pacities are discussed in relation to the in situ life history of the pollen.

The authors appreciate the aid of Dr. Duffield, formerly of the United States Forest Service, for help in obtaining the pollen and are indebted to Mr. W. Hirano of the Department of Botany, University of California at Berkeley for translating the Japanese papers by Okunuki.

Literature Cited

1. Buchholz, J. T. Suspension and early embryo of Pinus. Bot. Gaz. 66: 185–228. 1918.
2. Burk, D. A coloquial consideration of the Pasteur and neo-Pasteur effects. Cold Spring Harbor Symposia Quant. Biol. 7: 420–455. 1939.
3. Chamberlain, C. J. Gymnosperm Structure and Evolution. Pp. 1–484. University of Chicago Press, Chicago 1935.
4. Coulter, J. M. and Chamberlain, C. J. Morphology of Gymnosperms. Pp. 1–466. University of Chicago Press, Chicago 1917.
5. Doyle, J. and O'Leary, M. Pollination in Pinus. Sci. Proc. Roy. Dublin Soc. 21: 181–190. 1935.
6. Duffield, J. W. Techniques and possibilities for douglas-fir breeding. Jour. Forestry 48: 41–45. 1950.
7. Duffield, J. W. and Snow, A. G., Jr. Pollen longevity of *Pinus strobus* and *Pinus resinosa* as controlled by temperature and humidity. Amer. Jour. Bot. 28: 175–177. 1941.
8. Elser, E. and Ganzmüller, J. Die Chemische Zussamensetzung einigen Blütenstaubarten. Hoppe-Seyler's Zeit. physiol. Chem. 194: 21–32. 1931.
9. Ferguson, M. C. Contributions to the knowledge of the life history of Pinus with special reference to sporogenesis, the development of gametophytes and fertilization. Proc. Wash. Acad. Sci. 6: 1–202. 1904.
10. Giragossintz, G., Davidson, C. and Kirk, P. L. The micro determination of blood sugar by ceric sulfate titration. Mikrochemie 21: 21–34. 1936.
11. Hassid, W. Z. Determination of sugars in plants. Ind. Eng. Chem., Anal. Ed. 9: 228–229. 1937.
12. Hassid, W. Z., McCready, R. M. and Rosenfels, R. S. Determination of starch in plants. Ind. Eng. Chem., Anal. Ed. 12: 142–144. 1940.
13. Haupt, A. W. Oögenesis and fertilization in *Pinus Lambertiana* and *P. monophylla*. Bot. Gaz. 102: 482–498. 1941.
14. Hellmers, H. Metabolism in *Pinus ponderosa* pollen. Thesis, University of California, Berkeley 1950.
15. O'Kelley, J. C. External carbohydrates in growth and respiration of pollen tubes in vitro. Amer. Jour. Bot. 42: 322–327. 1955.
16. Okunuki, K. Über den Gaswechsel des Pollens. Bot. Mag. (Tokyo) 46: 701–721. 1932. (In Japanese.)
17. Okunuki, K. Über den Gaswechsel des Pollen von *Lilium auratum*, Lindl. Bot. Mag. (Tokyo) 47: 45–62. 1933. (In Japanese.)
18. Okunuki, K. Über den Gaswechsel der Pollen I. Acta Phytochimica 9: 267–285. 1937.
19. Okunuki, K. Über den Gaswechsel der Pollen II, III, IV. Acta phytochimica 11: 27–64, 65–80, 249–260. 1939.
20. Roe, J. H. A colorimetric method for the determination of fructose in blood and urine. Jour. Biol. Chem. 107: 15–22. 1934.
21. Umbreit, W. W., Burris, R. H. and Stauffer, J. F. Manometric Techniques and Related Methods for the Study of Tissue Metabolism. Pp. 1–203. Burgess Publishing Co., Minneapolis, Minnesota 1948.
22. Wilson, R. E. Humidity control by means of H_2SO_4 solutions. Jour. Ind. Eng. Chem. 13: 326–331. 1921.

