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Desensitization and Recovery of Phototropic Responsiveness in *Arabidopsis thaliana*[1]

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Phototropism is induced by blue light, which also induces desensitization, a partial or total loss of phototropic responsiveness. The fluence and fluence-rate dependence of desensitization and recovery from desensitization have been measured for etiolated and red light (669-nm) preirradiated *Arabidopsis thaliana* seedlings. The extent of desensitization increased as the fluence of the desensitizing 450-nm light was increased from 0.3 to 60 μmol m^{-2} s^{-1}. At equal fluences, blue light caused more desensitization when given at a fluence rate of 1.0 μmol m^{-2} s^{-1} than at 0.3 μmol m^{-2} s^{-1}. In addition, seedlings irradiated with blue light at the higher fluence rate required a longer recovery time than seedlings irradiated at the lower fluence rate. A red light preirradiation, probably mediated via phytochrome, decreased the time required for recovery from desensitization. The minimum time for detectable recovery was about 65 s, and the maximum time observed was about 10 min. It is proposed that the descending arm of the fluence-response relationship for first positive phototropism is a consequence of desensitization, and that the time threshold for second positive phototropism establishes a period during which recovery from desensitization occurs.

Light is known to induce adaptation in phototropism (Galland, 1991; Janoudi and Poff, 1991). During this process, plants undergo changes in their sensitivity and responsiveness to a light stimulus (Blaauw and Blaauw-Jansen, 1970; Galland and Russo, 1984; Iino, 1987; Galland 1991; Janoudi and Poff, 1991). The adaptation process consists of several steps: desensitization, a refractory period, recovery, and enhancement (Janoudi and Poff, 1991). Thus, an exposure to light initially causes desensitization, a total or partial loss of phototropic responsiveness (Iino, 1988; Janoudi and Poff, 1991). Following a refractory period, during which the level of responsiveness is constant, the plants recover and their phototropic responsiveness is enhanced (Janoudi and Poff, 1991).

In *Arabidopsis thaliana*, desensitization and curvature enhancement can be induced by blue light, whereas red light, via phytochrome (Janoudi and Poff, 1992), can induce enhancement but not desensitization (Janoudi and Poff, 1991). The degree of desensitization and the time needed for recovery of phototropic responsiveness depends on the fluence of the blue light used to induce desensitization.

The complexity of the fluence-response curve for phototropism may be a consequence of adaptation. In first positive phototropism, curvature increases with increasing fluence to a maximum, then decreases to a minimum extending into a zone of indifference at higher fluences (Fig. 1). Second positive phototropism can be exhibited at different fluences, dependent on the fluence rate of blue light, only after the irradiation times exceed a time threshold. We have previously reported that increasingly longer irradiation times are needed to induce second positive phototropism at increasingly higher fluence rates (Janoudi and Poff, 1990). Although it is not known what factors necessitate the existence of a time threshold for second positive phototropism, we have found that a preirradiation with red light decreases the time threshold for second positive phototropism and the width of the zone of indifference (Janoudi et al., 1992).

This study was undertaken to investigate the role of desensitization in first positive phototropism and in setting the time threshold for second positive phototropism. Toward this goal, we have determined the fluence and fluence-rate dependence of desensitization and the interaction of blue light and red light in desensitization and recovery. We report here that the degree of desensitization increases with increasing fluence and fluence rate of blue light preirradiation. Red light decreases the time required for recovery following a desensitizing blue-light pulse.

MATERIALS AND METHODS

Plant Growth

Seeds of *Arabidopsis thaliana* (L.) Heynh. strain Estland were sown in strips of microassay wells containing 0.7% (w/v) agar. Seed germination was potentiated by chilling at 5 ± 1°C in darkness for 3 d. Seeds were then exposed to white light for 18 h at 25°C. At the end of the white light irradiation, the strips were transferred into darkness at 25°C for 42 h, and then exposed to the appropriate photo stimulus. All manipulations were made in complete darkness because visible light is known to affect phototropic responsiveness (Steinitz et al., 1985; Janoudi and Poff, 1992).

