SOME EFFECTS OF RADIANT ENERGY IN RELATION TO ETIOLATION[1, 2]

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(WITH THREE FIGURES)

Numerous investigators have worked on the problem of determining the effect of small quantities of visible radiant energy on seedlings and other plants with stored foods. The literature is extensive and goes back over one hundred years. MacDougal (6) has thoroughly reviewed the work before 1900 while Trumpf (9) and Burkholder (1) have reviewed this and much of the later work.

The work before 1900 established the fact that visible radiant energy alone could be considered capable of initiating the morphological differences resulting when seedlings and plants with storage organs received small amounts of energy but not enough to carry on significant amounts of photosynthesis. Since 1900 more quantitative investigations have been carried out by Trumpf (9), Priestly (7), Withrow (13), and Went (11).

The following experiments were an attempt to give further information on the quantitative responses of certain seedlings to irradiation, and to find out more about the internal mechanism causing these responses.

Procedure

All the work was carried on in an air-conditioned darkroom. Photosynthesis was kept to a negligible level, either by exposing the plants to very low intensities of irradiation, or by giving them short daily exposures to higher intensities. Under these sets of conditions little or no chlorophyll was formed and the plant had to exist on stored food.

The temperature was kept between 20° and 22° C. except in one experiment where it was purposely lowered to 1° C. for 0.5 hour each day. Relative humidity was kept between 67 and 73 per cent.

The incandescent tungsten filament lamp was used as the source of radiant energy. The radiant energy from the lamp was filtered through 11 cm. of water and four thicknesses of red cellophane. The transmission of the cellophane was measured with a spectrophotometer and four layers were found to completely absorb all visible radiant energy below wavelength 5,850 Å. and to transmit uniformly about 60 per cent. of all wavelengths of visible energy above 6,200 Å. The plants were thus irradiated by energy in the yellow, orange, and red regions of the visible spectrum plus the near

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infra-red spectrum up to 11,000 Å. WITHROW'S (**13**) work demonstrated that when red kidney bean seedlings were exposed to radiant energy in the near infra-red beyond 8,000 Å. the responses described later did not occur. This was confirmed in one experiment when a few kidney bean plants were exposed for one half hour daily to the radiant energy of a 300-watt lamp in a deep bowl reflector placed 50 cm. above the tops of the plants. The radiant energy was filtered through 11 cm. of water and a plate of Corning infra-red transmitting glass number 254. This glass filter absorbs all visible radiant energy and transmits an average of 20 per cent. of the infra-red energy from 8,000 Å. to 11,000 Å. After a week of such exposure the plants appeared similar to the checks which had never been irradiated. The effective spectrum was therefore from 5,850 Å. to something less than 8,000 Å. All figures of irradiance are given in terms of this band.

The irradiance, or the intensity of irradiation, was varied by the use of different wattages of lamps, by varying the distance of the lamp from the plant, and by the use of neutral screens of white cloth. Differences in color temperatures of the various wattages of lamps used were not considered significant because of the restricted spectrum. The energy in this spectral region was very effective in causing the response and its use eliminated consideration of responses such as phototropism, which are correlated with the shorter wavelengths of the visible spectrum.

Most of the experiments were carried out using *Phaseolus vulgaris* variety red kidney, as the test plant, but from time to time other plants were used. The plants used were *Pisum sativum* variety Alaska, *Zea mays, Xanthium pennsylvanicum, Helianthus annuus,* and *Vicia faba.* Not all of the plants used responded to irradiation to the same degree, but the same general response was obtained from all. The general nature of the response can be observed in figure 1.

Most of the experiments were terminated seven to nine days after the seedlings appeared above the surface of the gravel used as the nutrient solution medium and about 13 to 15 days after the seed was sown. At the end of eight days of irradiation the cotyledons were quite shrunken and were nearly depleted of reserve foods. All experiments were repeated at least once.

A number of different problems was investigated. The first problem studied was to find how bean seedlings responded to increasing quantities of radiant energy of the longer wavelengths of the visible spectrum. The quantity of energy could be increased both by increasing the irradiance and by lengthening the time of irradiation. Both methods were used and the relationship between them was studied when both were varied so that the quantity of energy was kept constant. When irradiance was increased the time of irradiation was decreased and *vice versa.*

The second problem was to study the kinetics of the mechanism of the response. By varying the rate of cycles of alternating irradiation and non-irradiation over different lots of plants, but keeping the total energy constant, it was possible to obtain some information on the nature of the reactions involved in the response of the plant. By this technique it was also possible to differentiate between the mechanism involved in chlorophyll formation and the mechanism involved in the morphological response of bean seedlings, though both occur simultaneously under most conditions. Lowering the temperature at the time of irradiation and using a high irradiance gave information on the Q_{10} of the limiting reactions in the mechanism at the time of irradiation.

