Photocontrol of Hook Opening in Cuscuta gronovii Willd.1

Received for publication August 7, 1973 and in revised form November 12, 1973

RONALD F. KUJAWSKI2 AND F. H. TRUSCOTT
Department of Biological Sciences, State University of New York at Albany, Albany, New York 12222

ABSTRACT

Hook opening in seedlings of Cuscuta gronovii Willd. occurred only after prolonged exposures to blue, red, or far red light. Prolonged far red exposure was less effective than prolonged exposure to red or blue light. Brief far red irradiation inhibited the inductive effect of red light. The far red inhibition was in turn reversed by brief red irradiation. These effects suggest the involvement of two photosystems in the control of hook opening in Cuscuta gronovii Willd.: a phytochrome-mediated system and a separate high energy requirement.

The growth of dodder (Cuscuta gronovii Willd.) seedlings is characterized by the presence of a stem hook. Because cotyledons do not develop in dodder seedlings (22) this hook cannot be properly called an epicotyl or hypocotyl hook, although it resembles the stem hook characteristic of dicotyledonous plants. Our study of the photocontrol of hook opening in C. gronovii emerged from our investigations of the photocontrol of twining in this species (unpublished data). It was evident from preliminary studies that both hook opening and twining were photocontrolled and that these growth phenomena were physiologically distinct, requiring separate analysis with respect to the qualities of light necessary for each response.

MATERIALS AND METHODS

Seeds of Cuscuta gronovii Willd. (24) were prepared for germination by soaking them in concentrated H2SO4 for 30 or 60 min (6). Older seeds were observed to require a longer treatment with H2SO4 in order to attain germination success near 100%. Seeds were then rinsed at least 10 times in distilled water. These were scattered on the surface of moist sand in 11-inch diameter bowls. The bowls were covered with a thin sheet of plastic film to prevent evaporation and were maintained in growth chambers at 25°C in total darkness.

For examining the hook opening response of dodder, 6-day-old seedlings were used. The seedlings were transferred to glass dishes, 100 × 50 mm, and placed on one sheet of filter paper moistened with 5 ml of distilled water. Each dish contained 20 or 25 seedlings. Following exposure to various light regimes the dishes of seedlings were covered with a Petri dish lid and maintained in the dark. Seedlings were examined at 24-hr intervals for hook opening.

White light was supplied by eight 40-w warm-white fluorescent tubes and four 60-w incandescent bulbs in a Model MB-54 Percival growth chamber maintained at 25°C. The intensity of this light at the height of the plant material, as measured with a Weston illumination meter, Model 756, was approximately 1000 ft-c. Far red light was supplied by two or four 150-w flood lamps (General Electric 150 PAR/FL). The filter system used was a modification of the Klein system (11) and is described by Poell and Norris (17). Light was filtered through a far red filter (Carolina Biological Supply Far Red 750) plus 10 cm of ferrous ammonium sulfate solution (100 g/l). H2SO4 was added to a concentration of 1% (v/v) along with a piece of brass wire to retard the oxidation of the ferrous ammonium sulfate (13). The red light source consisted of two or four 150-w flood lamps. Light was filtered through a 3-mm thick sheet of Rohm and Haas red Plexiglas 2444 plus 10 cm of copper sulfate solution, 1% (v/v). Concentrated H2SO4, was added to the copper sulfate solution to a concentration of 0.2% (v/v). Blue light was obtained with four 150-w flood lamps and a 3-mm thick sheet of Rohm and Haas blue Plexiglas No. 2045 separated by 10 cm of copper sulfate solution, 1% (v/v), containing H2SO4 at 0.2% (v/v) concentration. A green safelight was used throughout the experiments when making observations and manipulating dark-grown seedlings for experimental treatments. The safelight consisted of two 15-w green fluorescent tubes (Sylvania F15T8/G) wrapped with several layers of green cellophane. Several samples of seedlings were used to test the influence of the safelight on hook opening. No effects due to the safelight were apparent.

The intensity of the incandescent lamps used in these lighting arrangements was controlled by a variable autotransformer. The setting on the variable autotransformer was selected arbitrarily to yield an incident energy level consistent with that used by other workers investigating photomorphogenesis (5) and are recorded below in conjunction with each of the several experiments reported here.

The spectral energy distribution of each light source was determined by an ISCO spectroradiometer, Model SR. The incident energy obtained from each light source was calculated by integrating the area beneath the spectral energy distribution curves with the aid of a compensating polar planimeter.

