

# Rapid Suppression of Extension Growth in Dark-Grown Wheat Seedlings by Red Light<sup>1</sup>

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## ABSTRACT

Continuous recordings were made using a linear displacement transducer to investigate short-term growth responses of intact dark-grown wheat (*Triticum aestivum* L. cv Maris Huntsman) seedlings to red light. To eliminate any effect of light prior to the experimental treatments, the seedlings were grown and mounted on the transducer apparatus in total darkness. The growth kinetics after irradiation were complex and appeared to consist of three successive phases of growth deceleration. When the tip of the intact coleoptile was irradiated with red light from two opposite fiber bundles (fluence rate:  $2 \times 64$  micromoles per square meter per second) for varying periods of time (10 seconds, 1 minute, 5 minutes, continuous), a decrease in extension rate was detectable after a latent period of 8 to 10 minutes. Up to 30 minutes after the start of the irradiation treatment, there was no difference in the kinetics of inhibition (about 20 to 25% inhibition) between the different lengths of irradiation. Extension rate reached a minimum (65% inhibition) at about 85 minutes, after which growth acceleration toward the dark control rate was observed. Far-red reversibility of the rapid effect of red light on growth was not observed, even when far-red light was given only 4 seconds after the end of 10 seconds red light. Short (15 seconds) far-red light did not induce a response.

Extension growth is one of the physiological responses which are obviously under control by light, operating both through phytochrome and through the blue-absorbing photoreceptor. Short-term responses to irradiation can provide information about early steps in the photocontrol of extension growth. Whereas rapid (*i.e.* latent period 20–60 s) effects of blue light are well known (2, 4, 9), the effects of phytochrome on extension are generally considered to be relatively slow. The most rapid phytochrome-mediated extension growth responses yet recorded are those of light-grown seedlings, in which growth rate is modulated by the relative proportions of R<sup>3</sup> and FR, with a latent period of 7 to 15 min (10, 11). With etiolated seedlings, the most rapid phytochrome-mediated response yet recorded is that of Vanderhoef *et al.* (13) who demonstrated a latent period of 20 min for the inhibition of maize mesocotyl extension. Pike *et al.* (12), using isolated tip segments of oat coleoptiles, found that R promoted extension after a latent period of 46 min. In the cited

experiments with etiolated seedlings, green light was used as a 'safelight'. It is known, however, that seedlings grown in the absence of light are exceptionally sensitive to irradiation (1, 8) and that exposure even to brief periods of green safelight is often sufficient to induce a physiological response. We argued, therefore, that any rapid effect of light on extension growth may have remained unnoticed by the previous investigators, as it might have occurred in response to exposure to the safelight. Accordingly, to avoid any light effect prior to the experimental treatments, the wheat seedlings used in this investigation were grown, prepared, and mounted onto the extension measurement apparatus, in complete darkness. The results demonstrate a R-mediated inhibition of extension growth with a latent period of 8 to 10 min, but evidence on the identity of the photoreceptor is as yet inconclusive.

## MATERIALS AND METHODS

**Plant Material and Growth Conditions.** Seeds of *Triticum aestivum* L. (cv Maris Huntsman, 1981) were presoaked for 2 h in tap water and grown singly in glass vessels (scintillation vials) in vermiculite for 4 d in complete darkness (3 d at 24°C, 1 d at 18°C). A seedling (coleoptile 12–17 mm in length) was selected in complete darkness and placed into a special Perspex cuvette which was then connected to the transducer system (linear displacement transducer, Sangamo-Weston Controls, England). A continuous water flow through the cuvette jacket was used to maintain the air temperature around the coleoptile constant at 20°C.

**The Transducer Apparatus.** The transducer system used by Morgan *et al.* (10, 11) was modified for growth experiments on intact coleoptiles (Fig. 1, A and B). The coleoptile was situated in a narrow glass tube in air, surrounded by a Perspex cuvette jacket to thermostat the system. The transducer needle (800 mg in weight) was connected to a small translucent conical cap (the end of a micropipette tip) which was placed on the tip of the coleoptile. The whole apparatus was enclosed in a Perspex chamber which was humidified, aerated, and maintained in darkness except for the stated light treatments. The growth rates were calculated from the original recorder traces in 5-min intervals.

**Light Sources.** Monochromatic light was produced by filtering light from a fiber light source (Schott KL 150 B, with a 5-mm, 3-way fiber optic bundle; Carl Zeiss Ltd., London, U.K.) through interference filters (25 mm diameter, Ealing Beck Ltd., Watford, U.K.). The interference filters were placed between the light source and the fiber probe. To get similar fluence rates for R and FR, neutral density filters were used. The tip region of the intact coleoptile was irradiated by two opposite fiber bundles (Fig. 1B); (the third fiber bundle was not used). The emission cone of the fiber bundles was approximately 60°, and thus, with light scattering in the water-filled cuvette, and in the coleoptile itself, more than the 5-mm tip region received light. The spectral photon distributions of the treatment light sources were measured using

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<sup>3</sup> Abbreviations: R, red light; FR, far-red light.

