

PHYSIOLOGICAL STUDIES OF LIGHT-INDUCED
GREENING IN FRESH MARKET POTATOES

By

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of LAURA GRUNENFELDER find it satisfactory and recommend that it be accepted.

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Abstract

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Exposure of tubers to light induces chlorophyll synthesis in cortical parenchyma tissue directly beneath the periderm, resulting in undesirable greening. There are two main issues associated with potato greening: human health and marketability. Human health is considered a major factor because of the parallel and independent development of toxic glycoalkaloids. Although no metabolic connection between greening and glycoalkaloid development has been established, green potatoes are considered less fit for consumption and are discriminated against by consumers. While produce managers routinely cull greened potatoes, there are currently no specific grading criteria, resulting in highly variable quality within and among stores. Accordingly, greening indices were developed for the cultivars White Rose, Dark Red Norland, Yukon Gold, Norkotah Russet and Reba. Tuber color changed rapidly, and the full range of greening (8-10 levels) for each cultivar was obtained within 6 to 10 days of exposure to light intensities found in retail stores. Fluorescence microscopy of sections of tuber tissue from 'White Rose' revealed chlorophyll development within the outer 15 layers of parenchyma cells directly beneath the periderm. The fluorescence coincided with development of chlorophyll and increased as periderm color changed from green stage 3 to 7. Regardless of cultivar, concentrations of total glycoalkaloids in the flesh samples never exceeded the upper limit for consumption of 20 mg/100 g fresh wt established by the FDA. Glycoalkaloids increased in parallel with greening, ranging from 6.9 mg/100 g dry wt prior to greening to 46.8 mg/100 g dry

wt (post-greening), well within the limits established by the FDA (approximately 1mg/g dry wt, assuming dry weight is 20% of fresh weight). Reduction of light intensity from 6.5 to 3.8 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ through packaging in perforated polyethylene bags had no effect on greening. However, the rates of chlorophyll accumulation and greening were less at 3.5 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ than at 10.2 and 17.9 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$. Chlorophyll development in tubers is thus highly sensitive to low light levels, likely as a consequence of phytochrome involvement. Reducing the photoperiod from 24 to 18 hours had no effect on the rate of greening. Low temperatures (7 and 10°C) slowed the greening process over a 5-day interval. Surveys implementing the greening scales showed a high degree of variation among and within retail outlets for the extent of greening, underscoring a need for more consistent grading procedures. The grading scales covered the full range of greening found in stores, demonstrating their potential utility as an aid to quality control. Collectively, these results emphasize the sensitivity of tubers to very low light intensities and illustrate the difficulties faced by retailers in developing displays and packaging that will extend shelf-life through attenuation of the greening process.

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General Introduction

Potatoes undergo a greening process when exposed to light, due to the transformation of amyloplasts to chloroplasts, along with assembly of the photosynthetic apparatus (Pavlista, 2001; Edwards, 1997). After a certain minimum time of exposure to light, chlorophyll production is induced in the cortical parenchyma tissues directly beneath the periderm (Petermann and Morris, 1985). The rate of chlorophyll synthesis is affected by many pre- and post-harvest stresses including wounding, light exposure, temperature and lighting conditions, as well as genotype (Smith, 1977; Percival, 1999; Reeves, 1988). The discoloration resulting from greening is largely a function of the green chlorophyll in conjunction with the natural skin (periderm) pigmentation of a particular cultivar.

The two main issues associated with potato greening are human health and marketability. Human health is a concern because of the independent and parallel development of steroidal glycoalkaloids in green tubers (Smith, 1997; Edwards and Cobb, 1999). Glycoalkaloids are a naturally occurring and toxic group of secondary plant compounds found commonly in the foliage and tubers of members of the Solanaceae. α -Chaconine and α -Solanine are the two most common and abundant of the solanaceous glycoalkaloids. They are found in all tissues of the potato plant and comprise approximately 95% of the total glycoalkaloids. In small amounts, glycoalkaloids contribute to potato flavor. Commercial cultivars commonly contain between 2 and 15 mg per 100 g fresh weight (Phlak and Sporns, 1992). However, at higher levels, consumption of steroidal glycoalkaloids by humans can result in symptoms ranging from nausea to coma and even death in extreme cases (Griffiths et al., 2001). Glycoalkaloids impart a bitter flavor to the potato when levels exceed 15 mg per 100 g fwt (fresh weight) and the FDA has established an upper limit of 20 mg per 100 g fwt for new cultivars (Phlak and Sporns, 1992).

Although no metabolic connection between chlorophyll and glycoalkaloid development has been established, green tubers are considered less fit for human consumption and are usually discriminated against by both consumers and processors. Control of glycoalkaloid levels in potatoes at the fresh market or retail levels is currently based on the development of green color as a secondary indicator. The external coloration of potatoes is intimately linked with chlorophyll production, a result of light exposure. Since light exposure was found to be the single most significant factor in the formation of solanine (a steroidal glycoalkaloid) in potatoes (as compared to tuber damage, fertilizer and pesticide applications, and soil moisture), the extent of greening is considered to be a qualitative and economical, if not reliable, indicator of glycoalkaloid levels in fresh market potatoes (Smith, 1977).

It has been estimated that between 14 and 17% of the U.S. potato crop is lost annually due to greening of tubers (see references in Morris and Lee, 1984). While produce managers routinely cull potatoes that have greened, the process is subjective and variable, due to the absence of specific grading criteria. Currently, very little information exists regarding the time course and extent of greening under retail/fresh market conditions. Therefore, at the request of the U. S. Potato Board, we developed greening scales based on changes in chlorophyll concentration and the associated changes in periderm color for subjectively sorting red-, russet-, and white-skinned cultivars for greening during handling and marketing. Additionally, a time-course of greening was developed for each cultivar at a light intensity commonly found in retail settings. The concentrations of glycoalkaloids associated with particular levels of greening in each cultivar were also characterized.

The thesis is organized into four chapters, each written as a separate manuscript. Chapter one characterizes the development of greening over time under light and temperature conditions

typically found in retail markets. This chapter describes development of the greening scales, which show the progression of greening for each cultivar, based on chlorophyll levels in relation to visible changes in the surface color of tubers. The location and depth of chlorophyll development in the cortical parenchyma tissue during greening was revealed by fluorescence microscopy. The second chapter evaluates and demonstrates the utility of the greening scales for quality control of tuber greening in fresh markets. Chapter three describes the development of glycoalkaloids with time during greening and relates glycoalkaloid concentrations to specific levels within each greening scale. The final chapter evaluates the extent to which packaging, light intensity, photoperiod and temperature can be used to attenuate the greening process.

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CHAPTER ONE

DEVELOPMENT OF GREENING SCALES

Abstract

Potatoes are often displayed under supplemental light in fresh market stores to increase their appeal to the consumer. In this environment, tubers undergo an undesirable greening process, which is due to the development of chlorophyll in the cortical parenchyma tissue directly beneath the periderm (skin). Produce managers routinely cull potatoes that have greened; however, the process is very subjective and variable, due to the absence of specific grading criteria. Also, little information exists regarding the time course and extent of greening under retail/fresh market conditions. The objectives of this study were to characterize the time course of greening/chlorophyll development for selected fresh market cultivars and to develop an objectively-based scale of greening for each cultivar that can be used by growers, shippers, and retailers to subjectively sort tubers based on changes in visible color. A survey of retail outlets showed that most potatoes are displayed at relatively low intensities of photosynthetic light, ranging from 2 to 10 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$. At 6.8 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$, chlorophyll concentrations in cells underlying the periderm of cultivars White Rose (WR), Yukon Gold (YG), Dark Red Norland (DRN), Russet Norkotah (RN) and Reba (R) increased linearly over a 5 to 7-day greening interval. The increased chlorophyll content affected changes in the L-value (darkness) and hue angle (color) of the periderm, characterizing the off color development unique to each cultivar. A greening scale was developed for each cultivar by subjectively selecting tubers from a population that had been greened for 0 to 7 days to achieve a visually perceivable continuum of greening that spanned 8 to 10 levels, depending on the cultivar. Periderm color and chlorophyll content were then quantified for tubers at each level of the greening scale. Chlorophyll content

increased linearly over the greening scales for WR, YG, and DRN ($R^2 = 0.95-0.98$, $P \leq 0.001$), calibrating the scales for chlorophyll concentration and indicating that the visible color changes were directly linked to chlorophyll development. The relationship between chlorophyll content and greening level of RN was quadratic with smaller increases in chlorophyll concentration over the final three greening levels. Fluorescence microscopy showed progressive increases in chlorophyll content in the outer 15 layers of cells of the cortex of WR tubers, from 3 to 7 days (green scale \cong 3 to 7) of exposure to light ($6.8 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$). The fluorescence was most intense in cells containing high concentrations of amyloplasts, likely reflecting chloroplast biogenesis from these plastids.

Introduction

When potato tubers are exposed to light, chloroplasts containing chlorophyll begin to develop in parenchyma cells of the cortex located directly beneath the periderm (Petermann and Morris, 1985). During the greening process, the grana of the chloroplasts are formed directly from vesicles or membranous tubular extensions, and chlorophyll pigments are assembled on the newly synthesized membranes (which develop during the conversion of amyloplasts to chloroplasts) (Anstis and Northcote, 1973). Unfortunately, there is little information on the transformation of amyloplasts to chloroplasts, despite the large amount of data available on the development of chloroplasts from etioplasts. The conversion of amyloplasts to chloroplasts involves biochemical and ultra-structural changes that include the development of thylakoids and the pigment-protein complexes which constitute the photosystems (PS I and PS II) (Edwards, 1997).

The final step in the production of chlorophyll is the conversion of protochlorophyllide (Pchl) to chlorophyll, which is dependent on a photochemical reaction in higher plants (von Wettstein, et. al., 1995). It is possible that Pchl is present in potato tubers, as it has been shown to be present in the roots of dark-grown plants (McEwen and Lindsten, 1992). However, potato tubers are underground stems, and no protochlorophyllide has been reported to date. Also in question is the possible role of phytochrome in the greening process of potatoes. It has been shown to be involved in the greening process of tomatoes, however, using aurea mutants (Kendror and Horwitz, 1990). Chloroplast development is part of the process of cellular differentiation and, as such, is possibly regulated by phytochrome and/or plant growth hormones. Use of a fluorescence microscope to determine the location and ontogeny of chloroplasts relative to amyloplasts in green tubers may provide some insight to the process of greening in fresh market potatoes.

The rate of chlorophyll synthesis is dependent on many pre- and post-harvest factors including wounding, light exposure, temperature and lighting conditions, as well as genotype (Smith, 1977; Percival, 1999; Reeves, 1988). While potato tuber greening occurs under a wide range of lighting conditions, the rates of both chlorophyll and glycoalkaloid accumulation were higher under sodium and fluorescent lighting compared to low- and high-pressure mercury lighting (Percival, 1999). The resulting discoloration of the tuber surface is largely a function of the green chlorophyll in conjunction with the natural skin (periderm) pigmentation of a particular cultivar.

Green potatoes are generally rejected by both potato processors and consumers. This is due in part to a perceived association between the development of chlorophyll in the tuber and the development of toxic glycoalkaloids. Although the two processes are likely independent,

both chlorophyll and glycoalkaloid concentrations increase in direct response to light exposure. The unsightly greening and discoloration caused by chlorophyll development also leads to discrimination by consumers, reducing marketability. A number of studies have used various methods to rate the level of greening; however, the bases for the subjective rating schemes were never described adequately nor related to objective criteria to the extent that would allow their use by industry (Reeves, 1988; Hardenburg, 1954; Folsom, 1947). Hence, there are currently no specific grading criteria for evaluating the extent of greening in fresh market cultivars. The objectives of this study were to characterize the process of greening in relation to color changes in a number of fresh market cultivars and to develop greening scales for each cultivar for use in quality control during handling, distribution and sale in retail markets.

Materials and Methods

Plant Materials and General Procedures

Potato (*Solanum tuberosum* L.) tubers (cvs. White Rose, Yukon Gold, Russet Norkotah, Dark Red Norland) were purchased in 50 pound boxes from a local grocery store directly off the supply truck. These potatoes thus represent those that would be subject to greening under the lighting conditions present in the retail market. The U.S. Potato Board provided samples of cv. Reba tubers. All tubers were stored at 4°C and 95% relative humidity in darkness prior to use.

Light intensities and temperatures for the various greening studies were chosen to match those typically found in grocery stores as determined by surveys of major retailers. Light intensity measurements from retail potato displays were compared among seven major grocery retailers in the local area (Fig. 1). All stores displayed the majority of their potatoes under relatively low levels of ambient light; either bagged (bagged/stacked bins) or piled without

packaging on shelves (loose bins) at room temperature. Most of the stores also had tubers in lighted displays on refrigerated shelves. At tuber level, the average light intensities in the low-light, non-refrigerated areas was $6.3 \mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ while the tubers stored in the high-light, refrigerated displays averaged nearly $28 \mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ (Fig. 1). Hence, the greening studies were conducted at room temperature (23°C) and $6.8 \mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ light intensity.

Light intensity was measured with a quantum sensor (Model LI-185B, Li-Cor, Inc., Lincoln, NB) as photosynthetic photon flux density (PPFD, $\mu\text{mol quanta m}^{-2} \text{sec}^{-1}$) in the 400-700 nm range. Photographs to document the extent of greening were taken using a Nikon Cool-Pix 950 digital camera (Nikon Corp, New York). A ventilation hood with fluorescent lighting (Sylvania RapidStart SuperSaver 34 W Cool White fluorescent tubes) was used as a lighting table for the greening experiments with a 24-hour photoperiod averaging $6.8 \mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ at tuber level, simulating standard retail conditions. The fluorescent lights had spectral peaks at approximately 360, 410, 435, 545 and 575 nm.

Color Assessment

Color of the undamaged surface of potato tubers was assessed using a Minolta Chroma Meter CIE 1976 (CIELAB) (Model CR-200, Minolta Corporation, Ramsey, NJ). CIE refers to the Commission Internationale de l'Eclairage (International Commission on Illumination). Changes in tuber color during greening were quantified using the CIELAB color model. CIELAB is an opponent color system that integrates L, a, and b color axes to define color in three dimensions. L-values represent the change in lightness on a scale from 0 (black) to 100 (white). A-values represent color change from red (positive values) to green (negative values), while b-values show the change in color from yellow (positive values) to blue (negative values).

The color at the origin of the three axes is gray. CIELAB uses a system of numerical coordinates to locate and thus define a particular color in a color sphere. Further information on CIELAB and the color sphere can be found at the following websites: <http://www.colourpeople.co.uk> and <http://adobe.com/support/techguides/color/colormodels/cielab.html>. CIELAB measurements were taken at three points on each potato tuber including the stem end, the middle and the bud end. Hue angles (a measure of color) were calculated as described by McGuire (1992) and Schreiner *et al.* (2003) using the following equation:

$$\theta = ((\text{ATAN}(b/a)/6.2832) \times 360);$$

$$\text{If } a > 0 \text{ and } b > 0 \text{ then } h = \theta$$

$$\text{If } a < 0 \text{ and } b > 0, \text{ then } h = 180 + \theta$$

$$\text{If } a > 0 \text{ and } b < 0, \text{ then } h = 180 + \theta$$

$$\text{If } a < 0 \text{ and } b < 0, \text{ then } h = 360 + \theta$$

Where h = hue angle

Development of Greening Scales

To establish the subjective greening scales for use by the industry, tubers of each cultivar were placed daily on a light table (24-hour photoperiod, $6.8 \mu\text{mol quanta m}^{-2} \text{sec}^{-1}$, 23°C) and greened for a period ranging up to 7 days. The tubers were set out in reverse chronological order (the potatoes that would be greening for the longest were placed first) so that all durations of greening could be sampled simultaneously at the end of the study. The tubers were re-randomized on the light table each day to minimize the effects of variation in light intensity. The color of each tuber was measured at zero time and at the end of greening. The tubers were photographed at the end of each study to document the variation in tuber greening due to light exposure time and among replicates. Tubers were selected visually according to the degree of greening to develop a continuous scale of greening for each cultivar. The greening scales ranged from 0 to 7 or 0 to 9, depending on the cultivar (0 = no greening, 7 or 9 = maximum greening).

Tuber color (CIELAB) was measured and chlorophyll was extracted and quantified from three replicates of tubers representing each level of the greening scale for each cultivar.

Chlorophyll Extraction and Measurement

After greening, four cores were cut at random from each potato tuber (perpendicular to the apical and basal axis) using a 15-mm-diameter cork borer. A thin slice (approximately 1-mm-thick), including the periderm, was cut from the end of each of the four cores, representing the surface of the tuber that was exposed to light (total surface area = 7.065 cm²). These four discs were collectively diced into smaller pieces, and immediately frozen in liquid nitrogen.

The chlorophyll extraction protocol was modified from the methods of Inskeep and Bloom (1985). The frozen tissue was ground to a fine powder using a mortar and pestle while still frozen. The frozen tissue (each sample derived from four discs) was then transferred to test tubes, and extracted with 3 mL of N,N-dimethylformamide (DMF). The extracts were vortexed and the tubes were covered in foil to exclude light and refrigerated at 4°C for 24-72 hours. The extracts were then vortexed and centrifuged for 15 minutes in a Damon/IEC Division HN-SII Centrifuge at approximately 2,500 g. The supernatant was centrifuged again for 10-15 minutes at 2,500 g and collected for chlorophyll determination.

Chlorophyll content was measured at A_{647} and $A_{664.5}$ using a Cary 100 Bio UV-Visible double beam spectrophotometer (Varian Instruments, Walnut Creek, CA). Chlorophyll a, chlorophyll b, and total chlorophyll concentrations were determined from the following equations (Inskeep and Bloom, 1985):

$$\text{Chl a} = 12.70 (A_{664.5}) - 2.79 (A_{647})$$

$$\text{Chl b} = 20.70 (A_{647}) - 4.62 (A_{664.5})$$

$$\text{Total Chl} = 17.90 (A_{647}) + 8.08 (A_{664.5})$$

$$A = \text{absorbance in 1.00-cm cuvettes and Chl} = \text{mg L}^{-1}$$

Fluorescence Microscopy

White Rose tubers were greened in reverse chronological order for 0, 1, 3, 5, and 7 days (24-hour photoperiod, $6.8 \mu\text{mol quanta m}^{-2} \text{sec}^{-1}$) as previously described. The tubers were then graded using the greening scale developed for cv. White Rose. Thin cross-sections of the light-exposed side of the tubers were mounted on glass microscope slides in water and covered with a glass cover slip. The samples were examined at 40x with a Lertz Aristoplan fluorescence microscope, and photographs were taken under halogen and blue light (excitation wavelength 450-490 nm; >490 nm cutoff) to determine the location and origin of the chloroplasts in the cortical parenchyma cells underlying the periderm.

Results

cv. White Rose

Time Course of Greening

At room temperature and relatively low light intensity (24-h photoperiod), White Rose tubers underwent a change in color (Fig. 2) that could be quantified using the CIE (Commission Internationale de l'Éclairage) L*a*b color system (CIELAB). L-value (measure of lightness) of the tubers decreased linearly over the 5-day interval, reflecting a darkening of the tuber surface with progressive greening (Fig. 3). In contrast, the hue angle (color) of the tubers increased over the greening period in direct response to linear increases in chlorophyll content of the underlying cortical tissue (Fig. 3).

Development of the Greening Scale

White Rose tubers that had been greened over a 5-day interval (Fig. 2) were sorted visually, based on the extent of greening, to create a ten-level scale for subjectively sorting

tubers as they move through the various distribution channels (Fig. 4). As tuber greening level increased from zero to nine, L-values and hue angles changed to reflect the values expected for each level of greening (Fig. 5). Moreover, chlorophyll content of the tuber tissue increased linearly over the entire subjective greening scale, effectively calibrating the scale for chlorophyll concentration. Note that green-9 tubers contained approximately 2.3-fold more chlorophyll than green-3 tubers (Fig. 5) and that the time required to green from stage three to nine was approximately 4 days (Fig. 6). At $6.8 \mu\text{mol quanta m}^{-2} \text{sec}^{-1}$, this particular lot of White Rose tubers advanced one level on the greening scale (Fig. 4) for every 17 h of light exposure. Hence, the ten-level greening scale (Fig. 4), together with the derived time course of greening (Fig. 6), provides a guide for quality control and estimating the remaining shelf life of White Rose potatoes in retail markets.

cv. Yukon Gold

Time Course of Greening

The greening of Yukon Gold tubers was assessed at 23°C and $6.8 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ light intensity (24-h photoperiod). The color changes over a 5-day interval of light exposure were relatively subtle and difficult to capture photographically (Fig. 7) as compared to the other cultivars studied. Nevertheless, the changes were easily quantified using the CIELAB color system. Yukon Gold tubers decreased in L-value over the 5-day interval, indicating a darkening of the tuber surface with progressive greening (Fig. 8). In contrast, the hue angle of the tubers initially decreased, reflecting the development of a visible reddish hue, followed by a linear increase with greening from day two through day five. These color changes were repeated in subsequent experiments, and are thus characteristic for this particular cultivar. Chlorophyll concentration more than tripled over the greening interval, increasing linearly with time (Fig. 8).

Greening of Yukon Gold tubers effects a general bronzing of the tuber surface, as opposed to development of a discernible green color. Tubers held in the dark over the 5-day period showed no change in color or chlorophyll content.

Development of the Greening Scale

Yukon Gold tubers greened over a 5-day interval (Fig. 7) were sorted by visible color, based on the extent of greening, to create an eight-level scale for grading tubers according to color (Fig. 9). As the greening level increased from two to seven, L-value decreased. Hue angle increased from green-3 to 7, characterizing the changes in color (Fig. 10). Chlorophyll content of the tuber tissue increased linearly over the greening scale, thus calibrating the scale for chlorophyll concentration and indicating that the visual perception of greening was directly proportional to the chlorophyll concentration in this cultivar (Fig. 10). Note that a green-7 tuber contains approximately 1.7-fold more chlorophyll than a green-3 tuber (Fig. 10). The time required to advance from green-3 to a green-7 was about 3 days (Fig. 11), averaging about 19 hours per level on the greening scale.

cv. Dark Red Norland

Time Course of Greening

The greening of tubers of this cultivar resulted in a dulling of the natural red color (Fig. 12) that appeared to darken the periderm but did not effect significant changes in L-value (measure of darkness), despite a notable increase in hue angle (Fig. 13). Chlorophyll concentration increased almost 5-fold over the 5-day greening period. Dark Red Norland potatoes held in the dark over the 5-day period showed no changes in color or chlorophyll content.

Development of the Greening Scale

Tubers greened over the 5-day interval (Fig 12) were sorted by visible color to create an eight-level scale for grading tubers according to color (Fig. 14). As the tuber greening level increased from zero to seven, L-values remained relatively constant, while hue angles increased through green-3 (Fig. 15). Chlorophyll content of tuber tissue increased linearly over the greening scale, calibrating the scale for chlorophyll concentration and, similar to the other cultivars, confirming that the color change was due to the accumulation of chlorophyll. A green-7 'Dark Red Norland' tuber contains approximately 1.8-fold more chlorophyll than a green-3 tuber (Fig. 15). The time required to green from stage three to seven was about 3 days, with a change in greening level every 17 hours (Fig. 16).

cv. Russet Norkotah

Time Course of Greening

The change in color of Russet Norkotah tubers at 23°C over a 6-day interval at 6.8 $\mu\text{mol quanta m}^{-2} \text{ sec}^{-1}$ light intensity (24-h photoperiod) was characterized by a decrease in L-value (darkening) and an increase in hue angle (Fig. 17). The chlorophyll content of tubers increased linearly with time to levels higher than those evident in the other cultivars; however, the perceivable changes in color were somewhat masked by the russet skin. This is partly reflected in the rather narrow range of change in L-value, relative to a cultivar such as White Rose. A disc of periderm was removed from the tubers to reveal the progressive development of chlorophyll in the underlying tissues. Russet Norkotah potatoes held in darkness over the 6-day period showed no changes in color or chlorophyll content.

Development of the Greening Scale

Russet Norkotah tubers greened over a 6-day interval were sorted visually to create an eight-level greening scale for this cultivar (Fig. 18). Little change in L-value was evident as tuber greening increased from zero to seven (Fig. 19). Hue angle increased as chlorophyll content of the tuber tissue increased over the greening scale. Increases in chlorophyll concentration of tubers over the latter part of the scale (green-5 to green-7) were less than that evident from green-0 to green-4. Nevertheless, the visual perception of color change closely agreed with increases in chlorophyll content of tuber tissue, effectively calibrating the scale for chlorophyll concentration. Green-7 tubers contained approximately 1.8-fold more chlorophyll than green-3 tubers (Fig. 19) and the time required to green from stage three to seven was about 3 days (Fig. 20). At $6.8 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$, this particular lot of Russet Norkotah tubers required about 27 h exposure to light to cause an incremental change in greening level over the range green-0 to green-3, as compared with only 16 h over the range green-4 to green-7. As with the other cultivars, the physiological status of ‘Russet Norkotah’ tubers, which no doubt varies among tuber lots, will likely influence the rate of greening and thus estimates of remaining shelf life.

cv. Reba

Time Course of Greening

Greening of Reba tubers was accomplished at 23°C and $6.8 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ light intensity (24-h photoperiod) over a 9-day period. As with Yukon Gold and Russet Norkotah tubers, changes in color were relatively subtle and difficult to capture photographically. Since changes in color could not be perceived beyond day six, the qualitative and quantitative evaluations of color were restricted to tubers that had greened over the initial 6-day period (Fig. 21). The relative lightness (L-value) of tubers was unaffected by chlorophyll accumulation in

the underlying tissues; however, the tubers did develop a distinctive green hue over the greening period (Fig. 22). Chlorophyll concentration increased relatively slowly over the first three days of greening, followed by a rapid increase through day six. Interestingly, the increases in chlorophyll concentration during greening of Reba and Russet Norkotah tubers (~11-fold and 20-fold, respectively) were substantially higher than those observed in the other cultivars, yet the associated change in perceivable color was not that striking. This is no doubt a consequence of the russet-skin characteristic of these cultivars. Differences in perceivable color per unit of chlorophyll among cultivars will dictate varying degrees of consumer discrimination of greened tubers that are unique to each cultivar.

Development of the Greening Scale

Reba tubers that had greened over the 6-day interval (Fig. 21) were sorted visually, based on the extent of greening, to create a ten-level scale for grading tubers according to color (Fig. 23). L-values remained constant across the greening scale, indicating no change in lightness of the tuber surface (Fig. 24). The increase in hue angle verified that the ten-level sequence of greening in tubers of this cultivar could be perceived visually. Chlorophyll content of tuber tissue increased slowly from green-0 to green-3 and then rapidly over the remaining greening levels. The resulting curvilinear relationship (Fig. 24) calibrates the scale, indicating that the visual perception of greening is proportional to chlorophyll concentration in this cultivar. Note that a green-9 tuber contains approximately 3.5-fold more chlorophyll than a green-3 tuber and that the time required to green from stage three to nine was about 4 days (Fig. 25). At $6.8 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ light intensity, this particular lot of Reba tubers changed greening level every 17 h.

Fluorescence Microscopy

Under white light, microscopic examination of tuber tissues revealed the closely packed phellem cells of the periderm overlying the much larger parenchyma cells of the cortex (Fig. 26). Under UV illumination, the natural fluorescence of suberin coating the walls of the phellem cells can clearly be seen in the periderm layer (Fig. 26). No fluorescence was detected in the cortical parenchyma cells at zero and one day of greening. However, fluorescence was visible following 3 days of greening, indicating chlorophyll development in the outer 10-15 layers of cells. The number of visible fluorescent areas increased through 7 days, reflecting a progressive increase in chloroplast development. Fluorescence was most intense in areas with high concentrations of amyloplasts, which likely reflects their conversion to chloroplasts (compare left and right panels in Fig. 26).

