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INTRODUCTION

In studies of the light-sensitivity of "dormant" lettuce seed previously reported (3)[1] it was noted that radiation of the longer wave lengths of visible light, characterizing the colors yellow, orange, and red, promoted germination, whereas radiation of the shorter wave lengths of visible light, characterizing the colors violet, blue, and green, inhibited germination. The material appeared to be unusually well adapted to the study of response to radiation, and steps were taken to establish the relative effectiveness of radiation of various wave lengths with respect to the germination of the seed.

While these studies were in progress, Johnston (6) at the Smithsonian Institution reported the results of a careful series of measurements of phototropic response of the etiolated oat coleoptile, which emphasized the fact that the shorter wave lengths of visible light were responsible for such bending. He interpreted this activity as due to an inhibitory effect of the shorter wave lengths upon the cells exposed to such radiation.

On account of the obvious analogy between the results obtained with the shorter wave lengths of light in respect to inhibition in germination and in phototropism the facilities of the two research divisions were combined in the furtherance of the germination study, the cooperative investigation leading to the results here presented.

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[1] Numbers in parentheses refer to list of literature cited, at the end of this paper.

REVIEW OF LITERATURE

With respect to light and germination three classes of seeds have been recognized for many years: (1) seeds germinating equally well in light or darkness, (2) seeds whose germination is hindered by light, and (3) seeds whose germination is favored by light. This classification has emphasized the variety of the responses that may occur and has proved satisfactorily descriptive for studies involving sunlight or other white light.

In 1883 Cieslar (2) reported with respect to certain seeds that yellow light favored germination, whereas violet light retarded it and rendered them appreciably dormant. This varying response to different wave lengths of light made it evident that in sunlight or other white light certain components were acting to promote germination, while certain other components were acting to retard it. Upon the relative effectiveness of these two groups of components, either through the radiant energies involved or through the particular sensitivity of the seed, one might presume to depend the gross effect of the white light with respect to germination. From the more technical standpoint, therefore, there were but two classes of seeds with respect to germination: (1) those whose germination was not influenced by light, and (2) those whose germination was influenced by light. Seeds of the latter class were designated " light-sensitive seeds," as contrasted with the widely occurring seeds of the class which germinate equally well in light and in darkness. The present considerations are confined to light-sensitive seeds.

Light-sensitivity as reported by Cieslar was limited for the most part to small seeds without reserve food materials. In the seeds of *Poa nemoralis, Agrostis stolonifera,* and *Nicotiana macrophylla* germination was reported as favored by white light, whereas the germination of seeds of *Viscum album* was reported as hindered by white light. In large measure the researches on light and germination by various workers in subsequent years have been concerned with the extension of these respective lists. In this respect it is to be noted that sensitivity to light is now generally recognized as a widely occurring characteristic of seeds.

The early distinction between the effect of " yellow " light and " violet " light gained precision through subsequent researches, and one finds in Molisch (9) the statement that yellow to red light promotes germination, whereas violet, blue, or green light inhibits germination. This information, however, has not been widely appreciated, and the more recent studies of light in relation to germination, such as those of Gardner (4), Lehmann (7), Maier (8), Nathammer (10) and Shuck (11), have concerned themselves for the most part with white light as a quantitative factor in germination.

Wholly unaware of the foregoing background of researches by German workers in this field, the senior author discovered that so-called " dormant " lettuce seed would germinate readily in white light, and further, that yellow, orange, or red light promoted this germination, whereas violet, blue, or green light inhibited it. It now appears that the germination response to radiation of specific wave lengths noted for dormant lettuce seed represents types of reactions of wide occurrence among seeds. This fact suggests that the further study of such responses may be warranted as promising results of both practical and theoretical significance in relation to germination and possibly also in relation to other aspects of growth. The results obtained by Flint (3) with green Wratten filters indicated that color alone was not a safe criterion to use in the interpretation of results obtained with filters, thus directing attention to their wave-length transmission.