Light Sources and Bilateral Irradiation

White light (50 μmol m^{-2} s^{-1}), provided by General Electric (Cleveland, OH) Delux cool-white fluorescent tubes, was used to potentiate seed germination. A slide projector equipped with a Sylvania (Danvers, MA) 900-W BVA tung-

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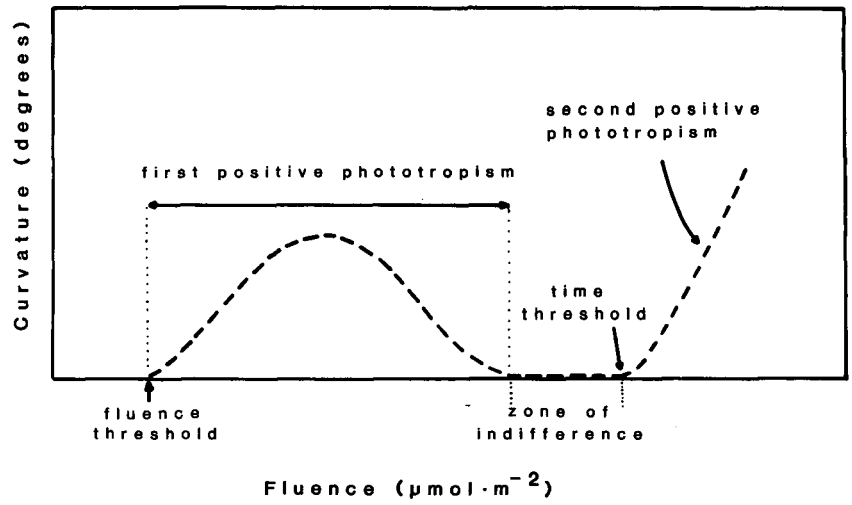

Figure 1. Diagrammatic fluence-response relationship for phototropism in *A. thaliana*.

sten-halogen lamp, in combination with the appropriate Corion (Holliston, MA) interference filter (10-nm half-band width; stray light blocked to >2000 nm) was used as the light source in the preirradiation and phototropism experiments. The duration of irradiation was controlled with a Uniblitz (Vincent Associates, Rochester, NY) shutter.

The bilateral irradiation was given by sequentially irradiating opposite sides of the seedlings with equal fluences and fluence rates. The time between the sequential irradiations was 10 to 15 s. Etiolated seedlings either received a bilateral red-light (669-nm) irradiation (enhancement treatment) or were kept in darkness 2 h before a bilateral blue-light (450-nm) irradiation (desensitization treatment) (Fig. 2). Phototropic curvature was induced with a unilateral blue-light irradiation given 10 to 1800 s after the desensitization treatment.

Curvature Measurement

Experiments were terminated 70 min after the end of the unilateral irradiation with 450-nm light. 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 was traced. Only seedlings that emerged upright (within a solid angle of 10°) from the agar were used. Curvature was measured as previously described (Steinitz and Poff, 1986).

RESULTS

Desensitization

Etiolated *Arabidopsis* seedlings respond with a curvature of about 10° when unilaterally irradiated with 450-nm light at 0.5 μmol m^{-2}. However, seedling phototropism decreased when the unilateral irradiation was preceded by a bilateral desensitizing irradiation with blue light (Janoudi and Poff, 1991). To measure a fluence-response relationship for desensitization, the amplitude of phototropic curvature was first enhanced by irradiation with red light to a curvature of about 36°. The phototropic responsiveness of etiolated seedlings was enhanced using a bilateral preirradiation with either red light at 10 μmol m^{-2} or blue light at 11 μmol m^{-2}. Following 2 h in darkness, the seedlings were bilaterally irradiated at various fluences of 450-nm light. The capacity of these seedlings for phototropism was then tested using a unilateral irradiation with 0.5 μmol m^{-2} of 450-nm light, administered within 30 s of the desensitizing blue-light irradiation. Those seedlings in which phototropism had been enhanced exhibited a decrease in responsiveness following the desensitizing irradiation with blue light (Fig. 3). The extent of desensitization increased as the fluence of desensitizing irradiation increased. The threshold fluence for induction of desensitization is about 0.02 to 0.05 μmol m^{-2}.