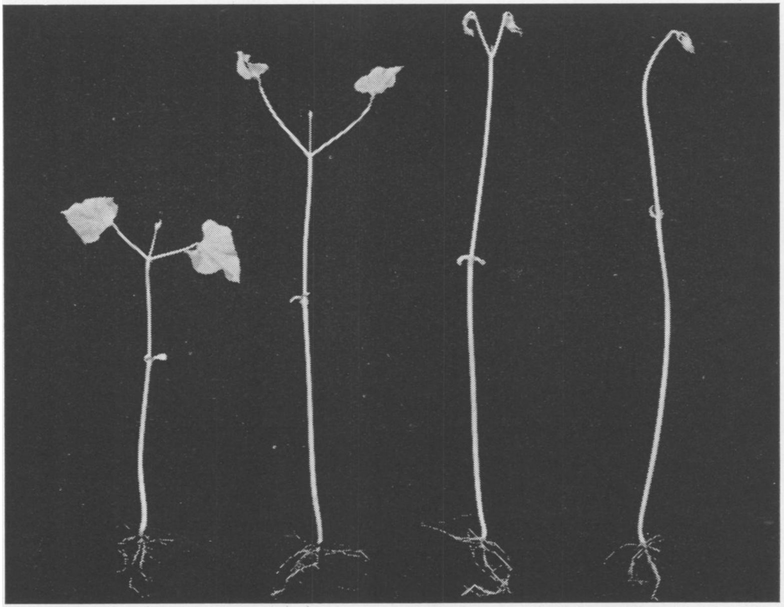

FIG. 1. The effect of the quantity of radiant energy. Left to right, 50,000 ergs/cm.^{2}/sec. for 15 minutes daily; 500 ergs/cm.^{2}/sec. for 15 minutes daily; 5 ergs/cm.^{2}/sec. for 15 minutes daily; and non-irradiated.

The third problem studied was to find out what pigment or pigment systems absorbed the visible radiant energy. Albino and normal maize seedlings were irradiated to see if chlorophyll or the carotenoid pigments were involved in the mechanism of the response.

The fourth problem studied was to find out if all parts of the plant were equally sensitive to visible radiant energy and to see whether other parts of the plant would respond if one part of the plant were irradiated. This was usually done by covering all but the part to be irradiated. In some cases various parts of the plants were removed and the effect of irradiation on the remaining parts was studied.

The fifth problem studied was the possible correlative relationship between the decrease in hypocotyl elongation associated with irradiation and auxin concentration in the hypocotyl.

Results

EFFECT OF VARYING QUANTITIES OF INCIDENT RADIANT ENERGY

An experiment was conducted to find the lowest irradiance that would still cause a perceptible response. When exposed to an irradiance of 0.05 erg/cm.²/sec. applied continuously, red kidney bean, pea, and broad bean all showed clear responses.

High irradiances with continuous irradiation to obtain the maximum response were not used since a few preliminary experiments showed that irradiances sufficient for photosynthesis would be required. Accordingly most of the work was done on the basis of exposures of 30 minutes or less daily. Little or no chlorophyll was formed under these conditions and the plants responded about the same as they did to continuous irradiation. With very little chlorophyll present, photosynthesis was negligible.

TABLE I

EXPOSURE TIME CONSTANT, IRRADIANCE INCREASED

TREATMENT	NO. PLANTS PER PLOT	AV. LENGTH HYPOCOTYL	AV. LENGTH FIRST INTERNODE	AV. LENGTH PETIOLE	AV. LENGTH X WIDTH BLADE
		cm.	*cm.*	*cm.*	*sq. cm.*
Non-irradiated	33	29.0	15.7†	0.7	....
5 H* 15 min. daily	34	24.8	18.1	3.1	....
500 H 15 min. daily	22	21.9	14.7	6.2	....
50,000 H 15 min. daily	13	15.7	10.0	4.3	....

* H = ergs/cm.²/sec.

† Elongation had not yet ceased.

Table I gives the data from an experiment in which the time of irradiation was kept constant at 15 minutes daily but the irradiance was increased 10,000 times in two steps from five ergs/cm.²/sec. to 50,000 ergs/cm.²/sec.

The bean seedlings gave definite responses up to the highest irradiance used. Those growing under the highest irradiance in the chambers were similar in form to the same age beans growing in the greenhouse in the winter, but were nearly devoid of chlorophyll.

As table I shows, hypocotyl length decreased as the irradiance increased. Internodes also decreased in length in the same manner. Petioles increased in length up to rather high irradiances but further increase caused a decrease in the length. The change in the appearance of the plants can be seen in figure 1. The change in morphological response with increase in irradiance was greater than if the response increased simply as the logarithm of the quantity of incident radiant energy. It appears that high irradiances were more effective per unit of energy than low irradiances.

The total quantity of radiant energy incident on the bean plants was varied in one experiment by keeping the irradiance constant at 50,000 ergs/cm.^{2}/sec. and varying the length of time the plants were exposed to this energy from one second daily up to 900 seconds daily. Table II shows the results of this experiment.

TABLE II

IRRADIANCE CONSTANT, EXPOSURE TIME INCREASED

TREATMENT	NO. PLANTS PER PLOT	AV. LENGTH HYPOCOTYL	AV. LENGTH FIRST INTERNODE	AV. LENGTH PETIOLE	AV. LENGTH × WIDTH BLADE
		cm.	*cm.*	*cm.*	*sq. cm.*
50,000 H 1 sec. daily	13	25.3	14.7	3.8	5.5
50,000 H 10 sec. daily	11	23.3	10.3	4.8	14.5
50,000 H 30 sec. daily	11	19.3	10.5	4.8	18.5
50,000 H 60 sec. daily	13	19.0	10.0	4.7	20.2
50,000 H 300 sec. daily	10	17.4	9.9	4.2	20.6
50,000 H 600 sec. daily	10	17.5	9.8	4.5	21.6
50,000 H 900 sec. daily	9	16.6	9.2	3.7	21.2

When the quantity of radiant energy was increased by lengthening the duration of the exposure, the plants given the greater amount of energy had shorter hypocotyls, shorter first internodes and longer petioles. Petiolar length decreased, however, when the total energy was increased above certain levels. Length and width of leaves increased rapidly with increases in energy at short exposures but increased throughout, as did all the other responses, even when the duration of exposure was long. These morphological responses increased less than if they increased as the logarithm of the quantity of incident radiant energy. This can be seen easily when length of hypocotyls is compared. There was a greater difference in the length of hypocotyls between the plants exposed 1 and 30 seconds than there was between those exposed 30 and 900 seconds.

