Effect of Temperature on Glycoalkaloid and Chlorophyll Accumulation in Potatoes (*Solanum tuberosum* L. Cv. King Edward) Stored at Low Photon Flux Density, Including Preliminary Modeling Using an Artificial Neural Network

Everard J. Edwards and Andrew H. Cobb*

Department of Life Sciences, The Nottingham Trent University, Clifton Lane, Nottingham NG11 8NS, United Kingdom

Potato tubers (cv. King Edward) were stored at 5, 10, 20, and 25 °C for up to 8 days in either low light (12 μmol of photons m^{-2} s^{-1}, photosynthetically active photon flux density) or darkness. Tubers were half-buried in potting compost to prevent light reaching the entire tuber surface. After 0, 2, 5, and 8 days samples were analyzed for photosynthetic pigment and glycoalkaloid content. Temperature clearly affected the accumulation of chlorophylls, with 20 °C causing maximal greening. The buried surface of tubers exposed to light also greened, although accumulating only 15−40% of the chlorophyll found in the upper, exposed tissues. However, there was no effect of the temperature or light exposure regimes employed on glycoalkaloid concentrations. Using this data, an artificial neural network was used to produce a preliminary model of the greening process. It was found that this closely resembled the actual results and could estimate intermediate data. It is concluded that there is no biosynthetic link between the low light-induced accumulation of chlorophyll and glycoalkaloids in this commercially important potato cultivar under the conditions described.

Keywords: *Artificial neural network; carotenoids; α-chaconine; chlorophyll; glycoalkaloids; light; potato; α-solanine; Solanum tuberosum; temperature*

INTRODUCTION

Exposure to light causes potato (*Solanum tuberosum* L.) tubers to green. This is due to the conversion of amyloplasts to chloroplasts (Anstis and Northcote, 1972) and the subsequent synthesis of chlorophyll and other photosynthetic pigments. Although chlorophyll is both tasteless and harmless, greened potatoes are deemed unfit for human consumption as tubers exposed to light also accumulate toxic steroidal glycoalkaloids (Conner, 1937). The two major alkaloids in the potato, comprising 95% of the total (TGA) are α-solanine and α-chaconine (Olsson, 1989). Both are triglycerides of the aglycon solanidine and have a toxicity to man higher than that of strychnine, about 2−5 mg kg^{-1} body weight (Morris and Lee, 1984). Consequently, the consumption of potatoes with high TGA concentrations can cause illness and even death (Willimot, 1933; Jadhav and Salunke, 1975). This has led to an upper limit of 200 μg of TGA g^{-1} fresh weight being commonly accepted within the industry.

Some studies of the response of potatoes to light have examined chlorophylls (Chl) and TGA in conjunction (Peterman and Morris, 1985; Kaaber, 1993; Griffiths *et al.*, 1994), but the results are often incomparable due to absolute concentrations of chlorophyll not being determined, and different sampling regimes being used for TGA analysis. While a number of publications have considered the effect of light quality and quantity on these responses (Percival *et al.*, 1994; Peterman and Morris, 1985; Baerug, 1962; Gull and Isenberg, 1960), only one recent paper has examined the role of temperature (Percival *et al.*, 1993). However, the latter study did not investigate the effects of temperature during Chl and TGA synthesis, but only the temperature during storage prior to light exposure.

Several earlier publications have documented the effect of temperature on tuber Chl formation, but disagree on the optimum temperature for storage. Ramaswarmy and Nair (1974) stated that maximum greening occurred at around 0 °C, agreeing with an earlier investigation by Larson (1950). However, Harkett (1975) found that 15−20 °C caused maximum Chl accumulation. Furthermore, it has been reported that low temperatures lead to a loss of carotenoids in potatoes stored in the dark (Bhushan and Thomas, 1990).

The authors also wished to produce a model of greening using this data and chose to use artificial neural networks (ANN) because of their ability to model data with a high degree of variation (Dayhoff, 1990).

ANN's have been used for a diverse range of modeling and predictive tasks, including taxonomic identification (Simpson *et al.*, 1992), predictive modeling of growth (Yee *et al.*, 1993), predictions of cardiac complications after surgery (Lette *et al.*, 1994), forecasting of share prices (Davalo and Niam, 1990), and the effects of microclimate on ozone injury (Balls *et al.*, 1996). This study describes the use of one such ANN to generate a model of the effect of temperature on the degree of greening in King Edward tubers exposed to light.

MATERIALS AND METHODS

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.

