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TECHNICAL MANUSCRIPT 614

RED LIGHT AND ETHYLENE EFFECTS ON BEAN HYPOCOTYL UNHOOKING

David M. Peterson
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MAY 1970

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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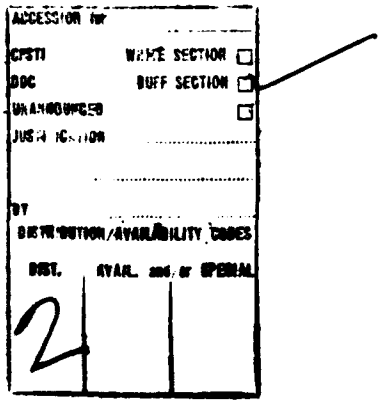

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PLANT SCIENCES LABORATORIES

Project 1B562602AD04

May 1970

ABSTRACT

Red light promotes the opening of the hypocotyl hook of *Phaseolus vulgaris* L. var. Black Valentine independently from its effect on decreasing ethylene production. This was shown by comparing red-light dose-response curves for hook opening and ethylene production. Also, for a given rate of ethylene production, achieved by treatment with indoleacetic acid, hooks treated with red light opened more than the controls.

Ethylene induces hook closing, and this effect is enhanced by prior red-light treatment. Ethylene does not affect phytochrome transformation, but acts in some way to prevent subsequent growth of the inner hook cells. There is no effect of red light or ethylene on the lateral transport of indoleacetic acid in the hook region.

I. INTRODUCTION*

The opening of the bean hypocotyl hook in response to red-light exposure and its inhibition by indoleacetic acid (IAA)[1,2] have recently been postulated to be under the control of ethylene.[3,4] Red light, which promotes hook opening, inhibits the production of ethylene by the tissue.[3-5] Conversely, IAA, an inhibitor of hook opening, stimulates ethylene production.[6-8] Moreover, hook opening is inhibited if hypocotyls are placed in an atmosphere containing 0.01 ppm or more of ethylene.[4] A similar relationship between red light and ethylene was shown for straightening of the plumular hook of etiolated pea seedlings.[5]

We have investigated this problem further, and on the basis of results presented below conclude that red-light promotion of bean hypocotyl hook opening is independent of its effect on decreasing the rate of ethylene production.

II. MATERIALS AND METHODS

Phaseolus vulgaris var. Black Valentine seeds were soaked 8 hours in running tap water and planted in vermiculite. They were grown 6 days in the dark at 25 C without additional watering. Standard hooks with a 2.5-cm shank as shown in Figure 1A[2] were excised from seedlings 14 to 18 cm long and placed upright in 50-ml Erlenmeyer flasks, supported by 12 ml of 1.2% agar. All manipulations were performed under a dim green safelight, which was shown to be photomorphogenically inert. The red light source consisted of a 100-watt incandescent bulb behind a Corning C.S. 2-60 glass filter and a 4-cm water heat trap. Energy at the level of the hooks was 3,000 ergs/cm^2 per sec. When hook angles were to be measured, the flasks were left open. Shadowgraphs were made of the hypocotyl hooks, usually 21 hours after light treatment, for subsequent measurement of the hook angles. For ethylene determinations, the flasks were closed with vaccine caps. Samples of the gas phase were withdrawn 21 hours later with a syringe for analysis by gas chromatography.[7] Ethylene production was expressed on the basis of dry weight of tissue. The dry weight averaged 6.5% of the fresh weight. Hypocotyls were treated with auxin by injecting 1 µl of various concentrations of IAA into the apical end of the standard hook with a microsyringe. Hypocotyls were treated with ethylene by placing the flasks in 10-liter desiccators[9] into which the appropriate concentration of ethylene was injected.

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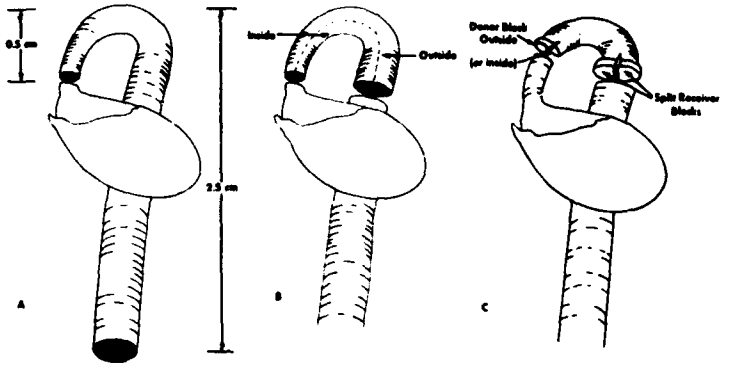

FIGURE 1. Portions of the Hypocotyls Used for Various Experiments. A) The standard hook, used for all experiments on hook angle measurement and most experiments measuring ethylene production. B) Separation of curved region into inside and outside portions for determination of ethylene production. C) Placement of donor and receiver agar blocks for experiments to measure transport of radioactive IAA.

