Linear Dichroism and Orientation of the *Phycomyces* Photopigment

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ABSTRACT The greater sensitivity of a cylindrical *Phycomyces* sporangiophore to blue light polarized transversely rather than longitudinally is a consequence of the dichroism and orientation of the receptor pigment. The abilities of wild type and several carotene mutants to distinguish between the two directions of polarization are the same. The **E**-vector angle for maximum response relative to the transverse direction is 42 ± 4° at 280 nm, 7° ± 3° at 456 nm, and 7° ± 8° at 486 nm. The in vivo attenuation of polarized light at these wavelengths is very small. The polarized light effect in *Phycomyces* cannot arise from reflections at the cell surface or from differential attenuations due to internal screening or scattering.

In 1934, Castle discovered that when a *Phycomyces* sporangiophore (spph) is illuminated from opposite sides by longitudinally (vibration of electric vector, **E**, parallel to the long axis of the spph cylinder) and transversely polarized light, the longitudinal beam must be 10–15% more intense than the transverse beam to prevent phototropic bending. He concluded that this effect is due to differential reflection losses (*see* Discussion) and not to dichroism of oriented receptors. This argument stood solidly until 1958, when Jaffe discovered that *Fucus* eggs germinate in a direction determined by **E** and stated that this result could be understood best in terms of anisotropic absorption of polarized light by linearly dichroic and oriented photoreceptors. He also suggested the same explanation for the effect in *Phycomyces*.

In order to settle this controversy, Shropshire, in 1959, measured the differential growth response of the spph to the two polarizations, at two wavelengths (380 nm and 450 nm), in air, and in a medium of approximately matching refractive index. He found that, in air, transversely polarized light was about 20% more effective at both wavelengths and that in the matching medium this greater effectiveness vanishes. Since dichroism is usually quite wavelength-dependent and since in a medium of matching refractive index reflections at the interface should be nearly eliminated, Shropshire

dismissed *Phycomyces*' sensitivity to polarized light as a consequence of differential surface reflections.

Jaffe (1960) contested the validity of these arguments. Using a theoretical analysis, he showed how Castle's phototropic result and Shropshire's growth response results can be explained by the dichroic oriented receptor hypothesis, and that reflection cannot account for the magnitudes that Shropshire observed for the differential growth responses in air. He reasoned that surface reflections are compensated quantitatively by multiple internal reflections within the spph. Jaffe's analysis, however, is valid only for the limiting cases of 0 and 100% internal light attenuation. Interpolating linearly between these limiting values, Jaffe concluded that reflection can only explain a 1.6% difference in effectiveness of the two polarized beams.

The validity of this linear interpolation, however, is questionable. The problem is better solved experimentally by utilizing mutants of *Phycomyces* aberrant in carotene production. These produce sporangiophores which differ markedly from the wild type in their optical density when grown on various media. The *carA5* (C2) and *carB10* (C5) mutants (Meissner and Delbrück, 1968; Ootaki et al., 1973) display a much reduced optical attenuation, approaching the limiting case of the transparent cylinder. *carR21* (C9) (Meissner and Delbrück, 1968) has an intermediate value of attenuation, greater than wild type's; and a newly selected mutant, *car-41* (C158), has a very large optical attenuation. Using these mutants, Jaffe's interpolation can be checked with a physiological range of attenuations for which the reflection hypothesis would predict a number of different values.

The existence of oriented screening pigments and structures within the cell could also give rise to the polarized light effect in *Phycomyces*. This can be checked by microspectrophotometric measurements of the in vivo optical attenuation of polarized light.

If the polarized light effect is due to the orientation and dichroism of the *Phycomyces* visual pigment, then the maximum response need not be for light polarized axially or transversely. It should be possible to measure the angle of orientation of the absorption transition moments by polarizing the stimulating light at angles away from the longitudinal or transverse directions.

Absorption by linearly dichroic oscillators is proportional to $\cos^2 \alpha$, where α is the angle between the **E**-vector and the absorption transition moment. A periodic variation in response, related to this function, should be observed when sweeping through various angles that **E** makes relative to the spph axis.

This paper discusses the measurement of the differential growth response of the wild type, and of the mutant strains C2, C5, C9, and C158 of *Phycomyces* to the longitudinal and transverse orientations of the **E**-vector of linearly polarized light. It describes measurements of the in vivo optical attenuation

of polarized light of wavelengths 306 nm, 320 nm, 446 nm, and 486 nm of the above strains. It concludes that the visual pigment is oriented and dichroic and details the measurement of the average orientations of the 280 nm, 456 nm, and 486 nm absorption oscillators of the visual pigment.

METHODS

Strains

Five strains of *Phycomyces blakesleeanus* were used in this study and are listed in Table I. The mutants differ from the wild type in β-carotene synthesis. C2 and C5 are colorless, C9 is red, producing lycopene instead of β-carotene, and C158 is "super yellow," accumulating five times the normal wild type concentration of β-carotene when grown on leucine-enriched medium.[1]

Growth Media

The media used are listed in Table II.

Culture Conditions

Spores of all five strains were diluted to 100 per milliliter of Hershey broth, heat shocked for 15 min at 48°C, and inoculated into 3 cm high, 1 cm diameter vials (4.5 × 1.4 cm vials for C158), containing 2 ml of various media. Wild type was grown on PDA and PDAY, C2 on LAC, C5 on PDA, C9 on PDAY, and C158 on GALY media. 20 of these vials were kept in closed glass jars at 20°C in room light of intensity 20 $\mu W/cm^2$. When the first spphs appeared the jars were opened and placed in growth chambers. Temperature was 22°C, the relative humidity 50–80% and the illumination was 4–5 $\mu W/cm^2$ from overhead tungsten lamps.

The spphs were harvested twice a day. The second through seventh crops of spphs, lengths 2.0–4.0 cm (wild type, C2, C5, C9) and 4.0–5.0 cm (C158), were used for all measurements.

Light Sources

Spphs were illuminated by a horizontal, collimated beam of light of desired spectral composition and polarization. For experiments utilizing visible light, a simple single lens projector with a tungsten or tungsten halogen lamp (Sylvania Electric Products, Inc., Lighting Products Div., Danvers, Mass.) served as the source. The lamp was powered by a voltage stabilizer (Raytheon Co., Microwave & Power Tube Div., Waltham, Mass.) and potentiometer (Superior Electric Co., Bristol, Conn., Powerstat) or a regulated DC power supply (Lambda Electronics Corp., Melville, N.Y., C-880M). The beam was filtered first by a heat-absorbing filter (Rolyn Optics Corp., Arcadia, Calif., KG3, 5 mm) and either a broad band blue color filter (Corning Glass Works, Science Products Div., Corning, N.Y., 5–61, 5 mm), a 455.5 nm interference filter (Ealing Optics Div., Ealing Corp., Cambridge, Mass., HBW = 10 nm), or

[1]I am gratefully indebted to Dr. Wan-Jean Hsu of the Fruit and Vegetable Chemistry Laboratory, A.E.S., U.S. Department of Agriculture, Pasadena, California 91106 for her measurements of the β-carotene content of C158 sporangiophores.

