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ORIENTATION AND LOCUS OF TROPIC PHOTORECEPTOR MOLECULES IN SPORES OF *BOTRYTIS* AND *OSMUNDA*

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ABSTRACT

Study of the tropic responses of *Botrytis cinerea* and *Osmunda cinnamomea* spores to blue light shows the photoreceptor molecules to be highly dichroic and oriented: in *Botrytis* their axes of maximum absorption lie perpendicular to the nearby cell surface; in *Osmunda*, parallel. The chief evidence lies in a comparison of their responses to plane polarized light—both germinate parallel to the vibration planes (defined by the axis of vibration of the electric vector and the axis of light propagation)—with those to partial illumination with unpolarized light: *Botrytis* grows from its brighter part; *Osmunda*, from its darker. The degree of orientation produced by polarized light corresponds, at high intensities, to that produced by the imposition of such large (about 100 per cent) intensity differences across a cell as to preclude all alternatives to oriented dichroic receptors. The photoreceptors of the *Botrytis* spore lie within the cell wall's inner half. The chief evidence lies in the component of its tropic responses to polarized light within the vibration plane: germination peaks about 10° off the vibration axis. This deviation arises from focusing which is effective only in the wall's inner half. At high intensities, anomalies appear in *Botrytis* which are interpreted as "centering," *i.e.*, a tendency toward growth from the center of two or more equally illuminated points of a cell rather than from one of them.

INTRODUCTION

Five years ago it was reported that zygotes of *Fucus*, if treated with polarized white light, subsequently showed a striking "polarotropic" response: they tended to germinate athwart the light's direction and in the plane of vibration of the E-vector (11). Analysis suggested that this response was a variant of the tropic response of these cells to unpolarized light mediated by an orientation of dichroic photoreceptor molecules parallel to the nearby surface of the cell (12). A survey then revealed polarotropic responses in all those tested cells of the lower plants which likewise respond tropically to unpolarized light (2).

In this paper we report a study of the polarotropic responses of the macroconidia or common asexual spores of the imperfect fungus and common plant pathogen *Botrytis cinerea* Pers., and of the spores of the fern *Osmunda cinnamomea* L. Particularly in the case of *Botrytis*, we have found the evidence for mediation of these responses through dichroic oriented photoreceptors to be so compelling as to lead us to emphasize these inferences

rather than the raw phenomena in the title and abstract of this paper.

MATERIALS AND METHODS

Getting and Growing Botrytis Spores

The strain of *B. cinerea* used came from the Institut für Mycologie der biologischen Bundesanstalt für Land- und Forstwirtschaft in Berlin-Dahlem, Germany. This fungus was maintained by serial culture on 5 mm deep layers of autoclaved 6.5 per cent Difco Sabouraud dextrose agar in 5 cm pyrex Petri dishes kept inverted, at 23°C, in the dark, stagnant interior of a small box. However, transfers were made at about the same time every day. Thus the cultures were briefly lighted and aired at 24 hour intervals.

Spores were harvested and sown for an orientation study as follows. With a tweezers one ripped some tufts of conidiophores from a week-old culture and agitated them over a substratum while blowing down from a height sufficient to avoid fogging. Then one inspected the substratum and repeated the sowing process several times until the desired cell concentration of about 20 cells/mm^2 was reached. The ovoid spores measured about 8 × 11 μ. Hence a concentration of 20 cells/mm^2 covered about 0.2 per cent of the illuminated area and introduced negligible scattering.

Unless otherwise noted, *Botrytis* spores were sown upon a dry hydrophobic surface, then covered with a 5 mm layer of 0.07 per cent Difco Czapek Dox broth and germinated at 23°C, for we find that, in contrast to dry hydrophilic surfaces (such as acid-cleaned glass), which entirely fail to fix these cells, hydrophobic ones rigidly immobilize them. To make the glass, quartz, and chromium substrata hydrophobic, we coated them with a layer of polystyrene a few tenths of a micron thick. (This was done by dipping the substratum in a 0.25 per cent solution of pieces of Falcon disposable Petri dishes in benzene, draining off the solution, and then evaporating off the residual solvent; the thickness cited was estimated from the interference colors shown by the dry film.)

To avoid overheating under intense infrared radiation (rays of 0.9 to 3.0 μ wavelength and $\geq$3.4 mwatt/cm^2), cells were blown upon a 100 μ thick layer of 6.7 per cent Eastman pigskin gelatine. This was then covered with a 35 μ thick film of polyethylene to allow respiratory gas exchange and the passage of infrared rays without drying. During irradiation the gelatine was cooled by supporting it with a 160 μ thick glass coverslip which in turn was bound through a glycerine film to a blackened water-cooled brass block. The spores were thus kept at 21 ± 2°C.

About 12 hours after being wetted, spores under the liquid medium were killed with ultraviolet rays. (They were placed 4 cm from a "sterilamp" for 10 minutes.) At this time an average of about nine-tenths of the cells had developed single germ tubes, one-tenth had not germinated, and a few per cent had formed two germ tubes. These latter figures were apparently independent of light.

Getting and Growing Osmunda Spores

The *Osmunda* spores used came from fronds collected near the Charles River during May in Waltham, Massachusetts. The plant was identified by Dr. Rolla Tryon of the Gray Herbarium at Harvard University. Soon after the fronds were collected, the spores were freed and then stored at 3°C in a dry state and in darkness. These spores were clearly divisible into two classes: one-third were relatively large, green, and densely filled cells most of which subsequently germinated; two-thirds were relatively small, brown, and empty cells which rarely germinated. Unless otherwise noted, the word "spore" will refer only to the former class of "large" spores. The experiments reported here were done 2 to 14 weeks after collection.

In most experiments, sowing was done by the method of Kofler (16): A pinch of dry spores was shaken in a clean, closed 5 cm Petri dish. The cover was then dropped lightly upon a clean Petri bottom bearing the substratum for germination. This loosened some spores from the cover, whence they settled upon the substratum. This was inspected and the process repeated until a concentration of about 5 spores/mm^2 was reached.

Usually, the dry *Osmunda* spores were sown upon a hard substratum coated with a thin layer of 0.1 per cent agar, immediately air-dried, rewetted by being covered with a 5 mm layer of 0.05 per cent pH 6.5 Knop solution (6, p. 644), and germinated at 23°C. This method immobilized most spores, the few loose ones being recognizable and ignored in the later orientation measurements, and avoided the substantial light scattering introduced by a thick gelled medium. (The adhesive agar layer was formed as follows: just before sowing, a 0.1 per cent solution of agar was boiled for a few minutes, cooled to room temperature, poured over, and then drained off the substratum.)

Since the orientation of the just germinated and thus least swollen cells was most accurately measurable, and since the germination rate varied with illumination, cultures were killed at times varying from 41 to 71 hours after rewetting the spores, this time being guided by inspection of dummy cultures. Like the *Botrytis* spores, the *Osmunda* cells were killed with ultraviolet rays. At this time 60 to 97 per cent of the spores had developed one rhizoidal outgrowth; the others had not germinated. No spores ever developed a chloronema.

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Light Control and Measurement

Optical components (if present) were arranged in the following order: (i) light source, a 500 or 1000 watt tungsten projection lamp powered by a voltage stabilizer; (ii) lenses; (iii) an aqueous water-cooled filter for infrared removal; (iv) baffles allowing only the escape of approximately horizontal rays; (v) a 45° prism or front surface mirror to redirect the ray vertically; (vi) solid filters for wavelength control; (vii) Wratten neutral density filters; (viii) either a polarizer or a striped surface to generate gradients; (ix) spores. In the main experiments, involving irradiances of ≤ 2.5 mwatt/cm^2, up to ten cultures received beams radiating from the same centrally placed source without the intervention of lenses; in accessory ones, involving more than 2.5 mwatt/cm^2, a single culture received rays concentrated by a lens system. For "vertical" illumination the beam(s) struck the cultures at 90° to the horizontal, but for "horizontal" illumination a beam descended upon one side of each culture chamber at about 6° to the horizontal. (These chambers were horizontally placed rectangular ones made of microslides.) Each beam, then, passed through a window so as to illuminate directly only the bottom of the chamber, whence it was totally reflected. Thus each spore was illuminated by two beams whose resultant direction was exactly horizontal, yet it is estimated that only 3 per cent of the cells were even partially shadowed by other spores.

