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Photoinhibition of Stem Elongation by Blue and Red Light[1]

Effects on Hydraulic and Cell Wall Properties

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ABSTRACT

The underlying mechanism of photoinhibition of stem elongation by blue (BL) and red light (RL) was studied in etiolated seedlings of pea (*Pisum sativum* L. cv Alaska). Brief BL irradiations resulted in fast transient inhibition of elongation, while a delayed (lag approximately 60 minutes) but prolonged inhibition was observed after brief RL. Possible changes in the hydraulic and wall properties of the growing cells during photoinhibition were examined. Cell sap osmotic pressure was unaffected by BL and RL, but both irradiations increased turgor pressure by approximately 0.05 megapascal (pressure-probe technique). Cell wall yielding was analyzed by *in vivo* stress relaxation (pressure-block technique). BL and RL reduced the initial rate of relaxation by 38 and 54%, while the final amount of relaxation was decreased by 48 and 10%, respectively. These results indicate that RL inhibits elongation mainly by lowering the wall yield coefficient, while most of the inhibitory effect of BL was due to an increase of the yield threshold. Mechanical extensibility of cell walls (Instron technique) was decreased by BL and RL, mainly due to a reduction in the plastic component of extensibility. Thus, photoinhibitions of elongation by both BL and RL are achieved through changes in cell wall properties, and are not due to effects on the hydraulic properties of the cell.

The photomodulation of stem elongation in etiolated seedlings has been intensively studied in several plant species (14). These studies clearly indicate that at least two photoreceptors, *e.g.* phytochrome and a blue photoreceptor (cryptochrome), are involved in the inhibition of stem elongation by light. The separate action of these photoreceptors can be distinguished by several criteria (6, 14, 18), such as differences in the time-course of RL[2] and BL inhibition. In etiolated (5, 6) as well as in light-grown (14) seedlings, BL inhibits elongation within seconds, while the inhibition by RL begins 15 to 90 min after the onset of irradiation.

Little is known about the effects of light on the different processes that regulate the rate of cell expansion. The rate of cell expansion (r) is a function of hydraulic cell parameters as well as of cell wall properties, and has been described by the following simultaneous equations:

$$r = \phi(P - Y) \quad (1)$$

and

$$r = L(\Delta\pi - P) \quad (2)$$

in which ϕ is the cell wall yield coefficient, Y is the yield threshold (the turgor pressure that must be exceeded for wall yielding to occur), P is turgor pressure, $\Delta\pi$ is the difference in osmotic pressure, and L is the cell hydraulic conductance (8, 21, 23). Thus, photoinhibition of elongation must occur through changes in one or more of the above parameters. Light might reduce growth rate by changing cell wall yielding properties (ϕ, Y), or by decreasing water uptake due to changes in hydraulic properties of the tissue, reducing P and wall stress at the same time. Recently, Cosgrove (10) reported that BL reduced the rate of elongation of etiolated cucumber hypocotyls by decreasing the wall yielding coefficient (ϕ), but not the yield threshold (Y). The purpose of the present study was to examine and compare the mechanisms by which RL and BL inhibited stem elongation in etiolated pea seedlings. Light effects on cell wall properties were studied *in vivo* by a wall relaxation (pressure-block) technique (9), and *in vitro* by stress/strain (Instron) analysis. Hydraulic parameters of the growing region were measured by vapor pressure osmometry (osmolality) and pressure-probe (turgor pressure) techniques.

MATERIALS AND METHODS

Plant Material

Pea (*Pisum sativum* L. cv Alaska) was obtained from W. Atleee Burpee Co., Warminster, PA. Seeds were sown in polyethylene vials (73 mm height, 26 mm diameter) filled with vermiculite, drenched with 100% Hoagland solution, and germinated in darkness at 28 ± 1°C. To reduce evaporative loss of water, vials with the growing seedlings were enclosed in plastic boxes and kept under high humidity. Seedlings selected for experiments were 5 to 7 cm tall, and their second internodes were 10 to 15 mm long. All measurements were made on the second internode. Seedlings were handled and selected under dim green light from a 40 W cool-

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[2] Abbreviations: RL, red light; BL, blue light; P, turgor pressure; π, osmotic pressure; ϕ, wall yield coefficient; Y, wall yield threshold; ϵ, volumetric elastic modulus; $\Delta\psi_g$, growth-sustaining water potential difference from xylem to epidermis.

white fluorescent lamp (9F 40/cw, Westinghouse, NY) with two green and one amber filters (Roscolene No. 874 and 813, Rosco, Port Chester, NY).

Light Sources

Broadband light source consisted of a 15 W cool-white fluorescent lamp (F 15 T 12/cw Philips, NY) filtered through different combinations of acetate filters:

BL

Three layers of blue filters (Celluloid No. 1654, Mazzuchelli, castiglione Olona, Varesse, Italy), provided 6 to 7 μmol m^{-2} s^{-1} at plant level. Unlike most blue acetates, this filter does not transmit above 700 nm.

