# Ethylene and Carbon Dioxide:

## Mediation of Hypocotyl Hook-Opening Response

Abstract. *Ethylene at low concentrations inhibits the light-induced opening of the bean hypocotyl hook; auxin inhibits the opening by inducing production of ethylene. Light causes a decrease in ethylene production and an increase in the production of carbon dioxide. Hook opening appears to be a response in which ethylene serves as a natural growth regulator and in which carbon dioxide may be involved also as a growth regulator through its antagonism of the action of ethylene.*

In the germination of many seeds the shoot emerges as a hook-shaped organ that grows in this form through the soil until it rises above the surface, at which time the hook straightens out. This straightening is a phytochrome-mediated light response that is inhibited by auxin at rather low concentrations. The straightening is due to rapid elongation of cells on the inner side of the hook elbow, this elongation being promoted by light and inhibited by auxin (*1*).

During a study of the physiology of this response in bean seedlings, we considered the possibility that ethylene might be involved, as had been suggested recently for hook opening in etiolated peas (*2*). Seeds of *Phaseolus vulgaris* (var. Black Valentine) were germinated 6 to 7 days at 26°C in total darkness at a relative humidity of 70 percent. As in previous work (*1*), the hook region of the hypocotyl was excised under a dim, photomorphogenically inactive, green safelight. About 10 hooks were placed in a 10-cm petri dish with 20 ml of test medium. At the end of the test period shadowgraphs were made of the hooks, and the angle of opening was measured on these shadowgraphs. The hooks we used were selected so that the initial angle of opening was 0° (meaning that the hook was curved through an angle of 180° from the direction of the straight portion of the hypocotyl).

In red light opening of the hooks was inhibited by ethylene (*3*) as well as by auxin [50 percent inhibition by 0.1 μg/ml of indoleacetic acid (IAA), complete inhibition by about 1 μg/ml; 15 to 25 percent inhibition by 0.01 part per million ethylene in air, complete inhibition by 0.1 to 0.5 ppm]. Higher concentrations of both auxin and ethylene induced a negative curvature (curvature in reverse of normal direction of opening). Inhibitions by both ethylene and auxin were partially reversed in air containing 7 percent $CO_2$. In view of the specific antagonism between $CO_2$ and ethylene (*4*), this suggested that inhibition of hook opening by auxin might be due to the induction, by auxin, of the production of ethylene (*5*).

That IAA induces the hooks to produce a volatile inhibitor of hook opening was shown by experiments in which half of the hooks in a closed illuminated vessel were treated with IAA, while half of the hooks in a second vessel were treated with plain agar blocks. Untreated hooks in the first vessel were strongly inhibited in opening, compared with those in the second vessel (*6*). This inhibition was largely prevented by the inclusion of mercuric perchlorate, an absorbent of ethylene, in the vessel. Mercuric perchlorate also induced considerable opening of hooks in a closed vessel in the dark, which suggests that the normal failure to open in the dark was due to production of ethylene.

Opening of hooks kept in a closed vessel in red light was almost completely inhibited by including KOH to absorb respiratory $CO_2$. This indicates that $CO_2$ antagonism of ethylene may be involved in normal hook opening, since ethylene and $CO_2$ should accumulate in the container in proportion to their rates of production and normal concentrations within the gas space of the tissue.

Production of ethylene and $CO_2$ by hypocotyl hooks was therefore assayed by gas chromatography (*7*). Representative results are given in Table 1 and are compared with the effects of treatments on the hook-opening response (*8*). Light did not influence evolution of ethylene from the straight (basal) portion of the hypocotyl, whereas light substantially reduced the release of ethylene from the elbow (curved) region of the hook. Time-course experiments showed that in red light the output of ethylene by hooks virtually ceased after about 8 hours, while in dark the output rate was steady for 20 hours (*9*).