Where all infrared rays had to be removed, the usual method involved immersion of the lamp in the center of a 0.3 M ferrous or 0.4 M cupric sulfate solution held in a glass cylinder; each horizontal beam then traversed 74 mm of this filter before emerging. The filter was adequately stabilized by 0.05 M H_2SO_4, together, in the case of the ferrous solution, with a layer of mineral oil above it and a piece of brass at its bottom.

The "standard blue" light used in most experiments was isolated by a Wratten 45A filter together with a suitable long wave cut-off filter. This "standard blue" peaked at 470 mμ, falling to one-half maximum intensity at 445 and 515 mμ and 1 per cent maximum at 425 and 550 mμ. Other relatively broad bands were isolated by combining suitable solid absorption filters and dichroic interference filters. Narrower bands in the region from 0.9 to 2.0 μ were isolated by using a Schott continuous running or wedge type interference filter.

Intensity was chiefly controlled with Wratten neutral density filters; some relatively fine control was also obtained by varying the lamp-to-culture distance.

Beams of wavelengths from 0.4 to 0.7 μ and from 0.7 to 2.8 μ were polarized by means of Polaroid type H and HR sheet polarizers respectively, except that a very intense beam lying between 2.0 and 3.0 μ was polarized with a gold on Kel-F Hertzian polarizer supplied by Dr. George Bird of the Polaroid Corporation.

Irradiances were measured with the aid of a thermopile calibrated against a United States Bureau of Standards lamp, or (for low intensities of visible light) with a photronic cell calibrated against the thermopile.

Partial Illumination

Partial illumination of the spores was made possible by immobilizing them upon substrata of fused quartz striped with 40 μ wide opaque bands (of $\backsim$0.1 μ thick chromium), 40 μ apart (see Fig. 2). When light was imposed only from below, any cells which lay upon a band's edge were so shadowed as to be subjected to an almost 100 per cent difference in light intensity; when light was also directed from above, they were subjected to a lesser and calculable relative difference. We define this as the quotient of the difference of intensities imposed on the cells' sides divided by the *higher* of these.

These substrata were in the form of squares, 25 × 25 × 1 mm, with a 25 × 19 mm area of one surface striped and the remaining quarter clear. In use, each square was fixed upon the bottom of a 5 cm Anumbra Petri dish (O. H. Johns Glass Co., Toronto, Canada) which served to contain the culture medium. When the effect of polarized light was to be compared with that of a 100 per cent difference of unpolarized light, a rectangle of Polaroid was taped underneath each Petri dish right under the clear quarter of its square. Thus the two effects of light intensity were compared upon spores germinating in the same culture.

Corrections for reflection from the unmetalized interfaces (such as that between the culture medium's top surface and the air) were very small, and were easily and directly applied. A correction for the light which traversed a cell from on top and was then reflected up through the cell by the chromium just beneath it was obtained by means of the following indirect argument: On substrata subjected to top lighting only, such reflection causes cell parts above chromium to receive *most* light; thus, the imposed differences and the resultant orientation should be in a direction opposite that produced when substantial light intensities come from below. Now, no doubt owing to their low transparency, *Osmunda* spores did not, in fact, exhibit any significant counterorientation under pure top lighting; so here the correction was negligible. However, the per cent orientations, V_1, of top lighted *Botrytis* spores in three experiments were -22 ± 6 per cent, -10 ± 5 per cent, and -12 ± 5 per cent. (The corresponding results of pure bottom lighting were $+86 \pm 3$ per cent, $+88 \pm 2$ per cent, and $+81 \pm 2$ per cent.) Then in each experiment

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we interpolated amid the orientation resulting from various ratios of top and bottom light intensities to find that ratio yielding an orientation opposite in direction and equal in degree to that produced by pure top lighting. We reasoned that the corresponding differences must also have been thus opposite and equal. From this it was easy to solve for the effective intensity of light reflected by the chromium and to correct the values of P, *i.e.*, the per cent differences imposed on the cells. The (absolute) errors thus introduced into the determination of P were about 2 to 5 per cent for $P < 50$ per cent and ≤ 2 per cent for $P > 50$ per cent.

Our analysis further indicates that for blue light two-thirds of the *transition* in light intensities imposed across a partially illuminated *Botrytis* or *Osmunda* spore occurred over 1.0 to 1.8 or 3.0 to 5.4 μ, hence over about 10 to 18 or 5 to 9 per cent of a cell diameter, respectively. *Diffraction* (estimated from a textbook exposition of Fresnel diffraction (23)), *raggedness* of the bands' edges (from sample fields observed under an oil immersion objective), and *divergence* (from measurements of the lamp filament's dimensions and of the filament-to-culture distance) made contributions of decreasing weight toward the blurring of the shadows' edges, while *scattering* (as deduced from the obvious clarity of the media together with the the minute fraction of the substrata's surface covered by spores) was negligible.

Measuring Orientation

We measured the initial directions of the outgrowths, as projected into the horizontal plane, with the aid of a Wild goniometer eyepiece modified by replacing the rotatable pointer with a pattern of fine parallel lines. Angles were recorded in 10° intervals. The initial direction of outgrowth of an *Osmunda* spore, which is a spherical cell, was defined as that from the center of the spore to the center of the base of the outgrowth. Since it was impractical to apply this criterion to the ovoid *Botrytis* spores, we measured the directions taken by the most basal measurable sections of their germ tubes.

In all cases we counted only cells more than one long spore axis from any other cell. A cell lying on a striping was counted only if the spore's outline as projected in the horizontal plane, and as it was estimated to have existed just before germination, was intersected by one and only one stripe's edge. The outgrowths showed such a strong tendency to form horizontally that the proviso of projection into the horizontal plane usually produced no trouble. The proviso of estimating the pregermination outline served for the exclusion of any spore whose projected outline intersected a stripe edge only through its outgrowth. The last proviso of intersecting only one edge was of significance only for the large *Osmunda* spores, since their diameter (65 μ) exceeded the width of the stripe (40 μ). We usually counted about 100 spores per distribution. At the time of counting, a few per cent of the *Botrytis* spores had sometimes formed two germ tubes; in such cases, the direction of the longer one was measured.

To characterize the degree of bipolar alignment of a distribution, the parameter V_2 was calculated:

$$V_2 = \sum p \cos 2\theta$$

where p = the percentage of all outgrowths lying at an angle θ to a reference axis (either the vibration axis of applied polarized light or the axes of the cells' outlines).

The corresponding unipolar vector used to characterize the response to an unpolarized light difference was

$$V_1 = \sum p \cos \theta$$

where p = the percentage of all outgrowths lying at an angle θ to that normal to the intersecting stripe's edge which pointed toward the clear band.

To characterize the degree of bimodal alignment of a distribution in response to horizontally directed light, the parameter L_2 was calculated:

$$L_2 = \sqrt{[\sum [p(\phi) + p(-\phi)] \sin 2\phi]^2 + [\sum p(\phi) \cos 2\phi]^2}$$

The character and statistical treatment of these parameters have been discussed in a previous paper (12).

Refractive Indices and Wall Thickness of Botrytis Spores

The average refractive index of the wall of the living cell was determined soon after wetting the fresh spores as 1.477 ± 0.002 at 470 mμ. The value was obtained by densely covering half of a glass block with dry spores, covering the whole block with the usual dilute aqueous medium, and then noting the critical angle at which light traversing the block first entered the cells rather than being totally reflected from the glass-cell interface. This critical angle was obtained by naked eye observation of the point at which the cell-covered half of the block suddenly became much brighter than the cell-free half. Confirmatory though less accurate values were obtained in the course of interference and phase microscope observations like those described below.

