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Investigations on Cause and Prevention of Greening of Potato Tubers

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INVESTIGATIONS ON CAUSE AND PREVENTION OF GREENING OF POTATO TUBERS[1]

By

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Introduction

Potato tubers have often been noticed to contain a recognizable amount of green pigment just under the skin. This condition, in the present study referred to as greening, is generally regarded as a more or less serious market defect. Such greening may therefore sharply reduce the retail sale of potatoes. Consumers not only object to the appearance of such tubers but have come to associate green potatoes with a bitterness in flavor.

Greening apparently may occur at any period in the normal life of the tuber upon exposure to light. This paper deals only with greening as it occurs in the post-harvest operations of handling, storage, and marketing. The kind of greening which takes place in the field in tubers still attached to the vine but not completely covered with soil and which is commonly referred to as "sunburn", will not be considered in this paper.

The Federal grades for potatoes (30)[2] regard sunburn as a defect on a par with mechanical injury and scab and other diseases but make no mention of light-greened tubers. Nevertheless, samples of potatoes containing tubers greened after harvest are generally considered off-grade when they reach the consumer.

That greening is a factor in consumer resistance to the purchase of potatoes is shown by the findings of Eberhard.[3] He carried out consumer-preference studies with Idaho potatoes in Los Angeles, Cincinnati, and Kansas City and found that the buyers object to green potatoes. He states that "a greened potato lacks the eye appeal and natural color so essential to successful merchandising."

In the Detroit market, Motts (24) found in the potatoes he examined, that 14 percent of the samples from Maine, 36 percent of the samples from Michigan, and 40 percent of the samples from Idaho contained some green tubers and that the defect was second in frequency to mechanical injury. Data from the California and Oregon markets (9) likewise show that greening can be a very serious trouble. Here as much as 23.3 percent and 27.2 percent of Oregon U. S. No. 1 and Oregon U. S. No. 2, respectively, were green at the time of taking the samples from retail stores. Up to 52.9 percent of the samples contained some green tubers.

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[1]Presented in partial fulfillment of the requirements for the degree of Master of Science to the University of Idaho Graduate School, June, 1949.

[2]Numbers in parenthesis refer to Literature Cited.

[3]Mr. Milton Eberhard, Assistant Agricultural Economist, University of Idaho. Personal communication. 1949.

No data are available regarding the extent to which greening takes place in the storage cellar, but it is common knowledge that when sorting after a period of storage, potatoes are often off-grade due to greening. It is also likely that a great deal of greening originates between the time of sacking at shipping point and time of retailing. Thus the California and Oregon studies referred to above (9) showed that if cotton sacks were used greening was the most common defect developing after shipping point inspection. Motts states that, "the development of green color beneath the skin in nearly all cases was due to over-exposure of the potatoes to light in the stores" (24). However, the present-day consumer wants to see what he buys and therefore most potatoes in retail stores are held in open bins or in open mesh bags and are thus exposed to light for as long a time as they remain in the store.

It is evident that light greening must be considered a serious defect and that the discovery of some means for its control would be very useful in the marketing of potatoes.

Some of the more severe cases of greening may be prevented merely by careful handling. However, to effectively prevent potatoes from becoming green during the necessary handling in shipping and retailing too little is known about the nature of greening and how it is affected by environmental conditions.

The purpose of this study was to learn more about environmental influences on greening, with principal reference to temperature, light intensity and duration, and humidity. Information was also sought on possible treatments of the tubers before or after they have become green to prevent greening or to remove it if already present.

Review of Literature

It has been frequently stated or implied in the literature that the appearance of greening in potato tubers is due to formation of chlorophyll (2, 6, 8, 16, 23). There is little or no conclusive support of this assumption based on chemical analysis. However, keeping in mind the apparent similarities between the formation of chlorophyll in other plant parts and the formation of the green pigment in potato tubers, it appears likely that the earlier writers have been correct in their assumption.

Although there have been no extensive studies demonstrating the depth at which chlorophyll may develop in the cortex of the tuber, Folsom (13) reports that in his experiments cortical greening reached a depth of 1/16 inch in the variety Katahdin. It was very shallow, however, in Chippewa and Green Mountain after 7 days exposure to daylight in a greenhouse.

Folsom (13) found no difference in the rate of greening among the three varieties Katahdin, Chippewa, and Green Mountain, but Mayfield et al. (20) observed that Bliss Triumph tubers developed less greenish-yellow color in storage than did tubers of Russet Burbank (Netted Gem).

Maturity of the tuber at time of exposure is mentioned in the literature as having some effect on the rate of greening. Thus Gram (15) and Dykstra (11) state that tubers harvested before maturity turn green more readily than mature ones. However, there have been no experiments published to prove this statement.

Conner (8), experimenting with different wavelengths in their effects on solanine formation in the potato tuber, observed greening after 20 days continuous exposure. This effect was caused only by wavelengths longer than 5500 A., i.e. yellow, orange, and red light.

However, a recent work by Frank (14) shows that blue light is also effective in chlorophyll formation. She worked with etiolated seedlings of **Avena byzantinum** var. **sativa** and was able to show that a narrow band about wavelength 4400 A. has greater relative effectiveness in formation of chlorophyll than the orange and red part of the spectrum which, however, has the widest effective bands. A certain amount of light energy applied over a wide range in the red end of the spectrum will thus have more quantitative effect than the same amount of energy applied over a wide range in the blue end of the spectrum. She was also able to show that the failure of earlier workers to detect this narrow but effective band in the blue part of the spectrum was due to the fact that the bands of light they used were too wide. This will inevitably lead to overlooking the effective band in the blue region because of its narrowness.

Wide variation has been reported in the length of exposure required to cause greening. Morgenstern (22) found in experiments on solanine formation that the tubers became green in 3 weeks in diffused daylight. Griebel (16), also working with solanine formation, found only slight greening in 25 days, but in another experiment he describes the tubers as distinctly green in 12 days. Bakken (2) states that potatoes exposed to light in a retail store began to turn green underneath the skin after 4 to 7 days. The differences may be due to different varieties used as the authors did not report with which varieties they were working. If tubers are exposed to direct daylight, greening can develop rapidly. Thus Folsum (13) was able to detect cortical greening after only 2 days exposure.

Folsum (13) also found that tubers at 70° F. exposed to light from fluorescent lamps developed greening more rapidly than tubers at 40° F. and under the same lighting conditions. Similar results were obtained by McCubbin (21). He found that tubers of the X889 strain of the Smooth Rural variety kept for 1 month at about 51° F. and exposed to light of an average intensity of 40 footcandles became dark green to purplish-black in color. Similar tubers kept for the same period at 36° F. and given the same illumination showed no such colors.

The convincing results mentioned above indicate that the apparent contradictory observations of the effect of temperature on greening reported by Mayfield et al. (20) may not be attributable to tempera-

ture. They observed in 7 years of storage tests that Russet Burbank (Netted Gem) tubers became greenish-yellow in color and acrid in flavor when kept in a cellar with average monthly temperatures ranging from 37.6° F. to 45.9° F. and the average monthly relative humidity ranging from 81.4 percent to 94.4 percent. The same variety stored in a cellar with temperatures from 55.0° F. to 60.0° F. and relative humidity from 42.2 percent to 56.9 percent, did not develop any green color or off-flavor. Tubers of the variety Bliss Triumph responded in a similar manner except that they did not develop acrid flavor in any case. The authors did not state whether the amount of light accidentally reaching the potatoes was the same in both cellars. If this is assumed to be the case these observations must be considered an indication of a promotive effect of high humidity on greening providing that temperature did not act in the opposite direction of that found by other workers mentioned above. No other report has been found in the literature regarding the effect of humidity on greening.