Recovery of Phototropic Responsiveness

The kinetics of recovery from desensitization were measured for enhanced and etiolated seedlings desensitized with

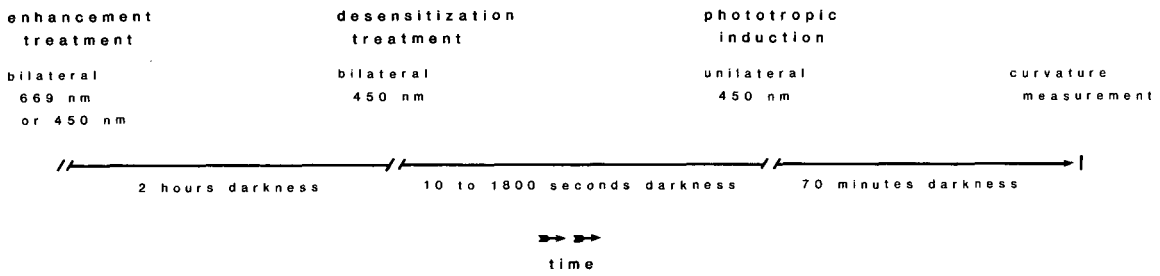

Figure 2. Schematic diagram of irradiation protocol used in this study.

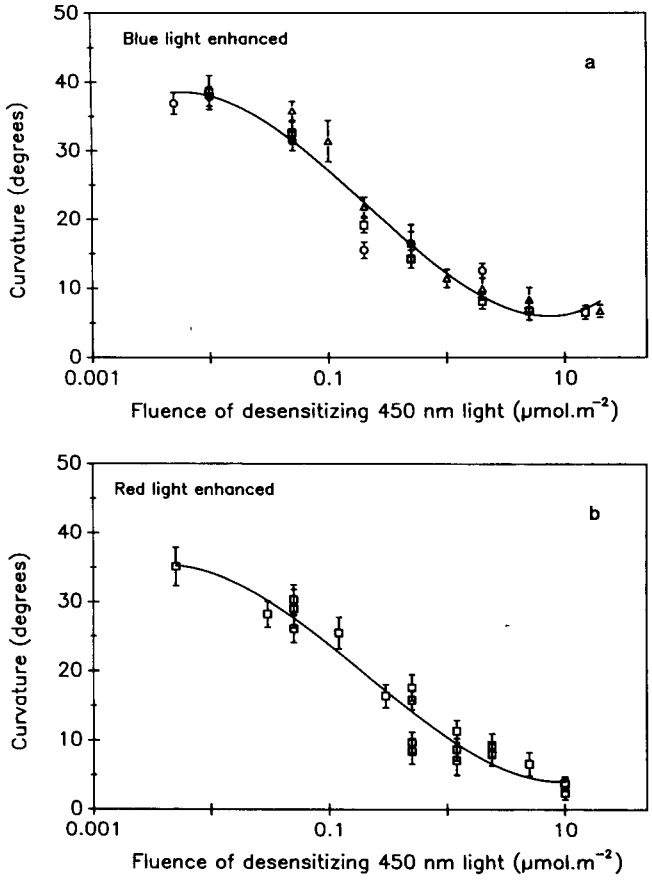

Figure 3. Fluence-response relationship for desensitization of phototropic curvature, in etiolated *A. thaliana*, induced by 450-nm blue light following enhancement with either red or blue light. The potential for phototropism was enhanced with an irradiation with (a) 450-nm light at 11 μmol m^{-2} or (b) 669-nm red light at 10 μmol m^{-2}. Following 2 h in darkness, the seedlings were exposed to desensitizing blue-light irradiation at the indicated fluences and fluence rates of 0.06 (○), 0.21 (△), and 0.36 (□) μmol m^{-2} s^{-1}. The capacity of the seedlings for phototropism was assessed by curvature measured 70 min after an inductive unilateral blue-light pulse at a fluence of 0.5 μmol m^{-2} administered within 30 s of the desensitizing exposure. Data points represent the mean curvature of ≥100 seedlings. Vertical bars represent ±1 SE.

blue light (450 nm) at fluences from 0.3 to 60 μmol m^{-2} and fluence rates of 0.3 or 1.0 μmol m^{-2} s^{-1}. The results (Fig. 4) show that phototropic capacity recovers as a function of time, but that the rate of recovery depends on the desensitization treatment.