QUANTITY OF RADIANT ENERGY PER DAY KEPT CONSTANT BY VARYING BOTH IRRADIANCE AND EXPOSURE TIME

As a result of several experiments, TRUMPF came to the conclusion that the quantitative response of bean seedlings to irradiation depended on the total daily quantity of radiant energy incident on the plant surface; that is, a low irradiance for a long time daily was as effective as a high irradiance for a short time daily, provided the total daily energy incident on the plant was the same.

Several experiments were run to repeat those results but using a far greater range of irradiances than had been used heretofore. In one experiment the irradiance was increased from 50 ergs/cm.^{2}/sec. to 50,000 ergs/cm.^{2}/sec. The radiant energy applied was 4,500,000 ergs/cm.^{2}/day. The results are given in table III.

TABLE III

TOTAL ENERGY CONSTANT, EXPOSURE AND IRRADIANCE VARIED

TREATMENT	NO. PLANTS PER PLOT	AV. LENGTH HYPOCOTYL	AV. LENGTH FIRST INTERNODE	AV. LENGTH PETIOLE	AV. LENGTH BLADE
		cm.	*cm.*	*cm.*	*cm.*
52 H continuously	32	20.0	12.6	5.9	3.5
500 H 150 min. daily	28	19.4	12.5	6.2	4.1
5,000 H 15 min. daily...	22	18.2	10.5	4.3	4.3
50,000 H 1.5 min. daily...	24	17.7	9.5	4.1	4.6

The response to irradiation increased with increase in irradiance even though exposure time was reduced accordingly to keep the daily quantity of radiant energy constant. Similar results were obtained when the daily quantity of radiant energy was one-tenth and also one-thousandth the amount used in table III. These results are in contradiction with those of TRUMPF but are confirmatory of the recent work of WENT.

In an attempt to measure how much more effective the high irradiances are than the low, several lots of bean seedlings were given different exposures at a high irradiance and then compared with a lot of seedlings that were given a lower irradiance but for a much longer time so that the total radiant energy was the same as the longest exposure of the high irradiance lots. The results of one such experiment are given in table IV. The starred treatments are those having the same total energy daily.

The bean seedlings irradiated at an irradiance of 50,000 ergs/cm.^{2}/sec. for 10 seconds daily received one-third the total daily quantity of energy of the low irradiance plot, yet these two lots of plants showed an overall

TABLE IV

MEASUREMENT OF GREATER EFFECTIVENESS OF HIGH IRRADIANCES

TREATMENT	NO. PLANTS PER PLOT	AV. LENGTH HYPOCOTYL	AV. LENGTH FIRST INTERNODE	AV. LENGTH PETIOLE	AV. LENGTH × WIDTH BLADE
		cm.	*cm.*	*cm.*	*sq. cm.*
18 H* continuously	24	21.0	13.5	6.8	13.0
50,000 H 1 sec. daily	13	25.3	14.7	3.8	5.5
50,000 H 10 sec. daily	11	23.3	10.3	4.8	14.5
50,000 H 20 sec. daily	11	19.1	10.9	4.6	14.5
50,000 H* 30 sec. daily	11	19.3	10.5	4.8	18.5

* These two plots have the same total incident energy.

response that was very similar. Since the only measurement of the effect of the radiant energy available was the ultimate form of the plant, it was assumed therefore, that irradiation at the high irradiance was approximately three times as effective per unit of energy as the low irradiance.

A COMPARISON OF DAILY IRRADIATION AND IRRADIATION ONLY ONCE DURING THE EXPERIMENT

Kidney bean seedlings responded efficiently to radiant energy applied for a single short exposure daily. Experiments were carried out to see if a single exposure to radiant energy at different times in the life of the plants was as effective as short daily exposures.

One lot of bean seedlings was irradiated with an irradiance of 500 ergs/cm.^{2}/sec. for 1.25 hours daily for six days and for 30 minutes on the seventh day making a total of eight hours of irradiation. Another lot was

TABLE V

A SINGLE IRRADIATION COMPARED WITH DAILY IRRADIATION

TREATMENT	NO. PLANTS PER PLOT	AV. LENGTH HYPOCOTYL	AV. LENGTH FIRST INTERNODE	AV. LENGTH PETIOLE	AV. FRESH WEIGHT LEAVES
		cm.	*cm.*	*cm.*	*gm.*
500 H 1.25 hr. daily	18	20.4	11.7	4.7	0.37
500 H 8 hrs. second day	18	23.1	13.6	3.9	0.26
500 H 8 hrs. fourth day	18	24.7	13.5	3.0	0.22
500 H 8 hrs. fifth day	18	27.5	9.8	2.4	0.22

irradiated at the same irradiance for eight consecutive hours the second day after the seedlings emerged and not thereafter; another lot was irradiated under the same conditions but only on the fourth day after emergence; and still another lot on the fifth day after emergence. All plants received the same total quantity of radiant energy but at different times during the experiment. The results are given in table V.