TABLE I

STRAINS OF *PHYCOMYCES BLAKESLEEANUS* USED IN THIS WORK

Strains C2, C5, C9, C158 were derived from spores of NRRL 1555 mutagenized by nitrosoguanidine as described in Heisenberg and Cerdá-Olmedo (1968).

Strain	Genotype	Phenotype
NRRL 1555	(−)	Wild type
C2	*carA5* (−)	Albino, <1% normal β-carotene, normal sensory responses
C5	*carB10* (−)	Albino, <0.1% normal β-carotene, normal phototropic threshold
C9	*carR21* (−)	Red, accumulates lycopene, normal phototropic threshold
C158	*car-41* (−)	Super yellow, accumulates β-carotene, negative phototropism, normal threshold, normal growth response

TABLE II

GROWTH MEDIA

Hershey broth (pH 7.2–7.4)	
Nutrient broth	8 g‡
Bacto peptone	5 g‡
NaCl	5 g
Glucose	1 g
Distilled H_2O	1 liter
PDA-Potato dextrose agar (4%)	
Potato dextrose agar	40 g/liter‡
0.05% Thiamine-HCl	1 ml
Distilled H_2O	1 liter
PDAY-Potato dextrose agar plus yeast	
PDA	1 liter
Yeast extract	1 g‡
LAC-Lactate medium	
* Agar	10 g‡
Ammonium lactate	5 g
Magnesium lactate	4 g
KH_2PO_4	3 g
$MgSO_4 \cdot 7H_2O$	1 g
$MnSO_4 \cdot H_2O$	0.2 g
0.05% Thiamine-HCl	0.1 ml
Distilled H_2O	1 liter
GALY-Glucose asparagine leucine plus yeast	
* Agar	10 g‡
* Glucose	30 g
L-asparagine	2 g
L-leucine	5 g
$MgSO_4 \cdot 7H_2O$	0.5 g
KH_2PO_4	1.5 g
0.05% Thiamine-HCl	0.5 ml
* Yeast extract	1 g‡
Distilled H_2O	1 liter

* Solutions autoclaved separately.

‡ Difco Lab, Detroit, Mich.

a 486 nm interference filter (Bausch & Lomb Inc., Scientific Instrument Div., Rochester, N.Y., HBW = 7 nm).

The ultraviolet source was a deuterium lamp (Schoeffel Instrument Corp., Westwood, N.J., model L-201) powered by a 30 W DC-regulated power supply (Schoeffel, model LPS-201). The beam was collimated by a quartz lens and passed through a 280 nm interference filter (Ealing Optics, HBW = 10 nm).

Polarization

The light was polarized by dichroic polarizing films (Polacoat, Inc., Cincinnati, Ohio, PL40 for 280 nm light; Polaroid Corp., Cambridge, Mass., HN22 for broad band blue light, HN38 for 455.5 and 486 nm light). The polarizer was mounted on an automatic device which rotated the filter 90° every 5 min. The initial orientation of the polarizer could be changed so that any angle of the E-vector relative to the spph axis could be obtained.

Intensity

The intensities of the polarized stimulus beams were independent of E-vector angle. The spectral composition of the light was unaffected by changes in polarization and intensity. Intensities were monitored throughout each experiment and were well-regulated showing a maximum drift of ±5%. The absolute intensities of the different lights used were: 1.6–1.9 $\mu W/cm^2$ for broad band blue, 1.3 $\mu W/cm^2$ for 486 nm, 1.2 $\mu W/cm^2$ for 456 nm, and 0.6 $\mu W/cm^2$ for 280 nm light. These intensities were measured with a photodiode (United Detector Technology Inc., Santa Monica, Calif., UDT PIN 5) and electrometer (Princeton Applied Research Corp., Princeton, N.J., model 130) and with a calibrated 935 phototube (RCA Corp., Lancaster, Pa., type 935) and electrometer (Eldorado Electronics, Concord, Calif., model 201). The beams were uniform in cross section to 2% for an area of 7 mm in diameter for the 280 nm light and 2 cm for the tungsten light. Spphs were always kept within these uniform areas.

Physiological Experiments

Phycomyces spphs respond to changes in light intensity by changing their growth rate. This is called the growth response (Bergman et al., 1969). A periodic 5 min step-up, 5 min step-down intensity change produces a sinusoidal change in growth rate with a 10 min period. Because the growth response has a latency, the sinusoidal response is phase shifted so that the maximum growth rate occurs halfway through the low intensity interval and the minimum rate, halfway through the high intensity interval (Fig. 1 *b*). If the stimulating light program consists of constant intensity but periodic changes in polarization from 5 min transverse to 5 min longitudinal, a similar response is observed. The maximum of the response occurs during the longitudinal interval (Fig. 2 *a*). In both cases the growth rate variation expresses a difference in intensity perceived by the spph. Comparing this intensity difference for various strains gives a measure of their relative responses to changes in the polarization of light.

A vertical spph was selected and fastened to the side of the vial with tape or silicone grease approximately 1 cm below the sporangium. The vial was placed on a platform rotated at 2 rpm and mounted on a micromanipulator permitting fine motions in three