Data in Table 1 show the strongly promotive effect of IAA on output of ethylene by elbow segments in both dark and light. Concentrations of IAA that increase the production of ethylene enough to offset the light-induced decrease in ethylene production by untreated hooks, largely counteract the influence of light on the opening response. This strongly implicates ethylene as the growth regulator that controls the opening response. This was supported by the finding that $CoCl_2$ (*1*) and cycloheximide, which induce opening in the dark and transitorily promote

Table 1. Effect of light and chemical treatments on the hook-opening response of bean hypocotyl and on the output of ethylene and $CO_2$. IAA, indoleacetic acid; 2,4-D, 2,4-dichlorophenoxyacetic acid; and GA, gibberellic acid.

| Treatment | Output | | Angle of opening (% of red light control) |
|---|---|---|---|
| | Ethylene ($10^2$ μl $hr^{-1}g^{-1}$)* | $CO_2$ (μl $hr^{-1}g^{-1}$)* | |
| *Hypocotyl hook (curved portion)* | | | |
| Darkness | | | |
| Water | 3.4 | 260 | 20 |
| IAA (0.175 μg/ml) | 10.6 | 275 | 14 |
| IAA (1.75 μg/ml) | 26.9 | 225 | 0 |
| $CoCl_2$ ($10^{-2}M$) | 0.12 | 195 | 53 |
| Cycloheximide (10 μg/ml) | 0.14 | 325 | 48 |
| Red light | | | |
| Water | 1.3 | 315 | 100 |
| IAA (0.175 μg/ml) | 4.8 | 375 | 37 |
| IAA (1.75 μg/ml) | 12.9 | 300 | — 9 |
| 2,4-D (10 μg/ml) | 17.7 | 370 | —22 |
| GA (100 μg/ml) | 1.4 | 345 | 138 |
| *Straight portion of hypocotyl* | | | |
| Darkness (water) | 1.5 | 160 | |
| Red light (water) | 1.4 | 200 | |

* Fresh weight.

opening in red light, inhibit the production of ethylene (Table 1).

Gibberellic acid, which consistently promoted the opening in red light (*1*), did not inhibit ethylene production, and its effect must therefore be regarded as independent of ethylene (Table 1).

Red light also induced a consistent increase in the rate of production of $CO_2$, as shown by data given in Table 1. In view of the previously mentioned evidence that respiratory $CO_2$ partly antagonizes the inhibitory action of endogenously produced ethylene in light-treated hooks, it seems probable that the promotion of $CO_2$ production by light may also be involved in the induction of opening by light. The effect of light on output of $CO_2$ is not simply a consequence of promotion by light of the growth of cells in the elbow, because the effect of light on respiratory $CO_2$ output was just as strong in a concentration of auxin that completely inhibited opening of the hook (Table 1).

It seems clear that ethylene, and possibly $CO_2$ also, serve as natural growth regulators in the hook-opening response of the bean hypocotyl. Regulation by ethylene may be of particular value in terms of the biological role of the hook-opening response. As long as the hook is growing underground the buildup of its endogenous ethylene that should result from confinement in soil would tend to prevent opening. Thus, opening could occur only after emergence from the soil, even though morphogenically effective intensities of light might penetrate some distance below the surface of the soil.

BIN G. KANG
CONRAD S. YOCUM
*Department of Botany, University of Michigan, Ann Arbor*
STANLEY P. BURG
*Department of Physiology, University of Miami School of Medicine, Miami, Florida*
PETER M. RAY
*Division of Natural Sciences, University of California. Santa Cruz*