The average refractive index of the cytoplasm of a living spore was determined as 1.38 ± 0.005 at 470 mμ. This value was obtained by measuring the phase shift undergone by light in traversing a cell's

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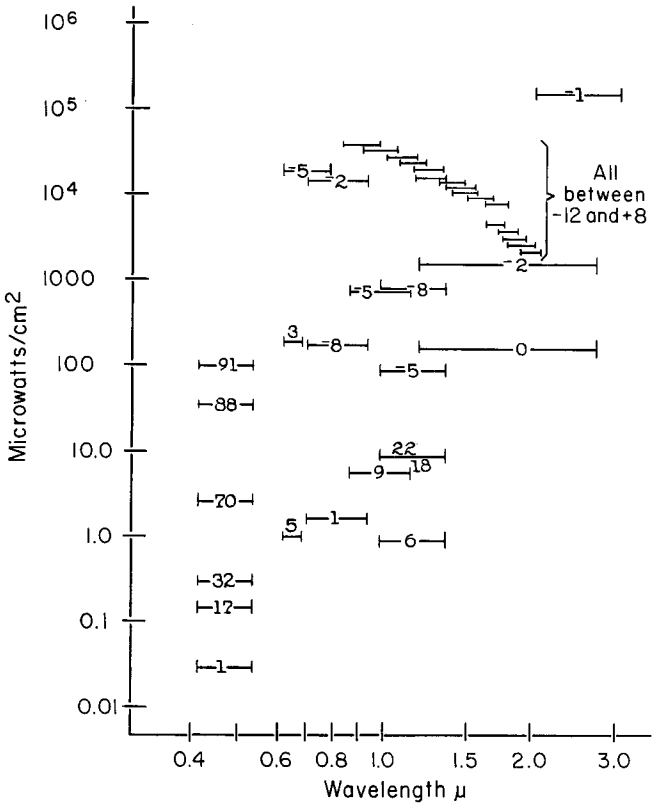

FIGURE 1

Spectral survey of *Botrytis* polarotropism.

diameter, using Dr. Paul Green's Cooke-Dyson interferometer microscope, and subtracting that part of the shift caused by the wall. These cells are so rigid that they do not flatten upon the substratum. Hence we equated the vertical cell diameter and wall thickness necessary in the calculations with horizontal ones measured with an ocular micrometer. During these measurements the cells lay upon a cellophane film through which they were fed with enough water to germinate later, but they were otherwise surrounded with various non-toxic oils of refractive indices from 1.44 to 1.50. Since these indices are close to that of the wall, the oils minimized corrections for phase shifts produced by the wall. The oils were appropriate mixtures of Cargille's non-drying type A immersion oil (n_D = 1.515), paraffin oil (n_D = 1.480), and Minnesota Mining and Manufacturing Company's Kel-F No. 3 fluorochemical oil (n_D = 1.407).

The thickness of the wall of the living, swollen, but yet ungerminated spore was measured as 0.55 ± 0.1 μ using a ×100 phase objective and an ocular micrometer. Halos were minimized by surrounding each measured spore with Kel-F No. 3 oil except for a narrow contact with the usual aqueous nutrient. Cells were thus enclosed with the aid of cellophane film as above, or by putting a drop of an oil suspension of dry spores in some aqueous medium, for some spores then appeared at a vertical oil-water interface with most of their surface in the oil.

RESULTS AND ANALYSIS

Preliminary Spectral Survey

In Fig. 1 we show a survey of the polarotropic responses of *Botrytis* spores to radiation of wavelengths from 0.4 to 3.0 μ. Each number in the body of this graph is the percentage of bipolar orientation (V_2) as calculated from the response of about 100 cells. Positive values of V_2 represent germination parallel to the vibration plane; negative ones, perpendicular to it. The standard deviations of V_2 were about ± 6 per cent. The lines around each value extend to the wavelengths where the irradiation band reached one-tenth of its peak value.

One sees a striking tendency to germinate parallel to polarized blue light which extends down to less than 0.1 μwatt/cm². (This is the response discovered by Bünning and Etzold (2) and illustrated here in Fig. 2.) However, no

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convincing deviations from randomness appeared at longer wavelengths, even when intensities a million times those giving responses in the blue were employed, for (excepting the 18 ± 6 per cent and 22 ± 5 per cent responses at 1.2 μ and 10 μwatt/cm^2) all the values lay within 2 standard deviations of zero.

In addition to the blue with *Osmunda*, we tested a broad band of red light (peaking at 660 mμ and falling to one-tenth maximum intensity at 610 and 700 mμ); here we also tested partial illumination. No significant deviations from randomness were found with intensities of this red light varying from 0.1 to 1000 μwatt/cm^2.

Comparison of Responses to Polarized Light and Partial Illumination

The responses of *Botrytis* and *Osmunda* spores to polarized blue light and to 100 per cent differences of unpolarized blue light are illustrated in Fig. 2. Both spores tend to germinate parallel to the plane of vibration, but *Botrytis* grows from its brighter part and *Osmunda* from its darker.

In Fig. 3 we show how the *degree* of these responses depends upon the light intensity. For both species, the two curves are nearly coincident, except that at intermediate intensities the polarotropic response falls a bit below that due to partial illumination. In other words, at least at high intensities, polarized light corresponds in its effect to the imposition of an approximately 100 per cent intensity difference across each cell.

In Fig. 4, we show comparisons at high intensities of the polarotropic responses (centrally dotted circles) with the responses to the whole range of relative differences. It is seen that *only* differences of about 100 per cent orient growth as completely as does polarized light; the degree of orientation falls linearly as the per cent difference falls. In other words, at least at high intensities, polarized light corresponds *uniquely* in its effect to that of an approximately 100 per cent difference.

We neglect "depolarization" by the birefringent cell walls for three reasons: (i) Inspection shows this birefringence to be feeble. (ii) The two cell meridians from which germination is most and least probable and which thus dominate the response pass just those rays which are not "depolarized" at all. (iii) Any "depolarizing" phase shift suffered by a given ray will be between components in the normal direction and a tangential one. The optic axis of the group of photoreceptor molecules hit by this ray must lie in the same normal direction. Thus the phase shift will be between components along and across the optic axis of the photoreceptors. As judged from the very low values of circular dichroism found for pigments *in vitro*, such a phase shift should scarcely affect absorption.

On the basis of these results we can virtually prove that the tropic photoreceptor molecules are highly dichroic and oriented as shown in the model cells of Fig. 5. In *Botrytis* these molecules are *anticlinal*, *i.e.*, their axes of maximum absorption lie perpendicular to the nearby cell surface, whereas in *Osmunda* they are *periclinal*. First we shall show that these arrangements *can* explain the results. Consider the "vibration poles," P and P', *i.e*, the poles of that model cell diameter which is in the vibration direction. Also consider the "vibration equator," which is the equator defined by the vibration poles. Examination of the model cells of Fig. 5 reveals the pattern of light absorption by the photoreceptors illustrated in the model cells of Fig. 6. In the *Botrytis*, or anticlinal, model, photoreceptor absorption is maximal at the vibration poles and falls to zero along the vibration equator. In the *Osmunda*, or periclinal, model, absorption is maximal along the vibration equator and falls to zero at the vibration poles. (The latter might also be called the *Fucus* model, since it was first proposed for this alga (12). In this manner, both the directions and the relative degrees of the polarotropic and of the partial illumination responses result from the tendencies of *Botrytis* and *Osmunda* spores to grow out in the regions where the photoreceptors absorb most and least light respectively.

The *exact* manner in which the rate of light absorption by the photoreceptors varies in going from the vibration pole to the vibration equator depends upon the radial position of the photoreceptors in the cells. We show on page 24 that the photoreceptors definitely lie near the surface of the *Botrytis* spore. Probably they likewise lie near the surface of *Osmunda*. On this basis we have drawn the graphs of Fig. 6. Let $\bar{A}$ be the specific rate of light absorption by the photoreceptors underlying any part of the cell's surface. Let the origin be at the model cell's center and PP' be on the x-axis. It readily follows that for the anticlinal and periclinal cases $\bar{A}$ is proportional to x^2 and $(1 - x^2)$ respectively, independently of the other coordinates.

