Photocontrol of Hypocotyl Elongation in Light-Grown Cucumis sativus L.1

RESPONSES TO PHYTOCHROME PHOTOSTATIONARY STATE AND FLUENCE RATE

VICTOR GABA*2 AND MICHAEL BLACK
Department of Biology, Queen Elizabeth College, (University of London), Campden Hill Road, London W8 7AH, England

Received for publication July 6, 1984 and in revised form July 10, 1985

ABSTRACT

The effects of the calculated photostationary state of phytochrome (ϕe) and the photon fluence rate on the elongation growth of the hypocotyl of light-grown seedlings of Cucumis sativus L. are examined. Two threshold responses to ϕe are found at values of 0.06 and 0.43. At ϕe = 0.06, there is no response at any fluence rate. In the ϕe range 0.1 to 0.43, elongation growth does not respond to changes in ϕe. Above the second threshold (ϕe = 0.43), there is a strong response to changes in ϕe. At all values of ϕe at and above 0.1, there is a response to fluence rate. A linear relationship can be demonstrated between a factor comprised of the logarithm of phytochrome cycling rate (a fluence-rate-dependent process) and ϕe, and the growth response.

It is clear that in light-grown plants of certain species, including cucumber seedlings, photocontrol of hypocotyl and stem elongation is exerted by a specific BL3 photoreceptor and by phytochrome (5, 7, 12, 25, 26). In the case of green cucumber seedlings, several features of photocontrol by a BL photoreceptor have been described, for example the action kinetics, the fluence-rate dependency, and the interaction with phytochrome (1, 7–9). Several aspects of control through phytochrome are also known, including the effect of prolonged irradiation with red light, the kinetics of action of the pigment, and photoconversion by both hypocotyl and cotyledons (4, 7). As far as control by the fluence rate of white light is concerned, it is probable that elongation responses of the light-grown cucumber hypocotyl are governed, at least partially, by a BL photoreceptor (7, 8, 10), but it is not yet clear to what extent phytochrome also participates; nor do we know how far differences in phytochrome photoequilibria are involved in control by the light environment. In the case of stems of several other species, it is well known that growth rates are controlled by both the photostationary state of phytochrome and by fluence rate (8, 12, 13, 20, 24). However, such examinations of the responses to these photobiological parameters have been performed using either: (a) light sources with no particular regard to the effect of the BL component (6, 12, 17, 19, 20, 24); or (b) a species (Sinapis alba), the hypocotyl of which, when de-etiolated, displays no specific response to BL (3). The work reported here is therefore the first which is directed towards the elucidation of the effects of ϕe and fluence rate in a BL-sensitive plant but in the absence of BL. The mechanism of control of elongation by fluence rate via phytochrome is still unclear, the evidence so far perhaps indicating that phytochrome cycling may be important (27). However, as another view holds that fluence-rate dependence is due to a loss of Pfr by dark (thermal) processes (13), we also examined the role of phytochrome cycling rate in fluence-rate dependent processes. A secondary issue relates specifically to the rapid response of the hypocotyl of light-grown Cucumis to BL, which apparently fails to induce a response characteristic of that to phytochrome, even though photoconversion might be caused by such a BL treatment (7). It has been hypothesized (8) that the lack of response to conversion of phytochrome by BL might be due to a threshold requirement in Cucumis, and a detailed investigation of the response to ϕe would reveal whether such a theory was tenable or not. In this paper, we examine these aspects of photocontrol by phytochrome and seek to clarify the role of this photoreceptor in the cucumber seedling’s response to light.