Daily irradiation was more effective than any single irradiation any time during the experiment. In comparing the various single exposure treatments with one another it is well to remember that the plants irradiated first had more time to develop and show the response to irradiation while those irradiated later had already elongated much before being irradiated and irradiation could not alter this. These also had the least time to respond before the experiment ended. This also holds for the last one or two irradiations where the plants were irradiated daily.

CYCLES OF IRRADIATION OTHER THAN ONCE EVERY 24 HOURS

The length of time between irradiations very greatly influenced the amount of the response. Several experiments were conducted to find the response of bean seedlings to periods of irradiation both more frequent and less frequent than once every 24 hours.

A number of experiments were run and the number of times the plants were irradiated varied from 144 times daily to once every three days. In all of these experiments irradiance was not varied but the duration of each period of irradiation was lengthened or shortened as the frequency of the cycles was short or long so that the plants received the same total quantity of radiant energy by the end of the experiment regardless of the frequency of the periods. For example, one plant received 7.5 minutes of irradiation

TABLE VI

CYCLES MORE THAN ONCE EVERY TWENTY-FOUR HOURS

TREATMENT	NO. PLANTS PER PLOT	AV. LENGTH HYPOCOTYL	AV. LENGTH FIRST INTERNODE	AV. LENGTH PETIOLE	AV. LENGTH × WIDTH BLADE
		cm.	*cm.*	*cm.*	*sq. cm.*
6000 H 5 min. every 24 hours	57	22.9	11.4	4.8	25.5
6000 H 10 min. every 24 hours	69	21.5	11.1	4.6	32.0
6000 H 7.5 min. every 12 hours	123	20.3	10.8	4.5	35.2
6000 H 15 min. every 24 hours	92	20.9	11.3	5.1	33.6
6000 H 22.5 min. every 36 hours	90	21.9	12.1	5.5	32.0
6000 H 30 min. every 48 hours	125	22.4	12.1	5.1	31.4

every 12 hours and another plant received 15 minutes of irradiation every 24 hours. Table VI shows the results of one such experiment where the cycles were multiples of 12 hours. To measure quantitatively the effect, two plots were run with shorter exposures than 15 minutes daily. If certain cycles of exposure were less effective than one exposure daily, the difference could be measured by comparing them with the plants given a daily exposure of less than 15 minutes.

Table VI shows that irradiation twice daily was the most effective and that as the time between irradiations was increased the response decreased even though the total quantity of radiant energy incident on the plant was the same. Irradiation 10 minutes daily produced about the same response as irradiation for 22.5 minutes every 36 hours even though the latter treatment had 50 per cent. more energy incident on the plants. Irradiation of between 5 and 10 minutes daily was as effective in causing the response as irradiation for 30 minutes every two days. That is, it was approximately twice as effective since about half the quantity of radiant energy was required to produce the same degree of response.

TABLE VII

CYCLES MORE THAN ONCE EVERY TWENTY-FOUR HOURS

TREATMENT	NO. PLANTS PER PLOT	AV. LENGTH HYPOCOTYL	AV. LENGTH FIRST INTERNODE	AV. LENGTH PETIOLE	AV. FRESH WEIGHT BLADE	CHLOROPHYLL PER GM. FRESH WEIGHT LEAF
		cm.	*cm.*	*cm.*	*gm.*	*mg.*
500 H 1.25 hours daily ...	42	22.0	12.6	5.9	0.31	0.25
500 H 0.5 min. 144 times daily	39	21.3	12.8	5.6	0.31	0.47
500 H 15 min. daily	25	24.4	12.8	5.1	0.25	0.10
500 H 6.25 seconds 144 times daily ...	40	21.6	13.6	5.7	0.27	0.26
Non-irradiated ...	39	30.0	11.0	0.8	0.10	0.01*

* Comparison was difficult and the figure is probably high.

In another experiment one plot was given 144 exposures daily of 6.25 seconds each, making a total exposure of 15 minutes daily. Another plot was given a single exposure of just 15 minutes daily. The results are given in the first five columns of table VII.

The plants irradiated 144 times daily had the shortest hypocotyls, but in all other respects they had the characteristics of plants that had received less energy; that is, the internodes were longer, the petioles were shorter and the leaves were smaller. It appeared that irradiation once every 10 minutes was less effective than irradiation once every 24 hours in many respects and more effective in some.

In another experiment, not given here in tabular form, an exposure of three times daily was compared with an exposure of once daily. The plants produced by these treatments were almost identical, indicating that they were of about equal effectiveness in producing morphological responses. The former were, however, somewhat greener.

Whenever irradiation was continued for more than 30 minutes daily at fairly high irradiances, or where plants were irradiated more than once daily, visible quantities of chlorophyll appeared in the leaves of kidney bean seedlings. The chlorophyll concentration in the leaves of plants irradiated many times daily was measured in one experiment. SHERTZ's (**8**) modification of WILLSTÄTTER and STOLL's method of extracting chlorophyll was used and GUTHRIE's (**2**) standard was made up for comparison purposes. The results were determined in a Klett colorimeter. Table VII, column six, shows the results of two separate series.