Bilateral irradiation of etiolated seedlings with desensitizing blue light at fluences of 1 μmol m^{-2} or higher caused an almost complete loss of responsiveness (Figs. 3–5). In contrast, a higher fluence of blue light, 10 μmol m^{-2}, was required for enhanced seedlings to reach a similar level of desensitization (Fig. 4; 10 μmol m^{-2}). At equal fluences, the degree of desensitization induced by blue light at a fluence rate of 1.0 μmol m^{-2} s^{-1} was greater (i.e. curvature was lower) than that induced at a fluence rate of 0.3 μmol m^{-2} s^{-1}.

The times required for detectable recovery (time at which curvature starts increasing) by etiolated and enhanced seedlings following a bilateral blue-light irradiation were estimated from Figures 4 and 5 and are presented in Figure 6 as a function of the fluence of desensitizing blue-light bilateral irradiation. In general, at both fluence rates tested, the recovery time increased as the fluence of blue light was increased from 0.3 to 60 μmol m^{-2} (Fig. 6). Moreover, in etiolated seedlings at equal fluences, recovery occurred earlier following irradiation with blue light at a fluence rate of 0.3 than at 1.0 μmol m^{-2} s^{-1}. Furthermore, at identical desensitizing fluences, enhanced seedlings began to recover more quickly than did etiolated seedlings (Fig. 6). The minimum time required for recovery to start in enhanced seedlings was about 65 s, following a bilateral irradiation with 450-nm light at a fluence of 0.3 μmol m^{-2}.

DISCUSSION

It is becoming apparent from the results of this study and earlier studies (Iino, 1988; Janoudi and Poff, 1990, 1991) that in phototropism light elicits multiple responses, some of which have opposite effects. The results of our study indicate that fluences of blue light that induce first positive phototropism can also induce desensitization. Similar fluences can also induce enhancement of phototropic curvature (Janoudi and Poff, 1991, 1992). Thus, blue light is perceived by the plant as a signal that induces and enhances phototropism while, concurrently, causing very rapid desensitization, which lowers the plant's responsiveness to blue light. We propose that the descending arm of first positive phototropism is a consequence of desensitization and that the time threshold for second positive phototropism establishes a time period during which recovery from desensitization can occur.

Several possibilities exist that could account for the decrease in curvature as fluence increases in first positive phototropism. One possibility is that the photoreceptor pigment is bleached increasingly, in a fluence-dependent manner, thus limiting perception of the stimulus and leading to decreased response. This possibility is difficult to reconcile with the observation that reciprocity is valid for a wide range of fluence rates throughout the ascending and descending arms of the fluence-response curve. Valid reciprocity implies that the number of photoreceptor molecules is sufficient to perceive all of the light stimulus irrespective of the rate at which the quanta are being delivered. In addition, diminishing the amount of available photoreceptor pigment should result in sensor adaptation (Galland, 1991). In sensor adaptation, the threshold for phototropism is shifted to higher fluences because of the decrease in photoreceptor pigment and a shift back upon recovery. However, *A. thaliana* exhibits response adaptation where the level of the response is diminished with no apparent change in the fluence threshold for phototropism (Janoudi and Poff, 1991).

A second possible explanation is that light penetrating the organ leads to phototropic stimulation of the shaded side, leading to a decrease in and eventually a lack of phototropic curvature toward the light source. There are insufficient data to either accept or reject this possibility.

