND THOMAS, P. (1990). Effects of gamma irradiation and storage temperature on lipoxygenase activity and carotenoid disappearance in potato tubers. *Journal of Agricultural and Food Chemistry.* **38**, 1586-1590.

BISWAL, B. (1995). Carotenoid catabolism during leaf senescence and its control by light. *Journal of Photochemistry and Photobiology B: Biology.* **30**, 3-13.

BOARDMAN, N.K., BJORKMAN, O., ANDERSON, J.M. AND GOODCHILD, D.J. (1975). Photosynthetic adaptation of higher plants to light intensity: Relationship between chloroplast structure, composition of the photosystems and photosynthetic rates. In: *Proceedings of 3rd International Congress on Photosynthesis.* Avron, A. (Ed). Elsevier, Amsterdam. pp 809-827.

BOFFEY, S.A. (1985). The chloroplast division cycle and its relationship to the cell division cycle. In: *The Cell Division Cycle in Plants.* Bryant, J.A. and Francis, D. (Eds). Cambridge University Press. pp 233-246.

BOMER, A. AND MATTIS, H. (1924). Der Solaningehalt der Kartoffeln. *Z. Unters. Nahr-u. Genussmittel.* **47**, 97-127.

BOSTOCK, R.M., NUCKLES, E., HENFLING, J.W.D. AND KUC, J.A. (1983). Effect of potato tuber age and storage on sesquiterpenoid stress metabolite accumulation, steroid glycoalkaloid accumulation, and response to abscisic and arachidonic acids. *Phytopathology.* **73**, 435-438.

BOYD, A.E.W. (1957). Field experiments on potato skin spot disease caused by *Oospora pustulans* Owen and Wakef. *Annals of Applied Biology.* **45**, 284-292.

BROUK, B. (1975). *Plants Consumed by Man*. Academic Press, London.

BURTON, W.G. (1955). Biological and economic aspects of the refridgerated storage of potatoes. *Procedings of the Institute of Refridgeration.* **51**, 168-72.

BURTON, W.G. (1958). The effect of the concentrations of carbon dioxide and oxygen in the storage atmosphere upon sprouting of potatoes at 10°C. *European Potato Journal.* **1**, 42-51.

BURTON, W.G. (1966). *The Potato*. 2nd Edn., Veenan, Wageningen.

BURTON, W.G. (1968). The effect of oxygen concentrations upon the sprout growth of the potato tuber. *European Potato Journal.* **11**, 249-265.

BURTON, W.G. (1974). Requirements of the users of ware potatoes. *Potato Research.* **17**, 374-409.

BURTON, W.G. (1989). *The Potato*. 3rd Edn., Longman Scientific and Technical, Harlow.

BURTON, W.G. AND HANNAN, R.S. (1957). Use of γ-irradiation for preventing the sprouting of potatoes. *Journal of the Science of Food and Agriculture.* **12**, 707-715.

BUSHWAY, R.J.M., BUREAU, J.L. AND KING, J. (1986). Modification of the rapid high-performance liquid chromatographic method for the determination of potato glycoalkaloids. *Journal of Agricultural and Food Chemistry.* **34**, 277-279.

CANO, M.P. (1991). HPLC separation of chlorophyll and carotenoid pigments of four kiwi fruit cultivars. *Journal of Agricultural and Food Chemistry.* **39**, 1786-1791.

CARMAN, A. S., KUAN, S. S., FRANCIS, O. J. AND KIRSCHENHEUTER, G.P. (1986). Rapid HPLC determination of the potato glycoalkaloids alpha-solanine and alpha-chaconine. *Journal of Agriculture and Food Chemistry.* **34**, 279-282.

CHAUBE, S. AND SWINYARD, C.A. (1976). Teratological and toxicological studies of alkaloidal and phenolic compounds from *Solanum tuberosum* L. *Toxicology and Applied Pharmacology.* **36**, 227-237.

CHENG, J., SAUNDERS, J.A. AND SINDEN, S. (1995). Colorado potato beetle resistant somatic hybrid potato plants produced via protoplast electrofusion. *In Vitro Cellular and Developmental Biology.* **31**, 90-95.

CHEN, S., DERRICK, P.J., MELLON, F.A. AND PRICE, K. R. (1994). Analysis of glycoalkaloids from potato shoots and tomatoes by four-

sector tandem mass spectrometry with scanning array detection: comparison of positive ion and negative ion methods. *Analytical Biochemistry.* **218**, 157-169.

CONNER, H.W. (1937). Effect of light on solanine synthesis in the potato tuber. *Plant Physiology, Lancaster.* **12**, 79-98.

CONOVER, J.C. AND PRYKE, J.A. (1987). Plastid and nuclear DNA in potato tuber tissue during greening. *Journal of Experimental Botany.* **38**, 1219-1227.

COSTES, C., BURGHOFFER, C., CARRAYOL, E., DUCET, G. AND DIANO, M. (1976). Occurance of carotenoids in non-plastidial materials from potato tuber cells. *Plant Science Letters.* **6**, 153-159.

CUNNINGTON, A.C., MAWSON, K., BRIDDON, A. AND STOREY, R.M. (1992). Low temperature storage for qualitity crops. *Aspects of Applied Biology.* **33**, 205-212.

DALE, M.F.B., GRIFFITHS, D.W. AND BAIN, H. (1992). Glycoalkaloids in potatoes - shedding light on an important problem. *Aspects of Applied Biology.* **33**, 221-225.

DALE, M.F.B., GRIFFITHS, D.W., BAIN, H. AND TODD, D. (1993). Glycoalkaloid increase in Solanum tuberosum on exposure to light. *Annals of Applied Biology.* **123**, 411-418

DAO, L. AND FRIEDMAN, M. (1996). Comparison of glycoalkaloid content of fresh and freeze-dried potato leaves determined by HPLC and colorimetry. *Journal of Agricultural and Food Chemistry.* **44**, 2287-2291.

DAVALO, E. AND NIAM, P. (1990). *Neural Networks.* Translated by Rawsthorne, A. Macmillan, London. pp. 111-112.

DAVIES, B.H. (1976). Carotenoids. In: *Chemistry and Biochemistry of Plant Pigments, Vol. 2.* Goowdwin, T.W. (ed). Academic Press, London. pp 38-165.

DAYHOFF, J. (1990). *Neural Network Architectures.* Van Nostrund Reinhold, New York.

DEAHL, K.L., SINDEN, S.L. AND YOUNG, R.J. (1993). Evaluation of wild tuber-bearing Solanum accessions for foliar glycoalkaloid level and composition. *American Potato Journal.* **70**, 61-69.

DEMMIG-ADAMS, B. (1990). Carotenoids and photoprotection in plants. A role for the xanthophyll zeaxanthin. *Biochymica Biophysica Acta.* **1020**, 1-24.

DEMMIG-ADAMS, B. AND ADAMS III, W.W. (1996). The role of xanthophyll cycle carotenoids in the protection of photosynthesis. *Trends in Plant Science.* **1**, 21-26.

ELTON, G.A.H. (1978). European diets in relation to standards of need. In *Diet of Man: Needs and Wants.* Yudkin, J. (Ed). Applied Science Publishers, London. pp 25-40.

FERREIRA, F., MOYNA, P., SOULE, S. AND VAZQUEZ, A. (1993). Rapid determination of *Solanum* glycoalkaloids by thin-layer chromatographic scanning. *Journal of Chromatography.* **653**, 380-384.

FEWELL, A.M. AND RODDICK, J.G. (1993). Interactive antifungal activity of the glycoalkaloids α-solanine and α-chaconine. *Phytochemistry.* **33**, 323-328.

FISHWICK, M.J. AND WRIGHT, A.J. (1980). Isolation and characterization of amyloplast envelope membrane from *Solanum tuberosum. Phytochemistry.* **19**, 55-59.

FITZPATRICK, T.J., HERB, S.F., OSMAN, S.F. AND MCDERMOTT, J.A. (1977). Potato glycoalkaloids: increases and variations of ratios in aged slices over prolonged storage. *American Potato Journal.* **54**, 539-544.

FAO (1993). *Production Statistics*. **47**, p 117.

FRIEDMAN, M. AND DAO, L. (1992). Distribution of glycoalkaloids in potato plants and comercial potato products. *Journal of Agricultural and Food Chemistry.* **40**, 419-423.

FRIEDMAN, M. AND LEVIN, C.E. (1992). Reversed phase HPLC separation of potato glycoalkaloids and their hydrolysis products on acidic columns. *Journal of Agricultural and Food Chemistry.* **40**, 2157-2163.

FRIEDMAN, M. AND MCDONALD, G.M. (1995). Acid catalyzed partial hydrolysis of carbohydrate groups of the potato glycoalkaloid α-chaconine in alcoholic solutions. *Journal of Agricultural and Food Chemistry.* **43**, 1501-1506.

FRIEDMAN , M. AND MCDONALD, G.M. (1997). Potato glycoalkaloids: chemistry, analysis, safety, and plant physiology. *Critical Reviews in Plant Sciences.* **16**, 55-132.

FRIEDMAN, M., RAYBURN, J.R. AND BANTLE, J.A. (1991). Developmental toxicology of potato glycoalkaloids in the frog embryo teratogenesis assay. *Food and Chemical Toxicology.* **29**, 537-548.

FRIEDMAN, M., RAYBURN, J.R. AND BANTLE, J.A. (1992). Structural relationships and developmental toxicity of *Solanum* alkaloids in the frog embryo teratogenesis assy - *Xenopus*. *Journal of Agricultural and Food Chemistry.* **40**, 1617-1624.

FRIEDMAN, M., MCDONALD, G. AND HADDON, W.F. (1993). Kinetics of acid-catalyzed hydrolysis of carbohydrate groups of potato glycoalkaloids α-chaconine and α-solanine. *Journal of Agricultural and Food Chemistry.* **41**, 1397-1406.

FUKUHARA, K. AND KUBO, I. (1991). Isolation of steroidal glycoalkaloids from *Solanum incanum* by two countercurrent chromatographic methods. *Phytochemistry.* **30**, 685-687.

GAFFIELD, W. AND KEELER, R.F. (1996). Induction of terata in hamsters by solanidane alkaloids derived from *Solanum tuberosum*. *Chemical Research in Toxicology.* **9**, 426-433.

GARDNER, G., GORTON, H.L. AND BROWN, S.A. (1988). Inhibition of phytochrome synthesis by the transaminase inhibitor, 4-amino-5-fluoropentanoic acid. *Plant Physiology.* **87**, 8-10.

VAN GELDER, W.M.J. (1989). Steroidal glycoalkaloids in *Solanum* species: consequences for potato breeding and food safety. PhD Thesis, University of Wageningen, The Netherlands.

VAN GELDER, W.M.J. (1990). Chemistry, toxicology, and occurrence of steroidal glycoalkaloids: potential contaminents of the potato (*Solanum tuberosum* L.). In: *Poisonous Plants Contaminating Edible Plants.* Rizk, A-F.M. (Ed). CRC Press, Boca Raton, Florida. pp117-156.

VAN GELDER, W.M.J. AND SCHEFFER, J.J.C. (1991). Transmission of steroidal glycoalkaloids from *Solanum vernei* to the cultivated potato. *Phytochemistry*. **30**, 165-168.

GOODWIN, T.W. (1980). *The Biochemistry of the Carotenoids*. Chapman and Hall, London.

GRANADO, F., OLMEDILLA, B., BLANCO, I. AND ROJAS-HIDALGO, E. (1992). Carotenoid composition in raw and cooked Spanish vegetables. *Journal of Agricultural and Food Chemistry.* **40**, 2135-2140.

GRIFFITHS, D.W., DALE, M.F.B. AND BAIN,H. (1994). The effect of cultivar, maturity and storage on photo-induced changes in the total glycoalkaloid and chlorophyll contents of potatoes (*Solanum tuberosum*). *Plant Science.* **98**, 103-109.

GROSS, J. (1991). *Pigments in Vegetables: Chlorophylls and Carotenoids.* Van Nostrand Reinhold, New York.

GULL, D.D. AND ISENBERG, F.M. (1958). Light burn and off flavor development in potato tubers exposed to fluorescent light. *Procedings of the American Society for Horticultural Science.* **71**, 446-454.

GULL, D.D. AND ISENBERG, F.M. (1960). Chlorophyll and solanine content and distribution in four varieties of potato tubers. *Procedings of the American Society for Horticultural Science.* **75**, 545-556.

GUSEVA, A.R. AND PASESHNICHENKO, V.A. (1958). A study of the biogenesis of potato glycoalkaloids by the method of labeled atoms. *Biokhimiya.* **23**, 385.

GUSEVA, A.R., BORIKHINA, M.G. AND PASESCHNICHENKO, V.A. (1960). Utilization of acetate for the biosynthesis of chaconine and solanine in potato sprouts. *Biokhimiya.* **25**, 213-214.

GUSEVA, A.R., PASESCHNICHENKO, V.A AND BORIKHINA, M.G. (1961). Synthesis of radioactive mevalonic acid and its use in the study of the biosynthesis of steroid glycoalkaloids from *Solanum*. *Biokhimiya.* **26**, 631-635.

HALDIMAN, P. (1996). Effects of changes in growth temperature on photosynthesis and carotenoid composition in *Zea mays* leaves. *Physiologia Plantarum.* **97**, 554-562.

HARKETT, P.J. (1975). The influence of temperature and skin colour on chlorophyll synthsis in potato tubers exposed to light. *Abstacts from Conference Papers of the 6th Trienniel Conference of the European Association for Potato Research.* pp 179-180.

HARTMANN, M.A. AND BENVENISTE, P. (1974). Effects of aging on sterol metabolism in potato tuber slices. *Phytochemistry.* **13**, 2667-2672.

HARVEY, M.H., MORRIS, B.A., MCMILLAN, M. AND MARKS, V. (1986). Potato steroidal alkaloids and neural-tube defects - serum concentrations fail to demonstrate a causal relation. *Human Toxicology.* **5**, 249-253.

HAWKES, J.G. (1985). Introduction to the revised impression of Salaman (1949).

HEFTMANN, E. (1967). Biochemistry of steroidal saponins and glycoalkaloids. *Lloydia*. **30**, 209-230.

HELLENAS, K. (1986). A simplified proceedure for the quantification of potato glycoalkaloids in tuber extracts by HPLC; comparison with ELISA and a colorimetric method. *Journal of the Science of Food and Agriculture.* **37**, 776-782.

HELLENAS, K-E., BRANZELL, C., JOHNSSON, H. AND SLANINA, P. (1995a). High Levels of glycoalkaloids in the established Swedish potato variety Magnum Bonum. *Journal of the Science of Food and Agriculture.* **68**, 249-253.

HELLENAS, K-E., BRANZELL, C., JOHNSSON, H. AND SLANINA, P. (1995b). Glycoalkaloid content of early potato varieties. *Journal of the Science of Food and Agriculture.* **67**, 125-128.