RL

Two red and one amber filters (Roscolene No. 821 and No. 813) provided 7 to 8 μmol m^{-2} s^{-1} at plant level.

Light sources used in growth transducer experiments were located on one side of the seedlings, with a mirror on the opposite side to minimize light gradients across the epicotyl tissue.

Elongation Measurements

Changes in stem length (second internode) were measured electronically using position transducers (Schaevitz Engineering, Pennsanken, NJ) connected to a microcomputer data acquisition system, and rates were obtained by electronic differentiation (6). Seedlings were connected to the transducer core assembly by a thin polyester thread, 1 to 2 mm below the hook. Seedlings were irradiated 4 to 6 h after attachment.

P

The apical 2 cm of the growing stem of intact seedlings was sealed in a plastic chamber to prevent evaporation. After 30 to 45 min of equilibration, the turgor pressure of about 10 different cortical cells was measured in the middle of the growing region (6–8 mm below the hook) with a computed-assisted version of a pressure probe with remote controls (11). In RL and BL treated seedlings, P was measured 2.5 to 3 h and 0.5 h after the onset of irradiation, respectively (*i.e.* during the time of maximal inhibition).

π

Tissue bulk osmolality was measured in sap expressed from the growing region (1 cm section below the hook) with a vapor-pressure osmometer (model 5500; Wescor, Logan, UT). Osmolality of RL and BL treated seedlings was measured 2.5 to 3 h and 0.5 h after the onset of the irradiation, respectively. Fresh or frozen/thawed tissue was used. Osmolality was converted to osmotic pressure dividing by 403 mOms kg^{-1} MPa^{-1}.

In Vivo Stress Relaxation

Stress was measured by the pressure-block technique (9). The apical 1.5 cm of the stem was sealed in a custom-made pressure chamber, and the position transducer was attached to the epicotyl 2 to 3 mm below the hook. Stress relaxation was initiated by raising the chamber pressure, so that it prevented elongation without causing shrinkage. Stem length was held constant within ± 2 μm. The pressure required to prevent (block) elongation increases with time and provides a measure of cell wall yielding properties. The pressure block unit was interfaced with a microcomputer programmed to regulate chamber pressure, so as to keep stem length constant. Growth rate was monitored by a chart recorder. In all treatments relaxation was initiated (onset of pressurization) 2.5 to 3 h after the plants were enclosed in the chamber. RL treated plants were sealed immediately after the light treatment (5 min RL). In BL treated plants, BL irradiation started 0.5 h before the onset of relaxation and continued during the experiment to prevent recovery from the BL inhibition during the relaxation measurement. Relaxations were started in all treatments after the plants reached stable growth rates. Time-course of changes in pressure during the relaxation was fitted to a third degree polynomial by the least squares method (10).

Stress/Strain Analysis

Measurements were carried out in frozen/thawed 1 cm stem segments taken from the growing regions just below the hook. Segments were immediately frozen and stored at −20°C. In RL-treated plants, segments were cut 2.5 h after a 5 min RL irradiation. In BL-treated plants, segments were cut at the end of a 0.5 h BL irradiation. Equivalent segments were taken from dark control plants. After thawing, segments were pressed for 5 min between paper towels and glass slides under a 300*g* weight to remove most of the cell sap. The flattened segments were mounted between clamps (5 mm between clamps) in a custom-made extensiometer interfaced to a microcomputer. The lower clamp was attached to a force transducer (B6–100, Kulite, Ridgefield, NJ), and the other one was attached to a sliding stage by a digital stepper motor (K82401-P2, Airpax, Chesire, CT). Vertical movement of the sliding stage was controlled by the microcomputer that also recorded the signals from the force transducer and displacement transducer. The 5 mm portion of the segment between the clamps was extended to a tension of 30*g* force in two cycles at 3 mm min^{-1}. Extensibility was calculated as the reciprocal slope of each load-extension curve (3). A second degree polynomial was fitted by the least squares method to the data of each of the two extension cycles, and the slope at the end of each cycle was computed from the fitted polynomial (10). Slopes are expressed as percent extension for 100*g*-force, and are corrected for the extension that occurs during the measurement. The plastic component of the extensibility was found from the difference between first (plastic + elastic) and second (elastic) extensibilities.