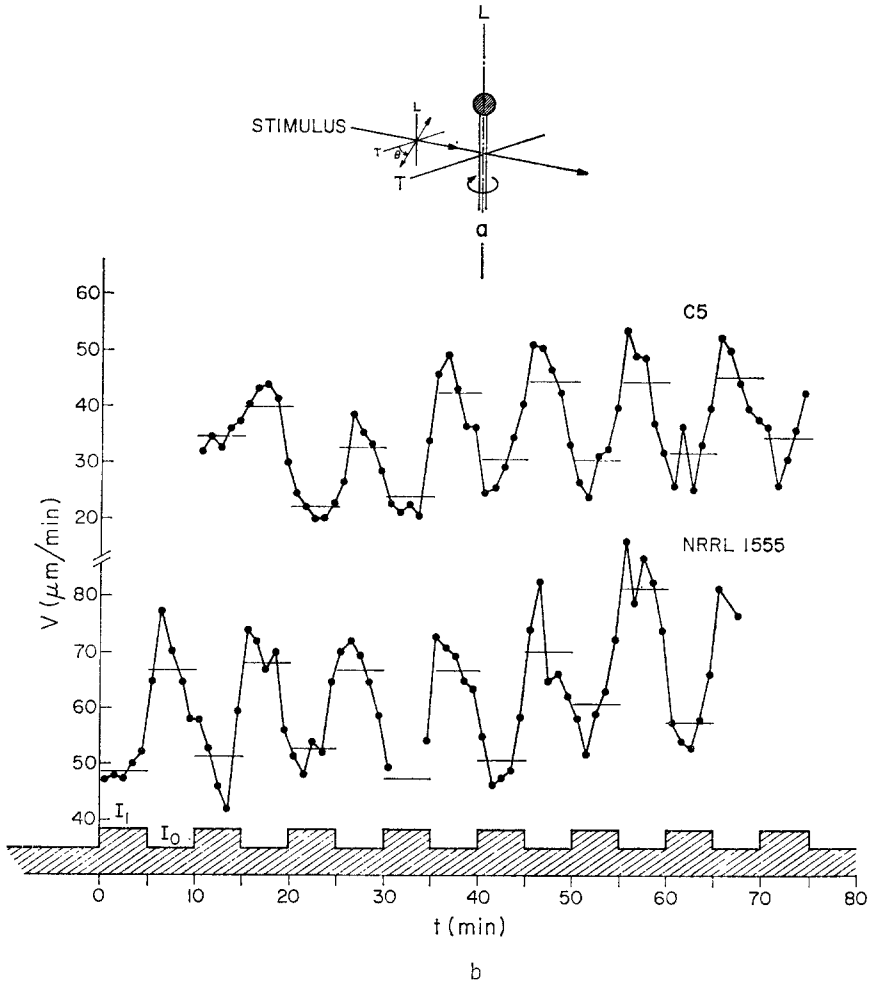

FIGURE 1. Illumination arrangement and growth responses. (*a*) Direction of irradiation and polarization of the stimulating light. The longitudinal and transverse directions are indicated by T and L, respectively. The angle of the **E**-vector relative to the axis is Θ. The sporangiophore is rotated in the beam at 2 rpm for symmetrical stimulation. (*b*) Growth rate, V, in response to a periodic light program of alternating intensities. Flux levels are $I_0 = 1.3\ \mu W/cm^2$ and $I_1 = 2\ I_0$. Growth is measured in 1-min intervals. The response of two strains is shown: C5 above, wild type (NRRL 1555) below. Horizontal bars indicate 5 min average growth rate during I_0 and I_1 periods. The light program is indicated by the hatched area. The height of this area is proportional to the intensity.

mutually perpendicular directions. By adjustments with two screws on the platform, the sporangium could be centered on the axis of rotation. This arrangement permitted spph position measurement, symmetrical illumination, and vertical growth.

The spph was centered in the beam and after adapting to the light program for 1 h, the position of the top of the sporangium was measured to $\pm 0.7\ \mu m$ using a measuring microscope fitted with a Filar micrometer (Gaertner Scientific Corp.,

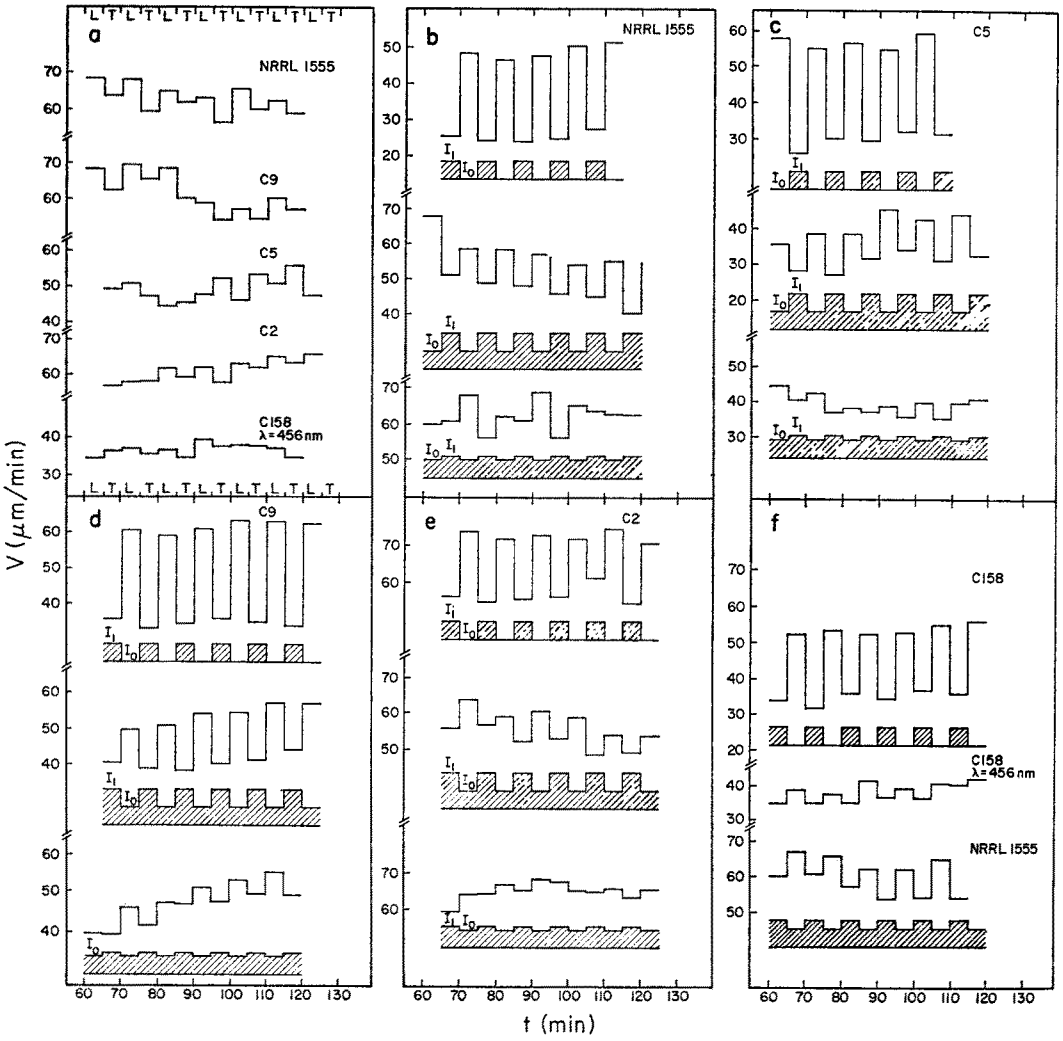

FIGURE 2. Growth responses to polarized light and intensity alternations. (*a*) Growth rate of five strains of *Phycomyces* in response to a periodic light program of alternating orientations of the **E**-vector of polarized blue light. **E**-vector orientations are longitudinal (*L*) and transverse (*T*). Intensity about 1.8 μW/cm^2. (*b–f*) 5 min average growth rate in response to a periodic light program of alternating intensities I_1, I_0 for five strains of *Phycomyces*. Light programs are indicated by hatched areas. $(I_1 - I_0)/(I_1 + I_0) = 1.00$ for upper curve, 0.33 for middle curve, 0.10 for lower curve. For C158 lower two curves are a comparison with the wild type of a response to $(I_1 - I_0)/(I_1 + I_0) = 0.2$.

Chicago, Ill.). This was done every 5 min at the moment the polarizer ceased rotating. Immediately after this, the angular deviation of the spph from the vertical was measured using a goniometer accurate to $\pm 2°$. This never exceeded 30° and was used for correcting the measured growth for its cosine error in the field of the microscope. The field of view was illuminated with physiologically inactive red light (Corning

Glass Works, type 2-59). An enclosure built around the apparatus kept the temperature constant to 1% and humidity constant to 5%. The temperature for the entire experimental series was kept at 20° ± 2°C. Fig. 3 shows the experimental arrangement.

The total growth during a number of periods of one **E**-vector orientation was compared with that during the orthogonal orientation. This gave a measurement of the differential growth response to polarized light. This response was calibrated in terms of an intensity difference of unpolarized light, as seen by the spph, by alternating between two intensities every 5 min. The base-level intensity was kept constant at 1.6 or 1.9 $\mu W/cm^2$. Alternating the voltage between two levels every 5 min produced the desired intensity change. The maximum change produced was 136% with a negligible change in spectral distribution. The ultraviolet calibration was done using neutral density filters (Ealing Optics).

