THE DEVELOPMENT OF CHLOROPHYLL IN SEEDLINGS IN DIFFERENT RANGES OF WAVE LENGTHS OF LIGHT*

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(WITH THREE FIGURES)

Introduction

While investigating the behavior of stomata in different kinds of light it was noticed that no chlorophyll developed in seedlings grown under some of the colored glass plates used in that work. The object of this investigation was to find out more fully, if possible, the wave lengths of light necessary for the development of chlorophyll in plants. A search of the literature yielded very little information on the subject. The only record of any other work of this kind is in Palladin's Plant Physiology (**4**), where Wiesner used double walled bell jars with different colored solutions to separate the spectrum into different parts. He reported that chlorophyll was formed in both the red-orange-yellow region, produced by potassium dichromate, and also in the green-blue-violet region produced by copper sulphate. The non-luminous heat rays were not effective. There is plenty of information on the effects of various wave lengths of light on photosynthesis, especially on those wave lengths which produce maximum photosynthesis. This is very well summarized by Spoehr (**6**).

Methods used

Seedlings were grown under colored glass plates and their color noted. No measurements of the amount of chlorophyll in the tissue were made. The relative greenness of the plants when compared with controls grown under all wave lengths of light was taken as a measure of the chlorophyll present. All plants were grown under similar temperature, humidity, and soil conditions, the variable being the different wave length of light. Seedlings of corn, wheat, oats, sunflower, radish, mustard, and bean were used in this work.

The colored glass plates used were the 6½ inch square ray filters manufactured by the Corning Glass Works, Corning, N. Y. The same series as used in the spectral glass houses of the Boyce Thompson Institute, reported by Popp (**5**), were employed with a few additions. The manufacturers'

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numbers of these plates are G86B, G38L, G38H, G34, G24, G124J, G584J, G55A, G585L, and G586A.

The transmission data for these glass plates were obtained from the Bureau of Standards Technologic Papers by COBLENTZ and EMERSON (**1**), GIBSON and MCNICHOLAS (**2**), and GIBSON, TYNDALL, and MCNICHOLAS (**3**). These data were converted by means of the chart and instructions given by GIBSON and MCNICHOLAS (**2**), values for the average thickness of the plates used. The values for combinations of two or more plates is the product of the data for each plate separately. All these data are given for an equal energy spectrum. The percentage of total transmission for each plate or each combination of plates was obtained by comparing the areas under the transmission curves when they were plotted on cross-section paper. These data were calculated for a spectrum extending from 300 mμ to 770 mμ, 770 mμ being the limit of visible spectrum as given by GIBSON and MCNICHOLAS (**2**). The transmission curves for these glass plates are shown in fig. 1 and fig 2. These data can be used only for plates of the thickness indicated in each case. The limits of transmission for each plate were checked by means of a wave length spectrometer loaned by the Physical Chemistry Department of the Ohio State University.

