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(WITH ONE PLATE)

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WAVE LENGTHS OF RADIATION IN THE VISIBLE
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OF LIGHT-SENSITIVE LETTUCE SEED

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INTRODUCTION

The purpose of this investigation is to fix definitely the wave lengths of radiation which maximally promote the germination of light-sensitive lettuce seed² and to study their relationships to the wave lengths of radiation most effective in other fundamental plant phenomena.

In a previous publication (Flint and McAlister, 1935) the wave lengths of radiation in the visible spectrum inhibiting the germination of light-sensitive lettuce seed were reported as occurring in two regions: one in the violet-blue-green region (with principal maxima at about 4400 and 4800 angstroms), and the other in the red-to-near-infrared region (with one maximum, at about 7600 angstroms). The earlier work of Flint (1934, a, b) had indicated the general range of the radiation promoting germination as 5200 to 7000 Å, but the curve of relative effectiveness of the radiation within this range was unknown. The accompanying graph (fig. 1), reproduced from a recent paper by Flint and McAlister (1936), illustrates the state of the study of radiation in relation to the germination of this particular seed at the beginning of the researches here reported for the same material.

It may be noted from figure 1 that at the intensities of radiation applied in the region 5200-7000 Å a germination of 100 percent was secured at the longer wave lengths. It follows that differences in the

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² Light-sensitive Arlington Fancy lettuce seed was used throughout the co-operative researches.

effectiveness of one part of this region over another were masked by the completeness of the germination.

In the study of the effectiveness of radiation in the violet-blue-green region it had been found that the principal critical wave lengths in the regions 4400 A and 4800 A were identical (within the range of experimental error) with those reported by Johnston (1934) and others for the phototropic response of oat coleoptiles. This analogy suggested that similar pigments were involved in the absorption in both instances, and that the results thus might well be of significance beyond their immediate application to the problem of dormancy in a particular kind of seed. This possibility has been made more prob-

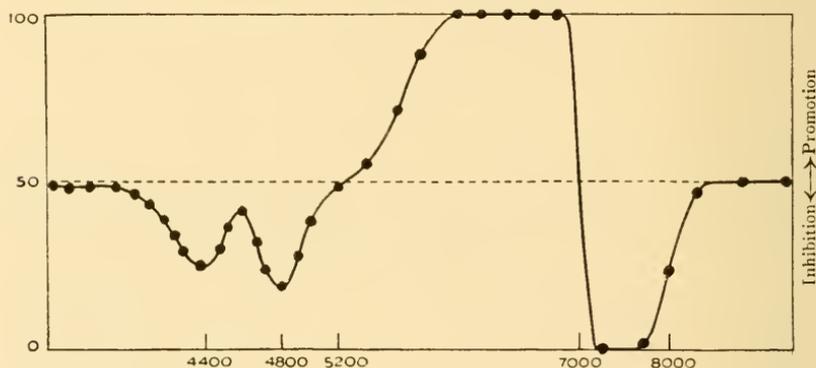


FIG. 1.—The action of radiation of specific wave-lengths in relation to the germination of light-sensitive lettuce seed. The percentages of germination in the spectrum (immediately following an exposure to red light sufficient to effect 50% germination) are indicated as ordinates, the wave-lengths of the spectral light being indicated as abscissae.

able through the recent work of Burkholder and Pratt (see Burkholder, 1936, p. 46), who found not only that radiation in the violet-blue region influenced the movement of *Mimosa*, but also that a band of radiation in the 7600 A region had a similar effect.

In view of the foregoing the study of the relative effectiveness of the wave lengths of visible radiation promoting the germination of light-sensitive lettuce seed seemed to warrant the continuation of this work, which led to the results here reported.

REVIEW OF LITERATURE

Cieslar (1883) reported that yellow light favored the germination of certain seeds, whereas violet light retarded it. In later years the phraseology became more precise, with "yellow to red" or "yellow, orange, and red" light as the promoting radiation, yet neither the wave

length range nor the relative effectiveness of bands of radiation within the range were established. Flint (1934) reported that the upper limit of the radiation inhibiting germination was at about 5200 Å, while the upper limit of the seemingly analogous phototropic response was set as about 5400 Å by Johnston (1934). The curves of the absorption spectra of carotin and xanthophyll as reported by McNicholas (1931) reach low points in the region 5000 Å to 5500 Å, with principal critical wave lengths of maximum absorption at about 4400 Å and 4800 Å (when allowance is made for the shift in wave length associated with the solvent). These correspond closely with the regions of maximum effectiveness of this radiation in inhibiting germination and inducing phototropism. The curves of the absorption spectra of the chlorophylls as given by Zscheile (1934) are uniformly low in the green region and Shuck (1935) has held that there was a band of this radiation which had no effect on germination. On the other hand the curve of the action of radiation of specific wave lengths in relation to the germination of light-sensitive lettuce seed as developed in the work of Flint and McAlister (see fig. 1) indicates an immediate transition of reaction in this region, though not as abrupt a change as that taking place at the long wave length end of the promoting influence at about 7000 Å.