RESPONSE OF THE HYPOCOTYL HOOK OF BEAN SEEDLINGS TO RADIANT ENERGY AND OTHER FACTORS[1,2,3]

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One of the characteristic morphogenic responses of dicotyledonous plants to visible radiant energy is the straightening or opening of the stem hook in the seedling stage. During germination in complete darkness, a hook develops in the hypocotyl which progressively moves into the epicotyl. In darkness this hook does not disappear up to the time the cotyledonary food reserves are exhausted in Black Valentine and Red Kidney bean seedlings. However, if the seedlings are exposed to low levels of red radiant energy, the hook opens completely within several days (5). This paper deals with the various factors which influence the opening of the excised hypocotyl hook of the bean seedling and its usefulness as a quantitative test organ for studies of the action spectrum and the kinetics of photomorphogenesis.

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Materials and Methods

Seedlings of *Phaseolus vulgaris*, var. Black Valentine, were grown in darkness in a subirrigated, gravel-culture chamber, in a constant condition room maintained at 25° C and 70 to 80 % relative humidity (5). No nutrient solution was used since tap water produced seedlings as uniform as any inorganic solution tried. The plants were harvested at 6 days from seeding and were selected for a uniform hook angle of

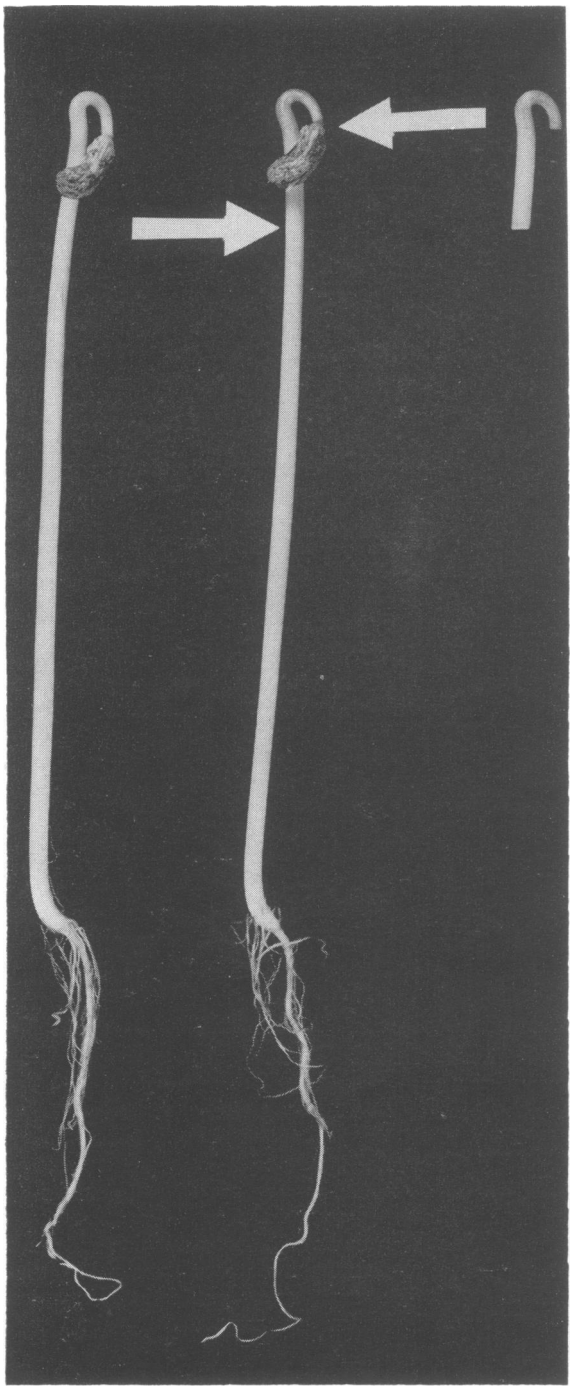


FIG. 1. Typical 6-day-old Black Valentine seedlings grown in gravel culture at 25° C. Arrows indicate the points at which the hook was excised.