Irradiation 144 times daily at least doubled chlorophyll concentration in the plants so irradiated as compared with those irradiated once daily. This was true when they were irradiated at 2,250,000 ergs/cm.^{2}/day as well as when irradiated at 450,000 ergs/cm.^{2}/day. The fact that chlorophyll concentration increased when they were irradiated many times daily, whereas the morphological response of the plants varied in no very consistent direction is evidence that chlorophyll formation and morphological response of kidney beans to irradiation are different mechanisms having different kinetics.

PLANT PIGMENTS AND THE RESPONSE TO IRRADIATION

Before any photochemical reaction can take place radiant energy must be absorbed. Some preliminary work was done in an endeavor to find out what pigment or pigment system was concerned in the absorption of the visible radiant energy. One method of attacking this problem was to use albino seedlings. Strains of albino beans or other dicotyledonous plants were not available, but a number of albino strains of maize were available and one of these was used.

Figure 2 shows the results obtained. The seedling on the left of each pair was an albino, that on the right was a normal seedling containing chlorophyll from the same parentage. The albino seedlings were completely white, indicating that they were free of apparent quantities of carotenoids as well as of chlorophyll. Both the albino and the chlorophyllous seedlings responded alike to the irradiation. Large amounts of seed were not available and only one fourth of the seedlings were albino so that only a few plants were available for comparison, but the results were clear enough.

The albino plants could respond to irradiation and responded to the same degree as their chlorophyll-containing sibs. No extractions were made of the albino maize seedlings to find out whether there were pigments present that could not be seen by casual observation.

The results obtained here do not specifically exclude chlorophyll or related substances from consideration as the radiant energy absorbing pigments, but they do show that if these are the pigments that absorb the radiant energy and initiate the response, the quantity of energy that is absorbed by the pigment is very small compared to the quantity that is incident on the plant surface.

FIG. 2. Albino maize seedlings at the left of each pair except the third where the normal green seedling only is shown. The treatments per pair are left to right: 100 ergs/cm.²/sec. continuously; 30 ergs/cm.²/sec. continuously; 10 ergs/cm.²/sec. continuously; and non-irradiated.

THE EFFECT OF TEMPERATURE AT THE TIME OF IRRADIATION

TRUMPF concluded that temperature had no effect on the morphological response to visible radiant energy. Several experiments were conducted to repeat and extend his work.

Several lots of bean seedlings were grown at 21° C. continuously until they began to appear above the surface of the gravel. One lot was irradiated daily before being kept at 1° C. for 0.5 hour; another was irradiated daily at the time the plants were being kept at a temperature of 1° C. Still another lot was not irradiated at any time but was kept at the temperature of 1° C. for 0.5 hour daily just to see what effect the low temperature had on general growth. Two other lots were grown at 21° C. continuously, one was irradiated and the other not. The results are shown in table VIII.

TABLE VIII

EFFECT OF TEMPERATURE AT TIME OF IRRADIATION

TREATMENT	NO. PLANTS PER PLOT	AV. LENGTH HYPOCOTYL	AV. LENGTH FIRST INTERNODE	AV. LENGTH PETIOLE	AV. LENGTH × WIDTH BLADE
		cm.	*cm.*	*cm.*	*sq. cm.*
2500 H 15 min. daily during 0.5 hr. at 1° C.	33	18.2	11.4	4.7	23.5
2500 H 15 min. daily at 21° C.	24	18.1	11.3	4.8	23.0
2500 H 15 min. daily 21° C. continuously	35	20.3	11.8	5.8	25.5
Non-irradiated but 0.5 hr. at 1° C. daily	20	28.9	12.2	......	....
Non-irradiated 21° C. continuously	20	29.6	13.3	......	....

There were no significant differences either in the type or the magnitude of the response between the beans irradiated at 1° C. and those irradiated at 21° C. provided both lots were kept 0.5 hour at 1° C. some time daily. The low temperature had a stunting effect as can be seen by comparing plants that were maintained at a constant temperature of 21° C. with those that received 1° C. for 0.5 hour daily. This stunting was evident whether the plants were irradiated or not. The slowing down of growth by the short exposures to low temperature apparently had no direct bearing on the mechanism of the response to irradiation since it occurred on irradiated and non-irradiated plants alike, and since the response to irradiation was not altered when the plants were irradiated at 1° C. or at 21° C. provided both were given the temperature of 1° C. at some time. These results confirm TRUMPF's work. The Q_{10} of the limiting reaction at the time the plants are being irradiated is unity or very close to unity.

LOCALIZATION OF THE RESPONSE TO IRRADIATION

Numerous experiments were conducted to see if any special part of the plant acted as a perceptor of the radiant energy or if each irradiated part of the plant acted separately as a perceptor of the irradiance. TRUMPF and WENT as a result of their work have come to the conclusion that each part of the plant acts separately.

Red kidney bean and pea variety Alaska were used as test plants. The first experiments were carried on at low irradiance, but the responses were small and inconclusive. Later experiments were carried on at higher irradiances, but extreme care had to be taken to prevent leakage of radiant energy.

In the early experiments the part of the plant that was not to be irradiated was wrapped in metal foil that was painted a dull black on both surfaces. This black wrapping was put on just before irradiation and removed after irradiation.

In later experiments with beans the part of the plant to be shielded from the radiant energy was slipped into a small cylindrical pasteboard carton with a removable lid. The slit in the lid was sealed up with black tape and the hole where the stem came through was sealed with modeling clay.

