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Short Term Phytochrome Control of Oat Coleoptile and Pea Epicotyl Growth[1]

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ABSTRACT

Continuous recordings of the effect of light on oat (*Avena sativa* L. cv. Victory) coleoptile and pea (*Pisum sativum* L. cv. Alaska) epicotyl growth were made. Using a single excised coleoptile 10 minutes of red light was found to promote growth after a latent period of 46 minutes. The stimulation was transient and was not far red-reversible. Blue and far red light also promoted growth with similar kinetics. The action of continuous red or far red light was similar to that of 10-minute light. The growth of the intact pea third internode (as well as excised segments) was strongly inhibited by red light, with a latent period of 80 minutes. This effect was far red-reversible, and far red and blue light caused only a slight inhibition of growth.

Knowledge of the latency period between R[4] irradiation of plants and expression of physiological or developmental changes is important in studying the mechanism of phytochrome action. Responses such as changes in membrane electrical potential may have latencies on the order of seconds (18), while several hours may be required for the appearance of certain inducible enzymes (17). Although growth is a complex process, possibly involving many limiting factors in different situations, there is much interest in understanding the mechanism by which phytochrome controls the growth of etiolated seedlings. There have been a few attempts to characterize the kinetics of such responses. The R-induced inhibition of intact pea stem elongation began about 6 h after irradiation (9), and a lag period of 4 h was reported for pea stem segments (3). The lag period for the R-induced growth promotion of apical oat coleoptile segments was less than 3 h (12). Recent work with optical and electronic transducers showed that auxin can promote the growth of pea stem and oat coleoptile segments within 15 min of application (7, 24).

We have utilized continuous recording with an angular displacement transducer to study the latent period and other aspects of growth regulation by light in oat and pea tissue. These results are compared to recent findings on the time courses of other phytochrome-mediated responses.

MATERIALS AND METHODS

General Procedures. All measurements were made with a Metripak 33-06 angular displacement transducer (Gould, Inc.) mounted on a microscope mechanical stage, so that the transducer arm could be adjusted to the horizontal at the beginning of each experiment. The transducer was connected to an Omni-Scribe recorder (Houston Instruments). All manipulations were carried out under green safelights (18) in a room maintained at 23 ± 2 C. Irradiations were given from the side, with a mirror behind the plant, using a 300-w projector and the following filters: red, Rohm and Haas Plexiglas 2423 and Corning filter 3-66 (intensity 3.0 mw/cm^2); far red, Corning filters 7-69 and 3-66 (1.4 mw/cm^2) (18); blue, Wratten filters 2A and 47B (0.8 mw/cm^2).

Experiments with Oats. Seedlings of *Avena sativa* L. cv. Victory were grown as described previously (19). Our initial experiments were conducted using segments in an Evans and Ray-type apparatus (7). Ten 1-cm apical segments were cut, the apical 1 mm was removed, and the segments strung on nylon line. A glass capillary tube was placed on top of the segment column, with the transducer arm resting on the flared-out top of the capillary. The growth chamber was filled with 1 mM K-phosphate (pH 6.2) containing 1.5% sucrose (12). The solution was circulated by air bubbled into the chamber.

In most of the experiments, a single 2-cm apical segment (tip intact) was used. A snug fitting plastic sleeve was placed around the middle of the segment, and the sleeve and plant were inserted through a hole in the lid of a foil-wrapped 30-ml vial filled with the buffered sucrose solution. The sleeve and plant were firmly held by the lid, with about 0.8 cm of the coleoptile protruding above the lid. A small translucent conical cap was placed on the tip of the coleoptile, and the transducer arm rested on this cap. For application of test solutions to the plants, we used a slightly larger cap, containing a wad of cotton soaked in the solution.

Experiments with Peas. Seedlings (*Pisum sativum* L. cv. Alaska) were grown as described before (20). For a few experiments 0.5-cm subapical internode segments were cut and a hole bored through the center with a 27-gauge needle (25). The segments could then be handled in the manner described for coleoptile segments. The buffer used was 5 mM K-citrate (pH 6.2) containing 1.5% sucrose and 20 μM $Co(NO_3)_2$. Other experiments with peas utilized intact plants in their Vermiculite-filled pots. Plants were selected with third internodes between 1 and 2 cm long. The plant was clamped at the second node. A cylindrical cap cut from plastic tubing was placed over the apical hook, and the transducer arm was placed on the cap.

