To the Graduate Council:

I am submitting herewith a thesis written by Edmond Ragland Badham entitled "The Effect of Light on Fruitbody Initiation in Psilocybe cubensis." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Botany.

Ronald H. Petersen, Co-Chairman

Larry Jones, Co-Chairman

I have read this thesis
and recommend its acceptance:

Accepted for the Council:

Vice Chancellor
Graduate Studies and Research

THE EFFECT OF LIGHT ON FRUITBODY INITIATION IN PSILOCYBE CUBENSIS

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Edmond Ragland Badham

March 1978

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ABSTRACT

The effect of light upon fruitbody initiation in Psilocybe cubensis (Earle) Sing. was studied. Initiation only occurred when cultures were illuminated. Photo-initiation could be inhibited by poor aeration of the cultures or the lack of vegetative maturity. Only low levels of light were necessary for initiation (23 ergs/ $sec/cm^2/day$ for five days). Wavelengths of 370, 440, and 460nm were the most effective in the induction of fruitbody initiation. Wavelengths greater than 500nm were ineffective. Certain abnormal forms are described which may be related to the build-up of gaseous products. The possible relationship between photo-initiation in fungi and phototropism in green plants is discussed.

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CHAPTER I

INTRODUCTION AND HISTORICAL REVIEW

It is common knowledge that fungi grow in damp, dark, and warm places. While it is undeniably true that some fungi prefer such habitats, there are many examples where these generalizations do not hold true. In fact, the observation concerning dark habitats is probably more related to water availability than any actual inhibition due to light per se. However, some fungi actually require light; for example, Elias Fries (1821) was aware that the mycelium of certain fungi remained sterile in darkness. In recent years fungal CO_2 fixation, phototropism, and initiation or inhibition of sporulation by fungi have been shown to be directly influenced by light. Effects of light have been studied in all the major groups of fungi, with the phycomycetes and Fungi Imperfecti having been studied most extensively. Both the effect produced by the light and the active wavelengths of light vary with the fungus, but the most commonly reported response is that of blue-light-mediated sporulation.

General reviews of the photobiology of fungi may be found in Carlile (1965, 1970), Leach (1971), and Page (1970). Hollaender (1956), Jagger (1967), Klein and Klein (1970), and Brickforth and Dunn (1972) reviewed general principles of light and photobiology.

In some instances the effect of light may be masked by other physiological conditions. It is wise, therefore, to review the physiology of the experimental organism. Although little information exists concerning the physiology of other light-requiring mushrooms, there is a good deal of literature regarding the common cultivated mushroom, Agaricus bisporus (much of this may be found in Mushroom Science, volumes 1-10). A good review of the physiology, including the effects of light in the Agaricales may be found in Reijnders (1963).

The Experimental Organism

The organism studied was determined to be Psilocybe cubensis (Figure 1). It matches a recent description (Smith, 1958). Synonymy is as follows:

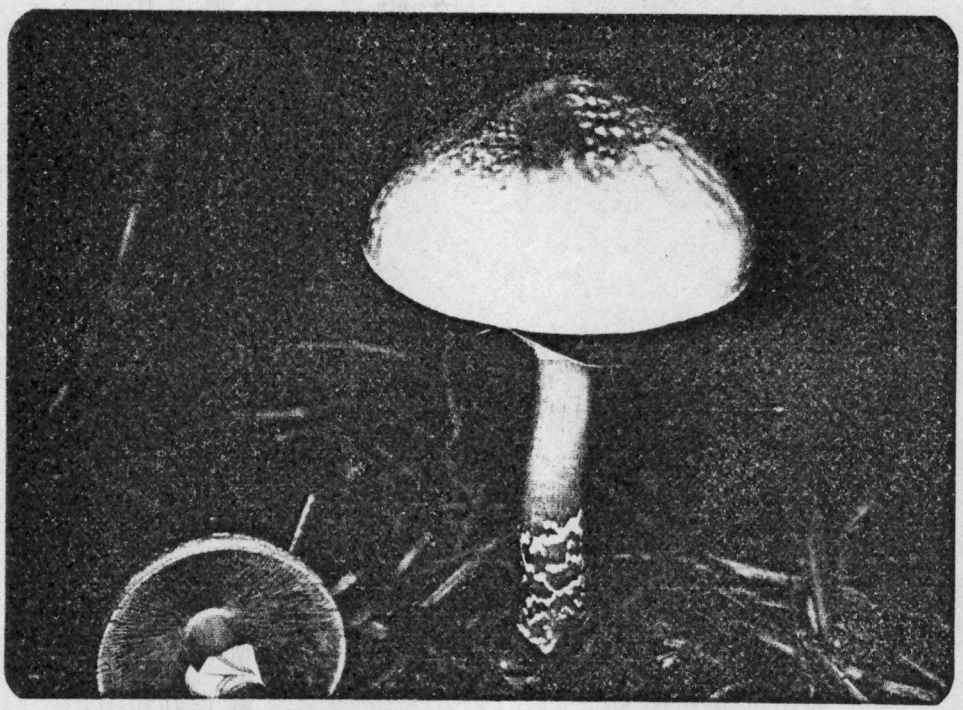
≡ Psilocybe cubensis (Earle) Sing., Sydowia 2: 37. 1948.

≡ Stropharia cubensis Earle, Est. Agron. Cuba 1: 240. 1906.

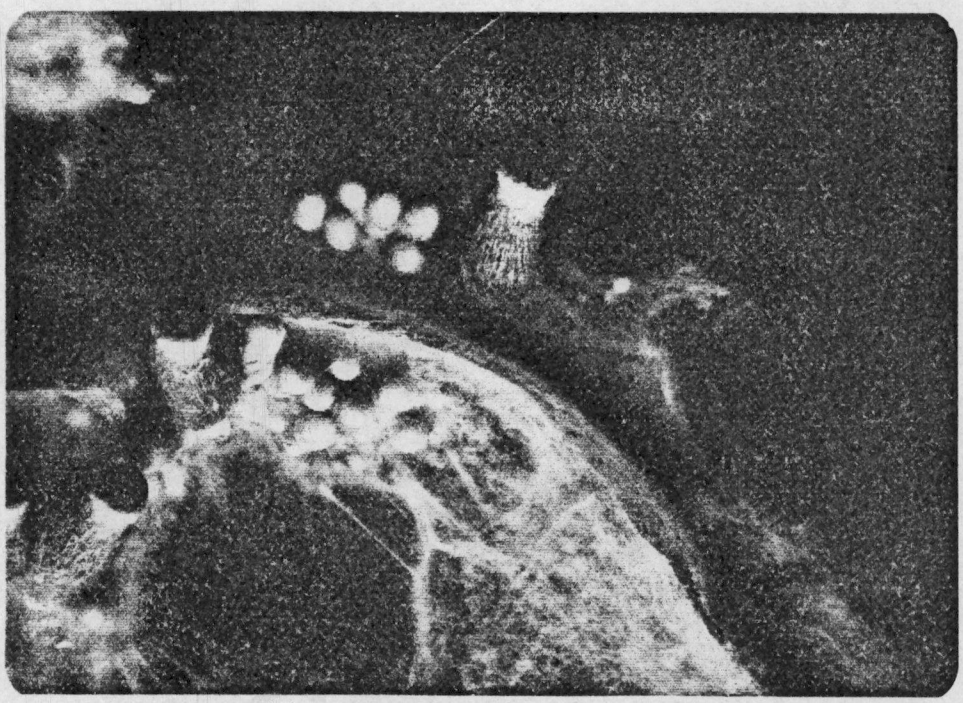
≡ Geophila cubensis (Earle) Heim, Les Champignons Hallucinogenes Du Mexique. Museum National D'Histroire Naturelle. Paris. 1958.

= Nematoloma caerulescens Pat., Bull. Soc. Mycol. Fr. 23: 78. 1907.

