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I. INHIBITION OF THE MESOCOTYL OF AVENA SATIVA BY CONTINUOUS EXPOSURE TO LIGHT OF LOW INTENSITIES

(With One Plate)

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INTRODUCTION

It is well known that growth of the mesocotyl of various grass seedlings is markedly influenced by illumination. Nevertheless, our understanding of the mechanism of this phenomenon is very fragmentary, despite the numerous studies that have been made since the effect was first noted by Cassini (1820) more than a century ago. One of the many aspects of the problem about which further quantitative information is desirable is that of the spectral effectiveness of the radiant energy causing the growth inhibition. The present experiments have been undertaken as a first approach toward the determination of the action spectrum for mesocotyl inhibition.

Review of the literature[1] leads to the conclusion that, in general, there has been inadequate appreciation of the dependence of the effects of radiant energy upon its intensity and spectral distribution, upon the duration of the irradiation, upon the developmental stage of the plant and possibly also upon other environmental and internal conditions. The present report is concerned exclusively with the results produced by light of known intensity and quality, applied throughout the entire period of growth of the mesocotyl. As far as the authors are aware the only comparable published experiments are those of Avery, Burkholder, and Creighton (1937).

EXPERIMENTAL PROCEDURE

Oats of the variety Markton[2] were used. After removal of the glumes the dormant grains were planted individually in small test

[1] An analysis of the literature relating to the physiology of the grass mesocotyl is being prepared for separate publication.

[2] The seeds were kindly supplied by T. R. Stanton, of the U. S. Department of Agriculture.

tubes (about 1 cm. in diameter and 7 cm. long) on slants of 1 percent agar made up with tap water. The seeds rapidly absorb water from the agar, and the seedlings develop very uniformly. This technique is convenient for irradiation studies in that the seeds, which have been found to be insensitive to light during the first few hours of germination, can be placed in the desired experimental environment within a few minutes after planting. No further attention or manipulation of the plants is required until the conclusion of the experiment when the seedling organs are measured. An 8-day growth period was chosen,

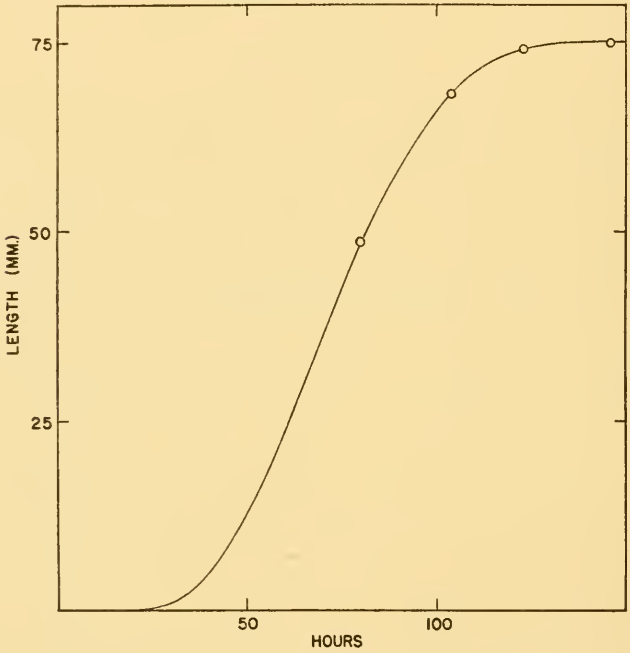

FIG. 1.—Growth curve of mesocotyl in complete darkness at 27.5° C.

since at the temperature used the mesocotyl and coleoptile have completed their growth in this length of time (see fig. 1).

The growth chamber consisted of a galvanized iron box divided into 4 compartments into each of which was placed a set of 25 to 30 culture tubes in a 400-ml. beaker. A layer of water on the floor of the growth chamber served to maintain the humidity of the air at saturation. The open top of the box was provided with flanges so that each compartment could be covered with an individual filter, without the light passing from one compartment to another. In practice three different light intensities were studied in a single experiment, the fourth group of plants serving as a dark control.

In order to provide a graded series of intensities at each wave length, light screens were made up of layers of colored cellophane enclosed between two sheets of glass. Various colors of cellophane are available and by choosing the appropriate number of layers almost any desired series can be obtained. The transmissions of these filters were determined for the particular spectral bands in which they were employed.

The spectral regions studied were isolated by means of two quartz-prism monochromators arranged in series. The wave-length spread of these bands was determined visually with a spectroscope.