Experimental Design. Potatoes were half-buried, longitudinally, in potting compost (Seed and Potting Compost, J. Arthur Bowers, Lincoln, U.K.) in trays (600 × 320 × 80 mm). Each tray contained 17 potatoes of which 5 were covered with a smaller opaque tray (225 × 175 × 50 mm) to act as dark controls. Three trays of potatoes were stored in each of four incubators (Mercia Scientific, U.K.) set to 5, 10, 20, and 25 °C. The photoperiod was 16 h light/8 h dark with 12 μmol m^{-2} s^{-1}, photosynthetically active photon flux density (PPFD). After 0, 2, 5, and 8 days of storage one tray of 17 potatoes per

S0021-8561(96)00732-7 CCC: $14.00 © 1997 American Chemical Society

treatment was analyzed for chlorophyll and carotenoid concentrations and glycoalkaloid content as described below.

Pigment Extraction and Quantification. A 6 mm diameter stainless steel cork borer was used to obtain three cores at random from the exposed to unexposed surfaces of each tuber. The outer 10 mm, including the periderm, from each core was combined, frozen in liquid nitrogen, and stored at −20 °C. All subsequent procedures were performed at 4 °C in the dark.

The method used for pigment extraction was derived from those of McKinney (1941) and Arnon (1949). Samples were extracted in 80% aqueous acetone (v/v) with approximately 2 mg of magnesium carbonate and 1 mg of sodium bisulfite g^{-1} sample. Chlorophylls *a* and *b* were calculated according to Lichtenthaler and Wellburn (1983). Total carotenoid concentration was estimated using the equations of Hendry and Price (1993).

Glycoalkaloid Extraction and Purification. A 10 mm diameter cork borer was used to obtain two cores from each potato, as above, these were bisected, and the matching halves from the two cores combined. Thus, samples consisted of either two half-cores from tissues exposed to light or two half-cores from unexposed tissues. Glycoalkaloids were then extracted and quantified according to Edwards and Cobb (1996).

Statistical Analysis. Data were initially analyzed using regression and ANOVA. Student's *t* test and the Kolmogorov−Smirnov test (a nonparametric test) were also used to provide further analysis in depth.

Neural Network Modeling. The entire data set for pigment accumulation together with the storage temperature, accumulated light dose, and length of exposure of each sample was used to train back propagation networks using the Neuroshell 2 software package (Ward Systems Group, Fredrick, MD) on a 386 DX personal computer (Tiny Computers, Salfords, Surrey, U.K.).

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 10% of the original data, the error between the network-predicted results, and the actual results of these data were logged. This process was repeated many times until the error of the network-predicted test results reached a minimum. The network was saved at this point and used as the greening model. The entire original data set was then presented to the network and the error between actual and predicted results used as an estimate of the effectiveness of the model.

Networks were also trained using only one output i.e. total Chl, Chl *a*, total carotenoids, etc., in order to determine if individual networks for each output performed better than a single network using all the results.

The mean squared error (MSE) of each network was used to determine the architecture that was most effective at modeling the actual data, and the correlation between actual and predicted data was used to compare the effectiveness of the network at predicting each output.

The network was then given a range of temperature inputs to examine its effect on greening in more detail than could be allowed from the original data.

RESULTS

Tubers stored in the dark exhibited no visible greening, while exposure to a low photon flux density resulted in considerable chlorophyll accumulation (Figure 1).

Photosynthetic Pigment Concentrations. Student's *t* and Kolmogorov−Smirnov tests showed no significant differences in chlorophyll concentrations between any of the control samples.

Temperature was observed to have a marked effect on Chl concentrations. Total Chl accumulation (Figure 1a) was evident after 2 days of light exposure in all exposed samples (significant at the 5% level in all samples except those stored at 5 °C). After 5 days of light exposure, the tubers stored at 20 °C contained 4.55 μmoles of Chl g^{-1} sample, more than double the total Chl concentrations of those stored at other temperatures. However by day 8 there was no significant difference between the amount of Chl in potatoes stored at 20 or 25 °C (5.84 and 5.54 μmol g^{-1} sample, respectively). By this time the samples stored at 10 °C had also shown a large increase in Chl content, although still only approximately 65% of the concentrations found in the samples stored at the higher temperatures. The samples stored at 5 °C showed a significant decrease in total Chl between 5 and 8 days of exposure.

An examination of the Chl *a* to *b* ratio and the concentrations of the individual chlorophylls (Figure 1, parts b and c) reveals that the majority of Chl in all samples was Chl *a* and that the Chl *a* to *b* ratio rose between 2 and 8 days of exposure, irrespective of storage temperature. 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 tubers stored at 20 °C resulting in Chl *a* to *b* ratios of 2.1 and 3.7, respectively.