Most of the data from the localization experiments show that these plants had longer hypocotyls than those of the plants in the other experiments. This was unavoidable because the irradiation procedure could not be carried on until the epicotyl and leaves had developed so that they were large enough to work with. This was usually two or three days after the plants had emerged above the surface of the gravel. The hypocotyl had elongated a great deal by this time.

TABLE IX

LOCALIZATION, IRRADIANCE 5000 H 15 MINUTES DAILY FOR LAST FOUR DAYS

TREATMENT	NO. PLANTS PER PLOT	AV. LENGTH HYPOCOTYL	AV. LENGTH FIRST INTERNODE	AV. LENGTH PETIOLE	AV. FRESH WEIGHT BLADE
		cm.	*cm.*	*cm.*	*gm.*
Non-irradiated	39	29.9	11.5	0.6	0.11
Hypocotyl only irradiated	8	30.2	12.5	0.8	0.13
Epicotyl only irradiated	9	28.7	13.2	1.3	0.14

TABLE X

LOCALIZATION, IRRADIANCE 5000 H 15 MINUTES DAILY FOR LAST THREE DAYS

TREATMENT	NO. PLANTS PER PLOT	AV. LENGTH HYPOCOTYL	AV. LENGTH FIRST INTERNODE	AV. LENGTH PETIOLE	AV. LENGTH × WIDTH BLADE
		cm.	*cm.*	*cm.*	*sq. cm.*
Entire epicotyl irradiated	15	31	12	2.9	8.1
Growing point only irradiated	12	31	12	2.5	7.0
Leaves only irradiated	13	31	13	1.6	4.9
Non-irradiated	29	32	13	......	......

Because of the individual treatment given each plant daily and because of the laborious technique involved, relatively few plants were used in any one experiment, but the experiments were repeated a number of times. Tables IX and X give the results of several typical experiments. Figure 3 illustrates table IX.

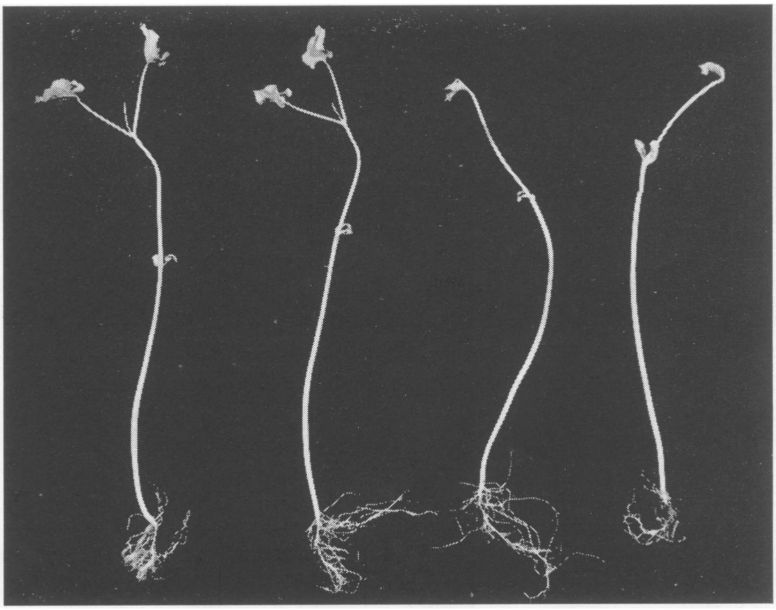

FIG. 3. Effect of localized irradiation of 5,000 ergs/cm.^{2}/sec. for 15 minutes daily. Left to right, entire plant irradiated; epicotyl irradiated; hypocotyl irradiated; and non-irradiated.

It was apparent that the hypocotyl of kidney bean was very insensitive to radiant energy. The cotyledons and the first internode also were not very sensitive. Most of the sensitivity of the kidney bean seedling to long wavelength visible radiant energy was confined to the young leaves and the growing point. Both seemed to act as effective perceptors, but the growing point appeared to be the most effective perceptor since a marked response resulted when it was irradiated even though its surface area was far smaller than that of the expanding leaves.

No conclusive results were obtained to indicate that the response was completely localized, that is to say that only that part which was irradiated responded to the irradiation. Adjacent parts seemed to be affected to some degree. The various plant parts did differ in their sensitivity to irradiation.

The removal of some organs of a non-irradiated plant has been found in some cases (**3, 5**) to have caused the remaining organs to develop differently than they otherwise would. To test this an experiment was conducted with kidney bean seedlings irradiated with 2,500 ergs/cm.^{2}/sec. for 15 minutes daily. As soon as the plants appeared above the gravel the cotyledons of some plants were removed and the epicotyl of others. One day later, as soon as they had developed enough to work with, the leaves were removed from still another lot of seedlings. Check lots, both irradiated and non-irradiated, were left. The results are given in table XI.

TABLE XI

LOCALIZATION, ORGANS REMOVED, IRRADIANCE 2500 H 15 MINUTES DAILY

TREATMENT	No. PLANTS PER PLOT	Av. LENGTH HYPO-COTYL	Av. LENGTH FIRST INTERNODE	Av. LENGTH SECOND INTERNODE	Av. LENGTH PETIOLE	Av. LENGTH X WIDTH BLADE
		cm.	*cm.*	*cm.*	*cm.*	*sq. cm.*
Non-irradiated	23	21.0	16.7	......	......	....
Entire plant irradiated	29	12.9	12.3	2.6	4.4	14.8
Leaves removed	18	12.6	14.0	3.3	......	....
Epicotyl removed	23	12.2	....	......	......	....
Cotyledons removed	13	10.9	8.2	0.2	1.3	2.9

The removal of an organ had three probable effects. Firstly, it relieved the remaining organs from the competition of the missing organ for the stored foods in the cotyledons, or in the case of the removal of the cotyledons it removed the main source of stored food itself. Secondly, perceptors of radiant energy were removed which had effects beyond the organ removed. Thirdly, the wounding may have caused unpredictable traumatic effects.