HENDRY, G.A.F. AND PRICE, A.H. (1993). Stress indicators: chlorophylls and carotenoids. In: *Methods in Comparative Plant Ecology.* Hendry, G.A.F. and Grime, J.P. (Eds). Chapman and Hall, London. pp 148-152.

HENDRY, G.A.F., HOUGHTON, J.D. AND BROWN, S.B. (1987). The degredation of chlorophyll - A biological enigma. *New Phytologist.* **107**, 255-302.

HILTON, R.J. (1951). Factors in relation to tuber quality in potatoes. II. Preliminary trials on bitterness in Netted Gem potatoes. *Sci. Agr.* **31**, 61-70.

HILTON, R.J. AND GAMBORG, O.L. (1957). Factors in relation to tuber quality in potatoes. IV. Tuber metabolic stage and its influence on total solanine. *Canadian Journal of Plant Science.* **37**, 407-412.

HLYWKA, J.J., STEPHENSON, G.R., SEARS, M.K. AND YADA, R.Y. (1994). Effects of insect damage on glycoalkaloid content in potatoes (Solanum tuberosum). *Journal of Agricultural and Food Chemistry.* **42**, 2545-2550.

HOOBER, J.K., KAHN, A., ASH, D.E., GOUGH, S. AND KANNANGARA, C.G. (1988). Biosynthesis of δ-aminolevulinate in greening barley leaves. IX. Structure of the substrate, mode of gabaculine inhibition, and the catalytic mechanism of glutamate 1-semialdehyde amino transferase. *Carlsberg Research Communications.* **53**, 11-25.

HOPKINS, J. (1995). The Glycoalkaloids: naturally of interest (but a hot potato). *Food and Chemical Toxicology.* **33**, 323-328.

HOWARD, H.W. (1970). *Genetics of the Potato.* Logos Press Ltd., London.

ILAG, L.L., KUMAR, A.M. AND SOLL, D. (1994). light regulation of chlorophyll biosynthesis at the level of 5-aminolevulinate formation in *Arabidopsis*. *The Plant Cell.* **6**, 265-275.

INSKEEP, W.P. AND BLOOM, P.R. (1985). Extinction coefficients of chlorophyll *a* and *b* in *N,N*-dimethylformamide and 80% acetone. *Plant Physiology.* **77**, 483-485.

ISHERWOOD, F.A. AND BURTON, W.G. (1975). The effect of senescence, handling, sprouting and chemical sprout suppression upon the respiratory quotient of stored potato tubers. *Potato Research.* **18**, 98-104.

ISHIZAKA, N. AND TOMIYAMA, K. (1972). Effect of wounding or infection by *Phytophthora infestans* on the content of terpenoids in potato tubers. *Plant Cell Physiology.* **13**, 1053-1063.

JADHAV, S.J. AND SALUNKHE, D.K. (1975). Formation and control of chlorophyll and glycoalkaloids in tubers of *Solanum tuberosum* L. and evaluation of glycoalkaloid toxicity. *Advances in Food Research.* **21**, 307-354.

JADHAV, S.J., SHARMA, R.P. AND SALUNKHE, D.K. (1981). Naturally occurring toxic alkaloids in foods. *Critical Reviews of Toxicology.* **9**, 21-104.

JADHAV, S.J., SALUNKHE, D.K., WYSE, R.E. AND DALVI, R.R. (1973). Solanum alkaloids: Biosynthesis and inhibition by chemicals. *Journal of Food Science.* **38**, 453-455.

JENSEN, K., TYGESEN, T.K., KESMIR, C., SKOVGAARD, I.M. AND SONDERGAARD, I. (1997). Classification of potato varieties using isoelectrophoretic focusing patterns, neural nets, and statistical methods. *Journal of Agricultural and Food Chemistry.* **45**, 158-161.

JOHNSTON, K.A. AND PIERCE, R.S. (1994). Biochemical and bioassay analysis of resistance of potato (*Solanum tuberosum* L.) cultivars to attack by the slug *Deroceras reticulatum* (Muller). *Annals of Applied Biology.* **124**, 109-131.

JONASSON, T. AND OLSSON, K. (1994). The influence of glycoalkaloids, chlorogenic acid and sugars on the susceptibility of potato tubers to wireworm. *Potato Research.* **37**, 205-216.

JONKER, H.H., KOOPS, A.J. AND HOOGENDOORN, J. C. (1992). A rapid method for the quantification of steroidal glycoalkaloids by reversed phase HPLC. *Potato Research.* **35**, 451-455.

KAABER, L. (1993). Glycoalkaloids, green discoloration and taste development during storage of some potato varieties (*Solanum tuberosum* L.). *Norwegian Journal of Agricultural Sciences.* **7**, 221-229.

KALDY, M.S. (1972). Protein yield of various crops as related to protein value. *Economic Botany.* **26**, 142-144.

KAMPS-HOLTZAPPLE, C. AND STANKER, L.H. (1996). Development of Recombinant single-chain variable portion recognizing potato glycoalkaloids. *ACS Symposium Series.* **621**, 485-499.

KANEKO, K., TANAKA, M.W. AND MITSUHASHI, H. (1976). Origin of nitrogen in the biosynthesis of solanidine by *Veratrum grandiflorum. Phytochemistry.* **15**, 1391-1393.

KEELER, R.F., YOUNG, S. AND BROWN, D. (1976). Spina bifida, exencephaly and cranial bleb produced in hamsters by the Solanum alkaloid solasodine. *Research Communications Chem. Pathol. Pharmacol.* **13**, 723-730.

KEUKENS, E.A.J., DE VRIJE, T., VAN DEN BOOM, C., DE WAARD, P., PLASMAN, H.H., THIEL, F., CHUPIN, V., JONGEN, W.M.F. AND DE KRUIJFF, B. (1995). Molecular basis of glycoalkaloid induced membrane disruption. *Biochimica et Biophysica Acta.* **1240**, 216-228.

KHANBARI, O.S. AND THOMPSON, A.K. (1994). The effect of controlled atmosphere storage at 4°C on crisp colour and on sprout growth, rotting and weight loss of potato tubers. *Potato Research.* **291**, 291-300.

KIRK, J.T.O. AND TILNEY-BASSET, R.A.E. (1978). *The plastids. Their Chemistry, Structure, Growth and Inheritance.* Elsevier, Amsterdam.

KOLASA, K.M. (1993). The potato and human nutrition. *American Potato Journal.* **70**, 375-384.

KOUKKARI, W.L. AND HILLMAN, W.S. (1966). Phytochrome levels assayed by in vivo spectrophotometry in modified underground stems and storage roots. *Physiologia Plantarum.* **19**, 1073-1078.

KOZUKUE, N., KOZUKUE, E. AND MIZUNO, S. (1987). Glycoalkaloids in potato plants and tubers. *HortScience.* **22**, 294-296.

KRINSKY, N.I. AND DENEKE, S.M. (1982). Interaction of oxygen and oxy-radicals with carotenoids. *Journal of the National Cancer Institute.* **69**, 205-210.

KUHN, R. AND LOW, I. (1954). Die konstitution des solanins. *Agnew. Chem.* **66**, 639-640.

KUMAR, A.M., SCHAUB, U., SOLL, D. AND UJWAL, M.L. (1996). Glutamyl-transfer RNA: at the crossroad between chlorophyll and protein biosynthesis. *Trends in Plant Science.* **1**, 371-376.

KVASNICKA, F., PRICE, K.R., NG, K. AND FENWICK, G.R. (1994). Determination of potato glycoalkaloids using isotachophoresis and comparison with a HPLC method. *Journal of Liquid Chromatography.* **17**, 1941-1951.

LARSON, E.C. (1949). Investigations on cause and prevention of greening in potato tubers. *Idaho, Agricultural Experimental Station, Research Bulletin.* **16**, 1-32.

LAUFER, B. (1938). The American plant migration. Part 1. The potato. *Mem. Field Mus. nat. Hist. (Anthropology).* **28** (Publ. 418), pp 132.

LAWSON, D.R., ERB, W.A. AND MILLAR, A.R. (1992). Analysis of Solanum alkaloids using internal standardization and capillary gas chromatography. *Journal of Agricultural and Food Chemistry.* **40**, 2186-2191.

LETTE, J., COLLETTI, B.W., CERINO, M., MCNAMARA, D., EYBALIN, M., LEVASSEUR, A. AND NATTEL, S. (1994). Artificial intelligence versus logistic regression statistical modelling to predict cardiac complications after noncardiac surgery. *Clinical Cardiology.* **17**, 609-614.

LICHTENTHALER, H.K. AND WELLBURN, A.R. (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions.* **11**, 591-593.

LILJEMARK, A. AND WIDOFF, E. (1960). Greening and solanine development of white potato in fluorescent light. *American Potato Journal.* **37**, 379-388.

LINNAEUS, C. (1753). *Species Plantarum.* Stockholme.

LINNEMAN, A.R., VAN ES, A. AND HARTMANS, K.J. (1985). Changes in the content of L-ascorbic acid, glucose, fructose, sucrose, and total glycoalkaloids in potatoes (cv. Bintje) stored at 7, 16 and 28 °C. *Potato Research.* **28**, 271-278.

VAN LOON, C.D. AND CROSNIER, J.C. (1982). 1955-1980, 25 ans de culture de la pomme de terre en Europe. In *EAPR 25 Jahre Europaische Gesellschaft fur Kartoffelforschung 1957-1982* B. Schhober *et al.* (Eds). European Association for Potato Research, Wageningen, pp. 33-53.

LOVE, S.L. HERRMAN, T.J., THOMPSON-JOHNS, A. AND BAKER, T.P. (1994). Effect and interaction of crop management factors on the glycoalkaloid concentration of potato tubers. *Potato Research.* **37**, 77-85.

MCEWEN, B. AND LINDSTEN, A. (1992). Characterization of protochlorophyllide and protochlorophyllide esters in roots of dark grown plants. *Physiologia Plantarum.* **84**, 343-350.

MCKINNEY, G. (1941). Absorption of light by chlorophyll solutions. *Journal of Biological Chemistry.* **144**, 315-323.

MAGA, J.A. (1980). Potato Glycoalkaloids. *CRC Critical Reviews in Food Science and Nutrition.* **12**, 371-405.

MAGA, J.A. (1994). Glycoalkaloids in the Solanaceae. *Food Reviews International.* **10**, 385-418.

MARTINS FARIAS, R. (1975). *New Archeological Techniques for the Study of Ancient Root Crops in Peru.* PhD Thesis, Univ. Birmingham.

MONDY, N.I. AND MUNSHI, C.B. (1990). Effect of nitrogen fetilization on glycoalkaloid and nitrate content of potatoes. *Journal of Agricultural and Food Chemistry.* **38**, 565-567.

MORAN, R. AND PORATH, D. (1980). Chlorophyll determination in intact tissues using *N,N*-dimethylformamide. *Plant Physiology.* **65**, 478-479.

MORGAN, M.R.A.; MCNENEY, R.; MATTHEW, J.; COXON, D.T. AND CHAN, H.W.S. (1983). An enzyme linked immunosorbent assay for total glycoalkaloids in potato tubers. *Journal of Agricultural and Food Chemistry.* **34**, 593-598.

MORRIS, S. C.; GRAHAM, D. AND LEE, T.H. (1979). Phytochrome control of chlorophyll synthesis in potato tubers. *Plant Science Letters.* **17**, 13-19.

MORRIS, S.C. AND LEE, T.H. (1984). The toxicity and teratogenicity of Solanaceae glycoalkaloids, particularly those of the potato (*Solanum tuberosum*): a review. *Food Technology in Australia.* **36**, 118-124.

MORRIS, S.C. AND PETERMAN, J.B. (1985). Genetic and environmental effects on levels of glycoalkaloids in cultivars of potato. *Food Chemistry.* **18**, 271-282.

MUCHARROMAH, BURTON, H.R. AND KUC, J. (1995). The effect of sterols on the phytoalexin, steroid glycoalkaloid, and sterol accumulation in potato tuber discs inoculated with *Phytophthora infestans* or treated with arachidonic acid. *Physiological and Molecular Plant pathology.* **47**, 13-27.

MURAJA-FRAS, J., KRSNIK-RASOL, M. AND WRISCHER, M. (1994). Plastid transformation in greening potato tuber tissue. *Journal of Plant Physiology.* **144**, 58-63.

NAIR, P.M., BEHERE, A.G., RAMASWARMY, N.K. (1981). Glycoalkaloids of *Solanum tuberosum* L. *J. Sci. Ind. Res.* **40**, 529-535.

NASH, R.J., ROTHSCHILD, M., PORTER, E.A., WATSON, A.A., WAIGH, R.D. AND WATERMAN, P.G. (1993). Catystegines in *Solanum* and *Datura* species and the death's-head hawk moth (*Acherontia atropus*). *Phytochemistry.* **34**, 1281-1283.

NEVIN, N.C. AND MERRETT, J.D. (1975). Potato avoidance during pregnancy in women with a previous infant with either ancephaly and/or spina bifida. *British Journal of Preventative and Social Medicine.* **29**, 111-115.

NIE, G.-Y. AND BAKER, N.R. (1991). Modifications to thylakoid composition during the development of maize leaves grown at low growth temperatures. *Plant Physiology.* **95**, 184-191.

NYMAN, M., SILJESTROM, M., PEDERSON, B., BACH KNUDSON, K.E., ASP, N.G., JOHANSON, C.G. AND EGGUM, B.O. (1984). Dietary fiber content and composition in six cereals at different extraction rates. *Cereal Chemistry.* **61**, 14-19.

OBERHUBER, W. AND EDWARDS, G.E. (1993). Temperature dependance of the linkage of quantum yield of photosystem II to CO_2 fixation in C_4 and C_3 plants. *Plant Physiology.* **101**, 507-512.

OEHME, F.W. (1978). The hazard of plant toxicities to the human population. In: *Effects of Poisonous Plants on Livestock.* Van Kampen, K.R. and James, L.F. (Eds). Academic Press, New York. pp. ?.

OHMURA, E., NAKAMURA, T., TIAN, R.H., YAHARA, S., YOSHIMITSU, H. AND NOHARA, T. (1995). 26-Aminocholestanol derivative, a novel key intermeadiate of steroidal alkaloids, from *Solanum abutiloides*. *Tetrahedron Letters*. **36**, 8443-8444.

OLSSON, K. (1986). The influence of genotype on the effects of impact damage on the accumulation of glycoalkaloids in potato tubers. *Potato Research.* **29**, 1-12.

OLSSON, K. (1989). Anatomical structure and chemical composition of potato tubers. In: *Impact Damage, Gangrene and Dry Rot in Potato, Important Biochemical Factors in Screening for Resistance and Quality in Breeding Material.* Olsson, K. (Ed). Swedish University of Agricultural Sciences, Sweden.