zero degrees and a length of 15 ± 1 cm. Only those with the hypocotyl doubled back and paralleled to itself (zero degree angle), as shown in figure 1, were used. The upper 2.5-cm section of the hypocotyl was excised and the terminal portion, including cotyledons,

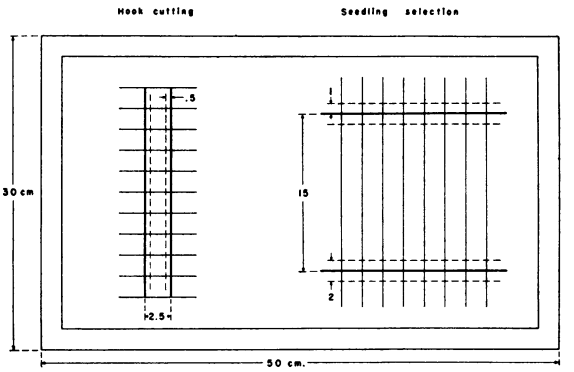


FIG. 2. Cutting template used over the green safelight for selecting bean seedlings and excising the hook.

leaves and buds, was removed unless otherwise noted. The length of the shorter segment of the hook was 0.5 cm.

The seedlings were selected and cut on a green safelight unit consisting of two 15-watt green fluorescent lamps mounted in a box faced with two panes of glass, between which were a green gelatin filter and two diffusing sheets of tracing paper. The seedlings were selected on the right hand grid and excised on the left hand grid of the template shown in figure 2. The grids were made symmetrical so as to permit working from either side of the safelight table. The filter transmitted between 500 and 550 mμ, with a peak at 525 mμ; the illumination was about 0.1 fc. An exposure of 60 minutes to the safelight unit produced no measurable opening of the hook from 6-day-old seedlings. In most cases the actual exposure time was less than 3 minutes. The safelight unit and the formulation of the green filter are to be described in detail elsewhere.

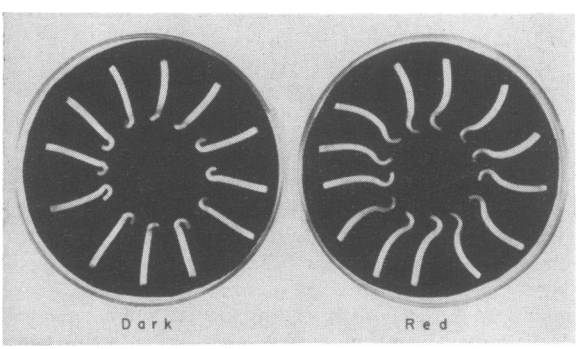


FIG. 3. Dark and irradiated hooks at the end of a 20-hr development period at 25° C. The angles of the control hooks kept in complete darkness are unchanged, although the hypocotyl developed an upward geotropic curvature, causing some of the hooks to bend away from the paper surface. Those on the right were exposed continuously for a period of 20 hrs to 0.1 μw/cm² of red energy 625 to 700 mμ. The hooks opened about 65°. For experimental purposes the hooks were placed on white filter paper; the black paper shown was used only for photographing.