The removal of the cotyledons stunted the plant, but did not greatly change the qualitative response to irradiation. That is, the plant without cotyledons resembled the unmutilated plant, but was simply smaller in all dimensions.

Removal of the epicotyl seemed to have little effect on the development of the hypocotyl. It was slightly smaller than the checks. Apparently the hypocotyl and cotyledons were sufficiently sensitive to irradiation to produce a response similar to that obtained when the entire plant was irradiated. Non-irradiated plants in which the epicotyl was removed soon after emergence seemed to have about the same length hypocotyls as normal non-irradiated plants.

Removal of the developing leaves had little effect on the hypocotyl, but seemed to favor the development of the first and second internodes. This is the only evidence that the removal of a competing organ favored the development of the remaining organs, but in this case a part was left behind which was very sensitive to radiant energy. The removal of the entire epicotyl removed the most effective perceptors. The removal of the leaves left the sensitive growing point intact. It is impossible to know whether the increased elongation of the first internode was simply a response to greater food supply, or was a response to an apparent lower effective irradiation brought about by a decrease in surface of perceptors of irradiation. The increased elongation of the second internode has usually been associated with more radiant energy, but again it may be due simply to a greater food supply.

GRAFTING EXPERIMENTS

A number of experiments were conducted in which a kidney bean seedling that had been irradiated for several days was grafted by the inarching technique to a non-irradiated bean seedling. The grafting operation was carried out in weak blue light since it was impossible to graft in total darkness. The radiant energy from a small wattage incandescent tungsten filament lamp was filtered through Corning glass number 511 which transmits the band 3,500 Å to 4,900 Å. Check plants showed no response when irradiated for the same period as the test plants.

Only a few grafts, of the many tried, were successful, but when they were successful, union took place in two or three days. Neither plant was irradiated after the graft was made. The graft union was made between hypocotyls in some cases and between epicotyls in others. Both plants developed normally according to the previous treatment; that is, they reacted exactly like plants that had been similarly treated but not grafted. There was no evidence to show that any material formed by the irradiation of the one plant and capable of causing growth changes crossed over the graft union and affected the plant that had not been irradiated. On the other hand nothing from the non-irradiated plant seemed to affect the growth of the irradiated plant. The grafting operation itself did not seem to stunt or alter growth.

AVENA CURVATURE ASSAYS OF HYPOCOTYLS OF IRRADIATED AND NON-IRRADIATED BEAN PLANTS

Emerging kidney bean seedlings were irradiated at 2,500 ergs/cm.^{2}/sec. for 15 minutes daily for five days. The hypocotyls of the irradiated plants and those of similar plants that had not been irradiated were still elongating at this time. Eleven hypocotyls from the irradiated plants averaged 19 cm. in length and weighed 30.8 grams. Six hypocotyls from the non-irradiated plants averaged 28 cm. in length and weighed 29.8 grams.

Approximately 30 grams of fresh hypocotyls cut in half lengthwise were immediately placed in 1,500 ml. of freshly prepared peroxide free ether and allowed to stand for 22 hours at 1° C. The ether was evaporated off in a steam bath and the extract taken up in a special agar preparation and diluted so that one ml. of the agar contained the extract of 10 grams of hypocotyls. Further dilutions were also made. The extract taken up in the agar was then tested for auxin activity on Avena coleoptiles in co-operation with LINK and EGGERS (**4**, **10**, **12**) and using their technique and equipment. Checks using several concentrations of pure synthetic indole acetic acid were also run at the same time. The degree of curvature with the indole acetic acid was linear with concentrations up to 40 γ per liter.

Two separate series were run with very similar results and the average of these is given in table XII. The data are presented both on the basis of the amount of Avena curvature when the extract of 10 grams of fresh hypocotyls was taken up in one ml. of agar and also on the basis of the amount of curvature that would result if one average hypocotyl were extracted and the entire extract taken up in one ml. of agar.

TABLE XII

AUXIN CONTENT OF HYPOCOTYLS

TREATMENT	NO. HYPOCOTYLS	AV. FRESH WEIGHT HYPOCOTYL	AV. LENGTH HYPOCOTYL	AVENA CURVATURE 10 GM. HYPOCOTYL/GM. AGAR	AVENA CURVATURE ONE HYPOCOTYL/ GM. AGAR
		gm.	*cm.*	*degrees*	*degrees*
Non-irradiated	12	5.1	27	6.8	3.5
2500 H 15 min.	21	2.8	20	14.7	4.1

On a fresh weight or length basis the hypocotyls of the irradiated plants contained much more ether extractable auxin than did the hypocotyls of the non-irradiated plants. When the results were calculated on the basis of the amount of ether extractable auxin per hypocotyl the differences were very small, but the hypocotyls of the irradiated plants still had slightly more auxin.