Careful operation of the cellar may reduce greening in storage according to several authors (4, 23, 28, 31). They recommend that precautions be taken to avoid exposure of stored tubers to light from open doors and that cellar light be installed in such a manner as to protect the tubers from direct exposure. The literature indicates also that more care in shipping and in handling in stores can largely avoid greening. Thus DeLoach and Sitton (9) came to the conclusion that much of the greening incident to marketing can be prevented if the potatoes are shipped in paper sacks and if displays that expose the product to much light are avoided. Bakken reports:

> "The retailers stack the filled bags upon the sidewalk in front of the stores, display the potatoes in large open boxes in the front window like oranges, or store them in a back room near a window where the light pours over them day after day until they are sold." (2)

It seems obvious that some of the more extreme cases of mishandling, such as this, can be eliminated by instructing the retail tradesmen, but it still will be necessary to keep the potatoes in light for some time as the customers demand to see what they buy.

Since complete prevention of greening thus appears impossible under present methods of potato marketing, a practical method of removing the green color of tubers after it has developed would be of great value. Work by Dustman and Duncan (10) and Reger (25) with apples seems to indicate a possibility in this field. They sprayed apples with solutions of sodium thiocyanate. Quantitative determinations of chlorophyll showed much lower content for the sprayed than the unsprayed fruits. Further, Brooks (5) found that it was possible to induce chlorosis with low concentrations of sulphur dioxide in the water plants **Elodea, Hydrodictyon, Nitella,** and **Spirogrya** without causing visible injury.

Morris and Afanasiev (23) claim that greening fades if the tubers are stored in darkness for several weeks. Folsom (13) found that greening faded faster in the dark at 75° F. than at 35° F. He states that green tubers of the variety Katahdin faded less than tubers of Chippewa and Green Mountain. He concludes:

> "Culinary tubers if exposed to light for 2 days may require over a month in warm dark storage for fading of the green, and if exposed to light for longer periods may require several months of warm dark storage for fading of the green."

However, his results are not quantitatively expressed and it is difficult to draw any conclusions from them.

Some authors state that greening should be correlated with formation of solanine (2, 6, 16, 17, 22). However, there is not complete agreement in this matter among findings of different workers. Thus Morgenstern (22) found that the tubers became green in 3 weeks in diffuse daylight and that the solanine content increased from 0.0113 percent to 0.0232 percent. Another sample exposed for 4 weeks turned green and the solanine content increased from 0.0064 percent to 0.0236 percent. Griebel (16) exposed tubers for 25 days and got very slight greening, but the solanine content increased from 0.0196 percent to 0.0393 percent in the same period. In other tests he describes the tubers as distinctly green after 12 days of exposure, but recorded no increase in solanine content. With potatoes harvested in 1923 he found (17) that the solanine content increased from 0.0052 percent to 0.0125 percent after 4 days of exposure without distinctive greening. However, none of the authors cited (16, 17, 22) stated the variety with which they were working and the light used was in all cases diffused daylight. Both these facts leave open several sources for variation and thus make it impossible to reach any explanation for the different results.

Bohmer and Mattis (6) removed the soil from tubers so that one half of each tuber, still attached to the plant, was exposed to light for 35 days. At harvest each tuber was divided into a greened and a non-greened portion. In the variety Kartoffel von Iserlohn the solanine content was 0.0038 percent in the non-greened part and 0.0187 percent in the greened part, but in the variety Modrow's Industrie the figures were 0.0020 percent and 0.0028 percent, respectively.

In experiments with the effect on the potato tuber of different wavelengths of light, Conner (8) found that the blue end of the spectrum was most active in the formation of solanine, but did not cause any greening, whereas the yellow-red end of the spectrum did not cause solanine formation but did cause visible greening. These findings suggest that the divergent results found by other authors (16, 17, 22) may be due not alone to differences in amount of light but also to differences in quality of the light used.

Experimental Work

General Methods of Procedure

Tubers of the potato (**Solanum tuberosum** L.) variety White Rose were used throughout most of this work. The varieties Katahdin and Russet Burbank were used in one experiment each.

The tubers of variety White Rose used in experiments 1, 3, 4, 8, 10, 14, and 15 were grown at the University of Idaho Branch Experiment Station at Sandpoint on silt-loam soil. The material used in experiments 2, 5, 6, and 9 was obtained from Robert Schooler, Genesee, Idaho, and was also grown on silt-loam soil. The Russet Burbank tubers used in experiment 11 were grown on irrigated, fine sandy loam soil at the University of Idaho Branch Experiment Station at Aberdeen, Idaho. The tubers of variety Katahdin used in experiment 7 were grown on silt-loam soil on the University of Idaho farm at Moscow.

Tubers approximately 2 inches in diameter, i.e. tubers weighing about 2½ to 3 ounces, were selected for the experiments and inspected very closely after washing to exclude tubers with even the slightest amount of greening already present. Only the more nearly flat side of the tubers was exposed during the experiment.

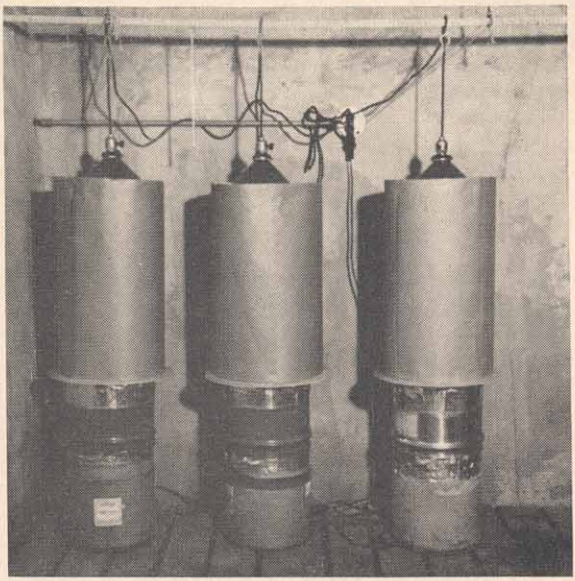

Figure 1.—General view of controlled-temperature chambers.

In order to be able to control some elements of the environment, three special chambers were constructed as shown in Figures 1 and

2. They consisted of a lower cardboard container with sockets for ordinary Mazda lamps mounted on a base. Above was a metal container as the experimental chamber. Both containers were lined with aluminum foil for insulation and for reflection of light. The lower container served as a heating element for the upper container through the metal bottom of which the heat was transmitted by radiation. To give the desired temperatures various combinations of ordinary Mazda lamps were used in the lower container.

The upper container served as the experimental chamber and had a false floor made of wood slats spaced three-quarters of an inch and raised 2 inches above the bottom of the container to place the potatoes in a position where the temperature could be kept uniform. The temperature range in this position was measured by a maximum-minimum thermometer.

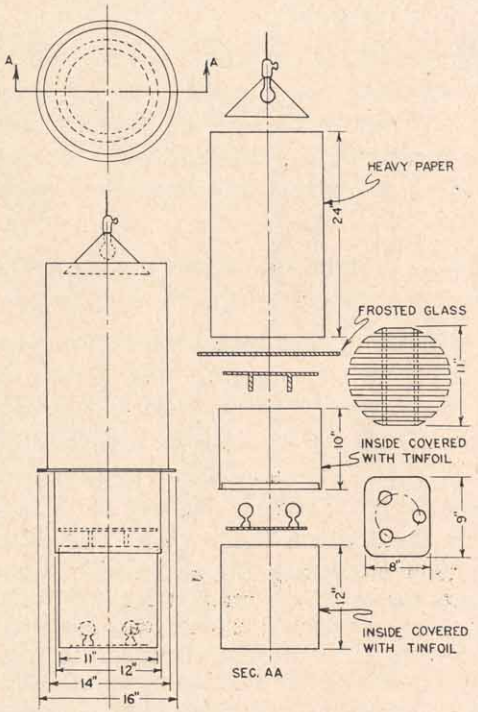

Figure 2.—Working-drawing of controlled-temperature chamber.

The thermometers were read twice every day and the temperature for a specific experiment is reported as average of the daily mean temperatures.

The relative humidity was measured in experiments 1 and 2 by humidistats placed on the false floor in the middle of the containers. The humidistats were read twice a day.

Mazda lamps used as a source of light were placed approximately 80 centimeters above the potatoes in the experiments and were shielded so as to keep the light for one container from interfering with another container. The intensity of light reaching the tubers was measured with a Weston Universal Light Meter by the indirect method recommended by the manufacturer (32). This method is based on the relationship between the units of incident light and that of brightness. A close up brightness reading of a white surface in the position of the potato tubers was made and by placing the "A" position on the exposure guide dial of the meter opposite the brightness indicated the intensity of illumination in footcandles was read opposite the "C" position.