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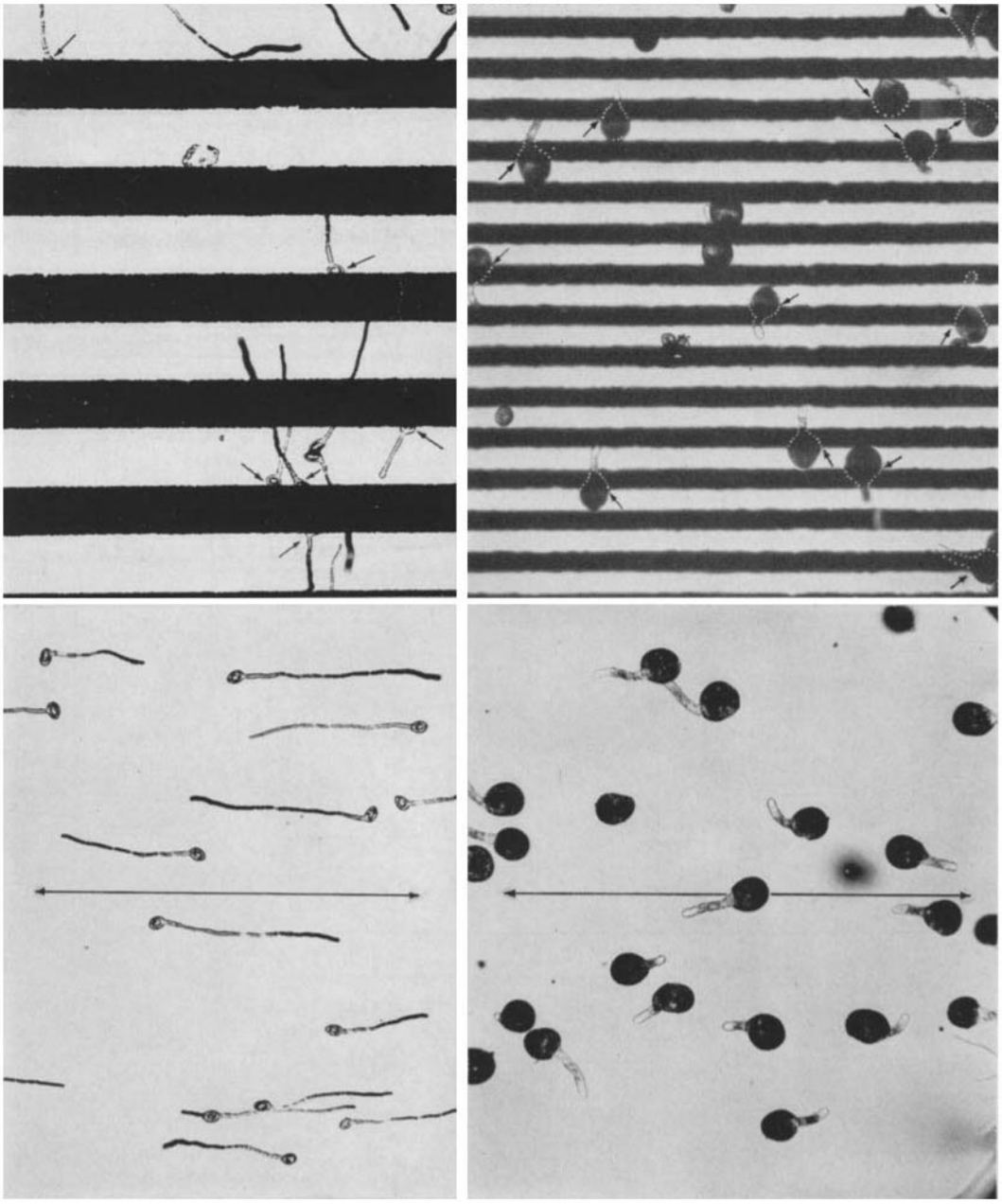

FIGURE 2

Photographs illustrating tropic responses of *Botrytis* and *Osmunda* spores to blue light. *Boirytis* (upper left) and *Osmunda* (upper right) spores were sown directly upon a pattern of opaque and clear stripes and lit from below with unpolarized light. Arrows point to cells which lay on boundaries and hence were partly lit. Cell outlines on or close to opaque stripes had to be drawn on the photograph for clarity, though they were sharply visible under the microscope. *Botrytis* spores tend to grow from their bright parts; *Osmunda*, from their dark parts. Stripes are 40 μ wide. Lower photographs show spores lit from below with plane polarized light. Double-headed arrows indicate the vibration axis. Spores of both species tend to germinate in this axis. Magnifications: upper and lower left, 184; upper and lower right, 72.

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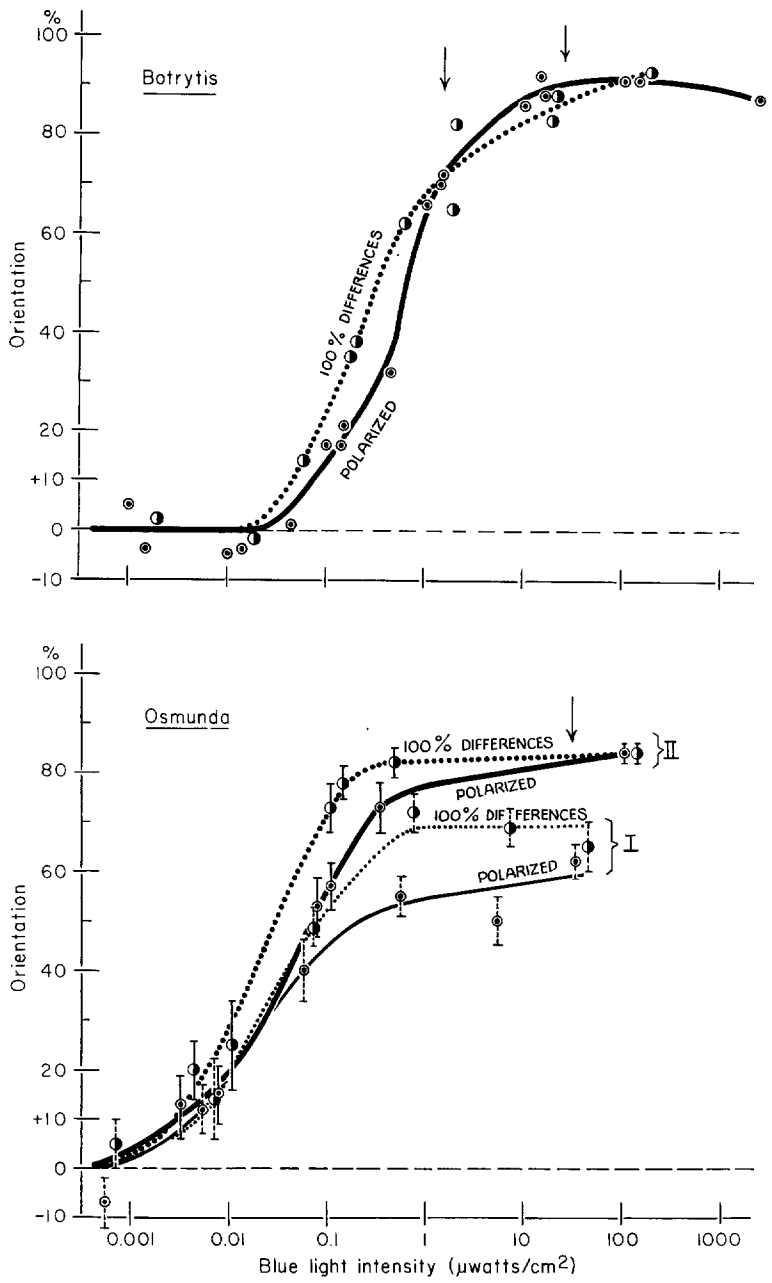

FIGURE 3

Above. Comparison of the response of *Botrytis* spores to polarized blue light with that to the imposition of 100 per cent differences of intensity across each cell, as a function of the average intensity. At the intensities marked by vertical arrows, the responses to lesser differences were tested (see Fig. 4). One microwatt/cm^2 added up to 1.9 × 10^3 erg/mm^2 in the 5.2 hours between the time when the spores were wet (and illumination began) and the time when half of them germinated.