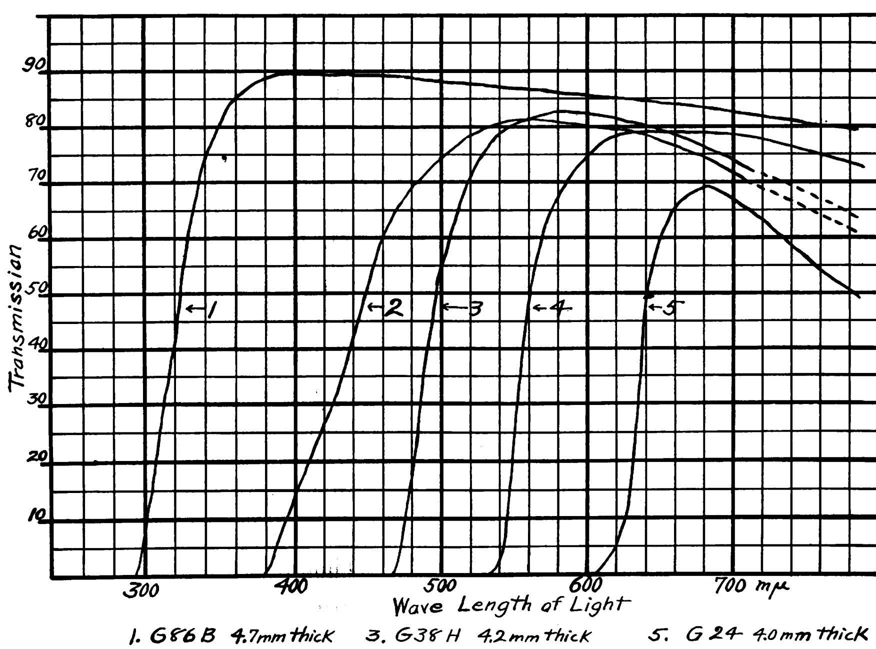

FIG. 1. Transmission curves of glass plates used in the experiments.

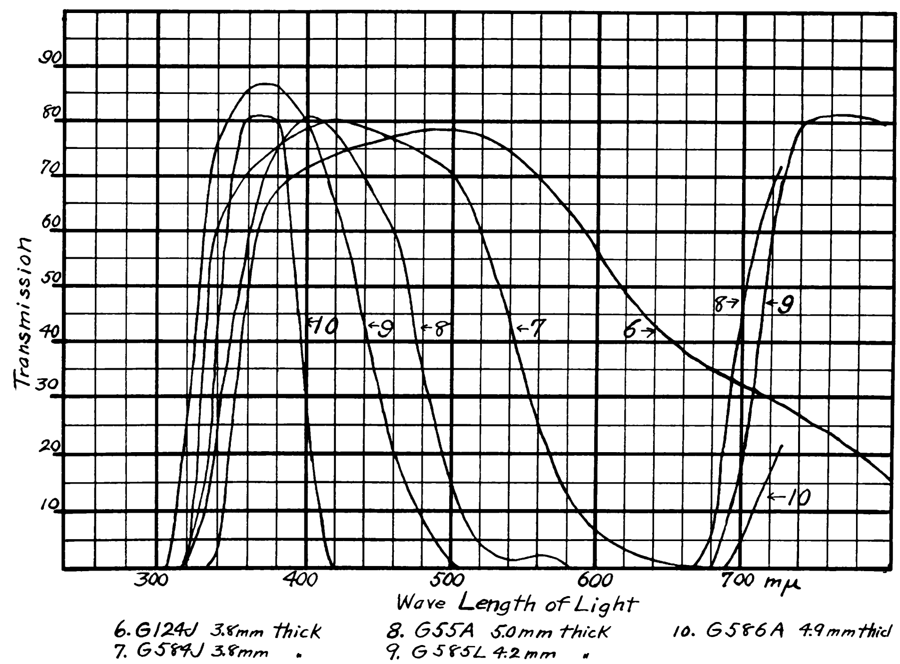

FIG. 2. Transmission curves of glass plates used in the experiments.

The data for the relative spectral distribution of radiant energy from the 400 watt Mazda C light were taken from GIBSON and MCNICHOLAS (**2**). The percentage of energy from the Mazda C light transmitted by each plate was found by calculation. The values of relative spectral distribution of energy from the Mazda C light were multiplied by the transmission value at each wave length and curves of these values plotted on paper. The areas under each curve were compared and reduced to percentages. These percentages are comparable when all plants are placed at equal distances from the light.

The plants were grown under ventilated, light-proof tin boxes which held four glass plates. The boxes were painted white (MgO paint) inside, and with aluminum paint on the outside. The seeds were planted directly in the soil when the experiments were conducted out of doors, and the tin boxes were sunk several inches in the soil to make a light-tight seal. In the greenhouse, flats of soil were used and the tin boxes were sunk for several inches into a large tray of soil. For the experiments under artificial light a large box was used. The plants were placed one meter from this light. Many experiments were tried, and many calculations of data were necessary for this work which has covered a period of several years at interrupted intervals. The results of only two typical experiments are given since similar results were obtained each time.