Discussion and Conclusions

The cultivars used in this study were specifically selected to represent the range in skin colors most commonly found in the fresh market. Color changes, as affected by chlorophyll development in relation to an array of skin colors, could thus be characterized. The color of tubers changed rapidly (within 6 days) as chlorophyll concentrations increased in response to light and temperature conditions identical to those found in local grocery stores. While the remaining shelf-life of the tubers of each cultivar can be estimated from the derived plots (relating greening scale to time) and associated equations, it should be noted that the physiological status of tubers, which no doubt varies among tuber lots, will likely influence the rate of greening and thus the estimates of remaining shelf life. Gull (1960) observed that potatoes stored for 3 months at 4.4°C were slightly more susceptible to light-induced greening

than tubers stored for 8 months. Physiological and chronological age, degree of periderm development, storage temperature, etc., all interact to affect tuber physiological status (Edwards, 1997; Smith, 1977), which in turn can affect the rate of greening.

There was a difference in the visible change in color between cultivars, with the white-skinned cultivar White Rose having the most apparent or discernible greening. This is attributed to variation among the cultivars in periderm thickness, color and presence of accessory pigments, all of which interact to affect the degree of discoloration. The varying discoloration will likely influence the degree to which consumers discriminate against greened tubers, and therefore will affect the cut-off or maximum level of greening, after which the store or consumer considers tuber color unacceptable. Because of this, it is to be expected that cut-off levels (based on the degree of visible greening or discoloration) will vary depending on cultivar.

Chlorophyll became visible by fluorescence microscopy to a very limited extent after 72 hours (3 days) of light exposure. Previous studies reported a lag phase of 20-24 hours after illumination of the tuber before any chlorophyll or amyloplast structural changes could be seen (Anstis and Northcote, 1973). A short delay is common for the transformation of plastids not specialized for photosynthesis.

In conclusion, we have developed grading scales for potato tuber greening that are based on objective criteria (chlorophyll concentration and color analysis) that can be used by the industry to subjectively grade potatoes for quality control and food safety. The scales were developed using lighting and temperature conditions that are consistent with conditions in fresh market retail stores, avoiding excessive light intensities and inconsistent light sources. The cultivars in this study were selected to represent a broad range in skin color commonly available

in the fresh market (white, yellow, red, russet). The utility of these scales in covering the range of greening of these cultivars in retail markets is demonstrated in chapter 2.

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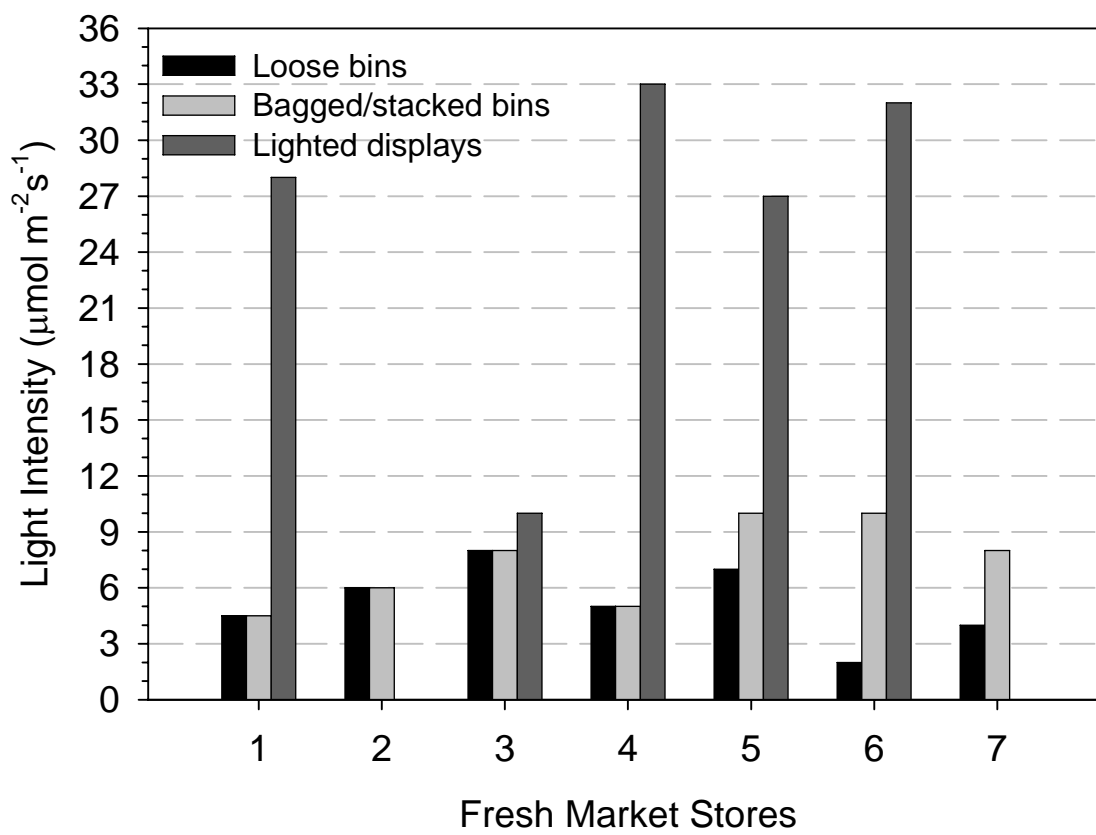


Fig. 1. Survey of light-intensities in displays of potatoes at local grocery stores. Light intensities were measured at tuber height in $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$.

Fig. 2. Progressive greening of White Rose tubers over a 5-day interval at 23°C. Five replicates of tubers were exposed to $6.8 \mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ light intensity from fluorescent bulbs (24 h photoperiod). Color and total chlorophyll content of the tubers are quantified in Fig. 3.

Days of Greening
($\sim 6.8 \mu\text{mol m}^{-2} \text{sec}^{-1}$)

White Rose



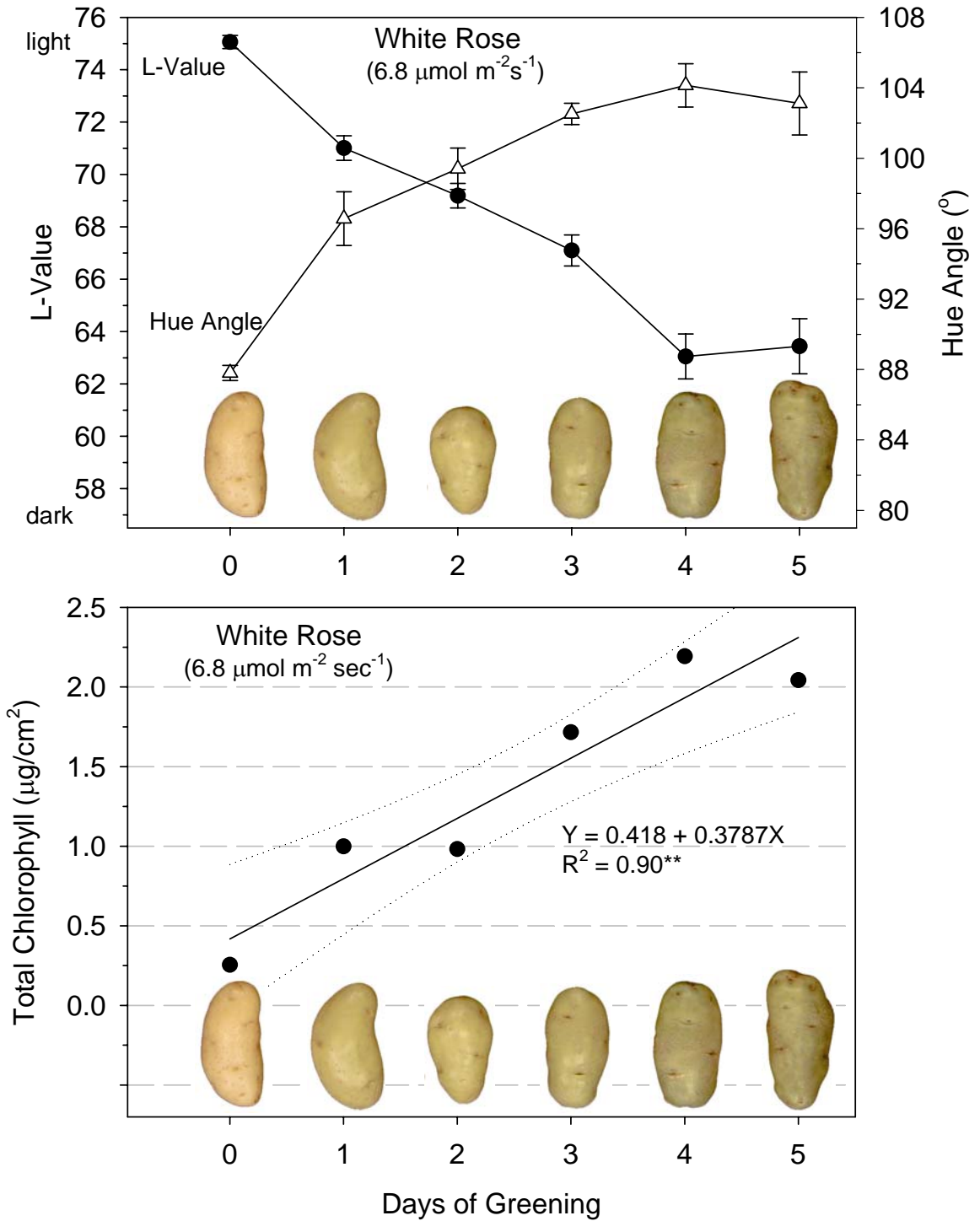


Fig. 3. Changes in L-value (relative darkness), hue angle and total chlorophyll content of White Rose tubers during 5 days of greening at 23°C. Tubers were incubated under cool white fluorescent light ($6.8 \mu\text{mol quanta m}^{-2}\text{sec}^{-1}$) for 24 h per day (see Fig. 2). Chlorophyll was extracted from 1-mm-thick x 1.5-cm-diameter discs of periderm from the side of the tubers facing the light. Dotted lines indicate 95% confidence intervals. Bars represent SE of the mean. $**P \leq 0.01$.

Fig. 4. Greening scale for White Rose tubers. Tubers were greened for 5 days under fluorescent light (see Fig. 2) and then sorted subjectively (based on visual differences in color) into ten greening levels. Color and total chlorophyll content of tubers in each greening level are quantified in Fig. 5.

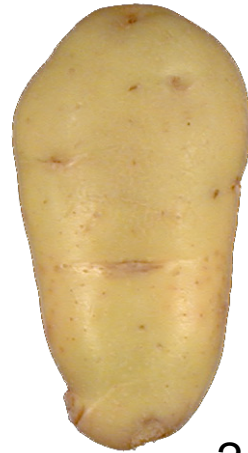
White Rose Greening Scale



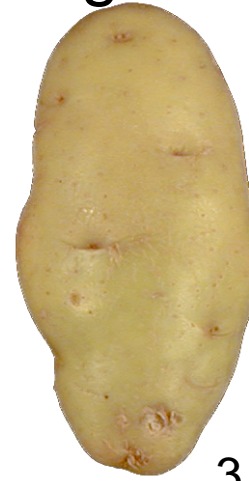
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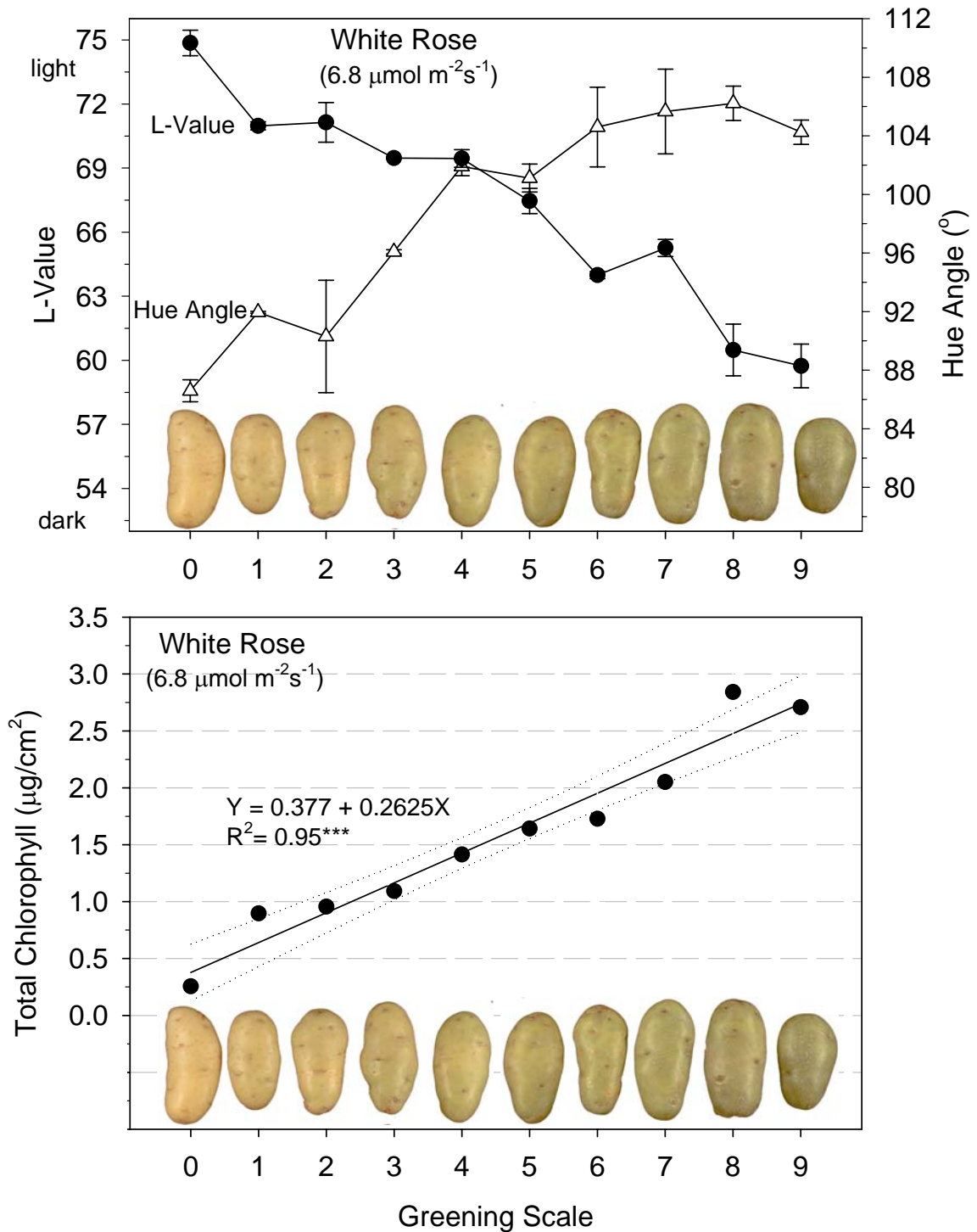


Fig. 5. Changes in L-value (relative darkness), hue angle and total chlorophyll content of White Rose tubers over ten levels of greening. Tubers were greened for 5 days at 23°C (see Fig. 2) and sorted into ten categories based on visual color differences (see Fig. 4). Note that the visual perception of greening in this cultivar is linear with respect to chlorophyll content of tubers. Dotted lines indicate 95% confidence intervals. Bars represent SE of the mean. *** $P \leq 0.001$.

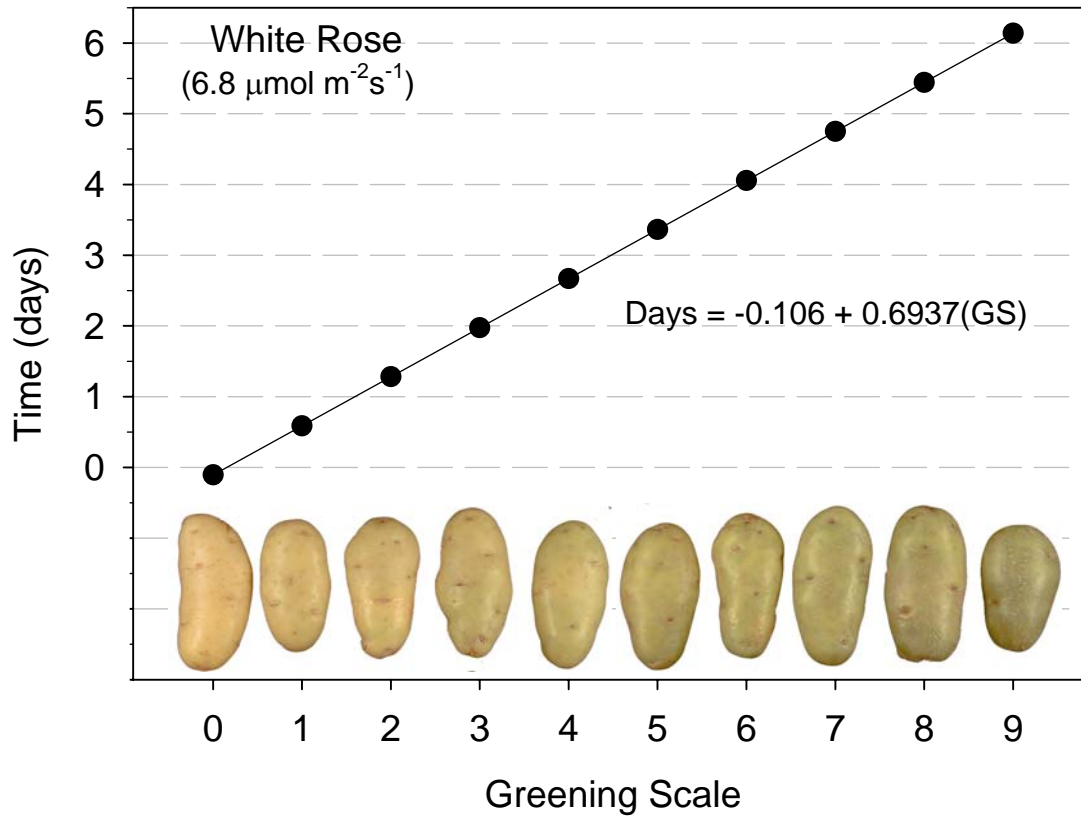


Fig. 6. Estimated time required for White Rose tubers to green over the ten-level greening scale. This graph was derived by calculating the chlorophyll content in tubers associated with each level of greening (Fig. 5 bottom) and then estimating the corresponding days of greening (from the linear equation at the bottom of Fig. 3). Note that about 17 h of constant (24-h) exposure to $6.8 \mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ light intensity are required for tubers to change one level on the greening scale. This relationship can be used to estimate the remaining shelf life for any given greening level. GS = Greening scale.

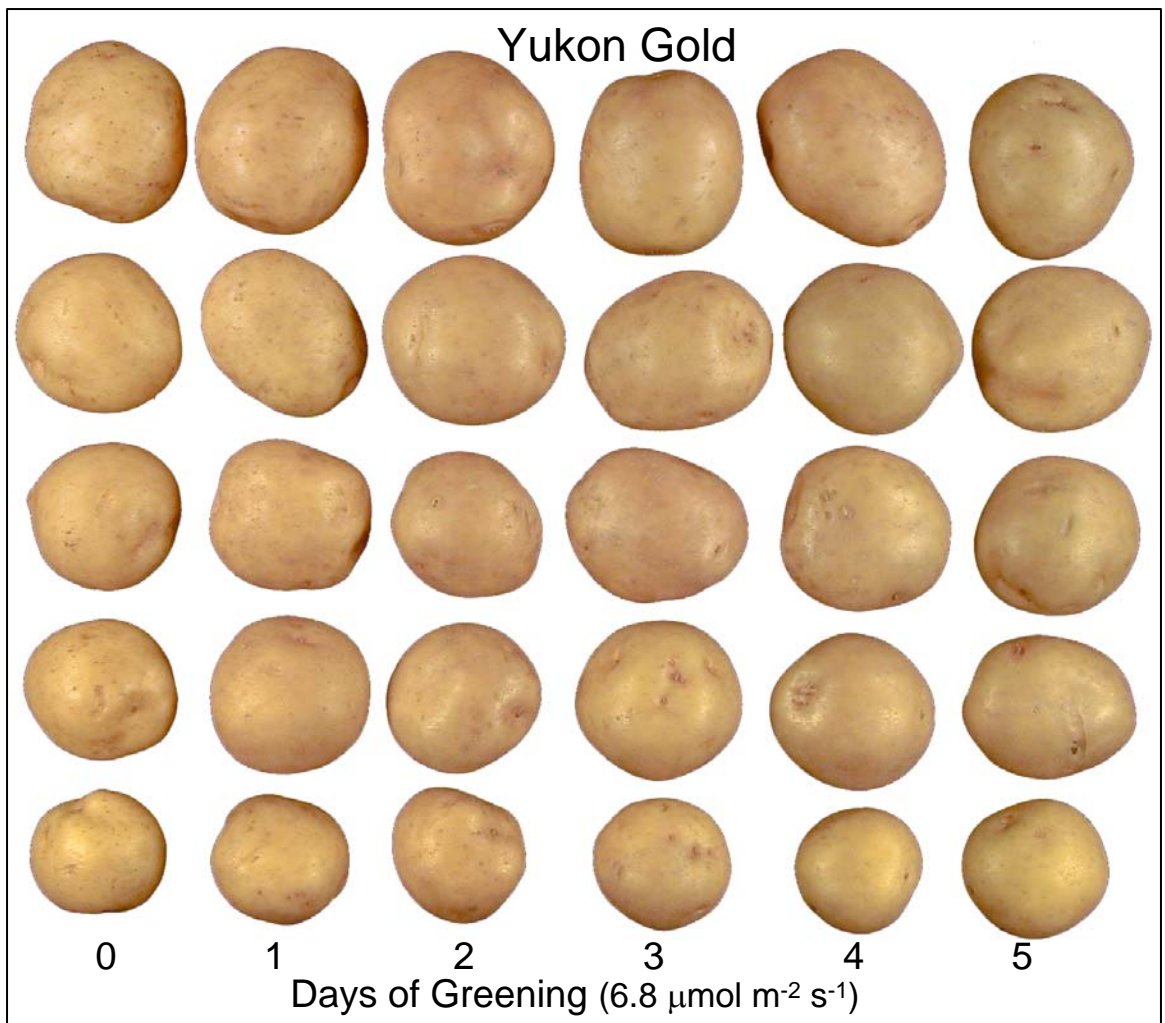


Fig. 7. Greening of Yukon Gold tubers over a 5-day interval at 23°C. Five replicates of tubers were exposed to $6.8 \mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ light intensity from fluorescent bulbs (24 h photoperiod). Color and total chlorophyll content of the tubers are quantified in Fig. 8.

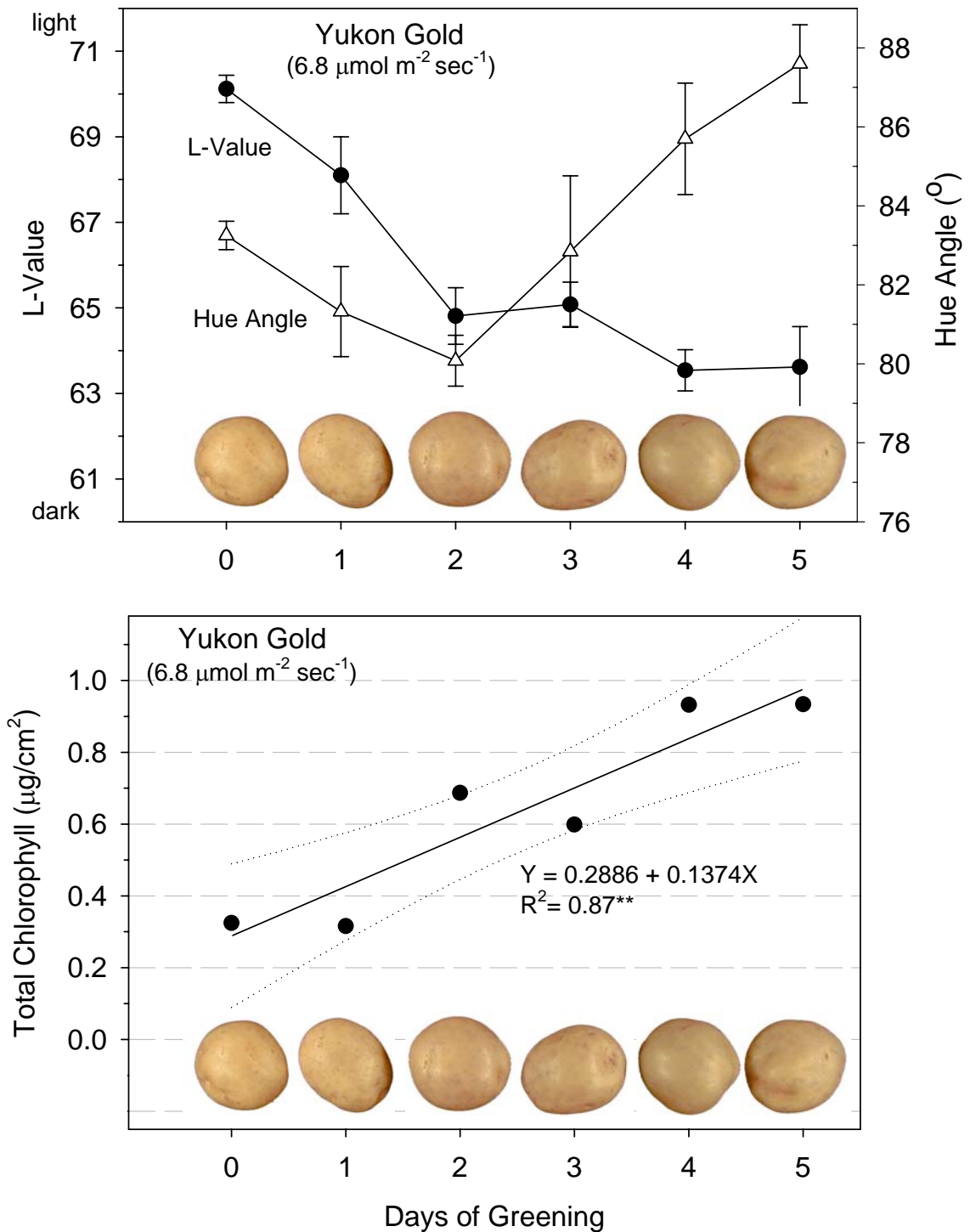


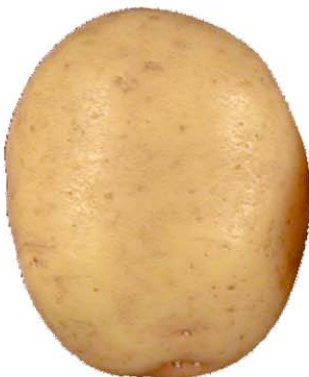
Fig. 8. Changes in L-value (relative darkness), hue angle and total chlorophyll content of Yukon Gold tubers during 5 days of greening at 23°C. Tubers were incubated under cool white fluorescent light ($6.8 \mu\text{mol quanta m}^{-2} \text{sec}^{-1}$) for 24 h per day (see Fig. 7). Chlorophyll was extracted from 1-mm-thick x 1.5-cm-diameter discs of periderm cut from the side of the tubers facing the light. Dotted lines indicate 95% confidence intervals. Bars represent SE of the mean. ** $P \leq 0.01$.

Fig. 9. Greening scale for Yukon Gold tubers. Tubers were greened for 5 days under fluorescent light and then sorted subjectively (based on visual differences in color) into eight greening levels. Color and total chlorophyll content of tubers in each greening level are quantified in Fig. 10.