Below. Comparison of the response of *Osmunda* spores to polarized blue light with that to the imposition of 100 per cent differences of intensity across each cell, as a function of the average intensity. At about the intensity marked by a vertical arrow, the responses to lesser differences were tested. (This estimate

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We shall now consider and exclude alternative explanations of the polarotropic responses.

1. *Dichroic filters:* It might be supposed that peripheral to the photoreceptors of each cell there lies a dichroic shell which passes only anticlinally or only periclinally vibrating light; however, if these really existed, then spores would *look* about like Fig. 6 when viewed through a polarizer. Since no such patterns are seen, this hypothesis is excluded.

2. *Differential scattering:* The spores grew in a shallow clear medium and covered a very small fraction of the beam's cross-sections (0.2 per cent with *Botrytis*, 3 per cent with *Osmunda*). Hence the total scattered light received by a spore could not have exceeded 0.1 to 1 per cent of that received directly, and therefore could not have produced intensity steps of more than about 0.1 per cent. Scattering, then, was negligible.

3. *Differential reflection:* At a cell's external surface differential reflection will more greatly weaken the polarized light before it enters the cell in the regions around E and E' of Fig. 5 than about the vibration poles, P and P'. Such reflection, then, will produce an intensity pattern in the cell which will favor germination athwart the vibration axis in *Osmunda* (where it is *not* in fact favored), but along it in *Botrytis* (where it is in fact favored). We may, therefore, exclude reflection as an explanation of polarotropism in *Osmunda* on qualitative grounds alone, but must consider its quantitative consequences in *Botrytis*.

We show below that in *Botrytis* most of the effective light is absorbed by photoreceptors 0.4 $\pm$ 0.2 μ below the cell surface on radii at 110 $\pm$ 20° to the direction of illumination. With the aid of a ray diagram like that in Fig. 8, we applied the Fresnel reflection equations to all the inter-

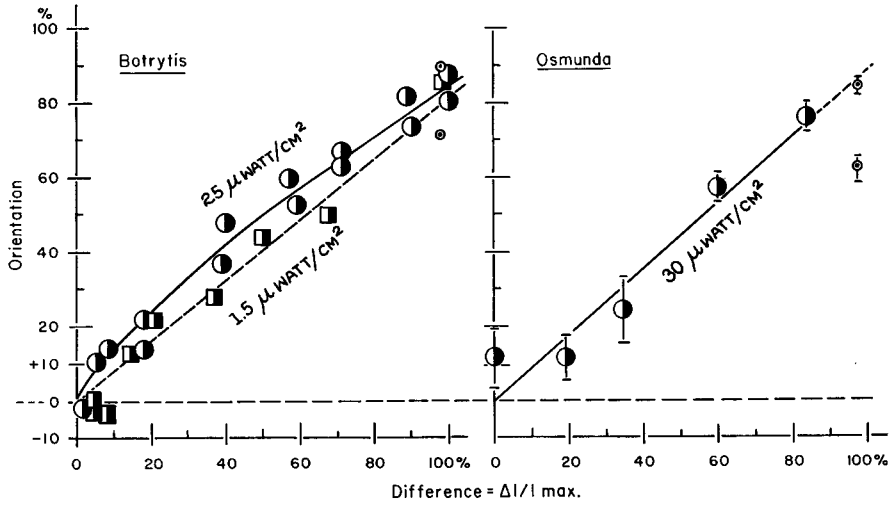

FIGURE 4

Comparison of the responses of *Botrytis* and *Osmunda* spores to polarized blue light with that to the imposition of an intensity difference across each cell, as a function of the relative size of this difference. These experiments were run at average intensities, marked by arrows in Fig. 3, which yielded nearly saturating responses when applied as 100 per cent differences or as polarized light; the centrally dotted circles show the orientation by polarized light at these intensities.

refers only to the blue and tropically effective component of the white light used there. See Fig. 4.) One-tenth microwatt/cm² added up to about 2×10^3 erg/mm² in the 2 to 3 days between wetting and killing the spores. Strong, non-orienting red light was also imposed on all cultures to support germination. In experiment I, the germination-supporting band included far red light (one-tenth maximum limits, 610 mμ and 820 mμ) and the medium was a 0.05 per cent Knop solution; in experiment II, the germination-supporting band excluded far red light (one-tenth maximum limits, 610 to 700 mμ), the medium was 1 per cent water agar, and (unlike experiment I) the orienting blue light was contaminated with a comparable intensity of far red light (one-tenth maximum limits, 710 to 820 mμ).

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faces traversed by the rays which strike these photoreceptors. We thus estimate that those in the meridians of the vibration plane, *i.e.*, those around *PP'* where germination is favored, received about 5 per cent more light than those in the orthogonal meridian. The data of Fig. 4 show that a 5 per cent difference will yield less than a tenth of the orientiation produced by polarized light. So reflection can be excluded as a major cause of polarotropism in *Botrytis*.

A Confirmatory Test of Photoreceptor Orientation in Botrytis with Unilateral Illumination

In the above experiments with vertical illumination, the directions of the outgrowths were practically restricted (by factors other than light)

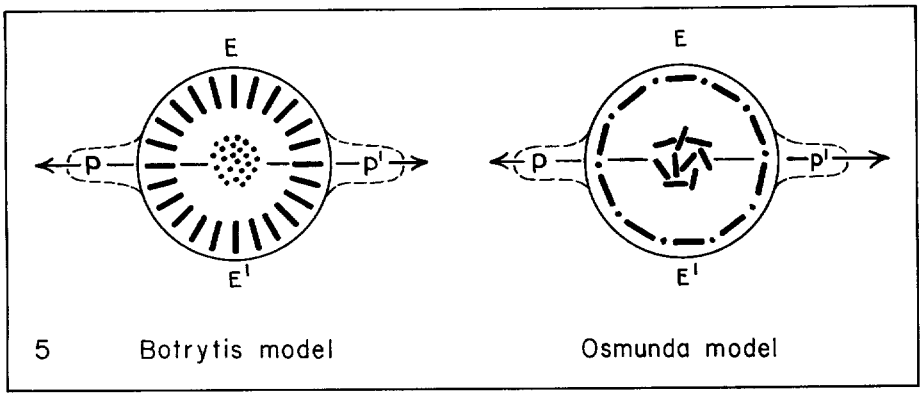

FIGURE 5

Diagrams of inferred photoreceptor orientation. A dash in a cell represents a photoreceptor molecule's axis of highest absorption; a dot shows this axis end on. Symbols in the centers of the circles represent molecules at those cell poles which face toward and away from the reader. The double-headed arrows represent the vibration axis of applied polarized light. The dashed humps indicate the directions of most frequent germination.

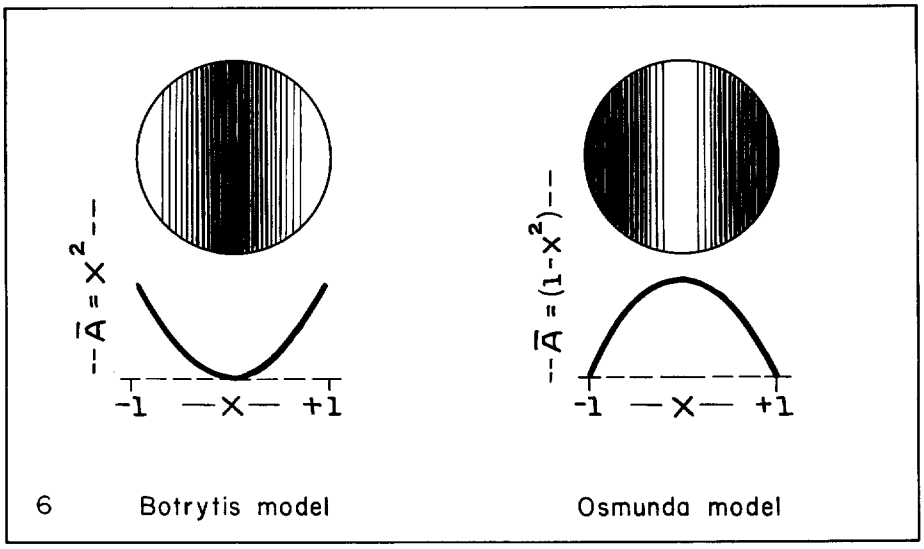

FIGURE 6

Patterns of the inferred relative rates, $\bar{A}$, of light absorption by the photoreceptors. In the blackest regions, they absorb least light. $\bar{A}$ is independent of y and z.