OLSSON, K. AND JONASSON, T. (1995). Genotypic differences in susceptibility to wireworm attack in potato: mechanisms and implications for plant breeding. *Plant Breeding.* **114**, 66-69.

OLSSON, K. AND ROSLUND, C-A. (1995). Changes in glycoalkaloid content of potato during long-trem storage. *Abstracts from the Joint Meeting of the Agronomy and Utilisation Sections of the European Association of Potato Research.* pp 41.

OU, K. AND ADAMSON, H. (1995). Chlorophyll accumulation in cotyledons, hypocotyls and primary needles of *Pinus pinea* seedlings in light and dark. *Physiologia plantarum.* **93**, 719-724.

ORGELL, W.H., VAIDYA, K.A. AND DAHM, P.A. (1958). Inhibition of human plasma cholinesterase *in vitro* by extracts of solanaceous plants. *Science.* **128**, 1136-1137.

PACTER, N.M. (1973). *Biosynthesis of acetate derived compounds.* John Wiley & Sons, London.

PALLETT, K.E. AND YOUNG, A.J. (1993). Carotenoids. In: *Antioxidants in Higher Plants.* CRC Press.

PARRY, A.D. AND HORGAN, R. (1991). Carotenoids and abscisic acid synthesis in higher plants. *Physiologia Plantarum.* **82**, 320-326.

PATIL, B.C., SALUNKHE, D.K. AND SINGH, B. (1971). Metabolism of solanine and chlorophyll in potato tubers as affected by light and specific chemicals. *Journal of Food Science.* **36**, 474-476.

PAUL, A.A. AND SOUTHGATE, D.A.T. (1978). *The Composition of Foods.* 4th Edn., HMSO, London.

PERCIVAL, G.C., HARRISON, J.A.C., DIXON, G.R. (1993). The influence of temperature on light enhanced glycoalkaloid synthesis in potato. *Annals of Applied Biology.* **123**, 141-153.

PERCIVAL, G., DIXON, G. AND SWORD, A. (1994). Glycoalkaloid concentration of potato tubers following continuous illumination. *Journal of the Science of Food and Agriculture.* **66**, 139-144.

PERCIVAL, G., DIXON, G.R. AND SWORD, A. (1996). Glycoalkaloid concentration of potato tubers following exposure to daylight. *Journal of the Science of Food and Agriculture.* **71**, 59-63.

PERCIVAL, G.C. AND DIXON, G.R. (1996). Effect of light intensity on glycoalkaloid content of potato tubers. *Abstracts of Conference Papers, Posters and Demonstrations from the 13th Trienniel Conference of the European Association for Potato Research.* 43-44.

PETERMAN, J.B. AND MORRIS, S.C. (1985). The spectral responses of chlorophyll and glycoalkaloid synthesis in potato tubers (Solanum tuberosum). *Plant Science.* **39**, 105-110.

PLHAK, L. AND SPORNS, P. (1992). Enzyme immunoassay for potato glycoalkaloids. *Journal of Agriculture and Food Chemistry.* **40**, 2533-2540.

PLHAK, L. AND SPORNS, P. (1994). Development and production of monoclonal antibodies for the measurement of solanidine potato glycoalkaloids. *American Potato Journal.* **71**, 297-313.

PORTER, W.L. (1972). A note on the melting point of α-solanine. *American Potato Journal.* **49**, 403-406.

POTATO MARKETING BOARD (1992) *Potato Storage.*

POTATO MARKETING BOARD (1995a) *Historical Data Summary from 1960.*

POTATO MARKETING BOARD (1995b) *Potato Consumption and Processing in Great Britain 1994.*

RAMASWAMY, N.K. AND NAIR, P.M. (1974). Temperature and light dependency of chlorophyll synthesis in potatoes. *Plant Science Letters.* **2**, 249-256.

RAMASWAMY, N.K., BEHERE, A.G. AND NAIR P.M. (1976). A novel pathway for the synthesis of solanidine in the isolated chloroplast from greening potatoes. *European Journal of Biochemistry.* **67**, 275-282.

RASCIO, N., PASQUA, G., DALLA VACCHIA, F. AND CASADORO, G. (1990). On the greening ability of lemon roots. *Cytobios*. **64**, 137-142.

RAYBURN, J.R., BANTLE, J A. AND FRIEDMAN, M. (1994). Role of carbohydrate side chains of potato glycoalkaloids in developmental toxicity. *Journal of Agriculture and Food Chemistry.* **42**, 1511-1515.

RENWICK, J.H. (1972). Hypothesis: anencephaly and spina bifida are usually preventable by avoidance of a specific but unidentified substance present in certain potato tubers. *British Journal of Social and Preventative Medicine.* **26**, 67-89.

ROBERTSON, E.J.; BAKER, N.R. AND LEECH, R.M. (1993). Chloroplast thylakoid protein changes induced by low growth temperature in maize revealed by immunocytology. *Plant, Cell and Environment.* **16**, 809-818.

RODICK, J.G. (1974). The steroidal glycoalkaloid α-tomatine. *Phytochemistry.* **18**, 1467-1470.

RODDICK, J.G. AND RIJNENBERG, A. (1986). Effect of steroidal glycoalkaloids of the potato on the permeability of liposome membranes. *Physiologia Plantarum.* **68**, 436-440.

RODDICK, J.G. AND RIJNENBERG, A. (1987). Synergistic interaction between the potato glycoalkaloids a-solanine and a-chaconine in relation to lysis of phospholipid-sterol liposomes. *Phytochemistry.* **26**, 1325-1328.

SALUNKHE, R.W., WU, M.T. AND JADHAV, S.J. (1972). Effects of light and temperature on the formation of solanine in potato slices. *Journal of Food Science.* **37**, 969-970.

SANFORD, L.L., DEAHL, K.L., SINDEN, S.L. AND LADD, T.L. (1990). Foliar solanidine glycoside levels in *Solanum tuberosum* populations selected for potato leafhopper resistance. *American Potato Journal.* **67**, 461-466.

SANFORD, L.L., DEAHL, K.L., SINDEN, S.L. AND LADD, T.L. (1992). Glycoalkaloid contents in tubers from Solanum tuberosum populations selected for potato leafhopper resistance. *American Potato Journal.* **69**, 693-703.

SANFORD, L.L. DEAHL, K.L., SINDEN, S.L. AND KOBAYASHI, R.S. (1995). Glycoalkaloid content in tubers of hybrid and backcross populations from a Solanum tuberosum X S. chacoense cross. *American Potato Journal.* **72**, 261-271.

SANFORD, L.L., DOMEK, J.M., CANTELO, W.W., KOBAYASHI, R.S. AND SINDEN, S.L. (1996). Mortality of potato leafhopper adults on synthetic diets containing seven glycoalkaloids synthesized in the foliage of various Solanum species. *American Potato Journal.* **73**, 79-88.

SCHOUTEN, S.P. (1992). Possibilities for controlled atmosphere storage of ware potatoes. *Aspects of Applied Biology.* **33**, 181-188.

SCHRIEBER, K. (1968). Steroid alkaloids: *Solanum* group. In: *The Alkaloids.* Manske, R.H.F. (Ed). **10**, Academic Press, New York. pp 1-192.

SHIH, M.J. AND KUC, J. α- And β-solamarine in Kennebec *Solanum tuberosum* leaves and aged tuber slices. *Phytochemistry.* **13**, 997-1000.

SHIH, M., KUC, J. AND WILLIAMS, E.B. (1973). Suppression of steroid glycoalkaloid accumulation as related to rishitin accumulation in potato tubers. *Phytopathology.* **63**, 8221-826.

SILVERMAN, R.B. AND LEVY, M.A. (1980). Synthesis of (*S*)-5-substituted 4-aminopentanoic acids: a new class of γ-aminobutyric acid transaminase inactivators. *Journal of Organic Chemistry.* **45**, 815-818.

SILVERMAN, R.B. AND NANAVATI, S.M. (1990). Selective inhibition of γ-aminobutyric acid transaminase by (3*R*,4*R*),(3*S*,4*S*)- and (3*R*,4*S*),(3*S*,4*R*)-4-amino-5-fluoro-3-phenylpentanoic acids. *Journal of Medical Chemistry.* **33**, 931-936.

SIMPSON, R., WILLIAMS, R., ELLIS, R. AND CULVERHOUSE, P.F. (1992). Biological pattern recognition by neural networks. *Marine Ecology Progress Series.* **79**, 303-308.

SINDEN, S.L. AND WEBB, R.W. (1972). Effect of variety and location on the glycoalkaloid content of potatoes. *American Potato Journal.* **49**, 334-338.

SINDEN, S.L. AND WEBB, R.W. (1974). Effect of environment on glycoalkaloid content of six potato varieties at 39 locations. *U.S. Department of Agriculture Technical Bulletin.* **1472**.

SINDEN, S.L., CANTELO, W.W., SANFORD, L.L. AND DEAHL, K.L. (1991). Allelochemically mediated host resistance to the Colerado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). *Memoires of the Entymological Society of Canada.* **159**, 19-28.

SMITH, D.C. AND FUCHS, P.L. (1995). A triply convergent total synthesis of a symchiral pyrrolidine-fused prostacyclin analog. *Journal of Organic Chemistry.* **16**, 2692-2703.

SPARENBERG, H. AND ULMANN, R. (1973). Inhibition of sprouting in potatoes by irradiation in the Netherlands: economic and commercial aspects. *Commission of the European Communities: Eurisotop Office Information Booklet.* **58**.

STANKER, L.H., KAMPS-HOLTZAPPLE, C. AND FRIEDMAN, M. (1994). Development and characterization of monoclonal antibodies that differentiate between potato and tomato glycoalkaloids and aglycons. *Journal of Agriculture and Food Chemistry.* **42**, 2360-2366.

SWINYARD, C.A. AND CHAUBE, S. (1973). Are potatoes teratogenic for experimental animals? *Teratology.* **8**, 349-358.

TEVINI, M. AND SCHONECKER, G. (1986). Occurence, properties and characterization of potato charotenoids. *Potato Research.* **29**, 265.

TEVINI, M., SCHONECKER, G., IWANZIK, W., RIEDMAN, M. AND STUTE, R. (1984a). Analyse, vorkommen und verhalten von carotenoidestern in Kartoffeln. *Arbeitsgem. Kartoffelforsch.* e.V. pp 42-47.

TEVINI, M., IWANZIK, W. AND SCHONECKER, G. (1984b). Analyse, Vorkommen und Verhalten von Carotinoiden in Kartoffeln und Kartoffelnprodukten. *Jahrbuch Forschungskreis Ernahrungsindustrie e.V.* pp 36-53.

THORNE, H.V., CLARKE, G.F. AND SKUCE, R. (1985). The inactivation of herpes simplex virus by some *Solanaceae* glycoalkaloids. *Antiviral Research.* **5**, 335-343.

TURNBULL, N.D. AND COBB, A.H. (1992). A study of tuber cell integrity and ultrastructure during prolonged storage. *Aspects of Applied Biology*. **33**, 113-118.

UPPAL, D.S. (1987). Varietal and environmental effect on the glycoalkaloid content of potato. *Qualitas Plantarum Plant Foods for Human Nutrition.* **37**, 333-340.

VALASINAS, A. AND FRYDMAN, B. (1992). Synthesis of (*R*)- and (*S*)-4,5-diaminovaleric acids. *Journal of Organic Chemistry.* **57**, 2158-2160.

VIRGIN, H.I. AND SUNDQUIST, C. (1992). Pigment formation in potato tubers (Solanum tuberosum) exposed to light followed by darkness. *Physiologia Plantarum.* **86**, 587-592.

WALMSLEY, J. (1991). Chlorophyll accumulation in photoperiodically-grown barley seedlings transfered to darkness: effect of time of dark transfer and daylength. *Photosynthetica.* **25**, 409-418.

VON WETTSTEIN, D., GOUGH, S. AND KANNANGARA, C.G. (1995). Chlorophyll biosynthesis. *The Plant Cell.* **7**, 1039-1057.

WIERENGA, J.M., AND HOLLINGWORTH, R.M. (1992). Inhibition of insect acetylcholine esterase by the potato glycoalkaloid a-chaconine. *Natural Toxins.* **1**, 96-99.

WILLIMOTT, S.G. (1933). An investigation of solanine poisoning. *The Analyst.* **58**, 431-438.

WOLF, M.J. AND DUGGAR, B.N. (1946). estimation and physiological role of solanine in the potato. *Journal and Agricultural Research.* **73**, 1-32.

WOOLFE, J.A. (1987). *The Potato in the Human Diet.* Cambridge University Press.

YAMAGUCHI, M., HUGHES, D.L. AND HOWARD, F.D. (1960). Effect of season, storage temperature, and temperature during light exposure on chlorophyll accumulation of White Rose potatoes. *Procedings of the American Society for Horticultural Science.* **75**, 529-536

YAMAGUCHI, M., PERDUE, J.W. AND MACGILLIVRAY, J.H. (1960). Nutrient composition of White Rose potatoes during growth and after storage. *American Potato Journal.* **37**, 73-6.

YEE, D., PRIOR, M.G. AND FLORENCE, L.Z. (1993). Development of predictive models of laboratory animal growth using artificial neural networks. *Computer Applications in the Biosciences.* **9**, 517-522.

ZHU, Y.S., MERKLE-LEHMAN, D.L. AND KUNG, S.D. (1984). Light induced transformation of amyloplasts into chloroplasts in potato tubers. *Plant Physiology.* **75**, 142-145.

ZIMOWSKI, J. (1992). Specificity and some other properties of cytosolic and membranous UDPGlc: 3b-hydroxysteroid glucosyltransferases from Solanum tuberosum leaves. *Phytochemistry.* **31**, 2977-2981.

ZITNAK, A. (1955). Factors influencing the initiation and rate of solanine synthesis in tubers of *Solanum tuberosum* L. PhD Thesis, University of Alabama, Canada.

ZITNAK, A. AND JOHNSTON, G.R. (1970). Glycoalkaloid content of B5141-6 potatoes. *American Potato Journal.* **47**, 256-260.

ZOBEL, M. AND SCHILLING, J. (1964). Behaiviour of solanine in potatoes with various preparation methods. *Z. Lebensm. Unters. Forsch.* **124**, 327-333.

APPENDIX 1

Pigment results from storage at different temperatures. µmol g^{-1} sample.