The growth during the high intensity period was compared to that during the low intensity period and the response plotted as a function of intensity change. This gave a measure of the sensitivity of each strain to intensity differences and a way to compare the different strains' sensitivities to polarized light.

The approximate phase of the response was determined using minute to minute measurements on a sample spph of each strain subjected to a 100% variation in light intensity as in Fig. 1 *b*. The timing of all the 5-min measurements was chosen to maximize the measured growth differences between stimulus intervals. Measuring once every 5 min is equivalent to averaging minute to minute data over 5 min intervals but increases the signal to noise ratio. This is especially important for the very small polarized light response.

Microspectrophotometry

In vivo absorbance measurements of the growing zones of live sporangiophores were made with a Cary Model 15 spectrophotometer (Applied Physics Corp., Monrovia, Calif.) with a microscope attachment as described by Zankel et al. (1967). This attachment was improved for measurements of absorption anisotropy and ultraviolet absorption. The modifications included exchange of objectives for ultraviolet transmitting glycerine immersion types (Carl Zeiss Inc., New York, N.Y.; 100×, 1.25 NA, 32×, 0.40 NA ultrafluor) and rotatable Glan Thompson polarizing prisms (Karl Lambrecht, Chicago, Ill.) mounted in the reference and sample beams.

The absorption spectra of C2, C9, and C158 as well as their absorbance differences between longitudinally (L) and transversely (T) polarized light at 450 nm and 500 nm were measured on the unmodified attachment. Specimens were prepared in the same way as described by Zankel et al. (1967). The absorbance differences between L and T light were measured for both the spph and the blank slide adjacent to the spph and then subtracted. The sample beam was polarized using an HN38 polaroid (Polaroid Corp.).

Absorption anisotropy was measured on the improved instrument at 455 and 486 nm for C158, and 306 and 320 nm for the wild type. The purpose of measurements at 306 and 320 was to obtain an extrapolated value of the anisotropy at 280 nm since the polarizing prisms did not transmit sufficient light for measurement at this wavelength. Both reference and sample polarizers were rotated simultaneously through

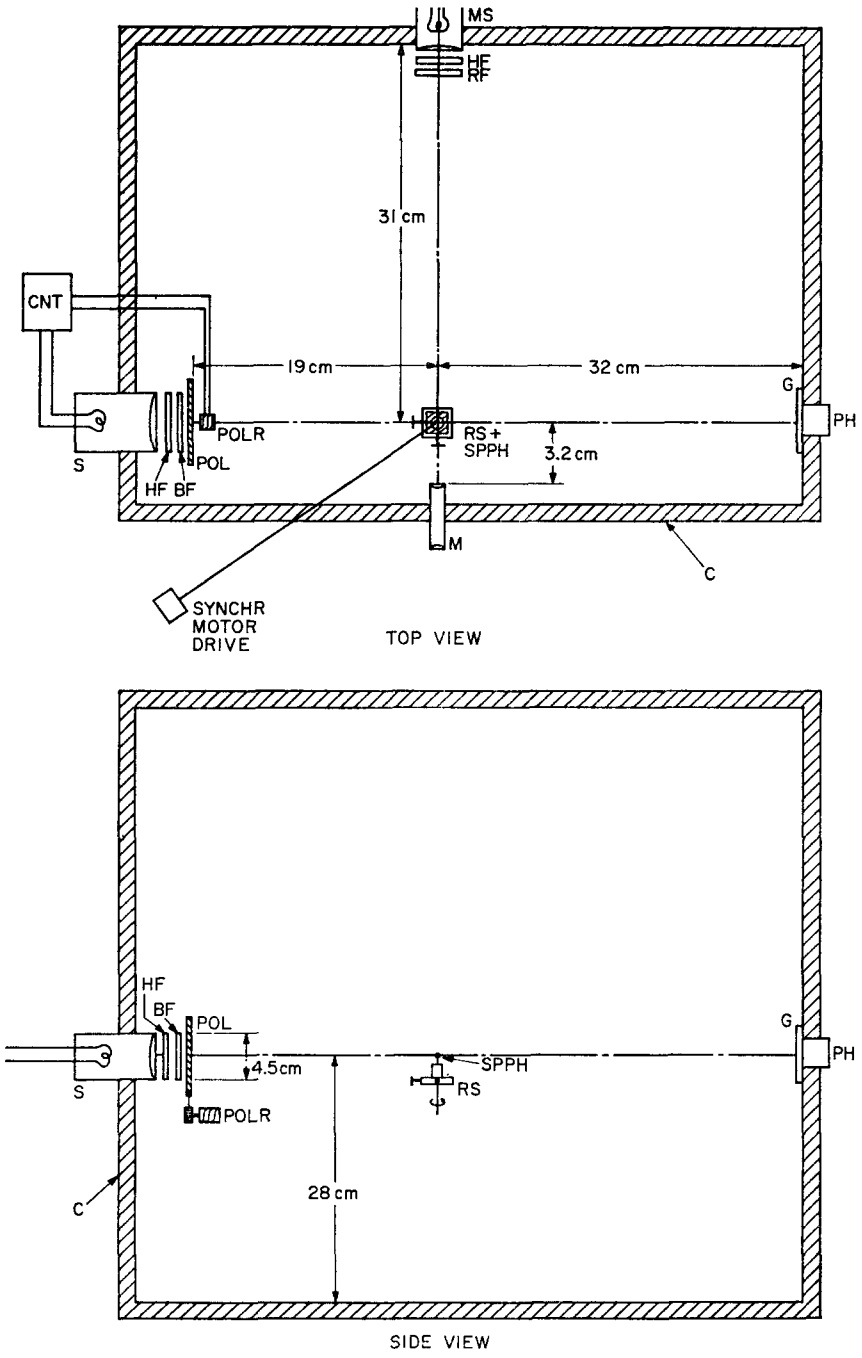

FIGURE 3. Apparatus for light programs. Legend: *BF*, blue filter; *C*, chamber with controlled temperature; *CNT*, polarizer rotation control or intensity switching control; *G*, glass plate; *HF*, heat absorbing filter; *M*, microscope; *MS*, microscope source; *PH*, photometer; *POL*, polaroid; *POLR*, polarizer rotator; *RF*, red filter; *RS*, rotating stage; *S*, stimulus source; *SPPH*, sporangiophore.

180° starting from an orientation perpendicular to the spph axis. The changes in absorbance were recorded in 15° increments. The same was done for the adjacent blank region of the slide. The results were subtracted to remove polarization artifacts of the instrument and mount.

The spectra including the ultraviolet region were measured using the modified attachment with the specimen mounted in distilled water, sandwiched between two quartz cover slips (Ammersil Inc., Hillside, N.J.).