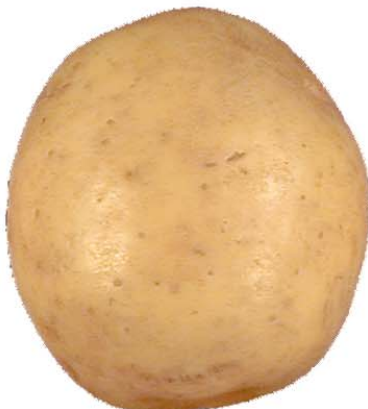
Yukon Gold Greening Scale



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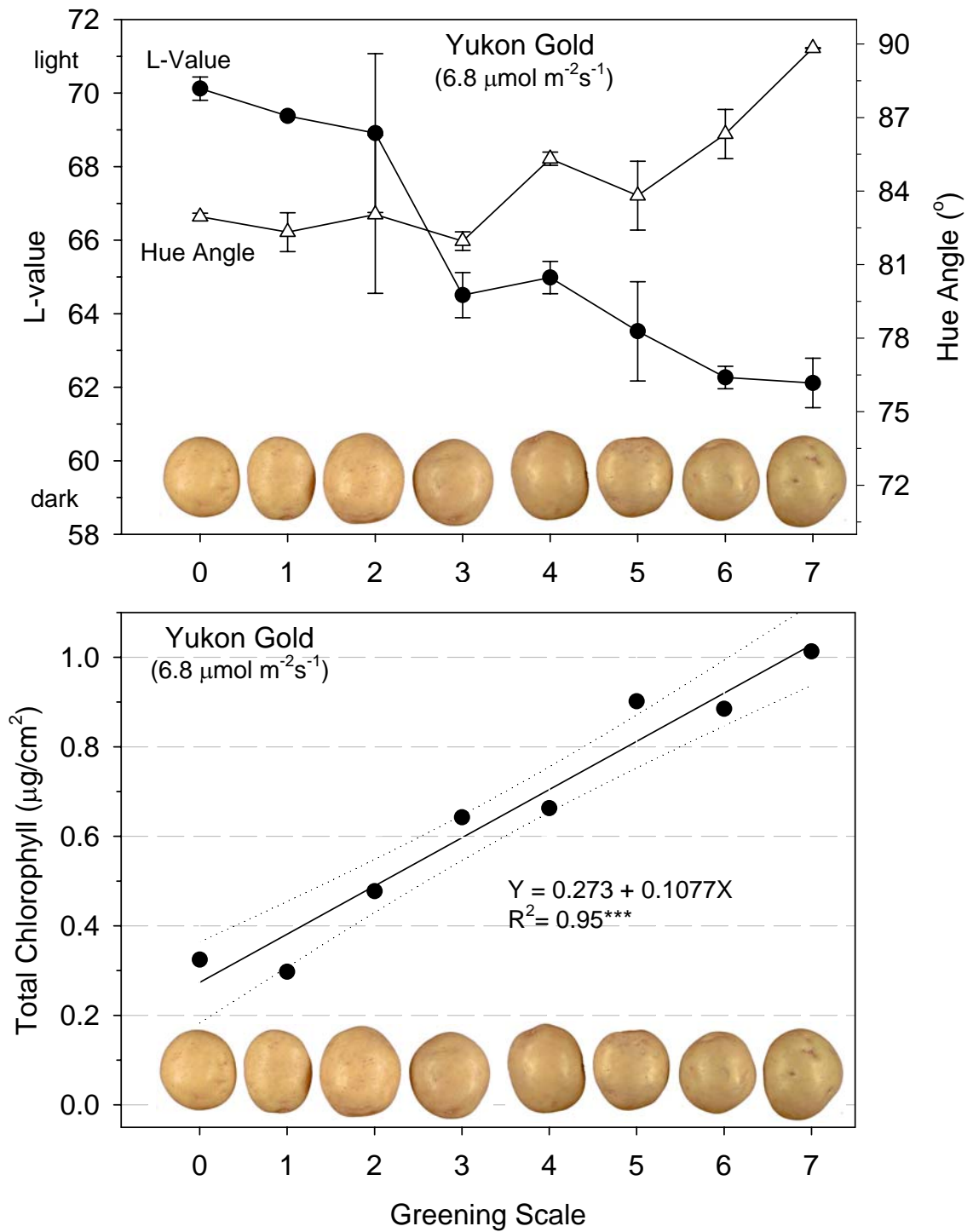


Fig. 10. Changes in L-value (relative darkness), hue angle and total chlorophyll content of Yukon Gold tubers over eight levels of greening. Tubers were greened for 5 days at 23°C (see Fig. 7) and sorted into eight categories based on visual color differences (see Fig. 9). Note that the visual perception of greening in this cultivar is linear with respect to chlorophyll content of tubers. Dotted lines indicate 95% confidence intervals. Bars represent SE of the mean. *** $P \leq 0.001$.

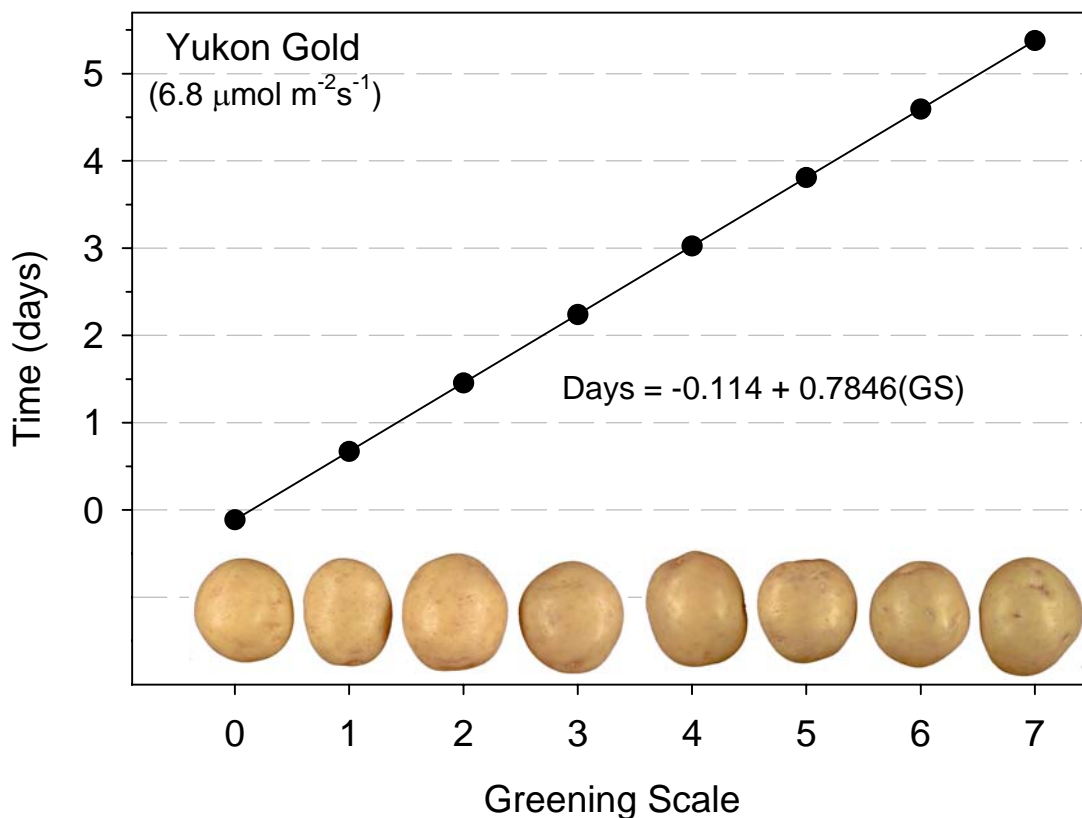


Fig. 11. Estimated time required for Yukon Gold tubers to green over the eight-level greening scale. This graph was derived by calculating the chlorophyll content in tubers associated with each level of greening (Fig. 10 bottom) and using these values to estimate (from the equation at the bottom of Fig. 8) the days of greening required to produce each level of chlorophyll. Note that about 19 h of constant (24-h) exposure to $6.8 \mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ light intensity are required for tubers to change one level on the greening scale. This relationship can be used to estimate the remaining shelf life, given a particular greening level.

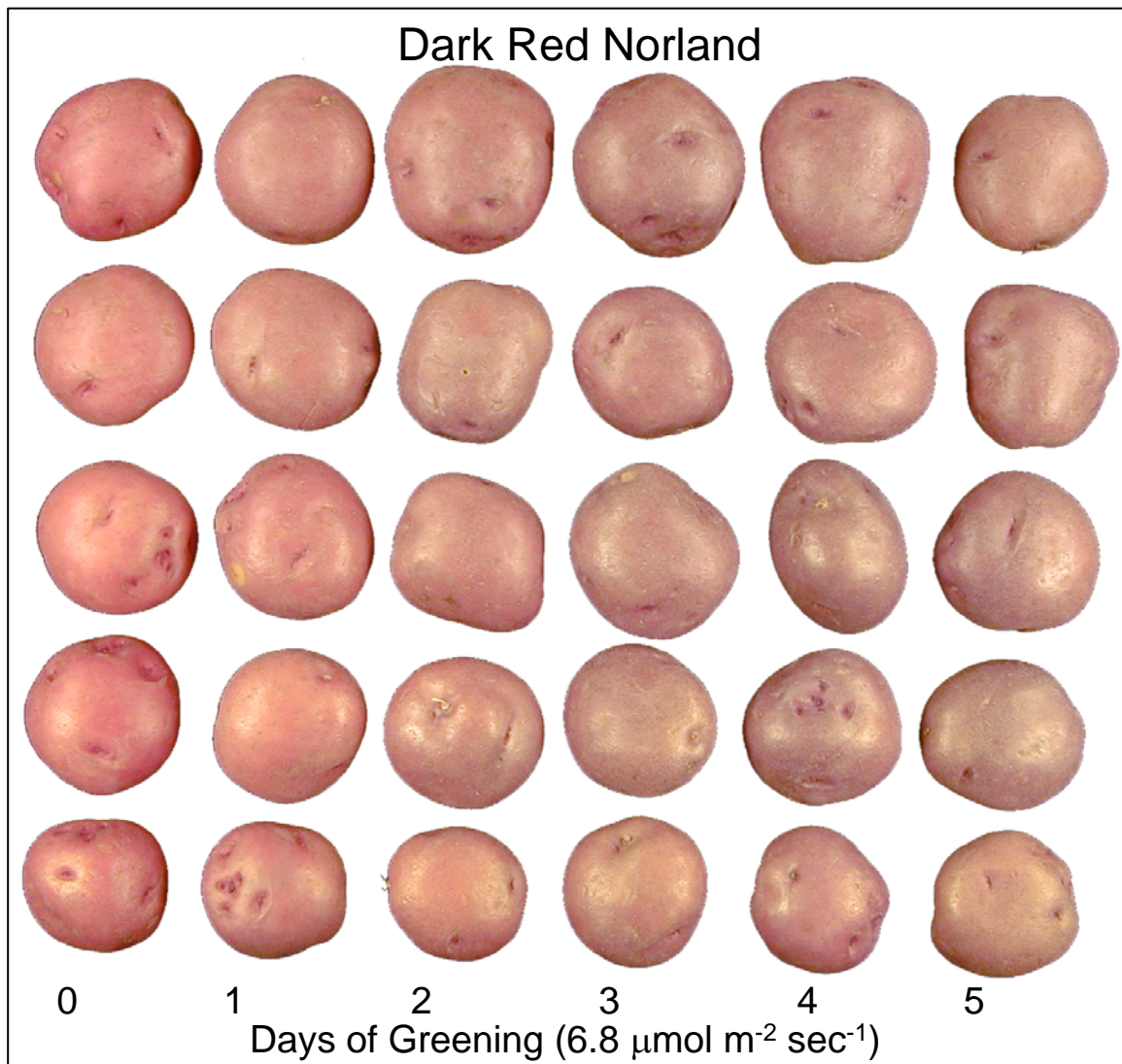


Fig. 12. Greening of Dark Red Norland tubers over a 5-day interval at 23°C. Five replicates of tubers were exposed to $6.8 \mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ light intensity from fluorescent bulbs (24 h photoperiod). Color and total chlorophyll content of the tubers are quantified in Fig. 13.

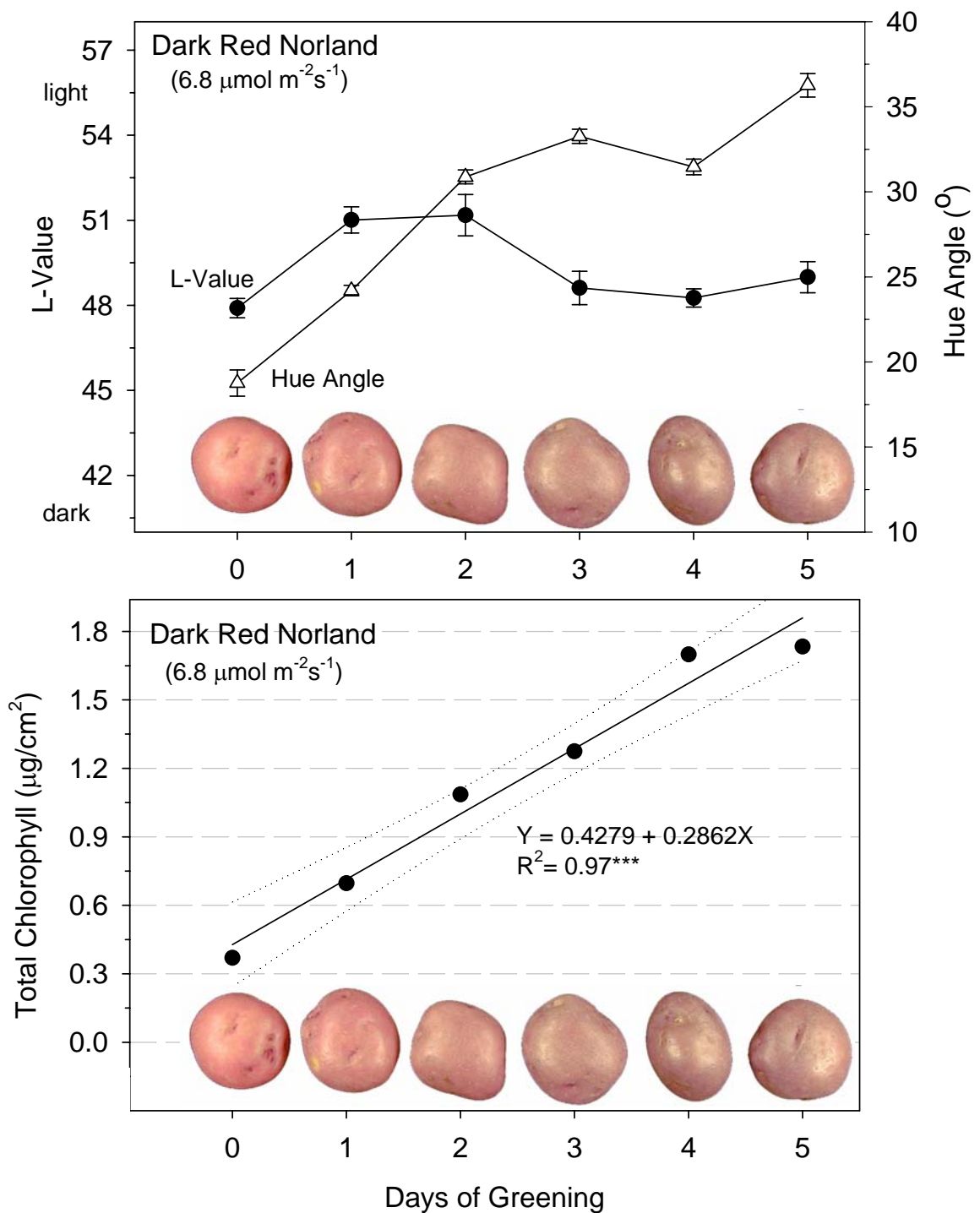


Fig. 13. Changes in L-value (relative darkness), hue angle and total chlorophyll content of Dark Red Norland tubers during 5 days of greening at 23°C. Tubers were incubated under cool white fluorescent light ($6.8 \mu\text{mol quanta m}^{-2} \text{sec}^{-1}$) for 24 h per day (see Fig. 12). Chlorophyll was extracted from 1-mm-thick x 1.5-cm-diameter discs of periderm from the side of the tubers facing the light. Dotted lines indicate 95% confidence intervals. Bars represent SE of the mean. *** $P \leq 0.001$.

Fig 14. Greening scale for Dark Red Norland tubers. Tubers were greened for 5 days under fluorescent light (see Fig. 12) and then sorted subjectively (based on visual differences in color) into eight greening levels. Color and total chlorophyll content of tubers in each greening level are quantified in Fig. 15.

Dark Red Norland Greening Scale



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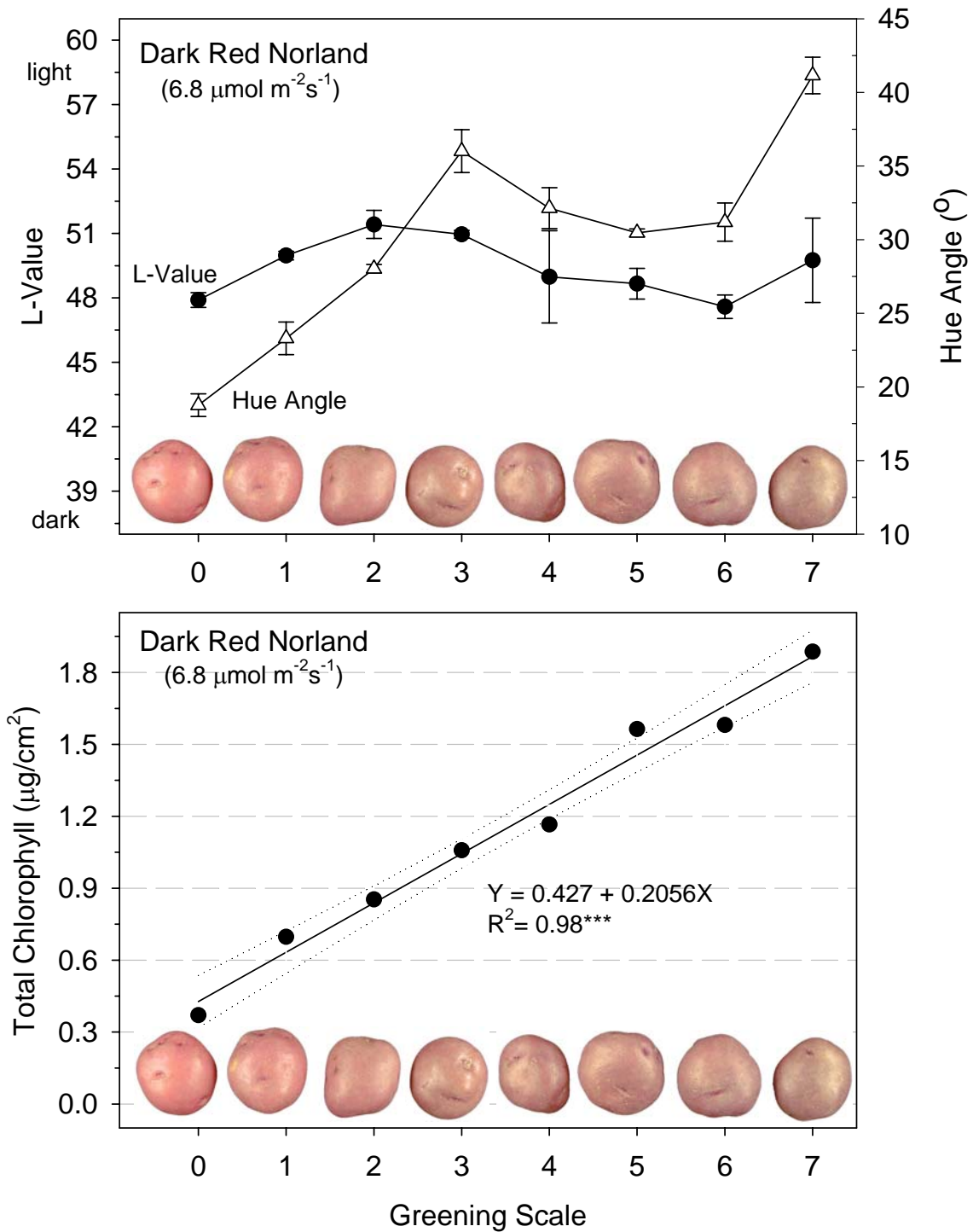


Fig. 15. Changes in L-value (relative darkness), hue angle and total chlorophyll content of Dark Red Norland tubers over eight levels of greening. Tubers were greened for 5 days at 23°C (see Fig. 12) and sorted into eight categories based on visual color differences (see Fig. 14). Note that the visual perception of greening in this cultivar is linear with respect to chlorophyll content of tubers. Dotted lines indicate 95% confidence intervals. Bars represent SE of the mean. *** $P \leq 0.001$.

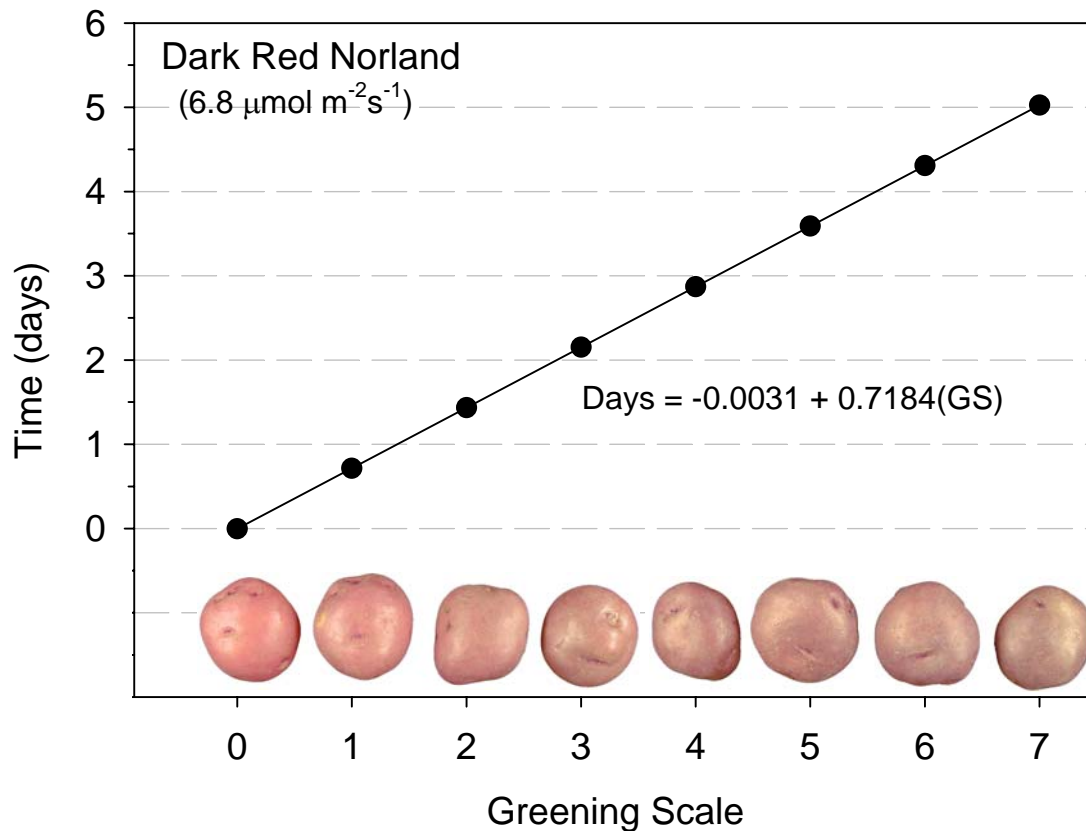


Fig. 16. Estimated time required for Dark Red Norland tubers to green over the eight-level greening scale. This graph was derived by calculating the chlorophyll content in tubers associated with each level of greening (Fig. 15 bottom) and using these values to estimate (using the linear equation at the bottom of Fig. 13) the days of greening required for tubers to produce those levels of chlorophyll. Note that about 17 h of constant (24-h) exposure to $6.8 \mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ light intensity are required for tubers to change one level on the greening scale. This relationship could be useful for estimating the remaining shelf life for any given greening level.

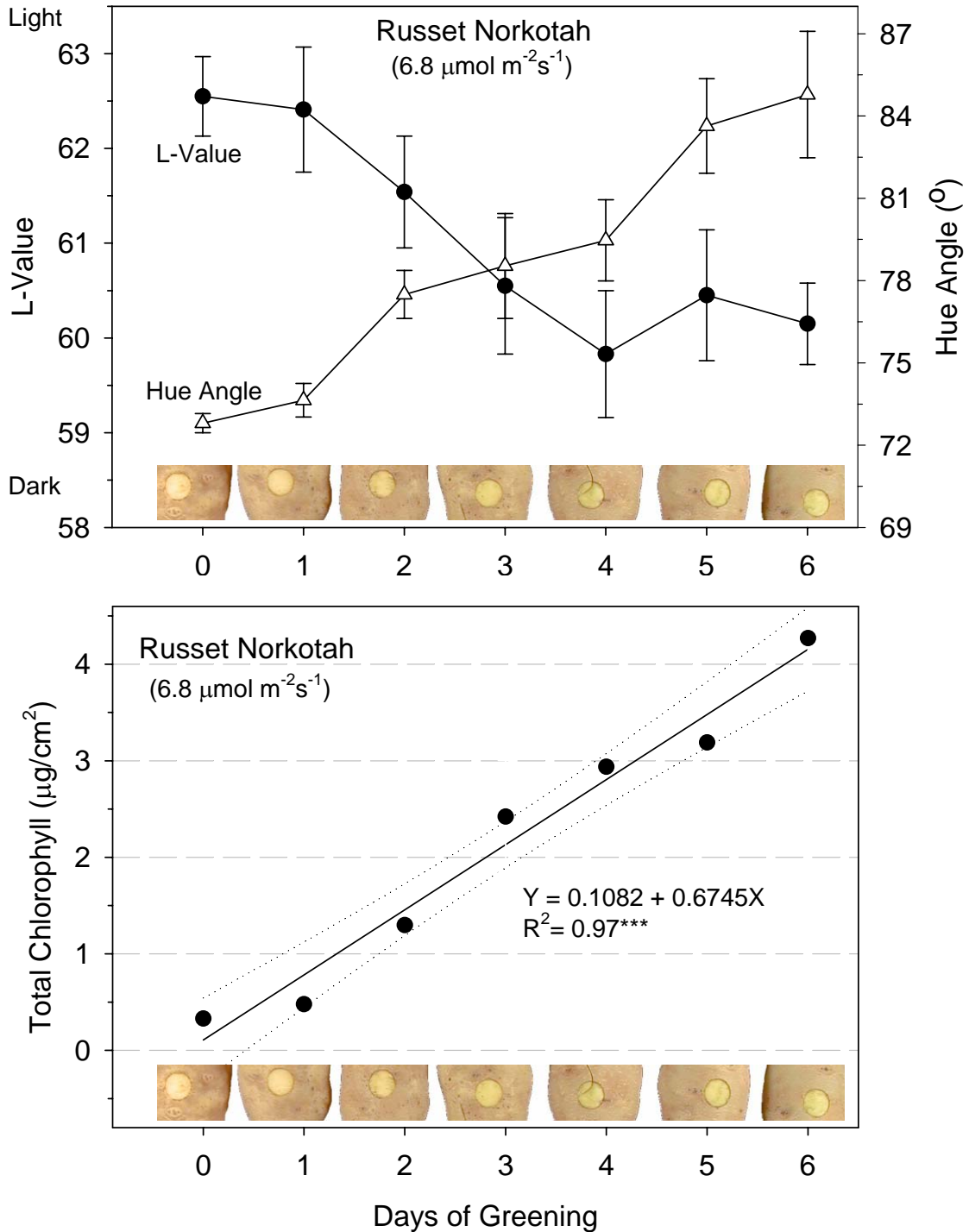


Fig. 17. Changes in L-value (relative darkness), hue angle and total chlorophyll content of Russet Norkotah tubers during 5 days of greening at 23°C. Tubers were incubated under cool white fluorescent light ($6.8 \mu\text{mol quanta m}^{-2} \text{sec}^{-1}$) for 24 h per day. Chlorophyll was extracted from 1-mm-thick x 1.5-cm-diameter discs of periderm from the side of the tubers facing the light. Dotted lines indicate 95% confidence intervals. Bars represent SE of the mean. *** $P \leq 0.001$.