5 Degrees

Upper Surface

		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	0.07	0.02	0.09	1.73	0.97	0.10
	Std Error	0.03	0.05	0.08	1.29	0.09	0.09
D2 Control	Mean	0.10	n/a	0.10	1.63	1.13	0.09
	Std Error	0.03	n/a	0.03	1.29	0.09	0.06
D2 Exposed	Mean	0.09	0.19	0.28	n/a	0.93	0.54
	Std Error	0.06	0.17	0.15	n/a	0.13	0.45
D5 Control	Mean	0.14	0.03	0.17	1.03	0.84	0.23
	Std Error	0.07	0.03	0.09	1.13	0.18	0.10
D5 Exposed	Mean	1.42	0.29	1.71	1.16	1.44	1.16
	Std Error	0.16	0.04	0.20	3.50	0.07	0.11
D8 Control	Mean	0.30	0.09	0.39	3.50	1.35	0.26
	Std Error	0.14	0.02	0.14	2.07	0.21	0.07
D8 Exposed	Mean	0.86	0.19	1.05	4.58	2.43	0.39
	Std Error	0.23	0.09	0.28	5.90	0.18	0.08

Lower Surface

		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	0.02	n/a	0.02	n/a	0.81	0.00
	Std Error	0.02	n/a	0.02	n/a	0.11	0.14
D2 Control	Mean	0.09	n/a	0.09	1.05	1.03	0.04
	Std Error	0.05	n/a	0.05	1.09	0.15	0.07
D2 Exposed	Mean	0.17	0.10	0.26	1.34	0.90	0.28
	Std Error	0.05	0.02	0.07	0.54	0.06	0.06
D5 Control	Mean	0.11	0.03	0.14	5.11	0.89	0.15
	Std Error	0.03	0.00	0.03	1.58	0.09	0.03
D5 Exposed	Mean	0.56	0.15	0.72	4.96	0.99	0.70
	Std Error	0.11	0.04	0.15	3.88	0.06	0.14
D8 Control	Mean	0.27	0.13	0.40	n/a	1.49	0.27
	Std Error	0.07	0.05	0.10	n/a	0.20	0.06
D8 Exposed	Mean	0.51	0.16	0.68	n/a	1.85	0.34
	Std Error	0.11	0.05	0.15	n/a	0.12	0.06

10 Degrees

Upper Surface

		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	0.07	0.02	0.09	1.73	0.97	0.10
	Std Error	0.03	0.05	0.08	1.29	0.09	0.09
D2 Control	Mean	0.20	0.31	0.50	0.65	1.34	0.31
	Std Error	0.07	0.12	0.19	0.05	0.22	0.11
D2 Exposed	Mean	0.37	0.24	0.61	1.43	1.98	0.31
	Std Error	0.05	0.08	0.13	0.87	0.09	0.06
D5 Control	Mean	0.11	0.03	0.14	0.24	0.96	0.11
	Std Error	0.05	0.06	0.09	0.56	0.13	0.08
D5 Exposed	Mean	0.45	0.13	0.58	10.74	1.44	0.37
	Std Error	0.13	0.05	0.16	7.16	0.13	0.08
D8 Control	Mean	0.39	0.22	0.61	2.84	1.69	0.29
	Std Error	0.23	0.10	0.32	1.59	0.34	0.09
D8 Exposed	Mean	2.92	0.79	3.71	3.74	3.31	1.09
	Std Error	0.38	0.11	0.48	0.20	0.22	0.08

Lower Surface

		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	0.02	n/a	0.02	n/a	0.81	0.00
	Std Error	0.02	n/a	0.02	n/a	0.11	0.14
D2 Control	Mean	0.19	0.34	0.52	0.77	1.22	0.43
	Std Error	0.05	0.12	0.17	0.17	0.11	0.14
D2 Exposed	Mean	0.17	0.10	0.26	1.97	1.59	0.17
	Std Error	0.02	0.03	0.04	0.73	0.11	0.03
D5 Control	Mean	0.11	n/a	0.11	1.38	0.93	0.10
	Std Error	0.04	n/a	0.04	1.49	0.12	0.05
D5 Exposed	Mean	0.23	0.06	0.29	2.41	1.06	0.22
	Std Error	0.08	0.04	0.12	1.27	0.09	0.08
D8 Control	Mean	0.26	0.02	0.29	0.00	1.29	0.16
	Std Error	0.16	0.07	0.23	8.51	0.22	0.11
D8 Exposed	Mean	1.15	0.36	1.51	3.37	1.95	0.73
	Std Error	0.24	0.07	0.30	0.38	0.15	0.10

20 Degrees

Upper Surface

		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	0.07	0.02	0.09	1.73	0.97	0.10
	Std Error	0.03	0.05	0.08	1.29	0.09	0.09
D2 Control	Mean	0.23	0.34	0.57	0.73	2.02	0.29
	Std Error	0.01	0.05	0.05	0.12	0.23	0.02
D2 Exposed	Mean	0.97	0.35	1.32	0.46	2.51	0.49
	Std Error	0.17	0.17	0.33	1.21	0.23	0.09
D5 Control	Mean	0.23	0.26	0.49	0.81	0.99	0.51
	Std Error	0.06	0.04	0.10	0.16	0.20	0.08
D5 Exposed	Mean	3.34	1.21	4.55	2.73	2.36	1.90
	Std Error	0.39	0.12	0.52	0.08	0.22	0.07
D8 Control	Mean	0.34	0.24	0.58	1.91	1.95	0.28
	Std Error	0.13	0.08	0.21	0.74	0.28	0.08
D8 Exposed	Mean	4.43	1.40	5.84	3.76	3.55	1.57
	Std Error	0.61	0.22	0.82	0.62	0.31	0.09

Lower Surface

		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	0.02	n/a	0.02	n/a	0.81	0.00
	Std Error	0.02	n/a	0.02	n/a	0.11	0.14
D2 Control	Mean	0.27	0.40	0.67	0.68	2.13	0.32
	Std Error	0.09	0.07	0.15	0.13	0.19	0.08
D2 Exposed	Mean	0.37	0.19	0.56	0.50	2.28	0.19
	Std Error	0.13	0.15	0.28	0.51	0.27	0.09
D5 Control	Mean	0.18	0.20	0.39	0.82	1.23	0.32
	Std Error	0.05	0.04	0.08	0.19	0.15	0.08
D5 Exposed	Mean	0.48	0.24	0.71	2.25	1.09	0.64
	Std Error	0.08	0.04	0.11	0.38	0.10	0.06
D8 Control	Mean	0.43	0.13	0.56	1.06	2.06	0.24
	Std Error	0.16	0.08	0.24	1.15	0.17	0.10
D8 Exposed	Mean	0.67	0.29	0.96	4.69	1.48	0.50
	Std Error	0.08	0.06	0.11	1.56	0.31	0.08

25 Degrees

Upper Surface

		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	0.07	0.02	0.09	1.73	0.97	0.10
	Std Error	0.03	0.05	0.08	1.29	0.09	0.09
D2 Control	Mean	0.17	0.33	0.50	0.27	1.90	0.31
	Std Error	0.06	0.18	0.24	0.37	0.34	0.16
D2 Exposed	Mean	0.65	0.12	0.76	0.32	1.84	0.43
	Std Error	0.05	0.03	0.04	3.61	0.11	0.03
D5 Control	Mean	0.03	0.04	0.07	0.00	0.76	0.02
	Std Error	0.02	0.06	0.08	0.58	0.18	0.19
D5 Exposed	Mean	1.30	0.53	1.83	2.92	1.50	1.22
	Std Error	0.19	0.11	0.27	0.33	0.15	0.17
D8 Control	Mean	0.03	n/a	0.03	n/a	1.70	n/a
	Std Error	0.03	n/a	0.03	n/a	0.09	n/a
D8 Exposed	Mean	3.74	1.80	5.54	2.11	4.45	1.25
	Std Error	0.38	0.16	0.50	0.13	0.27	0.07

Lower Surface

		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	0.02	n/a	0.02	n/a	0.81	0.00
	Std Error	0.02	n/a	0.02	n/a	0.11	0.14
D2 Control	Mean	0.27	0.57	0.84	0.49	1.78	0.52
	Std Error	0.06	0.06	0.09	0.12	0.24	0.10
D2 Exposed	Mean	0.16	0.12	0.28	1.23	1.66	0.16
	Std Error	0.04	0.03	0.07	0.65	0.12	0.04
D5 Control	Mean	n/a	n/a	n/a	n/a	0.49	n/a
	Std Error	n/a	n/a	n/a	n/a	0.12	n/a
D5 Exposed	Mean	0.16	0.10	0.26	0.88	1.00	0.26
	Std Error	0.04	0.04	0.07	0.55	0.09	0.07
D8 Control	Mean	0.21	0.08	0.29	1.26	1.63	0.16
	Std Error	0.16	0.05	0.21	0.66	0.13	0.11
D8 Exposed	Mean	0.54	0.62	1.16	1.06	2.27	0.56
	Std Error	0.07	0.09	0.16	0.18	0.20	0.09

n/a = Below limit of detection.

Glycoalkaloid results from storage at different temperatures. $\mu g\ g^{-1}$ sample.

5 Degrees

Upper Surface

		Solanine	Chaconine	TGA	S:C
D0 Control	Mean	41.15	72.21	113.36	0.62
	Std Error	5.43	14.78	18.63	0.11
D2 Control	Mean	14.68	36.35	51.04	0.40
	Std Error	3.19	7.37	10.17	0.07
D2 Exposed	Mean	14.76	37.32	52.09	0.38
	Std Error	4.57	6.66	10.74	0.08
D5 Control	Mean	15.32	42.35	57.67	0.38
	Std Error	7.31	9.11	15.54	0.09
D5 Exposed	Mean	19.26	58.79	78.06	0.36
	Std Error	2.39	9.19	11.48	0.03
D8 Control	Mean	17.86	54.64	72.49	0.30
	Std Error	6.30	6.83	12.66	0.07
D8 Exposed	Mean	10.79	36.06	46.85	0.31
	Std Error	2.36	5.34	6.84	0.05

Lower Surface

		Solanine	Chaconine	TGA	S:C
D0 Control	Mean	32.91	33.97	66.88	0.82
	Std Error	9.56	11.37	19.68	0.13
D2 Control	Mean	9.18	32.54	41.72	0.35
	Std Error	2.62	3.08	4.81	0.05
D2 Exposed	Mean	11.91	33.44	45.35	0.38
	Std Error	2.18	4.26	5.97	0.09
D5 Control	Mean	11.86	36.78	48.64	0.31
	Std Error	2.82	2.96	5.23	0.07
D5 Exposed	Mean	14.15	47.06	61.21	0.32
	Std Error	2.03	5.35	7.01	0.02
D8 Control	Mean	17.38	50.29	67.68	0.33
	Std Error	4.85	11.00	15.83	0.02
D8 Exposed	Mean	13.18	41.15	54.32	0.35
	Std Error	2.40	4.54	6.41	0.10

10 Degrees

Upper Surface

		Solanine	Chaconine	TGA	S:C
D0 Control	Mean	41.15	72.21	113.36	0.62
	Std Error	5.43	14.78	18.63	0.11
D2 Control	Mean	15.67	52.09	67.76	0.30
	Std Error	3.43	7.22	9.94	0.06
D2 Exposed	Mean	10.59	34.91	45.50	0.29
	Std Error	2.03	5.99	7.88	0.04
D5 Control	Mean	12.35	30.99	43.34	0.38
	Std Error	2.90	3.92	6.46	0.07
D5 Exposed	Mean	21.28	38.27	59.55	0.51
	Std Error	8.03	5.08	11.64	0.16
D8 Control	Mean	18.64	51.04	69.67	0.36
	Std Error	6.12	15.77	21.74	0.03
D8 Exposed	Mean	27.56	62.12	89.68	0.41
	Std Error	7.82	14.28	22.03	0.02

Lower Surface

		Solanine	Chaconine	TGA	S:C
D0 Control	Mean	32.91	33.97	66.88	0.82
	Std Error	9.56	11.37	19.68	0.13
D2 Control	Mean	15.67	44.33	60.00	0.41
	Std Error	4.16	11.99	15.90	0.11
D2 Exposed	Mean	13.96	31.96	45.91	0.44
	Std Error	1.94	5.63	6.98	0.05
D5 Control	Mean	15.32	42.99	58.31	0.36
	Std Error	3.48	9.29	12.47	0.05
D5 Exposed	Mean	15.74	38.19	53.93	0.43
	Std Error	4.04	9.63	13.42	0.07
D8 Control	Mean	24.35	47.79	72.14	0.53
	Std Error	6.59	17.81	23.72	0.19
D8 Exposed	Mean	14.82	39.88	54.71	0.43
	Std Error	3.37	8.14	11.15	0.12

20 Degrees

Upper Surface

		Solanine	Chaconine	TGA	S:C
D0 Control	Mean	41.15	72.21	113.36	0.62
	Std Error	5.43	14.78	18.63	0.11
D2 Control	Mean	4.94	24.21	29.15	0.23
	Std Error	1.97	8.00	9.91	0.01
D2 Exposed	Mean	17.78	38.34	56.12	0.41
	Std Error	5.29	9.67	14.74	0.06
D5 Control	Mean	20.96	42.85	63.81	0.49
	Std Error	7.20	14.06	21.25	0.01
D5 Exposed	Mean	25.47	62.41	87.88	0.39
	Std Error	6.99	5.95	11.38	0.10
D8 Control	Mean	20.89	48.71	69.60	0.44
	Std Error	4.58	9.31	13.24	0.05
D8 Exposed	Mean	13.06	35.24	48.29	0.32
	Std Error	2.84	5.71	8.26	0.05

Lower Surface

		Solanine	Chaconine	TGA	S:C
D0 Control	Mean	32.91	33.97	66.88	0.82
	Std Error	9.56	11.37	19.68	0.13
D2 Control	Mean	13.62	31.06	44.68	0.53
	Std Error	3.90	10.41	13.47	0.18
D2 Exposed	Mean	10.68	30.49	41.16	0.42
	Std Error	2.53	4.33	5.85	0.14
D5 Control	Mean	13.98	44.61	58.59	0.33
	Std Error	2.78	7.97	10.31	0.05
D5 Exposed	Mean	30.50	39.03	69.53	0.83
	Std Error	6.38	6.82	10.04	0.17
D8 Control	Mean	24.21	64.02	88.24	0.36
	Std Error	8.18	13.27	21.09	0.06
D8 Exposed	Mean	11.39	23.81	35.20	0.53
	Std Error	1.39	3.95	4.69	0.11

25 Degrees

Upper Surface

		Solanine	Chaconine	TGA	S:C
D0 Control	Mean	41.15	72.21	113.36	0.62
	Std Error	5.43	14.78	18.63	0.11
D2 Control	Mean	14.05	37.55	51.60	0.43
	Std Error	3.91	9.08	11.95	0.13
D2 Exposed	Mean	18.03	46.71	64.74	0.41
	Std Error	2.24	4.64	6.13	0.07
D5 Control	Mean	19.06	52.59	71.65	0.26
	Std Error	3.95	9.59	13.10	0.02
D5 Exposed	Mean	22.12	53.24	82.20	0.41
	Std Error	4.09	9.64	12.37	0.02
D8 Control	Mean	13.76	32.75	46.52	0.40
	Std Error	4.22	7.27	10.96	0.09
D8 Exposed	Mean	32.28	60.90	93.18	0.40
	Std Error	12.03	18.40	30.39	0.07