METHODS

The principal line of attack in this investigation involved the use of a spectrum, and to a large extent the work comprised successive improvements in the technique of utilizing the spectrum to the greatest

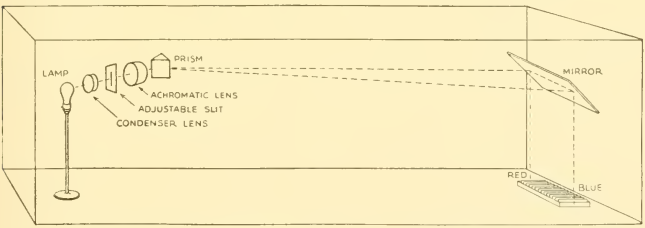

FIG. 1.—Schematic drawing of the apparatus used in the study of spectral light in relation to germination.

advantage in relation to germination. The set-up as finally elaborated (see fig. 1) consisted of a fixed light source, a condenser lens concentrating light upon an adjustable slit, an achromatic lens, a prism, and a silvered mirror. A light-proof house surrounded the set-up, with a partition at the slit (not shown in the figure). With this set-up, using a single filament 1,000 lumen 6.6 ampere Mazda street-lighting bulb as a light source, a spectrum was obtained which was about 1 foot long in the visible range.

For exposures of the material in the spectrum special boxes 10 x 4 x 1 inches were made of brass and provided with parallel center plates of monel metal about 4 x 1 inches, spaced at 0.4 inch. Two such boxes placed end to end thus more than filled the visible spectrum, and each of the 48 compartments was subjected to a band of radiation ranging in width from the order of 50 A in the low violet to that of 200 A in the high red. The spectrum and boxes were provided with a secondary light-proof housing, in which, at an elevation of about 1 foot, were installed two 20-inch milk-glass lumiline lights wrapped in red cellophane having no appreciable transmission for wave lengths shorter than 6000 A. These lights were so arranged that in conjunction with end-mirrors no shadows were cast in any compartment of the boxes. The intensity of illumination was regulated by a rheostat.

The focal plane of the spectrum obtained was located by inserting a plate of didymium glass between the condenser lens and the slit. The sharp absorption lines of didymium also provided a convenient means of establishing the wave lengths of all regions of the visible spectrum. Wave lengths in the near ultraviolet region were established by substituting a mercury arc as a light source and using uranium glass to pick up through fluorescence the lines characteristic of mercury. Wave lengths in the infrared region were established by following the absorption characteristics of water vapor with a thermopile. The radiation energies throughout the entire spectrum were established by means of a thermocouple.

The procedure in each experiment was as follows. Two boxes were placed in the spectrum and half filled with tap water, which served as the medium of germination. Dormant or light-sensitive lettuce seeds were then scattered into the compartments, surface tension bringing about a fairly uniform distribution of the seeds over the available water surface. About 100 seeds could be conveniently accommodated in each compartment.

After 2½ hours presoaking the seeds were given exposures of spectral light, of red lumiline light, or of both lights, depending upon the particular objective. The red lumiline light, by suitable modification of the duration or intensity of the exposure, was used for the most part to effect a 50 percent germination of the seeds independent of the spectral light—a feature ordinarily offering some difficulty, but entirely feasible with the material at hand, as had been attested by tables 2 and 3 of Flint's paper (3). Upon this base the nature and extent of any promoting or inhibiting influence of the spectral light was registered as a departure. After 24 hours the boxes were removed, and the seeds in each compartment were transferred immediately to numbered petri dishes, placed in a refrigerator at 3° C., and exposed to blue light (to prevent further germination), where they were kept until germination counts could be made.

In plotting the germination percentages against radiation the wave length falling on the median line of each compartment was taken as the wave length for the seeds of that compartment.

In plotting the inhibitory influence in the violet-blue-green region as corrected for energies involved, the curves were inverted to facilitate subsequent comparison with other data.

The transmission curves of the Wratten filters and of the ether extracts of lettuce seeds were made in the conventional manner with a double monochromator and a thermocouple.

RESULTS

In the experiments of Flint (3) two green Wratten filters were found to transmit light that promoted germination, and 10 green Wratten filters were found to transmit light that inhibited germination. The spectral transmission of all the green Wratten filters was studied, and the energy transmission curves were obtained by multiplying the percentage transmission by the energy radiated from a Mazda lamp at each wave length. The energy radiated by the lamp at each wave length was obtained from its known spectral energy curve. These energy values were used in conjunction with the inverse square law and the distances at which the respective filters (when used with the Mazda lamp) gave equal response with a Weston photronic cell. The energies transmitted by representative green Wratten filters are shown in figure 2.

It is to be noted from the curves of figure 2 that the two green filters which had been found to transmit light promoting germination (64 and 67) transmitted more of the ultraviolet and less of the long visible red than the green filters which had been found to transmit light inhibiting germination (56 and 60). This fact suggested that the promotion was associated either with a promoting influence in the ultraviolet or with an inhibiting influence in the long red or near infrared.