Russet Norkotah Greening Scale



Fig. 18. Greening scale for Russet Norkotah tubers. Tubers were greened for 5 days under fluorescent light and then sorted subjectively (based on visual differences in color) into eight greening levels. Color and total chlorophyll content of tubers in each greening level are quantified in Fig. 19.

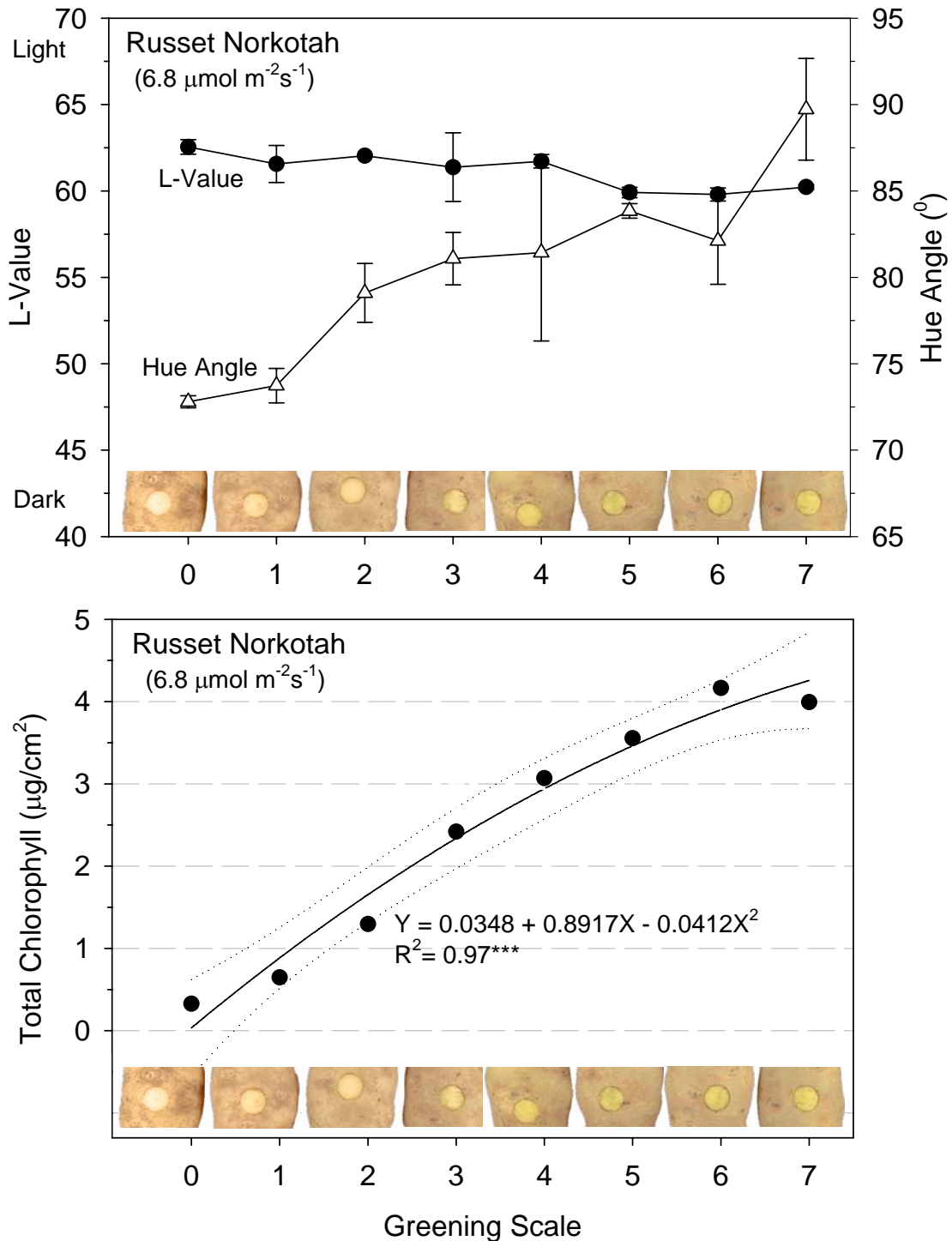


Fig. 19. Changes in L-value (relative darkness), hue angle and total chlorophyll content of Russet Norkotah tubers over eight levels of greening. Tubers were greened for 5 days at 23°C and sorted into eight categories based on visual color differences (see Fig. 18). Note that the ability to visually discern changes in chlorophyll content decreases over the latter (stages 6 to 7) part of the scale. Dotted lines indicate 95% confidence intervals. Bars represent SE of the mean. *** $P \leq 0.001$.

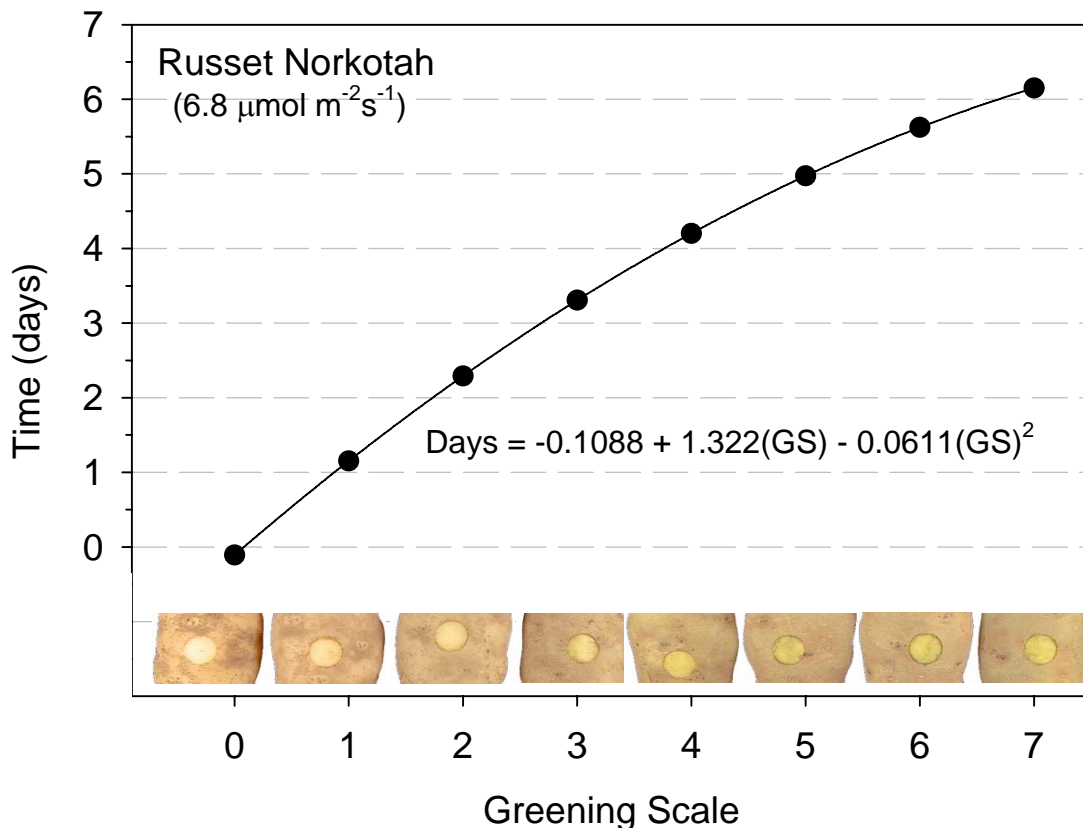


Fig. 20. Estimated time required for Russet Norkotah tubers to green over the eight-level greening scale. This graph was derived by calculating the chlorophyll content in tubers associated with each level of greening (Fig. 19 bottom) and then estimating (from the linear equation at the bottom of Fig. 17) the days of greening required to produce each level of chlorophyll. Note that approximately 27 h of exposure to light are required to induce incremental shifts in greening level from green-0 to green-3, as compared with only 16 h from green-4 to green-7. This is partly due to the reduced ability to visually perceive changes in tuber color of this russet-skin cultivar when chlorophyll concentration increases beyond about $3 \mu\text{g}/\text{cm}^2$. An estimate of the remaining shelf life of tubers exposed to $6.8 \mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ light intensity for any greening level can be derived from this graph.

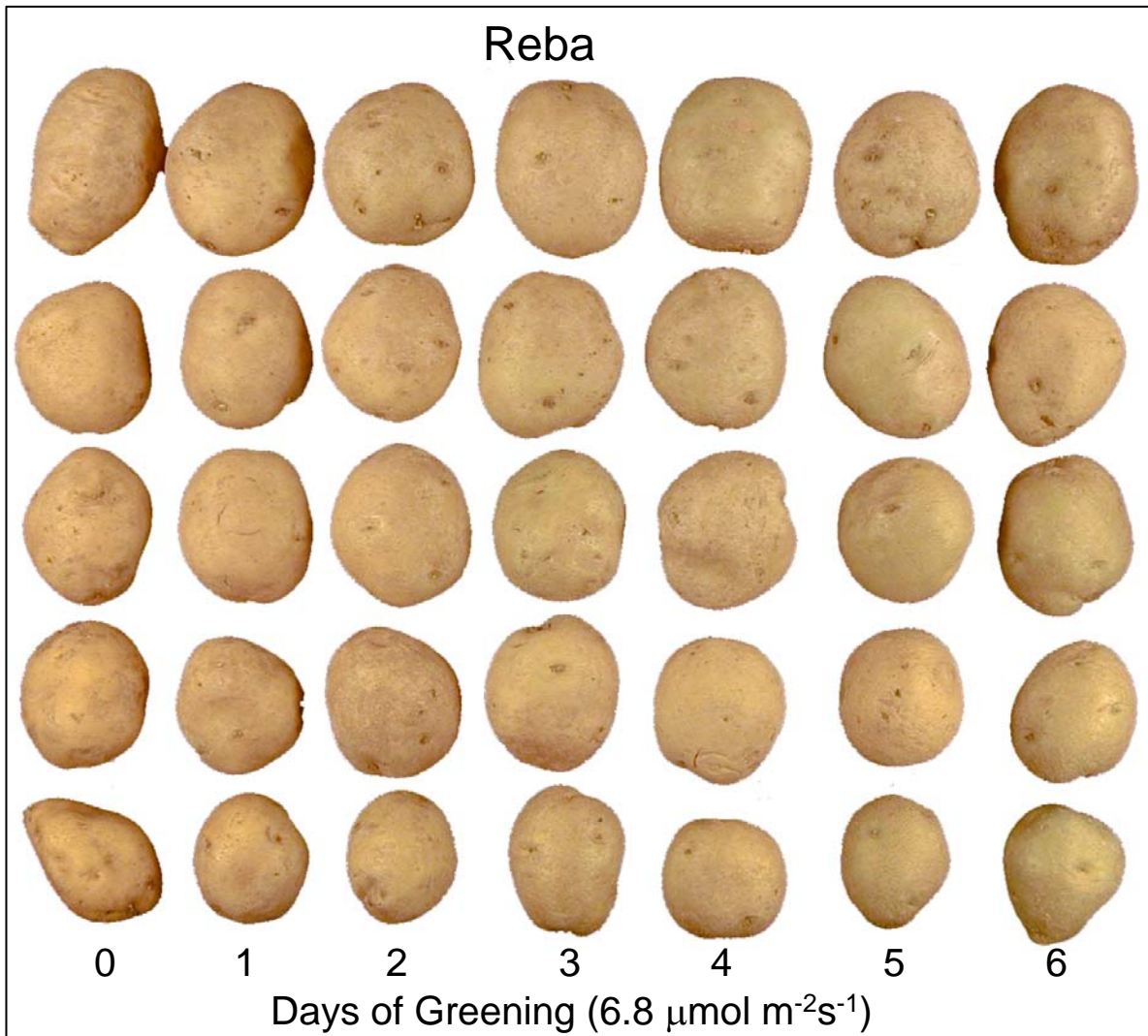


Fig. 21. Greening of Reba tubers over a 6-day interval at 23°C. Five replicates of tubers were exposed to $6.8 \mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ light intensity from fluorescent bulbs (24 h photoperiod). Color and total chlorophyll content of the tubers are quantified in Fig. 22.

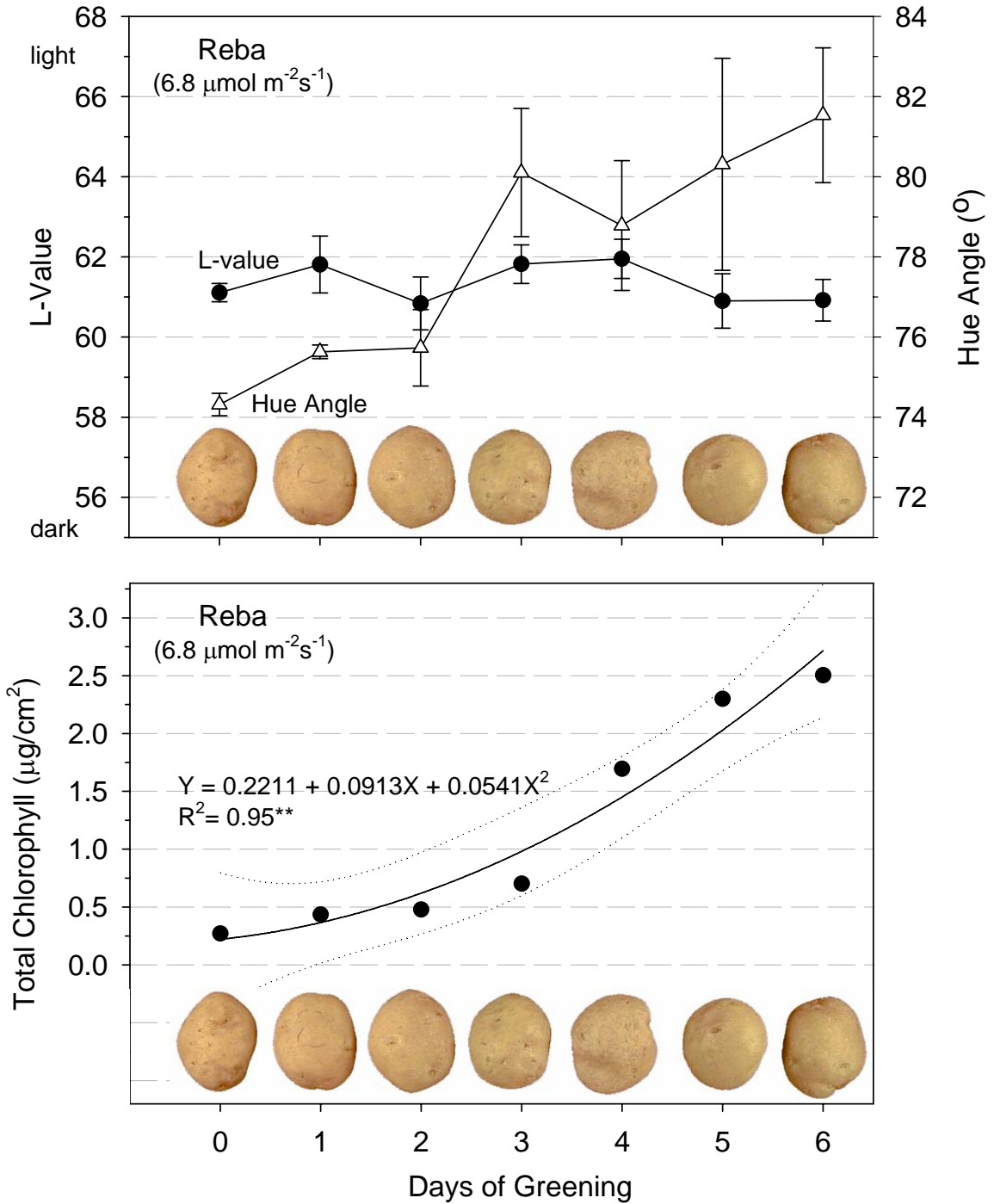
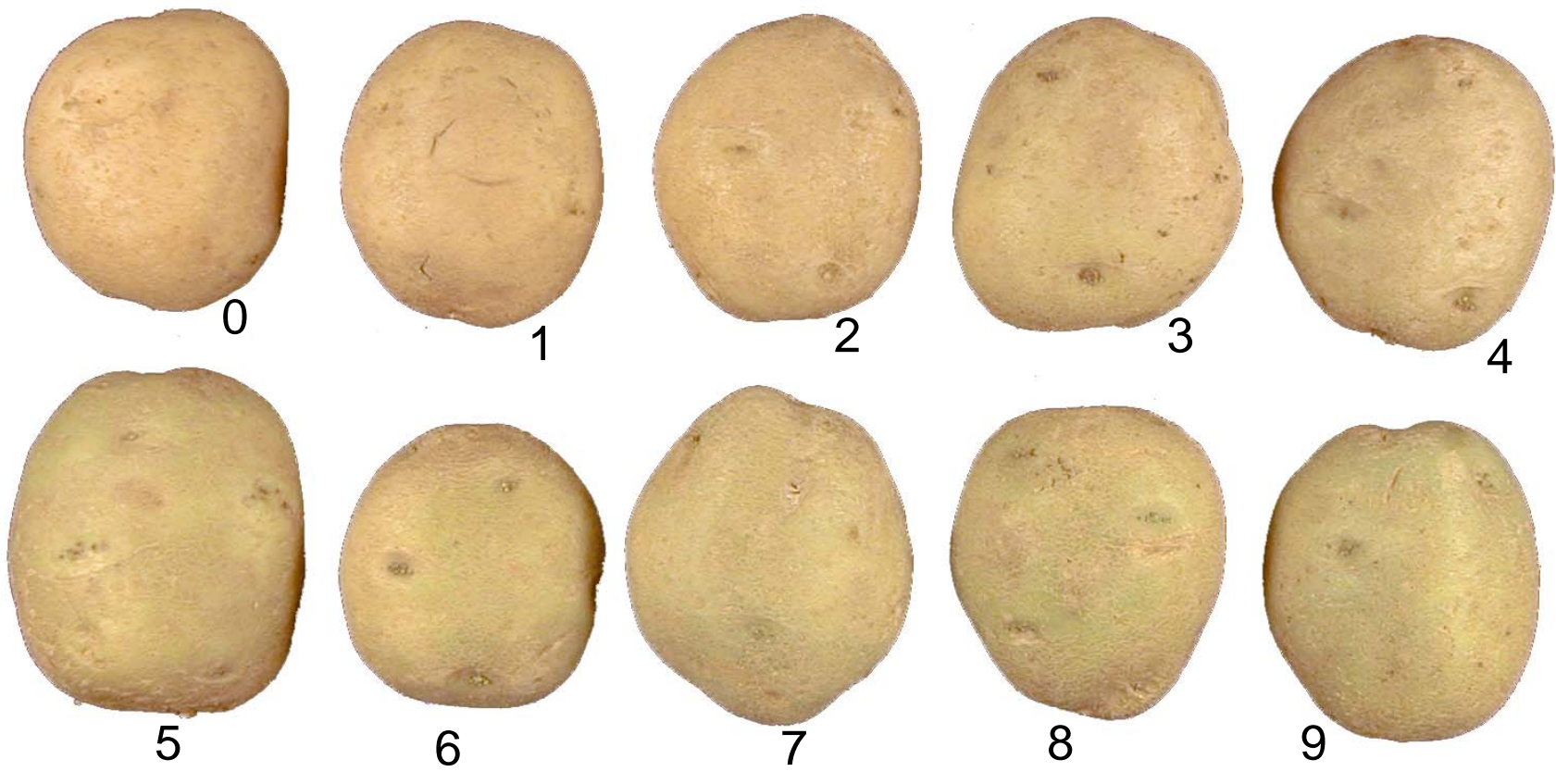


Fig. 22. Changes in L-value (relative darkness), hue angle and total chlorophyll content of Reba tubers during 6 days of greening at 23°C. Tubers were incubated under cool white fluorescent light (6.8 $\mu\text{mol quanta m}^{-2} \text{sec}^{-1}$) for 24 h per day. Chlorophyll was extracted from 1-mm-thick x 1.5-cm-diameter discs of periderm from the side of the tubers facing the light. Dotted lines indicate 95% confidence intervals. Bars represent SE of the mean. **P \leq 0.01.

Reba Greening Scale



48

Fig 23. Greening scale for Reba tubers. Tubers were greened for 6 days under fluorescent light (see Fig. 21) and then sorted subjectively (based on visual differences in color) into ten greening levels. Color and total chlorophyll content of tubers in each greening level are quantified in Fig. 24.

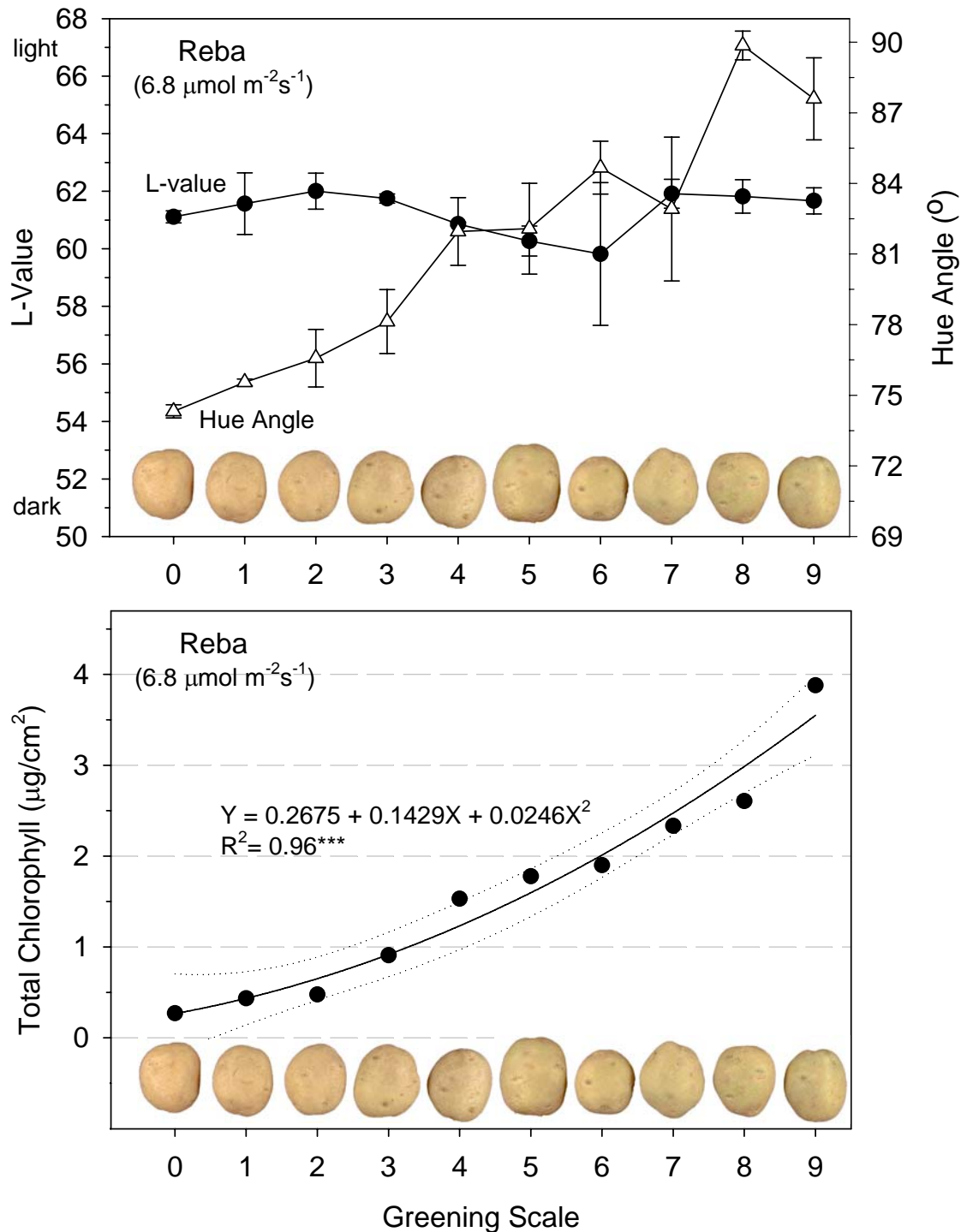


Fig. 24. Changes in L-value (relative darkness), hue angle and total chlorophyll content of Reba tubers over ten levels of greening. Tubers were greened for 6 days at 23°C and sorted into ten categories based on visual color differences (see Fig. 23). Dotted lines indicate 95% confidence intervals. Bars represent SE of the mean. $***P \leq 0.001$.

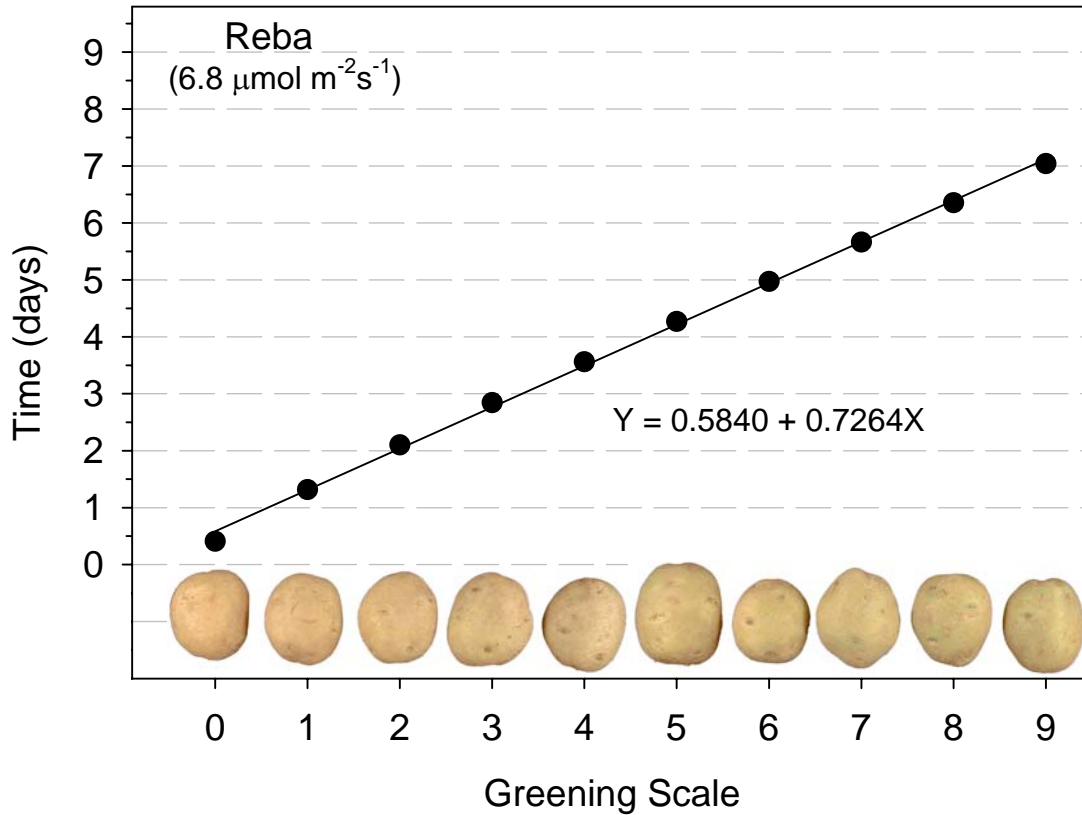
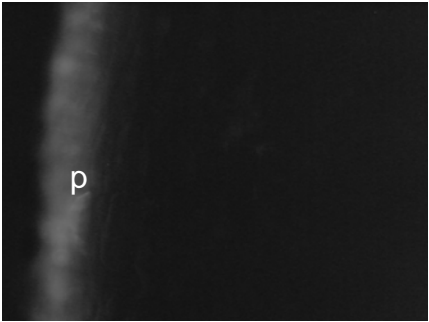
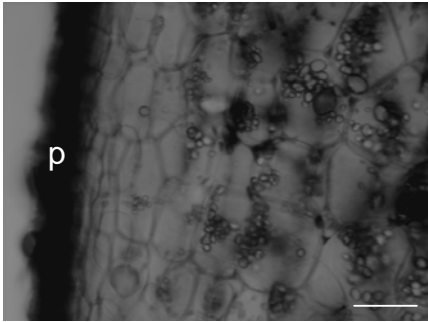


Fig. 25. Estimated time required for Reba tubers to green over the ten-level greening scale. This graph was derived by calculating the chlorophyll content in tubers associated with each level of greening (Fig. 24 bottom) and using these values to estimate (using the linear equation at the bottom of Fig. 22) the days of greening required for tubers to produce those levels of chlorophyll. Note that about 17 h of exposure to light ($6.8 \mu\text{mol quanta m}^{-2} \text{sec}^{-1}$) are required for tubers to change one level on the greening scale.