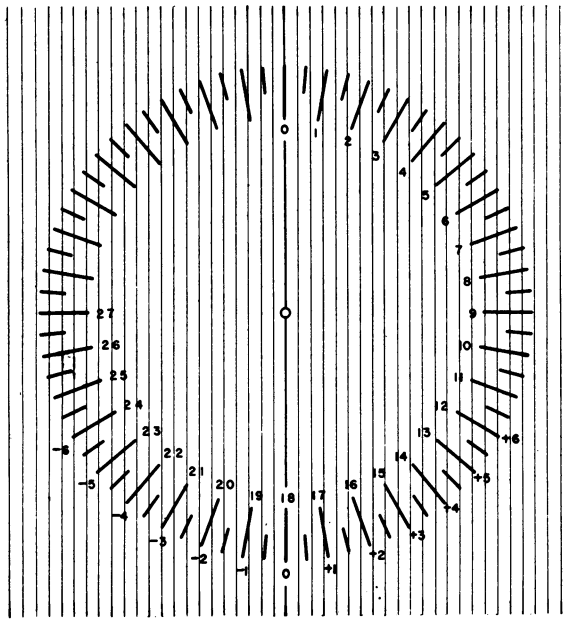


FIG. 4. Hook-measuring protractor indexed in 5° units. The upper part of the long leg of the hook was placed parallel to the line grid and moved across the grid until the short portion was parallel to one of the angles indicated. The data were recorded in terms of the 5° units of angle. By subtracting the recorded angles from 180°, the plus (opening) and minus (closing) values were determined.

Fifteen to 20 hooks were placed on filter paper in 15-cm Petri dishes containing 35 ml of distilled water. Typical dark and red-irradiated hooks at the end of a 20-hour development period are shown in figure 3. All hook angles were measured with the special circular protractor, indexed in 5° units (fig 4). In all tabulated data, a completely opened hook is indicated by a value of 180°, whereas a zero-degree angle represents the initial condition. In order to measure a hook angle, the upper portion of the longer leg of the hook was kept parallel to the line grid and moved across the protractor until the short leg was parallel to one of the radial lines. The average protractor readings multiplied by 10 for each dish were then subtracted from 180° to obtain the hook angle. This procedure eliminated the need of working with plus and minus values of angles when recording data. The tabulated positive angles indicate hook opening; negative values indicate hook closing.

Schneider (4) and Jacobs (3) have reported that additions of sugar to the media were either ineffective or inhibitory to growth of Avena-mescotyl and bean-hypocotyl sections respectively; these results are confirmed in so far as the opening of the bean hypocotyl is concerned. Buffers, mineral nutrients and combinations of them decreased the photoresponsivity, as compared to distilled water. Therefore, distilled water was used as the medium in all experiments reported here except where low concentrations of test substances were added.

RESULTS AND DISCUSSION

EFFECT OF TERMINAL ORGANS: The effect of the terminal organs (bud, leaves and cotyledons) on the hook response was obtained by placing dishes of hooks, with various combinations of organs present, in complete darkness and exposing to red energy (625 to 700 mμ) of 1.0 μw/cm^2. The results given in table I are the averaged data for two independent experiments. The responsivity of the hook to red energy is markedly reduced by the presence of any of the terminal organs. The smallest opening response was obtained with an intact hook containing bud, leaves and cotyledons, and the maximum response was obtained when all terminal organs were removed. The cotyledons were more active than the bud and leaves in inhibiting the hook opening. The presence of the cotyledons in complete darkness produced an appreciable closure of the hook as evidenced by the negative angles of opening. These results indicate that the terminal organs, particularly the cotyledons, provide a diffusible substance which causes the hook to tend to close. This response is opposite to that of the photoreaction. It will be shown later in this discussion that additions of low concentrations of indoleacetic acid to the culture medium produce an effect similar to that of the terminal organs. It can be speculated that these organs supply a diffusible auxin to the hook, which reduces its responsivity to red energy.

AGE OF SEEDLING: Hooks were excised from seedlings of 4, 5, 6 and 7 days of age to determine the most suitable stage for testing. Table II gives the results of the averages of three dishes for each age. The responsivity to red energy in terms of rate of opening per unit of incident energy increased with age. As the seedlings became older, the hook moved up the stem and by 7 days some of the hooks were in the epicotyl. Therefore, in all subsequent experiments, 6-day-old seedlings grown at 25° C were used to provide excised hypocotyl hooks.

TIME-COURSE OF HOOK OPENING: The time-course of the hook opening was determined by placing 10

TABLE I

EFFECT OF TERMINAL ORGANS ON THE ANGULAR RESPONSES OF THE HYPOCOTYL HOOK OF SIX-DAY-OLD BEAN SEEDLINGS

ORGANS PRESENT	ANGLE OF OPENING IN DEGREES *	
	DARK	RED **
Hook + bud, leaves and cotyledons	0	6
Hook + cotyledons	−7	8
Hook + bud and leaves	−2	26
Hook + bud	0	58
Hook alone	0	83

* Averaged data for 40 hooks (2 replications). Development time: 20 hours, 25° C.