A third possibility is that the level of another component of the signal transduction system, downstream of the pho-

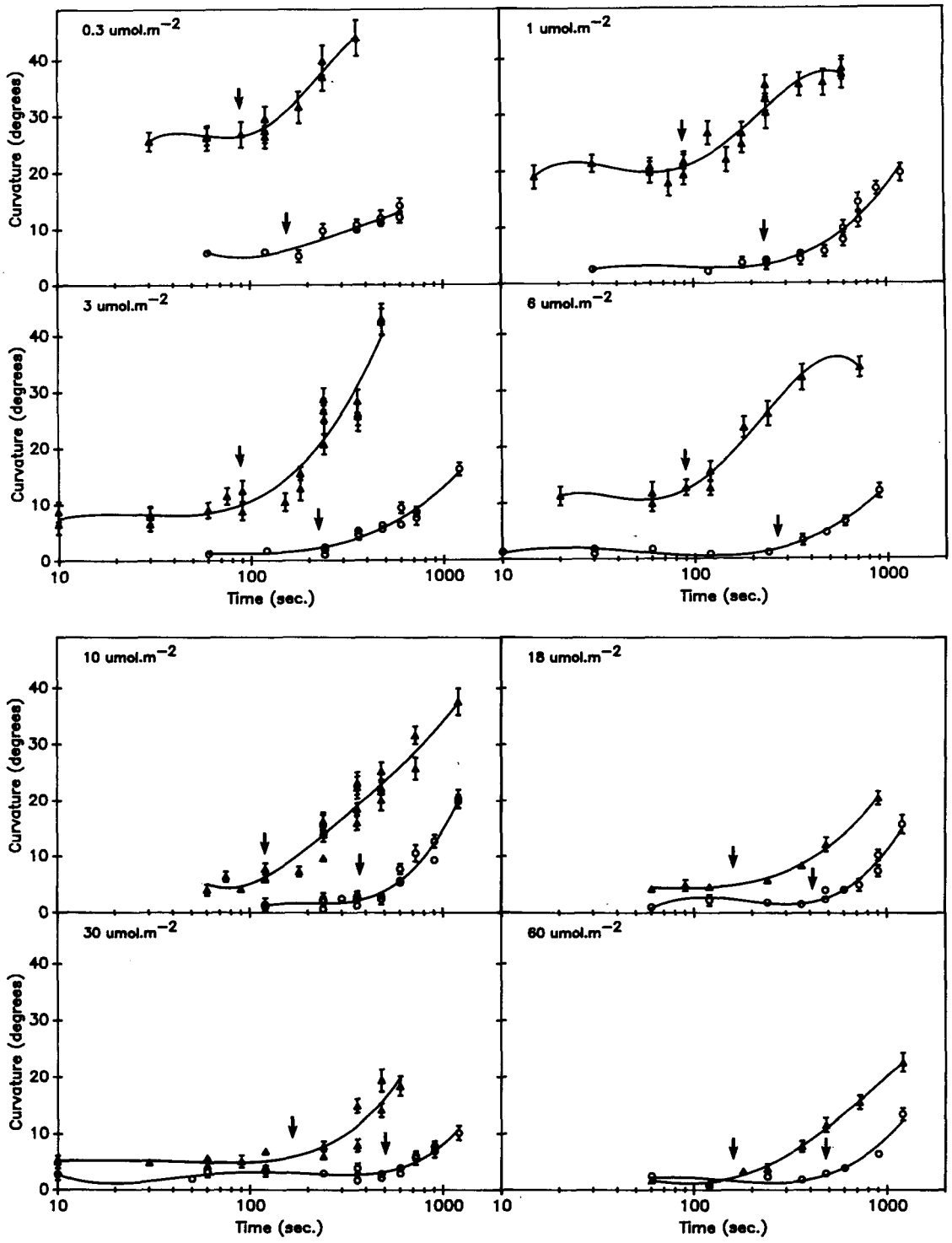

Figure 4. Kinetics for recovery of phototropism following a desensitizing blue-light pulse. Etiolated *A. thaliana* seedlings were either irradiated with bilateral 669-nm light (▲) at a fluence of 10 μmol m^{-2} or were kept in darkness (O) until a desensitizing irradiation with 450-nm light was given 2 h later. Desensitizing irradiation with 450-nm light was at a fluence rate of 0.3 μmol m^{-2} s^{-1}. All seedlings were then given a unilateral irradiation with 450-nm light at a fluence of 0.5 μmol m^{-2} at the indicated times after the 450-nm bilateral irradiation. Data points represent the mean curvature of ≥100 seedlings. Vertical bars represent ±1 SE.