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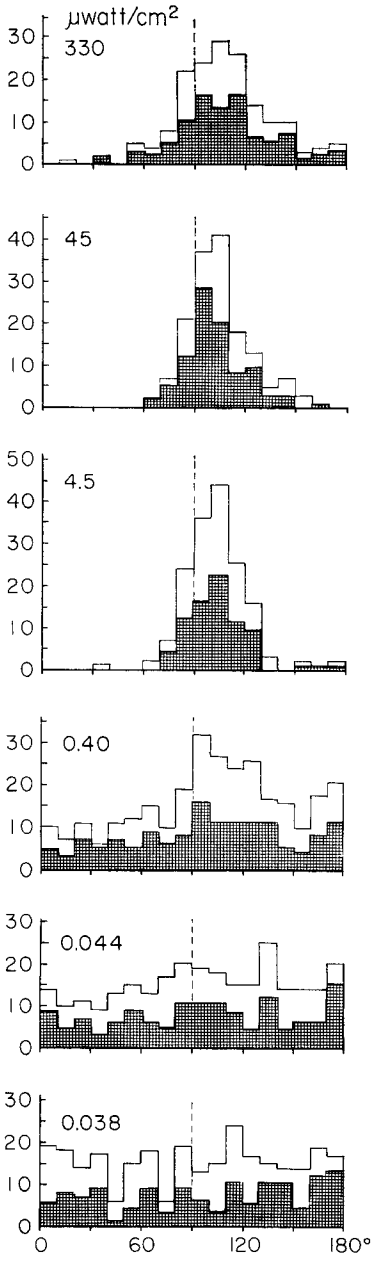

FIGURE 7

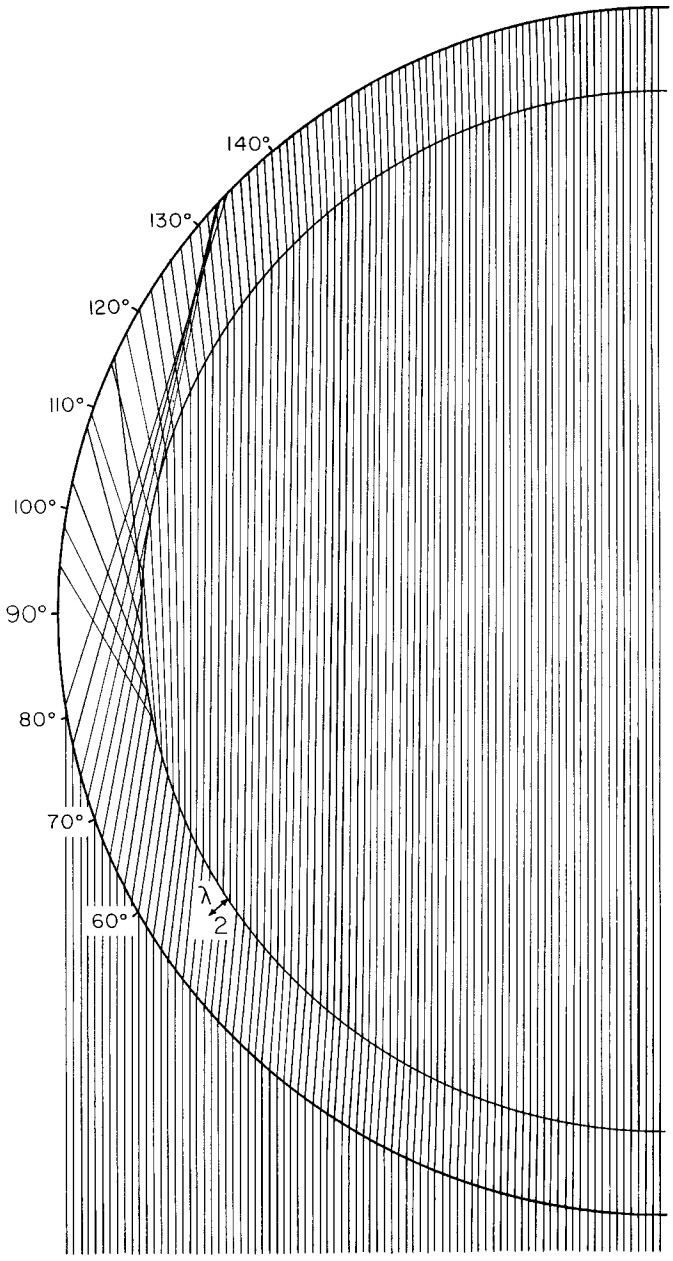

FIGURE 8

FIGURE 7. Tropic responses of *Botrytis* spores to horizontally directed and horizontally vibrating blue light. Ordinate indicates number of outgrowths found per 10° interval. Zero degrees points directly toward light. The concentrations at a bit more than 90° are clear. The shaded parts of the bars indicate the numbers of spores which grew to the left, and the unshaded parts, to the right, both as seen from the light source. The spores showed no significant preference for the left side over the right or *vice versa*.

FIGURE 8. Diagram of rays traversing a *Botrytis* spore. Neither rays leaving spore nor rays which have lost more than three-quarters of their energy through reflection losses are shown. Scale is shown by double arrow representing a half wavelength, or $\lambda/2 = 0.24\ \mu$.

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to the plane of the substratum, which is the plane *across* the beam. The angular distributions of outgrowths in the plane *of* the beam can be revealed by the response to light which is horizontally propagated as well as horizontally vibrating. If the photoreceptors are, indeed, dichroic and anticlinal, it follows that such illumination should favor germination in the horizontal plane and *at about 90° to the direction of propagation;* however, some deviation from 90° may be anticipated because of the deviation of the light by the relatively refractile cell.

The data shown in Fig. 7 verify this prediction. In it we show Cartesian plots of the whole distributions found in response to horizontally directed and horizontally vibrating beams. (Zero degrees is the direction directly toward the light.) There are obvious peaks at a bit more than 90°, *i.e.*, slightly away from the light, at all intensities eliciting much response. Specifically, the number of outgrowths is seen to jump at about 80 to 90°, peak at about 95 to 105°, and fall rapidly from about 110 to 130°. With the aid of the representative polar plot in Fig. 9, lower left, one can mentally combine these results with those yielded by vertically directed polarized light. One can thus obtain a surface representing the germination response of *Botrytis* spores to unidirectional polarized light, free of interference by either the substratum or gravity. As in the case of *Fucus* (12), this surface is shaped like a wing nut; the plane of the wings is the vibration plane and the tilt of the wings is slightly away from the light.

Locus of the Photoreceptors in Botrytis

In an effort to understand more closely the shift of the outgrowth peak beyond 90°, we drew the ray diagram of Fig. 8. In it we approximate the refractile heterogeneity of the system by dividing it into its three main regions: the medium, the cell wall, and the cytoplasm of refractive indices (at 470 mμ) of 1.338, 1.477, and 1.38 respectively. We approximate the ovoid shape of the cells by a sphere of a radius of 4 μ, which is the average minor semiaxis of the cells. We do this since the most pertinent rays are refracted by the relatively curved cell poles. The wall thickness is drawn as 0.55 μ.

Deviations of the rays from parallelism which might explain the shift of the germination peak beyond 90° are restricted to the inner half of the cell wall. In all parts of the cytoplasm except its peripheral few tenths of a micron, the deviations from parallelism are negligible. In the peripheral shell of the cytoplasm the rays bend so as to produce a significant peak in the radial or anticlinal component of the light at angles of about 75 to 85°, well in front of the observed germination peak. In the outer half of the wall, the rays are sharply focused between about 120 and 135°, well in back of the observed germination peak. In the inner half of the wall, however, rays are markedly concentrated between 80 and 125°, which is the region where germination peaks. Thus it seems apparent that anticlinal receptors located within the proximal half of the wall would absorb most light just where germination peaks.[1] Moreover, we have confirmed this impression with refined calculations; inspection proves to be a reliable indicator here because most of the energy in the region of focusing is in the normal or anticlinal component of the light (which is the one absorbed by the photoreceptors), because interference effects largely average out across the width of the wall's inner half, and because other "invisible" factors, such as reflection losses, are minor.