The experimental chambers were covered with a frosted glass plate resting on supports one-quarter of an inch above the walls of the container to permit limited air circulation. This frosted glass plate, being in the path of the light used for illumination, also served as a means of getting uniform light distribution on the potato tubers.

The experimental chambers were set up in a refrigerated storage where the temperature was kept nearly constant at about 40° F.

The amount of greening was measured in two ways, by matching the color of the tubers with a standard color chart, and by determining the amount of chlorophyll in the tubers. In matching the tubers with color charts the skin of the tubers was moistened with glycerine on the area selected for examination and the color was matched with the standard color charts published by Maerz and Paul according to the methods given there (19). The periderm was then removed, the surface again moistened with glycerine and the color of the underlying tissue, the cortex, matched with the standard. In preliminary tests it was found that it was easier to read the color when the surface was moistened and glycerine was found to give the most satisfactory results as it evaporated slowly and seemed to delay the browning naturally occuring when the cortex was injured and exposed to air.

As chlorophyll was assumed to be the component responsible for greening it seemed reasonable to use the amount of chlorophyll present in green tubers as a measure of the degree of greening. The method outlined by Compton and Boynton (7) for apple leaves was modified to apply to potatoes.

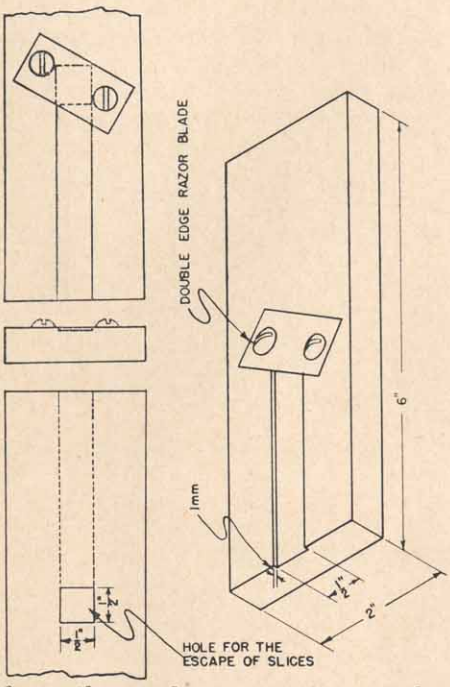

Figure 3.—Working-drawing of plan for slicing cylinders of potato into 1-mm. discs.

In the first part of the study 5 cylinders were cut with a corkborer 9.1 millimeters in diameter from each tuber to be tested. These cylinders were sliced into 1-millimeter discs on the plane shown in Figure 3. The first, second, and third millimeter layers of tissue from the surface were extracted separately in the following manner. Twenty discs, 5 from each of 4 tubers, were macerated in a test tube with a twin-edged spatula-shaped knife and extracted in darkness at a room temperature for 24 hours with approximately 8 ml. or 95 percent ethyl alcohol. This procedure will be referred to as method A. Later it was found desirable to change the procedure slightly in order to be able to sample each tuber individually. Ten cylinders were cut from each tuber and sliced as described above. The first 3 millimeters of tissue from the surface of each cylinder were extracted together, thus giving a sample of 30 discs from each tuber. This sample was macerated in a centrifuge tube with a propeller-like knife inserted into the tube and rotated by an adjustable-speed motor. The sample was extracted in 20 ml. of 95 percent ethyl alcohol in darkness at room temperature for 24 hours. This procedure will later be referred to as method B.

After the 24-hour period of extraction the extracts were filtered and made up to volume, 10 and 25 ml., respectively, for methods A and B. The relative density of the extracts as compared to a blank consisting of 95 percent ethyl alcohol was obtained by measuring the transmitted light in a Coleman Universal spectrophotometer at a wavelength of 6600 A. using a Coleman PC-5 filter which has its best transmittance in the range of 6500-8000 A.

To convert the density readings into concentrations of chlorophyll in the solution, the following procedure was followed. Six samples of green tubers of the White Rose variety were peeled and the peel-

ings chopped in a food chopper and extracted in darkness at room temperature for 24 hours with 95 percent ethyl alcohol. These extracts were then filtered and samples of 25 ml. transferred to ethyl ether by first shaking in a separatory funnel with 250 ml. distilled water and 30 gm. sodium chloride, and thereafter again shaken with 25 ml. ethyl ether for one-half hour. This extract was made to volume and read in the spectrophotometer at 6425 A. and 6600 A. and the concentration of chlorophyll calculated using the formulas and constants given by the Association of Official Agricultural Chemists (1). Various concentrations of chlorophyll in alcohol were then made up from the original extracts and read in the spectrophotometer. Fifty-six such readings at 6600 A. were plotted against the concentrations of semi-logarithmic graph paper. A straight line to best fit the points was arbitrarily drawn. The correlation between the line and the points was measured by the least squares method to be +0.974.

The methods and procedures applying to specific experiments are given together with the results on the following pages.

Presentation of Data

From the literature survey it appears that several problems were still unanswered and required investigation before any possible means of prevention or correction of greening could be suggested. This study was devoted to some of the problems considered to be of importance for further work. Phases studied fall into six parts: influences of environment; depth of which greening develops; effect of certain aspects of the previous history of the tubers; preventive and corrective treatments; fading of greening in dark storage; and relation of chlorophyll content to greening.

Influences of Environment

Several elements of environment probably have some influence on the amount of greening developing and the rate at which this greening takes place. The object of the experiments described in this section was to determine the influences of temperature, humidity, light intensity, and light quality on the rate and degree of greening of potato tubers.

HUMIDITY. Among the elements of environment considered in this study, humidity was the most difficult to keep constant when other conditions were varied. It was, therefore, considered important to know from the beginning any relationship between humidity and rate of greening in order to determine how much emphasis should be placed on keeping the humidity constant throughout the study. Two experiments were set up to test the effect of high and low relative humidity. Both experiments were carried out with the equipment shown in Figure 1. The high humidity was maintained by keeping a tray of water in the bottom of the experimental chamber. No measure was taken to lower the humidity in

the low-humidity treatment below that naturally occurring when the temperature was kept high in the container placed in a refrigerated room.

Experiment 1. Ten tubers of the variety White Rose were used in each treatment. At the end of each exposure period 5 tubers from each treatment were examined. The color was matched with the color charts and four cylinders were cut from each tuber to form the sample used for chlorophyll determination. The data are given in Table 1.

Table 1.—Chlorophyll content and color of White Rose potato tubers exposed to 9 footcandles of light in high and low relative humidity with temperatures of $58.4 \mp 0.81°$ F and $60.9 \mp 0.70°$ F., respectively.

Relative Humidity percent	Length of Exposure hours	Chlorophyll mg./100 cm.²	Color According to Maerz and Paul*	
			External Color	Color of Cortex
72.9 ±1.32	120	0.14	13K4, 13J4, 13J5	12K1, 20L1
50.3 ±1.79	120	0.17	13K4	12K1, 12L1, 19L3, 20L2, 20L3
72.9 ±1.32	360	0.36	14K3, 14K4, 14K5	20L3, 20L4, 20L5
50.3 ±1.79	360	0.34	14K3	20L2, 20L4

*See Literature Cited.

The figures for chlorophyll concentration are the total of the amounts found in the first, second, and third millimeter layers. Due to lack of replications it was not possible to test the reliability of the chlorophyll determinations statistically but the differences are not thought to be significant. The average temperatures in the high and low humidity chambers were $58.4 \pm 0.81°$ F. and $60.9 \pm 0.70°$ F. The difference, $2.5 \pm 1.07°$ F., was not considered significant.

Experiment 2. Each lot in the two humidity treatments, 88.0 ± 2.28 percent and 57.2 ± 2.07 percent relative humidity, consisted of nine tubers of the variety White Rose. The light intensity was 9 footcandles and the exposure period 240 hours. The average temperatures in the two chambers were $56.9 \pm 0.90°$ F. and $56.1 \pm 1.39°$ F. Sampling and extraction were carried out according to method B and the chlorophyll concentrations given in Table 2 were averages of individual determination of nine tubers.