The radiation source was a 1,000-lumen, 6.6-ampere Mazda street-series lamp operated from a 115-volt a.c. line, through a transformer, at 18 volts and 6 to 7 amperes. Line-voltage fluctuations were minimized by means of a voltage regulator. Gradual drifts in current due to ageing of the lamps could be detected by a sensitive ammeter connected in the circuit; this was read several times during each day and, when necessary, the current was adjusted by means of a variable resistor. The variation in current was never greater than a few hundredths of an ampere during a day and in many experiments no change could be observed over a period of several days.

The lamp was placed outside the dark room containing the growth chamber and the double monochromator so that only the radiant energy which passed through the instrument reached the plants. The beam of light from the exit slit was reflected downward onto the seedlings by means of a 45° silvered glass mirror.

The box containing the plants was mounted on a turntable rotated by a synchronous motor at 2 revolutions per minute, so that each set of plants traversed the light beam 4 times each minute. The rotation was considered necessary since the two sides of the box received light of slightly different wave-lengths owing to the widths (85 to 130 A.) of the bands isolated.

The intensities incident on the plants were calculated by means of the inverse-square law. This is justified since, at the distances used, the exit slit (1 by 10 mm.) can be considered as a point source without significant error. The relation between the intensity at the slit and that at 10 cm. distance from the slit was determined initially; from the values of this factor, of the length of the light path from the exit slit to the seeds, of the reflection loss due to the mirror and of the transmission of the filters, the intensity at the level of the seeds could be calculated. It is realized that an error is introduced through the use of vertical illumination, since, owing to the growth of the shoots, the effective intensity changes slightly during the course of the

experiment and is actually somewhat greater than that at the seed level. This error is smaller than the uncertainties in other measurements, however, and may be neglected.

Except for the experiments at 6600 A., the radiation intensity at the exit slit was measured by a vacuum thermocouple connected with a Leeds and Northrop type-HS galvanometer. The thermocouple and galvanometer combination were calibrated against a National Bureau of Standards standard of radiation, correction being made for the differences in wave-length distribution of the standard lamp and of the spectral regions isolated by the double monochromator. For the experiments at 6600 A. a barrier-type photocell ("Electro-

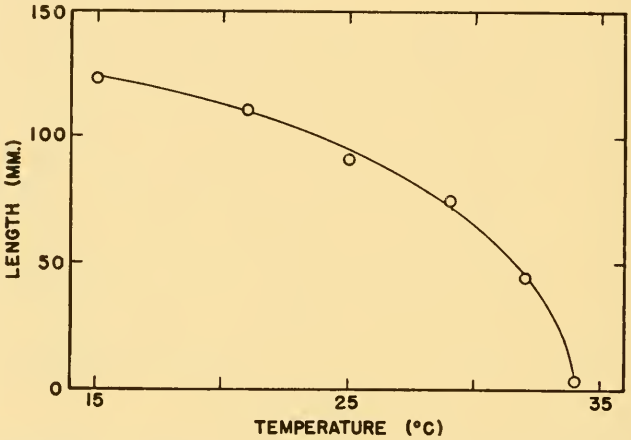

FIG. 2.—Influence of temperature on final lengths of mesocotyls grown in complete darkness.

cell") and high-resistance galvanometer, which had been calibrated against the thermocouple at this wave length, were used.

The relationship between the effects of temperature and of radiant energy on the development of the grass seedling is a subject that would appear to justify detailed investigation. Some preliminary results of such a study, shown in figure 2, emphasize the necessity of close control of temperature if comparisons are to be made among plants grown at different times. As a check on the adequacy of the temperature regulation in the present work a continuous thermograph record was obtained for each run. In the majority of these experiments the temperature was maintained at $27.5 \pm 0.2°$ C. In a few the temperature varied from this value over a 1- to 2-degree range. For this reason the length measurements of the plants in all the experiments are not strictly intercomparable. However, since there is no

evidence that the action spectrum is affected by small temperature differences, the values relative to the dark controls included in each run are comparable. The results, therefore, have been expressed in relative terms.

RESULTS

The mesocotyls of seedlings that are illuminated continuously from the time of planting do not attain as great a final length as do those grown in darkness. The extent of the growth inhibition is dependent upon the intensity and quality of the light. The results for a series of wave-length bands are summarized in table 1. These data are plotted in figure 3, using a logarithmic scale for the intensities; the curves were fitted by the method of least squares.

It will be seen that the inhibition is proportional to the logarithm of the intensity and that the slopes of the curves for different wave lengths are substantially equal. The curve for 7700 A. has been arbitrarily drawn parallel to the others since only the highest intensity employed was sufficient to cause measurable inhibition. Attention should be directed also to the great range of intensities over which the inhibitory effects are produced and to the existence of distinct threshold values for each wave-length band.