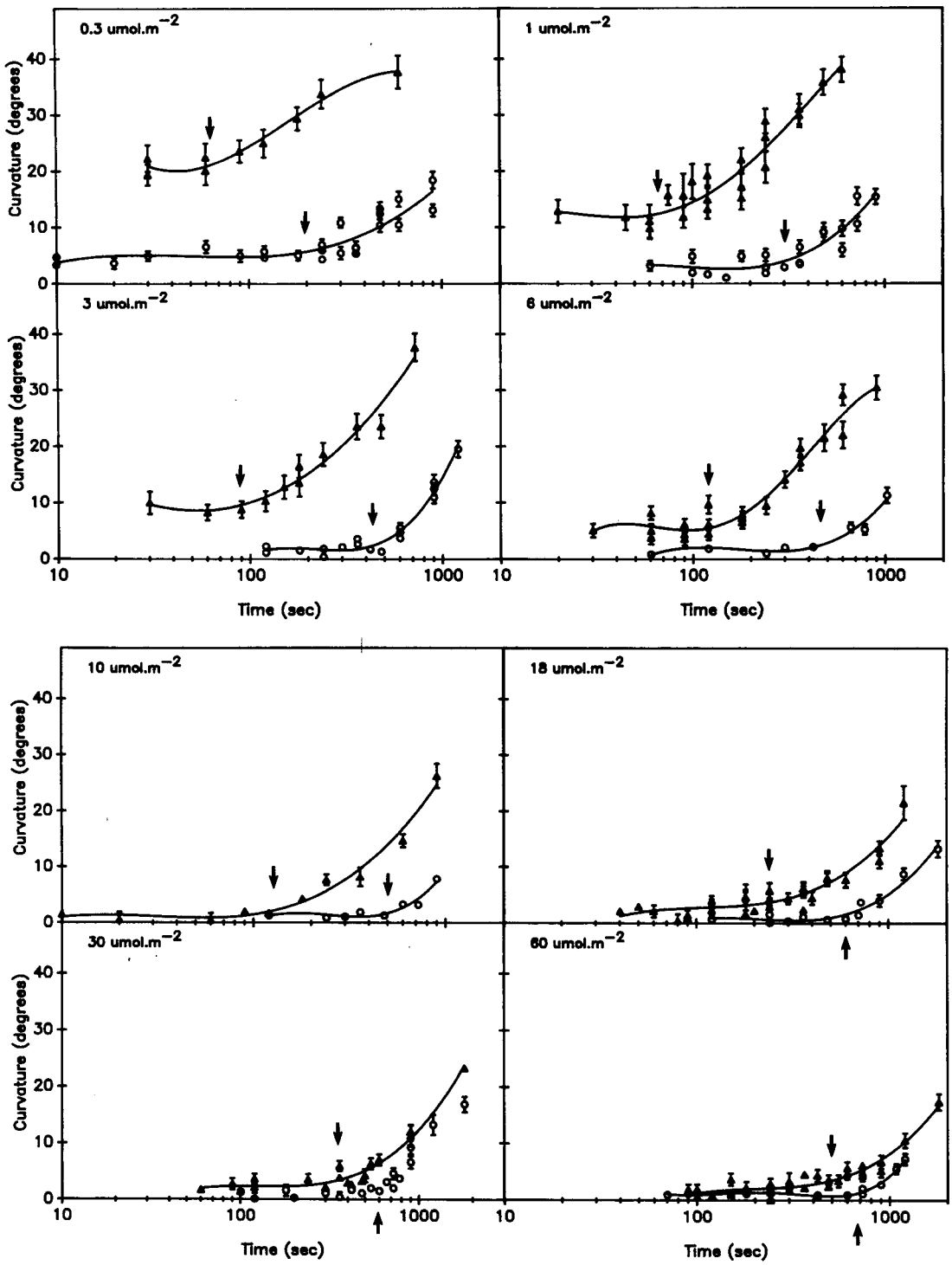

Figure 5. Same as in Figure 4, except that the fluence rate of bilateral 450-nm light was 1.0 μmol m^{-2} s^{-1}.

toreceptor pigment, is decreasing and becoming limited in response to the blue-light irradiation. This latter possibility would be consistent with the observed response adaptation (Janoudi and Poff, 1991) and with data reported here, showing that at each of the preirradiation fluences tested, a relatively constant level of response is maintained until recovery occurs. However, it is not clear why the seedling is desensitized further at a higher fluence rate.