Table 2.—Chlorophyll content of White Rose potato tubers exposed for 240 hours to 9 footcandles of light in high and low relative humidity.

Relative Humidity (percent)	Temperature (° F.)	Chlorophyll (mg./100 cm.²)
88.0 ±2.28	56.9 ±0.90	0.35 ±0.018
57.2 ±2.07	56.1 ±1.39	0.32 ±0.016
Difference	0.8 ±1.68	0.03 ±0.024

There was no statistically significant differences between the amounts of chlorophyll found under the two levels of humidity.

From these experiments it was concluded that humidity, within the range to be expected under ordinary conditions, has no significant influence on the rate of greening. The humidity prevailing in the rest of the experiments was therefore not considered and no care taken to keep it constant.

TEMPERATURE. Four experiments were carried out to clarify the effect of temperature on greening of potato tubers.

Experiment 3. In this experiment four tubers were examined from each of three temperature treatments at the end of each of three periods of exposure to 9 footcandles of light. Chlorophyll determinations were made according to method A described above. It is evident from the results of this experiment that the amount of chlorophyll in the tissue increased with length of exposure to light, and that chlorophyll accumulated most rapidly at the highest temperature. (Table 3, Figure 4.)

Table 3.—Chlorophyll content and color of White Rose potato tubers exposed to 9 footcandles of light at different temperatures.

Temperature ° F.	Length of Exposure hours	Chlorophyll mg./100 cm.²	Color According to Maerz and Paul	
			External Color	Color of Cortex
42.0 ±0.23	120	0.03	11K5, 12J5, 12L5, 12K6	11I1, 11J1
52.2 ±0.27	120	0.11	11J5, 11J6, 13J5	12J1, 12K1
68.1 ±0.31	120	0.17	13J3, 13J4, 13J5	12L1, 19K5, 20L1
42.0 ±0.23	240	0.03	12J5, 12K5, 12K6	11K1, 11L2
52.2 ±0.27	240	0.24	14J4, 14K3, 14K4	20L2, 20L4, 20L6
68.1 ±0.31	240	0.29	14K4, 14K5, 14L5	20L5, 20L6
42.0 ±0.23	360	0.09	12J5, 13J6	11K1, 12K1
52.2 ±0.27	360	0.34	14J1, 14K3, 14L3	20L6
68.1 ±0.31	360	0.34	14L3, 14K4, 14K5	20L6, 20L7

Experiment 4. Since the points used for plotting the curves in Figure 4 represent but single determinations of chlorophyll it was considered desirable to carry out another experiment at the same temperatures and with triplicate determinations. This made it necessary to confine the experiment to one exposure period, 240 hours. Method A was used in taking the samples. The average temperatures in this experiment (40.5 ±0.17° F., 50.9 ±0.48° F., and 64.6 ±1.01° F.) were slightly lower than in the preceding experiment, and the amounts of chlorophyll developed were accordingly slightly lower. There was a significant difference not only between the effect of the low and the two high temperatures but also between the two high-temperature treatments.

Experiment 5. The development of method B as outlined above under general methods of procedure made it possible to get 12 independent chlorophyll determinations from each temperature treatment. An experiment was carried out with temperatures 42.1 ±0.18° F., 55.7 ±0.19° F., and 68.4 ±0.95° F. which are at about the same levels as in the two preceding experiments. The light intensity and

period of exposure were the same, i.e. 9 footcandles and 240 hours, respectively. No significant difference was found between the high and the medium-temperature treatments.

Experiment 6. Since it was not possible from the foregoing experiments to get a clear-cut picture of how time and temperature acted together in their effects on chlorophyll formation in the tubers, an experiment on a larger scale was set up to clarify this interaction. Three large cardboard containers were placed in three different rooms with mean temperatures of approximately 41°, 51°, and 66° F. respectively. A single layer of tubers was arranged in circular fashion at the bottom of each container, with a Mazda lamp suspended 80 cm. over the center of the lot. The light intensity at the level of the tubers was 19 footcandles directly under the lamp, and 13 footcandles at the level of tubers in the periphery. The samples were taken in a modified random manner with emphasis on getting tubers which had been illuminated equally from each treatment. Samples of nine tubers were removed from each treatment after 72, 120, 240, 360, 480, and 600 hours of continuous exposure, and the chlorophyll content determined. The tubers developed chlorophyll slightly more rapidly in this experiment than in the three preceding ones (Tables 3, 4, 5, and 6.) This was probably due to the higher light intensity in this last experiment.

Table 4.—Chlorophyll content of White Rose potato tubers exposed for 240 hours to 9 footcandles of light at different temperatures.

Temperature (° F.)	Chlorophyll (mg./100 cm.^{2})
40.5 ±0.17	0.03 ±0.006
50.9 ±0.48	0.17 ±0.013
64.6 ±1.01	0.26 ±0.006

Table 5.—Chlorophyll content of White Rose potato tubers exposed for 240 hours to 9 footcandles of light at different temperatures.

Temperature (° F.)	Chlorophyll (mg./100 cm.^{2})
42.1 ±0.18	0.03 ±0.005
55.7 ±0.19	0.27 ±0.018
68.4 ±0.95	0.26 ±0.019

Table 6.—Chlorophyll content of White Rose potato tubers exposed to 13-19 footcandles of light at different temperatures.

Length of Exposure hours	Average Temperatures During Experiment		
	40.9 ±0.37 mg./100 cm.^{2}	51.4 ±0.82 mg./100 cm.^{2}	66.3 ±0.49 mg./100 cm.^{2}
72	0.02 ±0.003	0.05 ±0.002	0.16 ±0.009
120	0.01 ±0.005	0.09 ±0.009	0.19 ±0.022
240	0.02 ±0.004	0.32 ±0.014	0.33 ±0.025
360	0.04 ±0.005	0.48 ±0.046	0.42 ±0.011
480	0.10 ±0.014	0.61 ±0.010	0.41 ±0.019
600	0.18 ±0.011	0.71 ±0.037	0.45 ±0.10

A comparison between the results of this experiment, as expressed in Figure 5, and the results of experiment 3, expressed in Figure 4, shows that the same general trend exists in the curves up to 240 hours of exposure; namely, the highest amount of chlorophyll was developed in the highest temperature. However, at 240 hours of exposure the picture changes completely for the 51° F. and the 66° F. treatments. At this point the tubers in these two temperatures have developed about equal amounts of chlorophyll, but this amount, between 0.30 and 0.40 mg. per 100 cm.^{2}, was the maximum to develop at 66° F. since the amount did not increase

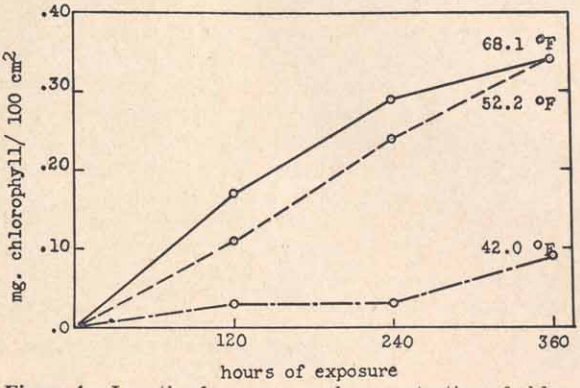

Figure 4.—Length of exposure and concentration of chlorophyll in tubers exposed at three levels of temperature (experiment 3).