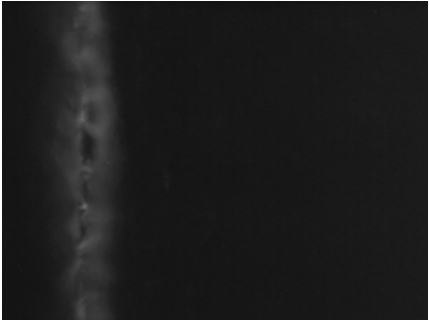
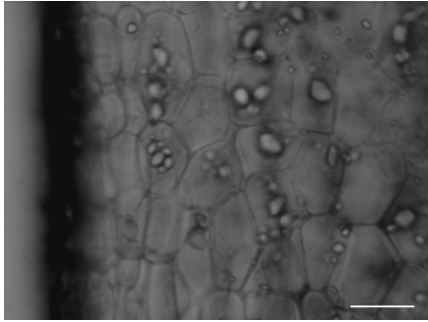
Fig. 26. Light (left panel) and fluorescence (right panel) micrographs of potato tuber periderm and underlying cortical tissue. White Rose tubers were greened at 23°C for 0 to 7 days at 6.8 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ light intensity. Note that the mature periderm (p) fluoresces due to the presence of suberin. Chlorophyll development is indicated by an increase in fluorescence associated with the conversion of amyloplasts (arrows). Bar = 30 μm .

White Rose

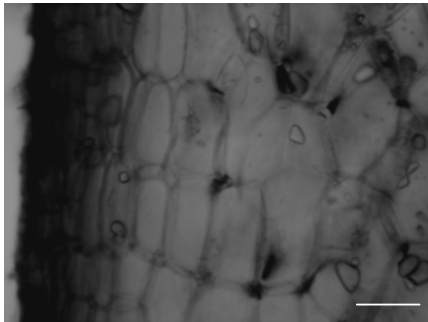
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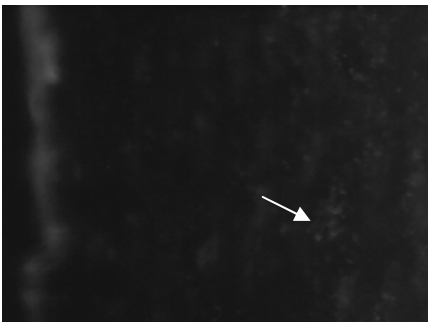
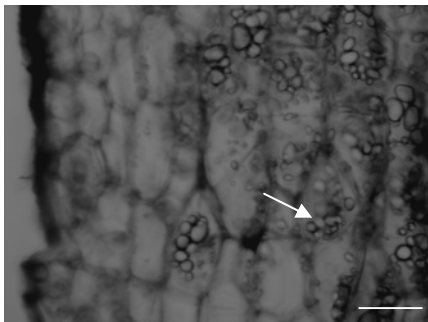
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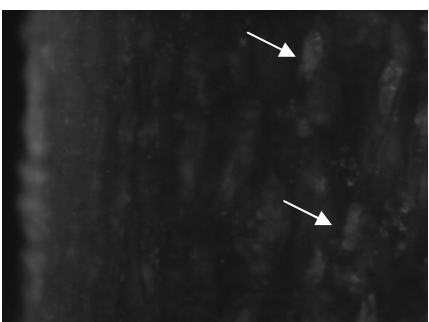
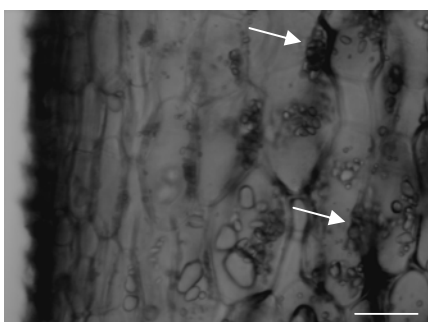
3 days



5 days



7 days



CHAPTER TWO

VALIDATION OF THE GREENING SCALES

Abstract

Subjective greening indices were developed in chapter one for use by the potato industry to grade or rate the extent of greening in fresh market potatoes (*Solanum tuberosum* L.). The focus of this study was to examine and validate the utility of the greening scales for grading tubers in retail environments. A survey of potatoes in local retail outlets showed a wide range in the extent of tuber greening, along with a large degree of variation in quality among stores. There was also a difference in the levels of greening among cultivars, likely due to differences in periderm color and consumer demand. The greening scales covered the full range of tuber greening observed in the retail outlets, validating their utility as a tool to facilitate greater quality control. The results underscore a need for the development and implementation of more consistent grading procedures, especially at the retail level.

Introduction

Exposure to light causes chlorophyll to develop in periderm and outer cortical tissues of potato, resulting in an undesirable discoloration of the tuber (Pavlista, 2001; Edwards, 1997). A perceived association between greening and the accumulation of toxic glycoalkaloids leads to discrimination by consumers, resulting in reduced marketability of tubers. Despite this, there are currently no specific grading criteria for evaluation of the extent of greening in tubers of fresh market cultivars. Therefore,

greening indices were developed (chapter one) for use by the industry to facilitate quality control in fresh market cultivars over a range of skin colors (Grunenfelder, 2005). The objective of the current study was to assess the utility of the greening scales developed for cvs. White Rose, Yukon Gold, Dark Red Norland, and Russet Norkotah. Tubers were randomly selected and purchased from local retail stores and graded for color using the appropriate greening scale. The range and extent of variability in greening were then evaluated for each cultivar, and the efficacy of the scales for use by retailers as a method for quality control was established.

Materials and Methods

Thirty tubers of each of four cultivars (White Rose, Yukon Gold, Dark Red Norland, and Russet Norkotah) were randomly selected from the shelves of local grocery retailers on four separate dates. The tubers of each cultivar were graded visually for color using the greening scales developed in chapter one. Objective color measurements (using CIELAB color system, as in chapter one) were taken to verify that the greening levels were consistent with the previously established scales. The percentages of tubers falling into each greening level are reported for each cultivar (n=120), averaged over the four stores and by individual store.

Results

A survey of the extent of greening of 'White Rose' tubers in local retail markets showed that the majority of tubers (89%) fell into the range of zero to three on the scale (Fig. 1, top), with the extent of greening never exceeding seven on the ten-level scale.

There was a relatively low percentage of tubers in the four to seven range. However, there was a large degree of variation among the individual stores in the proportion of tubers at each greening level (Fig. 1, bottom). The objective color measurements of L-value and hue angle fell within expected values for the respective greening levels for this cultivar (see chapter one; data not shown).

The Yukon Gold greening scale was used to evaluate the range of greening of tubers sampled from four retail markets. As with cv White Rose, the majority of Yukon Gold tubers (78%) fell into the range of green-0 to green-3, and the remaining tubers varied from green-4 to green-7 (Fig. 2, top). The proportion of tubers at each greening level varied among stores (Fig. 2, bottom). The L-values (measure of lightness) and hue angles of the tubers were within the range expected for each greening level (see chapter one; data not shown).

Approximately 34% of the 'Dark Red Norland' tubers showed a relatively high level of greening, thus falling into the range of green-4 to green-7 on the eight-level greening scale (Fig. 3, top). Moreover, there was wide variation among stores in the extent of greening of tubers of this red-skinned cultivar (Fig. 3, bottom). For example, approximately 50% of the tubers sampled from store D were graded as green-6 and green-7, indicating relatively high levels of chlorophyll. At the other extreme, most of the tubers from store B were graded as green-1 or green-2. The objective color measurements (L-value and hue angle) for the tubers were within the expected ranges (see chapter one; data not shown).

Green-1, green-2 and green-7 tubers accounted for 32%, 18% and 20%, of the Russet Norkotah tubers sampled; respectively (Fig. 4, top). This reflects a wide range in

the chlorophyll concentrations of Russet Norkotah tubers in retail markets. There was also a large degree of variation among stores in the proportion of tubers available at each greening level (Fig. 4, bottom). For example, store D had 45% green-7 tubers, whereas no tubers beyond green-2 were found in store B. This suggests a higher level of quality control for greening in store B. Objective color measurements (L-values and hue angles) were within the expected ranges for each greening level, with the exception of a few very green tubers that exceeded the values of the green-7 level and thus were outside the range of the eight-level scale.

Discussion and Conclusions

Tubers were sampled from four grocery stores and the extent of greening assessed to evaluate the utility of the greening scales developed in chapter one for each of the four cultivars. The majority of light-skinned, light-fleshed tubers fell into the range of zero to three, with the extent of greening never exceeding seven on the scale (Figs. 1 and 2). The relatively low percentage of tubers in the range from four to seven is likely a consequence of quality control efforts imposed by produce managers, in combination with consumer-dependent turnover of potatoes on the shelves. However, the darker skinned cultivars (red and russet) had a higher percentage of very green tubers (rating above a level 5 on the scales) (Figs. 3 and 4). This may be due to differences in periderm thickness and the presence of accessory pigments that mask the green color, resulting in less discrimination by consumers and less cullage at the retail level. Rather than developing a distinctive green color (as shown by cv. White Rose, Fig. 1), the red-skinned and russet tubers developed a bronze or “dirty” color, reflecting the combination

of periderm coloration (accessory pigments) with the underlying (green) chlorophyll. Interestingly, there was considerable variation among stores with regard to the proportion of tubers in each category, reflecting variable or inconsistent quality control (Figs. 1-4). Clearly, there is a need for the development and implementation of more consistent grading procedures and methods to reduce greening in retail markets. Overall, the greening scales developed in chapter one were demonstrated to be effective for subjectively rating the extent of tuber greening in retail outlets. The grading scales covered the full range of greening found in stores, demonstrating their potential utility as an aid to quality control.

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Fig. 1. Survey of the extent of greening of White Rose potatoes in retail outlets. Tubers were purchased from four stores and graded for color using the ten-level greening scale (tubers at top; ch. 1, Fig. 4). Thirty tubers were randomly sampled from each store. Upper graph shows the averaged percentage of tubers in each category. Lower graph shows the store-to store variation in greening.

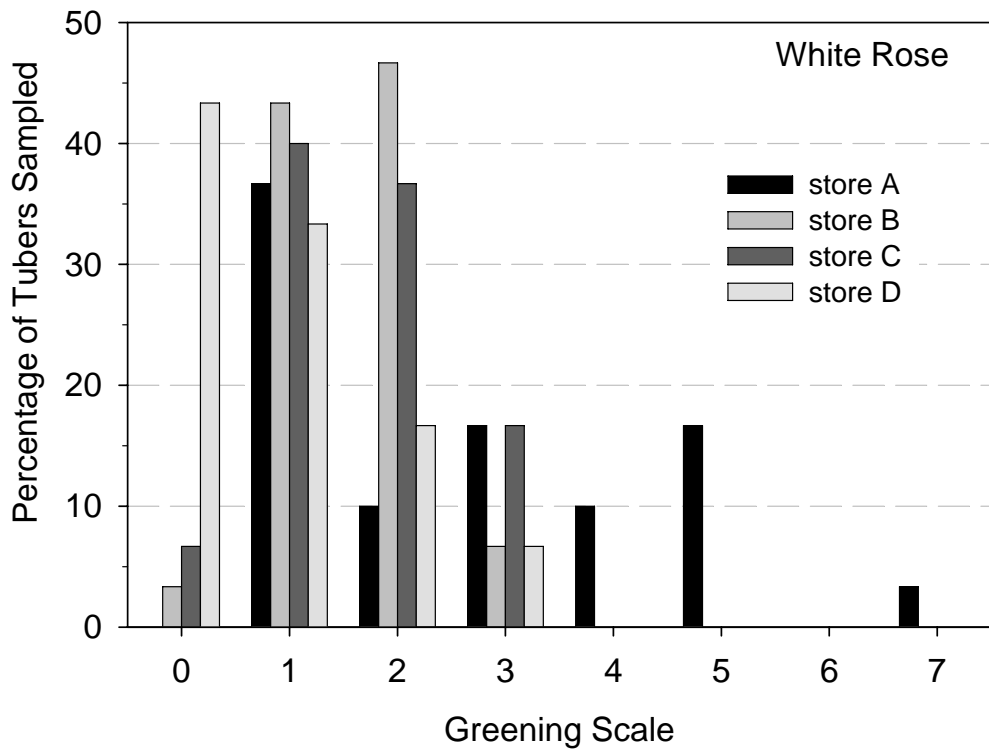
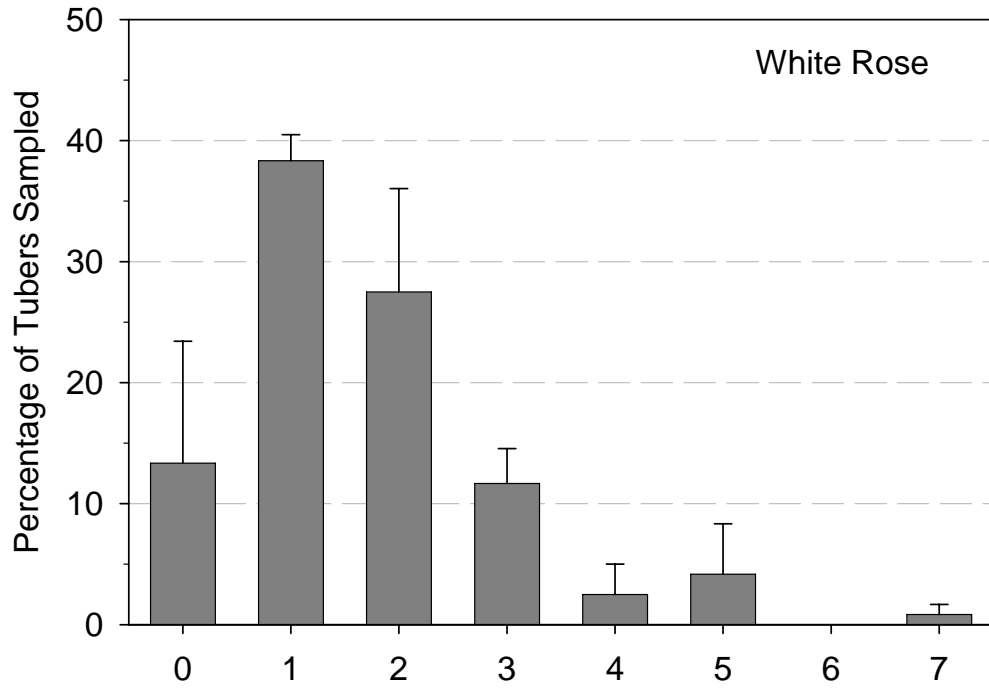


Fig. 2. Survey of the extent of greening of Yukon Gold potatoes in retail outlets. Tubers were purchased from four stores and graded for color using the eight-level greening scale (tubers at top; ch. 1, Fig. 9). Thirty tubers were sampled from each store. Upper graph shows the percentage of tubers in each category averaged over all stores. Lower graph shows the store-to-store variation in greening.

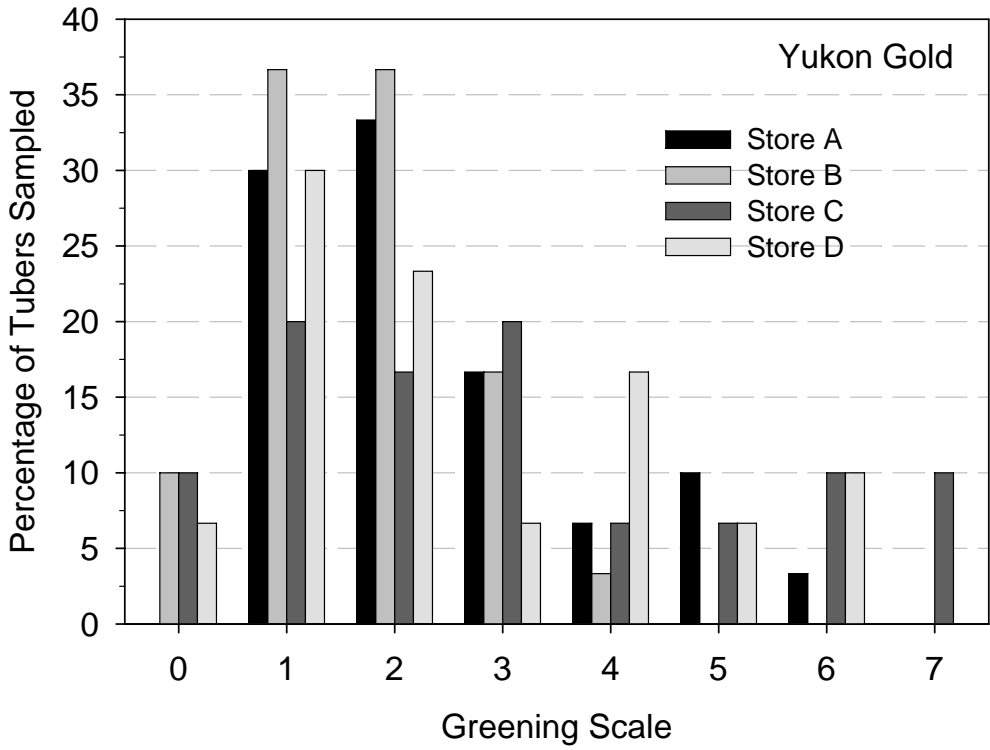
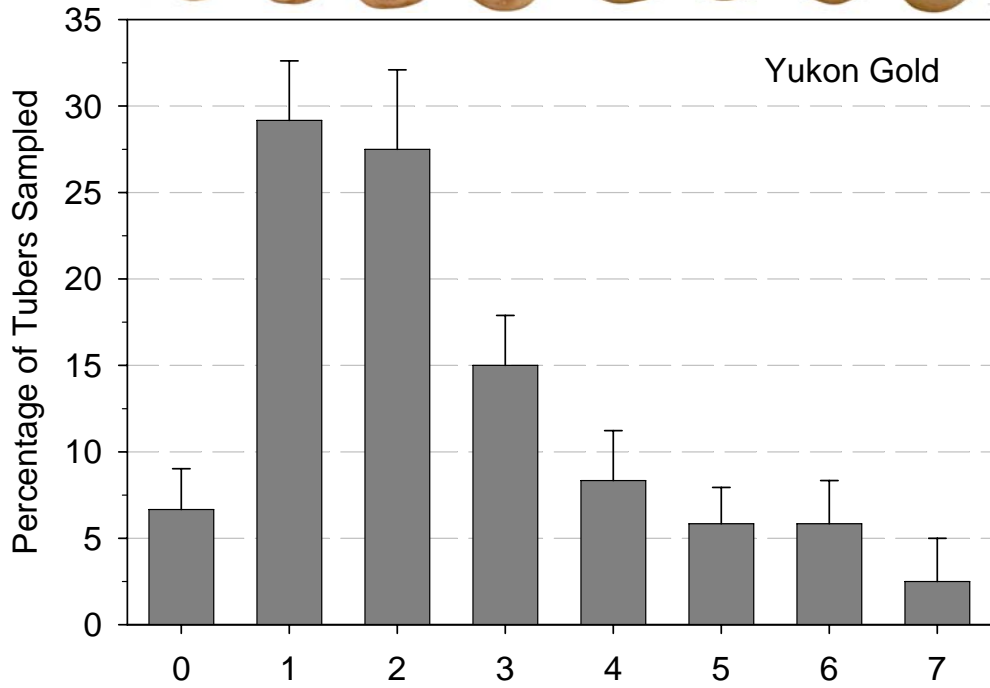


Fig. 3. Survey of the extent of greening of Dark Red Norland potatoes in retail outlets. Tubers were purchased from four stores and graded for color using the eight-level greening scale (tubers at top; ch. 1, Fig. 14). Thirty tubers were sampled from each store. Upper graph shows the percentage of tubers in each category averaged over all stores. Lower graph shows the store-to-store variation in greening.

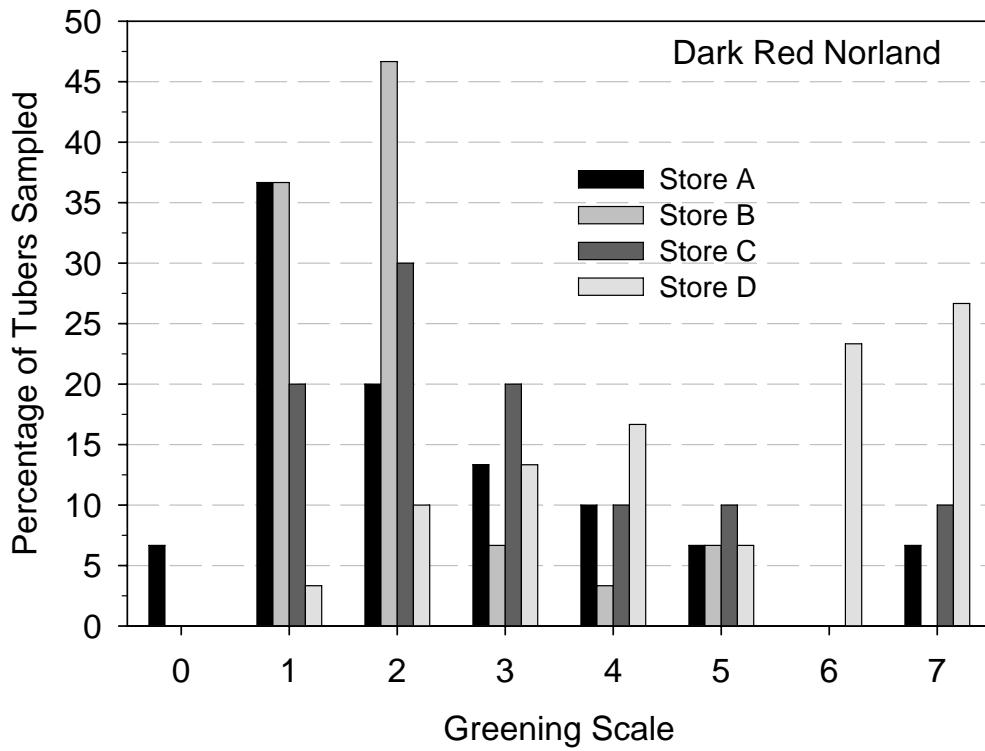
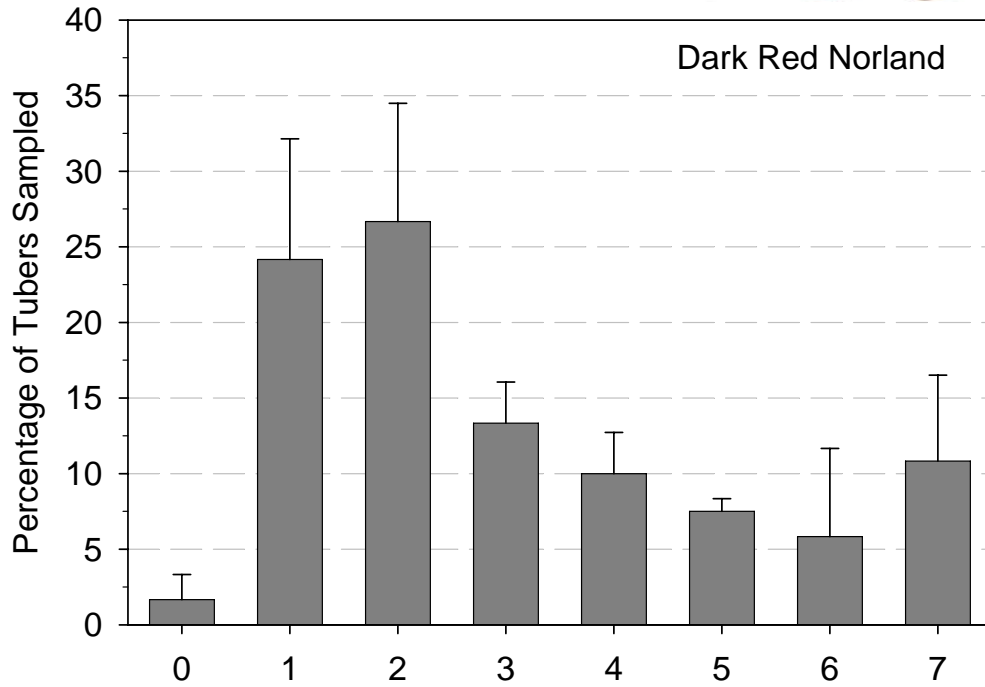
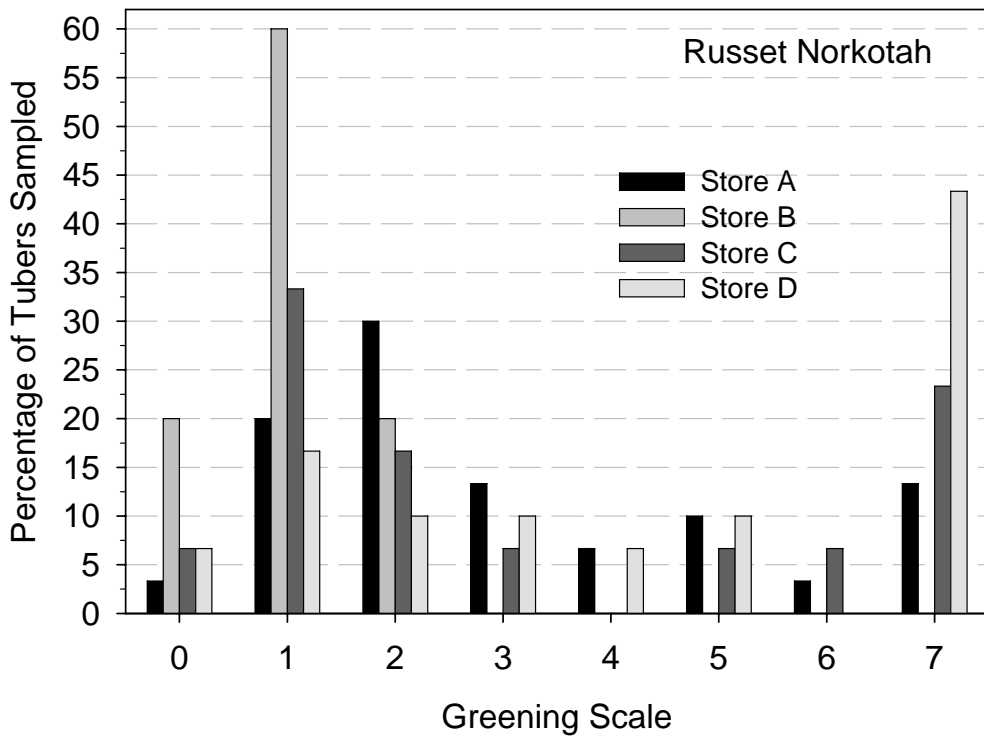
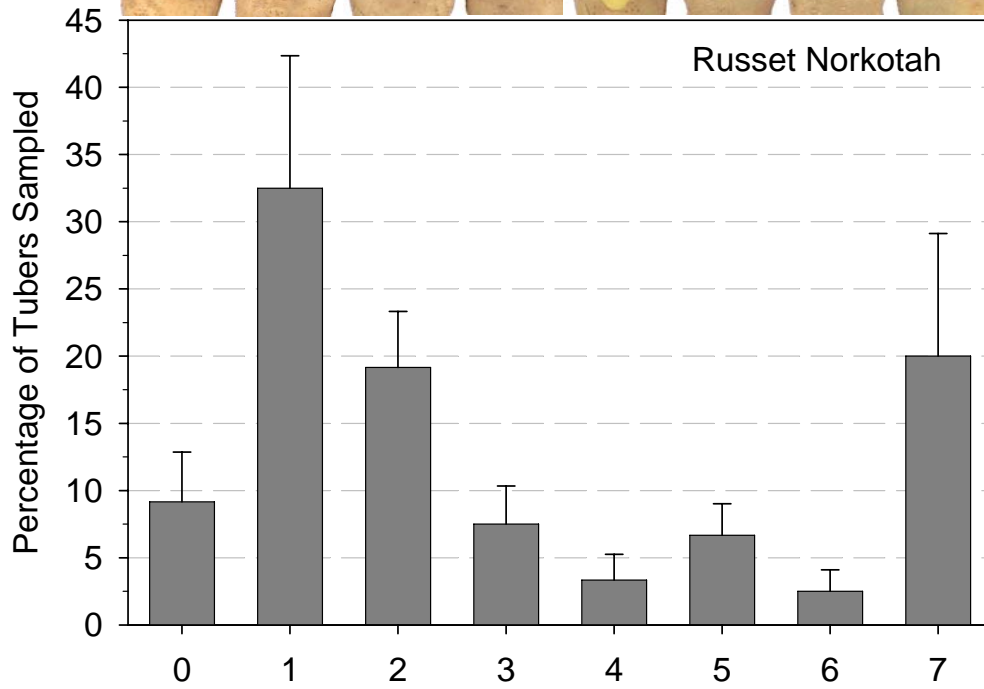


Fig. 4. Survey of the extent of greening of Russet Norkotah potatoes in retail outlets. Tubers were purchased from four stores and graded for color using the eight-level greening scale (tubers at top; ch. 1, Fig. 18). Thirty tubers were sampled from each store. Upper graph shows the percentage of tubers in each category averaged over all stores. Lower graph shows the store-to-store variation in greening.



