The Effect of Light upon Basidiocarp Initiation in Psilocybe cubensis

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THE EFFECT OF LIGHT UPON BASIDIOCARP INITIATION IN *PSILOCYBE CUBENSIS*

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SUMMARY

Formation of basidiocarp initials in *Psilocybe cubensis* occurred only when cultures were illuminated. Short durations of light (0.0025 sec of xenon-arc flash) were sufficient for initiation. Light-induced initiation was saturated at a dose of 0.345×10^4 ergs/cm^2 at 460 nm. UV and blue wavelengths of 370, 440, and 460 nm were the most effective. Green and red wavelengths greater than 510 nm were ineffective.

The requirement of light for basidiocarp initiation or development is well documented: Alasoadura, **1963**; Lu, **1965**; Manachère, **1970**, **1978**; Miller, **1967**; Miller and Palmer, **1977**; Plunkett, **1956**, **1961**. Chapman and Fergus (**1973**) found that the blue end of the spectrum at intensities above 1.5×10^4 ergs/cm^2/sec induced mature basidiocarp formation in *Coprinus domesticus* Fries, whereas green, red, and far red failed to induce initials. Kitamoto et al. (**1970**) studied initiation in *Favolus arcularis* (Fries) Ames and found that a half-maximal response is reached at 1.8×10^8 ergs/cm^2 (at 398 nm). Photoinduction was observed in the region between 350 and 560 nm and six peaks were described. Later Kitamoto et al. (**1973**) studied light-dependent pileus development in *Coprinus domesticus* and found the action spectra to be similar. Perkins and Gordon (**1969**) determined the action spectrum for basidiocarp initiation in *Schizophyllum commune* Fries. They showed that the dose response was linear up to a dose of approximately 1.1×10^5 ergs/cm^2 (at 440 nm). Spectral sensitivity peaked in the blue and UV. No light of wavelengths greater than 525 nm was photoinductive. Heim and Wasson (**1958**) reported that initials of *Psilocybe cubensis* (Earle) Sing. may be formed in darkness at elevated temperatures (27 C). Jackson and Alexopoulos (**1976**) stated that this species requires light for basidiocarp initiation (at 22–25 C).

The purpose of this study was to determine an action spectrum for basidiocarp initiation in *Psilocybe cubensis*.

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MATERIALS AND METHODS

Culture isolate.—The isolate of *P. cubensis* used in this study was obtained during 1975 by allowing a basidiocarp to deposit basidiospores onto agar. A subculture was deposited with ATCC (#36459). The original basidiocarp is deposited in the University of Tennessee Herbarium (TENN 40476).

Preparation of cultures.—An agar medium (Brodie, **1975**) containing relatively low levels of sugars (maltose, dextrose, sucrose), asparagine, peptone, yeast extract, in addition to salts was used. Six ml of medium were poured and slanted in plastic test tubes (16 × 125 mm) giving a surface area of 3 cm^2. Inoculation of these tubes was made from stock cultures which were grown in the dark for 1 mo in plastic Petri plates (15 × 100 mm) with taped lids. One 3 × 3-mm cube was taken from these and placed in each slant tube. The slant tube caps were not tightly closed. These transfers were made under "safe" light (15-watt incandescent light filtered by a red filter, Carolina Biological Supply red, 650). The cultures were grown for 3 wk in well-ventilated but light-tight boxes. Both cultures and light sources were kept in a controlled-temperature room at 21 C ± 2 and under relative humidity of 85% ± 15%. In most cases light treatments were given once per da for 5 da; the cultures were examined on da 6. Exposures were given at the same time each da. If manipulations were necessary they were accomplished under the same safe light described above.

Light sources and filters.—Two preliminary experiments were conducted to determine: 1) the general wavelengths of importance for fruitbody initiation, and 2) the duration of light necessary for initiation. In the first of these, dark-grown cultures were illuminated for 12 h per da by Cool-white fluorescent lamps at 100 ft-c. filtered with broad-band filters: UV (Kodak 18-A) peak at 350 nm, blue (Carolina Biological Supply) peak at 450 nm, green (CBS) peak at 550 nm, and red (CBS) peak at 650 nm. In the second preliminary experiment the white light of a xenon-arc lamp (Honeywell photoflash) at 0.0005 sec per da was used to initiate fruitbodies.

Based on preliminary observations further experiments were conducted to determine: 1) the relative effectiveness of different doses of light, and 2) the relative effectiveness of different wavelengths of light. Both of these experiments used a 300-watt General Electric quartz-iodine projector lamp in conjunction with a 0.25-m Jarrell-Ash monochromater with 2-mm slits. The ½ band width was 15 nm. The lamp

was placed 2.5 cm from the entrance slit and the cultures were placed at the exit slit. Usuing a spectroradiometer (International Light #783), equal light energies at different wavelengths were obtained by adjusting the voltage to the bulb. The light energy was adjusted to 23 ergs/sec/cm^2 at all wavelengths. Second-order spectra were eliminated for this calibration but were not eliminated when light was used to initiate cultures.