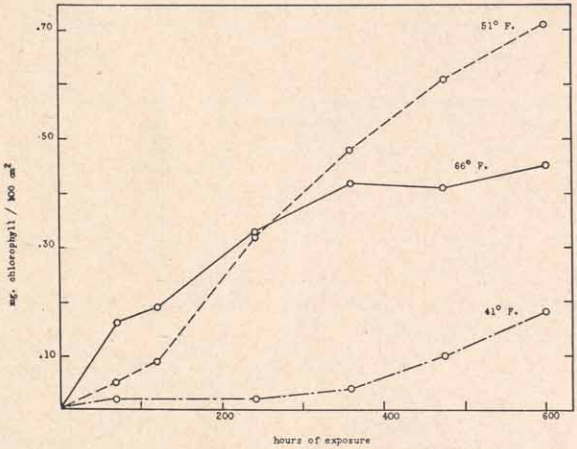

Figure 5.—Length of exposure and concentration of chlorophyll in potato tubers exposed at three levels of temperature (experiment 6).

above that with time. Under the 51° F. treatment, on the other hand, the chlorophyll continued to develop and the curve shows but little tendency to level off even at the end of the experiment

after 600 hours of exposure. The tubers held at 41° F. developed but little chlorophyll until between 300 and 400 hours of exposure.

LIGHT INTENSITY. In order to determine the effect of light intensity on the amount of greening, the following three experiments were carried out.

Experiment 7. Tubers of the variety Katahdin were exposed for 120, 240, and 360 hours to light intensities of 5, 9, and 28 footcandles in the controlled-temperature chambers. The average temperatures during the experiment were 62.0 ±0.45° F., 60.7 ±0.28° F., and 63.8 ±0.39 F. for the 5, 9, and 28 footcandle treatments, respectively. The results of the chlorophyll determinations and the color matchings are given in Table 7. It will be seen that the values are higher after 120 hours of exposure than after 240 hours. This does not seem logical and it is possible that the difference can be ascribed to experimental error. Method A was used for sampling and extraction and it was therefore not possible to test the reliability of the results statistically. Although the differences in average tempera-

Table 7.—Chlorophyll content and color of Katahdin potato tubers exposed to different light intensities at approximately 62° F.

Light Intensity footcandles	Length of Exposure hours	Chlorophyll mg./100 cm.²	Color According to Maerz and Paul	
			External Color	Color of Cortex
5	120	0.07	12I1, 12J2	11G2, 11H1, 11J1, 11K1
9	120	0.10	12J2, 13J4, 13J5, 13K2	11J1, 11K2, 12J1, 12K1
28	120	0.15	12J2, 13J4, 13K3, 14L6	12J1, 12K1, 12L2, 13L2
5	240	0.04	13J4, 13K3, 14K5	12J1, 12K1
9	240	0.08	13J4, 13K3, 13K5, 14K2	12K1, 13L2, 21L4
28	240	0.13	14L5, 14L7, 15L7	13L1, 13L2, 13L3
5	360	0.10	14K5, 14L4	12J1, 12K1, 12L1
9	360	0.15	13L3, 14L5, 14L6	12L1, 19L4, 19L6, 20L3
28	360	0.28	14L6, 15L5, 15L7	13L3, 21L2, 21L3, 21L6

tures between the containers were statistically significant at the five percent level, these slight differences are not thought to be responsible for the apparent illogical results.

Having determined, as pointed out earlier, that low temperature depressed greening at low light intensities, it became of interest to determine if high light intensity would increase the development of greening if the temperature was low. The following two experiments were carried out to test this possibility.

Experiment 8. Three different lots of tubers of the variety White Rose were used. They were exposed to 9, 28, and 80 footcandles of light, respectively, and all were sampled at intervals of 120, 240, and 360 hours. The average temperatures for the whole period were 42.8 ±0.16° F., 44.8 ±0.18° F., and 43.1 ±0.44° F. in the 9, 28, and 80 footcandle treatments, respectively. Method A was used in taking the samples. The analyses showed (Table 8) that the tubers in 9 and 80 footcandles developed little chlorophyll, whereas those in 28 footcandles developed considerly more.

Table 8.—Chlorophyll content and color of White Rose potato tubers exposed to different light intensities at approximately 43° F.

Light Intensity footcandles	Length of Exposure hours	Chlorophyll mg./100 cm.²	Color According to Maerz and Paul	
			External Color	Color of Cortex
9	120	0.03	No visible greening	No visible greening
28	120	0.04	No visible greening	No visible greening
80	120	0.04	No visible greening	No visible greening
9	240	0.06	12J4, 12J5, 13J6	11J1, 11K1
28	240	0.11	12J3, 12J5, 13J4, 13J5	10J1, 11K1, 12K1, 20L1
80	240	0.05	12H4, 12I3, 12J5	10G1, 11J1, 11K1
9	360	0.06	13J6, 14J6, 14K6	12J1, 12K1
28	360	0.14	13H5, 14J5, 14K5, 14L5	13L1
80	360	0.04	13H5, 13J6, 13K5	10K1, 11K1, 11L1

Unfortunately the average temperature in the 28 footcandle treatment was slightly higher, though not significantly so, than the average temperature in the 9 footcandle and 80 footcandle treatments. It is, therefore, not possible to determine whether the higher amount of chlorophyll in the 28 footcandle treatment is due to optimum illumination or to the slightly higher temperature. At any rate, it can be concluded that the high light intensity, 80 footcandles, did not promote the development of chlorophyll and the possibility exists that it reduced the rate of greening as compared to the effect of 28 footcandles. Time did not permit further testing of this possibility.

Experiment 9. In this experiment the light intensities were the same as used in the preceding experiment, but the average temperatures were somewhat lower, 38.4 $\pm$0.24° F., 37.7 $\pm$0.31° F., and 38.3 $\pm$0.40° F. for the 9 footcandle, the 28 footcandle, and the 80 footcandle treatments. After the end of the 240-hour exposure period the tubers were not visibly green and the chlorophyll test showed that no measurable amount of chlorophyll was present.

Although these experiments were not conclusive, they indicate that a light intensity as low as 5 footcandles can cause considerable greening at temperatures around 62° F. and that increasing the light intensities within the range used here and at the temperatures used in experiment 7 (60° to 64° F.) increases the amount of chlorophyll formed. However, there does not seem to be any effect from increasing the light intensity if the temperature is kept below approximately 39° F.

LIGHT QUALITY OR COLOR. Conners work (8) shows that only certain wavelengths are effective in developing greening in potato tubers. This suggests the possibility of screening some of the effective rays and still keeping the tubers in light which would permit inspection. As the emphasis in this study was laid on getting results easily duplicated in practice it was decided to use available color screens. Ordinary, colored cellophane was used. Two experiments were carried out to test the effects of these screens.

Experiment 10. Shallow trays with tubers of White Rose were covered with yellow, red, green, and blue screens and placed in a greenhouse and exposed to daylight. The temperature was approximately 60° F. during the nights, the day temperature being 15-25 degrees higher. The weather during the period of the experiment, March 10 to March 25, 1948, was mostly cloudy, many days being completely overcast. Approximate figures for light intensities reaching the tubers under the various screens at noon on a day considered average for the experiment were 4000 footcandles for the uncovered check, 1500 footcandles under the yellow screen, and 600 footcandles under the red, green, and blue screens. (Table 9.) The samples were taken according to method A. After 5 days of exposure one sample was taken from each treatment, after 10 days three samples, and after 15 days one sample.

Table 9.—Chlorophyll content and color of White Rose potato tubers exposed to daylight under cellophane screens of various colors.

Screen	Light Intensity footcandles	Chlorophyll mg./100 cm.²	Color According to Maerz and Paul	
			External Color	Color of Cortex
5 days exposure—				
None	4000	0.13	14B6, 14B7, 14D5, 14D6	13A6, 13K4, 14K5
Yellow	1500	0.11	13J6, 14J6, 14K6	11K1, 12J2, 12K1, 12K3
Red	600	0.10	13I5, 13J5, 13J6	11K1, 12J1, 13L2
Green	600	0.07	13J4, 13J5, 13K6	11K1, 12J1
Blue	600	0.10	13J5, 14J5, 14K6	12J1
10 days exposure—				
None	4000	0.41 ±0.021	14I3, 14J4, 14J6	14L3, 22L2
Yellow	1500	0.26 ±0.020	14K4, 14K5, 14K6	13L1, 14L2
Red	600	0.20 ±0.015	14J4, 14J5, 14J6, 14K5	12L1, 13L1, 20L3
Green	600	0.15 ±0.011	13J5, 13K4, 14K5, 14L6	12J1, 12K1
Blue	600	0.16 ±0.009	14J5, 14K6	12K1, 13L1
15 days exposure—				
None	4000	0.49	15C7, 15E6, 15J6, 15L6	14L3, 15H5, 15L3, 15L6
Yellow	1500	0.36	14K6, 14L6, 15J6, 15L8	14L1
Red	600	0.26	14J5, 14K5, 14K6	13L1, 14L1
Green	600	0.15	13K3, 14K3, 14K4, 14K5	12K1, 13L1
Blue	600	0.15	14J4, 14J5, 14K6	12K1, 12L1, 13L1

The color and chlorophyll determinations are given in Table 9. Tubers exposed to full daylight became almost purple over their entire surface. The tubers under the cellophane screens developed also a little red pigment but only around the eyes. This did not interfer with the chlorophyll determinations. The various screens reduced the rate of greening considerably. Most effective were the green and blue screens (Table 9).