Results

Table I gives the results of a series of experiments carried out in the greenhouse in the fall and winter of 1926–27. This series covered a period of six to seven weeks, since only five sets of plates and a control could be used at one time. The numbers of the glass plates are given with their limits of transmission, and the percentage of an equal energy spectrum transmitted by each. The development of chlorophyll is expressed by

TABLE I

TRANSMISSION DATA AND DEVELOPMENT OF CHLOROPHYLL

COMBINATIONS OF GLASS PLATES	LIMITS OF TRANSMISSION	TRANSMISSION OF AN EQUAL ENERGY SPECTRUM	DEVELOPMENT OF CHLOROPHYLL
	mμ	Per cent.	
G86B	296 – infra red	70.0	Green (control)
G38L	380 – " "	54.0	" as control
G38H	464 – " "	44.5	" " "
G34	528 – " "	33.5	" " "
G24	601 – " "	18.3	" " "
G24 + G55A	670 – " "	6.6	No trace of green*
G24 + G586A	680 – " "	5.7	" " " "
G24 + G585L	690 – " "	2.1	" " " "
G24 + G584J	601 – 680	Less than 0.1	Trace of green
G34 + G584J	528 – 680	1.0	Almost as green as control
G38H + G584J	464 – 680	6.7	" " " " "
G38L + G584J	380 – 680	14.4	Green as control
G34 + G55A	528 – 580	Less than 0.1	No trace of green
G38H + G55A	464 – 580	0.5	Trace of green
G38L + G55A	380 – 580	5.7	Almost as green as control
G38H + G585L	464 – 506	0.2	No trace of green
G38L + G585L	380 – 506	2.6	Almost as green as control
G38L + G586A	380 – 420	0.2	No trace of green
G586A	320 – 420	10.7	Trace of green
G585L	300 – 506	19.3	Almost as green as control
G55A	325 – 580	20.1	" " " " "
G584J	300 – 680	34.8	Green as control

* Faint trace of green in sunflower seedlings in one experiment

different amounts of the green color when compared with plants grown in full daylight. The plants which showed no trace of green were yellow in color similar to etiolated seedlings. These data seem to show two facts:

first, that there is no development of chlorophyll in radiant energy of wave lengths longer than 680 mμ; and second, that all wave lengths of the remaining visible and ultra-violet spectrum are effective if the intensity is high enough. Traces of green color were detected in all combinations where wave lengths shorter than 670 mμ were transmitted except in those where the percentage of total energy transmitted was very low. It must be remembered that the plants were grown in daylight and were not exposed to an equal energy spectrum, so that the transmission percentages are not strictly comparable. It was to get around this difficulty that the series under artificial light was conducted. Here the intensity is constant and the relative spectral distribution and total percentage transmitted can be calculated.

Figure 3 shows the relative spectral distribution of energy under the different combinations of plates used with artificial light. The relative

