Ethylene Is Not Involved in the Blue Light-Induced Growth Inhibition of Red Light-Grown Peas

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ABSTRACT

Although the growth of intact plants is inhibited by irradiation with blue light, the growth rate of isolated stem segments is largely unaffected by blue light. We hypothesized that this loss of responsiveness was a result of ethylene production as part of the wounding response. However, we found no interaction between ethylene- and blue light-induced growth inhibition in dark- or red light-grown seedlings of pea (Pisum sativum L.). Inhibition of growth begins in dark-grown seedlings exposed to blue light within 3 min of the onset of blue light, as was known for red light-grown seedlings. By contrast, ethylene-induced inhibition of growth occurs only after a lag of 20 to 30 min or more (dark-grown seedlings) or 60 min (red light-grown seedlings). Also, the inhibition response of red-light-grown seedlings is the same whether ethylene is present from the onset of continuous blue-light treatment or not. Finally, the spatial distribution of inhibition following blue light was different from that following ethylene treatment.

Blue light irradiation can inhibit stem elongation in dicotyledonous seedlings that have been grown in the dark or in red light by as much as 80% (1, 2, 6, 8). The elongation rate of excised stem segments, however, is much less sensitive to blue light. Cosgrove (3) found that blue light decreased the elongation rate of segments of dark-grown cucumber stem by approximately 35% if the segments were floated on distilled water. When the segments were floated in dishes containing large concentrations of auxin, blue light actually promoted rather than inhibited growth. Why cut sections have a diminished response to blue light is a puzzle.

One possibility is that the loss of responsiveness is a function of wounding. One of the many plant responses to wounding is increased ethylene production (12). Because ethylene is a well-known growth regulator, we hypothesized that this increase in ethylene production was somehow involved in the blue-light response.

This hypothesis was strengthened by the similarity of the kinetics reported for ethylene- and blue light-induced growth inhibitions. Warner and Leopold (11) found that 10 μL L⁻¹ ethylene induced a growth inhibition of approximately 60%. The inhibition began about 6 min after ethylene application in 4-d-old etiolated pea (Pisum sativum) seedlings. In comparison, we found that the latent time for blue light-induced growth inhibition in 6-d-old red light-grown pea seedlings was approximately 2 to 3 min (6). Both ethylene- and blue light-induced growth inhibitions are relieved after the stimulus is removed (6, 11).

In addition, it has been observed that when a blue-light response is seen in cut sections, the degree of inhibition caused by blue light is related to physiological conditions known to affect the rate of ethylene production. Shinkle and Jones (10) found that when cucumbers were grown in red light, blue light inhibited the growth of sections by approximately 50%, a much greater degree of inhibition than was seen in sections excised from dark-grown plants (2). Previous experiments with etiolated pea seedlings have demonstrated that exposure to red light results in the suppression of endogenous ethylene production (5). Such results are consistent with the view that blue light might act by increasing ethylene production. In such a case, the wound ethylene produced as a result of excising sections from the stem might saturate the inhibition. Such a saturation would cause an apparent lack of blue-light sensitivity in cut sections.

MATERIALS AND METHODS

Alaska pea (Pisum sativum L.) seedlings were grown for 6 d in continuous red light at a fluence rate of 1 μmol m⁻² s⁻¹ as described by Laskowski and Briggs (6) except that the peas used in section experiments were grown in clear (16 cm × 26.5 cm × 40 cm) plastic boxes rather than in individual beakers.

Section Experiments

Pea stem sections (1 cm long) were excised from the base of the third internode with a double-bladed cutter and placed in Petri dishes of distilled water located on a gently rotating shaker (approximately 40–50 rpm). There were 8 to 12 sections per treatment. Blue light was delivered at a fluence rate of 35 to 40 μmol m⁻² s⁻¹. Because a mirror was placed behind the sections, the actual fluence rate received by the plants was 70 to 80 μmol m⁻² s⁻¹. The light source was the same as that previously described (6).

For the experiments reported in Table II, the 1-cm region at the base of the third internode was delimited with small spots of carbon black in immersion oil. Plants were uprooted...
and placed upright, eight to a jar, with both their roots and cotyledons immersed in water. The length of the marked region was measured with a ruler both before and after a 6-h growth period.