The action spectrum is obtained by plotting the reciprocal of the intensity required to produce a given effect against the wave length. On the assumption that the number of quanta required for this response is the same at all wave lengths, comparison is more properly made on a quantum basis. This has been done in figure 4, although the correction is relatively small (table 2). The action curve shows a sharp peak at about 6600 A. and indication of a second maximum in the neighborhood of 6200 A.

DISCUSSION

The experiments here reported were undertaken to furnish a working curve as a basis for further investigation. The present results are to be regarded as constituting merely a first approximation to the action spectrum for mesocotyl inhibition since data are available for only seven relatively widely spaced wave lengths. Although these points have been connected by a curve, it is obvious that nothing is actually known of the intermediate regions. Other methods of study which are less time-consuming can be employed to obtain additional points on the curve and it is hoped to present the results derived by such technique at a later date.

TABLE 1.—*Light intensity and mesocotyl inhibition*

Wave-length band (A.)	Intensity at seed (ergs/mm.²/sec.)	Inhibition (%)
4250-4360	618,000. × 10^{-9}	66.9
	59,000.	45.7
	11,200.	30.1
	8,520.	33.2
	1,070.	5.4
	154.	0
	19.2	0
	2.78	0
5445-5552	78,000.	61.6
	75,700.	60.6
	22,000.	47.5
	21,300.	49.3
	13,100.	48.1
	6,200.	38.9
	6,010.	39.4
	3,950.	33.8
	1,120.	20.0
	1,040.	22.3
	312.	9.4
	82.6	0
	6.84	0
6155-6240	20,600.	67.3
	4,810.	60.3
	1,450.	46.9
	337.	33.1
	101.	23.7
	23.6	6.6
6435-6550	3,720.	57.6
	3,200.	59.7
	192.	30.1
	166.	29.6
	10.1	3.9
6545-6645	4,450.	69.2
	4,360.	68.7
	1,600.	62.0
	788.	57.0
	234.	43.4
	230.	44.8
	84.2	30.0
	41.6	26.6
	4.43	5.8
7030-7160	2,300.	66.9
	858.	35.4
	32.2	4.5
7640-7760	74,000.	11.0
	7,250.	0
	708.	0

