Plant Physiol. (1967) 42, 1077–1080

An Effect of Light on the Production of Ethylene and the Growth of the Plumular Portion of Etiolated Pea Seedlings[1]

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Received March 8, 1967.

Summary. The production of ethylene by etiolated pea epicotyls (*Pisum sativum* L., cv. Alaska) is confined to the plumule and plumular hook portion of the epicotyl, and occurs at a rate of about 6 $\mu l \cdot kg^{-1} \cdot hr^{-1}$. Such a rate is sufficient to give physiologically active concentrations of ethylene within the tissue. Exposure of etiolated seedlings to a single dose of red light caused a transient decrease in ethylene production and a corresponding increase in plumular expansion. Far-red irradiation following the red light treatment decreased the red effect to the level achieved by the far-red alone, suggesting that the ethylene production mechanism is controlled by phytochrome and thus that the ethylene intervenes as a regulator in the phytochrome control of plumular expansion.

A relationship between the production of ethylene and the inhibition of plumular expansion in etiolated pea epicotyls can be deduced from several recent observations. First, the rate of ethylene production in the growing epicotyl does not increase in proportion to the increasing mass or number of cells (5). This suggests that the production of ethylene may be restricted to a particular zone of tissue with a relatively constant mass or cell number. Second, application of low concentrations of ethylene to light-grown seedlings can maintain the very slow rate of leaf expansion characteristic of etiolated seedlings (3). Third, Burg and Burg (1) have shown that stem sections taken from light-grown pea seedlings produce less ethylene when treated with IAA than similarly treated sections from dark-grown plants. Finally, since this work was completed, Kang et al. (6) have reported that the red light-induced opening of excised bean hypocotyl hooks can be prevented by application of ethylene, and that the light reduces the production of ethylene by the hypocotyl hook segments.

Localization of Ethylene Production. Pea seeds (*Pisum sativum* L., cv. Alaska) were planted 1 cm deep in vermiculite contained in perforated plastic trays, which were allowed to stand in deionized water for 6 hours to soak the seeds. The drained trays were then placed individually in sealed metal containers and provided with a constant flow (10 liters$\cdot hr^{-1}$) of humidified air at 25°. The seedlings were transferred to the test chambers 4.5 days after planting. Each chamber (5) consisted of a glass cylinder, 3.5 cm inside diameter and 10 cm long, closed at top and bottom by a 1.0 cm thick closed-cell foam neoprene stopper. The upper stopper was fitted with glass inlet and outlet tubes; slits in the lower stopper supported and formed a gas tight enclosure around the epicotyls of 6 seedlings with various portions of the epicotyls protruding into the chamber. In one set of 5 chambers only the plumule and the plumular hook of the seedlings were enclosed. In another set the plumule, hook, and 1 cm of stem were enclosed, and in the third set the plumule, hook, and 3 cm of stem. Estimates of ethylene production were made about 2 hours after transfer by measuring the concentration of ethylene in the effluent air stream from the chambers (5). Refinements in our gas chromatographic technique have made it possible to obtain a peak 25 mm high above a background noise level of ± 3 mm with 0.001 ppm of ethylene in a 5 ml air sample. The experiment was conducted twice and the results were combined to give 10 samples of 6 epicotyls each for each of the 3 amounts of enclosed tissue (table I).

In our previous work (4) the rate of ethylene production was incorrectly reported as 0.006 mμl per epicotyl per hour. The correct figure is 0.06 (or 60 in the units of table I). From the values of table I, it can be seen that no additional ethylene is obtainable by enclosing more than the plumular part of the epicotyl.

The foam neoprene support surrounds a 10 mm portion of each epicotyl, in addition to that which protrudes into the chamber. The direct escape of ethylene produced in this portion, if any, will be prevented, and any such ethylene will diffuse up the stem into the chamber or down the stem to the exterior. Therefore, we can resolve the source of

[1] This investigation was supported in part by a research grant (EF-00156) from the Division of Environmental Engineering and Food Protection, United States Public Health Service.

Table I. *Localization of Ethylene Production in Pea Epicotyls*

Various portions of the epicotyl of intact, etiolated pea seedlings were enclosed in small cylindrical glass chambers, and the rate of ethylene production was determined by measuring the net concentration of ethylene in the effluent air stream as steady state conditions were approached (about 2 hrs after enclosure).