= Hypholoma caerulescens (Pat.) Sacc. & Trott. in Syll. Fung. 21: 212. 1912.



A.



B.

Figure 1. Psilocybe cubensis (Earle) Sing.

A. In nature 1/2 X.

B. In culture 5X.

= Stropharia caerulescens (Pat.) Sing. Ann. Mycol. 34: 340. 1936.

= Stropharia cyanescens Murr., Mycologia 33: 279. 1941.

= Panaeolus ovatus (Cooke & Massee) Aberdeen & Jones, Australian Journal of Science, 21: 1210. 1958. (Cited in Pollock, 1975).

P. cubensis fruits primarily on herbaceous dung. It commonly grows in cow and horse dung, but has also been reported from well manured soil and sugar cane mulch. Murrill (1941) described fruitbodies growing in a sawdust and manure pile and Kneebone (cited in Smith, 1958) reported that this species grew well in modified compost used for the cultivated mushroom, Agaricus bisporus. Presumably, the spores of P. cubensis are incorporated into the diet of animals as they graze and eventually germinate in the manure, where subsequently the fungus grows and fruits.

P. cubensis has been reported from Southeast Asia (North Vietnam, Cambodia, and Thailand), South America (Bolivia, Argentina), Central America (Cuba, Puerto Rico, British Honduras, and Trinidad), and North America. Its distribution in the United States includes: Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, Tennessee, and Texas (Thacz, 1977).

Fruitbody Development

Fruitbody development in P. cubensis was described by Heim (1958) and Reijnders (1963). Reijnders describes this species as being "bivelangiocarpic," meaning the fruitbody has two veils--a partial veil which is formed just below the gills between the margin of the cap and the stipe, and a general veil which encloses the entire primordium. These two veils combine to form a conspicuous persistent annulus. "Angiocarpy," the second half of Reijnders' term, indicates that the lamellae are enclosed until maturity. Besides a structural description of the development, Reijnders also provided a sequential description in which he described the species as being "probably hymenocarpic"; that is, the lamellar cavity forms first and only later the stipe or pileus becomes differentiated.

Pictorial representation of the development of P. cubensis was given by Heim (1958). Tracings of his photographs along with translations of his explanations follow in Figure 2. Heim's #1 in Figure 2 closely corresponds to my "stage of development" (discussed later, see Scoring) value of 2.5.

Cultural Physiology

Effects due to growth medium. Although many of the Agaricales and Aphyllophorales can easily be cultured vegetatively, only a much smaller number can be induced to

Figure 2. Description of the development of Psilocybe cubensis (translated from Heim, 1958).

1. Longitudinal section through the primordium of 0.8 mm diam. which shows the deep endogenous nature of the hymenium (hy) already formed in the lamellar cavity (lc). The general veil (gv) is strongly adnate to the cuticle (c) of the pileus and continuous along the stipe; and a tight covering or the pseudo-epicutis (pe) disappears rapidly. Already the zone of constriction (zc) is shown separating the pileus and the stipe.