Analysis of Data. The recorder tracings displayed the angle of displacement of the transducer arm as a function of time. Knowing the initial distance along the transducer arm from the axis of rotation to the plant, the growth increment at any time is the product of the initial arm length times the tangent of the angular displacement. The growth increment was then expressed as a per cent of the original height of the tissue. For columns of tissue segments, the original value was the height of the column. For the intact peas, we used the initial length of the third internode. For the single oat coleoptiles, we used one initial length of the coleoptile above the vial lid (the only region exposed to light). In all

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[4] Abbreviations: R: red light; FR: far red light.

cases we computed growth rates (in units of per cent increase in length per h) at hourly intervals by calculating the slope of a regression line through the per cent elongation data points at 12 min before the h, on the h, and 12 min after the h. Mean growth rates and standard errors were calculated for each set of replicates. Latent periods were estimated by finding the point of change of slope on the recorder tracings. In all cases time zero is the start of recording.

RESULTS

Oats. Our experiments with columns of apical segments immersed in buffer revealed a very pronounced spontaneous growth response (8) about 2.5 h after the beginning of recording. Irradiations were given 1.5 h after the start of recording, to allow time for the growth rate to stabilize. Any light-induced changes in growth rate were obscured by the spontaneous growth response, so this procedure was abandoned.

The apparatus using a single coleoptile, with its base in buffer and approximately the upper 0.8 cm exposed, was satisfactory, since growth rates were fairly constant over at least 8 h. Because of plant-to-plant variation in growth rate, we present average values for many plants in each treatment, rather than individual growth curves.

Table I shows the average growth rates for the various light treatments (given 2 h after the start of recording). At 3 h the R-treated plants showed double the growth rate of the dark controls. The stimulation then gradually declined. If the R was immediately followed by FR, there was no diminution of the growth promotion, although the return to the dark control growth rate was slightly more rapid. FR alone was about half as effective as R. The blue light-induced promotion of growth was as great as the R-induced promotion, but the growth rate returned to the dark control level more rapidly. The latent period for the response to R was 46 ± 1 min (mean ± SE); to R + FR, 45 ± 1 min; to FR and to blue, 42 ± 1 min.

For comparative purposes some trials were run using continuous R or FR irradiation. The maximum promotion (at 3 h) was quite similar to that in the 10-min irradiation trials. The growth rates did not decline as fast as in the 10-min irradiation trials, and at the end of recording they were still substantially above the dark control rates. The latent periods for the responses were not different from those stated above.

Experiments testing the response to auxin involved a cap containing cotton to aid in delivery of test solutions. In control experiments, 1 mM K-phosphate (pH 6.2) was applied. Preliminary experiments showed that 1 mM IAA was a suitable concentration; at 0.1 mM IAA, hardly any growth response occurred. The latent period, 16 ± 1 min, indicated rapid penetration and action. When IAA was applied at time zero, the growth rate was twice the

Table I. Growth Rates of Excised Oat Coleoptiles Given Different Light Treatments

The growth of 2-cm apical coleoptile segments was recorded with an angular displacement transducer. The upper 0.8 cm of the coleoptile was exposed to the air, while the remainder was immersed in a foil-covered vial of 1 mM $K\text{-}PO_4$ buffer, pH 6.2, + 1.5% sucrose. There were at least 15 replicates of each experiment, except for the continuous light treatments, of which there were 6 replicates. Light treatments were given 2 h after the beginning of recording. Growth was calculated as a percent of the initial length of the upper exposed section, and rates were calculated each hour as described in the text. Data are presented as mean ± SE.