A substantial series of exposures of moist dormant lettuce seed to various wave lengths in the ultraviolet ranging from the lower limits of the visible spectrum to below the ultraviolet characterizing solar radiation gave uniformly negative results, whereupon attention was directed to the infrared regions. Earlier studies with a spectrum by the senior author had given an approximate range of 5200 to 7000 A for the promoting effect, with a sharp falling off in germination in the long red. No inhibitory effect had been suspected as associated with this falling off, however, until the effort was made to

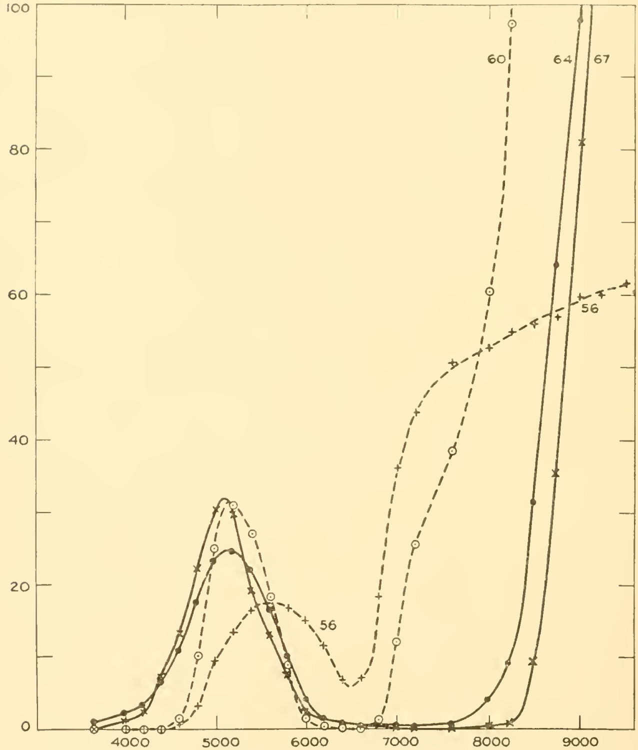

FIG. 2.—Energy transmission curves of green Wratten filters. The ordinates are relative energies transmitted at each wave length (indicated as abscissae). Numbers 56 and 60 are typical of the green filters transmitting light that inhibits germination. Numbers 64 and 67 transmit light that promotes germination.

explain the physical basis for the promoting effect of the light transmitted by the two aberrant green filters. The spectrum set-up previously used by the senior author had been considerably modified in these cooperative studies to permit a more precise measurement of the wave lengths to which seeds were exposed. With the apparatus and procedure described in the foregoing section such results as those given in figure 3 were obtained in the long red region.

Such results as those presented in figure 3 established the presence of a strong inhibitory influence in the region of 7600 A.

By applying the same methods to the violet, blue, and green regions of the spectrum, such results as those presented in figure 4 were obtained.

In conjunction with the foregoing experiments a study of ether extracts of lettuce seed was carried out. These extracts contained oil and pigments. Definite absorption in the region 4200 A to 5200 A was evidenced by the transmission curves. These remained bimodal, even after appreciable oxidation had taken place, and thus appeared suggestive in relation to the bimodal curve of inhibition given in figure 4. Definite absorption in the region 5200 A to 7000 A was also evidenced by the transmission curves, suggesting the presence of some precursors of chlorophyll and allied pigments. No appreciable absorption in the region 7600 A was noted.

DISCUSSION

The discovery of a strong inhibitory influence in the region of 7600 A, although made in an effort to explain the difference in the response to the light transmitted by certain green Wratten filters, and quite incidental to the study of the precise nature of the curve of inhibition in the regions characterizing violet, blue, and green light, may well transcend in importance the original objective of the cooperative studies. Since this discovery appears to offer a clearer approach to biological problems involving light, it has been given precedence in these considerations.

All the green Wratten filters used by Flint (3) were found to transmit in the 7600 A region, but the two filters transmitting light which promoted germination had such a low transmission in this region that the effects of the promoting regions—the yellow, orange, and red—predominated over the effects of the inhibiting regions—the long red, the violet, the blue, and the green. Since many blue and violet glass, liquid, or gelatin filters transmit in the region 7600 A, it follows that the newly discovered inhibitory band becomes a potential source of confusion as to the effectiveness of radiation in the more visible spectrum with respect to the germination of light-sensitive lettuce seed. Moreover, since the same type of light-sensitivity has been recognized as characterizing other seeds, this factor may well be of some general significance with respect to light-sensitivity in seeds.