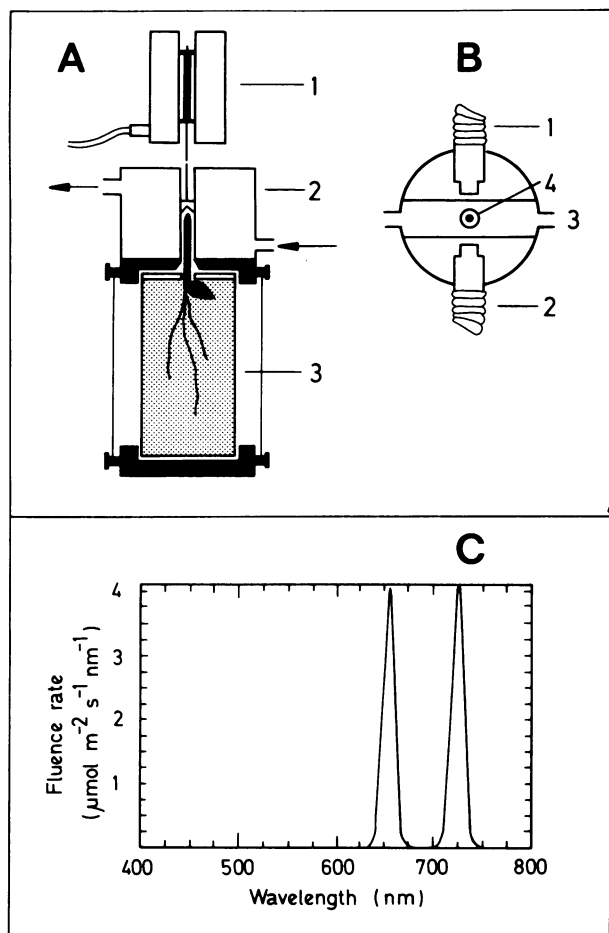


FIG. 1. A, Scheme of the apparatus used for continuous growth measurements of intact wheat seedlings. (1), linear displacement transducer; (2), cuvette-jacket with continuous water flow to maintain air around coleoptile at 20°C; (3), glass vessel containing seedling in vermiculite. B, Scheme of apparatus from above. (1, 2), fiber-optic bundles; (3), cuvette-jacket; (4), glass tube surrounding coleoptile. C, Spectral photon distributions of the treatment light sources.

identical geometry to that of the transducer apparatus, using a calibrated spectroradiometer (Gamma Scientific, San Diego, CA (Fig. 1C).

**Phytochrome Measurements.** Absorption measurements between 730 and 800 nm were made, using an Aminco dual-wavelength spectrophotometer type DW-2a. Photoreversible absorbance change after short-term irradiation was measured, using cuvettes with 2.5 mm sample thickness at 20°C. The actinic light was produced by a projector with adapted fiber optic probe (fluence rate for R:  $54 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). For *in vivo* spectrophotometry the coleoptiles were prepared in very dim green light.

## RESULTS AND DISCUSSION

After transfer to the transducer apparatus the seedlings required 3 to 4 h to recover. Recovery was taken as complete with the assumption of a constant growth rate (average  $12 \mu\text{m min}^{-1}$ ). Subsequently, the growth rate of dark-grown seedlings remained nearly constant in darkness for the next 3 h (Fig. 2). Irradiation treatments were routinely begun 4 h after transfer of the seedlings to the transducer apparatus.

Seedlings held previously in total darkness responded rapidly to exposure to R. Continuous R, pulses of 5 min or 1 min R (Fig. 3) or pulses of 10 s R (Fig. 4) yielded essentially similar

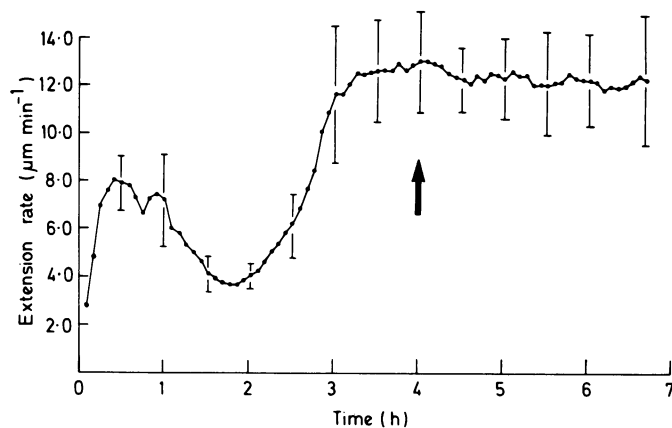


FIG. 2. Time course of extension rate of intact etiolated wheat seedlings (coleoptiles 12–17 mm in length) in darkness after transfer to the transducer apparatus. (Error bars indicate SD). ↑, Time zero for all light treatment experiments.