To determine the effect of red light on auxin transport, (1-C^{14})-IAA, specific activity 25.6 mc/mmole, was supplied unilaterally to the apical end of segments from the hook region at 5 x 10^{-6} M in 1.5% agar blocks containing 2% sucrose.[10] This concentration was the lowest that would yield sufficient counts in the receiver blocks. This concentration of IAA caused an 80% stimulation of ethylene production and a 20% reduction in hook opening. For experiments on the effect of ethylene on lateral transport, (5-H^3)-IAA, 16 c/mmole, was used at 1 x 10^{-7} M. This concentration had no effect on ethylene production or hook opening. Split receiver blocks were placed on the basal end of the segment. The arrangement of blocks on the tissue is shown in Figure 1C. Transport was continued for 3 hours, after which the radioactivity of the receiver blocks was measured by liquid scintillation counting. Single blocks were placed in 0.75 ml ethanol in a scintillation vial, and 15 ml of standard toluene - PPO - POPOP mixture were added prior to counting.

III. RESULTS

Both red-light-induced hook opening[11] and red-light-decreased ethylene production[3,5] were reversible by far-red light. Our results (Table 1) confirm the previous findings, and substantiate the conclusion that both responses are under the control of phytochrome.

TABLE 1. EFFECT OF RED AND FAR-RED LIGHT ON ETHYLENE PRODUCTION AND HOOK ANGLE[a]

Treatment	Ethylene Concentration, nliter/g dry wt at 21 hr	Hook Angle, degrees
Dark	104±6	40±2
Red	80±6	66±4
Red — Far-red	94±6	42±3
Far-red	98±5	41±3

a. Excised standard hooks were exposed to 100 mJ/cm^2 of red light, far-red light, or red followed by far-red light. For ethylene determinations, flasks were stoppered, and the ethylene concentrations in the gas phase were measured 21 hours later. For hook angle measurements, flasks remained open, and shadowgraphs were made 21 hours after exposure. Initial hook angle was 0 degrees. Standard errors are indicated.

The relationship of hook opening and ethylene production to the amount of red light energy was determined (Fig. 2). The various amounts of incident energy were obtained by varying the time of exposure with a constant-intensity source (Fig. 2A) or by varying the intensity with a constant exposure time of 135 seconds (Fig. 2B). Although the results using the two different methods were not identical, it is evident that there was not a close correspondence between ethylene production and hook angle at the different amounts of light energy. Thus, in Figure 2A the light effect on ethylene production was saturated at 10 millijoules (mJ) per cm^2, but hook opening was proportional to light energy up to 1,000 mJ/cm^2, the highest energy used. Figure 2B shows that the light effect on hook opening was saturated at 100 mJ/cm^2, as was previously found by Klein et al.[12] As in Figure 2A, ethylene production was decreased to a low rate at energies of less than 10 mJ/cm^2, although the high-intensity treatment (1,000 mJ/cm^2) caused a further decrease.

The effect of IAA on hook opening by bean hypocotyls in the dark or in red light was reported by Klein et al.[2] Kang and Ray[3] showed the effect of IAA on ethylene production by hypocotyls treated by dark and red light. To test the hypothesis that red light promotion of hook opening is mediated by ethylene, the above experiments were repeated, under slightly different conditions. The data on ethylene production were then plotted opposite the hook angles for each experimental condition. Figure 3A shows the hook angle obtained after injection of various amounts of IAA. As previously shown,[2] IAA inhibited hook opening, and hypocotyls receiving a red-light treatment (100 mJ/cm^2) opened more than those in the dark. Figure 3B shows the promotion of ethylene production by IAA. Hypocotyls treated with red light produced less ethylene than dark ones. The data were combined in Figure 3C; hook angle was plotted against ethylene produced for each treatment. The points for red-light-treated hypocotyls fall on a different curve from those for dark-treated hypocotyls. For any particular rate of ethylene production, red-light-treated hypocotyls opened more than the corresponding dark ones.