Lower Surface

		Solanine	Chaconine	TGA	S:C
D0 Control	Mean	32.91	33.97	66.88	0.82
	Std Error	9.56	11.37	19.68	0.13
D2 Control	Mean	11.44	29.58	41.01	0.41
	Std Error	4.09	6.48	9.14	0.13
D2 Exposed	Mean	15.21	34.38	49.59	0.74
	Std Error	1.79	4.18	4.79	0.34
D5 Control	Mean	14.61	41.44	56.05	0.26
	Std Error	2.40	6.17	8.53	0.01
D5 Exposed	Mean	29.47	67.74	97.21	0.42
	Std Error	4.38	9.04	13.32	0.02
D8 Control	Mean	16.59	29.22	45.81	0.41
	Std Error	3.16	7.92	7.88	0.07
D8 Exposed	Mean	18.50	39.41	57.91	0.46
	Std Error	3.22	5.60	8.27	0.06

APPENDIX 2

Pigment results from light exposure after varying storage duration. µmol g^{-1} sample

King Edward - Week 0

		Upper Surface						Lower Surface					
		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car	Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	n/a	n/a	n/a	n/a	0.60	n/a	0.05	0.04	0.09	0.23	1.22	0.05
	Std Error	n/a	n/a	n/a	n/a	0.20	n/a	0.05	0.04	0.09	0.52	0.26	0.05
D3 Control	Mean	0.02	n/a	0.02	n/a	0.40	0.04	0.02	n/a	0.02	n/a	0.36	0.03
	Std Error	0.02	n/a	0.02	n/a	0.03	0.04	0.02	n/a	0.02	n/a	0.08	0.03
D3 Exposed	Mean	2.12	0.37	2.49	4.74	1.52	1.59	0.43	0.05	0.48	1.34	0.58	0.82
	Std Error	0.32	0.10	0.42	1.21	0.21	0.08	0.06	0.03	0.07	0.56	0.06	0.10
D6Control	Mean	0.02	0.04	0.06	n/a	0.51	0.15	0.06	n/a	0.06	0.00	0.27	2.70
	Std Error	0.02	0.04	0.04	n/a	0.04	0.10	0.06	n/a	0.06	0.00	0.07	2.70
D6 Exposed	Mean	2.61	0.72	3.34	3.81	1.77	1.89	0.85	0.24	1.08	2.86	0.71	1.53
	Std Error	0.25	0.10	0.34	0.17	0.17	0.07	0.08	0.04	0.11	0.51	0.05	0.10
D10 Control	Mean	n/a	0.03	0.03	n/a	0.53	0.09	0.03	0.02	0.05	1.50	0.52	0.09
	Std Error	n/a	0.03	0.03	n/a	0.10	0.09	0.03	0.02	0.03	0.00	0.04	0.06
D10 Exposed	Mean	4.30	1.20	5.50	4.44	2.65	1.93	1.12	0.29	1.41	3.46	0.86	1.64
	Std Error	0.98	0.32	1.29	0.77	0.52	0.16	0.16	0.07	0.22	0.75	0.09	0.17

Brodick - Week 0

		Upper Surface						Lower Surface					
		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car	Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	0.03	n/a	0.03	n/a	0.37	0.04	0.06	n/a	0.06	n/a	0.44	0.11
	Std Error	0.03	n/a	0.03	n/a	0.07	0.04	0.03	n/a	0.03	n/a	0.06	0.07
D3 Control	Mean	0.02	0.02	0.04	0.27	0.29	0.21	n/a	n/a	n/a	n/a	0.27	n/a
	Std Error	0.02	0.02	0.04	0.27	0.05	0.21	n/a	n/a	n/a	n/a	0.04	n/a
D3 Exposed	Mean	2.84	0.49	3.32	19.78	1.95	1.71	0.26	0.04	0.30	0.15	0.64	0.54
	Std Error	0.23	0.09	0.28	13.99	0.14	0.12	0.04	0.03	0.04	0.11	0.11	0.08
D6Control	Mean	0.07	0.03	0.10	0.22	0.60	0.20	0.06	n/a	0.06	n/a	0.62	0.12
	Std Error	0.03	0.03	0.06	0.22	0.09	0.14	0.04	n/a	0.04	n/a	0.05	0.09
D6 Exposed	Mean	7.35	1.88	9.22	3.90	3.56	2.58	0.70	0.24	0.95	1.75	0.61	1.62
	Std Error	0.69	0.15	0.82	0.19	0.29	0.07	0.07	0.06	0.10	0.70	0.04	0.19
D10 Control	Mean	0.09	0.10	0.19	0.76	1.48	0.15	n/a	0.04	0.04	n/a	1.53	0.03
	Std Error	0.05	0.05	0.08	0.52	0.32	0.07	n/a	0.03	0.03	n/a	0.35	0.02
D10 Exposed	Mean	9.73	2.61	12.34	3.73	4.91	2.59	1.47	0.52	1.99	2.96	1.29	1.60
	Std Error	0.51	0.09	0.58	0.14	0.36	0.12	0.22	0.07	0.29	0.29	0.21	0.10

Record - Week 0

		Upper Surface						Lower Surface					
		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car	Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	0.02	n/a	0.02	n/a	0.88	0.03	0.02	n/a	0.02	n/a	0.85	0.02
	Std Error	0.02	n/a	0.02	n/a	0.09	0.03	0.02	n/a	0.02	n/a	0.06	0.02
D3 Control	Mean	n/a	n/a	n/a	n/a	0.67	n/a	0.04	n/a	0.04	n/a	0.76	0.05
	Std Error	n/a	n/a	n/a	n/a	0.07	n/a	0.02	n/a	0.02	n/a	0.06	0.03
D3 Exposed	Mean	4.48	0.89	5.37	5.06	4.03	1.34	0.84	0.23	1.06	3.07	1.88	0.60
	Std Error	0.26	0.05	0.30	0.20	0.19	0.06	0.07	0.05	0.06	0.58	0.13	0.06
D6Control	Mean	n/a	n/a	n/a	n/a	1.11	n/a	0.08	n/a	0.08	n/a	1.22	0.05
	Std Error	n/a	n/a	n/a	n/a	0.06	n/a	0.05	n/a	0.05	n/a	0.17	0.04
D6 Exposed	Mean	5.85	1.30	7.15	5.70	4.21	1.71	0.86	0.22	1.08	1.94	1.17	0.94
	Std Error	0.50	0.14	0.62	1.42	0.35	0.08	0.11	0.07	0.14	0.68	0.07	0.11
D10 Control	Mean	0.05	n/a	0.05	n/a	0.78	0.06	0.03	n/a	0.03	n/a	0.82	0.03
	Std Error	0.05	n/a	0.05	n/a	0.04	0.06	0.03	n/a	0.03	n/a	0.06	0.03
D10 Exposed	Mean	10.11	2.27	12.38	4.44	7.68	1.61	1.50	0.48	1.98	3.24	2.53	0.79
	Std Error	0.80	0.16	0.96	0.06	0.53	0.05	0.08	0.04	0.13	0.14	0.15	0.04

P. Dell - Week 0

		Upper Surface						Lower Surface					
		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car	Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	n/a	n/a	n/a	n/a	0.41	n/a	n/a	n/a	n/a	n/a	0.33	n/a
	Std Error	n/a	n/a	n/a	n/a	0.02	n/a	n/a	n/a	n/a	n/a	0.05	n/a
D3 Control	Mean	0.02	n/a	0.02	n/a	0.39	0.04	0.02	n/a	0.02	n/a	0.35	0.03
	Std Error	0.02	n/a	0.02	n/a	0.05	0.04	0.02	n/a	0.02	n/a	0.08	0.03
D3 Exposed	Mean	1.31	0.25	1.55	4.18	1.11	1.31	0.40	0.04	0.44	0.24	0.59	0.65
	Std Error	0.26	0.05	0.31	0.86	0.17	0.15	0.11	0.03	0.11	0.17	0.08	0.14
D6Control	Mean	0.06	0.34	0.41	0.11	0.25	0.00	n/a	0.07	0.07	n/a	0.44	0.14
	Std Error	0.04	0.28	0.27	0.11	0.07	0.00	n/a	0.05	0.05	n/a	0.13	0.07
D6 Exposed	Mean	2.57	0.55	3.12	5.33	1.58	1.96	0.28	0.10	0.38	1.29	0.49	0.96
	Std Error	0.25	0.08	0.30	0.54	0.13	0.08	0.05	0.03	0.07	0.41	0.05	0.30
D10 Control	Mean	n/a	n/a	n/a	n/a	0.26	n/a	n/a	n/a	n/a	n/a	0.24	n/a
	Std Error	n/a	n/a	n/a	n/a	0.05	n/a	n/a	n/a	n/a	n/a	0.03	n/a
D10 Exposed	Mean	2.90	0.54	3.43	5.09	1.68	2.04	0.22	0.03	0.25	0.77	0.55	0.45
	Std Error	0.21	0.08	0.28	0.68	0.09	0.10	0.04	0.02	0.05	0.43	0.04	0.11

n/a = below limit of detection.

King Edward - Week 10

		Upper Surface						Lower Surface					
		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car	Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	0.09	n/a	0.09	n/a	0.54	0.16	n/a	0.14	0.14	n/a	0.43	0.22
	Std Error	0.04	n/a	0.04	n/a	0.19	0.08	n/a	0.08	0.08	n/a	0.09	0.14
D3 Control	Mean	0.06	n/a	0.06	n/a	0.88	0.09	n/a	n/a	n/a	n/a	0.97	n/a
	Std Error	0.04	n/a	0.04	n/a	0.15	0.06	n/a	n/a	n/a	n/a	0.22	n/a
D3 Exposed	Mean	0.95	0.14	1.09	2.17	1.81	0.57	0.37	0.02	0.39	0.25	0.97	0.41
	Std Error	0.15	0.06	0.20	0.90	0.20	0.08	0.07	0.02	0.08	0.25	0.14	0.08
D6Control	Mean	0.11	0.02	0.13	0.25	0.76	0.16	0.05	n/a	0.05	n/a	0.69	0.07
	Std Error	0.06	0.02	0.07	0.25	0.04	0.08	0.03	n/a	0.03	n/a	0.08	0.04
D6 Exposed	Mean	2.74	0.61	3.34	4.04	2.19	1.42	0.77	0.23	1.01	2.45	0.83	1.23
	Std Error	0.42	0.15	0.56	0.73	0.24	0.12	0.08	0.03	0.10	0.40	0.06	0.11
D10 Control	Mean	0.02	n/a	0.02	n/a	0.62	0.02	n/a	n/a	n/a	n/a	0.58	n/a
	Std Error	0.02	n/a	0.02	n/a	0.02	0.02	n/a	n/a	n/a	n/a	0.02	n/a
D10 Exposed	Mean	4.86	1.81	6.67	2.66	3.17	2.09	1.32	0.71	2.03	2.05	1.03	1.95
	Std Error	0.59	0.20	0.78	0.14	0.35	0.06	0.16	0.08	0.23	0.27	0.09	0.15

Brodick - Week 10

		Upper Surface						Lower Surface					
		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car	Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	0.02	0.08	0.09	0.07	0.54	0.16	n/a	0.02	0.02	n/a	0.49	0.04
	Std Error	0.02	0.04	0.06	0.07	0.04	0.10	n/a	0.02	0.02	n/a	0.04	0.04
D3 Control	Mean	0.03	n/a	0.03	n/a	0.39	0.04	0.03	n/a	0.03	n/a	0.67	0.03
	Std Error	0.03	n/a	0.03	n/a	0.07	0.04	0.03	n/a	0.03	n/a	0.14	0.03
D3 Exposed	Mean	2.10	0.50	2.60	4.45	1.41	1.89	0.38	0.04	0.42	0.76	0.60	0.66
	Std Error	0.15	0.04	0.18	0.42	0.12	0.08	0.08	0.02	0.08	0.37	0.06	0.09
D6Control	Mean	0.04	n/a	0.04	n/a	0.63	0.07	0.08	0.02	0.10	0.13	1.01	0.07
	Std Error	0.02	n/a	0.02	n/a	0.17	0.05	0.06	0.02	0.06	0.13	0.36	0.03
D6 Exposed	Mean	6.93	1.75	8.68	4.93	3.39	2.61	0.83	0.16	0.99	2.78	0.87	1.18
	Std Error	0.73	0.20	0.91	1.21	0.39	0.11	0.13	0.05	0.16	0.64	0.14	0.13
D10 Control	Mean	0.08	0.02	0.10	0.00	0.83	0.12	n/a	0.07	0.07	n/a	0.68	0.15
	Std Error	0.05	0.02	0.05	0.00	0.24	0.05	n/a	0.05	0.05	n/a	0.15	0.09
D10 Exposed	Mean	11.48	3.41	14.89	3.36	5.89	2.52	0.81	0.23	1.04	3.93	0.97	1.09
	Std Error	1.06	0.28	1.32	0.10	0.50	0.08	0.06	0.03	0.08	0.40	0.08	0.06

Record - Week 10

		Upper Surface						Lower Surface					
		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car	Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	0.09	n/a	0.09	n/a	1.32	0.09	0.13	0.08	0.21	0.33	1.56	0.11
	Std Error	0.06	n/a	0.06	n/a	0.11	0.06	0.10	0.06	0.15	0.33	0.22	0.07
D3 Control	Mean	n/a	n/a	n/a	n/a	1.95	n/a	n/a	n/a	n/a	n/a	2.05	n/a
	Std Error	n/a	n/a	n/a	n/a	0.21	n/a	n/a	n/a	n/a	n/a	0.06	n/a
D3 Exposed	Mean	2.16	0.21	2.37	6.46	3.84	0.61	0.42	0.01	0.44	0.67	2.11	0.22
	Std Error	0.19	0.06	0.23	1.81	0.21	0.04	0.04	0.02	0.05	0.46	0.13	0.03
D6Control	Mean	n/a	n/a	n/a	n/a	1.27	n/a	n/a	n/a	n/a	n/a	0.98	n/a
	Std Error	n/a	n/a	n/a	n/a	0.12	n/a	n/a	n/a	n/a	n/a	0.11	n/a
D6 Exposed	Mean	5.09	1.11	6.21	4.63	3.37	1.83	0.63	0.08	0.71	2.64	1.09	0.64
	Std Error	0.42	0.10	0.51	0.14	0.21	0.07	0.06	0.02	0.08	0.81	0.07	0.06
D10 Control	Mean	0.03	0.03	0.07	0.10	1.32	0.04	n/a	n/a	n/a	n/a	1.36	n/a
	Std Error	0.02	0.03	0.05	0.10	0.18	0.03	n/a	n/a	n/a	n/a	0.13	n/a
D10 Exposed	Mean	4.86	1.81	6.67	2.66	3.17	2.09	1.32	0.71	2.03	2.05	1.03	1.95
	Std Error	0.59	0.20	0.78	0.14	0.35	0.06	0.16	0.08	0.23	0.27	0.09	0.15