RESULTS

A. *Differential Growth Response of Wild Type and Mutants to Longitudinally and Transversely Polarized Broad Band Blue Light*

Each of the strains showed responses typical of the alternating light intensity programs employed by Delbrück and Shropshire (1960). Let L and T denote longitudinally and transversely polarized light, respectively. Fig. 2 *a* shows the average growth rate during each T and L, 5-min half cycle for a typical spph of each strain. Figs. 2 *b-f* (lower set) show similar graphs for light programs in which the intensity was alternated between two levels, $I_1 > I_0$, differing by 20% $[I_1 - I_0)/(I_1 + I_0) \sim 0.1]$. Both sets appear very similar in both the magnitude and variability of the response. For comparison, the responses to intensity programs, with $I_1 = 2\ I_0$ (middle set) and with $I_0 = 0$ (upper set), are also shown in Figs. 2 *b-f*. The responses in these cases are much larger and show much smaller relative variation. Because of the small size of the polarized light response, its quantitative estimation required observation of several spphs (4–13) for many 10-min cycles (34–123).

From the measurements of total growth during each T and L half-cycle, the average growth rate for all T periods and all L periods was computed for each spph observed. One-half the difference of the rates during the two periods is called the amplitude of growth rate variation. The quotient of this quantity and the average growth rate is called the relative amplitude of growth rate variation, r. The average, $\bar{r}$, was calculated for several spphs by weighting each r by its variance, σ. Thus, spphs observed for many cycles showing little variation are much more influential in the calculation of the mean than noisy spphs observed for a few cycles. This procedure assumes there is no correlation between spph noise and its ability to respond. By comparing r from groups with large and small σ's, it is easily seen that the assumption is a valid one. The ratio of the averages of the two groups is 0.9 ± 0.4. Table III gives $\bar{r}$ for each strain used.

These values are to be compared with respect to the sensitivity of each strain to light intensity differences. An analogous quantity, $\bar{r}'$, was computed for the response of the spph to alternating 5-min intervals of light at intensities I_1 and I_0. Fig. 4 shows a plot of $\bar{r}'$ as a function of the differential in-

TABLE III

AVERAGE RESPONSES OF FIVE STRAINS TO ALTERNATING 5 MIN PERIODS OF LONGITUDINALLY AND TRANSVERSELY POLARIZED BLUE LIGHT

Strain	$\bar{r}$ (× 100)	Number spphs, cycles observed	$(\Delta I/I_0)_{eq}$ (× 100)	Number spphs, cycles observed for response curves
NRRL 1555	3.5±0.7	4, 34	22±5	33, 330
C5	3.6±0.2	5, 39	20±2	44, 417
C2	1.9±0.4	12, 71	32±7	53, 328
C9	3.8±0.3	13, 77	21±2	39, 232
C158 (λ = 456 nm)	1.3±0.3	32, 260	14±5	12, 96

tensity change, $(I_1 - I_0)/(I_1 + I_0)$, for each strain. This quantity can also be expressed as $[1 + 2\ (I_0/\Delta I)]^{-1}$ where $\Delta I/I_0 \times 100$ is the percent change in light intensity. The regions where $\bar{r}'$ appears to be linear depend on the strain and were selected by inspection. Straight lines were fitted to the data in these regions by the method of least squares.

Thus, if a spph sees a light intensity difference when it is exposed to alterations of **E**, $\bar{r}$ can be made equivalent to an $\bar{r}'$ and a corresponding $\Delta I/I_0$ can be assigned to it. This is the equivalent intensity difference perceived by the spph when the polarization is changed from T to L. It is given alongside $\bar{r}$ in Table III. The standard deviation of this quantity was determined by the propagation of the variance of $\bar{r}$ in the calculation of $\Delta I/I_0$ from the least squares parameters of the response curves.

In summary, all four strains see transversely polarized light about 20% more strongly than they do light polarized longitudinally. The transparent strains do not show a smaller effect and the optically dense strains show no larger effect.

B. *Microspectrophotometry*

The visible absorption spectra of wild type, C5, C2, and C9 are given on p. 135 in Bergman et al. (1969). Fig. 5 shows those of C158 and wild type. It also shows the UV absorption spectrum of the wild type. Both strains show a very high attenuation of light at specific wavelengths. This property has been used in section C. C9 and C2 were grown under different conditions than listed in Bergman et al. (1969) and consequently their spectra are shifted in the vertical direction slightly. The absorbance values at 450 and 500 nm for all the strains are given in Table IV.

C2, C9, C158, and the wild type were investigated for absorption anisotropy with respect to polarized light. Table V gives the optical density differences between longitudinally and transversely polarized light, $\overline{\Delta OD_{L-T}}$, as well as the maximum optical density difference $\overline{\Delta OD_{max}}$ encountered in measur-