Experiment 11. In this experiment tubers of the varieties White Rose and Russet Burbank were used. The trays were placed on a table and tilted slightly facing a north window and exposed to dif-

fused daylight. The average temperature during the experiment was 66.0 ±0.94° F. The exposure period ran from February 1 to March 6, 1949. The weather during the first 23 days of this period was very cloudy with rain and snow. The last 10 days were mostly sunny. The following approximate light intensities reached in tubers at noon in the various treatments: uncovered, 300 footcandles; yellow screen, 200 footcandles; and red, green, and blue screens 50 footcandles (Table 10).

Table 10.—Chlorophyll content of White Rose and Russet Burbank potato tubers exposed to diffused daylight under cellophane screens of various colors for 34 days at 66° F.

Screen	Light Intensity footcandles	Chlorophyll (mg./100 cm.²)	
		White Rose	Russet Burbank
None	300	0.58 ±0.057	0.46 ±0.025
Yellow	200	0.49 ±0.043	0.38 ±0.017
Red	50	0.32 ±0.045	0.27 ±0.041
Green	50	0.15 ±0.014	0.12 ±0.016
Blue	50	0.15 ±0.033	0.12 ±0.017

These were considerably lower than in the foregoing experiment, but the various screens reduced the light intensities proportionately. Four tubers of each variety in each treatment were sampled according to method B after 34 days of exposure. The chlorophyll determinations showed that the various screens exerted the same inhibitive effect on the development of greening as in the previous experiment. The green and blue screens reduced the rate of greening the most and the red screen was more effective than the yellow (Table 10). The number of tubers becoming objectionably green was evaluated throughout the experiment and these observations show the same general effects of the screens. (Table 11.)

Table 11.—Number of potato tubers being objectionably green after different periods of exposure to diffused daylight under cellophane screens of various colors at 66° F.

Length of Exposure days	Number of Tubers Out of Nine									
	Uncovered		Under Yellow Screen		Under Red Screen		Under Blue Screen		Under Green Screen	
	W.R.*	R.B.*	W.R.*	R.B.*	W.R.*	R.B.*	W.R.*	R.B.*	W.R.*	R.B.*
8	2	0	0	0	0	0	0	0	0	0
10	3	4	0	0	0	0	0	0	0	0
13	9	9	0	0	0	0	0	0	0	0
15	9	9	3	0	2	1	0	0	0	0
18	9	9	9	9	4	3	1	0	1	0
21	9	9	9	9	9	9	3	1	2	0
24	9	9	9	9	9	9	3	1	3	0
27	9	9	9	9	9	9	3	1	9	0
32	9	9	9	9	9	9	3	1	9	9
34	9	9	9	9	9	9	9	9	9	9

*W.R.—White Rose; R.B.—Russet Burbank.

It is realized that the screens also reduced the intensity of light at the same time that it changed its quality. However, the light intensities were equal under the red, blue and green screens and yet tubers under the red screen developed considerably more greening than tubers under the other two screens. This indicates that the blue and green lights are not so effective in promoting greening as is the red light.

That a difference in rate of development of visible greening exists between the two varieties tested, White Rose and Russet Burbank, is shown in Table 11. The figures given in Table 10 show that this difference exists not alone in visible color but also in amount of chlorophyll present in the tissue. The reliability of these figures was determined by Student's paired method, which gave a t-value of 3.469. This indicates by comparison with the t-table that the difference is statistically significant with odds of approximately 97.1.

Depth to Which Greening Develops

It is of considerable interest for the subsequent use of the tubers to know how far into the tissue the green pigment develops. With the technique used (method A) in the first part of the study it was possible to get an estimation of this point. The amount of chlorophyll in each of the first 3 millimeters of tissue from the surface was determined for each layer separately. Table 12 gives a summary of all the determinations where this technique was used. These figures agree very well with the general observation that the majority of the chlorophyll was present in the first millimeter and that the third millimeter layer had very little green pigment. It was seldom possible to see just by inspection any pigment in the third millimeter layer. The tissue farther from the surface than 3 millimeters was not analysed because visual inspection was not able to detect any pigment and it was known that if any at all was present it would be impossible to determine it with the technique used.

Table 12.—Chlorophyll concentration in the first, second, and third millimeter layers of tissue of potato tubers with various total amounts of chlorophyll.

Range of Total Chlorophyll mg./100 cm.²	Number of Determinations	First Millimeter Layer mg./100 cm.²	Second Millimeter Layer mg./100 cm.²	Third Millimeter Layer mg./100 cm.²	1st+2nd+3rd Millimeter Layers mg./100 cm.²
0—0.12	28	0.046	0.016	0.007	0.069
0.13—0.25	26	0.142	0.036	0.012	0.190
0.26—0.50	16	0.248	0.108	0.021	0.377
0—0.50	70	0.128	0.045	0.012	0.184

From this it can be concluded that in these experiments most of the development of greening was confined to the layer of tissue within 2 millimeters from the surface of the tubers.

Effect of Certain Aspects of the Previous History of the Tubers

MATURITY. It has been a common belief that potato tubers not fully mature when dug develop greening faster than mature tubers when they are exposed to light. However, no experiments on the subject have been reported. The following experiment was carried out to test this hypothesis.

Experiment 12. Tubers of the variety White Rose were dug August 12, 1948, when the vines were still green and healthy; on September 1 when the vines had started yellowing and showing signs of becoming mature, and on September 21 when the vines were completely dead. Three randomized plots were dug at each digging time. Four uniform tubers from each of the 9 plots were selected for exposure to 9 footcandles of light at a temperature of 69.0 $\pm$0.34° F. This treatment started on October 9, 1948, and continued for 240 hours. The 3 experimental chambers described above served as three replications of the light treatment. Method B was used in making the chlorophyll determinations with the modification that two tubers or 60 discs were analysed together, thus giving six separate determinations on tubers of each of the three different maturity levels. The results (Table 13) show no significant differences, at the 1 percent level, between the digging times. If the 5 percent level is taken as a criterion there is a significant difference between the amount of chlorophyll in tubers dug on August 12 and in those dug on September 1. However, it is the author's opinion that this difference should not be considered of any practical significance.

Table 13.—Chlorophyll content of White Rose potato tubers of different maturity after exposure to 9 footcandles of light for 240 hours at 69° F.

Treatments	Chlorophyll mg./100 cm.^{2}
Tubers harvested on 8-12-48	0.275
Tubers harvested on 9-1-48	0.328
Tubers harvested on 9-21-48	0.320
Least difference required for signifiance at the 5% level	0.041
At the 1% level	0.068

EXPOSURE TO SUN IN THE FIELD. It is sometimes a practice to leave the potato tubers on top of the ground for a few hours after they have been dug in order to get the skin to "set." It was considered important to know whether this exposure to strong sunlight would render the tubers more susceptible to greening when they were exposed later to lower light intensities.

Experiment 13 was set up to give information on this point. Tubers of variety White Rose were dug on September 21, 1948, in three randomized blocks. One sample from each block was placed in darkness immediately after digging whereas other samples were exposed to sunlight for 1, 2, and 4 hours. The tubers were marked

in order that the same side which was exposed to sun could be exposed to artificial light later. This light exposure was started in the experimental chambers on October 23, 1948, and lasted for 240 hours. The amount of chlorophyll present was determined according to method B. The analysis of variance was applied to the data and showed that there was no signficant difference between any two of the treatments. (Table 14.)

