A Common Fluence Threshold for First Positive and Second Positive Phototropism in Arabidopsis thaliana

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ABSTRACT

The relationship between the amount of light and the amount of response for any photobiological process can be based on the number of incident quanta per unit time (fluence rate-response) or on the number of incident quanta during a given period of irradiation (fluence-response). Fluence-response and fluence rate-response relationships have been measured for second positive phototropism by seedlings of Arabidopsis thaliana. The fluence-response relationships exhibit a single limiting threshold at about 0.01 micromole per square meter when measured at fluence rates from $2.4 \times 10^{-3}$ to $6.5 \times 10^{-3}$ micromoles per square meter per second. The threshold values in the fluence rate-response curves decrease with increasing time of irradiation, but show a common fluence threshold at about 0.01 micromole per square meter. These thresholds are the same as the threshold of about 0.01 micromole per square meter measured for first positive phototropism. Based on these data, it is suggested that second positive curvature has a threshold in time of about 10 minutes. Moreover, if the times of irradiation exceed the time threshold, there is a single limiting fluence threshold at about 0.01 micromole per square meter. Thus, the limiting fluence threshold for second positive phototropism is the same as the fluence threshold for first positive phototropism. Based on these data, we suggest that this common fluence threshold for first positive and second positive phototropism is set by a single photoreceptor pigment system.

Although phototropism has been intensively studied since its description by Darwin (4), this physiological process is poorly understood. A fluence-response relationship is one of the most basic measurements that can be made on a photobiological system, showing the relationship between the response of the organism and the number of quanta incident upon or absorbed by the organism. However, for phototropism, the fluence-response relationship is extremely complex (Fig. 1). The curvature that is produced in response to low fluence and short irradiation times is referred to as first positive curvature. Curvature increases with increasing fluence of blue light for approximately 1.5 orders of fluence above a threshold of about 0.01 $\mu$mol m$^{-2}$, and then decreases with increasing fluence for an additional 1.5 orders of fluence to an indifferent zone (14). Curvature increases again at higher fluence and longer irradiation times, and this is referred to as second positive curvature (Fig. 1). We do not understand the basis for the descending arm of first positive curvature, or the basis for second positive curvature, although several models have been proposed to account for these complexities (6, 7, 12, 13, 19).

In the ascending arm of the fluence-response for first positive phototropism, curvature is proportional to the number of quanta. That is, for a given curvature, there is a reciprocal relationship between the fluence rate of the light and the time of irradiation (fluence rate $\times$ time = a constant). Therefore, curvature in the ascending arm obeys the Bunsen-Roscoe law of reciprocity (3). In contrast, the apparent fluence threshold for second positive curvature varies, depending upon the fluence rate of the light. Thus, in second positive phototropism, the response is primarily a function of the time of irradiation. This time threshold, which can be calculated for second positive curvature, varies depending on the report but is in the range of 4 to 10 min (2, 5, 14, 18).

We have reasoned for second positive phototropism that the plant is measuring the amount of time over which it is irradiated, and that there must be a time threshold. However, we also reason that the plant cannot measure the duration of irradiation without measuring the light itself (i.e. fluence of blue light). Based on this reasoning, we have measured the fluence-response relationship for Arabidopsis thaliana under conditions which satisfy the apparent time threshold requirement for second positive phototropism. Under these conditions, we find that first positive and second positive phototropism have the same quantum threshold. Based on these data, we suggest that the fluence threshold for both first positive and second positive phototropism is set by a single photoreceptor pigment system.

MATERIALS AND METHODS

Plant Growth and Phototropic Stimulation

Seeds of Arabidopsis thaliana (L.) Heynh. strain 'Estland' were sown in strips of micro-assay wells containing 0.7% (w/v) agar as previously described (16). The micro-assay strips were placed in clear plastic boxes, lined with moistened paper to maintain a high RH. Seed germination was potentiated by chilling at 5 ± 1°C in darkness for 3 d, and then exposing to white light for 20 h at 25 ± 1°C at a RH greater than 90%. At the end of the white light irradiation, the boxes containing the micro-assay strips were transferred into darkness at 25 ± 1°C for 42 h, at the end of which time the seedlings were exposed to the phototropic stimulus. Since green light is

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known not to be phototropically ‘safe’ (17), all manipulations were performed in complete darkness.

**Light Sources**

White light at 50 μmol m⁻² s⁻¹, used to potentiate seed germination, was provided by two General Electric (Cleveland, OH) Delux Cool-White fluorescent tubes. A slide projector equipped with a Sylvania 900 W BVA tungsten-halogen lamp, in combination with a 450-nm interference filter (10-nm half-bandwidth) was used as the light source in the phototropism experiments. The fluence rate was varied with neutral density filters. Fluence rates were measured using a Li-Cor (Lincoln, NE) LI-190SA quantum sensor in combi-
Fluence-response curves were measured at variable times and fluence rates for first positive and second positive phototropism by *Arabidopsis thaliana* seedlings to 450-nm light. The fluence threshold at about 0.01 μmol m⁻² for first positive phototropism (Fig. 3) coincides with the single limiting threshold fluence at about 0.01 μmol m⁻² for second positive phototropism (Fig. 4).