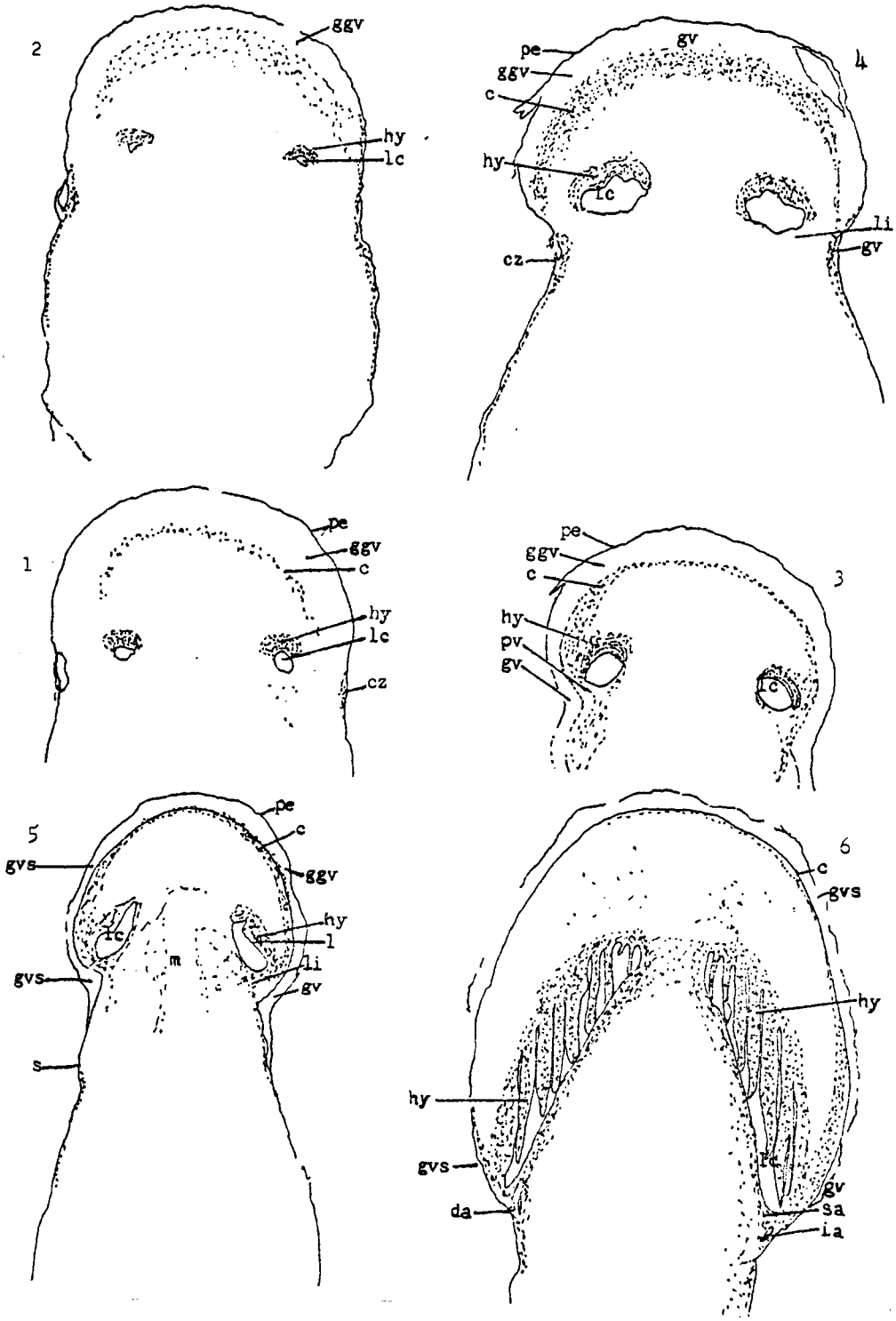
2. A longitudinal section through a primordium of 2.0 mm diam. showing the thickening of the gelatinized general veil (ggv) and its thinning out along the stipe.

3. A longitudinal section through a primordium of 3.0 mm diam. The hymenium and the lamellar cavity are completely formed, but still completely enclosed. The general veil, always thick on the cap and more scarce on the stipe, is still continuous. The lipsanenchyma (li) or partial veil (pv) is differentiated, well separated from the general veil (gv), and the pseudo-epicutis is a scarce covering on the adnate general veil.

4. A longitudinal section through a primordium of 2.0 mm diam. The same remarks as in #3, except the presence of a constriction zone (cz). The origin of bivelangiocarpy of this mushroom is clearly apparent.

5. A longitudinal section through a primordium of 4.0 mm diam. The pileus is completely differentiated but it is still attached to the stipe where elongation has scarcely begun; the double formation of the general veil and the partial veil is very evident. The lamellar chamber is now completely closed. On the cap the general veil is reduced and is now gelatinizing and deteriorating. The future covering of the pileus is evident. The medio-strata of the stipe (m) is shown. The general veil is still noticeable on the cap and high on the stipe as scales (gvs); it becomes obscure along the stipe.