Table 14.—Effect of exposure to sunlight immediately after digging on subsequent development of chlorophyll in White Rose potato tubers after exposure to 9 footcandles of light for 240 hours at 62° F.

Length of Exposure to Sunlight hours	Chlorophyll Content mg./100 cm.^{2}
0	0.345
1	0.314
2	0.259
4	0.307
Least difference required for significance at the 5% level	0.106

Preventive and Corrective Treatments

Treatment to prevent later greening would be a great advantage if it could be done before the potatoes are shipped.

Experiment 14. In a search for such treatment tubers of White Rose were treated with 50 percent ethylene gas for 24 hours and other tubers were soaked in 1 percent ammonium thiocyanate solution for 6 hours. These tubers were exposed, together with untreated controls, to 9 footcandles of light at an average temperature of approximately 58° F. The amount of chlorophyll found after 240 hours of exposure varied somewhat (Table 15) but the differences are not considered significant. The work was not carried further as it was evident that the treatments would not prevent greening.

Table 15.—Chlorophyll content of White Rose potato tubers exposed to 9 footcandles of light for 240 hours at approximately 58° F. after treatment with various chemicals.

Treatment	Temperature (° F.)	Chlorophyll (mg./100 cm.^{2})
None	56.8 ±0.35	0.19
50% ethylene gas for 24 hours	58.0 ±0.50	0.29
1% ammonium thiocyanate for 6 hours	58.6 ±0.24	0.21

As it possibly would be of some value to correct greening after it has developed some work was done on this phase of the problem. Sulphur dioxide is generally used as a bleaching agent and this compound was used here in the form of sulphurous acid. Green tubers of White Rose were soaked in solutions of the acid in a wide

range of concentrations and for various periods of time. In many cases it was possible to bleach the green pigment completely but it was not possible to find any treatment which would do this without injury to the tubers. The degree of injury ranged from complete killing of the tubers to very slight injury recognizable by the excretion of small droplets of sap from the lenticels.

Fading of Greening in Dark Storage

It has been stated in the literature that green tubers stored in the dark will fade faster at high temperature than at low temperature (13, 23). The following experiment was carried out to get more evidence on this point.

Experiment 15. Tubers of White Rose were exposed to daylight for 5 days in a greenhouse at 60-80° F. The weather was overcast during the exposure period. This experiment was carried out in connection with the experiment with chemical treatments reported above. After the exposure period half of the sample was treated with 50 percent ethylene gas for 24 hours and the other half with 1 percent ammonium thiocyanate, for 6 hours. Eight tubers were picked from each lot and the chlorophyll content determined according to method A. One half of each of the two lots were then stored for 26 days in darkness at 36° F. and the other half kept at room temperature at approximately 75° F. (Table 16) shows the chlorophyll content of the eight tubers in each treatment at the end of the dark period. The differences between the various treatments were small and inconsistent, which indicates that if any fading of greening takes place in dark storage it is too small to be of any practical value.

Table 16.—Chlorophyll content of greened White Rose potato tubers as effected by dark storage in 36° F and 75° F. for 26 days after chemical treatment.

Time of Measurement	Chemical Treatment Prior to Storage	
	Ethylene	Ammonium Thiocyanate
Before storing	0.23	0.21
After storing at 36° F.	0.30	0.21
After storing at 75° F.	0.22	0.25

Relation of Chlorophyll Content to Greening

In order to determine to what extent the findings in the chemical analysis of chlorophyll were correlated with the amount of greening as seen by inspection of the tubers the following test was carried out.

On two occasions in the course of the study, where two lots of tubers given the same treatments ranged from slightly above to slightly below a degree of greening which was considered to be barely objectionable from a practical standpoint, the tubers were divided into one lot regarded as objectionably green, and into a

second lot, not objectionably green. The chlorophyll in each of these tubers was determined. The results showed that all the tubers rated as objectionably green had a chlorophyll content above 0.15 mg. per 100 cm^2 and those rated as not objectionably green all had a chlorophyll content below 0.15 mg. per 100 cm^2. The average values for the two groups were 0.183 ± 0.0075 and 0.136 ± 0.0060 mg. per 100 cm^2.

These determinations are taken as evidence that there is a very close relationship between the readily visible green color of the tubers and the amount of chlorophyll present.

Discussion

The data presented show that greening of potato tubers is greatly influenced by temperature and at a given temperature is strongly dependent on time. Figure 5 gives an example of these facts. Here the chlorophyll content increased slowly at a low temperature (40.9° F.) throughout the experiment. At a medium temperature (51.4° F.) the chlorophyll increased relatively rapidly throughout the whole exposure period (600 hours) and the curve showed but little tendency to level off at the end of the experiment. However, at a high temperature (66.3° F.) the chlorophyll content increased most rapidly with time but reached a maximum after 360 hours of exposure and remained thereafter relatively constant throughout the rest of the experiment. The course of the curves for the first 120 hours shows the same general trend as those found by Lubimenko and Hubbenet (18) with wheat seedlings. Recently Smith (27) has reported results with barley seedlings, which also show similar trends, though his experiments were carried out for only 43 hours.

These similarities give reason to believe that chlorophyll formation in potato tubers is dependent upon temperature and time in a manner which is, in principle, similar to the way the formation of chlorophyll in etiolated seedlings is dependent upon these factors. On the basis the results reported herein have some significance in that they show that the temperature which promotes the most rapid development of chlorophyll, and thus can be termed optimum for this process, is not the temperature which promotes accumulation of the highest total amount of chlorophyll under prolonged exposure. Lubimenko and Hubbenet (18) found in their work that 26° C. (79.8° F.) was the optimum temperature for chlorophyll formation in etiolated wheat seedlings within the 72 hour limit of their experiment. They also assumed that the amount of chlorophyll accumulated at 26° C. after 72 hours of exposure was the total possible to accumulate at any temperature and length of exposure. However, by examining their results it will be seen that the curves obtained for 26° C. (79.8° F.) and 16° C. (60.8° F.) have a course which indicates that the two curves would have crossed each other if the experiment had been carried on long enough. Therefore, it occurs to the writer that Lubimenko and Hubbenet did not have sufficient

reason to believe that the amount of chlorophyll accumulated at the end of their experiment at the optimum temperature (26° C.) was the absolute maximum possible to accumulate. Rather, their work indicates that they would have got results similar to those reported herein had the exposure time been extended long enough.

On basis of the results shown in Figure 5 and with the apparent similarities of the work of Lubimenko and Hubbenet (18) and Smith (27) in mind it seems justified to postulate that that temperature which gives the most rapid accumulation of chlorophyll in plants does not necessarily lead to accumulation of the absolute maximum amount. It is likely that a definite temperature optimum exists at which the rate of development of chlorophyll is greatest within a certain short time. At temperatures above this optimum the chlorophyll may develop more slowly but soon reach a maximum amount which diminishes with increased temperature. This is indicated by the results obtained by Lubimenko and Hubbenet. At temperatures below this optimum, the rate of chlorophyll formation is also lower. However, the maximum amount to develop at these lower temperatures is higher than the amount accumulated at the temperature optimum for chlorophyll development within a short time. In the experiments reported here all the temperatures chosen were possibly below the optimum. This is indicated by the fact that in all cases the chlorophyll developed most rapidly at the highest temperature.

It seems logical to assume that there is an absolute maximum for the amount of chlorophyll that possibly can accumulate in the tissue of a certain species of plant. As far as the author is aware, the conditions of temperature and time which promote development of this maximum amount have not been established. However, the data at hand seem to suggest that this upper limit of chlorophyll will accumulate at a temperature slightly above the lower temperature limit (possible 38-39° F.) at which chlorophyll formation takes place and after an infinitely long period of exposure. The results obtained also lead the writer to believe that the nearer the temperature approaches a certain zero point for the process (possible 38° F.) the higher the maximum quantity of chlorophyll providing the exposure period is extended accordingly. Further experiments are needed to prove or disprove this hypothesis.