Our results indicate that recovery and curvature enhancement require several min, whereas desensitization has already been shown to require less than 2 s (Janoudi and Poff, 1991). Thus, during the short irradiation times in first positive

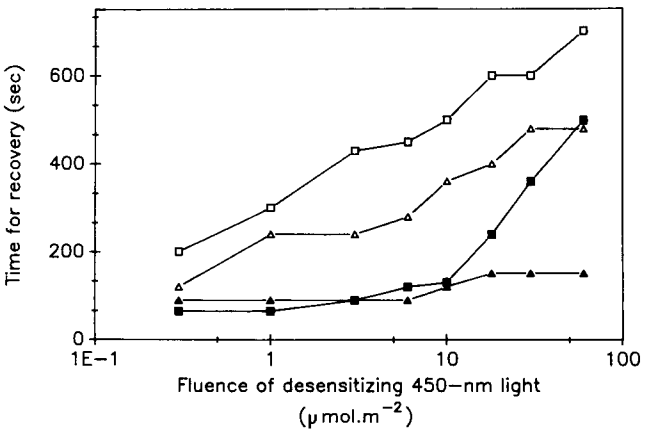

Figure 6. Time requirements for recovery of phototropic responsiveness following a desensitizing 450-nm bilateral irradiation at fluence rates of 0.3 (triangles) and 1.0 (squares) μmol m^{-2} s^{-1} and at various fluences in etiolated (△, □) and 669-nm red light-preirradiated (▲, ■) *A. thaliana* seedlings. Data points were estimated from the curves presented in Figures 4 and 5. Each data point represents the time threshold for observable recovery at each of the fluences of desensitizing 450-nm irradiation presented in Figures 4 and 5.

phototropism, a plant can respond to the curvature-inducing, and also desensitizing, light stimulus. Recovery and enhancement, however, require longer times and are not likely to have an appreciable effect during these short irradiation times. The shape of the fluence-response curve for first positive phototropism then reflects the net result of the more rapid components, curvature induction and desensitization, in phototropism.

The presence of a zone of indifference in the fluence-response relationship for phototropism may be partially due to the requirement for a time during which recovery from desensitization can occur. This time requirement for recovery is satisfied during the time threshold for second positive phototropism. These suggestions are supported by the observation that red light, which we found to decrease the time needed for recovery, also decreases the time threshold for second positive phototropism and the width of the zone of indifference (Janoudi et al., 1992). These recovery times are consistently shorter than the time threshold. Thus, although recovery occurs during this period, the time threshold is set by some process other than the recovery time.

The complexity increases in second positive phototropism, during which a plant is exposed to prolonged irradiations. The results of the current study indicate that exposure times typical for second positive phototropism are sufficiently long to allow for curvature enhancement as well as recovery to occur. Because the time needed for recovery is dependent on the fluence and fluence rate of the desensitizing pulse, and because the degree of curvature enhancement is time dependent, the fluence rate of blue light becomes a major determinant of the degree of phototropic curvature. At a given fluence, a plant exposed to blue light at a low fluence rate is less desensitized and has more time for recovery and enhancement than it does if the same fluence is given at a higher fluence rate. Differences in the levels of desensitization and enhancement may contribute to the differences in the degree of curvature obtained, at similar fluences, when different fluence rates are used.

In summary, in *A. thaliana* the degree of desensitization and the time required for recovery increase as the fluence and fluence rate of the desensitizing blue-light irradiation increase. Moreover, blue and red light, probably via phytochrome, decrease the time required for recovery.

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