RESULTS

Inhibition of Elongation by RL and BL

Stem elongation of etiolated Alaska pea seedlings was inhibited by both RL and BL. However, the seedlings exhibited different patterns of response to these irradiations (Fig. 1). Response to RL was relatively slow. After RL irradiation, a

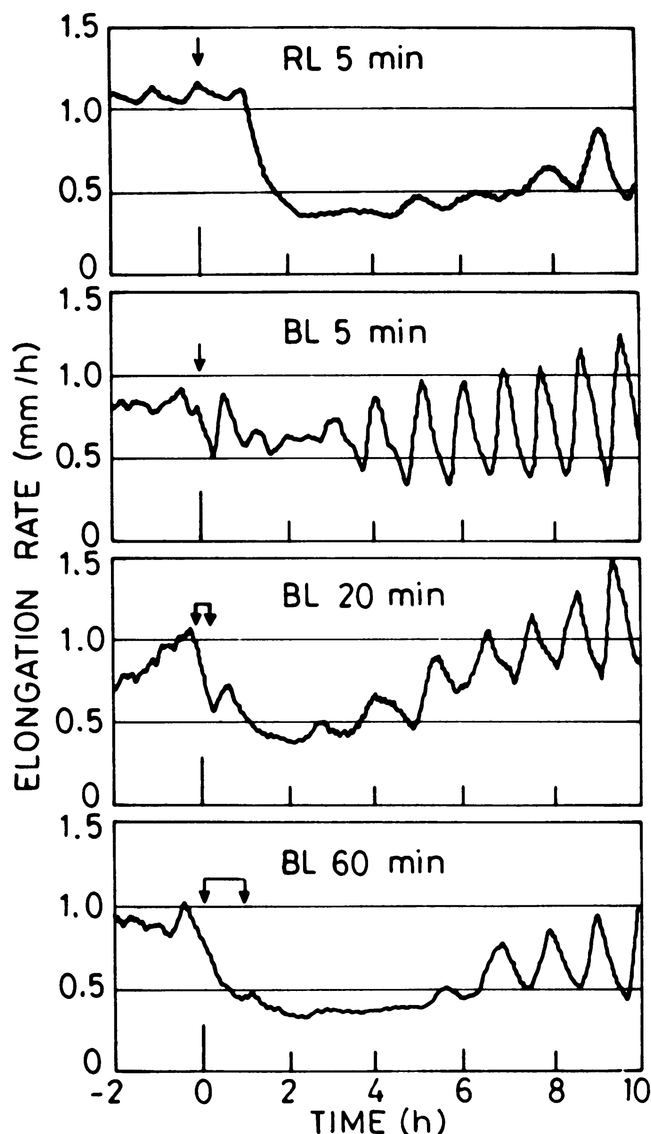

Figure 1. RL and BL effects on rates of elongation of etiolated Alaska pea. Elongation was measured continuously with LVDT position transducers. Seedlings were irradiated with RL (7–8 μmol m^{-2} s^{-1}) and BL (6–7 μmol m^{-2} s^{-1}) for different periods of time, 5 to 6 h after attachment to transducers. Representative time-courses for different treatments are shown.

lag period of about 60 min occurred before growth rate started to decrease (Fig. 2). The response times to attain 50 and 90% of maximal inhibition were 85 and 140 min, respectively, after the onset of irradiation. The new low growth rate remained stable for several hours, until it started to recover 6 to 8 h after the RL irradiation. At this time, progressively larger oscillations with about 1 h period started to appear. Analysis of these oscillations by time-lapse video (not shown) indicates these are true growth-rate oscillations, not artifacts due to the RL-enhanced nutation reported by Britz and Galston (2) in Alaska pea.

The time-course of the responses to RL was unaffected by the length of the irradiations we used (10 to 30 min), but the degree of growth rate inhibition was significantly (P = 0.01) increased the longer the exposure to RL (from 41–22% of growth rate in dark controls; see Fig. 2).

The effects of BL irradiations (range 1–60 min) were faster and more complex compared with RL (Fig. 1). The response to BL was compounded of a fast, short-term inhibition with a slower long-term inhibition. Onset of growth rate inhibition occurred about 1 min after the start of the BL (results not shown [5]), and 90% of the maximal short-term inhibition was reached 30 min later, irrespective of the length of BL irradiation (Fig. 2). A transient recovery of the growth rate was consistently observed after BL irradiations shorter than 60 min, and was followed by a subsequent decrease to a lower rate (long-term inhibition). Maximal long-term inhibition was attained about 130 min after the onset of irradiation, a time interval similar to that found for the same level of inhibition by RL. Longer BL irradiations resulted in significantly (P = 0.01) lower growth rates during the short- and long-term inhibition periods, as well as in lower rates reached during the recovery phase (Fig. 2). The transient recovery was absent in the 60 min BL treatment. In this treatment, however, a consistent further decrease in elongation was observed about 60 min after the onset of irradiation, indicating the presence of the long-term inhibition. In all BL treatments a gradual recovery of growth rate followed the long-term inhibition, with the simultaneous appearance of oscillations with the