TREATMENT	GROWTH RATE % INCREASE IN LENGTH/H 1 H	2 H	3 H	4 H	5 H	6 H
Dark	3.6 ± 0.3	2.6 ± 0.3	3.4 ± 0.3	2.8 ± 0.2	2.5 ± 0.2	2.2 ± 0.2
10 min Red	4.2 ± 0.4	3.0 ± 0.4	7.1 ± 0.4	5.2 ± 0.4	4.0 ± 0.4	3.1 ± 0.4
10 min Far red	3.6 ± 0.4	2.0 ± 0.2	5.0 ± 0.3	3.4 ± 0.3	2.9 ± 0.2	2.6 ± 0.3
10 min Red + 10 min Far red	3.9 ± 0.4	2.8 ± 0.3	7.7 ± 0.5	4.4 ± 0.3	3.4 ± 0.2	2.7 ± 0.3
10 min Blue	3.9 ± 0.3	2.2 ± 0.2	7.4 ± 0.5	3.6 ± 0.2	3.1 ± 0.2	2.1 ± 0.2
Continuous Red	3.4 ± 0.4	2.6 ± 0.2	7.8 ± 0.5	5.7 ± 0.3	4.5 ± 0.4	4.3 ± 0.3
Continuous Far red	3.1 ± 0.3	1.9 ± 0.4	5.4 ± 0.4	5.3 ± 0.5	4.0 ± 0.4	3.8 ± 0.6

Table II. Growth Rates of Excised Oat Coleoptiles Treated with Auxin

Measurements and calculations were made as described in Table I. Buffer (1 mM $K\text{-}PO_4$, pH 6.2) or IAA (10^{-3} M in buffer) was applied at the beginning of the experiment. Red light (10 min) was given 1 h after the beginning of the experiment. There were at least 10 replicates of each experiment. Data are expressed as mean ± SE.

TREATMENT	GROWTH RATE % INCREASE IN LENGTH/H 1 H	2 H	3 H	4 H	5 H	6 H
Dark + Buffer	3.7 ± 0.3	2.4 ± 0.2	2.5 ± 0.3	2.8 ± 0.3	2.6 ± 0.2	2.5 ± 0.2
Red + Buffer	4.2 ± 0.4	3.8 ± 0.4	4.2 ± 0.4	4.3 ± 0.3	4.2 ± 0.2	3.7 ± 0.3
Dark + IAA	9.2 ± 0.5	7.4 ± 0.4	6.2 ± 0.7	5.0 ± 0.6	4.0 ± 0.6	3.2 ± 0.6
Red + IAA	8.1 ± 0.5	7.0 ± 0.7	6.8 ± 0.8	5.8 ± 0.7	4.8 ± 0.7	3.4 ± 0.5

control rate at 1 h and triple at 2 h (Table II). The rate then gradually declined to approximately the control rate.

Comparing the dark + buffer and R + buffer (10-min irradiation at 1 h) experiments, there is an increase in growth rate (of less than 2-fold), though it is less than in the experiments of Table I, in which irradiation was given at 2 h. When IAA was applied at time zero and R at 1 h, the over-all growth pattern was similar to that seen with IAA alone. At 3, 4, and 5 h the R + IAA-treated plants showed a slightly higher growth rate than the dark + IAA-treated plants.

Peas. A few initial experiments were conducted with columns of 0.5-cm epicotyl segments strung on nylon line and immersed in growth medium. A set of typical growth curves is shown in Figure 1. The important result is that there is a marked inhibition of growth by R, with essentially complete photoreversibility by FR and no apparent effect of FR alone. The latent period for the response was about 1.5 to 2 h.

Table III presents the growth rates for intact plants, with irradiation given 1 h after the start of recording. The dark control growth rate remained fairly constant, with some increase toward the end. By 2 h after irradiation, the growth rate had begun to decline, and it reached less than one-third the control rate. The latent period for the decrease in growth rate was 80 ± 9 min. FR substantially reversed the growth inhibition, and the R + FR growth rates were little changed over 6 h. FR and blue light each produced a small inhibition, but the changes were too slight to permit the determination of a latent period.