Kang et al.[4] showed that the rate of ethylene production is highest in the curved portion of the hypocotyl. Because the cells along the inner circumference of the hook elbow are relatively short and unelongated compared with the cells along the outer circumference,[1] we subdivided the hook elbow into inner and outer halves as shown in Figure 1B. The rate of ethylene production was determined for these two halves (Table 2). The results show that the inner halves produced about twice as much ethylene as the outer halves in the dark. If red light treatment was given, ethylene production in both halves was decreased by an equal amount.

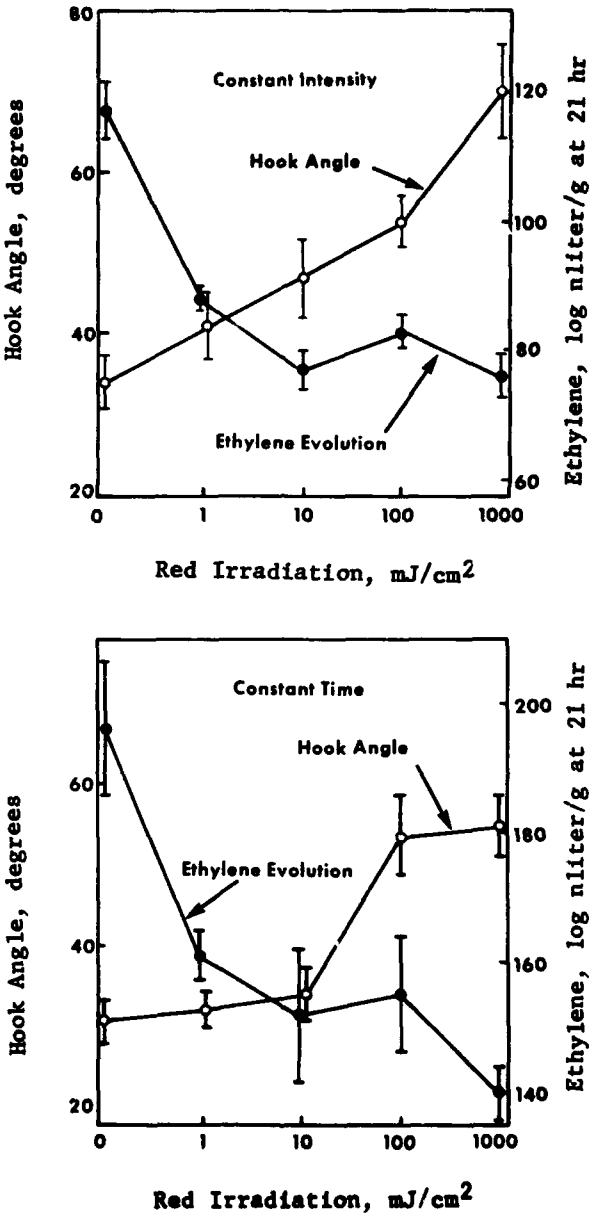

FIGURE 2. Effects of Amount of Red Irradiation on Hook Angle and Ethylene Production. After the light treatments, the hypocotyls were incubated in the dark for 21 hours. Hypocotyls to be used for hook angle measurements were left exposed to the air, and those for ethylene measurement were stoppered immediately after the light treatment. A) Hypocotyls exposed to a red light source of constant intensity for increasing periods of time. B) Exposure Time kept constant (135 sec) by varying the intensity of the source. Standard errors are indicated.

