STUDIES ON THE MECHANISM OF STEM GROWTH INHIBITION BY VISIBLE RADIATION [1, 2]

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Applied gibberellic acid specifically reverses the light inhibition of etiolated stem growth in certain species (4, 5). It therefore appears reasonable to suggest that light regulates stem elongation in these plants through some effect (or effects) on the gibberellin metabolism of the plant. Results presented in the present paper provide further support for this hypothesis. The effect of light on the gibberellin metabolism might be mediated through 1 of 3 general mechanisms. First, light may decrease the synthesis of endogenous gibberellin; or, second, it may cause the destruction (or diversion) of the natural gibberellin. Finally, light might make the tissue less responsive to a given amount of gibberellin. The results presented in the present paper make possible a partial resolution of these alternatives.

METHODS AND MATERIALS

Morse's Progress # 9 (Ferry-Morse Seed Co., Los Angeles), a strongly dwarfed variety of *Pisum sativum* was used for these experiments. The seeds were germinated and grown in vermiculite in plastic cups and continuously subirrigated with deionized water. The plants were grown in a darkroom maintained at 25 ± 1° C and a green safelight was used for watering and experimental manipulations. All plants were treated with gibberellin solutions (as a 4 μl alcoholic drop) while in the dark. Immediately after treatment those plants which were to be irradiated were transferred to continuous red light (150 ergs·cm^{-2}·sec^{-1}). Other radiation treatments are described in the particular experiment. A band of red radiation was obtained by filtering the radiation from pink 40-watt G.E. fluorescent tubes through 0.01-inch red cellulose acetate. Far-red radiation was obtained by filtering the radiation from 300-watt incandescent bulbs through Corning red-purple ultrafilters and 0.01-inch red cellulose acetate. The energy was 450 ergs·cm^{-2}·sec^{-1} standardized at 730 mμ. The total height of the plants from the soil level to the stem apex was measured in all cases. All experiments reported were repeated 2 or more times with substantially the same results.

EXPERIMENTAL RESULTS

To determine the time-course of growth following a single irradiation, 6-day-old dwarf pea seedlings were exposed to red radiation (2,000 ergs·cm^{-2}·sec^{-1}) for various lengths of time and the height of the plants was measured daily. The results of a typical experiment are illustrated in figure 1. It may be seen that even 10-minutes of irradiation results in a marked inhibition of growth. It is noteworthy that the growth inhibition by light continues for a period of several days. After 4 to 5 days the growth rate of the irradiated plants returns to the dark-grown rate, but the growth rate never exceeds that of the dark-grown plants. The same time-relation appears

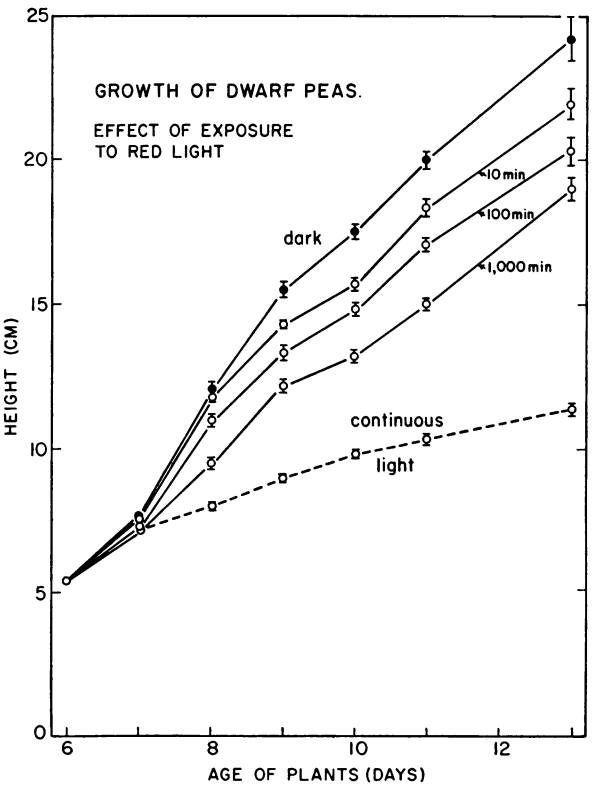

FIG. 1. Time-course of growth of dwarf pea seedlings following a single or continuous high intensity (2,000 ergs · cm^{-2} · sec^{-1}) red irradiation. The duration of the irradiation is indicated on the growth curves.