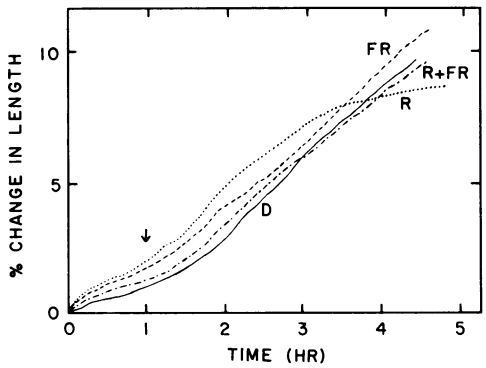

FIG. 1. Effect of light on pea epicotyl segment growth. Columns of 15 0.5-cm pea epicotyl segments were immersed in 5 mM K-citrate (pH 6.2) containing 1.5% sucrose and 20 μM $Co(NO_3)_2$. Growth was calculated every 4 min as a per cent of the initial length of the column. Irradiations were given 1 h after the start of recording (arrow). Representative curves are shown for dark (——), 5 min R (·····), 5 min FR (– – –), and 5 min R + 5 min FR (-·-·-).

DISCUSSION

Our chief goal was to determine latent periods for the effects of light on growth. Some information was also obtained on other aspects of the light control of growth.

The promotion of coleoptile growth by R is characteristic only of the apical region of younger coleoptiles (14), as shown in excised oat (12) and wheat (15) segments and in intact wheat and barley plants (14). In contrast, R inhibits the growth of entire intact rice, oat (21), wheat, and barley (14) coleoptiles, or of the basal region of intact wheat and barley coleoptiles (14), or of excised basal wheat segments (15). Blaauw *et al.* (4) reported a stimulation by R of the growth of very young intact oat coleoptiles.

Our experiments with excised apical coleoptile segments were confounded by the spontaneous growth response. Attempts to eliminate this response with agents such as 1 mM *p*-fluorophenylalanine (8) were not very successful. No such complications were evident in our single coleoptile experiments. The spontaneous growth response is probably an artifact of the use of excised segments immersed in solution, so alternate techniques should be considered whenever possible.

The sensitive recording technique indicated a latent period for the R response (Table I) considerably shorter than that reported previously for segments (12). The response to R was much slower than the response to IAA (Table III, and refs. 6 and 7), implying different mechanisms of action. The latent period was slightly longer than that for the R effect on $^{86}Rb^+$ uptake (between 15 and 30 min [20]), but was shorter than that for the R effect on the acidification of the medium (between 1 and 2 h [19]). The promotion of acidification was FR-reversible (19), but the growth promotion was not. In contrast to the promotion of growth by IAA, R acts through a mechanism not involving stimulation of H^+ efflux (22). Also, since R promoted $^{86}Rb^+$ uptake by both apical and basal coleoptile segments (20), while only promoting the growth of the former (14), there is no evidence linking the ion uptake and growth promotion effects. The latent period for the response to R provides no definitive information as to the possible site of action (on genes, membranes, enzymes, etc.).

The promotions of growth by 10-min irradiations were transient (Table I). In continuous light the decline was more gradual.

A notable feature of Table I is the lack of FR photoreversibility of the R effect. Other findings (4) argue against the possibility that there was an extremely rapid escape from photocontrol. In long term experiments with intact plants of the same variety, only a small (and sometimes nearly undetectable) proportion of the over-all photostimulation of coleoptile growth was FR-reversible (4). The total light dose we used (1.8 $w/s \cdot cm^2$) is within the range expected to show minimal photoreversibility (Fig. 2 in ref 4). Incomplete photoreversibility was also seen in the photoinhibition

Table III. Growth Rates of Intact Pea Third Internodes Given Different Light Treatments

The growth of the third internode of intact pea plants was recorded, with the plant clamped at the second node and the arm of the angular displacement transducer resting on the hook. There were at least 7 replicates of each experiment. Light treatments were given 1 h after the beginning of recording. Growth rates were calculated each hour, as described in the text. Data are presented as mean ± SE.