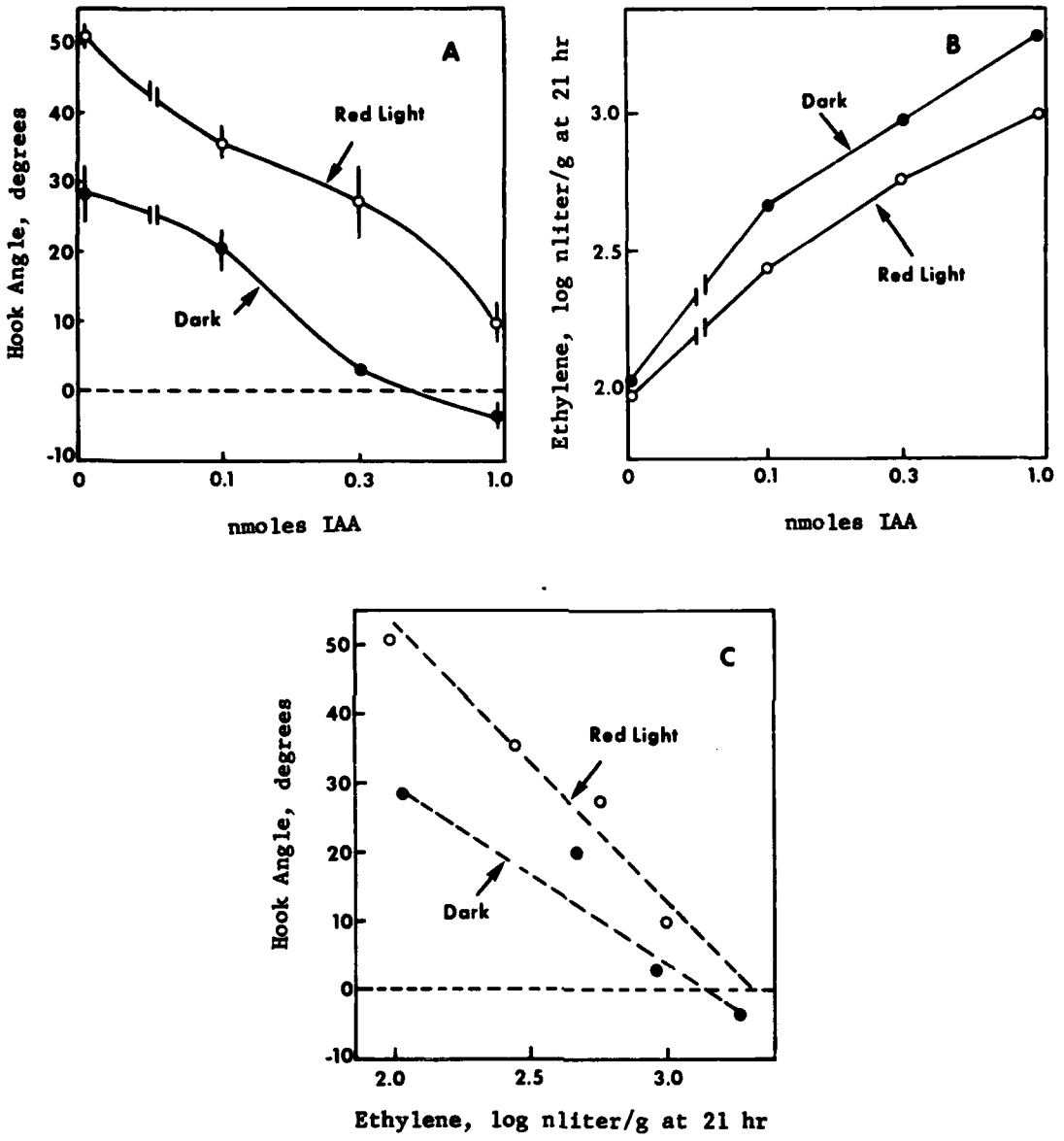

FIGURE 3. Relationship Between Hook Opening and Ethylene Production in Bean Hypocotyls at Various IAA Concentrations in Red-Light and Darkness. A) The inhibition of hook opening by indoleacetic acid in dark and red-light-treated (100 mJ/cm^2) hypocotyls. B) The promotion of ethylene production by indoleacetic acid in dark and red light-treated (100 mJ/cm^2) hypocotyls. C) A plot of hook angle vs. ethylene produced for hypocotyls receiving the same light and IAA treatments. The data are from Fig. 3A and 3B. Standard errors are indicated.

TABLE 2. EFFECT OF RED LIGHT ON ETHYLENE PRODUCTION BY INSIDE AND OUTSIDE REGIONS OF BEAN HYPOCOTYL HOOKS[a]

Treatment	Ethylene Production, nliter/g dry wt at 18 hr	
	Inside	Outside
Dark	491±42	288±24
Red light	390±48	187±18

a. The hook region only was excised and divided into equal inside and outside halves (see Fig. 1B). Ten each were placed in vials with 1 ml H_2O and exposed to 100 mJ/cm^2 red light or left in darkness. The vials were stoppered and incubated in the dark for 18 hours. The ethylene concentrations in the gas phase were then measured. Standard errors are indicated.