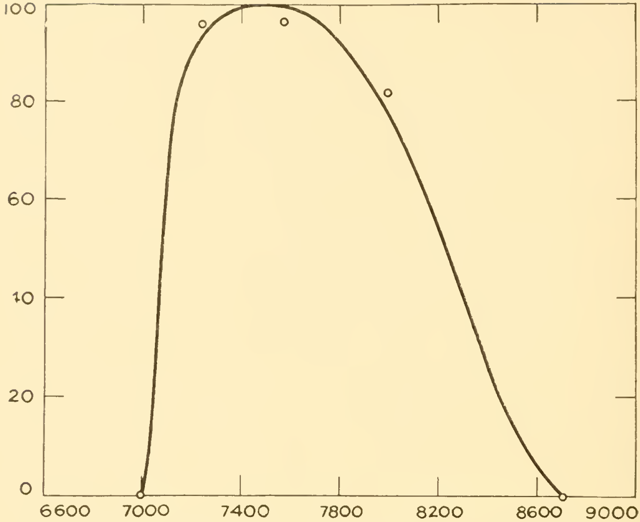

FIG. 3.—Curve of inhibition in the 7600 A region. The ordinates are percentage departures from expected germination values following exposure to promoting radiation, and are corrected for differences in the energy of the applied radiation indicated as abscissae.

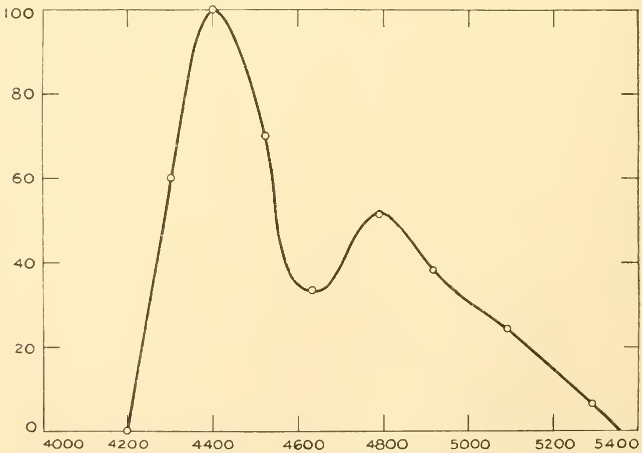

FIG. 4.—Curve of inhibition in the violet-blue-green region. The ordinates are percentage departures from expected germination values following exposure to promoting radiation, and are corrected for differences in the energy of the applied radiation indicated as abscissae. The maximum inhibition was arbitrarily taken as 100.

In connection with the later consideration of inhibitory influences associated with wave lengths characterizing violet, blue, and green light, it is to be noted that with both solar and Mazda radiation the energy at 7600 A is much greater than at the shorter wave lengths of the visible spectrum. In solar radiation there is a sharp absorption band in the 7600 A region interpreted as due to oxygen in the sun and water vapor in the earth's atmosphere. Notwithstanding this absorption, however, the energy of solar radiation at this wave length region is large. In consequence it would appear that under natural outdoor conditions and under customary indoor experimental conditions radiation of a wave length in the long red exerts a relatively powerful inhibitory influence upon the germination of dormant lettuce seed, although this influence is ordinarily more than counteracted by the promoting influence in the yellow-orange-red region. The extent to which the 7600 A region has an analogous effect upon other seeds and upon other phases of light-sensitivity is not known at this time, but because of the high energy and universal occurrence of the radiation, its potential significance becomes one of the most intriguing results of its discovery. Further studies of the possible effectiveness of this region in respect to the germination of other seeds and in respect to other phases of light-sensitivity are now in progress.

An examination of the germination responses to light of the wave lengths indicated in figure 4 reveals that there are two maxima of inhibition in the violet-blue-green region—a major one at 4400 A, and a somewhat subordinate one at 4800 A. It may now be noted that Bachmann and Bergann (1) and Johnston (6), studying the etiolated coleoptiles of *Avena sativa* Culberson, obtained curves of phototropic sensitivity having two maxima at about these same regions. The two types of data, the one indicating an inhibitory influence of light on the germination of seeds, the other an influence of light on the direction of growth of young etiolated shoots, have been brought together to facilitate comparison in figure 5.