TREATMENT	GROWTH RATE % INCREASE IN LENGTH/H 1 H	2 H	3 H	4 H	5 H	6 H
Dark	3.3 ± 0.3	3.2 ± 0.6	3.0 ± 0.4	3.4 ± 0.5	3.4 ± 0.5	3.9 ± 0.6
5 min Red	3.4 ± 0.4	4.0 ± 0.5	2.0 ± 0.2	1.7 ± 0.3	1.8 ± 0.3	1.1 ± 0.1
5 min Far red	3.5 ± 0.6	3.0 ± 0.5	2.3 ± 0.2	2.6 ± 0.2	2.4 ± 0.2	2.4 ± 0.2
5 min Red + 5 min Far red	2.8 ± 0.3	2.8 ± 0.3	2.4 ± 0.3	2.2 ± 0.3	2.7 ± 0.3	2.9 ± 0.4
5 min Blue	4.1 ± 0.4	2.9 ± 0.4	3.2 ± 0.4	3.1 ± 0.4	3.2 ± 0.5	3.0 ± 0.5

of growth of the intact oat mesocotyl (4) and intact rice coleoptile (21), in contrast, excised oat (cv. Clintland) (12) and rice (21) apical coleoptile segments showed nearly complete photoreversibility (light doses not given). If the theory developed from long term studies can be applied to our results, then our system behaves more like an intact coleoptile than an excised apical segment.

FR alone caused some promotion of growth, but the effect was much less than that of either R or R + FR. Blaauw *et al.* (4) suggested that either an extremely low per cent Pfr (as established in FR) saturates the growth response, or that phytochrome is not the only photoreceptor involved. There are several instances of action by Pfr levels too low to measure spectrophotometrically (11). The phytochrome photostationary state established by FR should be the same whether or not there was a preceding R treatment. Since the effects of FR and of R + FR were different (Table I), perhaps there is a complex interaction between phytochrome and another pigment. Yet, the latent periods and the pattern of decline from the peak growth rates were quite similar in all cases. The action of continuous FR (but not a short treatment) has been explained in terms of phytochrome (10). That blue light exerted an effect quite comparable to that of R is consistent with action either by phytochrome (21) or by another pigment (16), so additional work is needed to resolve this point.

IAA caused a rapid (6, 7) but transient increase in growth rate. Most published studies show continuous growth recordings for only a few h, so the transience has not been noted, although Köhler (13) noted a decline in growth rate to nearly the basal level after 3 h. Although we used a high IAA concentration, the total amount applied was small, in comparison to systems in which segments are continually immersed in a large volume of IAA at a lower concentration. We observed about a tripling of the growth rate; such a response required that less than 0.1 μM IAA be supplied to immersed segments and was far below the growth rate attainable with saturating levels of IAA (5). Because the coleoptiles were still receiving a supply of IAA from the intact apex, a high exogenous IAA concentration may have been needed to enhance growth.

When IAA and R were applied together, the effect was certainly less than additive. R and IAA, alone or together, produced only a transient growth stimulation. This similarity is in contrast to the marked differences in latent period.

R caused a decline in the growth rate of intact peas to about one-third the dark control value (Table III). The latent period (about 80 min) was appreciably shorter than that observed by Galston *et al.* (9). The inhibition continued for several h, but we did not continue recording long enough to observe the previously reported return to the control level (9). The latent period is much longer than that reported for the promoting action of IAA (1, 26), implying different mechanisms of action. Based on our limited studies the latent period for segments was longer than *in vivo,* but our technique indicates a latent period shorter than the 4 h previously reported for segments (3). R did not change the ability of pea epicotyl segments to take up $^{86}Rb^{+}$ or to alter the pH of the medium (20). The inhibition of growth by R does not depend on changes in either of these processes. The acid growth theory (22) does not seem applicable to control of growth by phytochrome.

The R-induced inhibition of growth was largely reversible by FR, and the effect of FR alone was generally similar to that of R + FR light (Table III). Long term experiments with peas generally show considerable FR photoreversibility (2, 23), although the effects of FR are somewhat dependent on the test conditions.

We found blue light to act very much like FR (Table III), in marked contrast to the similarity of blue and R effects in pea segment growth (2). In intact peas, then, it would seem that a short exposure to either blue or FR creates about the same photostationary state, unless some pigment other than phytochrome is involved. Meijer (16), using gherkin hypocotyls exposed to continuous light, reported an immediate growth inhibition by blue light, while R and FR both inhibited growth with a latent period of at least 30 min. There is thus no consistent explanation for the effects of blue light on growth (16). We are continuing to study the action of blue light and of IAA on peas.