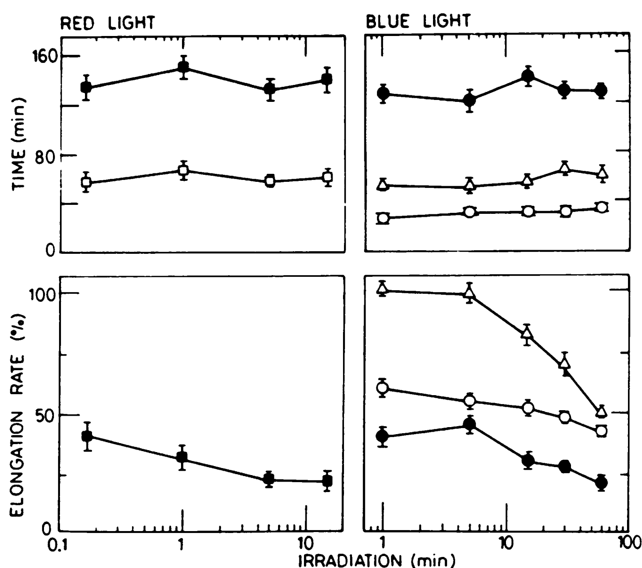

Figure 2. Effects of length of irradiation with RL (7–8 μmol m^{-2} s^{-1}) or BL (6–7 μmol m^{-2} s^{-1}) on the kinetics of epicotyl elongation in etiolated Alaska pea. Top panels: different parameters of the time-response patterns to RL and BL are presented. RL treatments: lag time to onset of inhibition (□), and time to 90% of maximal inhibition (■). BL treatments: time to 90% of maximal short-term (○) and long-term (●) inhibition; and time to maximal recovery in growth rate after short-term inhibition (△). Bottom panels: Relative inhibition of growth rates (as percentage of growth rates in continuous darkness: 0.81 ± 0.03 mm h^{-1}). RL treatments: (■), mean growth rate 3 to 6 h after RL irradiation; BL treatments: (○), growth rate at time of maximal short-term inhibition; (△), at time of maximal recovery from short-term inhibition; (●), during period of long-term inhibition.

same amplitude as those observed after RL treatments (Fig. 1).

The kinetics of the elongation response to RL and BL support the possibility that at least two photoreceptors are involved in the photoregulation of stem elongation in etiolated Alaska pea (6, 15). The fast inhibitory response is mediated by a pigment which absorbs only the blue region of the light spectrum, while the delayed long-term inhibition is mediated by a pigment absorbing in the red and blue regions. Thus, the response of Alaska pea to BL results from the consecutive inhibitory actions of these two light-sensitive systems.

Osmotic and Turgor Pressures

These hydraulic parameters were measured in: (a) dark grown seedlings, (b) seedlings 0.5 h after continuous BL irradiation (6–7 μmol m^{-2} s^{-1}), or (c) seedlings 2.5 h after a 5 min RL irradiation (7–8 μmol m^{-2} s^{-1}). These times and irradiations were chosen so as to result in similar values of maximal inhibition at the time the measurements were made (Figs. 1 and 2). Osmotic pressure was unaffected by the light treatments. However, BL and RL increased turgor significantly (P = 0.001) by 0.05 and 0.06 MPa, respectively (Table I).

Thus, inhibition of elongation by RL and BL is neither due to a reduction in the bulk cell osmotic pressure nor to a decrease in the turgor force acting on the cell wall. These results suggest that light may affect cell expansion through changes in the cell wall properties and that turgor increases as an indirect effect of slower growth rate.

Cell Wall Relaxation

Cell wall properties of the growing region in pea epicotyl were studied *in vivo* by the pressure-block technique (9). In this technique, water uptake by the growing region is prevented by applying the minimum external pressure that stops growth without causing shrinkage. Since cell wall loosening continues in the living tissue even in the absence of cell expansion, pressure has to be gradually increased to counteract cell wall relaxation and resulting water uptake. Thus, the time-course of pressure increase is a measure of cell wall relaxation.

From the representative stress-relaxation time courses shown in Figure 3 for dark, RL, and BL treated pea seedlings

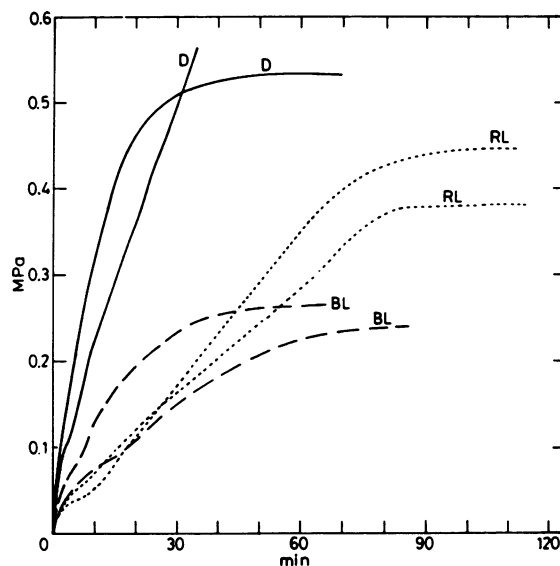

Figure 3. RL and BL effects on *in vivo* cell wall relaxation. Relaxation (pressurization) was started 2.5 to 3 h after sealing the upper 1.5 cm of the stem in the pressure chamber. RL (5 min, 7–8 μmol m^{-2} s^{-1}) was given just before sealing the seedlings. BL (6–7 μmol m^{-2} s^{-1}) started 0.5 h before the onset of relaxation and was given continuously to prevent recovery in rate of stem elongation. Representative time courses of relaxation are shown for each treatment.