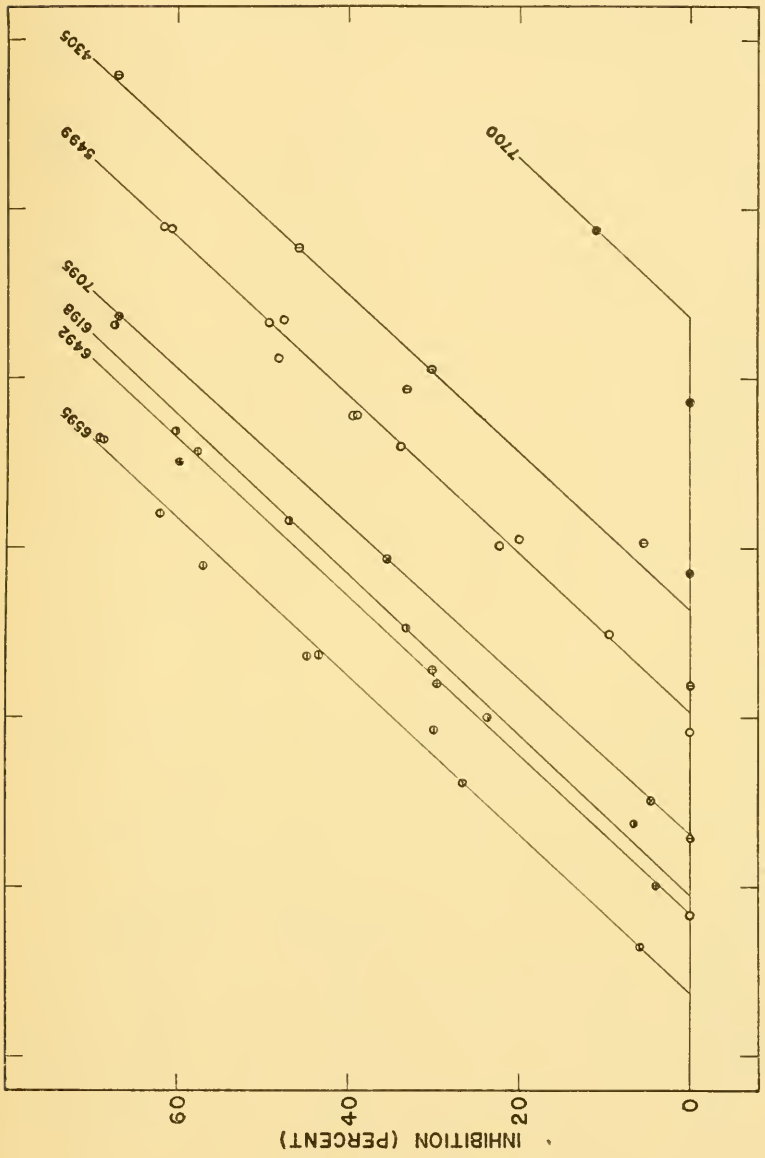

FIG. 3.—Effect of intensity on inhibition of mesocotyl growth by various wave lengths of light.

The true position of the peak shown at 6600 A. may possibly be at a somewhat longer wave length. There is some uncertainty also as to the exact location of the suggested second maximum.

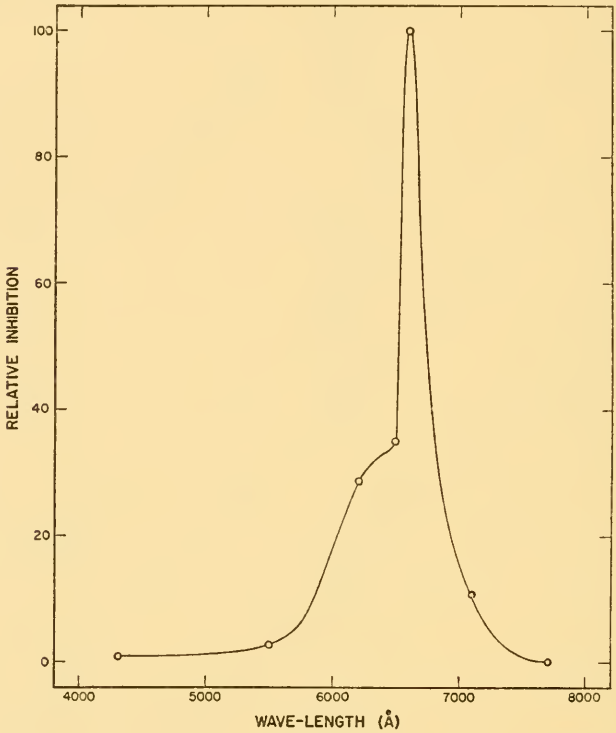

FIG. 4.—Spectral effectiveness curve for mesocotyl inhibition.

TABLE 2.—*Relative spectral effectiveness for mesocotyl inhibition*

Wave length (A.)	Relative effectiveness (Compared on energy basis)	Relative effectiveness (Compared on quantum basis)
4305	0.6	0.8
5499	2.2	2.4
6198	26.7	28.4
6492	34.0	34.5
6595	100.	100.
7095	11.6	10.8
7700	0.01	0.009

The absorption spectrum of the photoreceptive substance involved in the inhibition of growth by light may be expected to show a general resemblance to the action spectrum, provided that other pigments are

not present also. Since marked growth effects may be obtained by illumination of dark-grown seedlings for periods of only a few seconds, the light-sensitive system appears to be present in the completely etiolated seedling. Extracts of etiolated oats seedlings show absorption bands with maxima at about 625 and 660 mμ. Corresponding bands, at somewhat longer wave lengths, can be observed also on first illumination of etiolated oats leaves. Preliminary study has indicated that these bands are due to at least two substances; these might conceivably be the pigments which have been designated as protochlorophyll and chlorophyllogen. It is not possible to state, as yet, whether more than one pigment participates in the photoreceptive mechanism of mesocotyl inhibition.

The dark-grown oats seedling contains also relatively large amounts of carotenoid pigments which absorb strongly in the blue portion of the spectrum, where the provisional action spectrum shows no maxima. However, since the absorption by these yellow pigments might be expected to diminish the effectiveness of the shorter visible wavelength region and since the mesocotyl growth is in fact affected by light of such wave lengths, it seems very likely that the photoreceptive pigment possesses absorption bands in this region also.

The growth of the mesocotyl in darkness is the result of two processes: cell division and cell elongation. The data of Avery, Burkholder, and Creighton (1937) suggest that at low light intensities it is the process of cell multiplication that is inhibited, whereas cell elongation is affected only by higher intensities. If experiments such as those reported above are extended to higher intensities a more or less sharp inflection is found in the inhibition-intensity curves. It may be inferred that at intensities below this knee (i.e., for the region shown in the curves of figure 3) only cell division is influenced, whereas at higher intensities inhibition of cell stretching is involved also. Experiments designed to furnish cytological evidence bearing on this suggestion are now in progress. If the mechanism should prove to be as outlined the action spectrum determined at the lower intensities represents chiefly the inhibition of cell multiplication. It might be possible, by studies at higher intensities, to determine also the action spectrum for the cell-extension process.