Kommerell (1927), in studying quality of light and germination, had concluded that at any specified wave length the effectiveness of the radiation was proportional to the energy falling upon the surface of the seed. The work of Flint and McAlister, on the other hand, indicated that the effectiveness of the radiation was proportional to its absorption by the seed pigments. The investigations of Meischke (1936) support the latter viewpoint.

METHODS

The procedure and apparatus used in this investigation was essentially that described in detail in a previous paper (Flint and McAlister, 1935) except that blue light of controlled intensity was superimposed uniformly over the spectral region in which the seeds were being treated. By the well-known system of trial and error, using constant continuous spectral illumination, the blue light was so regulated in intensity that the maximum germination throughout the set of compartments in 24 hours was precisely 100 percent in some one compartment. With compartments 0.4 inch wide as used in the experiments previously reported, the conditions were then such that any increase in the intensity of the blue radiation gave less than 100 percent germination in this compartment. All experiments reported were at

room temperature less than 29° C., for at higher temperatures the Arlington Fancy lettuce seed used in these experiments would not germinate, irrespective of the light conditions. On account of the narrow range of wave lengths represented by the critical maxima obtained, no correction for relative energy was applied to the graphic representation of the data.

In the data as given in table I, it may be noted that there was a more or less progressive falling off in the peak germination and that there was some variation between the different series. Under the conditions of the experiments, with continuous operation of the spectral and superimposed light sources, a differential reduction in the efficiencies of the lights was to be expected.

RESULTS

A large number of experiments were carried out incidental to the attainment of the prescribed conditions. Under conditions such that an additional intensity of blue, when superimposed upon the spectral light, would reduce the percentage germination in the compartment yielding 100 percent germination, the following results in table I were obtained:

In conjunction with the results obtained as given in table I, an examination of the absorption spectra of lettuce seed extract in acetone was undertaken. The results of this study are given in plate I.

The foregoing results establish the critical wave lengths of the radiation promoting the germination of the seed used in these trials and appear to indicate the nature of the material responsible for the reaction. The relative effectiveness of radiation with wave lengths within

TABLE I.—*Percentage germination of light-sensitive lettuce seed in spectral green to infrared radiation with superimposed blue light.*
Compartment width 0.4 inch.

Series	Percentage germination at approximate wave lengths at center of compartments														
	4950	5080	5180	5300	5440	5650	5710	5850	6000	6210	6400	6700	7050	7500	8200
A	0	0	0	0	0	0	0	0	0	5.9	27.3	100.0	64.0	2.1	0
B	0	0	0	0	0	0	0	0	0	0	30.7	73.3	77.8	0	0
C	0	0	0	0	0	0	0	0	0	0	0	85.2	14.3	0	0
D	0	0	0	0	0	0	0	0	0	0	0	80.0	0	0	0
E	0	0	0	0	0	0	0	0	0	0	0	65.0	0	0	0
Average	0	0	0	0	0	0	0	0	0	1.18	11.6	80.7	32.03	0.42	0

the 5000-6000 A range has not been established in these trials, but in its entirety this radiation is obviously far less effective than that in the 6700 A region.

DISCUSSION

The results given in table 1 have been averaged and represented graphically as the full line curve in figure 2.

It is obvious from this diagram that the radiation most effective in promoting the germination of the seed had a wave length of the order of 6700 A. The nature and position of this curve invites comparison

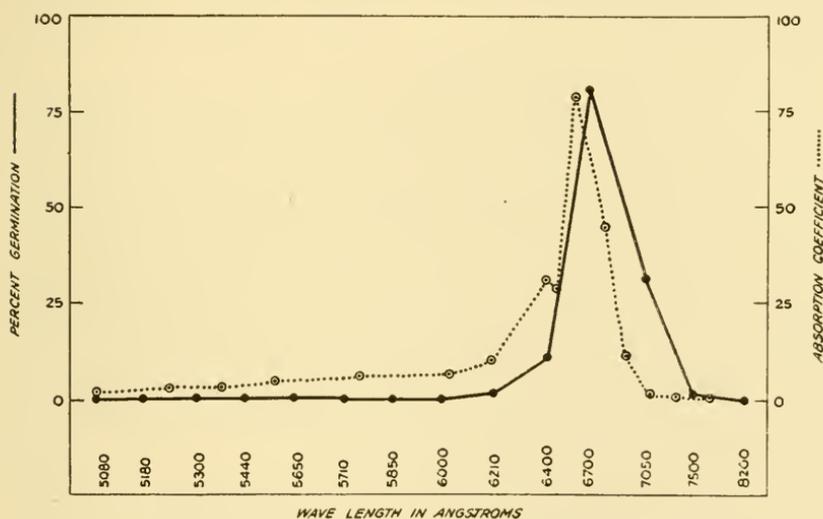


FIG. 2.—Curves of seed response and chlorophyll absorption.