and the summarized data for relaxation parameters in Table II, it is evident that the light treatments decreased the rate and the amount of cell wall relaxation. However, the time course observed for stress relaxation (Fig. 3) was more complex than those expected from the available theories (8, 21, 23) describing the wall yielding process. A simple exponential decay in the rate of relaxation was expected as turgor pressure approached yield threshold (7). However, in dark-treated as well as in light-treated pea seedlings, the increase in relaxation occurred in two phases after the onset of relaxation (pressuration).

During the first phase of the relaxation (up to about 5 min from onset of pressuration), the initial rate of relaxation was significantly (P = 0.001) inhibited by RL (54%) and BL (38%), in parallel with their inhibitory actions on stem elongation (Table II). Furthermore, the initial pressure required to stop growth (P_0) was also smaller in the light-treated seedlings.

Table I. *Hydraulic Cell Parameters as Affected by BL and RL Irradiations*

Measurements were made after 0.5 h of continuous BL (6–7 μmol m^{-2} s^{-1}), or 2.5 h after a 5 min RL irradiation (7–8 μmol m^{-2} s^{-1}). Values are means (SE). Means with different letters are significantly different at P = 0.001. (*n*: dark: 20, RL: 18, BL: 12).

	MPa			Significance
	Dark	RL	BL	
Turgor pressure (P)[a]	0.53 (0.01)[a]	0.59 (0.01)[b]	0.58 (0.01)[b]	P < 0.001
Osmotic pressure (π)	0.84 (0.01)	0.82 (0.01)	0.83 (0.02)	NS
Water potential (ψ)[b]	−0.32	−0.23	−0.25	

[a] Measured by pressure microprobe in cortical cells 6 to 8 mm below hook. [b] Calculated as $P - \pi$.

Table II. *Effects of RL and BL on in Vivo Cell Wall Relaxation of Etiolated Pea Seedlings (pressure-block technique)*

The apical 10 mm of the epicotyl was sealed in the chamber and relaxation (pressurization) was started 2.5 h later. RL (5 min; 7–8 μmol m^{-2} s^{-1}) was given just before mounting the seedlings in the chamber. BL (6–7 μmol m^{-2} s^{-1}) was turned on 30 min before start of relaxation, and seedlings were irradiated throughout the relaxation. Values are means (SE). Means with different letter are significantly different at P = 0.05. n = 10 in all treatments.

	Dark	RL	BL	Units	Significance[a]
Elongation rate	3.3 (0.4)[a]	1.2 (0.2)[b]	1.4 (0.2)[b]	% h^{-1}	***
Initial relaxation rate[b]	1.09 (0.14)[a]	0.50 (0.08)[b]	0.68 (0.13)[b]	MPa h^{-1}	***
Time to inflexion (T_i)[c]	4.9 (0.7)[a]	7.6 (0.6)[b]	5.6 (0.9)[a]	min	*
Relaxation rate after inflexion[d]	1.28 (0.16)[a]	0.54 (0.10)[b]	0.58 (0.10)[b]	MPa h^{-1}	***
P_o[e]	0.068 (0.01)[a]	0.026 (0.005)[b]	0.037 (0.007)[b]	MPa	***
P_f[f]	>0.55[a]	0.42 (0.03)[b]	0.27 (0.03)[b]	MPa	**
$(P_f - P_o) \approx (P - Y)$[g]	>0.48	0.43 (0.02)[a]	0.25 (0.02)[b]	MPa	**
Y	0.05	0.16	0.33	MPa	
Hydraulic conductance (L)[h]	0.63 (0.14)	0.75 (0.18)	0.46 (0.08)	MPa^{-1} h^{-1}	NS
Yield coefficient (σ)[i]	0.069	0.028	0.056	MPa^{-1} h^{-1}	