** Red irradiance of 1.0 μw/cm^2, 625 to 700 mμ.

TABLE II

RESPONSE OF THE HYPOCOTYL HOOK OF BEAN AS A FUNCTION OF SEEDLING AGE *

AGE IN DAYS	NO. OF REPLICATIONS	ANGLE OF OPENING IN DEGREES **
4	2	40
5	4	69 ± 6
6	4	84 ± 3
7	2	96

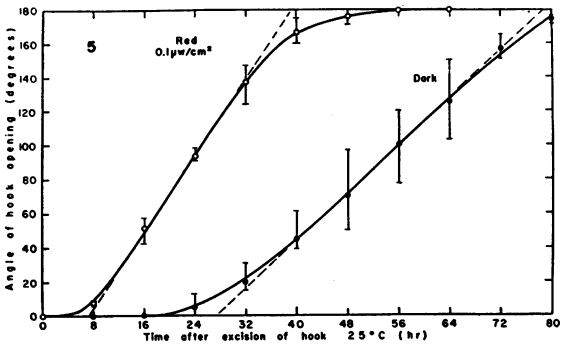
* Red irradiance of 1.0 μw/cm^2, 625 to 700 mμ; development period, 20 hours.

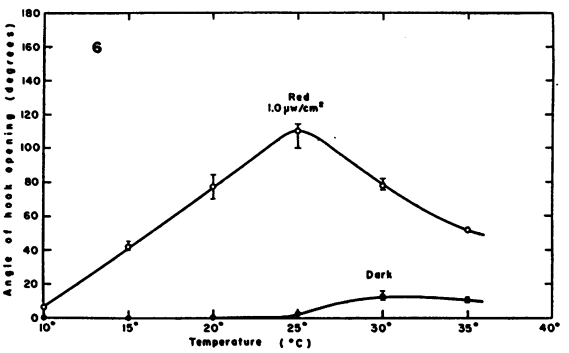
** Standard error is given only for the 5- and 6-day hooks where 4 replications of 20 hooks each were used.

dishes in a dark cabinet and exposing 10 others to a red irradiance of 0.1 μw/cm^2. At intervals of 8 hours, one dish each was removed from the dark and red cabinets and the hook angles measured. The data plotted in figure 5 show that in complete darkness there was a lag period of from 16 to 24 hours before hook opening began. Thereafter, the unhooking proceeded at a relatively constant rate, reaching approximately 180° after about 80 hours. For those hooks exposed to the red, the lag phase was much shorter, being about 4 hours, and the rate of unhooking was considerably greater as evidenced by a steeper slope of the time-course curve. Red energy markedly decreased the length of the lag phase and increased the rate of opening.

TEMPERATURE: The temperature response is given graphically in figure 6. The data for these curves were obtained by allowing the hooks to develop for a period of 24 hours in either complete darkness or exposed continuously to a red irradiance of 1 μw/cm^2 at a series of six temperatures, ranging from 10° to 35° C. Three replications were made at each temperature and the average data plotted; the vertical bars show the range of variation between the maximum and minimum results of all experiments performed. A 24-hour development period permitted the hooks in complete darkness to open to a measurable angle. The optimum temperature for the red-irradiated hooks was 25° C, while the optimum for those kept in complete darkness was 30° C. On the basis of these data, a constant temperature of 25° C was used in all subsequent experiments.