Portion of seedling enclosed in chamber	Total rate of ethylene production $10^6 \times mm^3 \cdot plant^{-1} \cdot hr^{-1}$
Plumule + plumular hook	55 ± 8
Plumule + hook + 1 cm of stem	52 ± 6
Plumule + hook + 3 cm of stem	57 ± 6

ethylene, at best, to within about 5 mm of a given point along the axis. It may be fairly stated that all of the detectable ethylene evolves from a zone of tissues beginning about 5 mm below the base of the plumular hook and extending upward through the plumule. At least 90 % of this ethylene is probably evolving from the plumule and the plumular hook alone and no more than 10 % from 5 mm of the stem just below the hook. The total fresh weight of the plumular portion of 4.5 day old seedlings is between 8 and 10 mg, so ethylene is being produced at a rate of about 6 $\mu l \cdot kg^{-1} \cdot hr^{-1}$. This is almost 3 times greater than the highest rate of production by pea epicotyls under conditions of mechanical stress (5), so the internal concentration must be well within the physiologically active range.

Effects of Light on Ethylene Production and Plumular Expansion. Germination and Manipulation of Plant Material. Seedlings to be used for the measurement of plumular expansion were grown in small plastic pots in the manner described above. About 10 % to 15 % of the seedlings from our seed lot had plumules which were abnormal in shape and size and which did not expand in the characteristic manner after exposure to light. These can be detected in the early stages primarily by their association with unusually short epicotyls. To avoid this source of variability, and because germination is less than 100 %, 15 seeds were planted in each pot, and only the 10 tallest seedlings in each pot were eventually used.

At appropriate intervals a sample of 2 pots, yielding 20 seedlings, was removed from the light-treated group, and likewise from the dark controls. The plumules were cut at the base of the third leaf, weighed individually, and the resulting data plotted as cumulative fresh weight against time (21). The relative growth curve (fig 1) was computed by determining the tangent of the original growth curves at frequent intervals and dividing the slope of the tangent of the treated plant growth curve by that of the controls.

Seedlings to be used to measure the effects of light on ethylene production were germinated as usual and then transferred to test chambers 3 days after planting. The transfer of the seedlings leads to a small transient decrease in ethylene production and an increase in plumular expansion beginning about 2 hours after transfer and returning to normal by 20 hours. While this leads to an increase in the absolute size of the plumule, the growth rate (as change in wt per unit time per unit initial wt) also returns to that of the seedlings left in the germination trays. Because of the above behavior, at least 24 hours were allowed to elapse between the transfer of the seedlings and the first measurements of growth and ethylene production. The chambers were then tested for leaks or abnormal levels of ethylene (usually associated with injured seedlings), defective samples were eliminated, and the dark-rate of ethylene production was determined for all remaining samples (usually 6 control and 6 treated chambers). These chambers were smaller than those previously described (2.4 cm inside diameter and 9 cm long), with glass tops to allow

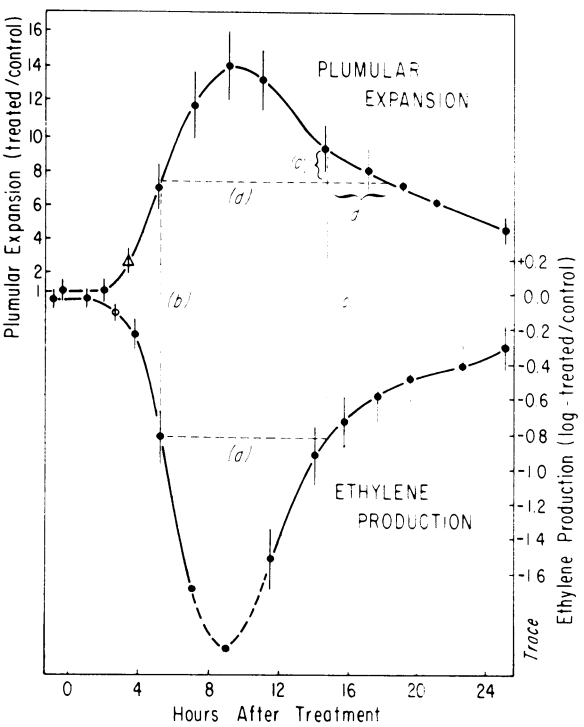

FIG. 1. The effect of a 60 second exposure of broad band red light (40,000 ergs$\cdot cm^{-1}$ total dose) on plumular expansion and ethylene production in etiolated Alaska pea seedlings. The curve of relative plumular expansion $\frac{[(d\ wt/dt \cdot wt)\ treated]}{[(d\ wt/dt \cdot wt)\ control]}$ was estimated from short segments of cumulative growth curves, similar to those found by Furuya and Thomas (2). Ethylene production was determined from the net concentration in the effluent air stream from chambers enclosing the upper part of the epicotyls. The relative ethylene production rate (treated/control) is presented on a log scale, since preliminary experiments showed leaf expansion to be inversely proportional to the log of the concentration of ethylene treatments (Goeschl and Pratt, unpublished).