The growth habits of dodder seedlings were examined by time-lapse photography. The photographic apparatus consisted of a 16-mm Arriflex movie camera controlled by an Arriflex intervalometer, Model 2. Frames were shot at 20-sec intervals. Incandescent light from one or two 75-w Ken Rad flood lamps served to illuminate the plant material. Films were analyzed using an LW photoanalyzer, Model 224-A, and sequences of events in the various plant responses were traced from projected film images.

RESULTS

The initial step in the investigation of hook opening was the definition of the process and of the criteria to be used in de-
terminating hook opening. Figure 1 shows the sequence of events in the hook-opening response of a dark-grown seedling following exposure to incandescent light. This diagram is traced from the projected image of the time-lapse movie of the hook opening of a single plant. The numbers on the horizontal axis indicate the elapsed time at each stage represented. Zero time is the beginning of a continuous exposure to incandescent light. In this sequence the original position of the elbow of the hook was marked with acrylic paint. The movement of this elbow up the stem relative to the original mark is evident. This type of movement is also characteristic of bean hypocotyl hook movement (18). The hook of bean moves up the hypocotyl into the epicotyl and opening occurs. In the case of Cuscuta gronovii, the hook appears to run off the end of the stem.

Under the continuous incandescent irradiation used for photographing the plant, hook opening occurred in about 12 hr. It will be shown that the response requires considerably longer time for completion in the dark following light induction.

Two ancillary aspects of seedling behavior in dodder complicate the study of the hook-opening process. Figure 1 illustrates a phenomenon of stem looping and shows the formation of three such loops during the 12.6-hr time span: at 2.6, 5.7, and 8.3 hr. The occurrence of such loops has been described by Lane and Kasperbauer (15) as a stage in the hook opening process. Although our analysis of time-lapse films showed that looping occurred several times during the hook-opening process, similar looping was observed in seedlings given no light exposure and in which hooks therefore remained closed. It was also observed in seedlings given brief red or blue irradiations of insufficient duration to bring about subsequent hook opening in the dark. Therefore, we do not regard looping as a part of the hook-opening process per se, but rather a separate event which accompanies the opening process and one which involves the same general region of the stem. Since the stem displays a rotating type of movement or circumnutation following hook opening, the looping which occurs during opening may well be more closely related to circumnutation than to the hook-opening process itself.

This looping movement, which in turn results in a highly variable angle at the hook elbow, negates the use of any quantitative measurement of the angle of hook opening such as that which is typically used in studying bean hook opening (14). Hence, along with earlier workers (15, 16), we have been forced to apply only qualitative criteria in determining the opened state of the stem hook.

The second complicating factor, and one not considered by earlier workers, becomes apparent when examining seedlings for the hook-opening response to brief light irradiations. It was observed that hooks considered to be open at the 48 hr observation were closed following a subsequent 24-hr dark incubation. Closer examination revealed the presence of a slight angle to the stem or an elbow as depicted in Figure 2. Such an elbow...
was not always obvious under the dim safelight used when making experimental observations. Observations made after the elbow was marked with acrylic paint revealed that the elbow often persisted and moved up the stem as did the more obvious hook configuration of a closed hook. Vertical stems with this slight elbow were rare in seedlings that received no light exposure, but occurred most often in stems given brief irrigations of red or blue energy. It is concluded that this growth pattern may be a consequence of geotropic effects rather than of the hook-opening process itself. In applying only qualitative judgement, it is necessary to rule out as "open" those stems which falsely appear to be without hooks (e.g., Fig. 2b). Thus, the hook is considered open when the entire stem assumes a vertical aspect and is free from the presence of any elbow-like constrictions.

That light is a controlling factor in hook opening is shown in Table I. Intact dark-grown seedlings were exposed to white light with an intensity of 1000 ft-c. Following exposure, seedlings were placed in the dark and observed for the hook-opening response at 24 and 48 hr after the initiation of the light exposures. The most obvious observation is the consistent lack of hook opening in the plants given no irradiation. There is a relationship between the percentage of hook opening and the total energy received. Prolonged irradiation induces the highest percentage of hook opening. Under conditions of continuous light exposure, at this intensity, 100% hook opening is accomplished in about 12 hr. Durations of exposure less than 12 hr have an inductive effect on hook opening. The seedlings given shorter durations of light irradiation complete the hook opening in the dark but at a slower rate. Continuous low intensity irradiation with blue, red, or far red light yielded a 100% hook-opening response after 24 hr with blue and an 83% response with red light as shown in Table II. The intensity of the irradiation for each quality of light was approximately the same, 100 μW/cm². Far red light had no promotive effect on the hook-opening response. Green safelight also had no effect on hook opening.