All the samples stored at 20 and 25 °C showed a significant increase in carotenoid content after 2 days of exposure, including the dark control samples (Figure 1, parts d and e). In the control samples these fell to initial concentrations by day 5, whereas carotenoid concentrations in the exposed samples remained steady and actually increased in the samples stored at 5 °C. Between 5 and 8 days carotenoid content rose in all samples, again including the dark controls. In the exposed tissues this increase was much greater than in the controls. However, the results after 8 days of light exposure did not mirror the Chl results. The tubers stored at 25 °C had a significantly higher carotenoid concentration than any of the other samples, 4.45 μmol g^{-1} sample.

The lower, unexposed surface of tubers exposed to light also showed some greening, despite receiving no direct light on the tissues themselves (Figure 1f). The pattern of accumulation with temperature approximately followed that of the upper, exposed tissues. Although, notably after 8 days of exposure the potatoes stored at 10 °C had the highest total Chl concentrations. However, even in these tubers Chl only reached 1.51 μmol g^{-1} sample, 40% of the highest concentration in the upper, exposed tissue. The tubers stored at 20 °C had final total Chl concentration of only 0.96 μmol g^{-1} sample, equivalent to 15% of the upper tissues. Carotenoid concentrations in the lower surface of exposed tubers exhibited no significant difference to controls.

Glycoalkaloid Concentrations. Examination of the data using ANOVA indicated some significant variations between or within treatments. Further analysis using Student's *t* test and the Kolmogorov−Smirnov test did not substantiate this. There was no variation in the TGA data significant even to the 10% level (Table 1).

Regression analysis of both the entire data set and the results for the upper surface of exposed tubers alone