It is only possible to speculate as to the probable causes for the increase in the upper limit of chlorophyll with lowering of the temperature. It seems likely that the decline in the maximum amount of chlorophyll to accumulate with a rise in temperature is due to the retarding effect of some metabolic process, probably in connection with increased respiration or enzymatic activity, which usurps material necessary for chlorophyll formation. This process, or processes, can be assumed to have a temperature coefficient lower than that for chlorophyll formation. If this is the case it will explain why it is possible to reach a higher level of chlorophyll in the tissue by lowering the temperature, as the chlorophyll formation will not decrease so much as the process usurping the material

under such conditions. There will thus be more material available for chlorophyll formation, and the pigment can accumulate to a higher level providing ample time is allowed.

In work on chlorophyll formation in plants it is preferable to get the formation to take place at a slow rate to be better able to study the interaction of temperature and time. This work suggests that potato tubers can be used for studies for this interaction as the development of chlorophyll in the tubers seems to be dependent on temperature and time in the same general fashion as in etiolated seedlings. Potato tubers are superior to seedlings in that the rate of development is slow and also in that the tubers are not dependent on photosynthesis and thus can be kept alive for a long time at the low light intensities required for this kind of studies. Further, by using potato tubers as the experimental object it is possible to get away from the complicating effect of growth of the tissue involved in chlorophyll formation. If growing seedlings are used, this effect can cause serious errors in experiments with long periods of exposure.

Recent observations have substantiated the earlier belief that chlorophyll is formed from a precursor, protochlorophyll, in the tissue upon exposure to light (cf. 27). This process is a rapid one (3) and seems to be strictly photochemical (18). It is shown to take place even at 0° C. (27). Formation of protochlorophyll takes place in the dark (26), and this process appears to be the one which is dependent on temperature. It is not definitely known whether it also is speeded up by light. The results of the experiment with various light intensities at low temperature (experiment 9) show that light does not promote the formation of protochlorophyll if the temperature is low. There was no measureable amount of chlorophyll even after 240 hours of exposure. From these facts it seems justifiable to conclude that the formation of protochlorophyll is primarily thermochemical and probably not photochemical at all, whereas the transformation of protochlorophyll into chlorophyll is entirely photochemical. However, the results do not entirely exclude the possibility that light has an accelerating effect on the formation of protochlorophyll, though this process is primarily dependent on temperature.

The data in Table 7 show that increasing the light intensity from 5 footcandles to 9 footcandles increased the chlorophyll formation considerably. A further increase in the light intensity to 28 footcandles had the same effect though not in direct proportion to the increase in light intensity. Although the possibility mentioned above, that light may have an accelerating effect on formation of chlorophyll, cannot be entirely excluded, these facts indicate that neither 5 footcandles nor 9 footcandles of light were enough to transform protochlorophyll into chlorophyll as fast as this former substance was formed at the temperature prevailing (62° F.). It is interesting to note that as low a light intensity as 5 footcandles is able to produce a considerable amount of chlorophyll.

Since it has been shown (8) that blue and green light did not produce greening of potato tubers it occurred to the writer that it should be possible to use light screens of these colors to prevent or reduce greening under ordinary light conditions. The results (Tables 9 and 10) show that it was possible to reduce greening considerably. The explanation for the failure of the screens to control greening completely may be that the screens used—one sheet of ordinary colored cellophane—presumably did not give absolutely monochromatic light. It is realized that the screens not alone changed the composition of the light but also reduced its intensity. However, it is shown that for equal light intensities the blue and green lights are much less effective in production of greening in potato tubers than is red light.

From a practical standpoint it may be of importance that the green and blue cellophane screens used delayed the appearance of objectionable greening as shown in Table 11. Under the diffused daylight conditions prevailing, the delay was approximately 10 days as compared to the uncovered check. Under higher light intensities and higher temperature the delay may not be so great as in this experiment, but the temperature chosen (66° F.) is considered normal for ordinary store displays and the light intensity (300 foot-candles) should be the upper limit of light that potatoes should be exposed to in proper handling. It thus seems possible to reduce greening by covering the potato tubers with either blue or green cellophane screens in places where it is desirable that they be open to inspection for a considerable period of time, as in store displays. The results also suggest the possibility of using green or blue cellophane windows in paper bags used for consumer packs.

Since the results show that little greening took place even at high light intensities if the temperature was kept below 40° F., the best way of preventing greening is probably to keep the temperature slightly below this point. This is ordinarily practiced in good storage cellars and it will probably be worthwhile to investigate further the effects of using this temperature throughout the entire period of handling the potatoes in shipping, wholesaling, and retailing. For the last phase of potato handling it appears possible to use refrigerated display cases of the type used in some vegetable marketing to keep the temperature of the tubers at the desired point (probably 40° F.) and thus be able to display the product without getting objectionable greening. However, more work will be needed before anything can be said about the practicability of this method.

Most of the results reported here are considered to be applicable to other potato varieties although they were obtained with one variety, White Rose. The possibility exists that there are genetic differences among varieties in their ability to develop greening, but it is not likely that it is great nor is there any reason to suppose that such genetic differences would materially alter the pattern of response to environmental forces demonstrated with the variety used here. It is known that all potato varieties develop chlorophyll in leaves and stems and also that the tuber is essentially a shortened

stem. A significant difference was found in the amounts of chlorophyll developed in tubers of White Rose and Russet Burbank when treated alike, the latter variety developing the lesser amount. This is, however, considered to be due to the thicker skin of this variety which presumably reduced the intensity of light reaching the chloroplasts where chlorophyll formation takes place.

Summary

The main object of the studies here reported was to determine the effect of some of the components of environment on post-harvest greening of potato tubers.

A rapid method for determining the amount of chlorophyll in potato tubers was developed on basis of the method used by Compton and Boynton (7) for determining chlorophyll in apple leaves.

The chlorophyll concentration in the tissue was shown to be closely correlated with visible greening.

A cheap and efficient controlled-temperature chamber for exposure of potato tubers to artificial light was devised.

The effect of temperature on greening was quite pronounced. Below 40° F. little or no greening took place. Of the temperatures tested the highest (68° F.) always caused the most rapid formation of chlorophyll at the light intensity used (9 footcandles), but the maximum amount at this temperature was accumulated after 240 hours of exposure and remained thereafter constant. At 51° F. the concentration of chlorophyll increased steadily throughout the experiment, the amount present after 240 hours equalling that at 68° F. and that after 600 hours of exposure being considerably higher.

On the basis of this information the theory put forth by Lubimenko and Hubbenet (18) is questioned; namely that the optimum temperature for chlorophyll formation (that temperature which promotes the process most rapidly) is also the temperature which promotes accumulation of the maximum amount possible for the material at hand. Instead, the theory is advanced that lowering of the temperature to a certain minimum (probably 38° to 39° F.) will increase the total amount of chlorophyll possible to develop providing the exposure period is extended sufficiently.

Increasing the light intensities increased the greening only within certain temperature ranges. Thus, at temperatures around 40° F. an increase in light intensity up to 80 footcandles did not cause any increase in chlorophyll formed, but at 62° F. an increase in light intensity from 5 footcandles to 28 footcandles caused a considerable increase in chlorophyll formed though not in direct proportion to the increase in light intensity.

The effect of covering the tubers with yellow, red, green, and blue cellophane screens was measured. The green and blue screens were the most effective, and about equally so, in reducing greening.

Tubers of the variety Russet Burbank were found to develop slightly but significantly less chlorophyll upon exposure to light than did tubers of the variety White Rose.

Humidity in the air surrounding the tubers had no effect on the amount of greening taking place.

Green potato tubers did not fade to any measurable extent in dark storage for 26 days at either 36° F. or 75° F.

Ethylene gas and ammonium thiocyanate solutions failed to prevent chlorophyll formation in potato tubers. Sulphurous acid bleached the green pigment but also injured the tubers.

The maturity of the tubers at harvest, and exposure to sunlight immediately after digging, had no effect on the amount of greening taking place upon subsequent exposure to artificial light.

Potato tubers are suggested as a new material for basic studies on chlorophyll formation in plants.

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