Discussion and conclusions

An increase in the quantity of radiant energy was always accompanied by an increased morphological response of the plants. The increase in amount of response with increase in quantity of energy was not linear, however, but gradually decreased and became smaller as the quantity of energy became larger and larger. It has been suggested that the response increases as the logarithm of the quantity of incident radiant energy. Such a simple relationship was not found.

The results given in tables III and IV show that high irradiances were more effective than low irradiances even though the total quantity of radiant energy was kept constant. These results are in contradiction to TRUMPF'S findings, but are in agreement with WENT's recent results. This departure from BUNSEN and ROSCOE's Law may be in part due to the masking effect of chlorophyll. Long exposures to low irradiances caused more chlorophyll to be formed than short exposures to high irradiances. Since chlorophyll absorbs radiant energy strongly in the region of the spectrum used it could act as a filter and decrease the effective irradiance beneath the plant surface. Table VII shows that measurable quantities of chlorophyll were formed under exposures as short as 15 minutes daily. Numerous observations showed that bean seedlings exposed for longer than 15 minutes daily greened much more than those given only 15 minutes daily. The latter appeared yellow in color, but the former were varying shades of yellowish green and green.

The plant response was greatest when the plants were irradiated twice daily. These results are similar to those found by WENT with peas. Irradiations less frequent than twice daily caused a lessening of the response even though the total quantity of radiant energy which the plant received during the experiment remained constant. As the number of irradiations increased over twice daily the amount of chlorophyll increased rapidly. The filtering effect of the chlorophyll and the consequent lower irradiance in the tissues may account for the decreased response of many plant parts under these conditions which were favorable to chlorophyll formation.

When the plants were irradiated less than twice daily the response decreased as the frequency of irradiation decreased. There is not enough information available to give a specific explanation of this phenomenon, though several general explanations may be found in the field of photochemistry.

The data show that the Q_{10} of the "light reaction" is as close to unity as the accuracy of this type of experiment would enable one to measure. If there were several photochemical reactions following one another rapidly in succession with chemical reactions in between, then during irradiation at high irradiances this entire chain of reactions would be slowed down to the rate of the slowest chemical reaction, especially at low temperatures since the rate of the chemical reactions would be decreased to one half or one third for every 10° C. temperature drop. The data indicate that the condition described above does not obtain and suggest that only one photochemical reaction is involved.

Previous published work indicated that only that part of the plant which is irradiated responds to the radiant energy. No clear evidence of complete localization of the response to radiant energy was found, though each part of the plant which was irradiated was capable of responding to radiant energy. The leaves near the tip and especially the growing point were the regions where the greatest overall response was obtained when they alone were irradiated. When the hypocotyl alone was irradiated, the response of any part of the plant except the hypocotyl was very small.

The grafting experiments gave no evidence that there was a substance formed in an irradiated bean seedling which would diffuse across a graft union in sufficient amounts to affect the growth of a non-irradiated bean seedling grafted to it or *vice versa.*

Albino maize seedlings which appeared to be free of chlorophyll and the carotenoid pigments responded to radiant energy very similarly to the normal seedlings of the same parentage. No extractions were made from the albino plants but it is probable that minute traces of pigments were present which could absorb the longer wavelengths of the visible spectrum.

The Avena curvature assay experiments were carried out specifically to find out whether a material causing Avena curvatures could be extracted and correlated with the quantity of radiant energy or the elongation of the internodes of kidney bean seedlings. In this study fresh, longitudinally split hypocotyls were extracted with cold, freshly prepared, peroxide free ether. There was evidence that some Avena curvature inhibitors were being extracted as well as auxin, since dilutions of one-tenth and one-fiftieth of that given in table XII gave curvatures greater than they would have been if the curvature response were as proportional to concentration as it was to indole acetic acid.

Ether extracts from kidney bean hypocotyls that were still elongating showed that variations in elongation of kidney bean hypocotyls due to irradiation with energy in the longer wavelengths of the visible spectrum are either negatively correlated with ether extractable auxin concentration as measured by Avena curvature or are non-correlated. The evidence obtained in this experiment does not indicate that auxin concentration alone can be used to explain the lessened elongation of hypocotyls of irradiated bean seedlings as compared to non-irradiated seedlings.

Summary

1. The amount of the morphological response of kidney bean seedlings increases at a decreasing rate with increases in incident radiant energy, but the amount of the response varies depending on the manner in which the energy is increased.

2. High irradiances cause more response than low irradiances even when the total radiant energy incident on the plants is the same.

3. The maximum morphological response is obtained when the plants are irradiated two or three times daily. Chlorophyll formation increases with increases in the number of cycles of irradiation up to 144 times daily.

4. Albino maize seedlings give the same morphological response to irradiation as do normal green maize seedlings.

5. The Q_{10} of the "light reaction" seems to be unity. The temperature at the time of irradiation does not in itself affect the magnitude of the response.

6. All parts of the plant can act to some extent as perceptors of radiant energy. Adjacent non-irradiated parts respond to some degree when any one part is irradiated. The leaves and especially the growing point are the most sensitive perceptors of radiant energy.

7. No effect is observed when an irradiated bean seedling is grafted to a non-irradiated seedling if both are not irradiated thereafter.

8. Ether extracts of hypocotyls of irradiated and non-irradiated bean seedlings indicate that both have about the same amount of auxin per hypocotyl, but the irradiated hypocotyls are smaller and the concentration of auxin in them is higher.

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