CHAPTER THREE
GLYCOALKALOID DEVELOPMENT DURING GREENING
OF FRESH MARKET POTATOES (*Solanum tuberosum* L.)

Abstract

Potato tubers develop chlorophyll as a result of exposure to light. Green potatoes are generally rejected by potato processors and consumers, partly due to a perceived association between chlorophyll content and the development of toxic glycoalkaloids. Although the two processes are independent, chlorophyll and glycoalkaloids increase in direct response to light and are therefore significant from both a health and economic perspective. Indices to subjectively grade fresh market potatoes for the extent of greening were recently developed under lighting conditions consistent with those of retail markets. A main objective of the present study was to characterize the changes in total glycoalkaloid (TGA) accumulation for cvs. White Rose, Yukon Gold, Dark Red Norland and Russet Norkotah with progressive greening over the respective greening scales, thus calibrating the scales for TGA content. On average, TGA concentrations in complete longitudinal sections of tubers (flesh samples) were highest in Dark Red Norland followed by Russet Norkotah, Yukon Gold and White Rose. TGA concentrations of flesh samples of White Rose and Yukon Gold tubers were somewhat variable and did not increase in direct proportion to greening level and chlorophyll content, particularly at higher levels of greening. On the other hand, TGA concentrations in Dark Red Norland and Russet Norkotah tubers were highly correlated ($P \leq 0.001$) with greening level and chlorophyll concentrations. When averaged over greening levels, skin samples (periderm plus 10-15 layers of attached cortical parenchyma cells) contained 3.4- to 6.8-fold higher

concentrations of TGAs than flesh samples, depending on the cultivar. The TGA concentration in periderm samples ranged from 37- to 160-mg/100 g dry wt. Regardless of greening level, concentrations of TGAs in the flesh samples (including attached periderm) never exceeded the FDA-specified limit for consumption. Discrimination by the industry against green tubers on the basis of perceived glycoalkaloid toxicity is therefore unfounded for the cultivars and greening levels studied.

Introduction

When potato tubers are exposed to light, a greening reaction occurs (Anstis and Northcote, 1973), along with a parallel increase in the amount of glycoalkaloids (Edwards, 1997). Glycoalkaloids are a naturally occurring and toxic group of secondary plant compounds common in members of the Solanaceae. Glycoalkaloids are found in all parts of the potato plant, with concentrations highest in the flowers and lowest in the tubers (Friedman and McDonald, 1997). Once formed, glycoalkaloids do not degrade and are not destroyed by heat or cooking (Anstis and Northcote, 1973).

Glycoalkaloids may be more toxic to humans than to other animals. A lethal dose is about 3-6 mg kg⁻¹ body weight (Morris and Lee, 1984). Compared to other common poisons, glycoalkaloids may be considered extremely poisonous. An acute toxic dose for glycoalkaloids was estimated by van Gelder (1990) as only 1.75 mg kg⁻¹ body weight. In comparison, strychnine and arsenic are acutely toxic at 5 and 8 mg kg⁻¹ body weight, respectively. Symptoms of glycoalkaloid poisoning include gastro-enteritis, abdominal pain, vomiting, depression, hallucinations, convulsions, fever, and in severe cases, death. In documented cases, symptoms did not appear for 12 hours after consumption of the

glycoalkaloids, which is unusual for plant toxins (Oehme, 1978). The mechanism of glycoalkaloid toxicity is two-fold: disruption of the phospholipids in membranes (Roddick, 1974) and inhibition of acetylcholinesterase (Orgell, et al., 1958), which results in depression of the central nervous system and the neurological effects observed during poisoning (hallucinations, convulsions, depression, etc.).

Although no metabolic connection between chlorophyll and glycoalkaloid development has been established, green tubers are generally considered less fit for human consumption and are discriminated against by the industry and consumers. It was estimated that between 14 and 17% of the U.S. potato crop is lost annually due to greening of tubers (see references in Morris and Lee, 1984). While produce managers routinely cull potatoes that have greened, the process is subjective and variable, due to the absence of specific grading criteria. Subjective greening scales, based on objective measurements of chlorophyll and color, were only recently developed for use by the industry to maintain a standard level of quality (Grunenfelder et al., 2005a). These greening scales were shown to be effective for assessing the levels of greening encountered in a range of cultivars in retail markets (Grunenfelder et al., 2005b). The objectives of the present study were to (1) calibrate the greening scales for glycoalkaloid concentrations (2) characterize chlorophyll/glycoalkaloid relationships for each cultivar and (3) assess the variability among cultivars for glycoalkaloid development.

Materials and Methods

Plant Materials and General Procedures

Potato (*Solanum tuberosum* L) tubers (cvs. White Rose, Yukon Gold, Russet Norkotah, Dark Red Norland) were purchased in 23 kg boxes from a local grocery store directly off the supply truck. These potatoes are therefore representative of those normally subjected to greening in stores. All tubers were stored at 4°C and 95% relative humidity in darkness prior to use. Light intensities and temperatures for the various greening studies (see below) were chosen to match those typically found in grocery stores, as determined in prior surveys of major retailers in the local area (Grunenfelder et al., 2005a). Light intensity was measured with a quantum sensor (Model LI-185B, Li-Cor, Inc., Lincoln, NB) as photosynthetic photon flux density (PPFD, $\mu\text{mol quanta m}^{-2} \text{sec}^{-1}$) in the 400-700 nm range. A Nikon Cool-Pix 950 digital camera (Nikon Corp, New York) was used to document the extent of greening.

Glycoalkaloids were measured for both flesh and periderm samples. For flesh samples, greened tubers were cut in half longitudinally (along the apical to basal axis). A thin slice (approximately 1.5 mm thick, periderm attached) was then cut from one half of the tuber with an electric slicer (Sunbeam Products Inc., Boca Raton, FL). Samples of periderm for chlorophyll analysis were taken with a cork-borer as described below. The remaining periderm from the light-exposed side of the tuber was then excised and used for glycoalkaloid analysis (see below).

Replication of Greening Scales

To reproduce the greening levels for each cultivar (see Grunenfelder, 2005a; greening scales shown in Figs. 1-4) tubers were placed daily on a light table (24-hour

photoperiod, $6.8 \mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ of PPFD at tuber level, 23°C) and greened for a period ranging up to 10 days. The Sylvania RapidStart SuperSaver 34 W Cool White fluorescent lights had spectral peaks at approximately 360, 410, 435, 545 and 575 nm, simulating standard retail conditions (Grunenfelder, 2005a). The tubers were set out in reverse chronological order (the potatoes that would be greening for the longest were placed out first) so that all durations of greening could be sampled simultaneously at the end of the study. The tubers were re-randomized on the light table daily to minimize the effects of variation in light intensity. Nine tubers were selected visually at the end of the study to represent each level of the greening scale (Grunenfelder et al., 2005a) for each cultivar (three replicates of three tubers for each level of the scales shown in Figs. 1-4). Objective color measurements (CIELAB units L^* , a^* , b^*) were taken with a colorimeter (Minolta Chroma Meter, model CR-200, Minolta Corp., Ramsey, NJ) and hue angle was calculated (see McGuire, 1992) to verify that the greening levels were consistent with the previously established scales. Chlorophyll and total glycoalkaloids were extracted and quantified from tubers representing each level of the greening scales (see below).

Chlorophyll Extraction and Measurement

After greening, four cores were cut at random from each potato tuber (perpendicular to the apical and basal axis) using a 15-mm-diameter cork borer. A thin slice (approximately 1-mm-thick) including the periderm was cut from the end of each core, representing the surface of the tuber exposed to light (total surface area = 7.065 cm^2 per tuber). The twelve discs from each sample (three tubers per replicate) were collectively diced into smaller pieces, frozen at -85°C and lyophilized. The lyophilized tissue was ground to a fine powder with mortar and pestle and chlorophyll was extracted

with 9 mL of N,N-dimethylformamide (DMF) (Inskeep and Bloom, 1985). The extracts were vortexed, covered with foil to exclude light and refrigerated at 4°C for 24-72 hours. The extracts were then centrifuged twice for 15 min at 2,500 g and A_{647} and $A_{664.5}$ were measured using a Cary 100 Bio UV-visible double beam spectrophotometer (Varian Instruments, Walnut Creek, CA). Chlorophyll a, chlorophyll b and total chlorophyll concentrations were calculated as described by Inskeep and Bloom (1985).

Determination of Glycoalkaloid Concentration

The total glycoalkaloid determination protocol was modified from Bergers (1990). Ground lyophilized potato tissue (500 mg periderm or flesh samples) was extracted in 10 mL of 80% EtOH at 85 to 90°C for 25 minutes. The extracts were filtered through Whatman #2 filter paper and reduced to 3 to 5 mL on a rotary evaporator at 50°C. Each extract was transferred to a 50-mL polypropylene centrifuge tube, rinsing twice with 3 mL of 10% (v/v) HOAc. The samples were then centrifuged at 10,000g for 30 minutes at 10°C. The supernatants were collected and the pH adjusted to 9 with NH_4OH . The extracts were refluxed at 70°C for 25 minutes and stored at 4°C overnight. The extracts were then centrifuged at 10,000g for 30 minutes at 10°C. The supernatants were discarded and the resulting pellets dissolved in 0.5 mL of 7% (v/v) phosphoric acid and stored at -20°C.

To quantify total glycoalkaloid (TGA) content, 200 μL of extract were added to 1 mL of 0.03% (w/v) paraformaldehyde in concentrated phosphoric acid. After developing for 20 minutes, A_{600} was measured and TGA concentrations were determined based on an α -solanine (Sigma-Aldrich, St. Louis, MO) standard curve. Results are expressed on a dry weight basis.

Data Analysis

Data were subjected to analysis of variance with greening levels and TGA concentrations as independent and dependent variables, respectively. Sums of squares were partitioned into linear, quadratic or cubic trends. Polynomial models and coefficients of determination are reported. Data are plotted with 95% confidence intervals.

Results

cv. White Rose

Chlorophyll concentration increased relatively slowly from green-0 to green-2, followed by a more rapid increase through green-9 (Fig. 1). This resulted in chlorophyll concentrations that were higher than those previously characterized (Grunenfelder et al., 2005a) at similar levels of greening for this cultivar. The increase in chlorophyll concentration was 17-fold over the 10-level greening scale in this study (from 0.25 to 4.27 $\mu\text{g}/\text{cm}^2$) versus 11-fold (from 0.25 to 2.75 $\mu\text{g}/\text{cm}^2$) in a previous study (Grunenfelder et al., 2005a). Despite the differences in chlorophyll content of tubers between the two studies, L-values and hue angles were comparable with those characterized previously at each level of the greening scale (data not shown), confirming the consistency with which tubers can be subjectively sorted based on color.

The changes in total glycoalkaloid (TGA) content of flesh (longitudinal slices including periderm) and peel (periderm plus 10 to 15 layers of cortical parenchyma cells) with greening level and chlorophyll concentration were best described by cubic polynomials ($P \leq 0.01$) (Fig. 1), which accounted for 73 to 90 percent of total variation.

Flesh TGAs increased approximately 2.3-fold from green-0 to green-3, remained relatively constant at an average of 16 mg/100 g dry wt from green-3 to green-7, then increased another 41% through green-9. A similar trend was apparent for the periderm (skin) TGA concentrations which were substantially higher than that of the flesh samples.

The increase in flesh TGAs from green-0 to green-9 was 3.1-fold (from 6.9 to 21.3 mg/100 g dry wt) (Fig. 1). Depending on cultivar, tuber dry matter ranged from 18 to 20% of fresh weight. Hence, on a 20% dry matter basis, the TGA content of a green-9 tuber was only about 4.3 mg/100 g fresh wt, a level that is one fifth of that established by the FDA as a limit for consumption (Edwards and Cobb, 1997; Sinden et al., 1984). Concerns regarding buildup of toxic glycoalkaloids in the flesh of green cv. White Rose potatoes under retail lighting conditions are thus unfounded. It is also improbable that the level of glycoalkaloids in green-9 tubers would adversely affect flavor. TGA-induced bitterness is generally not a problem until concentrations reach 13 to 15 mg/100 g fresh wt (Edwards, 1997).

As expected, glycoalkaloid concentrations were much more concentrated in the periderm than in the flesh samples (Fig. 1). Periderm concentrations increased from 77 to 137 mg/100 g dry wt as greening level increased from zero to nine, resulting in levels that were 11- and 6.4-fold higher, respectively, than the flesh concentrations in green-0 and green-9 tubers. On a fresh weight basis, periderm TGA concentrations increased from 15 to 27 mg/100 g fresh wt, exceeding the FDA-established limit and reaching levels that would likely affect flavor (Edwards, 1997). The concentrations (mg/100 g dry wt) of TGA in both the periderm and flesh samples of cv. White Rose tubers can be estimated

for the given levels of greening and chlorophyll content from the polynomial equations in Fig. 1.

cv. Yukon Gold

As with cv. White Rose, chlorophyll concentration increased relatively slowly as Yukon Gold tubers greened from zero to three, followed by a more rapid increase through green-7 (Fig. 2). The periderm chlorophyll concentrations were also higher than those previously characterized (Grunenfelder et al., 2005a) for similar levels of greening. Chlorophyll concentration increased 20-fold (from 0.20 to 4.17 $\mu\text{g}/\text{cm}^2$) from green-0 to green-7 (Fig. 2). This compares with a 3.4-fold increase characterized in the former study (Grunenfelder et al., 2005a). However, similar to cv. White Rose, the disparity in tuber chlorophyll content between studies did not affect the ability to select cv. Yukon Gold tubers representing each level on the scale. Changes in L-values and hue angles of tubers were comparable among studies with increasing level of greening (data not shown). Hence, at a particular level of greening, chlorophyll content may vary and is not a good indicator of the subjectively assessed color for cvs. Yukon Gold and White Rose.

TGA content of flesh samples was somewhat variable, increasing predictably over the first five greening levels, but unpredictably with further greening through level seven (Fig. 2). We speculate that TGA concentration remains relatively constant from green-4 to green 7 (as indicated by the dashed line in Fig. 2); however, further studies will be needed to fully characterize the relationship at the higher greening levels. The TGA concentration of tuber flesh increased 41% as tubers greened from zero to level four on the scale. In contrast to flesh samples, TGA concentration of the periderm was highly correlated with greening over the entire scale, increasing 139% as tubers greened from

level zero to seven. The relationship was best described by a quadratic polynomial ($R^2=0.90$, $P\leq 0.01$) with TGA concentration increasing only 34% over the first four greening levels and 75% as greening progressed to level seven. On average, TGAs were 5.2-fold more concentrated in the periderm than in the flesh of Yukon Gold tubers.

While no clear relationship was evident between tuber chlorophyll and TGA content of flesh samples above about $1.5 \mu\text{g}/\text{cm}^2$ chlorophyll (green-4), TGA concentration of the periderm increased linearly ($R^2=0.90$, $P\leq 0.001$) with chlorophyll over the entire greening scale (Fig. 2). Regardless of greening level, TGA content of the flesh remained well below that considered unsafe for human consumption. Concentrations in the periderm, however, reached approximately 20 mg/100 g fresh wt in green-6 and green-7 tubers, which is considered unsafe for consumption (Edwards and Cobb, 1997; Sinden et al., 1984). High TGA levels in the periderm are of lesser concern than high levels in the flesh, given that the flesh would dilute the periderm during consumption, and that the periderm is often removed prior to eating.

cv. Dark Red Norland

Total chlorophyll concentration increased at a constant rate of $0.22 \mu\text{g}/\text{cm}^2$ per greening level as tubers greened over the 8-level scale (Fig. 3). The rate of chlorophyll increase and the concentration of chlorophyll in tubers at each greening level were comparable with those characterized previously (Grunenfelder et al., 2005a). Chlorophyll content of tuber periderm increased 5.4-fold from green-0 to green-7. Changes in L-value and hue angle with greening level (data not shown) were also consistent with past studies (Grunenfelder et al., 2005a), demonstrating the close relationships between the objective measures of color (L-value, hue angle) and

chlorophyll, and the subjective perception of greening as assessed with the greening scale for this particular cultivar.

The changes in TGA content of flesh samples with greening level and chlorophyll concentration were best described by cubic polynomials (Fig. 3). Flesh TGA content increased approximately 74% from green-0 to green-2, 123% from green-2 to green-5, and only 15% from green-5 to green-7. The TGA increase was 4.5-fold (from 10.5 to 46.8 mg/100 g dry wt) over the 8-level greening scale, but the highest level in green-7 tubers was still less than half of the maximum safe concentration established by the FDA. Unlike cvs. Yukon Gold and White Rose, increases in TGAs paralleled increases in chlorophyll during greening. TGA content of complete longitudinal samples of Dark Red Norland tubers can thus be estimated from greening level and/or periderm chlorophyll concentration with the polynomial equations presented in Fig. 3.

As in the previous two cultivars, glycoalkaloid concentrations were much more concentrated in the periderm than in the flesh samples of Dark Red Norland tubers. Periderm TGA concentrations ranged from 38 to 159 mg/100 g dry wt, increasing linearly over the greening scale ($R^2= 0.99$, $P\leq 0.001$) and with chlorophyll concentration ($R^2= 0.97$, $P\leq 0.001$). Hence, the TGA concentration of skin samples can also be estimated based on greening level and chlorophyll concentration. As tuber greening increased beyond level three, TGA concentrations in skin samples exceeded the FDA limit of 20 mg/100 g fresh wt (assuming 20% dry matter). Peeling during processing and dilution by the flesh during consumption, however, would likely negate any potential danger posed by TGA toxicity. While TGAs increase in parallel with greening of Dark

Red Norland tubers, it is likely that concentrations would never get high enough to adversely affect human health under the lighting conditions present in retail markets.

cv. Russet Norkotah

Chlorophyll concentrations were highly correlated with greening levels of Russet Norkotah tubers, increasing 6-fold over the 8-level greening scale (Fig. 4). Moreover, the change in chlorophyll concentration per greening level, along with the concentration of chlorophyll in the periderm of tubers at each greening level, were consistent with those described previously (Grunenfelder et al., 2005a). L-values and hue angles fell within the expected ranges (data not shown), characterizing the progressive darkening and changes in color expected for the various greening levels of this cultivar (Grunenfelder et al., 2005a).

TGA concentration (flesh samples) increased 3.1-fold (from 8.2 to 25.7 mg/100 g dry wt) as tubers greened from zero to level-7 and the relationship was best described by a second order polynomial ($P \leq 0.001$) (Fig. 4). TGAs increased at a rate of 6.1 mg/100 g dry wt for every $\mu\text{g}/\text{cm}^2$ increase in chlorophyll content of the skin. Therefore, the subjective greening scale and chlorophyll content are good predictors of the TGA content in the flesh of tubers of this cultivar. The TGA content of flesh samples from green-7 tubers was about 5.2 mg/100 g fresh wt (assuming 20% dry matter), which is 4-fold lower than the FDA-specified limit for consumption.

On average, the TGA concentration was 3.8-fold more concentrated in the periderm than in the flesh samples of Russet Norkotah tubers. Periderm TGA concentrations ranged from 37.3 to 98.2 mg/100 g dry wt and increased quadratically with greening scale ($R^2 = 0.97$, $P \leq 0.001$) and linearly with chlorophyll concentration ($R^2 =$

0.98, $P \leq 0.001$). Hence, the TGA concentration of skin samples can also be estimated based on greening level and chlorophyll concentration. TGA concentrations in skin samples never exceeded the FDA limit of 20 mg/100 g fresh wt (assuming 20% dry matter). However, TGA levels in the skin of tubers that had greened beyond level three on the greening scale were probably high enough (>13 mg/100 g fresh wt) to affect flavor (e.g. increased bitterness) (Edwards, 1997). TGA levels in the flesh were too low to influence flavor. While TGAs increase in parallel with greening of cv. Russet Norkotah tubers, concentrations are not likely to reach toxic levels in response to the lighting conditions present in retail markets.

Discussion and Conclusions

In contrast to the relatively high light intensities (up to $250 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$, PPFD) and greening time periods (15-20 days) of past studies (Percival, G., 1999; Reeves, 1988; Sotelo and Serrano, 2000; Edwards and Cobb, 1997), the conditions employed in this study more closely simulated those present in retail environments. The results thus represent the extent of greening and TGA accumulation that could be encountered in stores and are therefore more applicable to the industry. The use of lower light intensities and shorter durations of exposure, however, resulted in smaller changes in TGAs than reported in past studies (Percival, 1999), reducing the ability to resolve differences in TGA among the greening levels, particularly for flesh samples.

Variability in light-induced chlorophyll and TGA accumulation among cultivars may be due in part to variations in tuber maturity, tuber size, thickness of skin and presence of accessory pigments. These pigments may act as “natural” light filters,

affecting the quality of light penetrating the outer periderm, thereby influencing the rates of chlorophyll and TGA accumulation. Indeed, Percival (1999) showed that chlorophyll and TGAs increased to a lesser extent under mercury vapor lighting compared with fluorescent and high pressure sodium lighting, all of which vary in emission spectra. Fluorescent and high pressure sodium lights emit ultraviolet (<300nm) and infrared (1300 nm) light, respectively, both of which stimulate TGA and chlorophyll synthesis (Percival, 1999).

TGA concentrations decrease in all parts of the potato plant (including tubers) as dry matter increases with maturity (Friedman and McDonald, 1997; Van Gelder, 1990). It is well documented that immature and small tubers have higher concentrations of glycoalkaloids, likely due in part to the high surface area to volume ratio of the smaller potatoes (Bomer and Mattis, 1924; Wolf and Duggar, 1946). Therefore, early potatoes (such as cv. Dark Red Norland) tend to have higher TGAs than later varieties. Stresses (both pre- and post-harvest) can also enhance tuber TGA concentrations (Friedman and McDonald, 1997). However, two of the most significant factors affecting TGA concentrations are light (Edwards, 1997; Percival, 1999) and cultivar (Edwards, 1997; Griffiths et al., 1994).

Previous surveys showed a wide range in the extent of tuber greening in retail markets (Grunenfelder et al., 2005b), with all of the greening levels found for the cultivars used in the present study. While the extent of correlation between greening level and TGA content was cultivar dependent, in general, higher TGA concentrations were associated with higher greening levels for all cultivars. Therefore, TGA content of fresh-market potatoes in stores is expected to be highly variable. Despite this variability,

the levels of TGAs that developed in the flesh of tubers in response to the lighting conditions characteristic of retail markets were within levels considered safe by the FDA (<20 mg/100 g fresh wt), and well under the toxic levels reported by Morris and Lee (1984).

In summary, tubers sorted subjectively using the greening scales displayed the expected degree of darkening (L-value) and color (hue angle) changes previously characterized for the greening levels. Chlorophyll content was directly correlated with greening level but varied for two of the cultivars (White Rose and Yukon Gold) between studies. These results indicate that chlorophyll is not the sole determinant of tuber surface color. The perceived color is undoubtedly determined by complex interactions among chlorophyll content, accessory pigments, periderm thickness and flesh color.

Greening scales for cvs White Rose, Yukon Gold, Dark Red Norland and Russet Norkotah were calibrated for TGA levels. While both flesh and skin TGA concentrations increased with greening level, the latter was more highly correlated with greening level than the former. Also, in cv. Yukon Gold, the TGA correlation was stronger in tubers with less greening and the variability increased at higher greening levels, especially after green-4. In contrast, TGAs increased in tubers of cvs. Dark Red Norland and Russet Norkotah with little variation over the entire greening scale. Variability in the extent of light-induced TGA development among the cultivars was also apparent. Before greening, TGAs ranged from 6.9- to 13-mg/100 g dry wt (flesh samples), in cvs. White Rose and Yukon Gold, respectively. After greening, TGAs in flesh samples of Yukon Gold reached a maximum of 19.4 mg/100 g dry wt, compared to 23.8 mg/100 g dry wt in cv. White Rose and 46.8 mg/100 g dry wt in cv. Dark Red Norland. Regardless of

cultivar, TGA concentrations in the flesh of even the greenest tubers were well below the limit established by the FDA. While the change in tuber color during greening certainly decreases the appeal of tubers to the industry and consumers, discrimination against greened tubers on the basis of perceived TGA toxicity is unfounded for the cultivars and greening levels studied.

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Fig. 1. Changes in total chlorophyll and glycoalkaloids in White Rose tubers over the 10-level greening scale (upper right, see Grunenfelder et al., 2005a). Tubers were incubated under cool white fluorescent light ($6.8 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$, PPF) for 24 h per day. Chlorophyll was extracted from 1-mm-thick x 1.5-cm-diameter discs of periderm from the light-exposed side of the tuber. Glycoalkaloids were extracted from a complete longitudinal section representing the entire tuber (flesh samples, left panel) or from periderm (right panel). Dotted lines indicate 95% confidence intervals. **,***F-values for the regressions were significant at $P \leq 0.01$ and 0.001 levels, respectively.