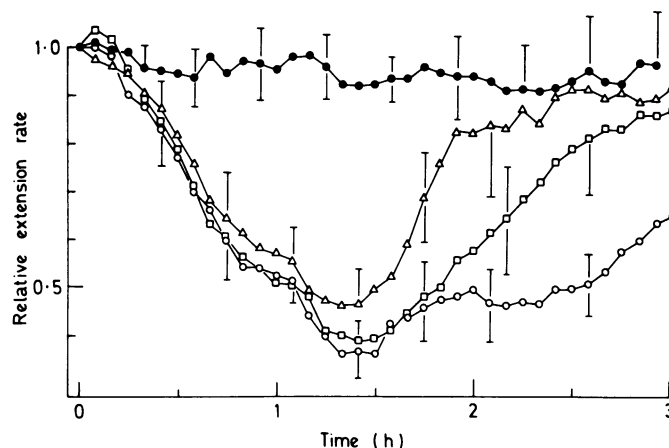


FIG. 3. Time course of extension rate of intact etiolated wheat seedlings after R-irradiation beginning at time zero. (●), Dark control,  $n = 6$ ; (○), continuous R,  $n = 5$ ; (□), 5 min R,  $n = 5$ ; (Δ), 1 min R,  $n = 7$ . Fluence rate:  $128 \mu\text{mol m}^{-2} \text{s}^{-1}$  (i.e.  $64 \mu\text{mol m}^{-2} \text{s}^{-1}$  from each fiber optic bundle). Extension rates are calculated relative to the rate in darkness before light treatment (=1). Dark extension rates varied from 12 to  $16 \mu\text{m min}^{-1}$ . Bars indicate SD.

response kinetics. In all cases, a pronounced shoulder of inhibition was observed at 40 to 65 min, followed by a peak of inhibition at approximately 85 min, after which extension rate tended to return to the dark level. The standard deviations of the plotted means are such that it is not possible statistically to differentiate between the effects of the R treatments used until about 120 min after the onset of irradiation. From this point, the continuous R responses are consistently greater than the effects of R pulses. Such complex kinetics may be explained by the temporal overlap of different growth reactions (inhibition and promotion), or by different sensitivities to light in different parts of the seedling. R has been shown to inhibit the growth of the basal parts of intact wheat and barley coleoptiles (6) and of excised wheat segments (7). A promotion of growth by R is characteristic of the apical region of coleoptiles, as shown for excised oat (5, 16) and wheat (3, 7) segments, and intact wheat and barley coleoptiles (6). The overall growth response to light depends on the developmental stage of the coleoptile, only very young coleoptiles exhibiting a general growth promotion by R (1); mesocotyl growth, on the other hand, is generally inhibited by R (8).

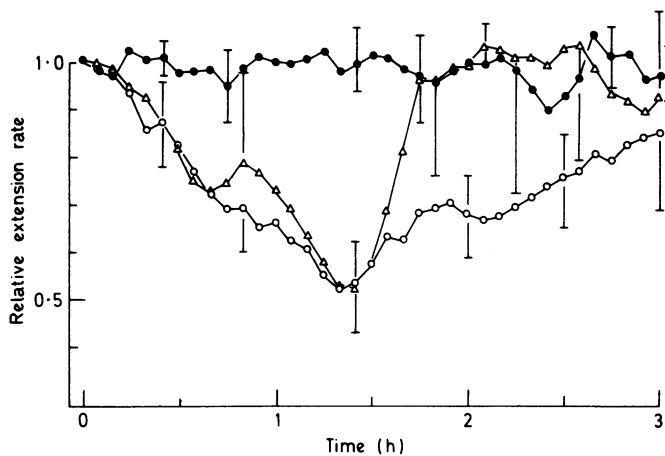


FIG. 4. Kinetics of extension of intact etiolated wheat seedlings after R, FR, and R/FR irradiation. (O), 10 s R (fluence rate:  $128 \mu\text{mol m}^{-2} \text{s}^{-1}$ ),  $n = 4$ ; (●), 15 s FR,  $n = 4$ ; (Δ), 10 s R/15 s FR (fluence rate for FR:  $124 \mu\text{mol m}^{-2} \text{s}^{-1}$ ),  $n = 5$ . Between R and FR irradiation a dark period of 3 to 4 s was inserted for filter change. Bars indicate SD.