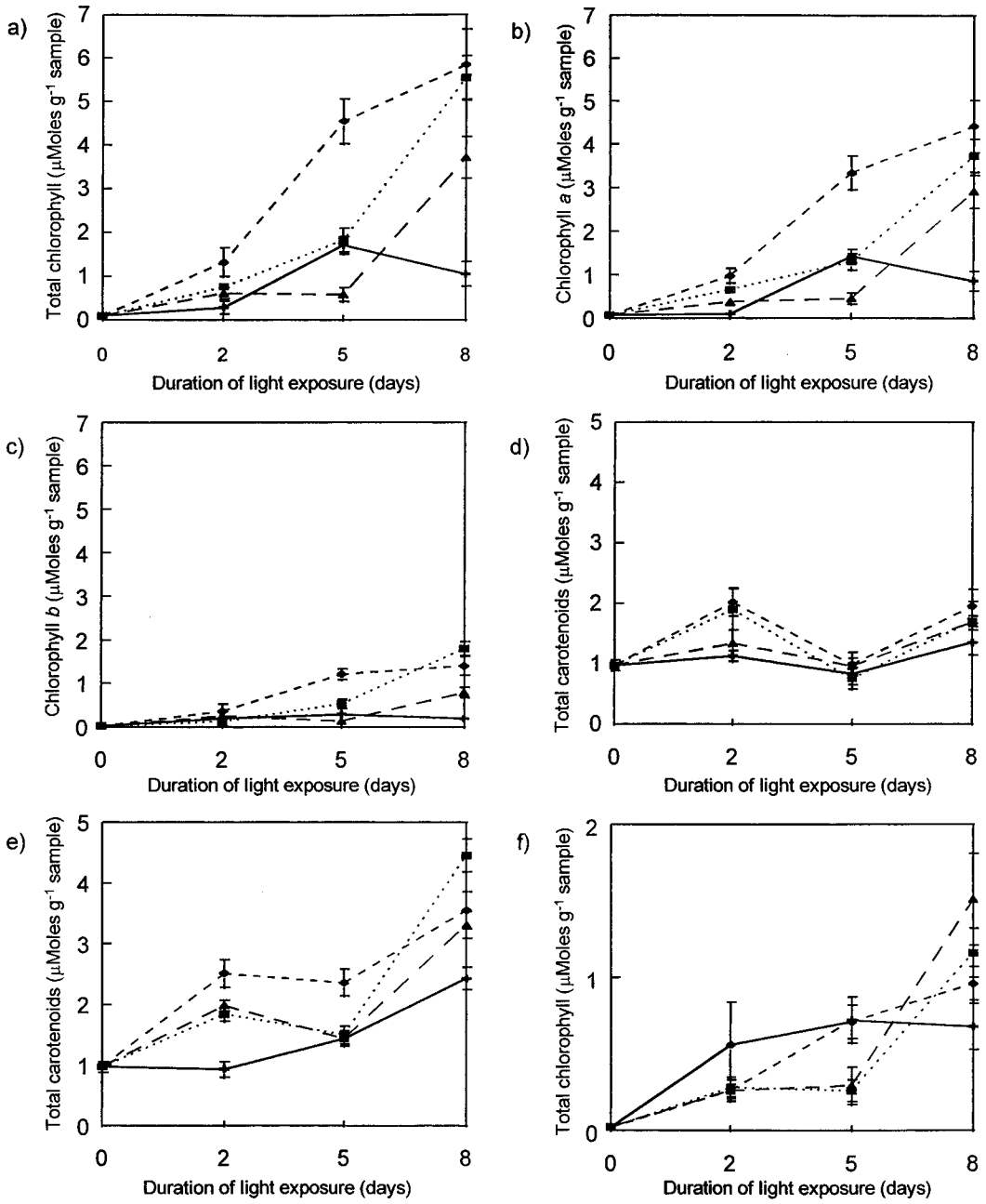

Figure 1. Accumulation of photosynthetic pigments in tubers exposed to 12 μmol of photons m^{-2} s^{-1} for 8 days at 5−25 °C. Samples stored at 5 °C (+), 10 °C (▲), 20 °C (◆), 25 °C (■). Each point is the mean of 12 (exposed) or 5 (control) samples ± standard error. (a) Total chlorophyll in upper surface of exposed tubers, (b) chlorophyll *a* upper surface of exposed tubers, (c) chlorophyll *b* in upper surface of exposed tubers, (d) total carotenoids in upper surface of control tubers, (e) total carotenoids in upper surface of exposed tubers, and (f) total chlorophyll in lower surface of exposed tubers.

Table 1. TGA Concentrations and α-Solanine:α-Chaconine Ratios in the Upper Half of Tubers Exposed to either 12 μmol of Photons m^{-2} s^{-1} or Darkness (control) for 8 Days at Four Different Temperatures (μg g^{-1} Sample)[a]

	5 °C	10 °C	20 °C	25 °C
		TGA Concentration		
control	72.49 ± 12.66	69.67 ± 21.74	69.60 ± 13.24	46.52 ± 8.26
exposed	46.85 ± 6.84	89.68 ± 22.03	48.29 ± 8.26	93.18 ± 30.39
		α-Solanine to α-Chaconine Ratio		
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

[a] Values are means ± standard errors where $N = 5$ for control and 13 for exposed samples. None of the results were significant to the 10% level using both tests.

showed no relationship between pigment and TGA concentrations ($R^2 = 0.044$ and 0.100, respectively).

Neural Network Model. 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. There was no connection between these two blocks, but both were connected with all inputs and outputs. One block applied a gaussian transformation function and the second a tan *h* transformation. This architecture

Table 2. Effectiveness of ANN in Predicting Actual Pigment Results

	Chl *a*	Chl *b*	total Chl	Chl *a*:*b*	total Car.	Chl:Car.
			Upper Surface			
R^2 [a]	0.73	0.63	0.73	0.52	0.71	0.51
MSE[b]	0.67	0.14	1.24	1.41	0.36	0.25
			Lower Surface			
R^2	0.43	0.46	0.43	0.32	0.51	0.42
MSE	0.09	0.03	0.19	1.65	0.19	0.06

[a] R^2 was calculated using regression of predicted versus actual results; values approaching 1 imply close correlation. [b] Low mean squared error (MSE) demonstrates a low absolute error. These results include data from both exposed and control tubers, where $N = 205$.

was found to be 22% more efficient at modeling the data than a standard three-layer back propagation architecture. The network predicted pigment content in the upper surface of tubers most effectively (Table 2). There was little difference between the predictions from the network trained using all outputs and networks trained using only one output for these samples. The predictions of pigment content in the lower surface of tubers showed a much lower correlation with the actual data. However, predictions from a single network using all outputs were a 90% improvement over networks trained with only one output.

The network was used to predict pigment content of samples stored at temperatures from 5−25 °C at 1 °C intervals (Figure 2). Generally the network predictions were accurate and allowed much more detail to be observed than the actual data. However, the network predictions lost accuracy when the duration of light exposure was close to zero.

DISCUSSION

Exposure of potato tubers to a low PPFD caused detectable greening within 48 h in all exposed samples, which was significant at temperatures as low as 10 °C.