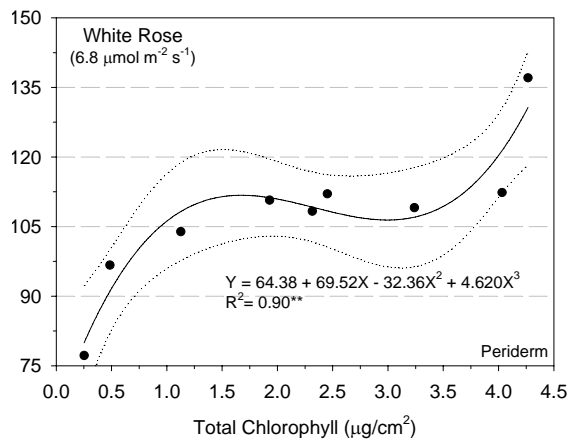
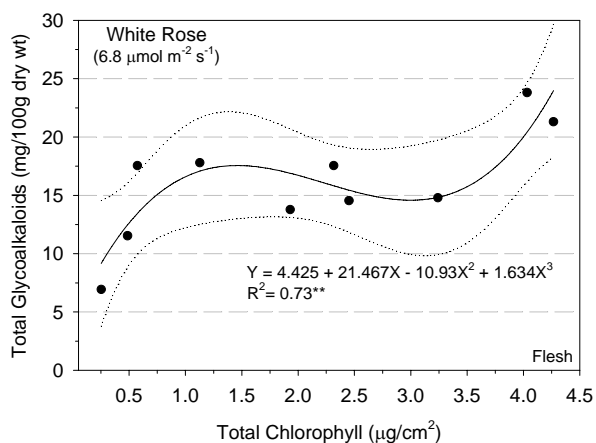
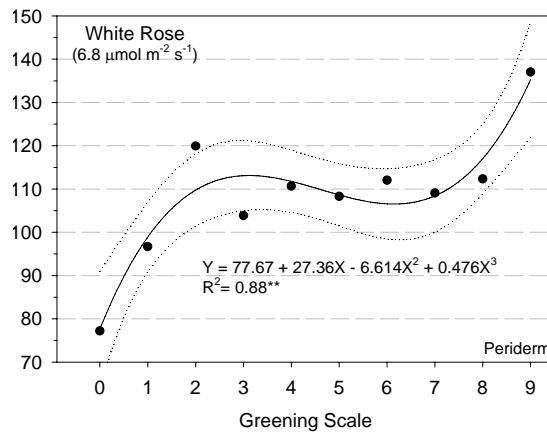
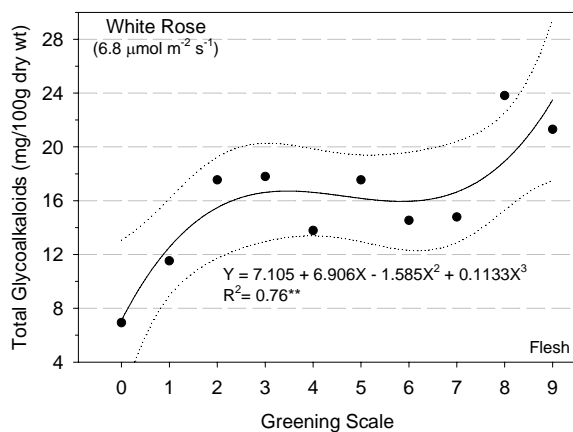
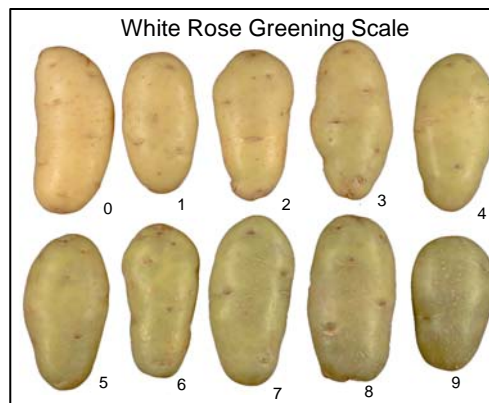
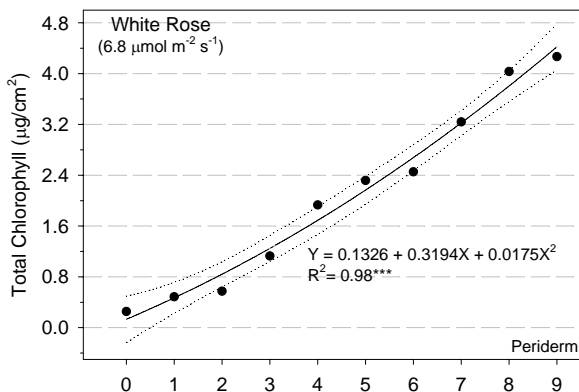


Fig. 2. Changes in total chlorophyll and glycoalkaloids (TGA) in Yukon Gold tubers over the 8-level greening scale (upper right, see Grunenfelder et al., 2005a). Tubers were incubated under cool white fluorescent light ($6.8 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$, PFD) for 24 h per day. Chlorophyll was extracted from 1-mm-thick x 1.5-cm-diameter discs of periderm from the light-exposed side of the tuber. TGAs were extracted from a complete longitudinal section representing the entire tuber (flesh samples, left panel) or from periderm (right panel). TGA regressions in flesh samples are valid for the first 5 greening levels only. Dashed lines indicate speculated trends at higher levels of chlorophyll from green-5 through 7. Dotted lines indicate 95% confidence intervals. *, **, ***F-values for the regressions were significant at $P \leq 0.05$, 0.01 and 0.001 levels, respectively.

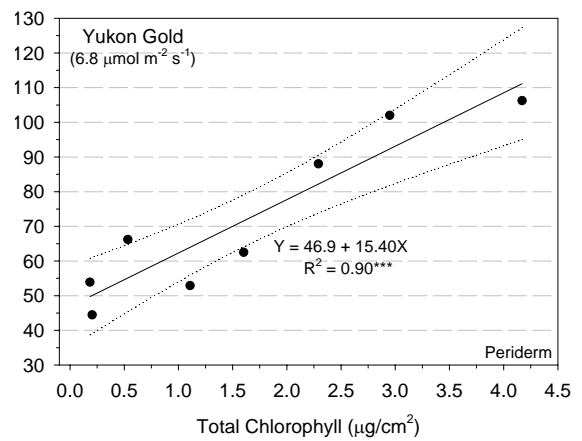
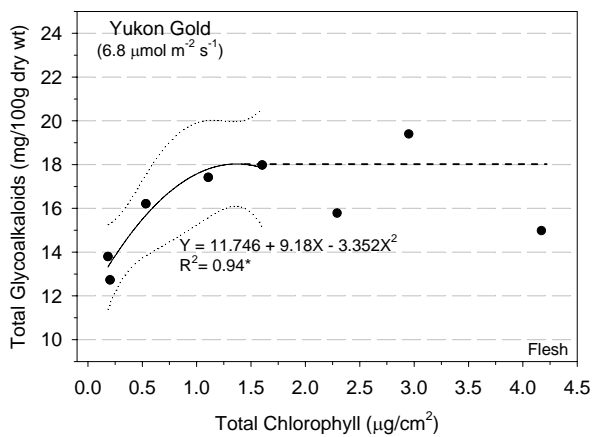
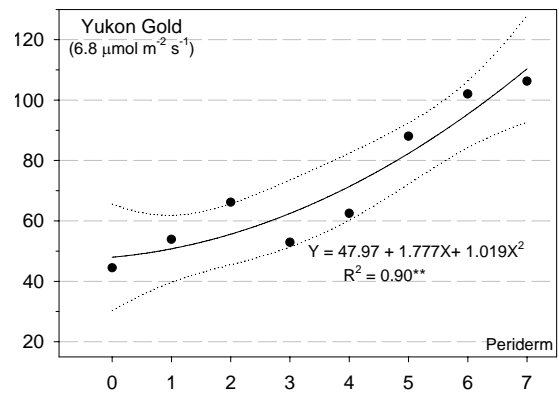
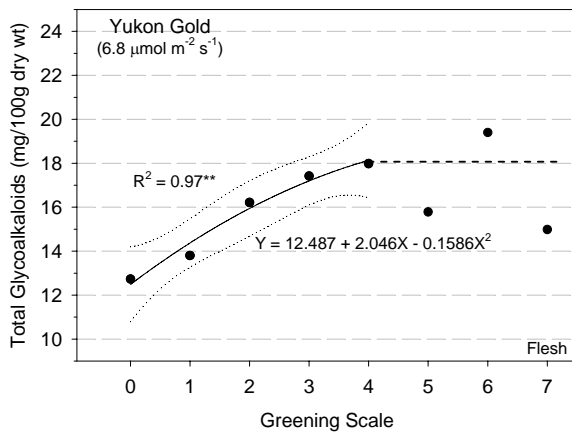
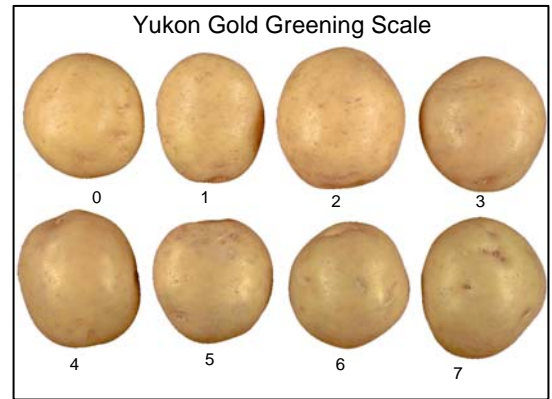
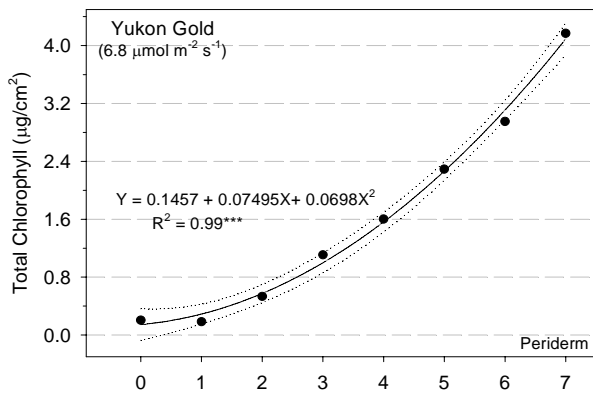


Fig. 3. Changes in total chlorophyll and glycoalkaloids in Dark Red Norland tubers over the 8-level greening scale (upper right, see Grunenfelder et al., 2005a). Tubers were incubated under cool white fluorescent light ($6.8 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$, PPFD) for 24 h per day. Chlorophyll was extracted from 1-mm-thick x 1.5-cm-diameter discs of periderm from the light-exposed side of the tuber. Glycoalkaloids were extracted from a complete longitudinal section representing the entire tuber (flesh samples, left panel) or from periderm (right panel). Dotted lines indicate 95% confidence intervals. ***F-values for the regressions were significant at $P \leq 0.001$.

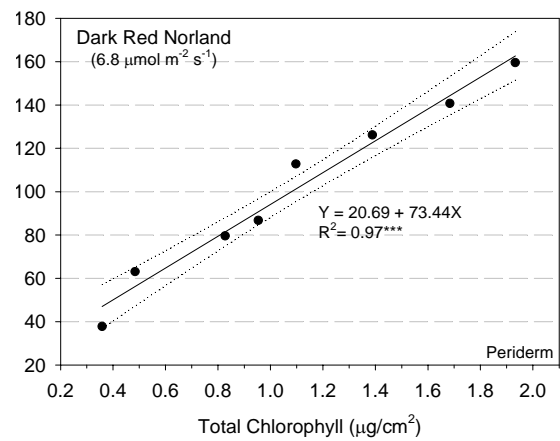
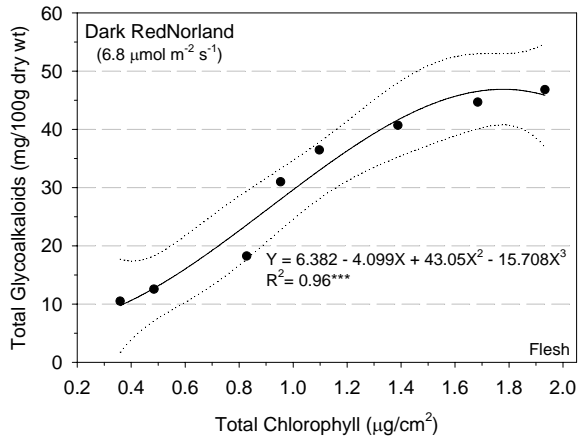
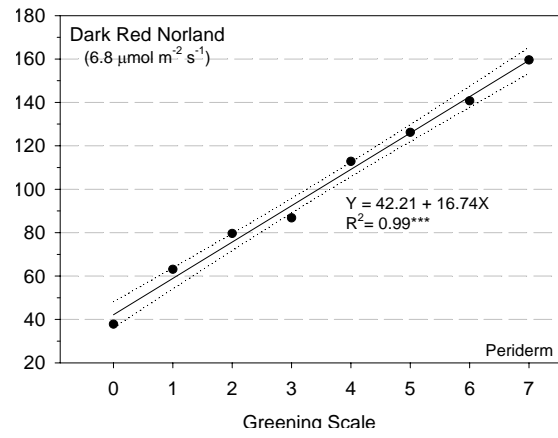
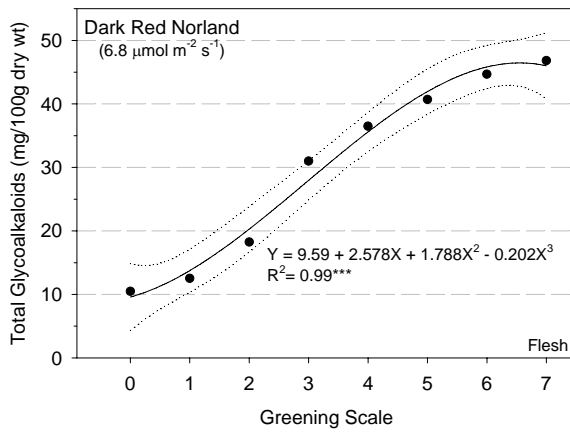
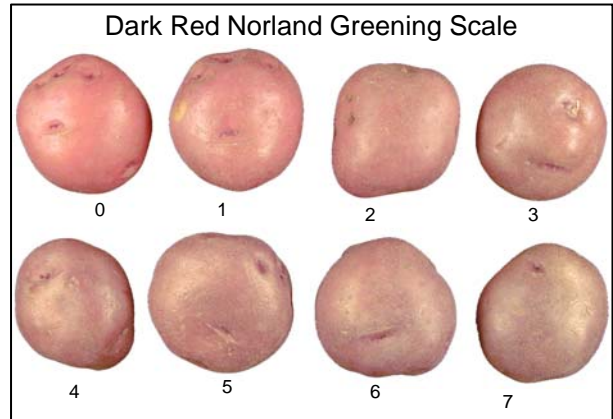
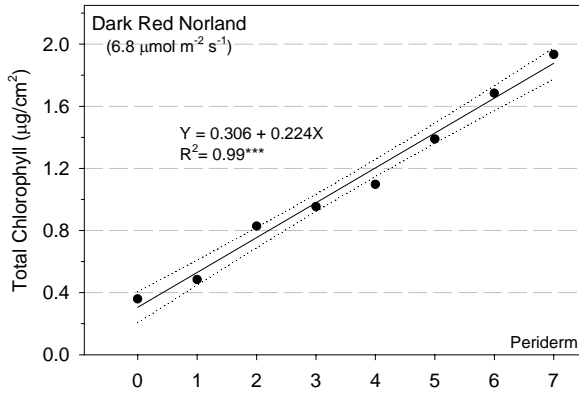
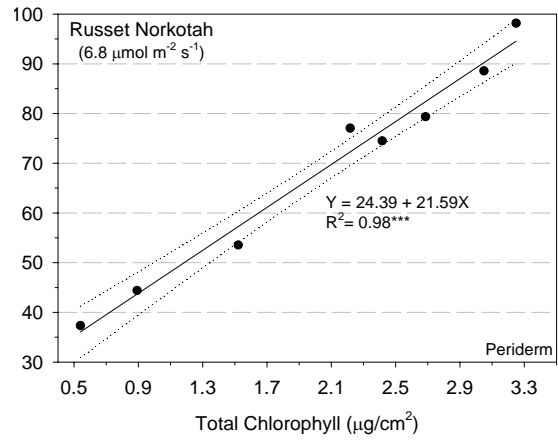
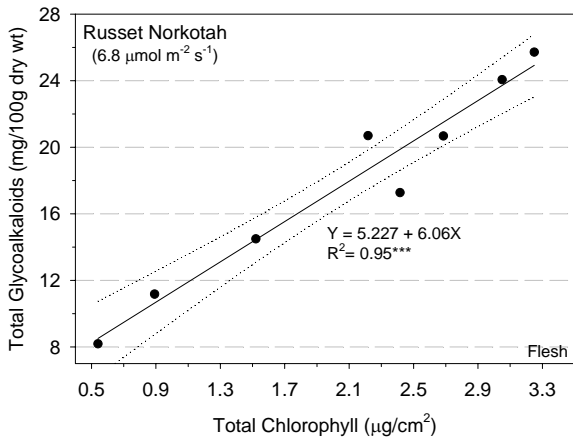
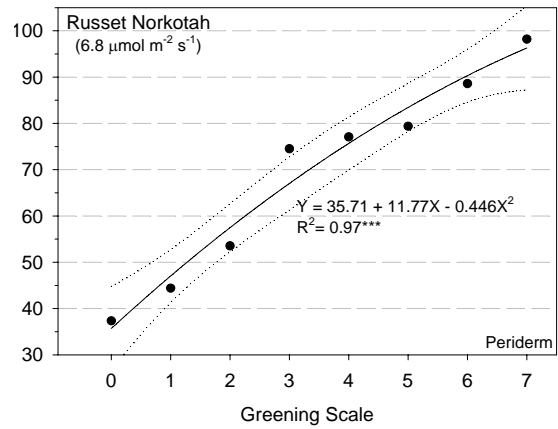
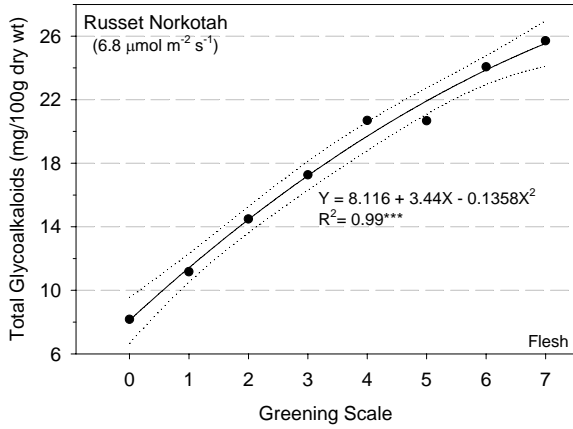
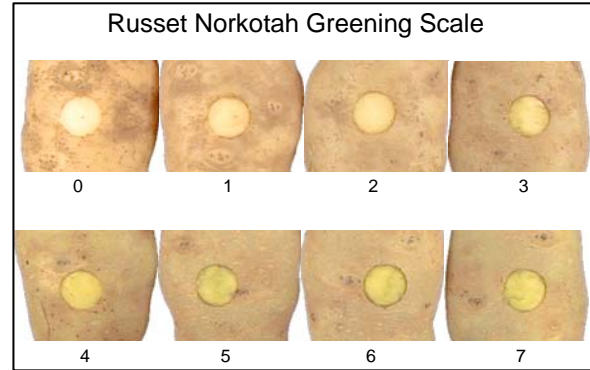
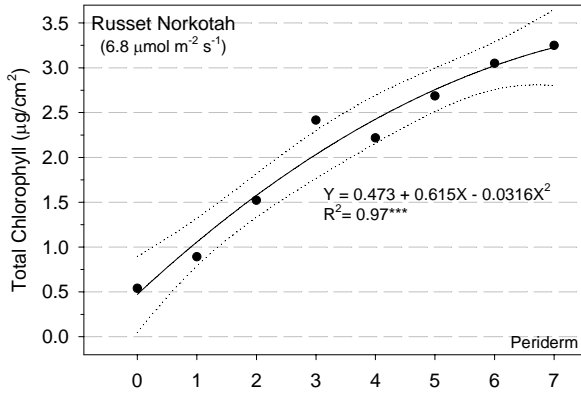


Fig. 4. Changes in total chlorophyll and glycoalkaloids in Russet Norkotah tubers over the 8-level greening scale (upper right, see Grunenfelder et al., 2005a). Tubers were incubated under cool white fluorescent light ($6.8 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$, PPF) for 24 h per day. Chlorophyll was extracted from 1-mm-thick x 1.5-cm-diameter discs of periderm from the light-exposed side of the tuber. Glycoalkaloids were extracted from a complete longitudinal section representing the entire tuber (flesh samples, left panel) or from periderm (right panel). Dotted lines indicate 95% confidence intervals. ***F-values for the regressions were significant at $P \leq 0.001$.



CHAPTER FOUR

THE EFFECTS OF PACKAGING, LIGHT INTENSITY, PHOTOPERIOD AND TEMPERATURE ON GREENING OF FRESH MARKET POTATOES

Abstract

Potatoes are often displayed under supplemental light in fresh market stores to increase their appeal to the consumer. In this environment, tubers undergo an undesirable greening process, due to the transformation of amyloplasts to chloroplasts in the cortical parenchyma tissue directly beneath the periderm (skin). Greening can be considered the response of potato tubers to light exposure, resulting in the production of chlorophyll, which is perceived as a green discoloration on the surface of the tuber. Consumers discriminate against greened tubers, reducing their value. The objectives of this study were to evaluate the effects of packaging, light intensity, photoperiod and temperature on the rate of greening in White Rose tubers. Reducing the light intensity from 6.8- to 3.8- $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ indirectly through packaging in perforated polyethylene bags of various colors did not attenuate greening in a simulated retail environment. For non-packaged tubers, reduction in light intensity from 18- to 10- $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ also had no effect on the rate of greening; however, greening was slowed significantly at 3.5 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$. Tubers darkened (L-values decreased) at a faster rate with a 24-h photoperiod than an 18-h photoperiod at 30 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$. However, the rate of tuber greening, as defined by hue angle, was unaffected by photoperiod. Greening and chlorophyll development were slower in tubers displayed at 7 and 10°C as compared with those at 15 and 20°C. Collectively, these results underscore the sensitivity of tubers to very low light intensity for greening and illustrate the technical difficulties faced by

retailers in developing displays and packaging that will extend shelf life through attenuating the greening process.

Introduction

When potato tubers are exposed to light, chloroplasts begin to develop from amyloplasts in parenchyma cells of the cortex located directly beneath the periderm (Petermann and Morris, 1985). The rate of chlorophyll synthesis is dependent on many pre- and post-harvest factors including wounding, light exposure, temperature and lighting conditions, as well as genotype (Smith, 1977; Percival, 1999; Reeves, 1988). While potato tuber greening occurs under a wide range of lighting conditions, the rates of both chlorophyll and glycoalkaloid accumulation are higher under high-pressure sodium and fluorescent lighting compared with low- and high-pressure mercury lighting (Percival, 1999). The resulting discoloration of the tuber surface is largely a function of the green chlorophyll in conjunction with the natural skin (periderm) pigmentation of a particular cultivar.

Green potatoes are generally rejected by both potato processors and consumers. This is due in part to a perceived association between the development of chlorophyll in the tuber and the development of toxic glycoalkaloids. Although the two processes are independent, both chlorophyll and glycoalkaloid concentrations increase in direct response to light exposure. However, for the cvs White Rose, Dark Red Norland, Yukon Gold, and Russet Norkotah, glycoalkaloids did not develop to toxic levels in the tuber flesh under simulated retail conditions within five days of greening (chapter three). Therefore, the unsightly greening and discoloration caused by chlorophyll development,

which varies among cultivars and leads to reduced marketability, is of primary concern to the industry. For a given interval of greening, the discoloration is most apparent in white skin cultivars such as White Rose (see chapter one). Hence, cv White Rose was used in the present study to evaluate the potential of packaging, light intensity, photoperiod, and temperature for reducing chlorophyll development and the overall greening response.

Materials and Methods

Plant Materials and General Procedures

White Rose potato (*Solanum tuberosum* L.) (selected because of the sensitivity of this cultivar to light exposure) tubers were purchased in 50 pound boxes from a local grocery store directly off the supply truck. All tubers were stored at 4°C and 95% relative humidity in darkness prior to use. Packaging materials were donated by Baker Produce in Kennewick, WA, and are consistent with packaging used in contemporary fresh markets.

Light intensities and temperatures for the various studies were chosen to correspond with conditions found in grocery stores as determined by surveys of major retailers (see chapter one). Light intensity was measured with a quantum sensor (Model LI-185B, Li-Cor, Inc., Lincoln, NB) as photosynthetic photon flux density (PPFD, $\mu\text{mol quanta m}^{-2} \text{sec}^{-1}$) in the 400-700 nm range. Unless otherwise noted, a ventilation hood with fluorescent lighting (Sylvania RapidStart SuperSaver 34 W Cool White fluorescent tubes) was used as a lighting table for the greening experiments. The light intensity was $6.8 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ at tuber level (24-h photoperiod), simulating standard retail

conditions. The radiation source (fluorescent tubes) had spectral peaks at approximately 360, 410, 435, 545 and 575 nm.

A dark room equipped with light tables (using the same fluorescent lighting) was used in the light intensity and photoperiod experiments. Temperature experiments were done in Conviron (125L, Winnipeg, Canada) controlled environment chambers under fluorescent lighting at $9.5 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$. Photographs to document the extent of greening were taken using a Nikon Cool-Pix 950 digital camera (Nikon Corp, New York).

Color Assessment

Color of the undamaged surface of potato tubers was assessed using a Minolta Chroma Meter CIE 1976 (CIELAB) (Model CR-200, Minolta Corporation, Ramsey, NJ). Changes in tuber color during greening were quantified using the CIELAB color model (see chapter one). CIELAB measurements were taken at three points on the light exposed side of each potato tuber including the stem end, the middle and the bud end. Hue angles (a measure of color) were calculated as described by McGuire (1992) and Schreiner *et al.* (2003).

Effects of Packaging

At $6.8 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ ambient light intensity outside the packages (control), the average light intensities inside GB (clear), BE (clear) and GS (opaque) perforated plastic bags (2.27 kg) were reduced to 6.45-, 4.83- and $3.8\text{-}\mu\text{mol quanta m}^{-2} \text{s}^{-1}$, respectively. Five replicates of White Rose tubers were placed randomly in each bag (with the control having no packaging) to be greened on the light table. The tubers were re-randomized daily inside the bags and on the light table. The tubers were greened in

reverse chronological order over the 5-day interval so as to have all greening stages available at the end of the study, as previously described (chapters one and three). Tuber color (CIELAB) was measured at zero time and at the end of greening. The tubers were photographed to document the effects of time of light exposure and packaging on greening. Total chlorophyll was extracted from periderm samples (see chapter one) and quantified by the methods of Inskeep and Bloom (1985). Chlorophyll concentration was expressed per cm^2 periderm and plotted against time.

Effects of Photoperiod

White Rose tubers were greened under fluorescent lights at $29.7 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ for 0 to 5 days using 18- and 24-h photoperiods. This relatively high light intensity is consistent with the intensity of light in the lighted cooler displays observed in local stores (see chapter one). Tubers (10 replicates) were placed daily at each photoperiod. The tubers were re-randomized daily under the lights to minimize variation in tuber greening due to positional differences in light intensity. The tubers were greened in reverse chronological order over the 5-day interval, in order to have all greening stages available at the end of the study. Tuber color (CIELAB) was measured at zero time and at the end of greening.