Figure 3 demonstrates the relation between red light dose and the hook-opening response of dodder seedlings. Dark-grown seedlings were exposed to 270 μW/cm² of red light for various periods ranging from 1 min to 4 hr and then allowed to develop in the dark. Observations were made 24, 48, and 72 hr after the initiation of light treatments. It can be seen that the magnitude of the response is dependent upon the total incident energy received.

A similar effect was obtained when examining the dose-response effect of blue light of 250 μW/cm² (Fig. 4). The energy dependence is again evident. If such blue irradiation is terminated with a brief red irradiation the percentage hook opening dramatically increases as shown in Table III. There is sufficient variability in the percentage of response among different seed batches (30–35% response in Fig. 4 with 14–16% response in Table III) to cause us to question the potentiating effect of the 5 min red exposure when observations are made at

### Table I. Effect of Light on Hook Opening

Dark-grown seedlings were exposed to white light of 1000 ft-c for durations as indicated. Exposed plants were dark-incubated and observed after 24 and 48 hr for hook opening. The response of 25 seedlings was observed at each treatment.

<table>
<thead>
<tr>
<th>Duration of Exposure (min)</th>
<th>Hook Opening</th>
<th>24 hr</th>
<th>48 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>8</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>4</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>8</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>60</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>360</td>
<td>72</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>480</td>
<td>96</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

### Table II. Effect of Continuous Light Irradiation on Hook Opening

Dark-grown seedlings were exposed to continuous light as indicated. Hook opening was determined after 24 hr of exposure. Radiant energy of each source was about 100 μW/cm². Blue, red, and far red light sources are given in the text. Sample size was 60 seedlings per light treatment.

<table>
<thead>
<tr>
<th>Experimental Treatment</th>
<th>Hook Opening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark</td>
<td>0</td>
</tr>
<tr>
<td>Far red</td>
<td>0</td>
</tr>
<tr>
<td>Blue</td>
<td>100</td>
</tr>
<tr>
<td>Red</td>
<td>83</td>
</tr>
</tbody>
</table>

### Table III. Terminal Red Irradiation and Hook Opening

Each datum is based upon a total of 50 seedlings. Observations were made at 24, 48, and 72 hr after initiation of light treatments.

<table>
<thead>
<tr>
<th>Experimental Treatment</th>
<th>Hook Opening</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hr</td>
<td>14</td>
</tr>
<tr>
<td>48 hr</td>
<td>62</td>
</tr>
<tr>
<td>72 hr</td>
<td>4</td>
</tr>
</tbody>
</table>

### Table IV. Reversibility of Light-induced Hook Opening

Each datum is based upon a total of 50 seedlings.

<table>
<thead>
<tr>
<th>Experimental Treatment</th>
<th>48 hr</th>
<th>72 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 HR red</td>
<td>48</td>
<td>56</td>
</tr>
<tr>
<td>4 HR red, 5 min far red</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4 HR red, 5 min far red, 5 min red</td>
<td>24</td>
<td>54</td>
</tr>
<tr>
<td>4 HR blue</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>4 HR blue, 5 min red</td>
<td>26</td>
<td>62</td>
</tr>
<tr>
<td>4 HR blue, 5 min red, 5 min far red</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4 HR blue, 5 min red, 5 min far red, 5 min red</td>
<td>2</td>
<td>24</td>
</tr>
</tbody>
</table>

1 Red energy = 270 μW/cm² (600-700 nm).
2 Far red energy = 450 μW/cm² (700-900 nm).
3 Blue energy = 250 μW/cm² (400-550 nm).
48 hr. However, the potentiating effect of red light following an exposure to blue or far red is dramatic when observed 72 hr following the light exposure. This effect was also observed for seedlings given a high energy far red irradiation followed by a brief red irradiation. The brief red irradiation alone did not yield a comparable hook opening response.

The photoreversibility of the system is demonstrated in Table IV. The inductive effect of a long duration of red light could be effectively reversed by a brief exposure to far red. The far red effect was in turn reversed by a brief exposure to red light. Similarly, the inductive effect blue light followed by brief red light is reversed by far red irradiation (Table IV).

The escape reaction from the photoreversibility could not be demonstrated even after a 24-hr interval between red promotive irradiation and the photoinhibitory far red irradiation.

**DISCUSSION**

The photomorphogenic responses of *Cuscuta gronovii* are affected by phytochrome. This conclusion is consistent with those of Zimmermann (25) for *Cuscuta pentagonii* and of Lane and Kasperbauer (15) for *Cuscuta indecora.* The results reported here add further information concerning the nature of these effects, and point out certain differences.