6. A deep longitudinal section through a primordium of 10.0 mm diam. The margin of the cap is still attached to the stipe by the double annulus (da), formed by the combination of the membranes of the superior annulus (sa) and the inferior annulus (ia). The general veil remains only on the cap as scales. The dermatic covering is free. The gills have grown completely within the lamellar cavity which is enclosed and the palisade hymenium is clearly visible. The expansion of the cap next, frees the double annulus and at the same time the elongation of the stipe develops rapidly.


2
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4
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gv
ggv
c
hy
lc
li
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lc
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gvs
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Figure 2

form fertile basidiocarps. Most such taxa are saprophytes, inhabiting wood, leaf mold, and dung.

Cultural characteristics of psilocybin-containing mushrooms are described by Heim (1958). The mycelium of P. cubensis is easily grown on a variety of substrates, including potato dextrose agar, oatmeal agar, malt extract agar, and Czapek's agar media. Additionally, it was grown in liquid shake culture by Catalfomo and Tyler (1974). The mycelial mat is usually white, but sometimes turns blue upon aging, injury, or cool temperatures. Heim (1958) reported that illuminating cultures on a 2-5% malt extract agar medium might cause them to become flesh colored. The rate of mycelial growth is the same in light as darkness.

Excessive carbohydrate concentrations have been shown to inhibit fruitbody initiation in other species (Robbins and Hervey, 1959; Maiello, 1977). Alasodura (1963) reported that greater than 4% malt extract in an agar medium may inhibit fruiting in Sphaerobolus sp., and that phosphate concentrations greater than 0.25% inhibit fructification on a 1% malt extract medium. Schwalb (1971) reported that in Schizophyllum commune fruitbodies developed synchronously in a low CO_2 atmosphere when the glucose concentration was 0.4-2.0%, but that synchrony was lost if the glucose concentration was raised to 4%.

Effects due to atmosphere. In addition to nutrient effects on fruitbody initiation, the atmosphere surrounding the culture plays an equally important role. Lambert (1933) first observed that excessive CO_2 can cause fruitbody abnormalities in Agaricus bisporus. Later Mader (1943) suggested the existence of a volatile unsaturated compound (in addition to CO_2) which inhibited initiation. Lockard (1962) investigated gases produced by metabolism other than CO_2 and detected acetaldehyde, ethylene, ethyl alcohol, and ethyl acetate. Other researchers (cited in Tschierpe, 1974) added methylethyl ketone, isobutanol, n-butanol, isoamyl alcohol, amyl alcohol, acetic acid, and isovalerianic acid to the list of volatile products of A. bisporus.

Tschierpe (1974) reported that a CO_2 concentration greater than 2% inhibited mycelial growth and a 32% CO_2 concentration caused growth to cease. This inhibitory effect has been shown to be due to excess CO_2 and not lack of O_2 (Tschierpe, 1974).

Although gases (in particular CO_2) in high concentrations are inhibitory to the initiation of fruitbodies, Ingold and Nawaz (1967) reported that a complete absence of CO_2 might be as detrimental as its overabundance. They found that Sphaerobolus sp. failed to initiate fruitbodies in air in which the CO_2 had been removed. Furthermore, Rast et al. (cited in Tschierpe, 1974) showed that

A. bisporus was capable of CO_2 fixation and suggested that certain amounts may have promoted growth. They established the optimum range of atmospheric CO_2 to be 100-6700 ppm. Long and Jacobs (1968) confirmed a CO_2 requirement and suggested an optimum range of 340-1000 ppm.

Effects due to vegetative maturity. Before basidiocarps may be initiated a certain maturity factor must be taken into account. Some taxa (i.e., Poria ambugata) are responsive to light after a minimal amount of vegetative growth (Robbins and Hervey, 1960). This fungus can form fruitbodies before the mycelium reaches vegetative maturity (vegetative maturity is considered to be attained when the mycelial mat entirely covers the substrate). Other fungi are unresponsive to light until after vegetative maturity (Lu, 1965). Durand (personal communication, 1977) believes that one of the factors which stimulates this reproductive potential is a lowering in the level of glucose. Aschan-Åberg (1960) suggested that a lowering of the nitrogen and potassium tends to favor fruitbody initiation.