Suboptimal temperatures are known to cause several effects in greening leaves, including a considerable reduction in Chl accumulation (Nie and Baker, 1991). Thylakoid biogenesis is also affected, and there is a reduction in plastid-encoded gene products (Robertson *et al.*, 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 which can result in photooxidative damage when PPFD is high (Oberhuber and Edwards, 1993). However, the use of low PPFD in this study would reduce the possibility of oxidation occurring, thus ensuring that any low temperature effect was not due to excess light energy.

The optimum storage temperature for Chl production was approximately 20 °C. Therefore, storage at both 5 and 10 °C can be considered suboptimal for tuber greening. Indeed, there was no accumulation of chlorophyll after 5 days in tubers stored at 5 °C and even some evidence of chlorophyll breakdown, shown by the significant reduction of Chl *a*. There was a concomitant decrease in the Chl *a* to *b* ratio which is indicative of senescence and chlorophyll breakdown (Hendry *et al.*, 1987).

These factors are important when considering the storage of potatoes during retail procedures and in the home. It is apparent that the display lifetime of stock is dependent 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. Potatoes are typically displayed for 7 days or less during retail and it is unlikely that this would be long enough to cause any reduction in quality associated with low temperature storage (Ewing *et al.*, 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 seedlings of barley (*Hordeum vulgare*), and a number of *Pinus* spp. (Ou and Adamson, 1995; Walmsley, 1991). Chl is not transported within plant tissues but synthesized within the chloroplast; therefore, it may be 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 pathway promoting light-independent Chl synthesis in unexposed areas of periderm. 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 would lead to significant accumulation of Chl.

As potatoes are an underground storage organ of a montane plant they would not be naturally exposed to the highest temperatures used in this study for significant lengths of time, and so these temperatures would be sources of stress to the tuber. In leaves, high carotenoid concentrations have been associated with environmental stresses (Pallett and Young, 1993), which may account for the observation that after 8 days of light exposure the carotenoid content of tubers is directly related to temperature, with the highest temperatures causing the highest carotenoid concentrations. Accumulation of carotenoids as part of a new photosynthetic apparatus of the amylochloroplasts 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 (Demig-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 values after 5 days in control tubers. This initial increase could possibly be a continuing response to earlier stress, e.g. storage under light before purchase or storage at high temperature. These responses indicate that with more study carotenoid concentrations could possibly be used as a monitor of tuber stress.

The photosynthetic pigment concentrations 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