Hook opening in seedlings of *C. indecora* was reported by Lane and Kasperbauer to be stimulated by brief exposures to red light. Although they could not clearly demonstrate the involvement of a high energy reaction, they felt that such a photoreaction was involved. The results reported here demonstrate the participation of a high energy photoreaction. This reaction was shown to be an energy-dependent reaction and could be stimulated by either blue, red, or far red light. Brief irradiations alone, such as those used by Lane and Kasperbauer, were not effective. Following prolonged irradiation with these light qualities, brief exposures to red or far red light yielded effects characteristic of phytochrome mediated morphogenesis.

Clearly two photoreactions are involved in hook opening. Klein et al. (14) demonstrated the involvement of two photoreactions in the hook-opening process in beans, a red-far red reversible reaction, and an energy-dependent reaction. A logarithmic relationship was found between the angle of opening and red energy. The nature of the HER was not discussed.

The HER found for hook opening in *C. gronovii* is atypical in that blue, red, and far red were shown to be effective. Most current interpretations of HER responses are based upon phytochrome as the photoreceptor (2, 7, 21). These models explain far red-induced responses. Pizzolongo (16) reported that photomorphogenesis of *Cuscuta pentagonii* seedlings was controlled by a blue light-induced high energy photoreaction. The blue-induced responses were reversed by exposures to wavelengths greater than 500 nm. He concluded that carotenoid pigments served as photoreceptors and mediated the responses. However, inasmuch as he did not separate the morphogenic events (i.e., hook opening versus subsequent coiling) in seedling growth, he did not observe phytochrome effects. The results reported by Pizzolongo are inconsistent with those reported by Zimmermann (25) and by Lane and Kasperbauer (15) mentioned earlier. They are, however, consistent with the involvement of blue light in photomorphogenesis reported here. Neither Zimmermann nor Lane and Kasperbauer investigated blue involvement in hook opening although Lane and Kasperbauer reported a blue-induced HER for potentiating of coiling.

Most recently, interest has focused upon photosynthesis as a contributor to HER responses (3, 4, 19, 20). Through the use of specific inhibitors of noncyclic and cyclic photophosphorylation, these workers demonstrated the involvement of photosynthesis in red HER and far red HER responses.

The nature of the high energy reaction involved in the hook-opening response of dodder is not known and has only been reported here. It is of interest to speculate on the possibility of photosynthetic involvement in the HER. The presence of photosynthetic pigments has been shown in other species of dodder. The presence of Chl *a* and carotenoids in *C. gronovii* was demonstrated by the authors (unpublished data). Kerstetter and Hull (10) found that *Cuscuta pentagonii* fixes CO₂ as a result of photosynthetic activity. Baccarini (1) reported similar findings for *C. australis.* The relation of any such high energy photosynthetic reaction to hook opening remains to be determined.

Klein (12), finding that an antiauxin promotes hook opening, proposed that red light-induced HER in hook opening of bean acts by reducing the level of auxin in the hook region. Kang and Ray (8) found that red light did not affect the amount of diffusible auxin released by hook tissue. Rather, they concluded that light induces hook opening by inhibiting the production of ethylene in hook tissue (9). The hypotheses proposed by these authors do not include a consideration of blue- or far red-induced HER and hook opening.

*Fig. 3.* Dose-response curve for hook opening of seedlings exposed to red light. Observations on hook opening were made at 24, 48, and 72 hr after initiation of light treatments. Each point is based upon the hook-opening response of 50 seedlings.

*Fig. 4.* Dose-response for hook opening of dark-grown seedlings exposed to blue light. Observations were made at 24, 48, and 72 hr after initiation of light treatments. Each point is based upon the hook-opening response of 50 seedlings.
Lane and Kasperbauer, (15) in describing the hook-opening process of dodder, noted that looping was observed and referred to this as a stage in the opening. We have observed such looping in stems opening in continuous light, in unopened stem hooks in continuous darkness, and in stems given brief non-inductive blue and red irradiations. We suggest that this looping not be considered as a criterion for hook opening. It appears that this movement is a geotropic response similar to that discussed above in relation to vertical stems possessing a slight elbow. Because these movements are most frequent following brief red or blue light irradiation, it seems probable that they are geotropic responses. Sensitivity to geotropic stimuli has been shown to occur following very low energy irradiations of these light qualities in other plant systems (23). The reversibility of this sensitivity was lost during a subsequent dark incubation period following the irradiation as discussed by Wilkins (23). Perhaps this accounts for the more obvious hooked configuration of dodder stems initially demonstrating looping.

LITERATURE CITED