Ethylene treatment of excised hypocotyl hook sections at levels of 0.01 ppm or more significantly inhibits hook opening.[4] At higher concentrations, the hook angle becomes negative (i.e. the hooks become tighter). We determined the effect of various concentrations of ethylene on hypocotyl hooks that had previously been exposed to 100 mJ/cm^2 red light or left in darkness. The results (Fig. 4) show that at low ethylene concentrations, hooks treated with red light opened more than the dark ones, as was expected. However, at higher concentrations of ethylene (0.01 ppm or more), the opposite occurred.

Does ethylene have an effect on the transformation of phytochrome, or is it inhibitory to the subsequent growth of the inner hypocotyl cells? In the previous experiments the red-light treatments were given in air, and the hypocotyls subsequently placed in an atmosphere containing ethylene. To answer this question, hypocotyls were placed in ethylene for a 2-hour pretreatment. Some were exposed to red light while still in the gas, and then all were vented and the ethylene was flushed away. After a growth period in air, hook angles were measured. The results shown in Table 3 indicate that ethylene treatment inhibited both light-induced and dark hook opening.

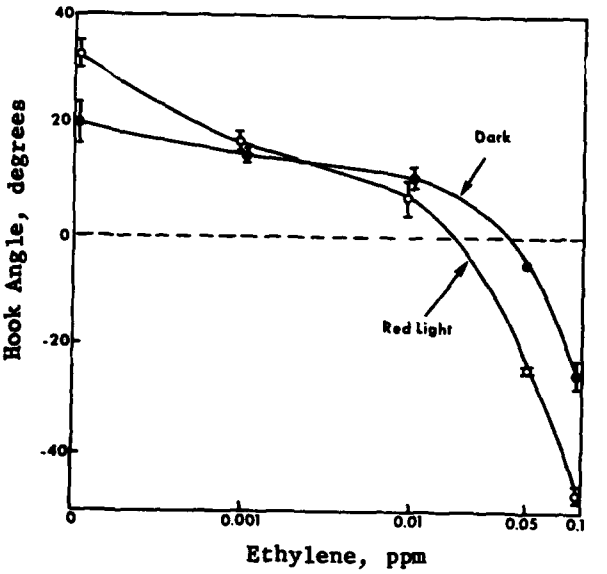

FIGURE 4. Effect of Exogenous Ethylene Concentration on Hook Angles of Hypocotyls Pretreated with 100 mJ/cm^2 Red Light or Left in Darkness. Standard errors are indicated.

TABLE 3. EFFECT OF RED LIGHT ON HOOK OPENING IN THE PRESENCE OF ETHYLENE[a]

Pretreatment	Hook Angle, degrees	
	Red Light	Dark
Air	63±1	47±2
Ethylene	52±3	31±3

a. Standard hypocotyl hooks were cut and placed upright in flasks. Some were pretreated for 2 hours with 1 ppm ethylene; others remained open to the air. Air- and ethylene-treated hooks were then exposed to 100 millijoules of red light per cm^2 or left in darkness, after which all were vented. After 21 hours' incubation in darkness, shadowgraphs were made for hook angle measurement. Standard errors are indicated.

Kang and Ray[3] showed that red-light treatment did not affect the yield of diffusible auxin from the hook tissue. We conducted experiments to determine if red light or ethylene had any effect on the lateral transport of IAA in the curved portion of the hypocotyl. Segments were cut from the hook region of the hypocotyls, and donor blocks of agar containing radioactive IAA were placed unilaterally on the apical cut surface as shown in Figure 1C. Split receiver blocks on the basal end of the segment were used to collect the transported radioactive IAA. The results are shown in Table 4. The first line indicates that in untreated hypocotyl segments in the dark, there was a net transport gradient from the outside to the inside of the hypocotyl. The results were the same if the hypocotyls were inverted. Neither prior red-light treatment nor ethylene had a significant effect on this lateral transport gradient (Exp. I and II). Since cutting the tissue induces the production of wound ethylene, the air control may have produced enough endogenous ethylene to exert an effect. Therefore, we determined the effect of 10% CO_2, an ethylene antagonist,[13] on lateral transport. Because CO_2 was without effect (Exp. III) we concluded that neither ethylene nor red light had an effect on lateral transport of IAA in the curved portion of the bean hypocotyl.