We infer, then, that the tropic photoreceptors in *Botrytis* must lie within the proximal half of the wall and, perhaps, the as yet vaguely defined border region between wall and cytoplasm. This conclusion is supported by the high degree of anticlinal orientation of the photoreceptors, for in contrast to the bulk of the cytoplasm the wall and plasma membrane are plainly rigid enough to thus fix these molecules; it is also easiest to imagine the path from light absorption to local wall growth if the photoreceptors lie in or close to this wall. Other ways of stating this conclusion are that the photoreceptors lie at 0.4 $\pm$ 0.2 μ

[1] Implicit in this whole paper has been the assumption that light absorption is governed by the electric rather than the magnetic vector. The *in vitro* evidence, though too scanty to warrant carefree generalization, all points this way. Moreover, considerations of symmetry show that if photoreceptor absorption in *Botrytis* were attributed to the magnetic field, then germination would be random in the plane of the E-vector except as modified by focusing. Thus the sharp concentrations of germination in two directions in the plane of the E-vector would have to be attributed *entirely* to focusing, an inference very difficult to square with the linear dependence of tropic response upon relative light intensity difference (14).

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in from the cell surface or at 90 ± 5 per cent of one cell radius out from its center.

Unilateral Unpolarized Light

Our goniometry is largely confined to the horizontal projection of the outgrowths. We have only a blurred indication of their vertical component, *i.e.*, we tally a spore as "up" if the horizontal projection of its outgrowth's base lies entirely within that of its outline. In the experiments so far reported, a combination of light and dark factors have largely confined germination to the horizontal plane: less than 1 per cent of the spores were tallied as "up."

torial maxima found in all the other responses to unilateral light.

We refer to the phenomenon represented by the third peak of Fig. 9, upper right, as "centering," for we believe that it arises not from a change in the photoreceptors but from a shift in the mechanism subsequent to light absorption, a shift from processes which select one of two most stimulated positions for growth to ones which select the center of these positions for growth. Two lines of data support this view. First, the data in Fig. 3 show no indication of any change

TABLE I

Tropic Responses of Botrytis Spores to Unilateral Illumination

Intensity (μwatts/cm^2)	Unpolarized light			Horizontally polarized light		
	Mode(s)	% up	% orientation (L_2)	Mode	% up	% orientation (L_2)
900	115°, (165°)	6	—			
330				105°	0.6	62 ± 4
120	115°	15	69 ± 3			
45				105°	0.0	81 ± 2
12	115°	7	67 ± 3			
4.5				105°	0.0	82 ± 2
1.1	115°	1	48 ± 3			
0.40				95°	0.0	24 ± 4
0.12	—	0.3	8 ± 4			
0.044				—	0.7	11 ± 4
0.010	—	0.0	8 ± 4			
0.0038				—	0.3	7 ± 4

More evidence of upward growth may be anticipated in the response to unilateral unpolarized light; for it follows from its symmetry that such light should stimulate equal germination from the whole zone slightly away from the light, including directions with large vertical components. This tendency should appear as "up" tallies and as a broadening and rearward shift of the peaks in the recorded horizontal projections of the distributions. In Table I we compare parameters of the responses of *Botrytis* to unilateral unpolarized light with those to horizontally polarized light (reported fully in Fig. 7). It shows the expected signs of upward growth (also see Fig. 9).

Centering

The response to the highest intensity of unilateral unpolarized light used (900 μwatt/cm^2) shows a new and surprising feature. Consider the polar plot of this response shown in Fig. 9, upper right. In it there appears a large, new, and distinct peak at the rear pole in addition to the subequa-

in photoreceptor orientation at 10^3 μwatt/cm^2. Secondly, Fig. 10 shows that the experimental distributions in response to 100 per cent differences at high intensities are far more centralized in the spores' lit parts than is the theoretical distribution yielded by equally frequent growth from all the apparently brightest points of the cell. (To sharpen the evidence, the results of Fig. 10 were obtained from those partly lit cells which were *more* than half lit.) The centralization of light—if any—was surely insufficient to explain the centralization of growth.

The Probable Cause of the Relative Weakness of Polarotropism in Dim Light

One might well suspect that this weakness arises from poor photoreceptor orientation in dim light; but further experiments point to a subtler mechanism which obviates this new assumption.

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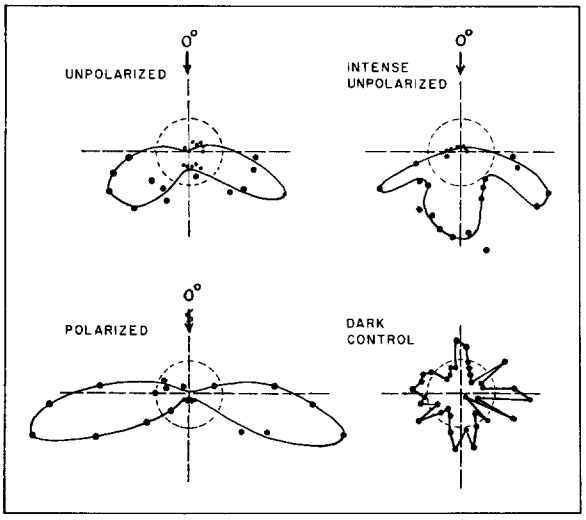

FIGURE 9

Polar plots illustrating the tropic responses of *Botrytis* spores to unilateral blue light. Each point indicates the percentage of outgrowths found in a 10° interval. Each reference circle represents a homogeneous distribution, *i.e.*, one in which $\frac{1}{36}$ or 2.8 per cent of the germ tubes grew out in every 10° interval. Arrows indicate light direction.

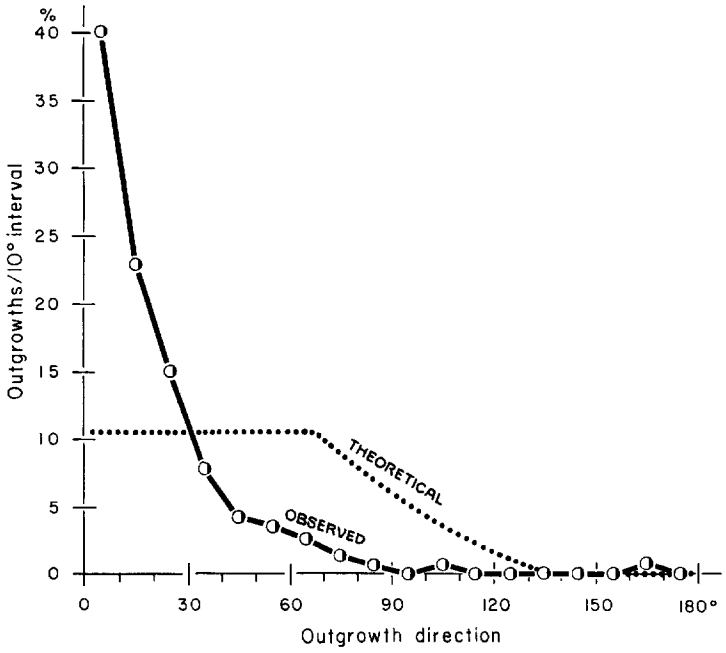

FIGURE 10

Evidence of centering. A comparison of the observed response of *Botrytis* spores to being half or more illuminated, with a non-centered distribution obtained theoretically, *i.e.*, yielded by the assumption of equally frequent growth from all the brightest points of the cells.