MATERIALS AND METHODS

Plant Material. Seeds of Cucumis sativus L. cv Ridge Greenline (Gunson, Witham, Sussex, England) were imbibed and planted on wet vermiculite. After growth in darkness for 90 h at 25°C, seedlings were selected for height (30–40 mm) and transplanted to fresh wet vermiculite in plastic boxes. The plants were then de-etiolated at 25°C for 30 h in white fluorescent light (‘daylight’ tubes: Thorn Electric) at 100 μmol m−2 s−1 as previously described (8). After de-etiolation, seedlings were marked with waterproof ink 20 mm below the cotyledonary node immediately before experimental irradiation (see below). In all experimental treatments, irradiation began with a 20 min exposure at high fluence rate to saturate the photoconversion of phytochrome and establish the requisite ϕe value. After the initial high-fluence-rate treatment, the plants received different fluence rates at the same (experimental) ϕe, for the remainder of the 24 h experimental period. Dark control seedlings received a saturating 20 min exposure to FR prior to being placed in darkness for the remainder of the 24 h period.

Measurement of Growth. The extension growth of the 20 mm marked hypocotyl unit was recorded 24 h after marking. The percentage inhibition (I) was calculated as follows:

\[ I = 100 - \left( \frac{X}{D} \right) \times 100 \]

where X and D are the mean growth increments in the light and dark, respectively. Each experiment involved the irradiation of plants of 1–10 replicates. The results presented are the means of three

1 Supported by the SERC and the Royal Society.
2 Present address: Department of Plant Genetics, Weizmann Institute of Science, Rehovot 76100, Israel.
3 Abbreviations: BL, blue light; FR, far-red light; H, phytochrome cycling rate; Pfr/Ptot, the ratio of Pfr to the total phytochrome content; ϕe, photostationary state of phytochrome; abc, calculated photostationary state of phytochrome.
or four replicates. Standard errors of these mean values are generally less than 3%, and at the most were 5%, and are not shown in the figures.

**Experimental Irradiations.** The seedlings were irradiated in a growth chamber of internal dimensions 2 m long, 0.3 m wide, and 0.4 m high. Controlled-temperature air (25°C) entered the cabinet from below the plants. Light for experimental purposes entered the cabinet through a window at one end. The light beam from tungsten halogen lamps (1× Rank Strand 1 kW T80 spotlamps and 2× 500 W Thorn Electric Sunflood lamps), passed through a water filter (150 or 300 mm thick) and a series of optical filters (see Table I) prior to entering the growth chamber. Different fluence rates of light were obtained at different positions in the cabinet and by the use of neutral density screens which did not alter the spectral photon distribution. The plants were placed in lines normal to the incident light beam. Blue light contamination (in the 300- to 500-nm range) in all light sources was estimated to be less than $1.5 \times 10^{-3} \text{ mol m}^{-2} \text{s}^{-1}$ and was insufficient to cause hypocotyl inhibition (1) or photomorph (22). The level of BL contamination in the light sources could not be measured directly by spectroradiometry, due to the level of internal light scattering in the radiometer unit. However, placing a 449-nm Schott interference filter in front of the spectroradiometer probe blocked the R and FR wavelengths and no measurable BL was found. Similarly, no BL could be seen when the source was viewed through Cinemoid filters and the interference filter.

**Measurement and Evaluation of Light.** Fluence rates and spectral photon distributions were measured with a computer-controlled Gamma Scientific (California) spectroradiometer (11), which also calculated the photosynthetic state of phytochrome ($\phi$) and the cycling rate or photosynthetic flux ($H$) as described by Barton and Frankland (2). Our spectroradiometer system yielded values in close agreement with those from the spectroradiometer and *in vivo* spectrometry methods of Barton and Frankland (2) (M. Barton, personal communication), but as noted by these workers, $\phi$ and $H$ may not be a completely accurate representation of the state of phytochrome inside the plant tissues, due to dark (thermal) reversion of Pfr. A similar technique has also been used by Ritter et al. (20) and Holmes et al. (13), and the term $\phi$ is equivalent to their $k_{0}/(k_{0} + k_{s})$. Additionally, the actual $\phi$ in green plants may be lower than $\phi_{i}$ due to preferential light absorption by Chl (13, 20).