In addition, second positive phototropism by *A. thaliana* seedlings to 450-nm light was measured as a function of fluence rate for different exposure times (10, 20, 40, and 60 min). The fluence rate-response curves (Fig. 5) at each exposure time show increasing curvature above some fluence rate threshold to a maximum, and then decreasing curvature with still higher fluence rates. The threshold fluence rate decreases with increasing exposure time. However, within the limits to which a threshold can be determined, the threshold fluence rates multiplied by the exposure times show a constant single fluence threshold at about 0.01 μmol m⁻² (Fig. 5). This fluence threshold is the same as the fluence threshold for first positive curvature (Fig. 3), and the same as the limiting fluence threshold for second positive phototropism from the fluence response curves (Fig. 4).

Finally, curvature of *A. thaliana* seedlings to 450-nm light at a constant fluence of 0.5 μmol m⁻² has been measured as function of the time of irradiation from 3 to 3600 s. The results (Fig. 6) show a constant curvature of about 9° for irradiation times from 3 to 600 s, and a rapid increase in amount of curvature for longer irradiation times.

**RESULTS**

Experiments were terminated 60 min after the end of the light stimulus, although this resulted in a variable time after the beginning of the light stimulus. The seedlings were then gently mounted on transparent adhesive tape with the direction of bending in the plane of the tape surface. The tape was inserted into a photographic enlarger and the hypocotyl curvature traced, and later measured using a protractor. Only seedlings that emerged upright from the agar were used.

**DISCUSSION**

Based on these data (Fig. 6), second positive curvature by *Arabidopsis thaliana* seedlings has a minimum time requirement (threshold) of about 10 min. This is consistent with the time thresholds which can be calculated from previously reported fluence-response relationships for *A. thaliana* (8, 9, 16), *Avena* (1), *Zea* (2), and *Pilobolus* (11).

Under conditions in which the time of irradiation exceeds the time threshold, it can be demonstrated that second posi-

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**Figure 5.** Fluence rate-response relationships for second positive phototropism of *A. thaliana*. Etiolated seedlings were irradiated with 450-nm light at the indicated fluence rates for A, 10 min; B, 20 min; C, 40 min; D, 60 min. Curvature was measured 60 min after the irradiation. Each data point represents the mean curvature of 90 to 110 seedlings ± 1 SE. Fluence rate is indicated on the lower abscissa of each panel. The corresponding fluence is indicated on the upper abscissa of each panel.
tive phototropism shows a single threshold fluence of about 0.01 \( \mu \text{mol m}^{-2} \) (Figs. 4 and 5). This fluence threshold coincides with the fluence threshold for first positive phototropism (8, 9, 16; Fig. 2). The Bunsen-Roscoe law of reciprocity (3) is valid for the threshold of second positive phototropism. However, based on the fluence-response curves measured for different fluence rates (Fig. 4), reciprocity ceases to be valid at fluences slightly above the threshold of 0.01 \( \mu \text{mol m}^{-2} \). Thus, at the threshold for second positive phototropism, when the time exceeds the time threshold, the plant is indeed measuring the number of quanta.

These results lead to a model for second positive phototropism with two thresholds, one in fluence and one in the time of irradiation. Although first positive phototropism appears to use more than one pigment at different wavelengths and fluence rates (10), the common threshold for first positive and second positive phototropism can be interpreted to mean that the element that limits first positive phototropism and sets its fluence threshold, is the same element setting the fluence threshold for second positive phototropism. Because the quantum threshold for second positive phototropism is the same as that for first positive phototropism, and because reciprocity is valid for both thresholds, it seems unlikely that the fluence threshold for first and second positive phototropism is set by different pigment systems.

Since Bunsen and Roscoe (3) defined the law of reciprocity, an emphasis has been placed on defining the set of conditions under which that law is valid for a particular biological response. Based on the results presented here, there clearly is much to be gained from a consideration of the possible mechanisms for the failure of reciprocity. Phototropism is not the only system in which a failure of reciprocity has raised major questions. Under conditions of short irradiation times, phytochrome-regulated photomorphogenesis obeys the law of reciprocity. However, under longer irradiation times, reciprocity fails and this failure has generated problems concerning the nature of the photoreceptor pigment(s) involved (15).

In summary, second positive phototropism exhibits a time threshold of about 10 min. In addition, if the irradiation times exceed the time threshold, second positive phototropism exhibits a limiting fluence threshold of about 0.01 \( \mu \text{mol m}^{-2} \). Thus, the limiting fluence threshold for second positive phototropism is insufficiently different from that for first positive phototropism to warrant postulating regulation by different photoreceptor pigment systems.

**LITERATURE CITED**