The authors take pleasure in acknowledging the cooperation in many ways of the other members of the Division of Radiation and Organisms and the technical assistance of E. R. Brydon and O. R. Zipf during part of this investigation.

SUMMARY

1. The relationship between growth inhibition of the mesocotyl of *Avena sativa* and the intensity of the radiant energy causing it has been determined for a number of relatively narrow wave-length bands in the visible spectrum. At low intensities the inhibition is proportional to the logarithm of the intensity.

2. From these data a provisional action spectrum of mesocotyl inhibition has been plotted. This shows a single peak at approximately 6600 A. and an indicated secondary maximum in the neighborhood of 6200 A.

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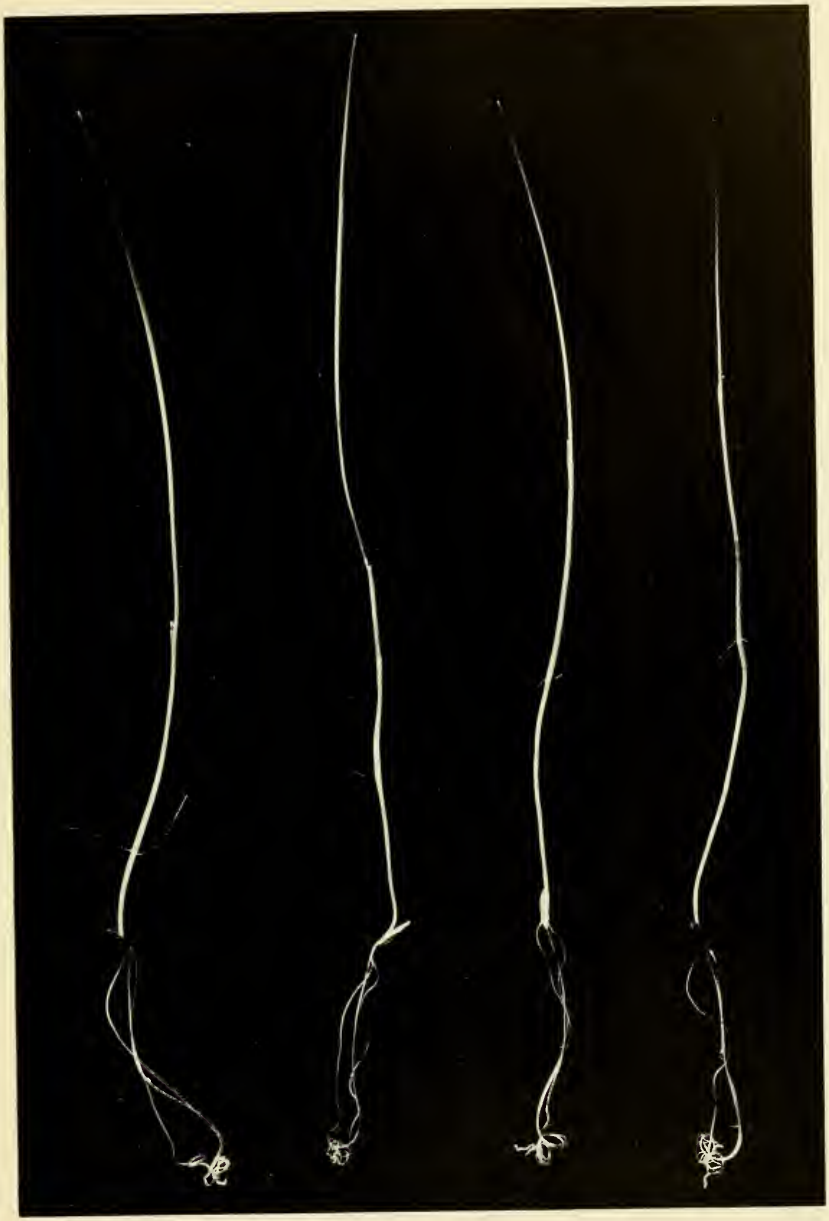

REPRESENTATIVES OF FOUR SERIES OF SEEDLINGS OF AVENA SATIVA GROWN UNDER VARIOUS LIGHT INTENSITIES

The apical limit of the mesocotyl is indicated by the adventitious roots at the coleoptilar node.