RED IRRADIANCE: The response to incident red irradiance was determined by exposing hooks to various irradiances for a period of 20 hours. Two sets of data are shown in figure 7; one determined in 1953 and a second determined in 1954 with a new crop of seed. The results are plotted on a log scale of irradiance. The angle of opening was proportional to the logarithm of the red irradiance (625 to 700 mμ) over the range of 0.001 to 10 μw/cm^2. The data for the two separate years were quantitatively consistent. This was one measure of the reproducibility of the hook response in the Black Valentine bean. A second test of reproducibility was established by repeating identical treatments as parts of other experiments





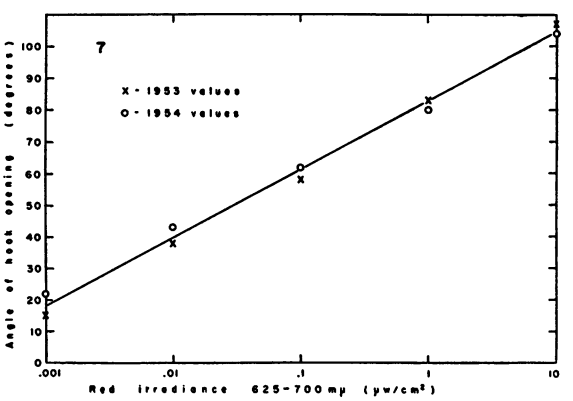


FIG. 5. Time-course of opening of excised hypocotyl hooks in complete darkness and exposed continuously to red energy of 0.1 μw/cm^2 at 25° C. The vertical bars indicate the range of maximum and minimum variations of all experiments on time-course. The hooks kept in complete darkness did not begin to open until about 20 hrs from the time of excision. The red-exposed hooks began to open at about 4 hrs.

FIG. 6. Temperature response of dark and red-irradiated hooks (625 to 700 mμ, 1.0 μw/cm^2). The development period was extended to 24 hrs in order to obtain appreciable opening of the dark hooks.

FIG. 7. Effect of red irradiance on the hook opening response. The data for the crosses were determined in 1953 and those for the circles in 1954. The angle of opening is approximately proportional to the logarithm of the irradiance.

over a period of 4 weeks. During this time a total of 400 hooks were treated with red energy at 2 μw/cm^2. The average response was 111°, with a standard error of the mean of $\pm$ 3°.

Hooks exposed for a few minutes of high intensity red energy and then allowed to develop in darkness for 20 hours open in the same manner as when exposed to continuous red energy. However, if, following the red exposure, the hooks are exposed to far-red energy, the effect of the first treatment is almost completely nullified. This can be repeated several times with the ultimate response determined by the last exposure. This promotion and blocking or reversal process is similar to that obtained with the germination of lettuce seed (1).

Auxins and Red Energy: The relationship between 3-indoleacetic acid (IAA) and red energy is presented graphically in figure 8 for concentrations of IAA from 10^{-8} to 10^{-5} M and red irradiance (625 to 700 mμ) from 10^{-3} to 10 μw/cm^2. In complete darkness, IAA caused closure of the hook, which increased in magnitude with concentration. All concentrations of IAA decreased the resultant effect of the photoreaction. This is consistent with the findings of Galston (2) that the sensitivity of the pea epicotyl to IAA was reduced by preirradiation of the seedling with red energy. Low concentrations of IAA between 10^{-8} and 10^{-6} M do not alter either the linearity of the opening response to the log of irradiance or the slopes of the curves above a 10° opening value. Below a 5 to 10° angle of opening, the response is not a linear function of the log of irradiance or energy. Since the two stimuli are opposing in the hook-opening response, each value of hook angle can be obtained by an infinite series of combinations of IAA concentration and irradiance. The excised hypocotyl hook can be used as an auxin assay by placing the hooks in the unknown extract of auxin and exposing to red energy of about 10 μw/cm^2. Under these conditions the auxin concentration expressed as moles per liter of IAA can be evaluated from the reduction in hook angle as compared to a water control. Concentrations as low as 10^{-9} M can be detected by this assay.