The responses of *Botrytis* spores to polarized light and to 100 per cent gradients were repeatedly compared. Sometimes, as in Fig. 11, the deficiency in polarotropism was markedly greater than that shown in Fig. 3. Now, the spores in the batch tested in Fig. 3 had been inspected and seemed to have grown out at random with respect to the axis of their own ovoid forms both in darkness and in light. However, the spores in the batches showing large deficiencies in polarotropism tended to germinate athwart this axis; thus those yielding Fig. 11 showed a value (V_2) of $-$ 55 $\pm$ 6 per

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cent of such orientation when grown in darkness. As judged from the symmetry of their external form, this represented a tendency inherent in *each* cell to grow with equal probability in *either* of the opposite directions orthogonal to its axis and parallel to the substratum.

We suggest that a variable degree of such a bipolar predetermination of growth was the chief cause as well as the correlate of the polarotropic deficiency found in dim light; it would work as follows: The dim light which yields submaximal responses overcomes the growth pattern inherent in the cells only to a quite limited extent. Nevertheless, a *unipolar* stimulus, such as the light gradients used here, would markedly orient a population just by favoring one of two equally predetermined directions in each cell. (Calculation under this assumption yields a figure of 64 per cent for the orientation, V_1.) On the other hand, a *bipolar* stimulus, such as polarized light, could *not* favor one of these two directions over the other in any cell. Thus at light intensities too low to overcome the inherent growth patterns, no polarotropism would occur despite the large effect of light gradients.

COMPARISON WITH OTHER DATA

Photoreceptor Orientation

In Table II we have assembled the chief information about photoreceptor orientation, in order of reliability, with the most certain inferences on top. Moreover, the widespread polarization sensitivity of the arthropods early suggested that their visual photoreceptors are oriented in *some* direction with respect to the ultramicroscopic tubules in the ommatidia (4). The same now seems probable for some cephalopod eyes (15).

Thus, with the peculiar exception of the *Funaria* spore in dim red light, photoreceptor molecules very generally appear to be highly oriented (though the available results yield no rule covering the direction of this orientation). This conclusion fits an emerging view of the molecular foci of cell action—a vision of highly organized membranes.

Cellular Locus of the Photoreceptors

We have concluded in this paper that the tropic photoreceptors of the *Botrytis* spore lie about one-half micron from its surface, either in the inner half of its wall or possibly in the as yet vaguely

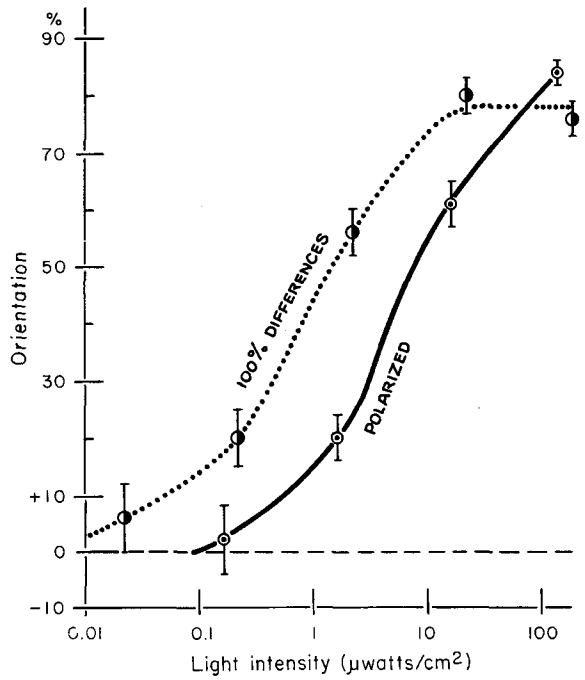

FIGURE 11

Phototropic responses of *Botrytis* spores which showed a strong tendency to germinate across the axis of their own ovoid outline.

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TABLE II

Chief Information Available on Photoreceptor Orientation

Organism	Group	Cell	Color of light	Photoreceptor		Reliability (references)
				Function	Orientation	
Frog, pike, etc.	Amphibia, fish	Rods	Green	Vision	Along lamellae	I (22, 3)
Botrytis	Imperfect fungi	Germinating spores	Blue	Tropism	Anticlinal	I*
Osmunda	Ferns	Spores forming rhizoids	Blue	Tropism	Periclinal	I*
Equisetum	Horsetails	Spores forming rhizoids	Blue	Tropism	Periclinal	II‡
Fucus, Pelvetia	Brown algae	Zygotes forming rhizoids	Blue	Tropism	Periclinal§	II (12)
Funaria	Mosses	Spores forming chloronemas	Red	Tropism	Random (dim light)	II‡
"	"	Spores forming chloronemas	"	"	Periclinal (bright light)	II‡
Mougeotia, Mesotaenium	Green algae	Vegetative	Red	Chloroplast taxis	Periclinal	II (8, 9)
Mougeotia	" "	"	Red	Photosynthesis (chlorophyll)	Along lamellae‖	III (21, 7)
Phycomyces	Bread molds	Sporangiophore	Blue	Tropism	Periclinal (hoop direction)	III (13)
Penicillium	Imperfect fungi	Vegetative filament	Blue	Tropism	Anticlinal	III (5)

In the column for reliability, I means most reliable; III means least reliable.

* From this paper.

‡ From our unpublished results.

§ A significant bit of accessory evidence, regrettably left out of the 1958 paper (12), is the report of Nienburg (20) that *Fucus* zygotes grow out from their dark regions when partially illuminated.

‖ Ruch (21) interprets his own data as arising from form dichroism, but the highest dichroic ratio he observed, which was 2.4 at 680 mμ, far exceeds the highest possible value of form dichroism in *Mougeotia*, which is 1.3 according to Goedheer (7). Furthermore, the latter has pointed out privately that form dichroism should not show the very strong wavelength dependence exhibited in Ruch's data. Finally, Menke's observations (17) of various types of weak anisotropy suggest the same alignment of porphyrins along the lamellae in a variety of plants.

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TABLE III

Doses Giving Half Maximal Alignment

Organism	Group	Cell	Light pattern	Dose giving 50% orientation	Authority
Botrytis cinerea	Imperfect fungi	Spore	Vertical, polarized	1.0×10^3 erg/mm^2	*
Osmunda cinnamomea	Ferns	Spore	Vertical, polarized	0.8×10^3 erg/mm^2	*
Equisetum palustre	Horsetails	Spore	Unilateral, unpolarized	$2.0‡ \times 10^3$ erg/mm^2	(19)
E. arvense	Horsetails	Spore	Vertical, polarized	2.0×10^3 erg/mm^2	§
Cystoseira barbata	Brown algae	Zygote	Unilateral, unpolarized	$0.3‡ \times 10^3$ erg/mm^2	(18)
Fucus furcatus	Brown algae	Zygote	Vertical, polarized	0.4×10^3 erg/mm^2	(12)

* This paper.

† Data converted on the basis that 1 meter-candle of white light from a tungsten lamp contains 4×10^{-3} erg/mm^2 sec of blue light (Weitz, 24).

§ Our unpublished results.

defined wall-cytoplasm boundary. Similar, though much cruder, evidence has been reported indicating that the photoreceptors of the zygote of the brown alga *Pelvetia* lie somewhere within a few microns of its surface (12). More generally, the high degree of periclinal or anticlinal orientation indicated by polarotropism in so many single cells suggests that their tropic photoreceptors lie in the relatively rigid and immobile wall or plasma membrane. This view also simplifies any imagined pathway between light absorption and a local effect upon wall growth, and even in multicellular flowering forms the auxin transport mechanism suggests a close relationship between the photoreceptors and cell membranes, while the successful replacement of weak blue light (as a morphogenetic stimulus) by mild rubbing suggests such a relationship in the immature mushrooms of *Coprinus* (1). Finally, we may note that for some of these reasons as well as less cogent ones, such a peripheral locus of the photoreceptors has long been a prevalent opinion.

Centering

Some moss spores (10) and fucoid zygotes (12) shift their direction of germination in response to unilateral light from their subequatorial zones to their rear poles as the light intensity rises. Probably these shifts are both covert cases of the same phenomenon and have the same general cause as those we call "centering" above.

Threshold Doses

In Table III we compare the doses of blue light per unit area reported to induce a half maximum alignment of the growth of various cells, when applied continuously from the start of development through the time of germination. The values (dose per unit area) are impressively similar for such widely different organisms.

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