[a] ***, **, *, Treatments effects were significant at P = 0.001; P = 0.01; P = 0.05, respectively; NS = no significant difference. [b] Calculated at 3 minutes, from the slope between 1 and 5 min in fitted cubic polynomial. [c] Time to inflexion in fitted cubic polynomial. [d] Calculated as the linear slope of the relaxation after the inflexion point. [e] The initial pressure required to stop growth. An estimate was obtained by extrapolating the slope between 1 and 5 min to time zero. [f] The pressure at the time when no further relaxation occurred, or where the slope of the relaxation fell below 0.1 MPa h^{-1}. Upper limit for the pressure chamber was 0.55 MPa. In dark controls P_f was >0.55 MPa in six seedlings, and 5.0, 5.2, 5.5, and 5.5 in the others. [g] $(P_f - P_o)$ values were calculated individually for each seedling. Thus, average $(P_f - P_o)$ differs slightly from the values calculated from the averages of P_f and P_o. [h] Growth-specific hydraulic conductance is calculated as relative growth rate/P_o for each seedling. [i] Values for σ were calculated from Equation 1, using mean values for r and Y from this table and values for P from Table I.

This initial pressure is needed to collapse the radial water potential difference ($\Delta\psi_g$) across the epicotyl (xylem to epidermis) caused by the continuous expansion of the growing cells (1, 8, 9). An estimate of P_0, and therefore of $\Delta\psi_g$, was obtained by extrapolating the initial relaxation time-course (between 1 and 5 min) to time zero (Table II). A higher P_0 was needed to stop the faster elongation of the seedlings in darkness (0.068 MPa) than to stop the slower elongation of the RL (0.026 MPa) and BL (0.037 MPa) treated seedlings. Furthermore, RL and BL irradiations reduced P_0 approximately to the same extent as they inhibited elongation rate (54 *versus* 64% for RL; 38 *versus* 58% for BL).

The rate of relaxation declined with time and in several instances (particularly in RL and B) a plateau was nearly reached before the rate of relaxation increased again (Fig. 3). An acceleration of relaxation occurred at about 5, 8, and 6 min after the onset of relaxation in the dark, RL, and BL treated seedlings, respectively, as estimated by the time to inflexion point in fitted cubic polynomials (T_i in Table II). This increase in relaxation rate has been interpreted as a response to growth inhibition and not to pressure *per se* (10). In each treatment, rates of relaxation after the inflexion point were similar to the rates during the initial relaxation (Table II). Therefore, the light treatments inhibited to about the same extent the initial and the following relaxation (46 *versus* 42% for RL; 61 *versus* 44% for BL).

Wall relaxation continues until the declining P reaches the Y. We estimate $(P - Y)$ as the final pressure (P_f: pressure when cell wall relaxation is completed) minus the initial pressure to stop growth (P_0) (9). RL and BL irradiations reduced $(P - Y)$ at least by 0.05 MPa (10%) and 0.23 MPa (48%), respectively, compared to the dark treatment. These values are underestimates since during relaxation the upper limit for the pressure chamber was reached in 6 of the 10 dark seedlings measured. Thus, their P_f was, in fact, somewhat higher than 0.55 MPa.

Since turgor pressure (P) was slightly (about 0.05–0.06 MPa) increased by the light treatments (Table I), the lower $(P - Y)$ values show that Y was increased by the light treatments. Calculated yield threshold (Y) values for dark, RL, and BL treatments were 0.05, 0.16, and 0.33 MPa, respectively. Relatively low Y values (about 0.05 MPa) were found also in dark grown Alaska pea (24) and cucumber (10). Thus, in RL and BL treated seedlings the lower rates of elongation and wall relaxation are associated with higher yield thresholds and lower values of $(P - Y)$.

To clarify this further, we estimated the yield coefficient ϕ using Equation 1 and the mean values for r, Y, and P found in Tables I and II. A small reduction (19%) in ϕ was seen in the BL-treated seedlings, whereas the RL-treated seedlings showed a 60% reduction in ϕ (Table II). A similar conclusion

can be reached by comparing the initial rates of relaxation (dP/dt) with the values for ($P - Y$), using the relation (7):

$$dP/dt = \epsilon\phi(P - Y) \quad (3)$$

where ϵ is the volumetric elastic modulus. Assuming ϵ is unchanged by the light treatments, we can calculate that RL decreases ϕ by more than 50%, whereas BL does not reduce ϕ. Thus, by these calculations BL appears to act largely by raising Y, whereas RL acts mainly by reducing ϕ (see further interpretation in "Discussion" section).

Another possible mechanism by which light may inhibit elongation is through the reduction of hydraulic conductance of the epicotylar tissue. Since $\Delta\psi_g$ is related to the rate of growth by $r = L\ (\Delta\psi_g)$ (9), the hydraulic conductance for radial water movement across the stem (L) could be calculated for each seedling, and mean values are presented in Table II. Calculated conductance values were not significantly different under the various light treatments. These values (range 0.46–0.75 $MPa^{-1}\ h^{-1}$) are comparable to those found in dark grown pea by the pressure-block technique (0.99 $MPa^{-1}\ h^{-1}$) (24), but lower than those estimated by measuring the half-time of tissue swelling (2.0 $MPa^{-1}\ h^{-1}$) (7). Note that L is an order of magnitude larger than ϕ, further evidence that water uptake does not substantially restrict expansive growth (1, 8, 9).