### References and Notes

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3. Inhibition of growth movements by ethylene was reported long ago by W. Crocker, A. E. Hitchcock, P. W. Zimmerman, *Contrib. Boyce Thompson Inst.* **7**, 231 (1935).
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6. In experiments of this type, about 20 hooks were supported by their bases in agar in a closed vessel (gas volume, about 250 ml.) Auxin treatment was given by applying, at the apical cut end of the hook, an agar block containing IAA.
7. S. P. Burg and E. A. Burg, *Plant Physiol.* **37**, 179 (1962).
8. In Table 1, gas output was measured by incubating hypocotyl tissue segments for 19 hours in 5 ml of test medium in a sealed 60-ml flask, a sample of air being withdrawn for gas chromatography at the end of the period. Red light treatment was continuous illumination, during incubation period, with about 250 erg $cm^{-2}$ $sec^{-1}$, wavelength >600 mμ. Angle of opening of red light controls was 95° to 110° in different experiments from which data on hook opening quoted in Table 1 were drawn.
9. Similar results with pea epicotyls have been obtained by J. D. Goeschl (private communication).

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## Dieldrin: Degradation by Soil Microorganisms

Abstract. *An attempt was made to discover microorganisms that degrade dieldrin, an extremely stable chlorinated hydrocarbon insecticide. Examination of more than 500 isolates from soil that had been heavily contaminated with various insecticides revealed the existence of a few microbes that are very active in degrading this compound to various metabolites.*

Certain chlorinated hydrocarbon insecticides are known to be very stable and to persist in soil for many years (*1*). Among them, dieldrin is the most stable and hazardous insecticide in our environment (*2*); this persistence suggests that it is of low biochemical reactivity and also makes plausible the view that its potent effect upon insects and mammals depends upon a physical complex with their nervous systems, rather than a chemical reaction (*3*). Most chlorinated hydrocarbon insecticides have little effect upon bacterial and fungal growth (*4*), and many microbial changes brought about by application of these insecticides to soil may be attributable to other secondary effects (*5*).

Recently, Chacko *et al.* (*6*) demonstrated that several actinomycetes can degrade 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT), but no microorganisms tested by these authors were capable of degrading dieldrin. Korte *et al.* (*7*) also found a number of microorganisms that slowly degraded all representative chlorinated cyclodiene insecticides except dieldrin. Studies on the degradation activity of dieldrin by biological systems other than microbes have been equally unsuccessful, although there are reports that dieldrin can be degraded very slowly in vivo by some mammals (*8*) and insects (*9*). None of these degradation activities can be regarded as significant enough to reduce the actual toxicity of dieldrin by these systems, for the amount of metabolites in each instance was extremely small.

In this study we attempted to survey the degradation activities of various microbial isolates from samples of insecticide-treated soil in the hope of finding certain microorganisms that degrade dieldrin.

Soil samples that contained dieldrin-degrading microbes were obtained from five major locations: two from apple orchards in northeastern Ohio (Tope's orchard, Fredericksburg, and Synder's orchard, Wooster); two from the dieldrin factory yards of Shell Chemical Company near Denver, Colorado; one from an orchard area near the University of Wisconsin, Madison; and two from a peach orchard near Fort Valley, Georgia.

Microorganisms were isolated from soil samples by adding 1 g of soil to 99 ml of sterile water and shaking the mixture vigorously for 3 minutes. From this stock, serial dilutions of $10^{-2}$ and $10^{-3}$ were made in sterile petri dishes and mixed with soil-extract agar of the type described by Allen (*10*). Plates were incubated at 30°C for 4 days; they were then examined, and individual colonies were selected and streaked singly on soil-extract agar. After incubation, an additional and similar transfer was made to ensure purity of the culture. For dieldrin incorporation, each isolate was first inoculated in 10 ml of a solution of yeast extract and mannitol, as described by Fred and Waksman (*11*), and maintained at 30°C for 57 hours. This mixture was then incubated with 0.01 μmole of $C^{14}$-dieldrin (universally labeled, specific activity 9.4 mc/mmole), added with 10 μl of acetone, in a screw-capped 20-ml test tube at 30°C for 30 days without shaking. The reaction was stopped by adding a 0.1-ml portion of 20 percent trichloroacetic acid to the tube, and the contents were immediately extracted twice, each time with an equal amount of chloroform that had been dried over anhydrous sodium sulfate. The distribution of radioactivity between the aqueous and solvent phases was re-