**Ethylene Treatments**

The effect of ethylene on the growth of pea seedlings (Figs. 1, 3, and 4) was determined by measurements taken from negatives of time-lapse photographs (see ref. 6). For each experiment, a single seedling—complete with the 30-mL beaker in which it was potted—was placed in a closed Plexiglas chamber. Ethylene was added at the times indicated by injection through the serum cap located at the top of the chamber.

**Determination of Growth Rates in Dark-Grown Plants**

Following imbibition in green light (fluence rate less than 0.1 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)), peas were grown for 4 d inside a completely dark box. A humid atmosphere was maintained by placing an open beaker of water inside the box. Before an experiment, seedlings were removed from the box in a completely dark room. Small spots of carbon black were placed at the top and bottom of the youngest internode with the aid of an IR light source and a Find-R-Scope (FjW Industries, Mt. Prospect, IL). The IR light was provided by a 25-W incandescent light bulb contained in a tin can. To prevent visible light from escaping through the sides of the can, the can was covered with a thick layer of black cloth. The top (front) of the can was covered with multiple layers of colored plastic. Using an Li-1800 Spectroradiometer (Lambda Instruments, Lincoln, NE) to measure the light, we found that the maximum emission between 300 and 1100 nm was 0.3219 \( \mu \text{mol m}^{-2} \text{s}^{-1} \text{nm}^{-1} \), which emitted at 1038 nm; only 0.0009 \( \mu \text{mol m}^{-2} \text{s}^{-1} \text{nm}^{-1} \) was emitted at 760 nm. This same light source provided the illumination for photography. High-speed IR film (Kodak No. 2481, HIE 135–36; Eastman Kodak Co., Rochester, NY) was used to record the images. Marked plants were placed, one per experiment, in the Plexiglas treatment chamber 30 min prior to the initiation of photography.

For studies of kinetics and fluence dependence of blue-light responses in dark-grown plants using position transducers, seedlings were germinated and grown as described previously (6) but in complete darkness. At 4 d after planting, seedlings were attached to position transducers as described previously (6), either using dim green working light or IR light and the Find-R-Scope. No differences were found between responses of plants prepared under the different illumination conditions. Seedlings were allowed to equilibrate for 1 h after being attached to the transducer, and then were given a blue-light irradiation of 30 s to 10 min. For the fluence-response curve, growth rates were determined at 6-min intervals and the effect of blue light was expressed as a percentage of the dark control. For the kinetics of the response, 10 min of blue light was given at a fluence rate of 30 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) (with mirror in place, the total fluence rate was 60 \( \mu \text{mol m}^{-2} \text{s}^{-3} \)). Fluence rates were measured using a Licor Quantum-Photometer (Li 185-A, Lambda Instruments). Growth rates were determined from chart recorder traces of transducer output.

**RESULTS AND DISCUSSION**

Continuous irradiation of 6-d-old red light-grown pea seedlings with 80 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) blue light decreases the growth rate of cells located in a 1-cm region at the base of the third internode (Table I). Excised sections from this part of the plant, placed in water containing 1 or 10 \( \mu \text{mol IAA} \), grow at a rate comparable to that found in the intact plant (Table I). Continuous irradiation with blue light, however, fails to inhibit the elongation of these excised internodes (Table I). Even when 2-cm sections are cut, or when the entire third internode including the apex is removed from the plant, the growth rate of the marked 1-cm region remains unaffected by blue light (Table I).

Additional experiments were performed to determine whether some factor other than the cut itself was responsible for the loss of the blue light-induced growth inhibition. To observe the effects of handling, plants were gently uprooted, marked, and placed upright in a jar of water. Both the elongation and light responsiveness of these plants were similar to those seen in intact plants (Table II). Because handled plants retained both high growth rates and a sensitivity to blue light and cut tissue had neither, it was of interest to know whether plants with low growth rates somehow lost the capability of responding to blue light. Placing uprooted plants in a vase containing 0.15 M mannitol decreased the elongation of the tissue at the base of the third internode as strongly as any of the surgical treatments (compare Table II with Table I); however, blue-light irradiation inhibited the elongation of mannitol-treated seedlings just as it did the control seedlings (Table II). Hence, the loss of blue-light sensitivity appeared to be a specific result of cutting rather than a nonspecific effect of decreased growth.