Table III. *Stress-Strain Analysis*

Effects of RL (5 min; 7–8 μmol $m^{-2}\ s^{-1}$) and BL (30 min; 7–8 μmole $m^{-2}\ s^{-1}$) on the components of epicotyl extensibility (elastic and plastic) in etiolated pea seedlings. One cm segments were taken from the growing region below the hook 2.5 h (RL) and 0.5 h (BL) after the onset of irradiation. Measurements were made in frozen/thawed sections. Values are means (SE) of five separate experiments. Total n = 30 to 35 in each treatment.

Extensibility Component	Dark	Light	Light/Dark	Significance[a]
	%/100 *g*			
RL irradiation				
Total	16.7 (0.6)	14.9 (0.6)	0.84	**
Elastic	10.5 (0.3)	10.0 (0.3)	0.95	NS
Plastic	6.2 (0.3)	5.0 (0.4)	0.80	**
BL irradiation				
Total	23.5 (0.6)	19.6 (0.4)	0.83	***
Elastic	12.5 (0.3)	11.6 (0.3)	0.93	NS
Plastic	11.0 (0.4)	8.2 (0.3)	0.75	***

[a] ***, **, *: Treatments effects were significant at P = 0.001; P = 0.01; P = 0.05, respectively; NS = no significant difference.

Stress Strain Analysis

The possibility that RL and BL reduced cell wall yielding by changing its viscoelastic properties was studied by the Instron technique (stress/strain analysis). The plastic and elastic components of cell wall extensibility were measured in frozen/thawed sections collected 0.5 and 2.5 h after the onset of BL and RL irradiations, at about the time when maximal inhibition of elongation was reached (Figs. 1 and 2). Relatively large, significant (P = 0.01), unexplained differences in extensibility components were found between sowings, even though growth conditions and processing techniques were practically identical. Nevertheless, consistent differences between dark and light treated seedlings were found in each experiment and were analyzed by a two-way ANOVA. In both the RL and BL series of experiments, the interactions [sowing × light treatment] were not significant for each extensibility component. Since the RL and BL effects are superimposed on the between-sowing variability, only the main effects for the light treatments are presented in Table III. RL and BL reduced by 20 and 25%, respectively, the plastic component of extensibility, without affecting the elastic component. Long-term cell wall extension (creep) of frozen/thawed section under pH 6.8 or 4.5 was unaffected by previous BL or RL irradiations of the etiolated seedlings (results not shown).

DISCUSSION

In the present work we compared the mechanisms by which RL and BL inhibit stem elongation in etiolated pea seedlings. Although the time courses of the elongation response to RL and BL were quite different (Figs. 1 and 2) and are probably mediated by two different photoreceptors (5, 13), the inhibition by both light sources has a common ground: it is caused by a reduction in cell wall yielding. Hydraulic parameters that determine elongation, such as bulk osmotic pressure (π, Table II) and hydraulic conductance (L, Table II) were unaffected by BL or RL. The central role of cell wall properties in the photomodulation of stem growth is emphasized by the fact that turgor pressure (P, Table I) was increased 0.05–0.06 MPa by the light treatments, though they inhibited elongation. The likeliest explanation for the rise in turgor after BL or RL is that cells with a slower relaxation rate approach osmotic equilibrium more closely, and so reach a higher turgor. In our case, elongation rate was inhibited by about 75% (compared to dark controls) at the time of turgor measurement (*e.g.* 2.5 and 0.5 h after RL and BL, Fig. 2). This increase in turgor gives an estimate of the radial water potential difference ($\Delta\psi_g$) supporting cell elongation in the epicotyl in darkness—*i.e.* a 0.05 to 0.06 MPa rise in turgor, associated with about 75% growth inhibition, indicates a $\Delta\psi_g$ of about 0.06 to 0.07 MPa. These values are in close agreement with P_0, the pressure needed to stop growth in the pressure-block system (P_0 = 0.068 MPa, Table II). Such an increase in turgor was not observed in cucumber seedlings after BL (10), probably because of a much smaller $\Delta\psi_g$ supporting growth or a more stringent regulation of turgor pressure in this species.

In pea the effects of BL and RL on wall relaxation are apparently due to different mechanisms. RL reduces relaxation mainly by lowering the wall yield coefficient (ϕ) with a concomitant smaller increase in the yield threshold (Y), while most of the inhibitory effect of BL was due to an increase in Y (Table II). However, the BL effect on Y is evidently not universal, since in cucumber BL appears to affect ϕ but not Y (10).