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to hold for the longer irradiations. Continuous irradiation results in a linear rate of growth much slower than the dark controls.

Far-red radiation will completely reverse the effects of brief red irradiation of the dwarf pea (table I). Plants were exposed to 15 minutes of red radiation (2,000 ergs·cm^{-2}·sec^{-1}) followed, in the cases indicated, by far-red radiation for 30 minutes. The time interval between the 2 irradiations was approximately 10 minutes. It is clear that these plants respond to red and far-red radiation, in terms of total growth, in a manner identical to the response of individual internodes of other species (2, 3).

In order to establish more critically the nature of the interaction between light and gibberellin, quantitative experiments were run comparing the response of the plants to added gibberellin in light and darkness.

Gibberellic acid treatment will prevent inhibition of pea stem growth by radiation (in both dwarf and tall varieties) and it will also reverse the dwarf character in peas as well as other species (1, 4, 8). These facts make it possible to compare directly the gibberellin sensitivity of irradiated dwarf plants with those grown in darkness. The results of a typical experiment are illustrated in figure 2 a. The inhibition as a result of irradiation is obvious at low doses or no gibberellin treatment. At saturating doses of gibberellin the plants attain the same maximum growth whether irradiated or grown in darkness.

In order to compare directly the response of irradiated plants with those maintained in darkness, growth promotion has been calculated as the percent of growth at a saturating dose of gibberellin. The grouped results of 4 separate experiments are illustrated in figure 2 b. In 2 of these experiments gibberellic acid (Eli Lilly and Co.) was used and in the other 2 gibberellin A (Merck and Co., Inc.) was used. No significant difference between the response to gibberellic acid and to gibberellin A was ever observed.

At saturating doses of gibberellin the irradiated and non-irradiated plants are found to be of equal height, while without gibberellin irradiation markedly

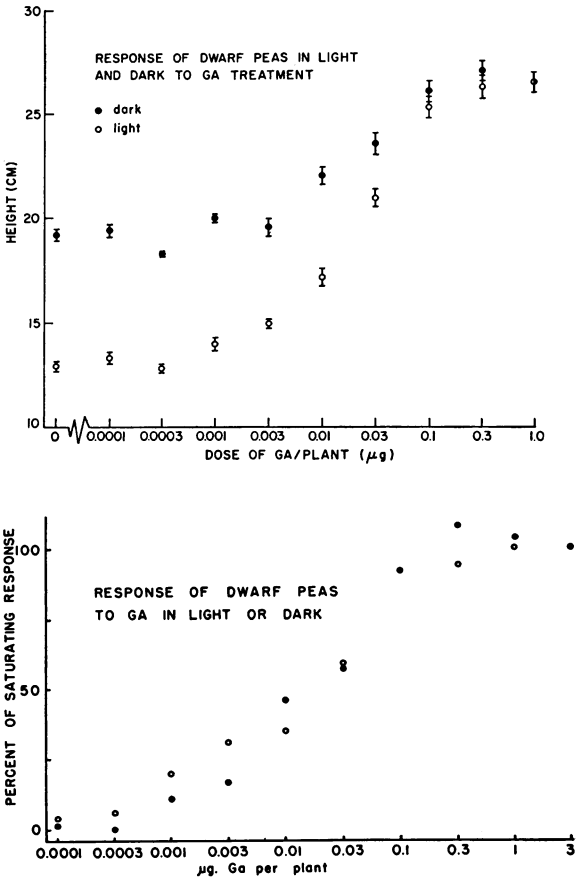

FIG. 2a (*top*). The height of red irradiated and dark-grown dwarf pea seedlings 4 days after treatment with gibberellic acid at the doses indicated.

FIG. 2b (*bottom*). The effect of gibberellin on growth in light (open circles) and darkness (closed circles). The abscissa represents the promotion of growth in each case as a percentage of the response to a saturating gibberellin treatment. Average of 4 separate experiments.