<table>
<thead>
<tr>
<th>$\phi$</th>
<th>Water Filter</th>
<th>CuSO$_4$.5H$_2$O in 17-mm-Thick Tank</th>
<th>Gel Filters</th>
<th>No. of layers</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.06</td>
<td>300</td>
<td>g dm$^{-3}$</td>
<td></td>
<td>2 of No. 58 + 1 of No. 20 Cinemoid*</td>
</tr>
<tr>
<td>0.1</td>
<td>150</td>
<td></td>
<td></td>
<td>2 of No. 58 Cinemoid + 1 of No. 92 Chromoid</td>
</tr>
<tr>
<td>0.27</td>
<td>300</td>
<td></td>
<td></td>
<td>2 of No. 58 + 1 of No. 15 Cinemoid</td>
</tr>
<tr>
<td>0.37</td>
<td>150</td>
<td></td>
<td></td>
<td>3 of No. 58 + 1 each of No. 25 and 26 Cinemoid</td>
</tr>
<tr>
<td>0.43</td>
<td>150</td>
<td></td>
<td></td>
<td>3 of No. 58 + 1 of No. 26 Cinemoid</td>
</tr>
<tr>
<td>0.54</td>
<td>150</td>
<td></td>
<td></td>
<td>3 of No. 58 Cinemoid</td>
</tr>
<tr>
<td>0.62</td>
<td>300</td>
<td>2.5</td>
<td></td>
<td>3 of No. 58 Cinemoid</td>
</tr>
<tr>
<td>0.69</td>
<td>300</td>
<td>10</td>
<td></td>
<td>3 of No. 58 Cinemoid</td>
</tr>
<tr>
<td>0.73</td>
<td>150</td>
<td>15</td>
<td></td>
<td>2 of No. 58 Cinemoid</td>
</tr>
<tr>
<td>0.77</td>
<td>Grolux fluorescent tubes with a layer of Cinemoid No. 14 as in Ref. 7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Cinemoid and Chromoid were obtained from Strand Electric Engineering Co.

**RESULTS**

The basic experimental procedure was to vary the fluence rate of light at each $\phi$ value. Seedlings respond to both fluence rate and $\phi$ values above 0.06, but light of $\phi$ of 0.06 has no inhibitory effect at any fluence rate (Fig. 1). The responses elicited by $\phi$ values of 0.1 and 0.27 are similar, with growth inhibitions from about 3% to about 25% over about a 10$^3$-fold range of fluence rates. Photosynthetic states of 0.37 and 0.43 are slightly more effective than the lower $\phi$ values and again the response increases (from approximately 10-45% inhibition) with a 10$^3$-fold increase in fluence rate. At $\phi$ values above 0.43, inhibition is markedly dependent on both the photoequilibrium and the fluence rate, when the latter is above approximately 3 μmol m$^{-2}$ s$^{-1}$. Photosynthetic states from 0.69 to 0.77 produce high levels of inhibition, and display closely similar fluence-rate dependencies.

Fluence-rate dependency has been considered in terms of phytochrome cycling rates (2, 15, 20, 23, 24, 27, 29). Calculation of the data shown in Figure 1 in terms of cycling rate is presented in Figure 2. The lowest $\phi$ value again produces no growth inhibition at any cycling rate. All $\phi$ values from 0.1 to 0.43 induce equivalent responses at equivalent cycling rates. Differences between the curves for these $\phi$ values, which are apparent in Figure 1, are eradicated when cycling rates rather than fluence

![Fig. 1](https://example.com/fig1.png)

**Fig. 1.** Plot of percentage inhibition (of dark control elongation) against photon fluence rate at various values of $\phi$. The response to light was measured after 24 h continuous irradiation. The numbers on the lines refer to the values of $\phi$ established in those plants.