The values of ϕ calculated in this study are based on the relation $r = \phi(P - Y)$ and our calculation assumes that Y and ϕ remain constant during relaxation, so that we may relate them back to the values of r and P extant before the start of relaxation. The complex, nonexponential dynamics of relax-

ation (Fig. 3) bring this assumption into question. Other results also indicate that Y in pea epicotyls can shift quite substantially during relaxation (9, 12, 26; DJ Cosgrove, unpublished data). If so, then the interpretation of ϕ takes on a more complex character, in that it also incorporates the ability of the tissue to shift Y to lower values. A full understanding of how the dynamics of relaxation relates to long-term measures of ϕ and Y remains a challenge for the future. Despite these uncertainties, our results nevertheless point out that, although both RL and BL inhibit wall relaxation, they do so by different biophysical mechanisms. This suggests to us that two different biochemical processes are involved in wall relaxation (or at least in the control of wall relaxation).

The possibility that ϕ and Y can be influenced differentially by various factors is supported by other studies with pea seedlings. Removal of cotyledons inhibited stem elongation due to slower cell wall relaxation, caused mainly by an increase in Y (24). Auxin enhanced elongation and wall relaxation without affecting Y (7). Gibberellin (or gibberellin synthesis inhibitors) regulate elongation through concomitant changes in ϕ and Y (12).

In pea the decrease of wall relaxation after BL and RL is associated with a reduction in the plastic component of extensibility as determined in frozen/thawed sections by stress/strain (Instron) analysis (Table III). This fact may indicate that the BL and RL effects on elongation are due to changes in the viscoelastic properties of the cell wall. However, the relative reduction of the plastic extensibility by light was small (20–25%, Table III), compared to the inhibition of elongation (about 75% in LVDT measurements, Fig. 2; about 60% in seedlings mounted in the pressure-block system, Table II). Furthermore, in cucumber, the fast regulation of wall yielding by BL occurred without detectable changes in the viscoelastic properties of the cell walls (10). On the other hand, in several studies a correlation has been found between elongation rate and mechanical cell-wall properties as measured *in vitro* by Instron analysis (5), stress-relaxation (17, 30), or *in vivo* by extensibility measurements (19, 20). It is noteworthy that the changes in viscoelastic properties of the wall usually lag behind the changes in elongation rate and/or are of a smaller magnitude (3). For example, in maize mesocotyls, no correlation was found for up to 7 h between mechanical extensibility and changes in elongation rate induced by red or far-red irradiations, but changes in plastic extensibility were observed 20 h after irradiation (29).

In our case, the relatively small change in plasticity observed at the time of maximal inhibition of elongation by RL and BL (Table III; Fig. 2) may indicate that wall relaxation is photomodulated by metabolic processes unrelated to those determining the viscoelastic properties of the cell wall. Alternatively, since the quantitative relationship between cell wall plasticity and elongation rate is unknown, it is conceivable that relatively small changes in plasticity may be sufficient to produce larger changes in growth rate. This is a crucial dilemma in the interpretation of wall mechanical assays, and persists today despite the common use of this technique for more than 25 years.

In view of the different kinetics of BL and RL inhibition of elongation (fast *versus* slow response; Figs. 1 and 2), and the fact that ϕ and Y were differentially affected by these irradiations, we conclude that the photomodulation of growth and wall relaxation by BL and RL are mediated by different processes activated by different photosystems. This conclusion is also supported by the finding that prolonged (60 min) BL irradiation results in a two step inhibition of growth in which the first step (due to BL photoreceptor) does not saturate the response during the second step (due to RL photoreceptor) (Figs. 1 and 2). Thus, the responses are additive and superimposed.

Although RL and BL modulate growth through different mechanisms, they both act by inhibiting wall relaxation, and not by altering the hydraulic properties of the growing tissue. This is consistent with the view that the major limitation for cell expansion rate is the yielding properties of the wall (9). The results, furthermore, suggest that there are at least two points or processes controlling wall relaxation.

The RL inhibition is slow enough that changes in auxin availability or sensitivity may mediate the response, as hypothesized for RL responses of grass coleoptiles and mesocotyls (16, 22). In accordance with such a mechanism, both RL (Table II) and auxin (7) were found to modify pea stem growth principally via a change in ϕ. Further evidence for auxin mediation and its significance could be obtained by comparing the pattern of light-regulated genes (28) with auxin-regulated genes (15, 27). Such comparison might also help to identify genes involved in the control of cell wall expansion.

In contrast to RL inhibition, BL suppresses growth so quickly (within 30 s [5]) that a more rapid and reversible mechanism of growth control is required. One obvious candidate is inactivation of the plasma-membrane proton pump, with a consequent alkalinization of the apoplast and growth inhibition a la acid-growth hypothesis (4, 26). In cucumber seedlings, BL induces a large (100-mV) membrane depolarization which may be related to pump inhibition (25). At present, however, no direct evidence for the molecular nature of the growth control by BL in pea stems is available.

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