An examination of figure 5 reveals that within the range of experimental error the two types of plant response to light show identical critical wave lengths. Johnston (6, p. 14) and others interpret phototropic response as an index of growth retardation on the theory that the light on the exposed side of the shoot inhibits elongation, while on the opposite unexposed side elongation is relatively uninfluenced. The results here reported obviously tend to sustain the correctness of this interpretation. Both of the foregoing types of plant response to light involved etiolated structures, and further studies are in progress relating to the types of plant response characterizing green tissues.

In résumé, the results obtained with the Wratten filters, taken in conjunction with the curve of violet-blue-green inhibition given by the spectral data and with the newly discovered inhibitory influence in the 7600 A region, make it more than ever obvious that the effects obtained through the use of any color filter may not safely be interpreted without an analysis of its spectral transmission.

The results presented in this paper indicate the general relative effectiveness of radiation of various wave lengths in the visible spectrum found to inhibit the germination of light-sensitive lettuce seed.

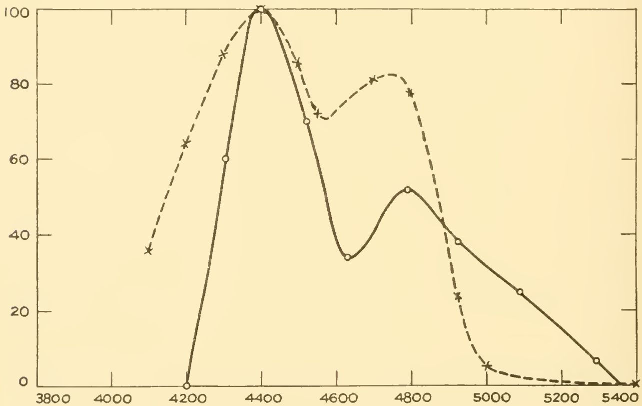

Fig. 5.—Curve of inhibition in the violet-blue-green region compared with the curve of phototropic response of oat coleoptiles obtained by Johnston. Heavy line represents the inhibition; dotted line the phototropic response.

Further studies designed to yield quantitative results as to the relative effectiveness of both promoting and inhibiting radiation are now in progress.

SUMMARY

Announcement is made of the discovery of a band in the region of 7600 A which inhibits the germination of light-sensitive lettuce seed far more effectively at the energies characterizing both solar and Mazda radiation (in this region) than do similar inhibitory influences previously noted in the regions 4200 A to 5200 A.

The relative effectiveness of radiation in the violet, blue, and green regions of the spectrum—at 4200 A to 5200 A—in inhibiting germination in light-sensitive lettuce seed is found to be the same as its relative

effectiveness in bringing about phototropic response in etiolated coleoptiles of oats. Both phenomena may be represented by bimodal curves showing critical wave lengths in the regions 4400 A and 4800 A.

LITERATURE CITED

1. Bachmann, Fr., and Bergann, Fr.
1930. Über die Werkigkeit von Strahlen verschiedener Wellenlänge für die phototropische Reizung von *Avena sativa*. Planta, Arch. Wiss. Bot., vol. 10, pp. 744-755.

2. Cieslar, Adolph
1883. Untersuchungen über den Einfluss des Lichtes auf die Keimung der Samen. Pp. 270-295.

3. Flint, Lewis H.
1934. Light in relation to dormancy and germination in lettuce seed. Science, vol. 80, pp. 38-40.

4. Gardner, W. A.
1921. Effect of light on germination of light-sensitive seeds. Bot. Gaz., vol. 71, pp. 249-288.

5. Gassner, G.
1915. Beitrage zur frage der Lichtkeimung. Zeitschr. Bot., vol. 7, pp. 609-661.

6. Johnston, Earl S.
1934. Phototropic sensitivity in relation to wave length. Smithsonian Misc. Coll., vol. 92, no. 11, pp. 1-17.

7. Lehmann, E.
1918. Über die minimale Belichtungzeit welche die Keimung der Samen von Lythrum salicaria auslöst. Ber. Deutsch. Bot. Ges., vol. 36, pp. 157-163.

8. Maier, Willi
1933. Das keimungsphysiologische Verhalten von Phleum pratense, L., dem Timotheegras. Jahrb. Botanik, vol. 78, pt. 1, pp. 1-42.

9. Molisch, Hans
1930. Pflanzenphysiologie als Theorie der Gärtnerei. P. 323.

10. Nathammer, Ann.
1927. Keimungphysiologische Studien unter Hervorhebung des Lichtkeimungsproblems. Biochem. Zeitschr., vol. 185, pp. 205-215.

11. Shuck, A. L.
1935. Light as a factor influencing the dormancy of lettuce seeds. Plant Phys., vol. 10, pp. 193-196.