Effects of Light Intensity

White Rose tubers were greened for 0 to 5 days at low light ($3.5 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$), medium light ($10.2 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$) and high light ($17.9 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$) intensities. Five replicates of tubers (for each light intensity) were placed daily on the light tables in reverse chronological order over the 5-day interval. Daily re-randomization of the tubers on the light tables minimized variation attributable to

differences in light intensity at tuber level. Tuber color (CIELAB) was measured at zero time and at the end of greening. Total chlorophyll was quantified and plotted against time.

Effects of Temperature

White Rose tubers were greened for 0 to 5 days in controlled environment chambers (Convion) equipped with cool white fluorescent bulbs ($9.5 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$) at 7°C, 10°C, 15°C and 20°C. Three tubers were placed daily at each temperature in reverse chronological order, and were re-randomized on a daily basis. Tuber color was measured before and after greening. Total chlorophyll was quantified and plotted against time.

Results and Discussion

Evaluation of Packaging

Clear and opaque perforated polyethylene bags (Fig. 1) were tested for their abilities to attenuate tuber greening. The extent of greening at $6.8 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ in this particular lot of White Rose tubers was less than that recorded in the previous study (chapter one) and consequently the changes in color of tubers were difficult to resolve visually over the 5-day greening interval (Fig. 1). This indicates that physiological differences among tuber lots can significantly affect the greening process. Despite the reductions in light intensity caused by the packaging, tubers greened at the same rate as non-packaged tubers over the 5-day period (Fig. 2).

Effects of Photoperiod

In an attempt to limit greening and prolong the shelf life of potatoes, some produce managers use specially designed blankets to shield tubers from light for short

periods of time after regular business hours. This effectively alters the photoperiod to which the tubers are exposed. To determine whether reduction of photoperiod can attenuate the greening process, White Rose tubers were incubated under fluorescent light ($29.8 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$) at 23°C for 5 days with an 18- or 24-h photoperiod. L-values of tubers declined significantly faster under continuous illumination (Fig. 3), indicating a more rapid darkening of the tuber surface compared with tubers under the 18-h photoperiod. However, photoperiod had no effect on the actual rate of greening (change in hue angle) over the 5-day interval. Tubers increased from green-0 to green-4 regardless of photoperiod. Hence, reducing the photoperiod from 24 to 18 hours did not slow the greening process enough to affect a meaningful increase in shelf life.

Effects of Light Intensity

Greening of White Rose tubers was significantly slower at $3.5 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ than at either 10.2 or $17.9 \mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ light intensities over a 5-day interval at 23°C (24-h photoperiod) (Fig. 4). However, tubers held under the two higher light intensities greened at similar rates. The higher light intensities are more consistent with those found in retail settings. Note that the differences in greening induced by the low and higher light intensities could not be resolved until after 3 days (Fig. 4). Moreover, the chlorophyll content of these tubers at 3 days was sufficient to warrant a stage three to four assignment on our greening scale (see chapter one). After 5 days of greening at the lowest light intensity ($3.5 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$), White Rose tubers had a chlorophyll content ($2 \mu\text{g/cm}^2$) (Fig. 4) that was consistent with that of green-7 tubers (chapter one), compared to tubers at the higher light intensities (10.2 and $17.9 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$), which reached a level 9 on the greening scale. For this experiment, lowering the light

intensity slowed the progression of greening from about stage three to seven over 5 days of greening. Since stage three to four tubers represent a relatively small fraction of tubers in retail outlets (see chapter two), lowering the light intensity would likely not be effective in managing the greening problem. The relative insensitivity of tubers to light intensity supports the involvement of phytochrome in the greening response, which is therefore invoked at very low light intensities.

Effects of Temperature

Differences in the greening responses of tubers due to temperature were not apparent early in the study. Regardless of temperature, chlorophyll content increased to approximately $1.0 \mu\text{g}/\text{cm}^2$ (green stage 2, see chapter one) over the first two days of greening (Fig. 5). Temperature-induced differences in chlorophyll and tuber color were only apparent over the final 3 days of greening, possibly due to the storage of these particular tubers (4°C) for 2 to 3 weeks prior to the study. At $9.5 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ light intensity, tubers developed chlorophyll and greened faster at 20 and 15°C than at 10 and 7°C (Fig. 5). At the lower temperatures, chlorophyll concentration, hue angle and L-values remained relatively constant from day two to five. Low temperature thus attenuated the greening response. From a practical standpoint, use of low temperatures to slow greening and increase the shelf life of potatoes in retail markets would have to be justified economically, relative to the vast array of higher-margin produce that compete for refrigerated shelf space.

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Fig. 1. Time course of greening of White Rose potatoes at 23°C as affected by packaging in 5 lb polyethylene bags. Tubers packaged in the perforated bags were placed under 6.8 $\mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ light intensity provided by cool white fluorescent bulbs (24 h photoperiod). Light intensities were 6.45 and 4.83 $\mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ inside the two clear bags, GB and BE, respectively. Light intensity inside the colored GS bag was 3.8 $\mu\text{mol quanta m}^{-2} \text{sec}^{-1}$. Color and total chlorophyll content of the tubers are quantified in Fig. 2.

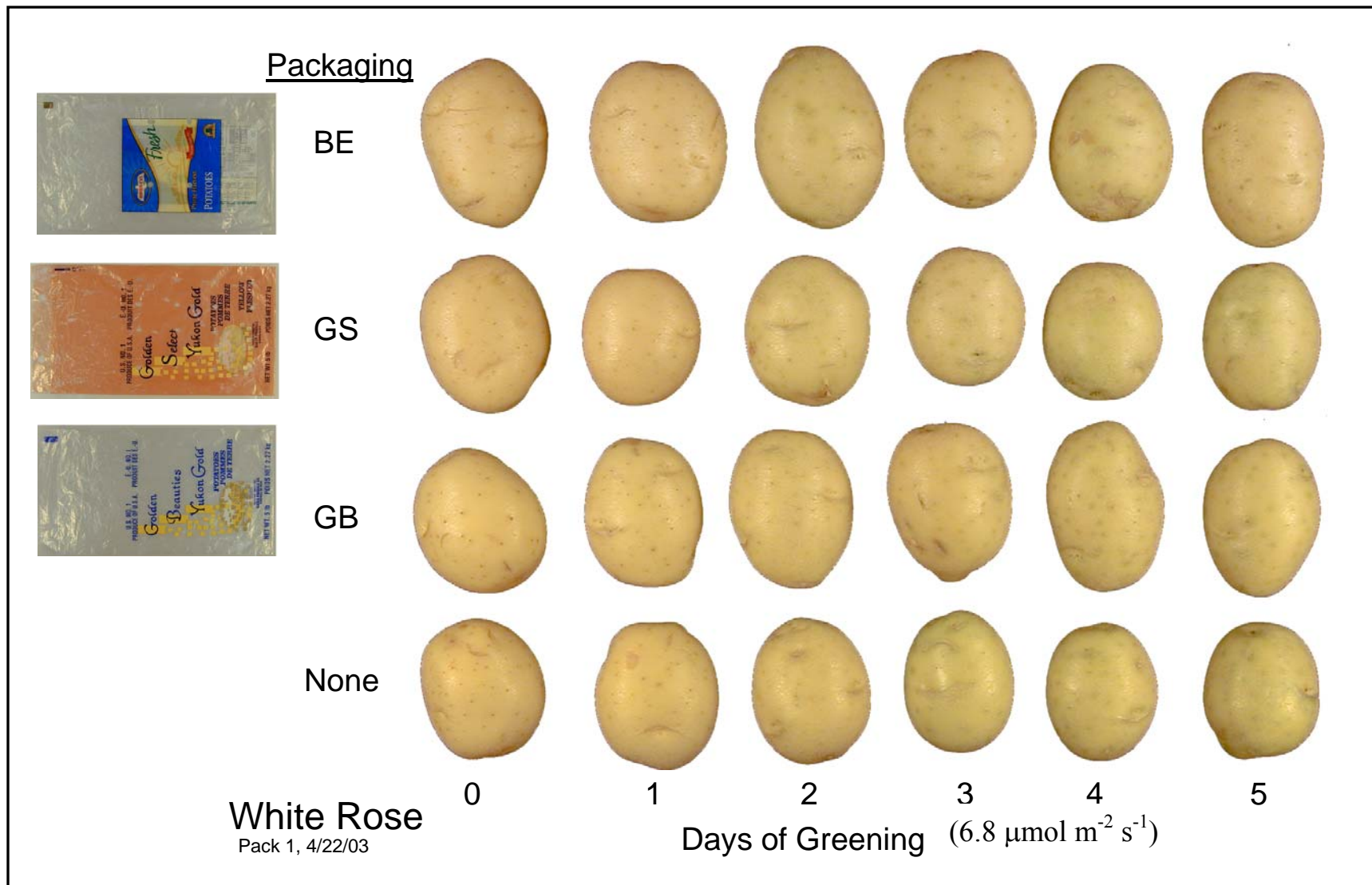


Fig. 2. Effects of packaging on changes in L-value, hue angle and total chlorophyll content of White Rose tubers during 5 days of greening at 23°C. Light intensities were 6.45 and 4.83 $\mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ inside the two clear bags, GB and BE, respectively. Light intensity inside the colored GS bag was 3.8 $\mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ (24-h photoperiod). Tubers are shown in Fig. 1. Inset shows total chlorophyll averaged over all packaging treatments. Bars show SE of means.

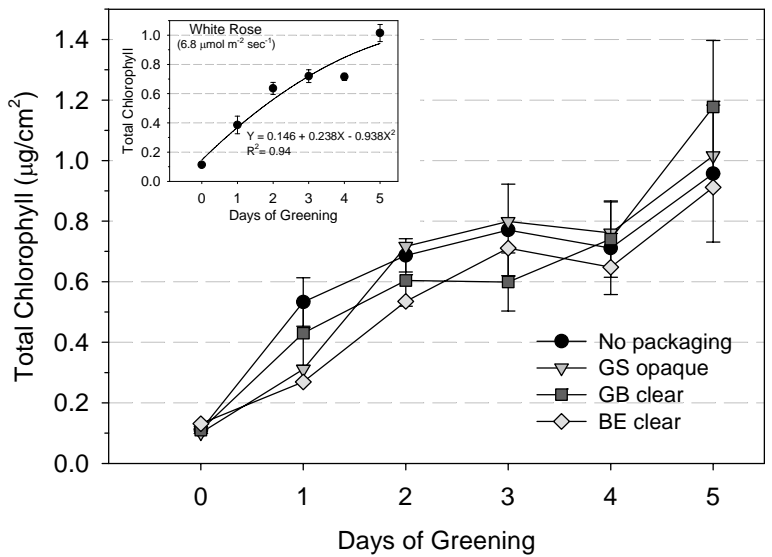
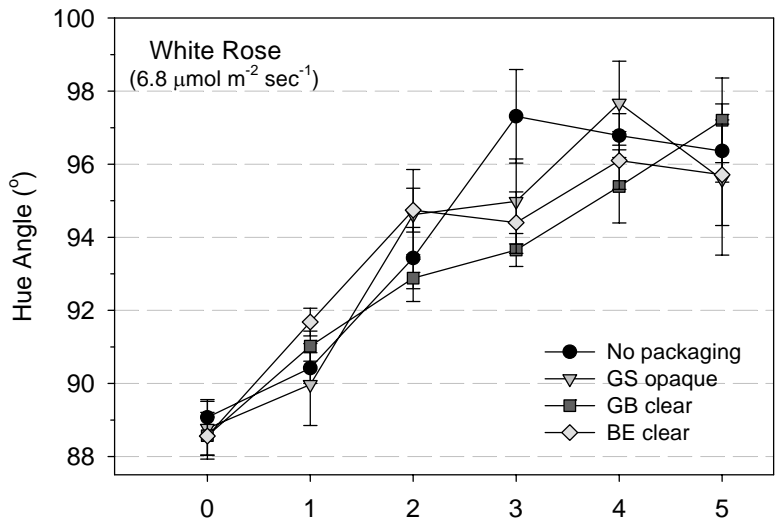
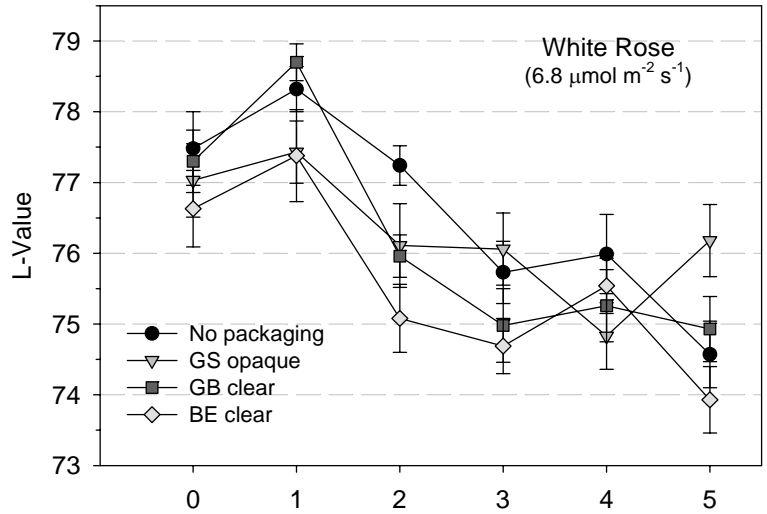


Fig. 3. Effects of photoperiod on the greening response of White Rose tubers at $29.8 \mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ light intensity (23°C). Tuber greening was assessed as change in L-value (relative lightness) and hue angle. Greening levels are shown in the inset tubers. Bars show SE of means.

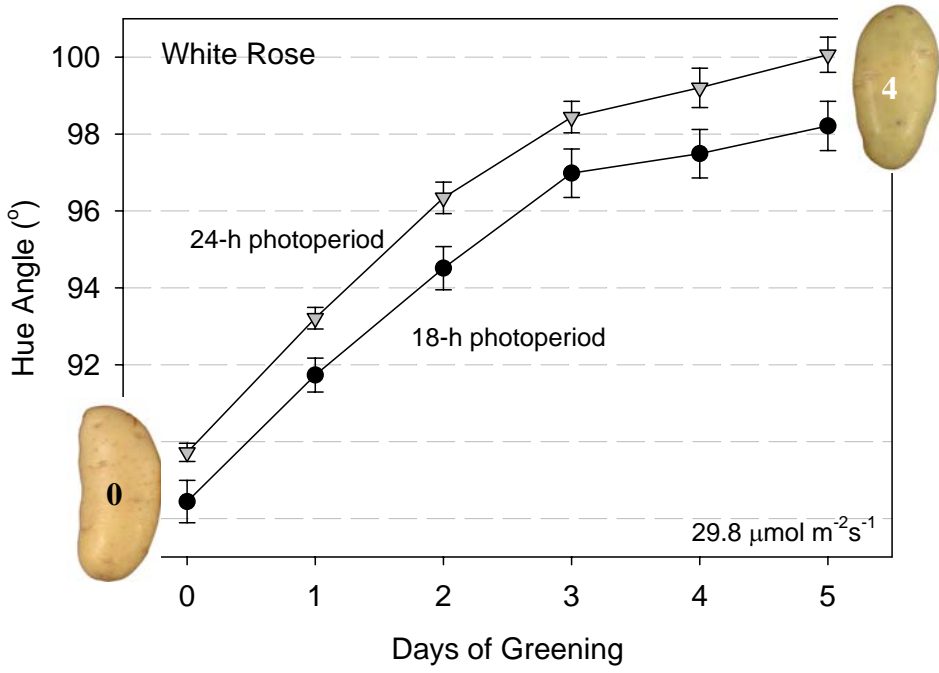
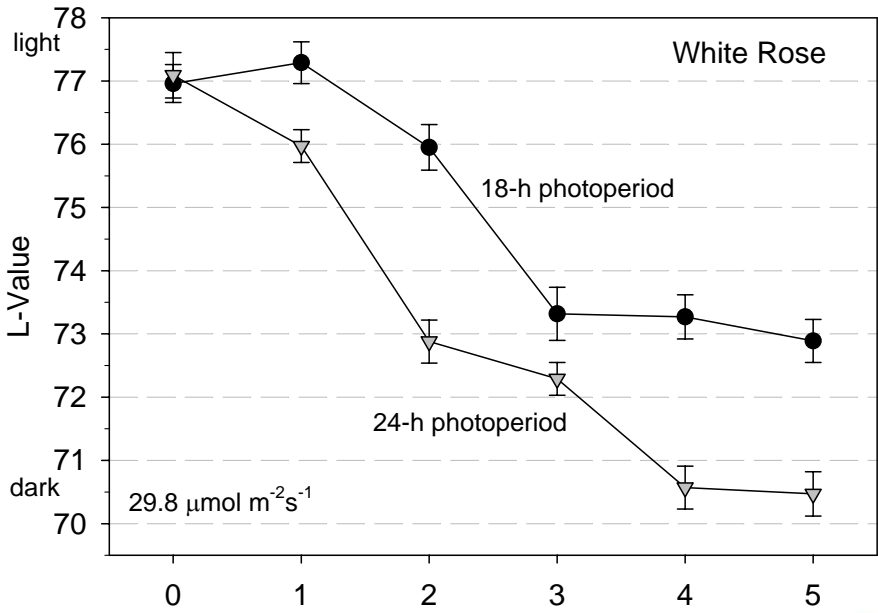


Fig. 4. Changes in L-value, hue angle and total chlorophyll content of White Rose tubers over time at different light intensities. Tubers were incubated under cool white fluorescent light (24-h photoperiod). Chlorophyll was extracted from 1-mm-thick x 1.5-cm-diameter discs of periderm from the side of the tubers facing the light. Greening levels are shown in the inset tubers. Bars show SE of means.

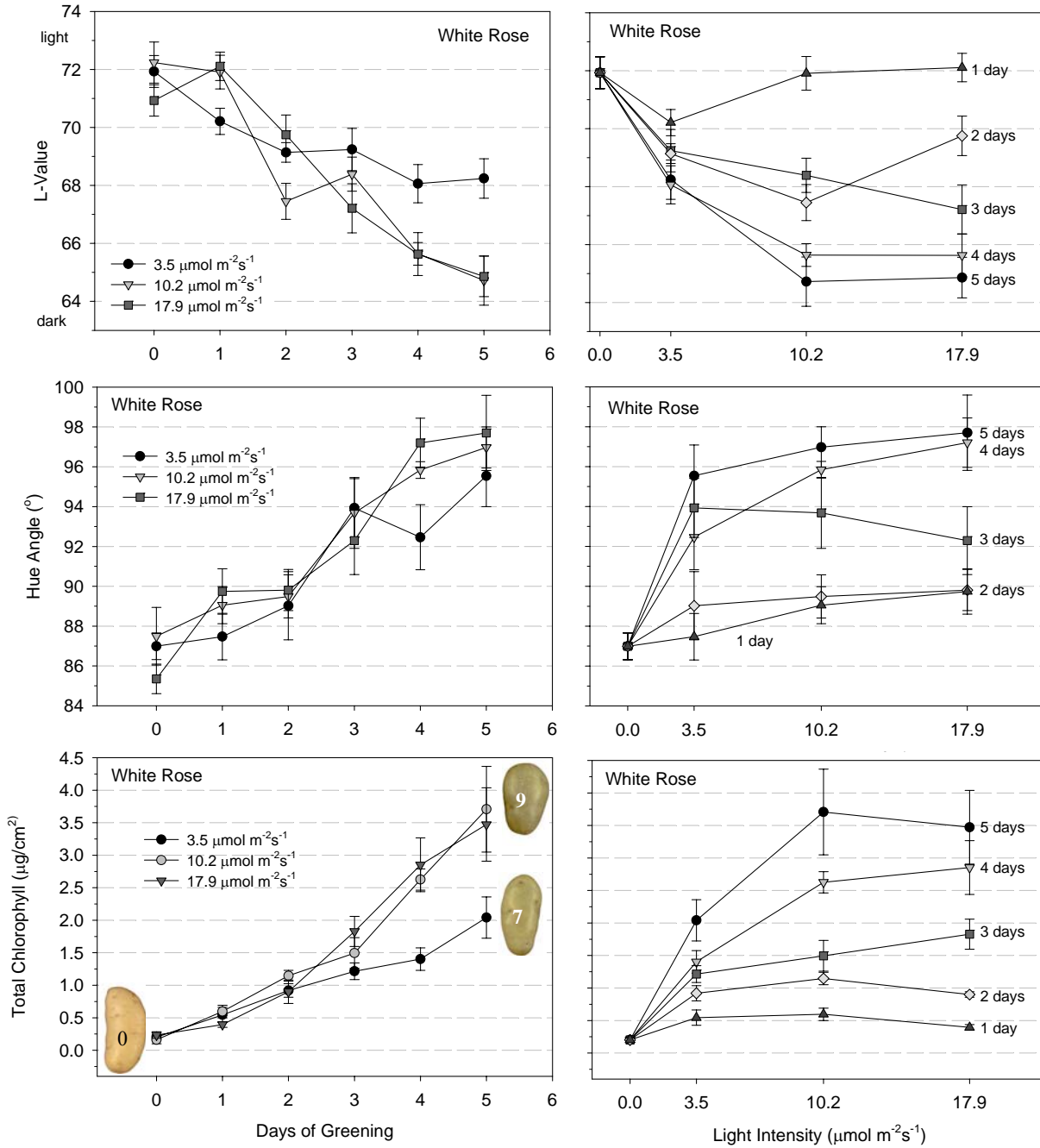
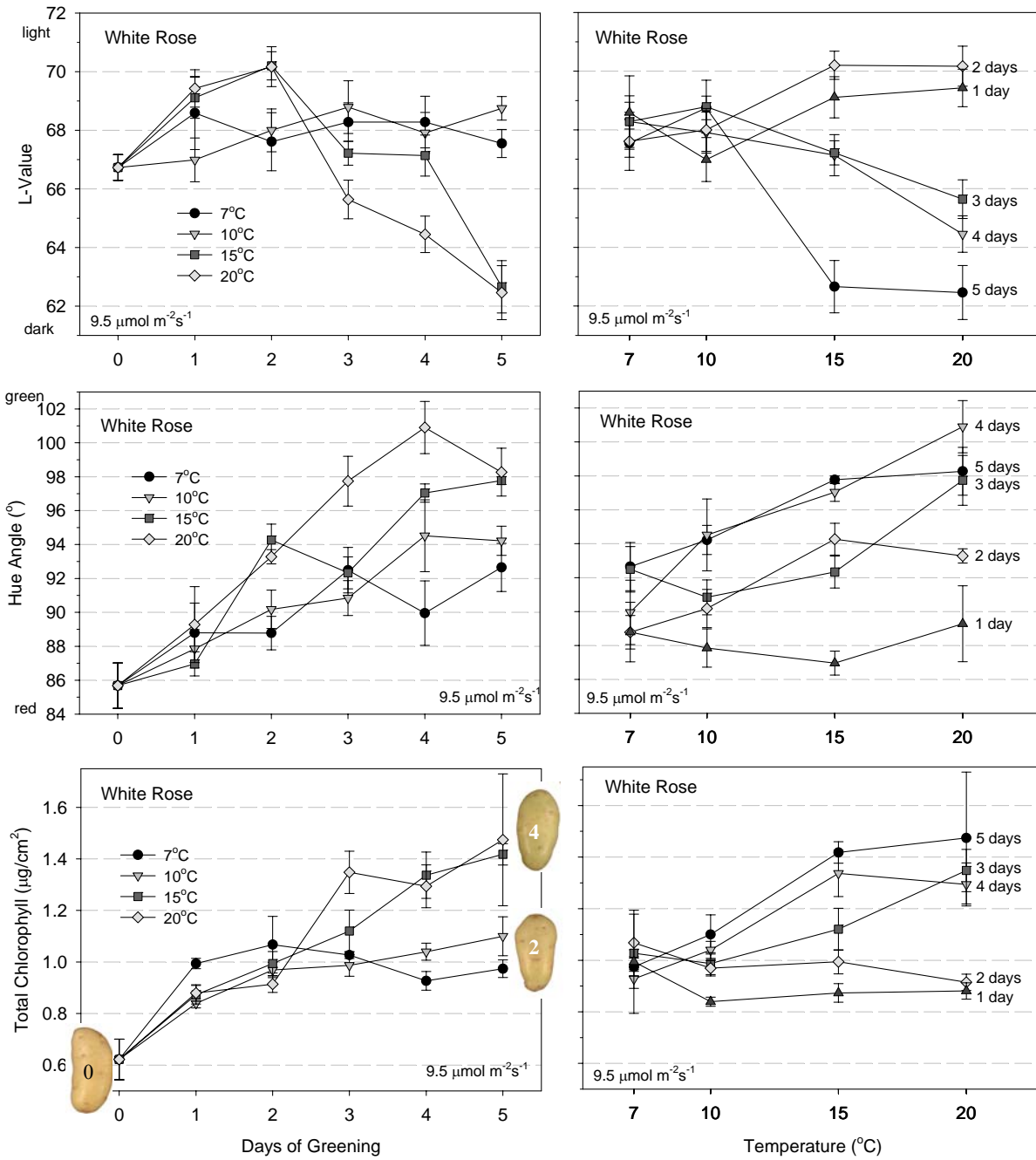


Fig. 5. Changes in L-value (relative darkness), hue angle and total chlorophyll content of White Rose tubers over time at different temperatures. Tubers were incubated under cool white fluorescent light (24-h photoperiod) at $9.5 \mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ light intensity. Chlorophyll was extracted from 1-mm-thick x 1.5-cm-diameter discs of periderm from the side of the tubers facing the light. Greening levels are shown in the inset tubers. Bars show SE of means.



General Summary and Conclusions

Tuber color changed rapidly (within 6 days) as chlorophyll concentration increased in response to light intensities and temperatures that were identical to those found in potato displays in local grocery stores. The visual perception of greening was most apparent in the white-skinned cultivar, White Rose, as compared to the red- and russet-skin cultivars. Variation among cultivars in periderm thickness, color and presence of accessory pigments interact to affect the degree of discoloration during greening, which will no doubt influence the degree of discrimination by consumers for potatoes that have greened. Therefore, cut-off values on the various greening scales, beyond which tuber color is unacceptable, will vary among cultivars. While the remaining shelf-lives of ‘White Rose’, ‘Yukon Gold’, ‘Dark Red Norland’, and ‘Russet Norkotah’ tubers may be roughly estimated from the derived plots (and associated equations) relating greening scales to time, the physiological status of a particular lot of potatoes will affect the rates of greening and chlorophyll development.

There is a high degree of variation among retail outlets for the extent of greening in potatoes on the shelves, underscoring a need for the development and implementation of more consistent grading procedures. The greening scales developed in this project were shown to be effective for subjectively grading tubers from local markets for color, demonstrating their potential utility as an aid to quality control.

Periderm samples contained much higher levels of total glycoalkaloids (TGAs) than flesh samples (between 4 and 7 times higher). Flesh concentrations were more variable in certain cultivars (White Rose and Yukon Gold) because of dilution (more dry matter). Total glycoalkaloids increased in parallel to greening (both greening scale and chlorophyll content),

but were well within the limits established by the FDA for the flesh samples of all cultivars studied.

Reducing the light intensity directly or indirectly through packaging in perforated polyethylene bags had no effect on the rate of greening. Tubers are highly sensitive to low levels of light for chlorophyll development, which is likely a consequence of the involvement of phytochrome in the response. Reducing the photoperiod from 24 to 18 hours did not slow the greening process, suggesting that covering tubers in retail markets with specially designed, light tight 'blankets' will probably not be an effective method for attenuating greening. Reduced temperature slowed the rate of greening; however, use of refrigerated shelf space for bulk potatoes in retail markets must be justified economically. Collectively, these results underscore the sensitivity of potato tubers to low levels of light, illustrating the difficulties faced by retailers in developing displays and packaging that will extend tuber shelf-life through attenuation of greening.