![Fig. 2](https://example.com/fig2.png)

**Fig. 2.** Plot of percentage inhibition of elongation against phytochrome cycling rate ($H$) at various values of $\phi$. The data here were recalculated from those of Figure 1. Numbers on lines refer to the $\phi$ of
rates are used. The differences in inhibition due to \( \phi_c \) values above 0.43, seen in Figure 1, are again evident. When the response to \( \phi \), at fixed cycling rates is derived from Figure 2, the effect of changes in \( \phi_c \) can be discerned more clearly (Fig. 3). Two thresholds of action of phytochrome photostationary state are seen, 0.06 and 0.43. In the \( \phi \) range 0.10 to 0.43, there is no response to changes in \( \phi_c \), but only to the cycling rate. Above \( \phi \) of 0.43 there is a strong response to both \( \phi_c \) and \( H \), though at higher \( \phi \) (>0.69) changes in magnitude of \( \phi_c \) become less important.

An empirical equation relating \( \phi \) and \( H \) to the growth response can be constructed using the approach of Bartley and Frankland (2), who analyzed seed germination using an equation with empirically derived constants. Our equation (Eq. 1), which incorporates cycling rate \( (H) \) and \( \phi_c \), yields a value which is directly related to the inhibition of hypocotyl elongation growth (Fig. 4).

\[
\text{Inhibition} = a \left( \phi_c - b \right) f \left( \phi_c - b \right) + c \log H + d \tag{1}
\]

where the constant \( a \) is 161, \( b \) is 0.43, \( c \) is 12, and \( d \) is 51. This equation is constructed to take into account the failure of seedlings to respond to changes in \( \phi_c \) between 0.1 and 0.43 and that changes in \( \phi_c \) above 0.43 have a more powerful effect on the growth response than changes in log \( H \). The equation can be used predictively to relate \( \phi \) and cycling rate to growth inhibition. We should note, however, that the equation is valid only in the absence of \( BL \).

**DISCUSSION**

We have shown here for the first time in a light-grown \( BL \)-sensitive plant, that there are cycling-rate-dependent responses (Fig. 2) which operate only through phytochrome, for they occur in the absence of \( BL \). In addition, such plants have strong responses to \( \phi \), above a threshold value of 0.43 (Fig. 3). It is noteworthy that between the two threshold values of \( \phi \) of 0.1 and 0.43, there is the unusual situation whereby only changes in cycling rate elicit a growth response (Figs. 2 and 3). In addition, we have previously found that the \( Pfr/Ptot \) ratio in \( BL \) measured in vivo in \( Cucumis \) is at maximum 0.4 (7, 10) which is the threshold value for response to \( \phi_c \) (Fig. 3). This indicates that \( BL \) is incapable of photoconverting enough phytochrome to induce a strong response and would explain why long term \( Pfr \)-type responses are not produced by a \( BL \) pulse (7), i.e. the threshold theory (8) appears to be correct.

Threshold responses to \( \phi \), have been reported previously in the control of hypocotyl elongation (13, 20, 27, 28) and other responses (21, 30). Graded responses to \( Pfr/Ptot \) ratio have also been noted in several species (6, 13, 17, 19, 20, 27). The two thresholds may represent two sites of \( Pfr \) action in cucumber, and once the second threshold \( ( \phi_c = 0.43 ) \) is exceeded the response becomes quantitatively different by varying continuously with \( \phi_c \). At each site of phytochrome action cycling-rate-dependent processes must also operate.

As the growth response of green cucumber seedlings varies with the logarithm of the fluence rate, the response would not appear to depend solely on differences in photosynthesis, as the latter varies directly with the fluence rate. Fluence-rate-dependent inhibition is also found even when the cotyledons are covered (using the method described in 4) indicating that photosynthetic activity of these organs (the major photosynthetic site) is not required. Photosynthesis does, however, contribute to the photoinhibition of elongation growth, since the inhibition is slightly reduced by \( DCMU \); but we do not yet know the extent to which photosynthesis might be involved in the elongation response. Experiments with the herbicide norflurazon, which blocks carotenoid synthesis and produces bleached plants, do not clarify the role of photosynthesis in \( Cucumis \). Such white \( Cucumis \) plants lack most of the response to red light, including the effect via the cotyledons, but this may be due to nonspecific damage as norflurazon greatly retards seedling growth and development even in darkness (10). Nevertheless, inhibition in norflurazon-bleached seedlings of other species is dependent on fluence rate.
demonstrating that photosynthesis is not a primary part of the response (3, 20).