P. Dell - Week 10

		Upper Surface						Lower Surface					
		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car	Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	n/a	n/a	n/a	n/a	0.35	n/a	n/a	n/a	n/a	n/a	0.31	n/a
	Std Error	n/a	n/a	n/a	n/a	0.02	n/a	n/a	n/a	n/a	n/a	0.07	n/a
D3 Control	Mean	0.06	0.10	0.16	0.63	0.38	0.46	n/a	n/a	n/a	n/a	0.33	n/a
	Std Error	0.04	0.05	0.07	0.41	0.05	0.22	n/a	n/a	n/a	n/a	0.03	n/a
D3 Exposed	Mean	0.82	0.21	1.03	3.72	0.73	1.32	0.21	0.03	0.23	0.85	0.39	0.54
	Std Error	0.15	0.08	0.21	1.07	0.08	0.14	0.04	0.02	0.05	0.53	0.03	0.09
D6Control	Mean	0.02	n/a	0.02	n/a	0.20	0.07	n/a	n/a	n/a	n/a	0.27	n/a
	Std Error	0.02	n/a	0.02	n/a	0.05	0.07	n/a	n/a	n/a	n/a	0.04	n/a
D6 Exposed	Mean	2.60	0.49	3.09	5.56	1.63	1.86	0.50	0.05	0.54	0.56	0.65	0.82
	Std Error	0.33	0.08	0.40	0.89	0.17	0.14	0.09	0.03	0.12	0.38	0.07	0.14
D10 Control	Mean	n/a	n/a	n/a	n/a	0.35	n/a	n/a	n/a	n/a	n/a	0.38	n/a
	Std Error	n/a	n/a	n/a	n/a	0.07	n/a	n/a	n/a	n/a	n/a	0.03	n/a
D10 Exposed	Mean	4.08	1.06	5.14	3.88	2.18	2.32	0.38	0.09	0.47	1.86	0.62	0.77
	Std Error	0.45	0.12	0.57	0.16	0.19	0.09	0.04	0.03	0.06	0.58	0.03	0.08

King Edward - Week 20

Upper Surface

		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	0.03	n/a	0.03	n/a	0.73	0.04
	Std Error	0.03	n/a	0.03	n/a	0.06	0.04
D3 Control	Mean	n/a	n/a	n/a	n/a	0.86	n/a
	Std Error	n/a	n/a	n/a	n/a	0.04	n/a
D3 Exposed	Mean	0.66	0.11	0.78	2.11	1.62	0.31
	Std Error	0.31	0.06	0.37	0.98	0.24	0.11
D6Control	Mean	n/a	n/a	n/a	n/a	0.71	n/a
	Std Error	n/a	n/a	n/a	n/a	0.07	n/a
D6 Exposed	Mean	0.79	0.17	0.95	3.05	1.18	0.64
	Std Error	0.25	0.06	0.30	0.93	0.13	0.18
D10 Control	Mean	0.04	0.06	0.10	0.28	0.88	0.11
	Std Error	0.02	0.04	0.07	0.18	0.11	0.07
D10 Exposed	Mean	3.01	0.63	3.65	5.35	2.57	1.21
	Std Error	0.73	0.19	0.91	1.81	0.40	0.15

Lower Surface

		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	0.03	n/a	0.03	0.62	0.88	0.03
	Std Error	0.03	n/a	0.03	0.48	0.06	0.03
D3 Control	Mean	n/a	n/a	n/a	n/a	0.91	n/a
	Std Error	n/a	n/a	n/a	n/a	0.06	n/a
D3 Exposed	Mean	0.23	0.03	0.26	0.69	1.03	0.23
	Std Error	0.08	0.02	0.10	0.48	0.13	0.06
D6Control	Mean	n/a	n/a	n/a	n/a	0.74	n/a
	Std Error	n/a	n/a	n/a	n/a	0.13	n/a
D6 Exposed	Mean	0.46	0.12	0.58	1.23	0.95	0.46
	Std Error	0.26	0.06	0.30	0.56	0.22	0.12
D10 Control	Mean	0.05	0.04	0.09	0.13	0.76	0.12
	Std Error	0.03	0.03	0.04	0.13	0.04	0.06
D10 Exposed	Mean	0.59	0.21	0.80	1.79	1.11	0.62
	Std Error	0.13	0.06	0.19	0.42	0.10	0.12

Brodick - Week 20

Upper Surface

		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	n/a	n/a	n/a	n/a	0.93	n/a
	Std Error	n/a	n/a	n/a	n/a	0.05	n/a
D3 Control	Mean	0.05	n/a	0.05	n/a	1.05	0.05
	Std Error	0.04	n/a	0.04	n/a	0.14	0.03
D3 Exposed	Mean	1.67	0.21	1.88	5.67	2.54	0.76
	Std Error	0.10	0.05	0.14	1.29	0.15	0.06
D6Control	Mean	n/a	n/a	n/a	n/a	0.83	n/a
	Std Error	n/a	n/a	n/a	n/a	0.11	n/a
D6 Exposed	Mean	4.93	1.13	6.06	4.38	3.45	1.81
	Std Error	0.43	0.09	0.50	0.21	0.34	0.10
D10 Control	Mean	0.00	0.03	0.03	n/a	0.73	0.04
	Std Error	0.00	0.03	0.03	n/a	0.11	0.04
D10 Exposed	Mean	10.54	2.41	12.95	4.37	6.10	2.14
	Std Error	0.40	0.07	0.46	0.12	0.31	0.05

Lower Surface

		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	n/a	n/a	n/a	n/a	0.90	n/a
	Std Error	n/a	n/a	n/a	n/a	0.19	n/a
D3 Control	Mean	0.02	0.06	0.08	0.22	1.03	0.09
	Std Error	0.02	0.04	0.05	0.22	0.08	0.06
D3 Exposed	Mean	0.38	0.06	0.43	1.14	1.51	0.31
	Std Error	0.03	0.02	0.04	0.46	0.12	0.04
D6Control	Mean	n/a	n/a	n/a	n/a	0.75	n/a
	Std Error	n/a	n/a	n/a	n/a	0.13	n/a
D6 Exposed	Mean	0.56	0.14	0.70	2.75	0.95	0.75
	Std Error	0.05	0.03	0.07	0.66	0.09	0.06
D10 Control	Mean	0.04	0.05	0.09	0.80	0.67	0.13
	Std Error	0.03	0.04	0.04	0.00	0.09	0.05
D10 Exposed	Mean	1.13	0.14	1.27	2.78	1.39	1.02
	Std Error	0.36	0.03	0.35	0.70	0.13	0.33

Record - Week 20

Upper Surface

		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	0.02	n/a	0.02	n/a	1.62	0.01
	Std Error	0.02	n/a	0.02	n/a	0.10	0.01
D3 Control	Mean	0.03	0.02	0.05	0.31	1.32	0.04
	Std Error	0.03	0.02	0.05	0.31	0.12	0.04
D3 Exposed	Mean	1.02	0.01	1.03	0.68	1.94	0.53
	Std Error	0.12	0.01	0.12	0.68	0.14	0.05
D6Control	Mean	0.05	n/a	0.05	n/a	1.69	0.03
	Std Error	0.03	n/a	0.03	n/a	0.08	0.02
D6 Exposed	Mean	4.71	1.05	5.76	4.55	3.92	1.51
	Std Error	0.30	0.07	0.36	0.15	0.26	0.10
D10 Control	Mean	0.03	0.04	0.07	0.14	1.69	0.03
	Std Error	0.03	0.04	0.07	0.14	0.15	0.03
D10 Exposed	Mean	8.09	1.99	10.08	4.17	7.20	1.41
	Std Error	0.50	0.16	0.62	0.19	0.43	0.06

Lower Surface

		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	0.02	n/a	0.02	n/a	1.85	0.01
	Std Error	0.02	n/a	0.02	n/a	0.15	0.01
D3 Control	Mean	0.02	n/a	0.02	n/a	1.31	0.01
	Std Error	0.02	n/a	0.02	n/a	0.21	0.01
D3 Exposed	Mean	0.02	n/a	0.02	n/a	0.99	0.02
	Std Error	0.02	n/a	0.02	n/a	0.08	0.02
D6Control	Mean	n/a	n/a	n/a	n/a	1.84	n/a
	Std Error	n/a	n/a	n/a	n/a	0.15	n/a
D6 Exposed	Mean	0.44	0.02	0.47	0.53	1.25	0.37
	Std Error	0.08	0.02	0.09	0.37	0.10	0.07
D10 Control	Mean	0.02	0.02	0.04	1.00	1.17	0.02
	Std Error	0.02	0.02	0.02	0.00	0.12	0.02
D10 Exposed	Mean	0.60	0.24	0.83	3.59	2.16	0.40
	Std Error	0.06	0.05	0.10	0.91	0.16	0.06

P. Dell - Week 20

Upper Surface

		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	n/a	n/a	n/a	n/a	0.61	n/a
	Std Error	n/a	n/a	n/a	n/a	0.05	n/a
D3 Control	Mean	n/a	n/a	n/a	n/a	0.68	n/a
	Std Error	n/a	n/a	n/a	n/a	0.13	n/a
D3 Exposed	Mean	0.59	0.07	0.66	2.26	1.02	0.60
	Std Error	0.12	0.02	0.14	0.91	0.08	0.09
D6Control	Mean	0.02	0.06	0.08	0.00	0.29	0.25
	Std Error	0.02	0.04	0.03	0.00	0.03	0.11
D6 Exposed	Mean	1.30	0.17	1.47	4.80	1.11	1.32
	Std Error	0.12	0.03	0.14	0.89	0.09	0.07
D10 Control	Mean	n/a	n/a	n/a	n/a	n/a	n/a
	Std Error	n/a	n/a	n/a	n/a	n/a	n/a
D10 Exposed	Mean	3.66	0.61	4.27	6.31	2.25	1.87
	Std Error	0.31	0.07	0.38	0.35	0.13	0.08

Upper Surface

		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	n/a	n/a	n/a	n/a	0.51	n/a
	Std Error	n/a	n/a	n/a	n/a	0.06	n/a
D3 Control	Mean	0.02	n/a	0.02	n/a	0.51	0.05
	Std Error	0.02	n/a	0.02	n/a	0.03	0.05
D3 Exposed	Mean	0.08	0.06	0.14	0.32	0.50	0.35
	Std Error	0.03	0.05	0.06	0.32	0.05	0.18
D6Control	Mean	0.03	0.03	0.06	0.00	0.43	0.12
	Std Error	0.03	0.03	0.04	0.00	0.06	0.08
D6 Exposed	Mean	0.16	0.02	0.17	0.44	0.53	0.34
	Std Error	0.03	0.01	0.04	0.32	0.04	0.08
D10 Control	Mean	n/a	n/a	n/a	n/a	0.41	n/a
	Std Error	n/a	n/a	n/a	n/a	0.04	n/a
D10 Exposed	Mean	0.26	0.04	0.30	0.26	0.60	0.51
	Std Error	0.02	0.03	0.04	0.19	0.04	0.07

King Edward - Week 30

Upper Surface

		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	n/a	n/a	n/a	n/a	0.51	n/a
	Std Error	n/a	n/a	n/a	n/a	0.02	n/a
D3 Control	Mean	0.02	n/a	0.02	n/a	0.51	0.04
	Std Error	0.02	n/a	0.02	n/a	0.06	0.04
D3 Exposed	Mean	0.68	0.17	0.85	3.57	1.10	0.68
	Std Error	0.19	0.05	0.23	1.29	0.11	0.10
D6Control	Mean	n/a	n/a	n/a	n/a	1.12	n/a
	Std Error	n/a	n/a	n/a	n/a	0.11	n/a
D6 Exposed	Mean	2.22	0.54	2.76	5.51	1.75	1.34
	Std Error	0.51	0.15	0.65	2.28	0.25	0.19
D10 Control	Mean	0.04	0.02	0.06	0.18	0.72	0.08
	Std Error	0.02	0.02	0.04	0.18	0.03	0.05
D10 Exposed	Mean	3.74	1.07	4.82	3.63	2.95	1.40
	Std Error	0.86	0.25	1.11	0.71	0.44	0.17

Lower Surface

		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	n/a	n/a	n/a	n/a	0.48	n/a
	Std Error	n/a	n/a	n/a	n/a	0.02	n/a
D3 Control	Mean	0.13	n/a	0.13	n/a	0.53	0.26
	Std Error	0.07	n/a	0.07	n/a	0.03	0.15
D3 Exposed	Mean	0.26	0.09	0.35	0.53	0.82	0.35
	Std Error	0.11	0.04	0.12	0.21	0.06	0.11
D6Control	Mean	0.04	0.02	0.06	0.15	1.20	0.05
	Std Error	0.02	0.02	0.04	0.15	0.05	0.03
D6 Exposed	Mean	0.99	0.11	1.10	1.22	0.84	1.08
	Std Error	0.48	0.04	0.47	0.39	0.08	0.33
D10 Control	Mean	0.05	0.06	0.11	0.31	0.69	0.17
	Std Error	0.03	0.06	0.09	0.21	0.03	0.15
D10 Exposed	Mean	0.51	0.14	0.65	3.30	0.99	0.63
	Std Error	0.10	0.03	0.13	0.93	0.07	0.13

Brodick - Week 30

Upper Surface

		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	0.17	n/a	0.17	n/a	0.78	0.21
	Std Error	0.05	n/a	0.05	n/a	0.07	0.04
D3 Control	Mean	n/a	n/a	n/a	n/a	0.80	n/a
	Std Error	n/a	n/a	n/a	n/a	0.16	n/a
D3 Exposed	Mean	1.31	0.19	1.50	6.31	2.02	0.76
	Std Error	0.07	0.02	0.08	0.83	0.12	0.04
D6Control	Mean	0.02	n/a	0.02	n/a	0.79	0.03
	Std Error	0.02	n/a	0.02	n/a	0.14	0.03
D6 Exposed	Mean	5.70	1.48	7.18	3.89	4.12	1.74
	Std Error	0.25	0.08	0.32	0.10	0.16	0.03
D10 Control	Mean	0.02	n/a	0.02	n/a	0.99	0.02
	Std Error	0.02	n/a	0.02	n/a	0.07	0.02
D10 Exposed	Mean	9.15	2.38	11.53	3.84	5.41	2.14
	Std Error	0.37	0.06	0.42	0.09	0.23	0.06