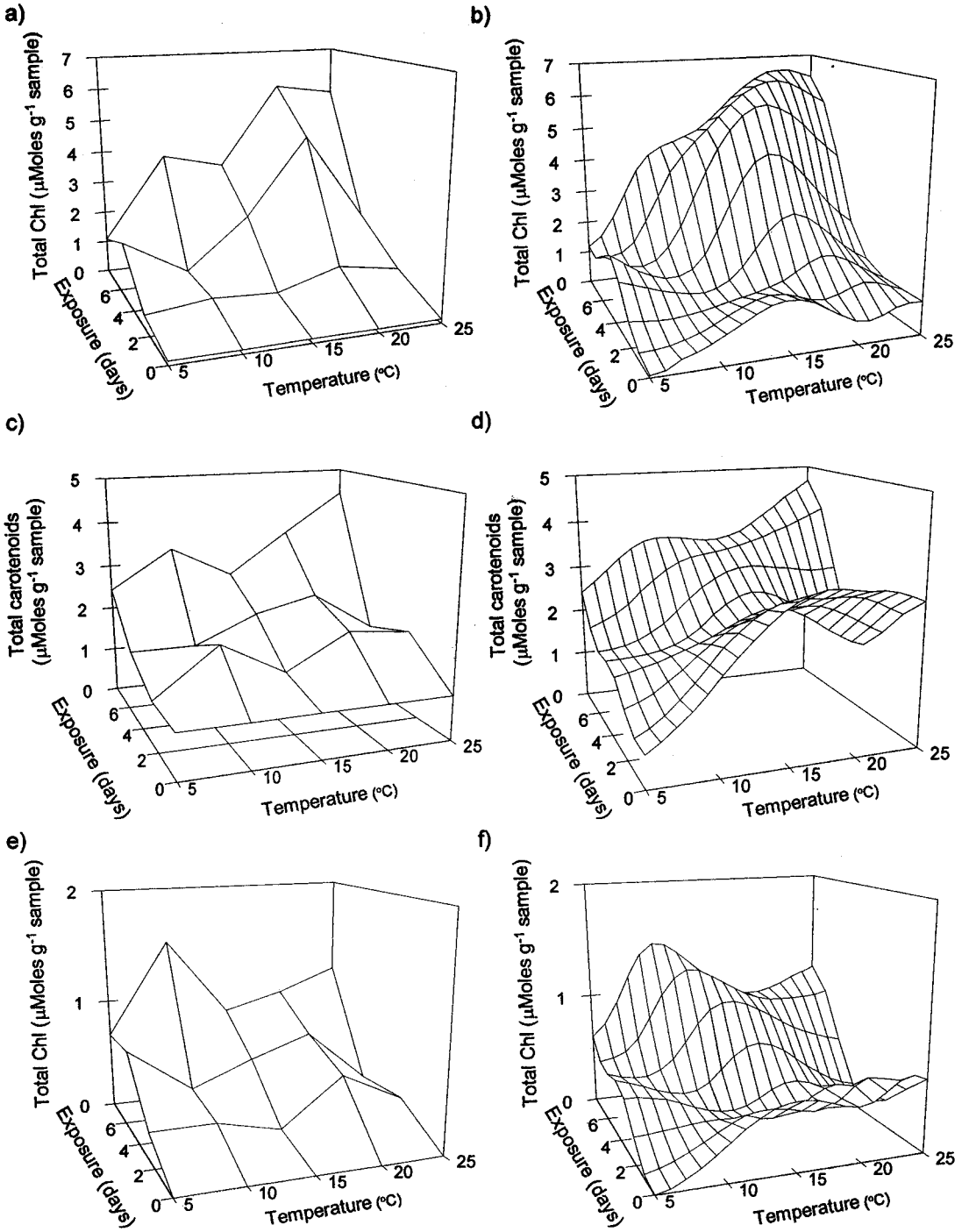

Figure 2. Comparison of actual data and neural network predictions. (a) Actual total Chl in upper surface of exposed tubers, (b) network-predicted total Chl in upper surface of exposed tubers, (c) actual total carotenoids in upper surface of exposed tubers, (d) network-predicted total carotenoids in 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.

variable, it was used to model the data. Comparison of the predicted and actual results showed that while the predictions followed the actual data closely there were some discrepancies. When the duration of exposure was low, the network predictions deviated more markedly from the actual data, and at 8 days of exposure the network tended to overestimate the pigment concentration. 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 modeling of variable data, may be reducing the accuracy of the predictions at either end of the exposure period.

This model is only preliminary, and a more widely encompassing and accurate model needs to be produced to be useful. In order to do this, exposure of potatoes in a number of different environmental regimes needs to be carried out and the data added to the network model. Factors including light quantity and quality as inputs would also improve the model.

As any inputs can be given to the network, specific environmental regimes could be examined using the model, allowing a more detailed analysis than is possible using either the original data or conventional statistics. This may also be used to further test the validity of the model, by exposing tubers to light in a specific environ-

mental regime previously unseen by the network and comparing the prediction to the actual result.

It is evident that exposure to low photon flux density 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, the extent of light-enhanced TGA accumulation is known to be cv. specific (Percival *et al.*, 1996), and it is likely that cv. King Edward potatoes do not accumulate TGA strongly. It is also possible that TGA concentrations are not affected by the low photon flux density used in this study, as light quantity is known to be important in the alteration of TGA pools (Percival and Dixon, 1996; Hilton and Gamborg, 1957). No attempt was made to model the TGA data with an ANN.

The α-solanine to α-chaconine ratios appear to be stable and were not affected by any treatment (Table 2). This provides some evidence that the high degree of variation and lack of significance of the TGA data is natural and not due to experimental error. It also suggests that tuber glycoalkaloids are stable and not rapidly metabolized, even with increasing metabolic activity.

Ramaswarmy *et al.* (1976) suggested that glycoalkaloids are formed within the chloroplast as a direct product of photosynthesis. The present study contradicts this, certainly at low photon flux densities. The lack of TGA accumulation despite significant Chl production suggests that the two 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, e.g. Dale *et al.* (1993).

ABBREVIATIONS USED

ANN, artificial neural network; Chl, chlorophyll; MSE, mean squared error; PPFD, photosynthetically active photon flux density; TGA, total glycoalkaloids.

ACKNOWLEDGMENT

We thank Dr. G. Balls for technical assistance with the setting up of the ANN used.

LITERATURE CITED

Anstis, B. J. P.; Northcote, D. H. Development of chloroplasts from amyloplasts in potato tuber discs. *New Phytol.* **1973**, *72*, 449−463.