**Table I. Elongation of the 1-cm Region at the Base of the Third Internode of 6-d-old Red Light-Grown Pea Seedlings over a 6-h Period**

<table>
<thead>
<tr>
<th>Type of Section</th>
<th>Length of Marked Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td>Intact plants in beakers ((n = 6))</td>
<td>10.3</td>
</tr>
<tr>
<td>1 cm sections in 1 ( \mu \text{M} ) IAA</td>
<td>9.8 ± 0.1</td>
</tr>
<tr>
<td>1 cm sections in 10 ( \mu \text{M} ) IAA</td>
<td>10.2 ± 0.1</td>
</tr>
<tr>
<td>2 cm section in 1 ( \mu \text{M} ) IAA ((\text{growth of the central 10 mm is reported}))</td>
<td>9.4 ± 0.1</td>
</tr>
<tr>
<td>Apex and entire third internode, no added IAA</td>
<td>10.1 ± 0.1</td>
</tr>
</tbody>
</table>

* Six plants per treatment.  
* Seven plants per treatment.

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Table II. Elongation of the 1-cm Region at the Base of the Third Internode of 6-d-old Red Light-Grown Pea Seedlings over a 6-h Period

<table>
<thead>
<tr>
<th>Plant Material</th>
<th>Length of Marked Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td>Intact, uprooted plants in water</td>
<td>10.4 ± 0.2</td>
</tr>
<tr>
<td>Intact, uprooted plants in 0.15 M mannitol</td>
<td>9.9 ± 0.1</td>
</tr>
</tbody>
</table>

Seven plants per treatment.

Effect of Ethylene on the Elongation of Red Light-Grown Pea Seedlings

Addition of 10 μL L⁻¹ or 100 μL L⁻¹ of ethylene to the air surrounding a red light-grown pea seedling inhibits elongation of the apical 5 mm of the third internode with a detectable effect at approximately 60 min after application (Fig. 1). Over the course of 9 h during ethylene application, this apical region expanded approximately 75% less than control tissue (Fig. 1). More basal regions of the pea stem were much less sensitive. Over the same 9 h, elongation of tissue in the middle of the third internode was inhibited by approximately 40% (Fig. 1). At the base of the third internode, application of 10 μL L⁻¹ ethylene was no longer sufficient to saturate the response. This pattern of inhibition was the opposite of that found for blue light (6), in which case the least inhibition was seen in the upper third and the most in the lower.

These data differ substantially from those of Warner and Leopold (11). Working with 4-d-old dark-grown pea seedlings, they found the latent time for ethylene action to be 6 min when 10 μL L⁻¹ ethylene were supplied and 18 min when only 5 μL L⁻¹ ethylene were applied. Measurements of the latent time for ethylene action made in other laboratories vary from 15 to 60 min, depending on the tissue (see ref. 4). It was possible that the difference in timing of blue light- and ethylene-induced growth inhibition existed because we were working with red light-grown plants, whereas Warner and Leopold (11) had been working with dark-grown plants. Hence, we attempted to reconcile our findings with those of Warner and Leopold by examining the effect of both blue light and ethylene on dark-grown pea seedlings. We found that the blue-light response of 4-d-old dark-grown pea seedlings is very similar to that of red light-grown seedlings. The fluence dependence of the blue-light inhibition of elongation (Fig. 2) shows threshold and saturation at fluences within a

Figure 1. Effect of ethylene on the spatial distribution of growth along the axis of 6-d-old red light-grown pea seedlings. Seedlings were placed in air-tight Plexiglas chambers 30 min before the experiment began. Thereafter, photographs of control and ethylene-treated plants were taken at 15-min intervals. Ethylene was applied 60 min after the start of photography (arrow). Each curve represents the average of four or five individual plants. The error bars represent SD.

Figure 2. Fluence-response curve for blue light-induced inhibition of elongation in 4-d-old dark-grown pea seedlings. Inhibition during 12 min after onset of blue light was calculated as percentage inhibition from an average growth rate over 18 min before blue-light irradiation. Growth rates were determined at 6-min intervals. Error bars indicate SE for 10 replicate treatments.
half-order of magnitude of those reported for red light-grown pea seedlings. The lag time for the blue light-induced inhibition of elongation is also at least as rapid in dark-grown seedlings as that observed in red light-grown peas. Figure 3 shows that blue light causes inhibition of elongation within 3 min after the onset of irradiation.