TABLE 4. EFFECT OF SEVERAL PARAMETERS ON LATERAL TRANSPORT OF AUXIN[a/]

Experiment		Donor Inside		Donor Outside	
		In Receivers, cpm	Lateral Movement, %	In Receivers, cpm	Lateral Movement, %
I.	Dark	137±15	33±5	114±19	47±3
	Red light	168±19	27±5	134±24	49±2
II.	Air	783±127	30±6	583±79	52±5
	50 ppm Ethylene	836±54	31±7	410±75	49±7
III.	Air	292±30	19±1	283±39	54±7
	10% CO_2	442±73	15±3	300±4	51±5

a. Donor blocks of 1.5% agar containing C^{14}-IAA, 5 x 10^{-6} M, 26.5 mc/mmole (Exp. I) or H^3-IAA, 1 x 10^{-7} M, 16 c/mmole (Exp. II and III) were placed unilaterally on either the inside or outside (see Fig. 1C) of the apical end of a segment from the hook region of a bean hypocotyl. Split receiver blocks were placed on the basal end of the segment. Transport took place for 3 hours, with the segments suspended in normal (horizontal) position. Receiver blocks were counted separately. Per cent lateral movement is the per cent of the total radioactivity recovered in the receiver laterally opposite from the donor block. Standard errors are indicated.

IV. DISCUSSION AND CONCLUSIONS

There is no doubt that the unhooking response of bean hypocotyls is controlled by phytochrome[11,12] (Table 1). Evidence also favors the view that, in some plant tissues, ethylene production is controlled by phytochrome[3,5] (Table 1). The question asked here is whether hook opening and ethylene production are causally related, or whether they are two independent effects. Evidence presented in this paper favors the latter. Very low red-light energy treatments give a near-maximal effect on ethylene production, but greater amounts of red light cause hooks to open more (Fig. 2). Red-light-treated hooks open more for a given level of ethylene production than ones kept in darkness (Fig. 3C). In the presence of 0.01 ppm ethylene or more, hooks pretreated with red light show a growth promotive effect (although in the opposite side of the hook from the usual). This result (Fig. 4) is inconsistent with a hypothesis of ethylene mediation of the red-light response. The inner halves of the hook region produce almost twice as much ethylene as the outer halves on a dry weight basis (Table 2). After red-light treatment the rate of production is decreased in both halves, but red-light-treated inner halves still produce more ethylene than outer halves kept in the dark. Obviously, the rate of ethylene production is not simply related to growth rates of these cells, because in red-light-treated hypocotyls, the inner hook region cells are growing much faster than the outer ones.[14]

Hook closing by high auxin levels was shown by Klein et al.,[2] and is undoubtedly an ethylene effect. Hook closing by ethylene itself was reported by Kang et al.[4] The enhancement by red light of this hook closing response to ethylene (Fig. 4) was also shown by Kang and Ray,[3] although higher ethylene levels were required, probably because they used continuous red light. We have no satisfactory explanation for this effect, but can only suggest that perhaps red light normally promotes growth of the outer cells to a lesser extent than the inner. In the presence of ethylene, the growth of the inner cells may be completely inhibited, but the outer ones are insensitive to the gas. This explanation would account for the enhanced hook closing by red light in the presence of ethylene.

There was no effect of red light on the yield of diffusible auxin from hook tissue.[14] We performed experiments to see if either red light or ethylene would affect the lateral transport of auxin in the hook region, as a possible explanation for their morphogenic effects. The unilaterally placed donor block and split receiver blocks (Fig. 1C) were used to offset the effect of a shorter distance of transport along the inner surface of the curved segment. There was a net transport of radioactivity towards the inside of the hook (47 versus 33% transport outwards), but there was no effect of either red light or ethylene on the distribution of radioactivity in the receiver blocks (Table 4). The CO_2 experiment was performed to determine if wound-produced ethylene might be masking any possible effect of added ethylene, but results were not significantly different from those with the air control.

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Unclassified
Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author) Department of the Army Fort Detrick, Frederick, Maryland, 21701	2a. REPORT SECURITY CLASSIFICATION Unclassified 2b. GROUP

3. REPORT TITLE

RED LIGHT AND ETHYLENE EFFECTS ON BEAN HYPOCOTYL UNHOOKING

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

5. AUTHOR(S) (First name, middle initial, last name)

David M. Peterson
Gerald R. Leather

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
May 1970	19	14

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO. 1B562602AD04	Technical Manuscript 614
c. Task-Work Unit 02-001	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d. DD 1498 Agency Access. DA OL 0003	CMs 6698, AMXFD-AE-T 49836

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14. Key Words

Ethylene
Red light
Bean hypocotyl hook

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