Arnon, D. I. Copper enzymes in isolated chloroplasts. *Plant Physiol.* **1949,** *24*, 1−17.

Baerug, R. Influence of different rates and intensities of light on solanine content and cooking quality of potato tubers. *Eur. Potato J.* **1962**, *5*, 242−251.

Balls, G. R.; Palmer-Brown, D.; Sanders, G. E. Investigating microclimatic influences on ozone injury in clover (*Trifolium subterraneum*) using artificial neural networks. *New Phytol.* **1996**, *132*, 271−280.

Bennett, J. Biosynthesis of the light harvesting chlorophyll *a*/*b* protein. Polypeptide turnover in darkness. *Eur. J. Biochem.* **1981**, *118*, 61−70.

Bhushan, B.; Thomas, P. Effects of gamma irradiation and storage temperature on lipoxygenase activity and carotenoid disappearance in potato tubers. *J. Agric. Food Chem.* **1990**, *38*, 1586−1590.

Conner, H. W. Effect of light on solanine synthesis in the potato tuber. *Plant Physiol.* **1937**, *12*, 79−98.

Dale, M. F. B.; Griffiths, D. W.; Bain, H.; Todd, D. Glycoalkaloid increase in *Solanum tuberosum* on exposure to light. *Ann. Appl. Biol.* **1993**, *123*, 411−418.

Dayhoff, J. E. *Neural Network Architectures: An Introduction*; Van Nostrand Reinhold: New York, 1990.

Davalo, E.; Niam, P. *Neural Networks*; translated by Rawsthorne, A.; Macmillan: London. 1990; pp 111−112.

Demig-Adams, B.; Adams, W. W., III The role of xanthophyll cycle carotenoids in the protection of photosynthesis. *Trends Plant Sci.* **1996**, *1*, 21−26.

Edwards, E. J.; Cobb, A. H. An Improved High Performance Liquid Chromatographic Method for the Analysis of Potato (*Solanum tuberosum*) Glycoalkaloids. *J. Agric. Food Chem.* **1996**, *44*, 2705−2709.

Griffiths, D. W.; Dale, M. F. B.; Bain, H. The effect of cultivar, maturity and storage on photo-induced changes in the total glycoalkaloid and chlorophyll contents of potatoes (*Solanum tuberosum*). *Plant Sci.* **1994**, *98*, 103−109.

Gull, D. D.; Isenberg, F. M. Chlorophyll and solanine content and distribution in four varieties of potato tubers. *Proc. Am. Soc. Hortic. Sci.* **1960**, *75*, 545−556.

Haldiman, P. Effects of changes in growth temperature on photosynthesis and carotenoid composition in *Zea mays* leaves. *Physiol. Plant.* **1996**, *97*, 554−562.

Harkett, P. J. The influence of temperature and skin colour on chlorophyll synthesis in potato tubers exposed to light. *Abstr. Conf. Pap. 6th Trienn. Conf. Eur. Assoc. Potato Res.* **1975**, 179−180.

Hendry, G. A. F.; Houghton, J. D.; Brown, S. B. The degradation of chlorophyll—a biological enigma. *New Phytol.* **1987**, *7*, 255−302.

Hendry, G. A. F.; Price, A. H. Stress indicators: chlorophylls and carotenoids. In *Methods of Comparative Study*; Hendry, G. A. F., Grime, J. P., Eds.; Chapman and Hall: London, U.K., 1993; pp 148−152.

Hilton, R. J.; Gamborg, O. L. Factors in relation to tuber quality in potatoes. IV. Tuber metabolic stage and its influence on total solanine. *Can. J. Plant Sci.* **1957**, *37*, 407−412.

Jadhav, S. J.; Salunkhe, D. K. Formation and control of chlorophyll and glycoalkaloids in tubers of *Solanum tuberosum* L. and evaluation of glycoalkaloid toxicity. *Adv. Food Res.* **1975**, *21*, 307−54.

Kaaber, L. Glycoalkaloids, green discoloration and taste development during storage of some potato varieties (*Solanum tuberosum* L.). *Norw. J. Agric. Sci.* **1993**, *7*, 221−229.

Larsen, E. C. *Science* **1950**, *111*, 206.

Lette, J.; Colletti, B. W.; Cerino, M.; McNamara, D.; Eybalin, M.; Levasseur, A.; Nattel, S. Artificial intelligence versus logistic regression statistical modelling to predict cardiac complications after noncardiac surgery. *Clin. Cardiol.* **1994**, *17*, 609−614.

Lichtenthaler, H. K.; Wellburn, A. R. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.* **1983**, *11*, 591−593.