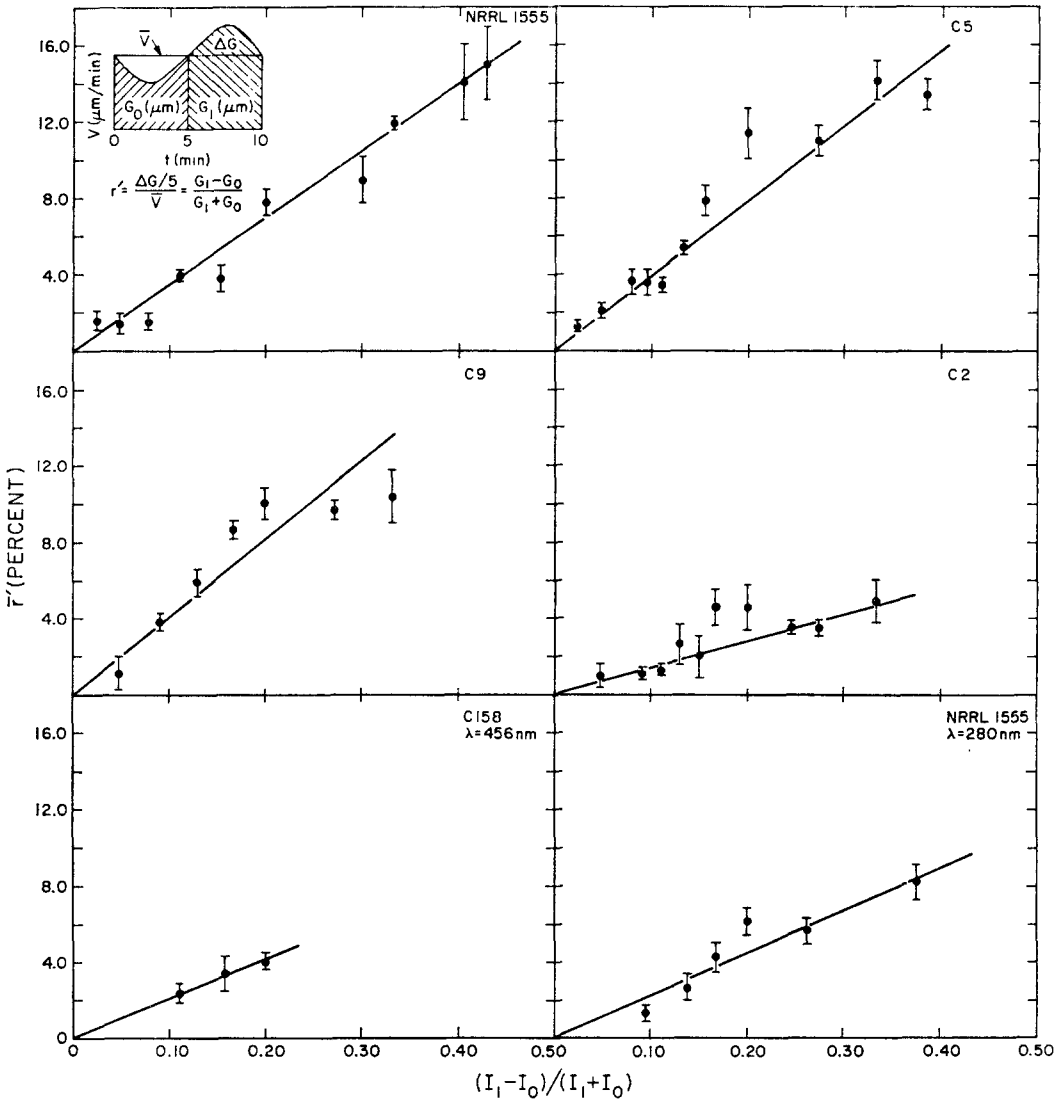

FIGURE 4. Growth response versus intensity differentials. $\bar{r}'$ is the relative amplitude of the growth rate change during a periodic stimulus program consisting of 5 min of intensity I_0 alternating with 5 min of I_1. It is taken as the total growth, G_0 in μm, for all the I_0 periods, minus growth, G_1, for all the I_1 periods divided by the total growth $G_1 + G_0$. This quantity is the ratio of the amplitude of the growth rate change ($\Delta G/5$) and the average growth rate ($\bar{V}$) during the entire run. The mean for several sporangiophores, $\bar{r}'$, is plotted as a function of $(I_1 - I_0)/(I_1 + I_0)$. The strain is indicated in the upper right of each graph along with the wavelength of light if not broad band blue.

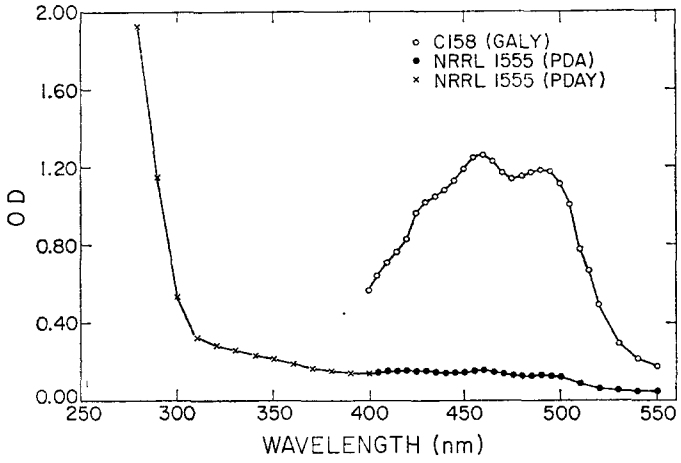

FIGURE 5. Sporangiophore absorption spectra. Visible absorption spectra of C158 grown on GALY medium and wild type grown on PDA; ultraviolet spectrum of wild type on PDAY. Optical density (OD) is plotted as a function of wavelength.

TABLE IV

IN VIVO ABSORBANCE VALUES AT 500 nm AND 450 nm FOR FIVE STRAINS

Strain	Growth medium	OD_{450}	OD_{500}
NRRL 1555	PDA	0.14	0.12
C5	PDA (from Bergman et al., 1969)	0.05	0.04
C2	LAC	0.06	0.04
C9	PDAY	0.16	0.16
C158	GALY	1.19	1.11

Experimental precision = ±0.01.

ing OD as a function E-vector angle. In all cases the anisotropy was very small, at most 0.02 ± 0.01.

C. E-*Vector Angle for Maximum Response at 280 nm, 456 nm, 486 nm*

Because of the cylindrical symmetry of the spph, receptor dipoles in the proximal half of the cell, whose projections in the *T-L* plane (Fig. 1 *a*) are oriented at an angle α to the transverse axis, will have corresponding symmetrically opposite dipoles in the distal half with orientation $-\alpha$. Intuitively, this means that the net absorption of normally incident polarized light will be maximum only at values of Θ equal to 0° or 90°; that is, when E bisects the angle 2α between the two sets of dipoles. For $\alpha \leq 45°$ absorption will be maximum at $\Theta = 0°$, and for $\alpha \geq 45°$ it will be maximum at $\Theta = 90°$. Quantitatively, the absorption is proportional to $\cos^2(\Theta - \alpha) + \cos^2(\Theta + \alpha)$, which has extrema at $n\pi/2$ where $n = 0,1,2\ldots$. Thus *regard-*

TABLE V

POLARIZED LIGHT ABSORPTION ANISOTROPY

Strain	Growth medium	λ	$\overline{\Delta OD}_{L-T} \times 10^2$	$\overline{\Delta OD}_{max} \times 10^2$
		nm		
NRRL 1555	PDAY	306	2±1	2±1
	PDAY	320	1.0±0.5	1.5±0.7
	PDA	463	0±1	—
C2	LAC	450	1.2±0.5	—
	LAC	463	0.6±0.8	—
	LAC	500	0.9±0.3	—
C9	PDAY	450	0.3±0.2	—
	PDAY	500	0.6±0.1	—
C158	GALY	455	0.9±0.7	1.4±0.9
	GALY	486	0.2±0.4	0.8±0.5

TABLE VI

E-VECTOR ANGLES FOR MAXIMUM RESPONSE

Strain	Growth medium	Wavelength (λ)	Transmission T	Maximum response ($\bar{r}$)	Angle of max response (θ_{max})	$(\Delta I/I_0)_{eq}$
		nm	%	%	*degrees*	%
NRRL 1555	PDAY	280	1.2	1.4±0.3	42±4	18±8
C158	GALY	456	5.0	1.3±0.3	7±8	14±5
C158	GALY	486	6.3	1.8±0.2	7±2	—

less of what α is, the spph will always show a maximum response at $\Theta = 0°$ or 90°.

This difficulty was circumvented by choosing wavelengths and strains displaying high optical densities. At 280 nm the absorbance of wild type is 1.9 ± 0.1; at 456 nm and 486 nm the absorbance of C158 is 1.3 ± 0.2 and 1.2 ± 0.2, respectively. For these high absorbancies the influence of the distal region becomes negligible and $\bar{r}$ will show a dependence on Θ.

Let $\bar{\alpha}$ be the average α for all the receptor absorption dipoles in the proximal half of the spph. Receptor absorption for light polarized at angles Θ and Θ + 90° to the transverse axis will be proportional to $I_1 = \cos^2(\Theta - \bar{\alpha})$ and $I_0 = \sin^2(\Theta - \bar{\alpha})$, respectively. Since r' is proportional to $(I_1 - I_0)/(I_1 + I_0)$ in the intensity cycling experiments, the corresponding r in these experiments will be proportional to $\cos 2(\Theta - \bar{\alpha})$. Thus differential absorption, D, will be maximum when $\Theta = \Theta_{max} = \bar{\alpha}$, have the period π, and possess the property: $D(\Theta) = -D(\Theta + 90°)$. These properties are also common to the measured differential growth response, r. The r was measured for light programs consisting of 5 min alternation between light polarized at the angle Θ and the angle Θ + 90°. For each Θ, 5 spphs were observed over a total of approximately 45 cycles. Θ took on values from 0°–75° in 15°

increments. Thus, six values of $\bar{r}$ were obtained corresponding to the six angles.