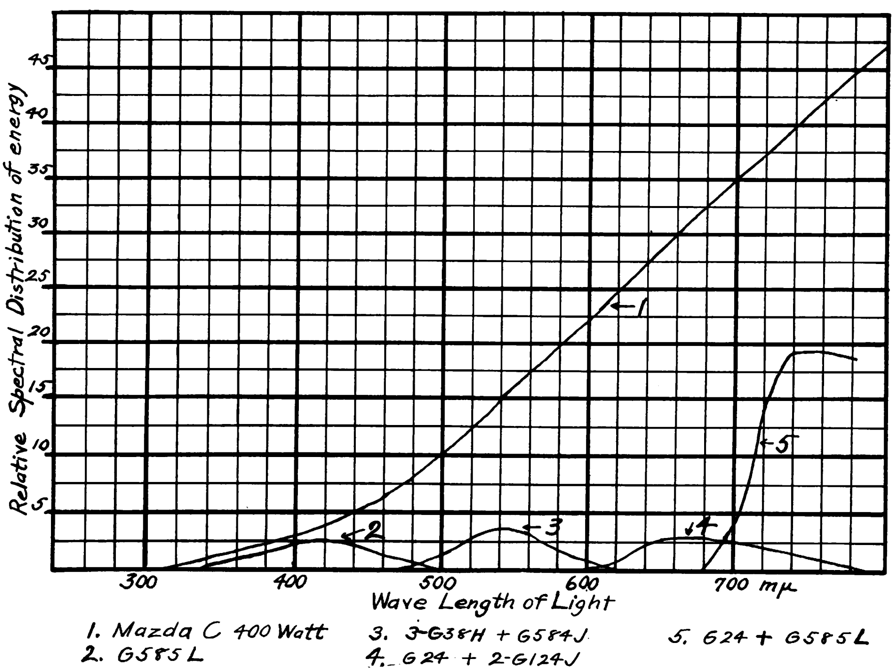

FIG. 3. Relative spectral distribution of energy under different combinations of glasses used.

spectral distribution of energy from the Mazda C 400 watt lamp is also given. The combination 3-G38H + G584J and 624 + 2-G124J were chosen to give values as near as possible to those for G585L. The plants under these plates were all the same distance from the light.

Table II gives the results of an experiment under artificial light. The combinations of plates used, their limits of transmission, and the relative percentage of the total energy from the Mazda C transmitted by each are given. The seeds were germinated and the seedlings grown in the dark to a height of several inches before the light was supplied. The time required for the development of the green color under the different lights is given.

TABLE II

TRANSMISSION DATA AND DEVELOPMENT OF CHLOROPHYLL

Combinations of glass plates	G585L	3–G38H + 584J	G24 + 2–G124J	G24 + G585L
Limits of transmission	320–506 mμ	464–630 mμ	600–800 mμ	680–infra red
Percentage of energy from 400 watt Madza C transmitted	3.3	3.1	4.0	18.0
Time	Relative development of chlorophyll in the seedlings			
Feb. 10, '27	Yellow	Yellow	Yellow	Yellow
9:30 A. M.	Yellow	Yellow	Yellow	Yellow
10:15 A. M.	Yellow	Yellow	Yellow	Yellow
11:15 A. M.	Yellow	Yellow	Yellow	Yellow
12 Noon	Yellow	Yellow	Faint trace green	Yellow
1:00 P. M.	Yellow	Faint trace green	Trace green	Yellow
2:00 P. M.	Trace green	Trace green	Green	Yellow
3:00 P. M.	Trace green	Trace green	Green	Yellow
4:00 P. M.	Trace green	Green	Green	Yellow
Feb. 11, '27				
10:00 A. M.	Trace green	Green	Green	Yellow

These data show, as in the other experiments, that even after 24 hours there is not the faintest trace of green in light of wave lengths longer than 680 mμ. The relative intensity of this radiant energy is three times that of the other parts of the spectrum in which chlorophyll development is very pronounced. The data also show that, for approximately equal energy values in the remaining visible and ultra-violet spectrum, there is a difference in their effect on chlorophyll development. This effect seems to be that the red rays are more effective than the green, and the green more than the blue. When the effective energy under the G24 + 2-G124J combination is considered to extend only to 680 mμ, since longer wave lengths of radiant energy have no effect, the relative transmission percentage becomes 2.5 per cent. instead of 4.0 per cent. as compared with 3.1 per cent. in the green and 3.3 per cent. in the red. The relative effectiveness of the red region is still more apparent.

The fact that there is no development of chlorophyll beyond 680 mμ is very striking because the center of the band of maximum absorption by chlorophyll and the band of greatest efficiency of radiant energy in photosynthesis are at about 675 mμ (SPOEHR). It seems, therefore, that the effectiveness of radiant energy in chlorophyll development increases with the wave length up to about 680 mμ and then ceases abruptly.

Conclusions

1. Wave lengths of radiant energy longer than 680 mμ are not effective in the formation of chlorophyll in seedlings of corn, wheat, oats, barley, beans, sunflowers, and radish.
2. All other regions of the remaining visible and ultra-violet spectrum (to 300 mμ) are effective provided the energy value is sufficient.
3. For approximately equal energy values in these regions, the red rays are more effective than the green, and the green more than the blue.
4. The effectiveness of radiant energy appears to increase with wave length to about 680 mμ and then to end abruptly.

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