MacKinney, G. Absorption of light by chlorophyll solutions. *J. Biol. Chem.* **1941**, *144*, 315−323.

Morris, S. C.; Lee, T. H. The toxicity and teratogenicity of *Solanaceae* glycoalkaloids, particularly those of the potato (*Solanum tuberosum*): a review. *Food Technol. Aust.* **1984**, *36*, 118−124.

Morris, S. C.; Graham, D.; Lee, T. H. Phytochrome control of chlorophyll synthesis in potato tubers. *Plant Sci. Lett.* **1979**, *17*, 13−19.

Nie, G.-Y.; Baker, N. R. Modifications to thylakoid composition during the development of maize leaves grown at low growth temperatures. *Plant Physiol.* **1991** *95*, 184−191.

Oberhuber, W.; Edwards, G. E. Temperature dependence of the linkage of quantum yield of photosystem II to CO_2 fixation in C_4 and C_3 plants. *Plant Physiol.* **1993**, *101*, 507−512.

Olsson, K. 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: Uppsala, Sweden, 1989; pp 8−21.

Ou, K.; Adamson, H. Chlorophyll accumulation in cotyledons, hypocotyls and primary needles of *Pinus pinea* seedlings in light and dark. *Physiol. Plant.* **1995**, *93*, 719−724.

Pallett, K. E.; Young, A. J. Carotenoids. In *Antioxidants in Higher Plants*, Alscher, R. G., Hess, J. L., Eds.; CRC Press: London, 1993; pp 59−90.

Percival, G. C.; Dixon, G. R. Effect of light intensity on glycoalkaloid content of potato tubers. *Abstracts of Conference Papers, Posters and Demonstrations of the 13th Triennial Conference of the European Association for Potato Research*; Wageningen; The Netherlands. 1996; pp 43−44.

Percival, G.; Dixon, G.; Sword, A. Glycoalkaloid concentration of potato tubers following continuous illumination. *J. Sci. Food Agric.* **1994**, *66*, 139−144.

Percival, G.; Dixon, G.; Sword, A. Glycoalkaloid concentration of potato tubers following exposure to daylight. *J. Sci. Food Agric.* **1996**, *71*, 59−63.

Percival, G. C.; Harrison, J. A. C.; Dixon, G. R. The influence of temperature on light enhanced glycoalkaloid synthesis in potato. *Ann. Appl. Biol.* **1993**, *123*, 141−153.

Peterman, J. B.; Morris, S. C. The spectral responses of chlorophyll and glycoalkaloid synthesis in potato tubers (*Solanum tuberosum*). *Plant Sci.* **1985**, *39*, 105−110.

Ramaswarmy, N. K.; Nair, P. M. Temperature and light dependency of chlorophyll synthesis in potatoes. *Plant Sci. Lett.* **1974**, *2*, 249−256.

Ramaswarmy, N. K.; Behere, A. G.; Nair, P. M. A novel pathway for the synthesis of solanidine in the isolated chloroplast from greening potatoes. *Eur. J. Biochem.* **1976**, *67*, 275−282.

Robertson, E. J.; Baker, N. R.; Leech, R. M. Chloroplast thylakoid protein changes induced by low growth temperature in maize revealed by immunocytology. *Plant, Cell Environ.* **1993**, *16*, 809−818.

Simpson, R.; Williams, R.; Ellis, R.; Culverhouse, P. F. Biological pattern recognition by neural networks. *Mar. Ecol. Progr. Ser.* **1992**, *79*, 303−308.

Virgin, H. I.; Sundqvist, C. Pigment formation in potato tubers (*Solanum tuberosum*) exposed to light followed by darkness. *Physiol. Plant.* **1992**, *86*, 587−592.

Walmsley, J. Chlorophyll accumulation in photoperiodically-grown barley seedlings transferred to darkness: effect of time of dark transfer and daylength. *Photosynthetica* **1991**, *25*, 409−418.

Willimott, S. G. An investigation of solanine poisoning. *Analyst* **1933**, *58*, 431−438.

Yee, D.; Prior, M. G.; Florence, L. Z. Development of predictive models of laboratory animal growth using artificial neural networks. *Comput. Appl. Biosci.* **1993**, *9*, 517−522.

Received for review September 24, 1996. Revised manuscript received January 17, 1997. Accepted January 27, 1997.[⊗] This study was partially funded by the Potato Marketing Board of the United Kingdom and The Nottingham Trent University.

JF9607324

[⊗] Abstract published in *Advance ACS Abstracts,* April 1, 1997.