Effects due to light. Generalizations about photobiological effects among basidiomycetes are difficult at best. The growth of some mushrooms are not affected by light, e.g., Agaricus bisporus (Singer, 1961). In others, growth is inhibited by light, e.g., Armillaria mellea (cited in Marsh, 1959). Yet other fungi require light for

some facet of fruitbody initiation or development: Poria ambugata (Robbins and Hervey, 1959); Cyathus stercoreus (Lu, 1965); Spaerobolus stellatus (Alasoadura, 1963); Coprinus lagopus (Madelin, 1956); Panus fragilis (Miller, 1967); Polyporus brumalis (Plunkett, 1956); Collybia velutipes (Plunkett, 1953); Pleurotus ostreatus (Marsh, 1959).

Alasoadura (1963) was the first to attempt to define the active area of the spectrum for basidiocarp initiation in Sphaerobolus sp. using equal quanta of light at different wavelengths. Using "daylight" fluorescent lamps and interference filters at 20nm intervals, he showed that fruitbody initiation was stimulated between 400 and 520nm. Wavelengths below 400nm were not tested. Because he detected two peaks--one at 440nm and one at 480nm--he suggested that β and γ carotene were the receptors. These compounds had already been isolated from this fungus by Friederichsen and Engel (cited in Alasoadura, 1963).

In the most complete study yet published on photoinitiation of a basidiomycete (Schizophyllum commune), Perkins and Gordon (1969) came very close to providing an action spectrum of fruitbody initiation. Using a quartz-iodine lamp and interference filters as well as a spectrograph, they proved the Bunsen-Roscoe law of reciprocity held over a wide range. The dose-effect curve was linear up to a dose of approximately 1.1×10^5 ergs/cm^2 (at 448nm).

Spectral sensitivity peaked in the UV (around 360nm) and blue wavelengths. They also found that temperatures (2°-38° C) at the time of illumination had little effect upon the photobiological event of photoinitiation. Finally, they suggested that the activity illicited by wavelengths shorter than 400nm was more compatible with flavin light absorption than carotenoid light absorption because the latter absorbs weakly in the ultra-violet.

In a very thorough summary article by Briggs (1976), the suggestion is made that the blue light receptor in green plants and fungi is the same. An action spectrum with a peak near 370nm, a shoulder near 420nm, a maximum at 445-460nm, and another peak (or shoulder) between 470-490nm was described for positive phototropism of Avena coleoptiles; phototropism of Phycomyces sporangia; carotenoid synthesis and suppression of circadian rhythm of conidiation in the fungus Neurospora; light induced oxygen uptake in Chlorella; phototropism in the germlings of a liverwort, Sphaerocarpus; and even carotenogenesis in the bacterium Mycobacterium. Briggs discussed possible receptors and suggested that flavins were responsible.

Cailleux (cited in Heim, 1958) reported that the primordia of P. cubensis may be formed in darkness at elevated temperatures (27° C). Jackson and Alexopolus (1976) stated that P. cubensis requires light for basidiocarp initiation (at 22°-25° C).

CHAPTER II

MATERIALS AND METHODS

Source of Cultures

The culture used in this study was isolated in 1975 by allowing a fresh mushroom (identified as Psilocybe cubensis) to deposit spores onto agar. The culture is assumed to be dikaryotic. I have collected the same taxon at three different locations: Columbus, Mississippi; Whiteville, North Carolina; and Lauringburg, South Carolina. The primary characteristics used to distinguish this mushroom were its large purple-brown spores which have a germ pore, its tendency to stain blue if cut or bruised, and its filamentous cuticle. This taxon matched a detailed description in Smith (1958) and Heim (1958). I conducted all experiments with the Whiteville isolate (TENN. No. 40476), and this culture has been deposited at the American Type Culture Collection.