direct exposure of the epicotyls to light. Six such assemblies were placed in a controlled temperature cabinet at 25° for eventual light treatment, and another 6 in a similar cabinet in continuous darkness. The individual chambers were connected to a manifold of capillary flow meters supplying an inflow of 40 ml/hr of humidified air to each chamber. Determining changes in the rate of ethylene production by measuring the concentration of ethylene in a 40 ml per hour effluent from a 40 ml free volume chamber results, theoretically, in a 2-fold decrease in the apparent rate of such changes. This has been verified experimentally by supplying an empty chamber with known but constantly changing amounts of ethylene and measuring the resulting changes in effluent concentration. A correction factor based on these measurements was used in calculating the changes in ethylene production.

Light Sources. Transplanting and other manipulations were conducted under a dim green light, consisting of a Luxo drafting lamp with 2 15-watt fluorescent tubes filtered with two 3 mm thick layers of green Plexiglas, 2 similar layers of blue Plexiglas, and 3 layers of 0.4 mm sheets of amber acetate. This system gives a peak emission at 525 mμ (50 mμ width of one-half peak emission); it is very dim, and does not cause photomorphogenic effects with as much as 30 minutes of exposure.

A bank of 6 24-inch, 20-watt, GE warm-white fluorescent bulbs was equipped with a filter consisting of two 3 mm sheets of red Plexiglas (No. 2423), three 0.4 mm sheets of amber acetate, and a 3 mm pyramid pattern Plexiglas diffuser. The emission spectrum of this system was estimated from the absorption spectrum of the complete filter and the published emission spectrum of the lamps. Measurements at the plant level, with an Eppley thermopile and the appropriate cut-off filters, indicate about 660 ergs $cm^{-2} \cdot sec^{-1}$ of radiation distributed in a spectrum essentially the same as that shown by Furuya and Thomas (2). The plants to be treated were illuminated for 60 seconds at a distance of 80 cm. This exposure gave approximately 40,000 $ergs \cdot cm^{-2}$, or about 4 times the saturating dose for the subsequent transient growth response (2).

The far-red light source consisted of 5 300-watt clear internal reflector spot lamps filtered through 10 cm of water and two 3 cm thick sheets of Westlake Plastic's FRF 700 far-red filter material. At plant level (50 cm below the filter) the intensity was about 150,000 $ergs \cdot cm^{-2} \cdot sec^{-1}$ total energy, of which about 10,000 $ergs \cdot cm^{-2} \cdot sec^{-1}$ is in the region between 710 and 745 mμ.

Effect of Red Light. The 60 sec red light treatments caused a marked deceleration of ethylene production beginning possibly within 2 hours and becoming statistically significant by 2.5 hours (fig 1). Closely following the decrease in ethylene production was an acceleration in the rate of plumular expansion which was just significant at 3.5 hours. As in the data of Furuya and Thomas (2), there was still very little difference in the absolute size between the light- and dark-grown plumules, but a plot of the growth rates from several measurements before and after this time shows a sharp inflection, possibly as early as 2.5 hours. By 6 hours the evolution of ethylene had diminished to 8 to 10 % of that of the dark controls, and by 9 hours only trace quantities of ethylene could be detected from most samples and none at all from the others. At the same time the growth rate reached a maximum.

Twelve hours after the light treatment, all of the samples began to produce measurable quantities of ethylene again, and the rate of production increased until, by 24 hours, ethylene evolution had increased more than 10-fold above the minimum. Individual sets of plants were discarded at various times during the following hours as some of the plants began to push against the tops of the chambers. However, ethylene evolution continued to increase in all remaining sets of plants and, in 2 sets which remained until 32 hours, the production of ethylene reached nearly 80 % of the control level.

As the plants recovered their ability to produce ethylene in the dark, there was also a deceleration of plumular expansion. By 48 hours after treatment (not shown on fig 1) the rate of plumular expansion had decreased to the level of the dark controls. This recovery of ethylene production, and accompanying reduced growth rate, are of considerable interest, since they may be considered as aspects of re-etiolation. This entire experiment was repeated using 4-day-old seedlings, and all relationships were the same as above, but the overall response was somewhat slower.