The fluence-rate-dependence of hypocotyl inhibition must involve processes other than photosynthesis or the photoconversion of phytochrome to establish a particular photoequilibrium, as in these experiments the photoconversion was saturated before fluence-rate-dependency was tested. Cycling between the two forms of phytochrome has been suggested as a basis of fluence rate effects in photomorphogenesis (2, 14–16, 23, 24, 27, 29). In the present experiments, the relationships between inhibition, \( \phi _a \), and fluence rate are clarified when the data are considered in terms of phytochrome cycling rate. Our findings differ from those for green Chenopodium rubrum in which inhibition varies with \( \phi \) between 0.2 and 0.77 at all cycling rates (20). It is unclear if this difference is species dependent, if it is caused by screening high concentrations of Chl and other pigments in Chenopodium, or if it results from the presence of BL in the experimental treatments employed. There are conflicting reports of whether (13) or not (27) there is fluence-rate-dependent inhibition of Sinapis alba hypocotyl elongation at \( \phi \) of less than 0.4.

An empirical equation (Eq. 1) has been constructed which linearly relates \( \phi \) and \( H \) with the growth response (Fig. 4). This equation does not prove a relationship between \( \phi _a \), \( H \), and growth response, but it does demonstrate that it is possible to relate measurable parameters of the light environment, in terms of the photochemical behavior of the phytochrome system, to a plant response, as in the data of Bartley and Frankland (2). It has predictive value and can be used to explore the interaction between \( \phi _a \), \( H \), and other factors in photocontrolled growth such as responses to the specific BL photoreceptor, as well as for comparisons of the responses of dark- and light-grown plants.

The above findings suggest how \( \phi \) and \( H \) participate in the perception of natural light by Cucumis. In open, unshaded conditions, fluence rate effects at \( \phi = 0.55 \) (a typical value of \( \phi \) in full sunlight [25]) might be due to differences in cycling rate, as would also happen in nonvegetational shade. Vegetational shade will permit more growth because of reduced \( \phi _a \) and \( H \), but when \( \phi \) has fallen below 0.43 differences in cycling rate become the only controlling factor (Fig. 3). However, these features will probably be modified by temperature changes (28).

While perception by phytochrome—invoking both \( \phi \) and \( H \)—is important in responses to shade as outlined above, we note that a blue-absorbing receptor also has a key role. Indeed, since the regulation of hypocotyl elongation growth by a BL photoreceptor is much more sensitive to fluence rate from its threshold of 3 \( \mu \)mol m\(^{-2}\) s\(^{-1}\) to 220 \( \mu \)mol m\(^{-2}\) s\(^{-1}\) (10) this receptor, rather than phytochrome, might well be the dominant fluence-rate detector (7, 24).

Acknowledgements—The authors thank Dr. M. Bartley for the use of his unpublished data and Drs. B. Frankland and R. Perry and Professor J. Gressel for advice. For technical assistance, we thank Nasim Patel and the late Graham Klein, to whom this paper is dedicated.

LITERATURE CITED

4. BLACK, M JE SHUTTLEWORTH 1974 The role of the cotyledons in the photocontrol of hypocotyl extension in Cucumis sativus L. Planta 117: 57–66
5. EVANS, LT, SB HENDRICKS, HA BORTHWICK 1965 The role of light in suppressing hypocotyl elongation in lettuce and Petrocea. Planta 64: 201–218
7. GARA, V, M BLACK 1979 Two separate photoreceptors control hypocotyl growth in green seedlings. Nature 278: 50–54