Preparation of Cultures

An agar medium (Table 1) and plastic test tubes (16 x 125 mm) with screw caps were used. Six ml of media was poured into each tube and subsequently slanted at an angle of 25° on a horizontal surface. Inoculation was taken from stock cultures which were grown for one month in large Petri plates (15 x 100 mm) with taped lids.

TABLE 1

BRODIE MEDIUM (MODIFIED AFTER BRODIE, 1975)*

Ingredients	Amount
Bacto agar	20g
Maltose	5g
Dextrose	2g
Peptone	.2g
L-asparagine	.2g
Yeast extract	2g
Magnesium sulphate	.5g
Calcium nitrate	.5g
Potassium phosphate, dibasic	.5g
Ferrous sulphate	.01g
Distilled water	1 liter

*After autoclaving for 20 minutes, the medium has a pH of 6.0-6.5.

One $3mm^2$ plug was taken from these between 1 and 2.5cm from the edge of these stock cultures and placed in each slant tube. The slant tube caps were not tightly closed (see Chapter III). The illumination for this manipulation was provided by a 15 watt incandescent lamp covered by a red filter (curve labeled red in Figure 3).

The experimental organism was grown in the dark for three weeks in 30 x 30 x 50cm cardboard boxes. Two layers of black felt cloth enclosed an open side of the box allowing for proper ventilation. From 40-120 cultures were stored at one time in the boxes. Both cultures and light sources were kept in a cold room ($8m^2$) at 21° C ± 2° and relative humidity of 85% ± 10%. Two fans circulated the air within the room. The cold room was aired to the fresh air of the lab each day for 10 minutes.

Light treatments within each experiment were given at the same time each day. If manipulations were necessary, they were accomplished under the same safe light described above. Each experiment had controls which were exposed to the same environmental conditions but had no light treatments.

Light Sources

The primary light source was a 300 watt General Electric, quartz-iodine projector lamp used in conjunction with a .25m Jarrell-Ash monochromator with 2mm slits. The lamp was placed 2.5cm from the entrance slit and the