with the curve of the absorption spectrum of the chlorophylls in this region, and the analogy is impressive. Here is an instance, then, in which, within the limits of experimental error, the apparent critical wave length of radiation promoting germination coincides with the major absorption in this region by a pigment common to all green plants. The approximate order of correspondence is indicated in figure 2. In Zscheile's work the leaf pigments were extracted with ether. In the germination study the seed pigments were acting in their natural environment, which was an aqueous solution. Under these circumstances the wave length shift apparent in figure 2 is about the amount to be expected.

In plate 1 the upper spectrogram is taken from Willstätter and Stöll and represents the absorption of an acetone extract of leaf pigments.

The center spectrogram represents the absorption of an acetone extract of seed pigments. The lower spectrogram represents the emission of the mercury arc, and provides a wave-length scale for the center spectrogram. It is apparent from this plate that the pigments absorbing the longer wave lengths of visible radiation in the seed are similar to those pigments found in the leaf by Willstätter and Stöll.

Since the leaf pigments absorbing the longer wave lengths of visible radiation are for the most part identified as chlorophyll, it follows from the data represented in figure 2 and plate 1 that (within the limits of experimental error) the radiation most effective in promoting germination in the seed is that most effectively absorbed by chlorophyll in the same region.

Unfortunately, these experiments were terminated before it was possible to make a positive identification of the pigments responsible for the relatively intense absorption for wave lengths of radiation shorter than 5400 Å that is apparent on plate 1. It is certain that this is not due to the blue absorption of chlorophyll alone, for it is too intense compared to the red absorption and also extends too far toward the longer wave lengths. Thus, the presence of a yellow pigment is indicated, and the close agreement between the critical wave lengths of radiation inhibiting germination and those absorbed by the carotenes is impressive. However, a more extensive study of the absorption spectrum of the yellow pigment found in these seeds is necessary before more can be said.

The close analogy between the critical wave lengths of radiation influencing seed germination and the critical wave lengths of radiation absorbed by plant pigments places a distinct emphasis upon what is perhaps a new and promising viewpoint. In the seeds the violet-blue light promotes a set of physiological responses quite different from the set which is promoted by orange-red light. It follows that in green plants the physiological response to violet-blue light may be quite different from the response to orange-red light. If such is the case, the study of quality of light offers increasing promise in relation to the problems of photosynthesis, photoperiodism, seed maturation, seed germination, plant distribution, and so on.

In conjunction with an increasing interest in qualitative light effects the results here reported obviously emphasize the desirability of a more adequate knowledge of the quality of light at the earth's surface and of the modification of that quality with time of day and year, with latitude, with altitude, and with water vapor in the earth's atmosphere.

SUMMARY

Radiation of wave lengths ranging from about 5200 Å to about 7000 Å characterizing the colors yellow, orange, and red, promotes the germination of light-sensitive lettuce seed; but within this range the longer wave lengths are by far the most effective.

The critical wave length of the radiation promoting germination within the most effective range is approximately 6700 Å.

Within the limits of experimental error the radiation most effective in promoting germination is that most abundantly absorbed by chlorophyll in the same region.

The absorption characteristics of an acetone extract demonstrate the presence of chlorophyll in the seed.

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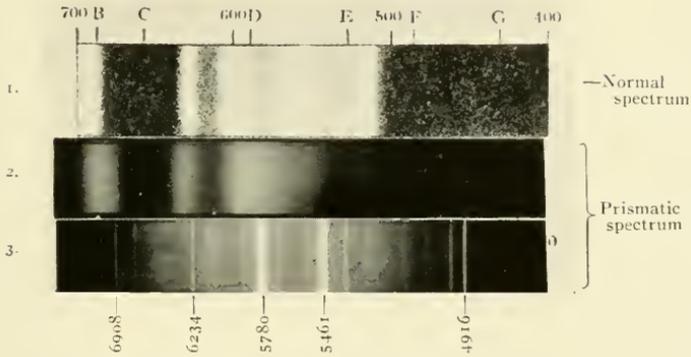
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COMPARISON OF LEAF AND SEED PIGMENTS

1. Spectrogram of acetone extract of leaf pigments.
(Willstätter and Stoll.)
2. Spectrogram of acetone extract of seed pigments.
3. Mercury comparison spectrogram.