LITERATURE CITED

1. Barkley GM, ML Evans 1970 Timing of the auxin response in etiolated pea stem sections. Plant Physiol 45: 143–147
2. Bertsch WF 1963 The photoinhibition of growth in etiolated stem segments. III. Far red reversibility of blue light effects in *Pisum.* Am J Bot 50: 754–760
3. Bertsch WF, WS Hillman 1961 The photoinhibition of growth in etiolated stem segments. II. Growth caused by sugars in *Pisum.* Am J Bot 48: 504–511
4. Blaauw OH, G Blaauw-Jansen, WJ Van Leeuwen 1968 An irreversible red light-induced growth response in *Avena.* Planta 82:87–104
5. Cleland RE 1972 The dosage response curve for auxin-induced cell elongation: a reevaluation. Planta 104:1–9
6. de la Fuente RK, AC Leopold 1970 Time course of auxin stimulation of growth. Plant Physiol 46: 186–189
7. Evans ML, PM Ray 1969 Timing of the auxin response in coleoptiles and its implications regarding auxin action. J Gen Physiol 53: 1–19
8. Evans ML, MR Schmitt 1975 The nature of spontaneous changes in growth rate in isolated coleoptile segments. Plant Physiol 55: 757–762
9. Galston AW, AA Tuttle, PJ Penny 1964 A kinetic study of growth movements and photomorphogenesis in etiolated pea seedlings. Am J Bot 51: 853–858
10. Hartmann KM 1966 A general hypothesis to interpret "high energy phenomena" of photomorphogenesis on the basis of phytochrome. Photochem Photobiol 5: 349–366
11. Hillman WS 1967 The physiology of phytochrome. Annu Rev Plant Physiol 18: 301–323
12. Hopkins WG, WS Hillman 1965 Response of excised *Avena* coleoptile segments to red and far-red light. Planta 65: 157–166
13. Köhler D 1956 Über die Beziehungen zwischen der Länge von Haferkoleoptilen und der Wachstumsgeswindigkeit ihrer isolierten Ausschnitte. Planta 47: 159–164
14. Lawson RV, RL Weintraub 1975 Effects of red light on the growth of intact wheat and barley coleoptiles. Plant Physiol 56: 44–50
15. Lawson RV, RL Weintraub 1975 Interactions of microtubule disorganizers, plant hormones, and red light in wheat coleoptile segment growth. Plant Physiol 55: 1062–1066
16. Meijer G 1969 Rapid growth inhibition of gherkin hypocotyls. Acta Bot Néerl 17: 9–14
17. Mohr H 1972 Lectures on Photomorphogenesis. Springer-Verlag, New York
18. Newman IA, WR Briggs 1972 Phytochrome-mediated electric potential changes in oat seedlings. Plant Physiol 50: 687–693
19. Pike CS, AE Richardson 1977 Phytochrome-mediated hydrogen ion excretion by *Avena* coleoptiles. Plant Physiol 59: 615–617
20. Pike CS, AE Richardson 1979 Red light and auxin effects on ^{86}rubidium uptake by oat coleoptile and pea epicotyl segments. Plant Physiol 63: 139–141
21. Pjon CF, M Furuya 1967 Phytochrome action in *Oryza sativa* L. I. Growth responses of etiolated coleoptiles to red, far-red, and blue light. Plant Cell Physiol 8: 709–718.
22. Rayle DL 1973 Auxin-induced hydrogen ion secretion in *Avena* and its implications. Planta 114: 63–73
23. Thomson BF 1959 Far red reversal of internode-stimulating effect of red light on peas. Am J Bot 46: 740–742
24. Warner HL, AC Leopold 1971 Timing of growth regulator responses in peas. Biochem Biophys Res Commun 44: 989–994
25. Yamamoto R, K Maki, Y Yamagata, Y Masuda 1974 Auxin and hydrogen ion actions on light-grown pea epicotyl segments. I. Tissue specificity of auxin and hydrogen ion actions. Plant Cell Physiol 15: 823–831