Action Spectra for Photogrowth and Phototropism in Protonemata of the Moss

*Physcomitrium turbinatum*

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**Summary.** Protonemata of *Physcomitrium* were grown in a sucrose-mineral nutrient, liquid medium. Even in this medium containing organic nutrient, the growth rate of lateral branch, chloronemal filaments showed a light dependence which was linear with log intensity. Intensities necessary to give a constant growth rate (45 μ/1.75 hrs.) were determined at selected wavelengths. The resulting action spectrum paralleled the in-vivo absorption spectrum of a single filament in the red region, showing a major peak at 680 nm. Growth rate and absorption approached zero in the far-red (730 nm). However, significant growth activity occurred at 365—400 nm where absorption was low, and negligible growth was found at 440—500 nm where absorption was high.

The action spectrum for the positive, directional photo-orientation of growth was determined by the null-point method in which the effectiveness of each selected wavelength was compared to a 665-nm standard in simultaneous, bilateral irradiation. In contrast to growth, the major peak of phototropic activity was found at 730 nm with significant activity extending to 800 nm. A minor peak was at 680 nm. There was some activity in near ultraviolet but not at longer blue wavelengths.

It is concluded that the blue-absorbing system responsible for phototropism in virtually all other groups of plants is inactive or absent in *Physcomitrium*. Instead growth and orientation seem to be dependent upon an interaction between the photosynthetic and phytochrome systems. Further, the data suggest that the physiological activity of phytochrome in photo-orientation of growth does not derive from a certain amount of Pfr or Pfr/Pt ratio but rather it derives from the simultaneous excitation and consequent cycling of Pt and Pfr.

**Introduction**

The apical cell of a moss protonemal filament is large (20 × 100—200 μ), chlorophyllous, uninucleate, and highly dependent upon light stimuli for both rate and orientation of growth. The fact that moss protonemata exhibit a strong tendency to grow toward a unilateral light source has been recognized at least since the time of PRINGSHEIM and PRINGSHEIM (1935). Yet, in spite of the analytical advantages inherent in the study of a single cell and indication that a unique phototropic response to red
light is involved (Jaffe and Etzold, 1965) the details of the photoreponses have not been explored. Detailed action spectra are still necessary for identifying the active pigment or pigments.

Perhaps at least part of the reason for this apparent neglect has been the lack of a suitable experimental method for obtaining reproducible data. Some of the inherent problems are described in the next section and a method is presented which may be generally useful in obtaining such data with filamentous systems. Detailed action spectra for growth and phototropism are presented for the moss *Physcomitrium turbinatum*.

**Methods and Materials**

*Experimental Plant Material.* Moss protonema exists in two main habits, chloronema and caulonema. These filament types, which show physiological differences, may be distinguished on the simple morphological point that a chloronema has perpendicular cross walls while a caulonema has oblique cross walls. Further discussion of differences may be found in Boe’s (1961) review.

The spore gives rise to chloronemata which under suitable conditions differentiate into caulonemata. Caulonemal filaments may become heterotrichous by giving rise to a lateral branch filament (Seitenzweige, Seitenfädlen) at the distal end of each cell. [Photographs may be seen in Nebel and Naylor (1968) and Kofler (1959).] Implications of terminology to the contrary, the lateral branch filaments have characteristics of chloronemata, not caulonemata. The characteristics of caulonemata pertain only to the principal axes (Hauptfädlen). The present study deals exclusively with the lateral-branch, chloronemal type of filament.

When a protonemal inoculum of *P. turbinatum* is placed on fresh agar medium and incubated under light, it soon gives rise to a tuft of lateral, chloronemal filaments commensurate with regeneration of new caulonemal filaments (Nebel and Naylor, 1968). These lateral filaments show a positive phototropic response whether they grow in air, under liquid medium, or under paraffin oil. In air the lateral filaments soon become irregularly branched. In liquid medium they grow in a uniform, non-branched fashion to a length of several millimeters. Therefore, methods were designed to grow the filaments under liquid medium since the growth in this condition is more readily quantitated.

*Method of Measuring Phototropism.* In subterminally growing systems such as the *Avena* coleoptile or the *Phycomyces* sporangiophore, a slight asymmetry in growth leads to a comparatively large angular displacement of the tip. Little growth is required in the system to produce a significant bending response. In an apically growing system such as protonemata, however, asymmetry in the tip growth does not per se lead to any comparable displacement. A large amount of symmetric growth must follow before the initial growth asymmetry becomes morphologically apparent. Since symmetric growth is also highly dependent upon light, illumination required for the requisite symmetric growth completely overrides any brief unilateral light stimulus.

The only practical alternative is to examine the phototropic orientation under an equilibrium condition. Hence a null-point method was used. The basis of this method has been described in detail by Debruck and Shropshire (1960). Briefly, one gives a continuous, unilateral, standard irradiation which provides an acceptable growth rate and also a significant phototropic response. From the opposite side one simultaneously gives a test irradiation and varies its intensity until the fila-