For dose-response experiments monochromatic light (460 nm) was used at eight different dosages to initiate fruitbodies. All cultures were illuminated once per da for 5 da and scored on da 6. For treatments providing doses greater than 0.345×10 ergs/cm^2 (23 ergs/sec/cm^2 per da for 5 da) an increase in the time of illumination was used to increase the dose. For treatments less than this amount neutral-density filters (Kodak) were used to decrease the dose. Ten cultures per treatment were used.

For spectral-sensitivity experiments approximately 30 cultures per light treatment were illuminated each da at 23 ergs/sec/cm^2 for 30 sec with monochromatic light of wavelengths from 370 to 510 nm and scored on da 5, 6, 7, or 8. Most replicates were scored on da 6 and those scored on other da were normalized to 6-da data for the interpretation of the results.

Scoring.—For preliminary experiments cultures were scored for the presence of basidiocarp initials (here defined as the first "knots" of hyphae which are visible to the unaided eye and which can, under appropriate conditions, become basidiocarps). For further experiments an attempt was made to use a more precise method of scoring which involved: 1) the size and degree of development and 2) the number of initials per total area of the slanted tube. Numerical values for these categories were assigned as follows: size and degree of development—O, rhizomorphs only, 1, flat aggregations of hyphae, 2, spherical initials 600 μm diam, 3, spherical initials 1,000 μm diam, 4, initials pyramidal in shape; for density of initials in the tubes—1, 1–3 initials, 2, 4–8 initials, 3, 9–30 initials, 4, 31+ initials. A combination of these factors (the sum of the scores) was here termed the "degree of initiation" and was a useful quantitative index to the experimental induction of basidiocarp initiation.

RESULTS

Preliminary experiments.—When cultures were illuminated with light from Cool-white lamps filtered by broad-band filters only blue and

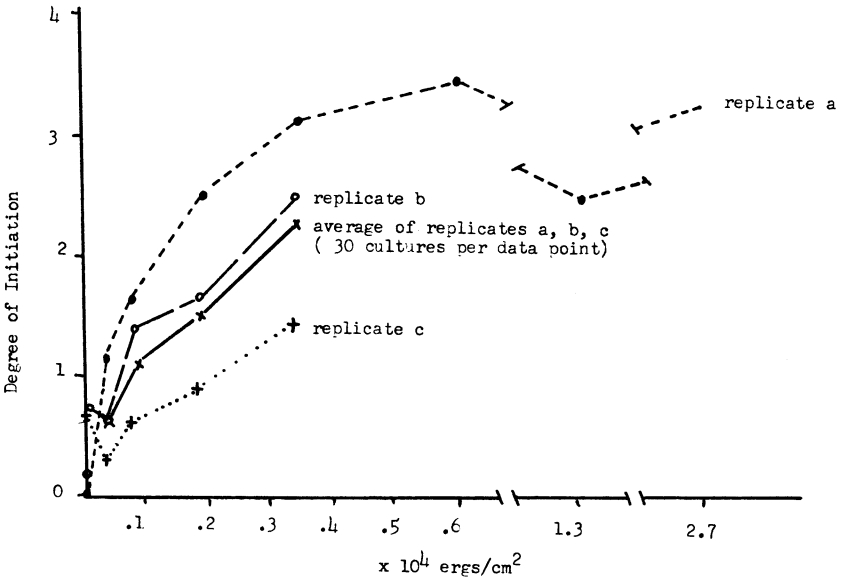

FIG. 1. Dose-response of *Psilocybe cubensis* to light. Eight different doses of monochromatic light (460 nm) used to initiate fruitbodies in dark-grown cultures. Average response approximately linear between 0.086×10^4 and 0.345×10^4 ergs/cm^2.

UV-illuminated cultures formed fruitbody initials. When other cultures were illuminated with one 0.0005-sec flash/da for 5 da initials were present on the 6th da.

Dose-response experiments.—This experiment was conducted to determine in what range of light doses a direct proportion exists between the light dose and the degree of initiation. Such a relationship, if it is linear, is called the Bunsen-Roscoe law of reciprocity. Proof of this relationship is essential to determination of action spectra because if, for example, a spectral-sensitivity study was conducted at doses above saturation all wavelengths would be equally active. As seen in FIG. 1 the average response is nearly linear in the range of 0.086 and 0.34×10^4 ergs/cm^2 at 460 nm. Also based on replicate "a," response is saturated at doses greater than 0.345×10^4 ergs/cm^2. Further studies concerning spectral sensitivity were conducted in the range of doses described above.