TABLE I

TIME-COURSE OF GROWTH OF DWARF PEA SEEDLINGS FOLLOWING A SINGLE IRRADIATION AS INDICATED BY THE TREATMENT *

TREATMENT	DAYS AFTER TREATMENT 0	1	2	3	4	TOTAL GROWTH
	height of plants (cm)					
Dark	4.0 ± 0.3	6.5 ± 0.3	10.6 ± 0.4	14.2 ± 0.3	17.4 ± 0.3	13.4
Red (15 min)**	4.1 ± 0.2	6.5 ± 0.2	9.9 ± 0.4	12.8 ± 0.4	15.6 ± 0.4	11.5
Red + far-red (30 min)	4.1 ± 0.3	6.0 ± 0.2	10.8 ± 0.3	14.1 ± 0.2	17.4 ± 0.3	13.3
Far-red (30 min)†	4.0 ± 0.2	6.4 ± 0.3	10.8 ± 0.4	14.3 ± 0.3	17.4 ± 0.2	13.4
Red (24 hr)‡	4.1 ± 0.2	5.7 ± 0.3	7.5 ± 0.2	10.2 ± 0.2	12.3 ± 0.4	8.2

* Also indicated is the standard deviation of the mean. The dark-grown seedlings were treated at the age of 6 days.

** Intensity 2,000 ergs · cm^{-2} · sec^{-1}.

† Intensity 450 ergs · cm^{-2} · sec^{-1}.

‡ Intensity 150 ergs · cm^{-2} · sec^{-1}.

inhibits growth. At intermediate doses of gibberellin, the height of the irradiated plants is less than in darkness (see fig 2 a), but the promotions of growth are equal fractions of maximum growth promotion.

Discussion

The results illustrated here indicate that the effective natural gibberellin is reduced as a result of irradiation. Successive increments of added gibberellin, then, give response curves in the 2 conditions (dark vs. irradiated) which approach a common maximum value.

If radiation was, in fact, acting on some growth process other than the gibberellin system, or if the radiation acted to reduce the number of active sites for gibberellin action, the growth at saturating doses of gibberellin could not be equal. Furthermore, if radiation acted to interfere with gibberellin action (e.g., causing a competition for active sites or reducing the affinity for active sites) then saturation would not occur at the same gibberellin dose, as is shown to occur. Irradiation results in an approximately 70 % reduction in growth rate under the conditions used here. If the radiation were acting, e.g., by reducing the affinity for gibberellin, then a 50 % reduction in growth rate might result from a 50 % reduction in affinity for the gibberellin, and 2 times as much gibberellin would have to be applied to reach maximum growth. The data are probably not sufficiently accurate to detect such a difference with certainty. However, it should be noted that the observed deviations from the theoretical results are, especially at low doses, in the opposite direction from that expected for an effect on affinity.

Evidence supporting the general view that it is the concentration of gibberellin which is affected by the radiation, rather than its action at the site of growth comes from the work of Idle (3). He has found that irradiation of the tip (in *Vicia faba*) is even more effective in the inhibition of growth than irradiation of the growing region. Furthermore, the effectiveness of irradiation of the tip persists far longer. Gibberellin has been shown to be produced in the stem tip of the pea seedling (6).

These results provide further evidence that irradiation reduces stem growth through a reduction in the level of the natural gibberellin of the plant. This is true for *Pisum sativum* and presumably for other species but in other cases, e.g., Phaseolus (6), the situation is clearly more complex.

In a paper published in 1941, Went reported the results of experiments in which dwarf peas were irradiated briefly with orange light (9). The time-course of growth was not followed, but the average length of each internode as well as total height was determined at the end of the experiment. The results showed that little or no inhibition of stem growth occurred as a result of a single brief irradiation, but the radiation did cause a substantial change in the relative lengths of the various internodes. Generally, that internode which was elongating at the time of irradiation was shorter and the internodes laid down subsequently were longer than in the dark control.

Went's results are subject to 2 different interpretations which could not be resolved at that time. It might be that the light causes a temporary reduction in growth which is later compensated by an increase in the growth rate. This would be reflected in the length of the various internodes. These same experimental results would be observed if the irradiation temporarily stimulated node formation but had no effect on stem elongation. The results presented in the present paper make it clear that this 2nd interpretation is the correct one. The response of these plants is, then, analogous to that of *Phaseolus vulgaris,* in this respect (e.g., (2, 3, 6)).