These six values were plotted against Θ. The function $r_{max}\cos 2(\Theta - \Theta_{max})$ was least squares fitted to the data. From this fitted curve the angle of maximum response, Θ_{max}, and the maximum response, r_{max}, were obtained. These results are shown in Fig. 6.

In all the physiological experiments, the reading errors and errors due to fluctuations in growth rate were minimized by running experiments over many cycles. The largest error came from interspecimen variation. This variation, however, can be tolerated in light of the conclusions drawn. The intensity difference experiments showed consistency in the response curves. χ^2, the sum of the squares of deviations of the data points from the least squares line, was close to one for all strains, indicating a good fit. Other errors were relatively small and therefore did not interfere with the spph response or its measurement.

The experiments were not all done at the same intensity levels. This fact probably has little significance. The response is a function of the quotient of (intensity x time) and adaptation level (Bergman et al., 1969) and the

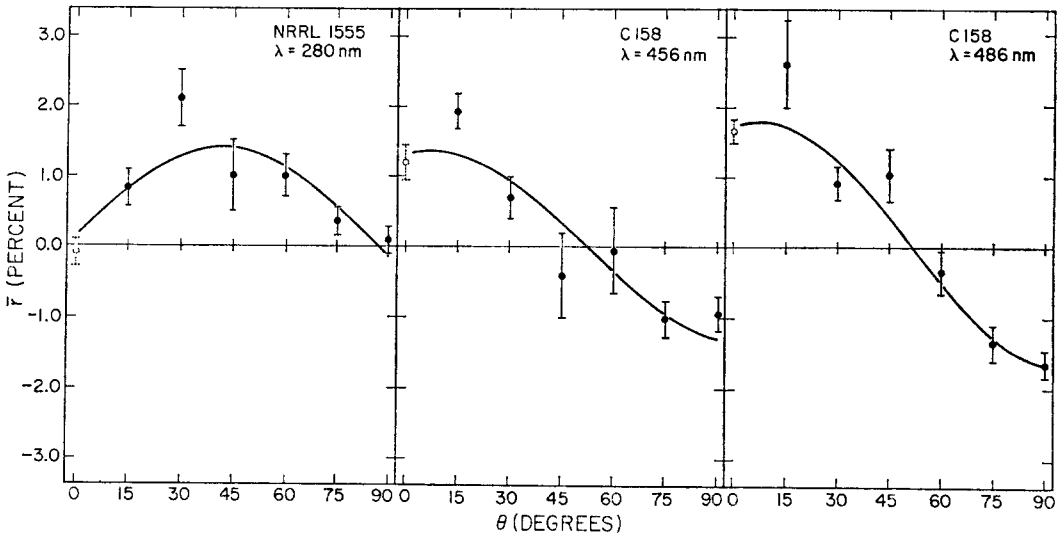

FIGURE 6. Polarized light responses versus Θ, at high optical density. Relative amplitude of growth rate change as a function of **E**-vector angle relative to the transverse axis of the sporangiophore. The light program consists of a periodic alternation between **E** oriented at Θ and $\Theta + 90°$. $\bar{r}_{max}\cos^2(\Theta - \Theta_{max})$ is fitted to the data points by the method of nonlinear least squares. Θ_{max}, the phase angle, is the angle at which maximum response occurs. r_{max} is the maximum value of the fitted curve. Since an alternation of **E** between $\Theta = 0°$ and 90° is the same as on alternation between 90° and 180°, except for phase, r (90°) is shown again as a dotted data point, i.e., r (0°) $= -r$ (90°).

spphs were fully adapted to the intensity levels of the experiments. Consequently, the stimulus produces the same value in any intensity difference or polarization experiment for all intensity levels in the linear range (0.02–5 $\mu W/cm^2$). The levels utilized never deviated from each other by more than about a factor of 2.

DISCUSSION

The Fresnel Effect

A beam of light impinging on a surface is partially reflected and partially refracted. For a spph, this means that some light gets into the cell and some is reflected away. The quantity of light that is reflected or refracted is determined by the Fresnel reflection coefficients which depend on the refractive indices of the two media, the angle of incidence, and the polarization of the beam. Castle (1934), using these parameters, calculated the absorption of longitudinally and transversely polarized light entering the cell perpendicularly from opposite sides. His graphic integration showed that on the first lap across the spph, the ratio of the light absorbed in the opposite halves of the cell would equal 1 (i.e. phototropic balance) if the longitudinally polarized beam were 20% more intense than the transversely polarized beam. In order to perform the integration, Castle confined the calculation to the limiting case of zero light attenuation but did not consider what would happen on the next lap after the first internal reflection.

Jaffe (1960) took this consideration into account and showed that it should not be neglected. In a homogeneous transparent cylinder, he showed that because of successive internal reflections within the spph, the sum of the light quanta absorbed by a receptor pigment present in low concentration in the series of successive traverses will be independent of reflectivity, for any beam with a given angle of incidence. Specifically, the total absorption, summed over successive laps, is

$$A = K(1 - R) + K(1 - R)R + K(1 - R)R^2 + \cdots = K \quad \text{for} \quad K \ll 1,$$

where A = absorption, K = fraction absorbed during each lap between reflections, and R = reflection coefficient. For small R, the infiinte series converges rapidly and can be approximated by the first two terms corresponding to only one internal reflection. The first internal reflection compensates for the effect of surface reflection. The net effect is that the same amount of light is seen by the spph irrespective of the amount lost at incidence.

Jaffe's theorem applies to the case of zero light attenuation. If there is attenuation, there will be incomplete compensation due to internal reflections. In wild type spph there is 36% light attenuation. Based on the absorp-

tion due to β-carotene content alone (Bergman et al., 1969), 18% is due to absorption and the remainder due to scattering. Absorption removes light quanta from the cell while scattering redistributes them. Thus, absorption is more effective than scattering in destroying the compensatory effect of internal reflections. The Jaffe theorem, therefore, cannot be applied directly to the wild type. Even so, Jaffe estimated that the effect of different reflections for T and L would be small, equivalent to a 1.6% intensity difference, and therefore could not account for the large experimental result measured by Shropshire.

In C2 and C5 the optical attenuation is equal to 14 and 12%, respectively, entirely due to scattering. Consequently, this attenuation plays a relatively smaller role than it would if it were due to absorption. This implies that light losses will play a *much* smaller role in producing the differential growth response in these strains than in wild type.

If reflection losses were the cause of the polarized light effect, then they should be much smaller in the C5 and C2 strains. Our experiments show that the responses of the C5 and C2 are as large or larger than those of the wild type.

The average optical density of C9 from 400–500 nm is approximately 20% higher than that of the wild type. Consequently, the reflection hypothesis would predict a greater sensitivity to polarized light for this strain than for wild type. However, C9 sees polarized light as effectively as wild type, and C158, an even more optically dense strain, shows a slightly smaller effect.

In conclusion, these experiments substantiate Jaffe's contention that the reflection hypothesis cannot be correct. Reflection losses can have only a minute effect on the responses of *Phycomyces* spphs to polarized light.

Anisotropic Attenuation

We consider next the possibility that the polarized light effect could be a result of anisotropy of the optical attenuation due to screening pigments or to scattering structures.