Summary

A method for the quantitative measurement of the photomorphogenic response of plants is presented, using the hypocotyl hook of bean seedlings. Varia-

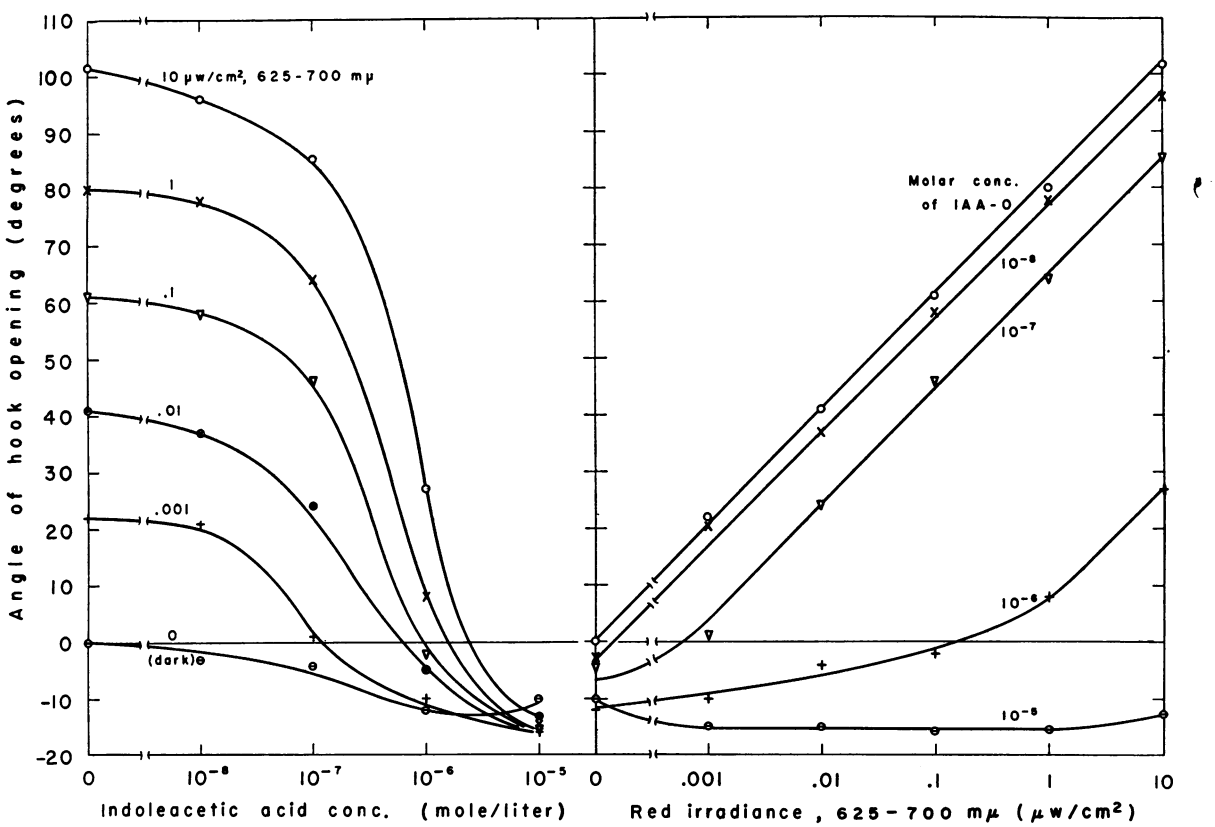


Fig. 8. Effect of various combinations of red irradiance and IAA concentrations on the opening of the bean hook. The family of curves on the left present the angle of hook opening as a function of the logarithm of the molar concentration of IAA for various red irradiances. Curves on the right present the same data plotted with hook opening as a function of red irradiance for various IAA concentrations. The hooks were exposed to the red irradiance and IAA concentrations for the complete 20-hr development period.

bility is small and the response to red radiant energy is very reproducible. The material is easy to handle and manipulate. The assay is reliable between 10^{-3} $\mu w/cm^2$ and 10 $\mu w/cm^2$ of red (625 to 700 mμ) and the photoresponse is directly proportional to the logarithm of the irradiance.