Lower Surface

		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	0.05	n/a	0.05	n/a	0.81	0.04
	Std Error	0.03	n/a	0.03	n/a	0.17	0.03
D3 Control	Mean	0.03	n/a	0.03	n/a	0.79	0.02
	Std Error	0.03	n/a	0.03	n/a	0.18	0.02
D3 Exposed	Mean	0.21	n/a	0.21	n/a	1.21	0.18
	Std Error	0.03	n/a	0.03	n/a	0.09	0.02
D6Control	Mean	n/a	n/a	n/a	n/a	0.96	n/a
	Std Error	n/a	n/a	n/a	n/a	0.10	n/a
D6 Exposed	Mean	0.55	0.16	0.71	2.38	1.48	0.48
	Std Error	0.04	0.04	0.08	0.71	0.08	0.04
D10 Control	Mean	0.06	0.04	0.05	3.31	1.14	0.04
	Std Error	0.04	0.02	0.05	1.45	0.11	0.04
D10 Exposed	Mean	0.59	0.20	0.79	2.77	1.34	0.61
	Std Error	0.06	0.03	0.08	0.37	0.11	0.06

Record - Week 30

Upper Surface

		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	0.03	0.04	0.06	0.14	1.42	0.03
	Std Error	0.03	0.04	0.06	0.14	0.26	0.03
D3 Control	Mean	0.07	0.04	0.11	0.14	1.67	0.06
	Std Error	0.03	0.04	0.06	0.14	0.09	0.03
D3 Exposed	Mean	1.22	0.17	1.39	5.95	1.72	0.76
	Std Error	0.16	0.04	0.20	1.32	0.12	0.10
D6Control	Mean	0.13	0.08	0.21	0.92	2.13	0.10
	Std Error	0.04	0.04	0.06	0.49	0.08	0.03
D6 Exposed	Mean	4.48	1.20	5.67	3.78	3.22	1.72
	Std Error	0.55	0.14	0.67	0.17	0.28	0.09
D10 Control	Mean	0.04	n/a	0.04	n/a	1.36	0.04
	Std Error	0.04	n/a	0.04	n/a	0.09	0.04
D10 Exposed	Mean	8.50	2.21	10.71	3.80	5.63	1.92
	Std Error	0.72	0.14	0.86	0.11	0.45	0.06

Lower Surface

		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	0.10	n/a	0.10	n/a	1.28	0.06
	Std Error	0.10	n/a	0.10	n/a	0.17	0.06
D3 Control	Mean	n/a	n/a	n/a	n/a	1.60	0.00
	Std Error	n/a	n/a	n/a	n/a	0.03	0.00
D3 Exposed	Mean	0.20	n/a	0.20	n/a	1.16	0.17
	Std Error	0.04	n/a	0.04	n/a	0.08	0.03
D6Control	Mean	0.08	n/a	0.08	n/a	2.18	0.04
	Std Error	0.02	n/a	0.02	n/a	0.20	0.01
D6 Exposed	Mean	0.40	0.04	0.44	0.75	1.34	0.34
	Std Error	0.03	0.03	0.05	0.41	0.09	0.04
D10 Control	Mean	n/a	0.02	0.02	n/a	1.27	0.02
	Std Error	n/a	0.02	0.02	n/a	0.10	0.02
D10 Exposed	Mean	0.86	0.32	1.18	3.11	1.74	0.70
	Std Error	0.09	0.04	0.13	0.44	0.17	0.07

P. Dell - Week 30

Upper Surface

		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	0.02	0.04	0.05	0.09	0.48	0.12
	Std Error	0.02	0.04	0.05	0.09	0.09	0.12
D3 Control	Mean	0.08	0.00	0.08	2.42	0.61	0.12
	Std Error	0.04	0.00	0.04	2.26	0.06	0.06
D3 Exposed	Mean	0.73	0.21	0.94	1.89	1.16	0.78
	Std Error	0.09	0.06	0.14	0.50	0.07	0.08
D6Control	Mean	n/a	0.02	0.02	n/a	0.48	0.04
	Std Error	n/a	0.02	0.02	n/a	0.05	0.04
D6 Exposed	Mean	2.31	0.67	2.98	3.43	1.66	1.74
	Std Error	0.29	0.07	0.35	0.22	0.14	0.16
D10 Control	Mean	n/a	0.04	0.04	n/a	0.39	0.05
	Std Error	n/a	0.04	0.04	n/a	0.08	0.05
D10 Exposed	Mean	4.84	1.17	6.01	4.26	2.68	2.19
	Std Error	0.51	0.13	0.63	0.18	0.22	0.12

Lower Surface

		Chl a	Chl b	T. Chl	Chl a:b	T. Car	Chl:Car
D0 Control	Mean	n/a	0.03	0.03	n/a	0.51	0.06
	Std Error	n/a	0.03	0.03	n/a	0.11	0.06
D3 Control	Mean	0.09	0.09	0.18	0.51	0.75	0.24
	Std Error	0.03	0.04	0.06	0.22	0.07	0.08
D3 Exposed	Mean	0.13	0.10	0.23	0.51	0.71	0.32
	Std Error	0.01	0.03	0.03	0.16	0.02	0.04
D6Control	Mean	n/a	n/a	n/a	n/a	0.55	n/a
	Std Error	n/a	n/a	n/a	n/a	0.09	n/a
D6 Exposed	Mean	0.12	0.05	0.16	0.29	0.64	0.26
	Std Error	0.03	0.03	0.05	0.15	0.06	0.07
D10 Control	Mean	n/a	n/a	n/a	n/a	0.44	n/a
	Std Error	n/a	n/a	n/a	n/a	0.05	n/a
D10 Exposed	Mean	0.26	0.03	0.29	0.53	0.67	0.47
	Std Error	0.07	0.02	0.07	0.28	0.06	0.12

Glycoalkaloid results from light exposure after varying storage duration. $\mu g\ g^{-1}$ sample

King Edward - Week 0

		Upper Surface				Lower Surface			
		Solanine	Chaconine	TGA	s:c	Solanine	Chaconine	TGA	s:c
D0 Control	Mean	27.73	76.64	104.37	0.39	37.89	52.86	90.76	1.00
	Std Error	6.21	17.71	20.76	0.07	7.35	13.82	13.81	0.36
D3 Control	Mean	19.74	42.48	62.21	1.00	24.39	53.84	78.23	1.03
	Std Error	4.72	20.90	24.78	0.39	6.25	20.76	24.24	0.45
D3 Exposed	Mean	21.37	71.08	92.45	0.39	19.19	48.93	68.11	0.62
	Std Error	2.63	12.52	13.18	0.06	2.08	8.88	10.77	0.16
D6Control	Mean	16.34	57.42	73.76	0.68	27.48	78.27	105.75	0.46
	Std Error	2.09	15.32	17.22	0.40	14.34	51.47	65.80	0.05
D6 Exposed	Mean	50.89	122.09	172.99	0.55	19.10	47.99	67.10	0.88
	Std Error	12.08	23.59	34.89	0.16	5.60	14.27	18.18	0.25
D10 Control	Mean	21.36	65.89	87.25	0.39	16.89	46.22	63.11	0.46
	Std Error	5.58	20.31	25.57	0.07	2.23	12.87	15.03	0.10
D10 Exposed	Mean	40.84	83.83	124.67	1.06	15.64	49.43	65.07	0.33
	Std Error	16.62	23.76	39.88	0.52	1.35	4.21	4.98	0.03

Brodick - Week 0

		Upper Surface				Lower Surface			
		Solanine	Chaconine	TGA	s:c	Solanine	Chaconine	TGA	s:c
D0 Control	Mean	31.26	50.34	81.60	0.65	65.21	83.51	148.72	0.77
	Std Error	6.03	11.69	17.60	0.05	14.19	9.06	20.91	0.14
D3 Control	Mean	47.97	89.07	137.04	0.88	62.02	127.65	189.67	0.46
	Std Error	13.73	22.88	34.63	0.36	21.17	33.62	54.51	0.03
D3 Exposed	Mean	157.44	229.32	386.76	0.65	62.64	100.04	162.69	1.79
	Std Error	30.92	36.27	63.55	0.07	16.19	25.19	40.15	0.93
D6Control	Mean	51.06	95.33	146.39	0.55	48.15	85.58	133.73	0.49
	Std Error	4.88	6.51	8.08	0.06	18.29	17.86	36.03	0.09
D6 Exposed	Mean	349.05	445.47	794.52	0.74	76.55	132.82	209.37	0.52
	Std Error	76.99	71.65	148.22	0.03	23.44	27.84	50.35	0.06
D10 Control	Mean	99.56	162.90	262.47	0.58	51.22	104.32	155.54	0.44
	Std Error	22.35	26.77	49.03	0.04	16.33	22.83	38.66	0.06
D10 Exposed	Mean	473.75	653.74	1127.49	0.71	75.73	154.87	230.60	0.49
	Std Error	69.09	81.22	144.94	0.06	11.56	19.07	29.13	0.06

Record - Week 0

		Upper Surface				Lower Surface			
		Solanine	Chaconine	TGA	s:c	Solanine	Chaconine	TGA	s:c
D0 Control	Mean	47.15	79.48	126.64	0.59	29.26	49.16	78.42	0.59
	Std Error	12.14	21.51	33.37	0.05	5.89	8.45	14.17	0.03
D3 Control	Mean	35.42	91.67	127.09	0.39	29.75	57.07	86.82	0.52
	Std Error								
D3 Exposed	Mean	51.53	100.49	147.73	0.45	43.84	73.37	106.25	0.49
	Std Error	2.71	6.58	8.02	0.01	1.13	3.30	5.17	0.04
D6Control	Mean	47.77	96.45	144.23	0.48	38.71	93.19	131.90	0.40
	Std Error	21.72	39.68	61.03	0.07	16.03	31.91	47.65	0.03
D6 Exposed	Mean	67.53	122.50	190.03	0.52	20.35	38.60	58.95	0.53
	Std Error	15.09	23.73	38.73	0.03	4.57	6.87	10.98	0.06
D10 Control	Mean	42.70	48.12	90.82	1.13	47.53	66.60	114.12	0.72
	Std Error	1.67	12.90	11.56	0.45	22.70	33.09	55.79	0.02
D10 Exposed	Mean	222.31	268.42	490.72	0.68	44.52	87.44	131.96	0.49
	Std Error	99.33	80.91	180.10	0.10	8.52	12.11	20.42	0.04

P. Dell - Week 0

		Upper Surface				Lower Surface			
		Solanine	Chaconine	TGA	s:c	Solanine	Chaconine	TGA	s:c
D0 Control	Mean					81.94	213.71	295.65	0.38
	Std Error					24.14	28.81	52.96	0.06
D3 Control	Mean	87.57	171.42	258.99	1.11	58.12	130.34	188.47	0.50
	Std Error	14.08	50.71	62.03	0.67	12.31	37.53	49.83	0.06
D3 Exposed	Mean	41.11	91.07	132.18	0.66	69.21	134.90	204.11	0.50
	Std Error	9.58	22.09	31.45	0.18	8.27	12.55	20.58	0.02
D6Control	Mean	61.25	135.86	197.11	0.47	63.11	123.42	186.53	0.51
	Std Error	14.21	33.06	46.62	0.03	10.86	20.14	30.87	0.02
D6 Exposed	Mean	177.33	383.75	561.08	0.45	79.45	174.38	253.83	0.43
	Std Error	27.21	48.12	74.76	0.02	17.43	32.67	49.63	0.03
D10 Control	Mean	56.80	135.03	191.84	0.43	47.20	99.89	147.09	0.47
	Std Error	6.88	18.26	25.04	0.01	4.90	10.91	15.56	0.02
D10 Exposed	Mean	341.57	602.78	944.34	0.53	63.35	130.84	194.19	0.49
	Std Error	72.91	84.82	156.71	0.03	4.10	10.32	13.80	0.02

King Edward - Week 10

		Upper Surface				Lower Surface			
		Solanine	Chaconine	TGA	s:c	Solanine	Chaconine	TGA	s:c
D0 Control	Mean	38.19	98.87	137.06	0.46	47.87	114.29	162.16	0.45
	Std Error	5.26	21.32	25.70	0.09	10.49	31.33	41.49	0.04
D3 Control	Mean	32.91	85.36	118.27	0.48	58.27	75.41	133.67	0.78
	Std Error	5.62	25.70	30.10	0.10	11.70	16.21	27.60	0.05
D3 Exposed	Mean	22.67	70.93	93.51	0.32	22.96	62.52	89.60	0.36
	Std Error	2.17	5.73	8.00	0.02	2.18	7.41	9.70	0.02
D6Control	Mean	26.61	67.21	93.82	0.43	18.62	65.24	83.86	0.30
	Std Error	4.30	11.70	13.39	0.08	2.21	11.91	14.08	0.02
D6 Exposed	Mean	33.87	89.54	123.41	0.39	22.53	62.42	84.95	0.37
	Std Error	3.94	10.97	14.52	0.02	1.47	6.11	7.57	0.01
D10 Control	Mean	39.35	82.16	121.51	0.47	30.34	79.60	109.93	0.40
	Std Error	7.93	6.93	14.30	0.06	2.10	7.17	6.33	0.05
D10 Exposed	Mean	52.52	149.05	201.57	0.36	34.32	87.06	123.42	0.43
	Std Error	10.99	22.67	31.99	0.05	3.09	10.15	12.02	0.05

Brodick - Week 10

		Upper Surface				Lower Surface			
		Solanine	Chaconine	TGA	s:c	Solanine	Chaconine	TGA	s:c
D0 Control	Mean	76.35	164.68	241.03	0.47	90.77	186.62	277.39	0.48
	Std Error	17.46	32.50	49.13	0.04	20.69	32.34	52.47	0.04
D3 Control	Mean	138.36	308.45	446.81	0.44	97.47	234.72	332.19	0.50
	Std Error	26.32	26.69	51.88	0.05	8.24	47.42	55.26	0.12
D3 Exposed	Mean	149.01	228.53	377.53	0.63	67.93	140.19	208.12	0.50
	Std Error	35.26	55.54	89.89	0.05	11.88	24.60	36.12	0.02
D6Control	Mean	108.59	228.20	336.79	0.47	82.80	171.81	254.61	0.48
	Std Error	15.39	22.33	37.55	0.02	17.54	31.35	48.86	0.01
D6 Exposed	Mean	367.37	469.49	836.87	0.76	190.10	312.85	502.95	0.57
	Std Error	65.37	66.05	129.43	0.06	57.35	77.30	134.14	0.04
D10 Control	Mean	96.08	195.86	291.94	0.49	83.68	180.53	264.21	0.47
	Std Error	9.52	15.11	24.51	0.01	5.09	15.54	20.63	0.01
D10 Exposed	Mean	561.06	661.84	1222.91	0.77	142.34	268.11	410.45	0.53
	Std Error	104.48	78.31	181.31	0.06	20.38	35.92	55.75	0.02