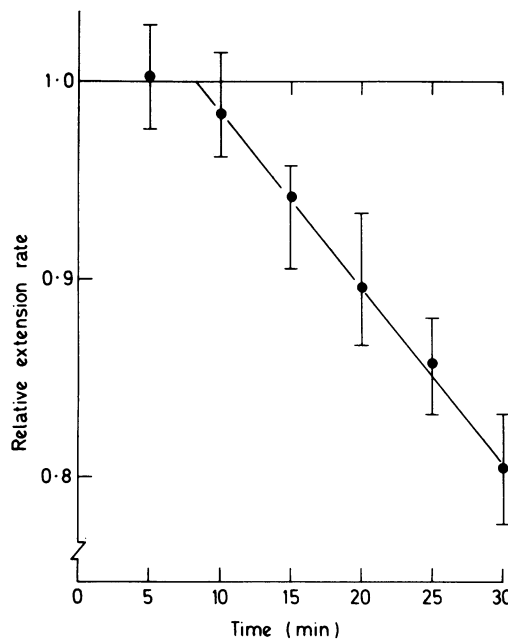


FIG. 5. An attempt to define accurately the latent period for R-mediated inhibition of etiolated wheat seedling growth. The points represent the means of all the different time courses for the first 6 time points (i.e. 30 min) after the onset of irradiation. The bars indicate the ranges of the data, and are not intended as error estimates. The line is calculated by regression analysis using time points 2 to 6 only.

Operationally, the responses presented here appear to consist of two overlapping, transient decelerations of growth, peaking at about 45 and 85 min, respectively, followed by a longer term inhibition seen only under continuous R. Calculations based on the data for 5-min pulses indicate that the net effect on seedling length, measured over a 12-h experimental period (the minimum commonly used in straight growth assays) would be a maximum of 6% inhibition. Thus, although the growth inhibition seen here is sizeable on a continuous measurement basis (maximum 65% inhibition), its longer term effect is virtually negligible. It is, of course, quite possible that further inhibition (or promotion) of growth in response to R could occur after the end of the 3-h experimental period used here.

Accurate determination of the latent period is difficult because of the inherent and inescapable variability encountered with transducer determinations of growth rates of single plants. If all the available data for the first 30 min are assumed to come from the same population, then a plot of the overall means indicates that the latent period is near 8 min (Fig. 5). This estimate is based on the rather unlikely assumption that the onset of inhibition is abrupt; the actual latent time, therefore, is probably nearer to 5 min. Thus, this appears to be the most rapid R-mediated growth response yet reported, although it is still relatively slow compared with the latent times of blue light effects (2, 4, 9). Using plants mounted onto transducers under dim green safelights and allowed to extend in darkness for a further 4 h, we have observed substantial, transient, growth inhibition induced by R pulses, but with a latent period of approximately 30 min (G. M. Jackson and H. Smith, unpublished data).

The photoreceptor responsible for these rapid growth decelerations is not yet known. It has not been possible to demonstrate R/FR photoreversibility using pulses of light. FR given immediately after 5-min or 1-min pulses of R did not prevent the early growth inhibitions. Even pulses of 10 s R ( $1.28 \text{ mmol m}^{-2}$ ) followed as quickly as possible by 15 s FR ( $1.86 \text{ mmol m}^{-2}$ ) showed no differences in early kinetics from the R pulse alone (Fig. 3). Measurements of spectral photoreversibility using segments of isolated coleoptile tips (including young leaves) demonstrated that 15 s FR was sufficient to remove Pfr to levels below those detectable by *in vivo* spectrophotometry (data not shown). Although the lack of photoreversibility in these experiments argues against a role for phytochrome, two possibilities cannot be rigorously excluded. First, extension growth may escape from photoreversibility very rapidly; approximately 4 s was needed to change filters between R and FR irradiations. Warner and Ross (15) reported loss of reversibility of R-induced increase in wall extensibility of maize coleoptiles within 2 s, although reversibility of extension growth was only lost over 2.5 min (14). The second alternative is that the responses are saturated at the very low Pfr (or Pfr/[Pr + Pfr]) levels established by 15 s FR after R (s 13). This possibility could only be viable if 15 s FR after dark produced insufficient Pfr to induce a response (Fig. 3). Measurements of such low levels of Pfr are not possible with current techniques. Seedlings mounted onto transducers under dim green light show growth inhibition by R pulses which is fully reversible by FR (G. M. Jackson and H. Smith, unpublished data).

From these data, we may conclude that rapid effects of R on extension growth may be detected using wheat seedlings which have received no prior light. As yet, definitive evidence on the identity of the photoreceptor responsible for the rapid effects is not available. The earliest response is not fluence dependent above about  $100 \mu\text{mol m}^{-2}$  and is not R/FR reversible within 20 to 30 s of the onset of irradiation. Planned experiments on the wavelength and fluence rate dependence of the rapid responses should help to identify the photoreceptor.

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