Spectral-sensitivity experiments.—These experiments were used to determine the effect of different wavelengths of light on fruitbody initia-

tion (action spectrum). The average degree of initiation at the various wavelengths is shown in FIG. 2.

Observations of cultures.—Initiation did not occur in cultures that were not ventilated. Gases present in the atmosphere or those produced by metabolism may be responsible for this effect. Light has no effect on initiation before vegetative maturity is completed, i.e., the agar surface is covered by mycelium. In one case atypical initials were formed which increased in size as chestnut-colored spheres up to 5 mm diam. These structures contained spores and were grown at 10 C. In some sealed cultures one-celled conidia borne on clamped hyphae were noted (probably "ramifications acremoniformes," Heim and Wasson, **1958**). Abnormalities in basidiocarp development were also noted, i.e., morchelloid forms (McKnight, **1971**; Watling, **1971**). Sometimes when primordia were developed under low levels of light a blue zone subtended the pileus. This zone is in the exact location of the phototrophic zone described by Plunkett (**1961**). The blue pigment has been related to the presence of hallucinogenic indoles (Singer, **1958**) and its location might provide a clue to the role of these substances.

DISCUSSION

This study provides another addition to the growing list of Basidiomycetes that require light for initiation or development. The low level

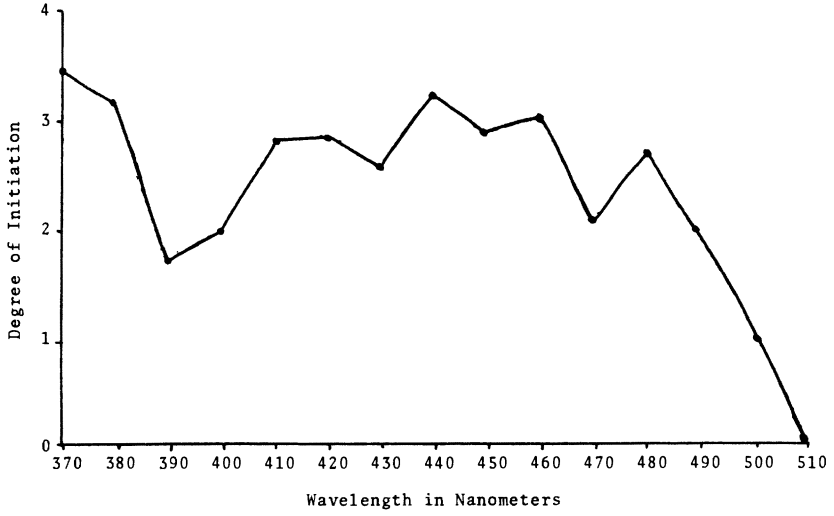

FIG. 2. Spectral sensitivity of photoinduction of basidiocarp initiation in *Psilocybe cubensis*.

of light necessary for initiation of basidiocarps in *Psilocybe cubensis* is in agreement with the requirements of other Basidiomycetes that have been studied and emphasizes the need for prudence in suggesting that certain mushrooms do not require light for fruitbody initiation (Heim and Wasson, **1958**). Spectral-sensitivity studies show that at least two areas of the spectrum stimulate fruitbody initiation, the blue and the UV. This is characteristic for many photoresponses of fungi (Tan, **1978**). Both action spectra determined for fruitbody initiation by Kitamoto et al. (**1972**) and Perkins and Gordon (**1969**) show relatively strong activity at 370 nm. Similarly strong activity at 440 and 480 nm (Kitamoto, **1972**) and 420 and 480 nm (Perkins and Gordon, **1969**) are in agreement with the present study. However, the peaks at 400 and 520 nm and the lack of activity at 460 nm (Kitamoto, **1972**) are very unlike the results obtained in this study.

The observation that a lack of ventilation can inhibit basidiocarp initiation is well documented in the literature (Plunkett, **1956**; Tschierpe, **1974**). Additionally, the effect of maturity upon the ability of cultures to respond to photoinitiation has been previously reported (Lu, **1965**).

Briggs (**1976**) suggested that the blue-light reactions in green plants and fungi are similar. If this is so then many of the known blue-light-mediated effects in green plants need to be reviewed for fungi. Of particular concern to this study is the connection between blue-light reactions and red-light reactions (phytochrome system) demonstrated by Chon and Briggs (**1966**). Since red light did not initiate fruitbodies it was considered appropriate as a "safe" light. This may be an incorrect assumption. Finally, although development after initiation seems to require light, it is not known if the action spectrum required is similar to that needed for initiation of basidiocarps.

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