Back scattering by fibers, having diameters much smaller than the wavelength of light, is greatest for light polarized parallel to the fiber axis (Kerker, 1969). As a consequence, the transversely oriented chitin fibrils (20 nm in diameter; Roelofsen, 1951) of the spph cell wall would allow more longitudinal rather than transverse light into the cell, a situation producing a polarized light effect opposite in sign to the one measured.

Direct microspectrophotometric measurements on the growing zones of live spphs have shown that the anisotropy of the attenuation amounted to at most a 5% difference of longitudinally as opposed to transversely polarized light.[2] Clearly, this cannot explain differences of 20–30%.

[2] See Addendum.

Oriented Dichroic Receptors

The remaining hypothesis to account for the polarized light effect is the preferential absorption of transversely polarized light by the photoreceptor molecules. This implies that the molecules are linearly dichroic and have an angular distribution of their transition dipoles favoring transverse absorption.

A linearly dichroic molecule is one which is anisotropic with respect to absorption of linearly polarized light. The simplest case of such a molecule is one with only a single axis of absorption. It can be thought of as a dipole capable of vibrating in one direction only. The electric vector of light of the proper frequency can set up oscillations along the axis of the dipole but not in any other direction. Consequently, only the component of the **E**-vector which is parallel to the dipole will set up oscillations; an **E**-vector perpendicular to the axis will have no effect. The direction of the dipole corresponds to the quantum mechanical transition dipole moment and the oscillations correspond to absorption.

A dichroic effect can only be measured if the molecules have some degree of orientation; i.e., a non-uniform spatial distribution. What sort of orientation is necessary in order to produce the required degree of absorption anisotropy (1.2:1 for transverse absorption:longitudinal absorption) measured in *Phycomyces*? In the cylindrical microvilli of crayfish rhabdoms (Waterman et al., 1969), a ratio of 1:2 for transverse:longitudinal absorption was measured microspectrophotometrically. This result was explained by postulating receptor dipoles fixed and tangent to the cylindrical membrane of the microvilli but randomly oriented in the tangential plane. Such an orientation, implicated in nearly all plants sensitive to polarized blue light, predicts the measured preference for longitudinal absorption. This is perhaps the simplest nonrandom orientation that can be expected in the cylindrical case.

In *Phycomyces* the situation must be different, since the transverse component is absorbed more effectively. This means that the dipoles cannot be ordered as in the microvilli, but must have a predominantly equatorial alignment. These directions and some possibilities are illustrated in Figs. 7 and 8.

In Jaffe's theoretical analysis, it is inferred that aligned tangential ordering for the visible wavelengths is the most likely since radial ordering cannot explain the fall of the dichroic ratio in a medium of matching refractive index. The series of experiments measuring the polarized light effect as a function of Θ can be interpreted as a measure of tangential ordering. If the dipoles are assumed to lie on a cylindrical surface at the angle ϕ with respect to the azimuthal direction, then Θ_{max}, the angle at which maximum differential absorption occurs, will show the following dependence on ϕ (Jesaitis, 1973).

$$\tan 2\,\theta_{max} = \frac{8 \tan \phi}{\pi\,(1-2 \tan^2 \phi)}.$$

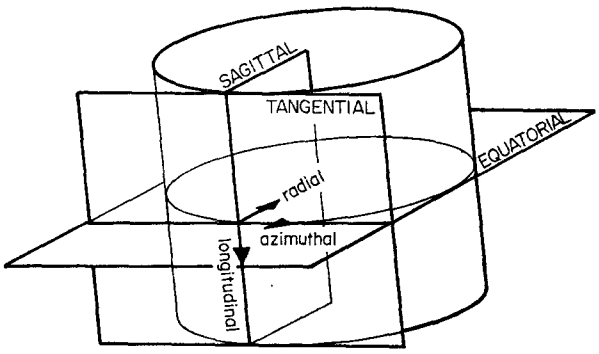

FIGURE 7

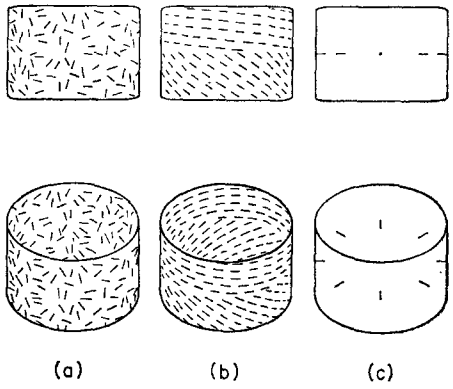

FIGURE 8

FIGURE 7. Definition of directions used in reference to the spph cylinder. The three orthogonal planes containing the receptor dipole components are shown. The tangential plane is tangent to the cell surface and parallel to its cylindrical axis. The equatorial plane is perpendicular to the cylindrical axis. The saggital plane contains this axis and the diameter. The intersection of these planes define the radial, azimuthal, and longitudinal component directions.

FIGURE 8. Oblique and normal views of hypothetical receptor transition dipole orientation. (*a*) Random orientation in the tangential plane as postulated for the receptor in the microvilli in crayfish rhabdoms. (dichroic ratio 1:2, transverse: longitudinal; Waterman et al., 1969). (*b*) Ordered tangential orientation at an angle 5.5° or 33° to the azimuthal axis. Such orientations will produce a maximum dichroism at $\Theta = 7°$ and 42°, respectively. (*c*) Radial orientation. (*b*) and (*c*) or a mixture of the two may correspond to the average receptor orientation in *Phycomyces*.

Substituting the measured values for the 486, 456, and 280 nm dipoles in this expression, and solving for the respective ϕ's gives the tangential orientation angles of these dipoles. They are 5.5°, 5.5°, and 33.5°, respectively. However, both the 280 nm dipole and the visible dipoles could have a partially radial orientation and in that event the inferences concerning the orientations of the dipoles are no longer cogent. If the tangentiality assumption is dropped the data are insufficient to draw such definite conclusions. Some further data are needed. For instance, if the assumption is introduced that the receptor molecule is a flavine, then one can take from the fluorescence polarization measurements of Kurtin and Song (1968) and Siódmiak and Frackowiak (1972), that the angle between the 280 dipole and those in the blue is about 60°. It can be shown (Jesaitis, 1973) that this constraint is compatible with our measurements and two possible sets of orientation for the dipoles can be derived. Corresponding calculations for carotenes cannot be made since no analogous fluorescence polarization measurements are available. Thus, positive conclusions regarding the chemical identity of the receptor molecule cannot be drawn from the present data.

We may summarize by saying that *Phycomyces* spphs have 15–20% greater

sensitivity to light polarized transversely than longitudinally, at 280, 456, and 486 nm. By exclusion of other causes this effect is shown to arise from a linearly dichroic and oriented photopigment. The pigment molecules are probably aligned so that the transition moment for absorption in the blue is nearly equatorial and tangential.

ADDENDUM

Note added July 7, 1973: Experiments done in collaboration with Professor R. D. Allen at the Biology Department, S.U.N.Y. at Albany, measuring anisotropy of single layer cell walls gave values of 2–3%, and of the sign predicted by Kerker (1969) for form dichroism. The dichroism disappeared upon imbibition with glycerol. (Work supported by NSF Grant GM-18854 to R. D. A.)

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