Record - Week 10

		Upper Surface				Lower Surface			
		Solanine	Chaconine	TGA	s:c	Solanine	Chaconine	TGA	s:c
D0 Control	Mean								
	Std Error								
D3 Control	Mean	30.50	62.22	92.72	0.49	21.09	47.78	68.87	0.44
	Std Error	4.30	7.98	12.05	0.03	2.71	5.18	7.79	0.02
D3 Exposed	Mean	55.01	91.64	146.64	0.60	31.28	53.74	85.02	0.61
	Std Error	8.85	11.09	19.24	0.05	3.63	7.34	10.42	0.05
D6Control	Mean	24.46	56.99	81.44	0.45	30.72	58.27	88.99	0.50
	Std Error	4.41	12.48	16.63	0.05	7.85	7.00	14.58	0.09
D6 Exposed	Mean	143.68	192.38	336.05	0.70	40.45	70.98	111.43	0.58
	Std Error	29.56	28.95	57.89	0.04	6.04	10.90	16.83	0.03
D10 Control	Mean	21.40	47.15	68.56	0.44	27.18	48.84	76.02	0.56
	Std Error	5.55	7.38	12.66	0.05	0.63	3.56	4.07	0.03
D10 Exposed	Mean	120.50	180.12	300.61	0.63	41.59	67.80	109.39	0.59
	Std Error	23.92	27.36	50.92	0.04	6.69	8.10	14.61	0.04

P. Dell - Week 10

		Upper Surface				Lower Surface			
		Solanine	Chaconine	TGA	s:c	Solanine	Chaconine	TGA	s:c
D0 Control	Mean	74.57	172.53	247.10	0.43	109.86	216.42	326.28	0.49
	Std Error	8.04	15.00	22.52	0.02	27.79	45.54	72.94	0.03
D3 Control	Mean	155.62	282.12	437.74	0.56	140.44	269.59	410.03	0.52
	Std Error	9.18	27.65	35.63	0.03	16.53	25.93	41.82	0.02
D3 Exposed	Mean	227.8502	399.113843	626.964	0.6	126.9698	270.630787	397.6	0.4
	Std Error	27.13375	33.0179014	59.165	0	33.37183	43.367092	73.32	0.1
D6Control	Mean	112.81	222.63	335.45	0.48	116.27	218.51	334.78	0.51
	Std Error	31.64	38.34	69.92	0.05	25.81	25.84	50.73	0.06
D6 Exposed	Mean	329.07	548.01	877.08	0.59	143.44	292.07	435.51	0.46
	Std Error	42.78	49.26	91.21	0.03	33.67	41.70	74.81	0.03
D10 Control	Mean	97.91	224.47	322.38	0.43	113.05	257.08	370.13	0.45
	Std Error	21.26	34.87	54.08	0.04	16.21	36.23	48.69	0.04
D10 Exposed	Mean	433.44	625.98	1059.42	0.67	90.83	225.54	316.38	0.41
	Std Error	59.06	65.32	123.42	0.03	13.00	24.12	34.76	0.04

King Edward - Week 20

		Upper Surface				Lower Surface			
		Solanine	Chaconine	TGA	s:c	Solanine	Chaconine	TGA	s:c
D0 Control	Mean	13.44	43.94	57.38	0.31	13.02	36.85	49.87	0.38
	Std Error	1.54	5.74	7.03	0.03	1.26	5.09	5.67	0.07
D3 Control	Mean	33.79	88.85	115.88	0.32	33.26	95.90	129.17	0.37
	Std Error	7.17	22.09	31.22	0.01	5.47	16.79	21.13	0.07
D3 Exposed	Mean	35.62	91.49	120.64	0.39	23.42	50.83	71.90	0.39
	Std Error	6.98	21.35	28.19	0.04	4.40	11.62	15.94	0.06
D6Control	Mean	93.97	245.71	339.68	0.37	36.66	125.37	162.02	0.29
	Std Error	20.01	37.84	57.85	0.02				
D6 Exposed	Mean	24.18	70.20	94.38	0.35	30.59	78.42	109.01	0.39
	Std Error	1.52	5.61	7.00	0.01	2.33	5.49	7.59	0.01
D10 Control	Mean	16.41	59.34	75.75	0.28	31.86	86.19	118.05	0.37
	Std Error	1.32	2.12	2.87	0.02	10.42	23.25	32.75	0.06
D10 Exposed	Mean	41.20	67.52	108.72	0.64	46.20	75.24	121.44	0.61
	Std Error	9.76	17.48	26.87	0.07	9.51	14.50	23.42	0.06

Brodick - Week 20

		Upper Surface				Lower Surface			
		Solanine	Chaconine	TGA	s:c	Solanine	Chaconine	TGA	s:c
D0 Control	Mean	76.98	129.59	206.57	0.58	60.75	118.86	179.61	0.55
	Std Error	14.43	18.21	32.18	0.04	5.23	23.34	27.84	0.06
D3 Control	Mean	84.69	146.91	231.60	0.57	69.70	113.58	183.27	0.63
	Std Error	15.92	24.52	39.92	0.04	14.05	23.34	37.23	0.05
D3 Exposed	Mean	107.45	217.31	324.76	0.49	62.89	116.56	179.45	0.53
	Std Error	9.74	14.97	24.43	0.02	9.25	12.15	21.05	0.03
D6Control	Mean	85.55	154.49	240.04	0.54	80.89	148.81	229.69	0.50
	Std Error	21.87	36.38	58.10	0.02	25.99	32.43	58.21	0.05
D6 Exposed	Mean	153.38	235.75	389.13	0.58	58.29	111.39	169.68	0.54
	Std Error	37.31	43.64	80.67	0.04	12.68	26.24	38.91	0.01
D10 Control	Mean	115.20	193.11	308.31	0.62	107.65	170.56	278.21	0.72
	Std Error	23.60	37.05	56.50	0.10	16.97	45.56	60.94	0.12
D10 Exposed	Mean	259.06	393.11	652.17	0.65	75.83	108.70	184.53	0.83
	Std Error	34.06	41.70	73.83	0.04	14.30	15.47	27.26	0.16

Record - Week 20

		Upper Surface				Lower Surface			
		Solanine	Chaconine	TGA	s:c	Solanine	Chaconine	TGA	s:c
D0 Control	Mean	35.80	67.06	102.86	0.55	30.87	56.32	87.19	0.60
	Std Error	4.75	11.70	16.44	0.03	6.11	16.40	22.50	0.04
D3 Control	Mean	56.91	80.33	137.24	0.60	21.98	44.81	66.79	0.49
	Std Error	25.45	28.30	53.73	0.08	3.17	4.21	6.55	0.06
D3 Exposed	Mean	40.05	66.77	106.83	0.62	31.29	50.96	82.25	0.63
	Std Error	6.33	8.56	14.71	0.03	2.33	4.20	6.38	0.03
D6Control	Mean								
	Std Error								
D6 Exposed	Mean	36.75	71.27	102.08	0.53	29.88	61.28	72.77	0.58
	Std Error	6.13	10.33	17.26	0.04	4.37	10.78	16.18	0.03
D10 Control	Mean	55.24	84.76	140.01	0.68	72.66	96.31	168.97	0.98
	Std Error	14.43	18.93	31.27	0.08	24.42	53.61	73.81	0.28
D10 Exposed	Mean	54.93	135.71	190.64	0.41	64.27	152.61	216.88	0.42
	Std Error	4.14	1.34	2.80	0.03	8.80	21.42	30.23	0.00

P. Dell - Week 20

		Upper Surface				Lower Surface			
		Solanine	Chaconine	TGA	s:c	Solanine	Chaconine	TGA	s:c
D0 Control	Mean	99.84	252.12	351.95	0.39	75.07	200.19	275.26	0.38
	Std Error	17.22	29.75	45.29	0.03	13.99	40.28	54.10	0.02
D3 Control	Mean	140.39	322.57	462.96	0.42	75.51	245.55	321.06	0.29
	Std Error	32.13	26.52	57.69	0.07	23.95	39.16	62.46	0.06
D3 Exposed	Mean	74.12	176.37	250.50	0.40	69.70	159.33	229.03	0.43
	Std Error	11.98	20.20	31.97	0.02	9.59	15.88	24.38	0.03
D6Control	Mean								
	Std Error								
D6 Exposed	Mean	144.22	288.75	432.97	0.47	102.83	195.94	298.78	0.49
	Std Error	35.01	46.36	80.59	0.04	22.48	23.35	45.31	0.04
D10 Control	Mean	113.57	224.40	337.97	0.50	79.07	164.02	243.09	0.51
	Std Error	17.90	33.26	50.34	0.03	21.02	33.12	50.41	0.09
D10 Exposed	Mean	255.94	467.26	723.20	0.52	79.72	203.04	282.76	0.38
	Std Error	40.64	51.67	92.02	0.02	10.83	13.94	24.16	0.03

King Edward - Week 30

		Upper Surface				Lower Surface			
		Solanine	Chaconine	TGA	s:c	Solanine	Chaconine	TGA	s:c
D0 Control	Mean	37.20	107.45	144.65	0.35	36.75	93.87	130.62	0.39
	Std Error	1.61	9.94	11.14	0.02	6.57	8.49	14.15	0.04
D3 Control	Mean	81.38	127.71	209.09	0.59	109.94	106.76	216.70	1.02
	Std Error	27.51	26.12	51.52	0.12	35.94	12.56	41.81	0.34
D3 Exposed	Mean	22.20	55.23	77.43	0.41	16.51	45.51	62.02	0.38
	Std Error	2.83	5.97	8.47	0.03	1.49	5.12	6.23	0.03
D6Control	Mean	15.52	77.44	92.96	0.19	26.28	87.39	113.67	0.29
	Std Error	4.79	10.35	14.78	0.04	4.79	13.31	18.05	0.01
D6 Exposed	Mean	30.26	83.54	113.79	0.39	29.91	81.00	110.91	0.37
	Std Error	3.63	11.59	15.02	0.02	4.08	9.43	13.23	0.02
D10 Control	Mean	36.35	107.53	143.88	0.34	47.16	129.94	177.10	0.35
	Std Error	5.05	13.01	17.93	0.01	9.04	20.72	29.73	0.01
D10 Exposed	Mean	33.89	78.48	112.37	0.45	28.30	62.41	90.71	0.47
	Std Error	6.15	11.19	16.44	0.06	3.67	7.24	9.95	0.05

Brodick - Week 30

		Upper Surface				Lower Surface			
		Solanine	Chaconine	TGA	s:c	Solanine	Chaconine	TGA	s:c
D0 Control	Mean	111.03	196.99	308.02	0.56	88.57	180.64	269.21	0.51
	Std Error	20.48	33.81	54.09	0.01	15.82	40.46	56.00	0.03
D3 Control	Mean	54.95	123.45	178.40	0.44	64.74	139.29	204.03	0.45
	Std Error	13.76	26.63	39.99	0.04	14.65	17.72	31.48	0.07
D3 Exposed	Mean	69.36	137.15	206.51	0.51	62.74	121.18	191.66	0.48
	Std Error	6.34	8.79	13.68	0.04	7.19	14.84	19.51	0.05
D6Control	Mean								
	Std Error								
D6 Exposed	Mean	73.44	143.78	217.22	0.51	39.96	85.18	125.14	0.48
	Std Error	9.90	18.72	27.63	0.03	4.98	9.62	14.36	0.02
D10 Control	Mean	58.87	102.42	161.28	0.61	55.79	102.07	157.86	0.55
	Std Error	9.11	20.69	27.41	0.08	8.16	16.08	24.00	0.04
D10 Exposed	Mean	160.15	186.03	346.18	0.90	74.27	106.45	180.72	0.76
	Std Error	42.43	29.21	68.61	0.16	22.78	17.44	38.43	0.16

Record - Week 30

		Upper Surface				Lower Surface			
		Solanine	Chaconine	TGA	s:c	Solanine	Chaconine	TGA	s:c
D0 Control	Mean	89.12	123.14	212.25	0.56	71.36	108.93	180.29	0.48
	Std Error	56.20	56.56	112.74	0.09	49.35	52.28	101.62	0.09
D3 Control	Mean	41.82	66.18	108.00	0.63	25.88	35.30	61.18	1.02
	Std Error	2.50	3.21	5.66	0.01	4.93	8.61	13.42	0.31
D3 Exposed	Mean	34.66	65.65	100.31	0.60	32.38	58.86	91.24	0.63
	Std Error	4.13	7.55	9.28	0.09	3.29	7.93	9.12	0.07
D6Control	Mean	44.97	65.20	110.18	0.72	27.76	38.36	66.12	0.73
	Std Error	18.80	29.86	48.60	0.05	5.91	7.30	12.96	0.05
D6 Exposed	Mean	36.46	54.73	91.19	0.64	29.73	35.53	65.26	0.92
	Std Error	5.77	6.38	11.93	0.05	6.18	6.37	12.14	0.20
D10 Control	Mean	56.44	108.59	175.75	0.63	35.88	76.72	118.54	0.56
	Std Error	13.34	16.03	25.76	0.09	11.82	21.69	34.54	0.06
D10 Exposed	Mean	42.33	82.33	124.66	0.61	42.15	66.73	108.88	0.73
	Std Error	4.94	13.75	17.84	0.06	14.43	21.78	35.81	0.08

P. Dell - Week 30

		Upper Surface				Lower Surface			
		Solanine	Chaconine	TGA	s:c	Solanine	Chaconine	TGA	s:c
D0 Control	Mean	75.51	160.16	235.67	0.47	93.71	159.23	252.95	0.64
	Std Error	11.92	16.87	25.98	0.05	13.73	29.37	40.52	0.09
D3 Control	Mean	65.12	166.95	232.07	0.39	68.72	161.98	230.70	0.41
	Std Error	7.99	16.95	22.53	0.04	17.06	23.06	39.80	0.04
D3 Exposed	Mean	94.71	198.22	292.93	0.48	71.19	141.41	212.60	0.70
	Std Error	10.72	15.30	24.66	0.03	9.28	19.42	27.18	0.22
D6Control	Mean	95.41	157.93	253.33	0.90	82.86	171.17	254.04	0.85
	Std Error	35.68	47.62	82.05	0.35	21.24	48.76	68.34	0.36
D6 Exposed	Mean	98.37	278.83	377.19	0.33	69.80	172.39	242.18	0.37
	Std Error	32.29	27.49	54.60	0.09	19.48	18.05	34.37	0.07
D10 Control	Mean	91.33	201.78	293.11	0.44	86.66	147.42	234.08	0.92
	Std Error	22.62	18.46	36.80	0.09	23.31	42.26	65.35	0.34
D10 Exposed	Mean	245.58	385.77	631.35	0.75	110.51	154.04	264.55	0.87
	Std Error	64.82	66.60	128.61	0.15	21.35	17.02	32.09	0.19