The present author has suggested in an earlier paper (4) that the endogenous gibberellin might be reversibly inactivated by light. This idea was proposed as the result of the 1st interpretation of Went's results in which it was assumed that brief irradiations resulted in a temporary decrease in growth rate followed by an increase to restore the original height. There is no longer a question of a reversible inactivation of the natural gibberellin. It appears clear that the natural gibberellin is either destroyed (or diverted) or its synthesis is inhibited in response to visible radiation.

It may be suggested that the effect of radiation is probably on gibberellin synthesis. This is indicated by the duration of the response of the plant to brief irradiations. The results reported here show that the effects of a single brief irradiation last as long as 4 or 5 days. Since the steady-state concentration of natural gibberellin in the plant is almost certainly very low (consider the extraction experiments which have been reported, and the fact that these experimental plants are gibberellin-limited dwarfs) it would appear highly unlikely that the long term effects observed could be due to a photo-destruction of the gibberellin present at any one time. Destruction of the gibberellin as a result of irradiation could only be envisaged if the radiation resulted in the establishment of a gibberellin-destroying system which would continue long after the radiation had been removed. This latter possibility cannot, of course, be ruled out.

It may be concluded that visible radiation affects stem growth in such plants as Pisum by reducing the natural gibberellin level, either by causing the formation of a gibberellin-destroying system or by retarding the synthetic processes which result in the formation of gibberellin.

It has been shown here that the red : far-red pigment system is effective in the inhibition of stem growth in this plant. The low intensities utilized for continuous irradiation presumably preclude participation by the "high intensity" pigment system of Mohr (7). Whether growth inhibition as a result of continuous low intensity irradiations is due exclusively to the red : far-red pigment system is not established. However, it is known that gibberellic acid

may prevent stem growth inhibition as a result of both low and high intensity continuous irradiation in this species (1, 4).

Summary

Exposure of a dwarf pea (Morse's Progress # 9) to a single brief irradiation with red light results in an inhibition of growth which persists for several days. After 4 or 5 days the plants resume growth at the dark-grown rate. Far-red irradiation will negate the red effect.

The effect of a series of gibberellin doses on the growth responses of irradiated or dark-grown peas has been determined. In the absence of applied gibberellin the growth of irradiated plants is inhibited compared to that in darkness. At saturating doses of gibberellin the irradiated and dark-grown plants are the same height. The proportionate responses of irradiated and dark-grown plants are equal at equal doses of gibberellin.

It is concluded that visible radiation probably inhibits stem growth in this species through an effect on the level of endogenous gibberellin.

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GROWTH RESPONSES OF ALASKA PEA SEEDLINGS TO VISIBLE RADIATION AND GIBBERELLIC ACID[1, 2]

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Inhibition of stem growth is a general response of etiolated plants to irradiation, but the mechanism of radiation action is still little understood. We know the action spectra for both the low energy (2, 12) and a high energy (8) inhibition of stem elongation. The effective pigments are, however, not yet identified. It has also been shown that the application of gibberellic acid will specifically reverse both low intensity and high intensity radiation inhibition in certain species (4, 6, 11). It was felt that a more detailed description of stem inhibition by visible radiation and its reversal by gibberellic acid in a single species might provide a background for a more complete and general understanding of these phenomena.

Materials and Methods

Seeds of *Pisum sativum*, variety Alaska (Ferry-Morse Seed Co., Los Angeles) were soaked in deionized water for 4 to 5 hours in darkness, then planted in vermiculite in plastic cups. The seedlings were grown in a dark room maintained at 25 ± 0.5 C. The pots were continuously subirrigated with deionized water. For the experiments reported here 5 uniform plants were selected per pot and at least 2 pots of plants were used for each experimental treatment. The experimental treatments were usually started when the seedlings were 4 days old, 3 to 4 cm above the vermiculite level.

The sources of radiation were the same as those previously described, i.e., colored fluorescent tubes filtered through colored cellulose acetate. Measurement of radiation intensity was with a calibrated photo-cell, also previously described (6). In the experiments reported here the red and blue radiation were never used simultaneously in the same room. The temperature under the lights was maintained at 25 ± 0.5° C, measured with a mercury thermometer.

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