The technic consists of excision of the hook from 6-day-old seedlings of Black Valentine bean and exposure to red energy (625 to 700 mμ). The angle of hook opening is a quantitative measure of the photomorphogenic effect. The presence of bud, leaves or cotyledons, particularly the latter, causes an effect opposing the photoreaction, as does also 3-indoleacetic acid.

The photoresponse increases with age of the seedling up to seven days, but at this age the hook has progressed up the stem close to the cotyledonary node; therefore, 6-day-old seedlings were found most suitable. In complete darkness no opening could be observed for 16 to 24 hours following excision; with red energy, the lag period was 4 hours and the subsequent opening was at a markedly increased rate as compared to the dark. The maximum opening in the red occurred at 25° C; in the dark at 30° C.

Literature Cited

1. Borthwick, H. A., Hendricks, S. B., Toole, E. H. and Toole, V. K. Action of light on lettuce-seed germination. Bot. Gaz. 115: 205–225. 1954.
2. Galston, Arthur W. and Baker, Rosamond S. Studies on the physiology of light action. V. Photo inductive alteration of auxin metabolism in etiolated peas. Amer. Jour. Bot. 40: 512–516. 1953.
3. Jacobs, W. P. Control of elongation in the bean hypocotyl by the ability of the hypocotyl tip to transport auxin. Amer. Jour. Bot. 37: 551–554. 1950.
4. Schneider, Charles L. The effect of red light on growth of the Avena seedling with special reference to the first internode. Amer. Jour. Bot. 28: 878–886. 1941.
5. Withrow, R. B., Klein, W. H., Price, L. and Elstad, V. Influence of visible and near infrared radiant energy on organ development and pigment synthesis in bean and corn. Plant Physiol. 28: 1–14. 1953.

SOME FACTORS AFFECTING ABSORPTION AND TRANSLOCATION OF ZINC IN CITRUS PLANTS[1,2]

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Zinc deficiency occurs almost universally in citrus plants. In its more severe stages this disorder seriously reduces tree growth and fruit production. Soil treatments have not been altogether successful in solving this problem. Foliar sprays of zinc are commonly used in the form of oxide, sulfate, hydroxide, or carbonate, materials which are frequently incorporated in sprays applied for pest control purposes. Variability of results raises a number of questions concerning factors affecting absorption and translocation of zinc. The experiments reported here served to identify some of these factors and suggest procedures for additional experiments. The variables studied were the following: 1) placement of material on leaves, 2) leaf age, 3) concentration of zinc used, 4) leaf vs. root application, and 5) amount and chemical form of zinc applied to soil.

Materials and Methods

In these studies the radioactive isotope zinc-65 was used as a tracer in following the movement of zinc in citrus plants. At the time of shipment the solution contained 3.78 mg Zn per mc, in the form of $ZnCl_2$. In this report, the symbol Zn* is used to denote a mixture of Zn^{65} with the naturally occurring isotopes. Concentrations of Zn^{65} are expressed in terms of activity at the time of shipment. The experiments covered a span of approximately one half-life (250 days).

Elapsed time between treatment and harvest is indicated in the description of each experiment.

Three types of plants were used: 1) 3-year-old navel orange trees growing out-of-doors in solution cultures; 2) Koethen sweet orange seedlings, 15 to 25 cm tall, grown in the greenhouse in solution cultures or in pots of soil; 3) rooted cuttings of Eureka lemon, 15 to 25 cm tall, grown in the greenhouse in solution cultures.

Except where otherwise noted, nutrient solutions were maintained in the pH range 4.0 to 4.5. Their composition was the same as that reported for previous studies (3). All plants were adequately supplied with zinc prior to the time of treatment.

Preparation of Plant Material for Analysis: In a preliminary study it was found, in agreement with Smith et al (1), that a solution of Dreft detergent acidified to 0.3 *N* with HCl was more effective in removing zinc applied to the leaf surface than was Ivory soap or a 10 % solution of sodium ethylene diamine tetra-acetate (NaEDTA) adjusted to pH 5.

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[2] Paper No. 903, University of California Citrus Experiment Station, Riverside, California.
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