When we examined 4-d-old dark-grown plants that had been treated with 10 μL L⁻¹ ethylene, we found the latent time for ethylene action to be between 20 and 30 min (Fig. 4). It is unlikely that our failure to observe a more rapid response to ethylene was caused by the method we used to measure growth because our instrumentation was sufficient to detect a rapid inhibition of growth after blue-light irradiation that was of similar magnitude to the ethylene response observed before irradiation (11). The fact that we found a lag time of 20 to 30 min instead of 6 min (11) may be a consequence of differences in seed lots, the manner in which the plants were handled, the amount of lead time allowed between moving the plants and beginning the ethylene treatment, or other as-yet unidentified differences in the growth conditions. In addition, our plants were handled under IR light, whereas plants in Warner and Leopold's experiment were subject to irradiation with a "dim green light." Although their use of a green light may have triggered a light-induced growth response in the very low fluence range (7), it does not explain the fact that the lag times they observed varied in length depending on the concentration of ethylene applied. It would be interesting to discover if such a very low fluence response could, in some way, increase the sensitivity of pea seedlings to ethylene. It is possible that ethylene could cause an increase in tissue sensitivity to light-induced growth inhibition analogous to the effect of auxin on the light sensitivity of oat coleoptiles reported by Shinkle and Briggs (9). If so, the effect of green light is specific to the response of ethylene, as the response of dark-grown peas to blue light was unaltered by handling under dim green work light (data not shown).

Figure 3. Effect of blue light on the growth rate of dark-grown 4-d-old peas. Plants were exposed to 80 μmol m⁻² s⁻¹ blue light for 10 min; the interval during which the light was on is marked by arrows. This curve represents the average ± se for 10 replicate experiments.

Figure 4. Effect of ethylene on the growth of 4-d-old dark-grown pea seedlings. Elongation was calculated by measuring the distance between a mark placed at the top of the plant, just below the hook, and a mark placed at the base of that same internode. These distances were obtained from a series of time-lapse photographs. All manipulations, including photography, were performed with the aid of IR light. The data represent the averages of eight experimental and seven control plants.

In any event, the data we collected clearly separate the growth-inhibitory effect of ethylene from blue light-induced growth inhibition. The lag time between ethylene application and significant growth inhibition is much longer than the time required for blue light-induced inhibition. In addition, ethylene inhibited the growth of the apical portion of the

Figure 5. Effect of ethylene on blue light-induced growth inhibition in a marked region located at the base of the third internode of 6-d-old red light-grown pea seedlings. Seedlings were placed in air-tight Plexiglas chambers and photographed immediately. Plants that did not increase in length during the initial 30 min were removed from the population. From 60 min on, plants were exposed either to 250 μmol m⁻² s⁻¹ blue light (n = 8) or to 250 μmol m⁻² s⁻¹ blue light plus 100 μL L⁻¹ ethylene (n = 8). The relative increase in length prior to addition of blue light has been normalized to 1 for both curves. The curves are shown ± se.
third internode more strongly than it did the base. This relationship is the opposite of that seen after blue-light irradiation, where the base of the third internode is inhibited more strongly than the top (6). Therefore, we conclude that blue light does not act by inducing rapid ethylene production.

Because we had observed that cut sections were less sensitive to blue light than intact plants, we also wanted to know if the presence of ethylene could act as an inhibitor of blue-light sensitivity. To test this hypothesis, we simultaneously exposed pea seedlings to blue light and ethylene. When given alone, 250 μmol m⁻² s⁻¹ continuous blue-light treatment was sufficient to decrease the growth rate of a marked region located at the base of the third internode (Fig. 5). After the application of ethylene alone, there is a 40- to 60-min lag period during which growth the rate is unchanged (Fig. 1). During this period, ethylene had no effect on the ability of plants to undergo blue-light-induced growth inhibition (Fig. 5). Hence, it was concluded that ethylene does not inhibit the blue-light response and, hence, that the production of wound ethylene is not the cause of diminished blue-light sensitivity in cut pea stem sections.

LITERATURE CITED