Effect of Far-red Light. The transient increase of plumular expansion induced by red light has been shown previously (2) to be controlled by phytochrome. If the endogenous production of ethylene is involved in regulating plumular expansion, then it too must respond to the action of phytochrome. To test this, seedlings were enclosed in chambers as described above and given treatments of red, far-red, and red followed by far-red light. Preliminary tests with the far-red light source had shown that it would induce a growth response equivalent to about 40 % of the maximum red light response.

The magnitude of the response was the same in the range from 10 to 120 seconds of exposure, so 20 seconds of far-red light was chosen as a convenient working time. This exposure gives a total dose of about 2×10^5 $ergs \cdot cm^{-2}$ of active far-red light. A red light dose of about 6600 $ergs \cdot cm^{-2}$ (10 sec) was chosen to produce between 70 and 80 % of the maximum response.

A set of 5 chambers with 6 plants in each chamber was used in each of the following treatments: A) dark control, B) red light exposure of

Table II. *Far-red Reversal of Red-Light induced Changes in Ethylene Production and Plumular Expansion*

	Ethylene production $10^6 \times mm^3 \cdot plant^{-1} \cdot hr^{-1}$	Plumular growth $mg \cdot hr^{-1} \cdot mg^{-1}$ tissue
(A) Dark	52 ± 7	0.011 ± 0.002
(B) Red	10 ± 2	0.074 ± 0.009
(C) Far-red	22 ± 3	0.033 ± 0.006
(D) Red : far-red	19 ± 3	0.037 ± 0.006

6600 $ergs \cdot cm^{-2}$, C) far-red light exposure of 2×10^5 $ergs \cdot cm^{-2}$, and D) an exposure to red followed by far-red light at the same dosages given in the separate treatments. Measurements of ethylene production and growth were made at about 2-hour intervals to be sure that all of the treatments followed the same time course. As in the earlier experiment, the responses reached a maximum between 8 and 10 hours after treatment. The rate of growth shown in table II is based on the weight increase between 9 and 10 hours, and ethylene production was measured at 9.5 hours.

As seen in table II, and as suggested by preliminary tests, the far-red light induces a small decrease in ethylene production and a partial increase in plumular expansion. These effects of far-red light can be expected since the absorption spectrum of the P_R form of phytochrome extends into the region of the spectrum covered by our light source. The similarity in behavior between the far-red and red/far-red treatments (table II) suggests that the red light effect on ethylene production is indeed far-red reversible and, therefore, that it is controlled by the phytochrome system. Recalculation of the data of table II to the form of figure 1 shows that in each treatment the changes in rate of plumular expansion are nearly proportional to the log of the changes in ethylene production.

Discussion

It is clear from these results that a good correlation exists between the photo-induced decrease in ethylene production and increased plumular expansion. In considering the possibility of a causal relationship between the changes in ethylene production and growth, we might expect the light-induced changes in ethylene production to precede the corresponding changes in growth. This is emphasized because of the evidence (1) that the growth of pea stem segments does not appear to respond to ethylene until after 3 hours. We may test for such a time sequence by selecting any 2 points of equal ethylene production in the descending and ascending phases (fig 1), such as the points intersected by line (a). Vertical lines (b) and (c), constructed from these points, intersect the growth curve at slightly different levels, depicted by segment (d'). We must point out, however, that the ethylene production curve is based on the gross amount of ethylene evolving from the plumular region, and that we do not know to what extent its production may be further localized within that region. Further, the fresh weight and volume:surface ratios are increasing at various rates during the experiment, which makes it difficult to relate the ethylene and growth data on a strictly comparable basis. We have, however, estimated the ethylene production rates on a fresh weight basis and from them in turn have estimated the possible internal concentrations as previously described (3). Allowing the widest latitude, we have found that the time delay may be as short as 1 hour with respect to the chosen interval (fig 1) and may be, therefore, statistically insignificant. In no way, however, do the results suggest that the growth response can precede the ethylene response.

That the correlation between the changes in ethylene production and plumular expansion is more than coincidental is suggested by the fact that the far-red or the red/far-red causes an acceleration of growth which can be accounted for quantitatively by the decrease in ethylene. These same data also demonstrate the control of ethylene by the phytochrome system which is an important step in establishing a causal relationship, since the growth response had been shown previously to be controlled by the phytochrome system.

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