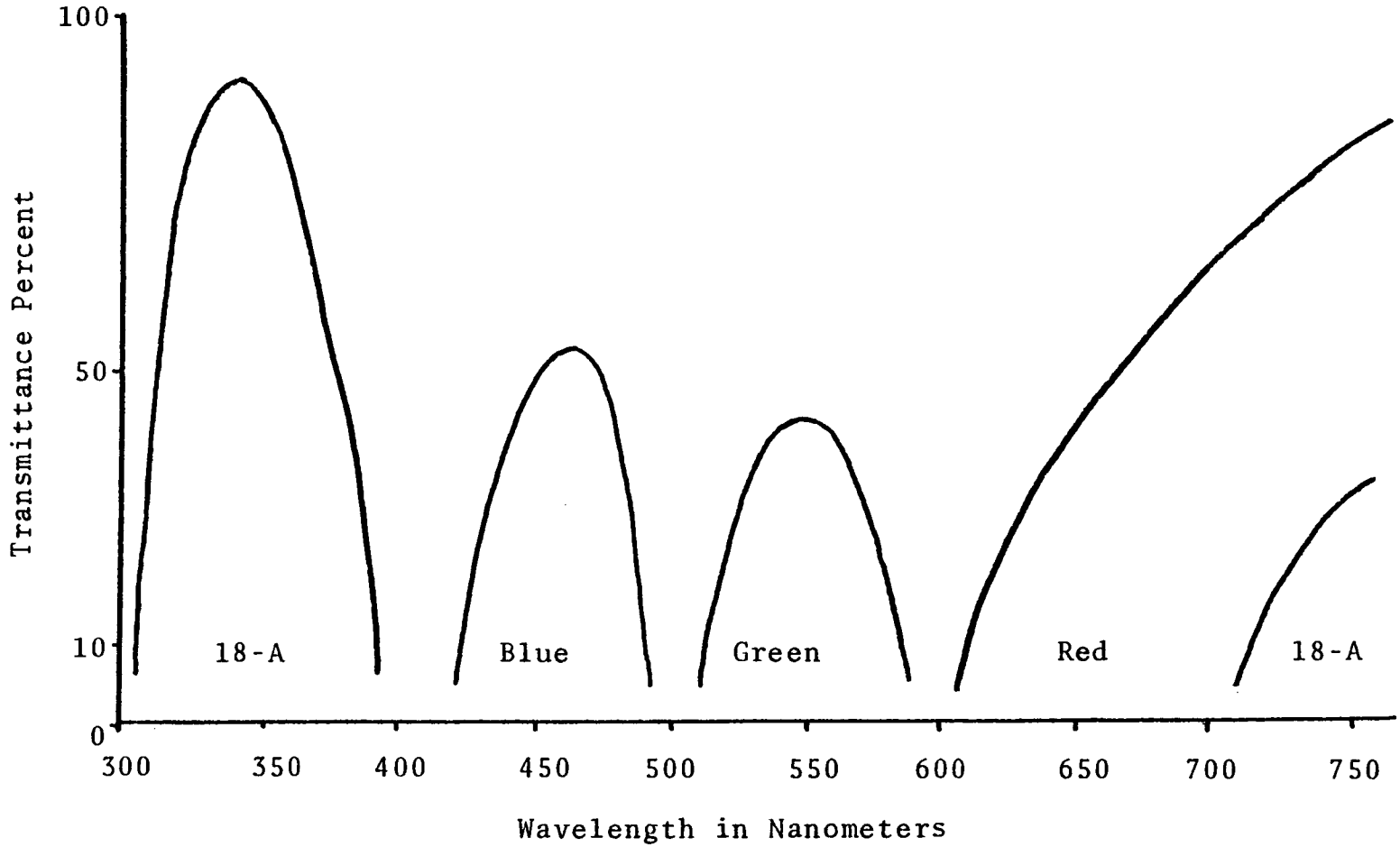


Figure 3. Light transmittance of broad band filters (as measured on Cary 14 spectrophotometer).

cultures were placed directly at the exit slit. For wavelengths less than 450nm the 3000Å blaze was used. For wavelengths of 450nm or greater, the 6000Å blaze was used. Using a spectroradiometer (International Light #783), equal light energies at different wavelengths were attained by adjusting the voltage to the bulb (see Table 2). The half band width of the monochromator was approximately 15nm. The light energy was 23 ergs/sec/cm^2 at all wavelengths. The lamp and monochromator were cooled by a centrifugal fan and were enclosed by two layers of black felt cloth which rendered the system light-tight except the exit slit. In experiments involving the monochromator, the light from the monochromator was used to position cultures and to mark them so that they could be positioned similarly on following days.

Some preliminary experiments utilized a Xenon arc flash (Honeywell photoflash). The flash lasted .5 milli second. The flash was placed 6cm from the cultures.

TABLE 2

VOLTAGES USED TO GIVE IDENTICAL LIGHT ENERGIES AT VARIOUS WAVELENGTHS OF A QUARTZ-IODINE LIGHT SOURCE*

Wavelength (nm)	Volts	Grating (Å)
370	120	3000
380	109	3000
390	104	3000
400	99	3000
410	96	3000
420	93	3000
430	90	3000
440	85	3000
450	79	6000
460	72	6000
470	67	6000
480	62	6000
490	58	6000
500	56	6000
510	56	6000

*Calibrated with an International Light Spectroradiometer #783.

CHAPTER III

RESULTS

Preliminary Experiments

Experiment 1 (effect of atmospheric composition on initiative potential). This experiment was conducted to determine the effect of different degrees of aeration on the ability of cultures to form fruitbody initials. Cultures were grown as stated above except where stated otherwise. Illumination was provided by diffuse lab light ("cool white" fluorescent lamps) of approximately 100 ft-c. The temperature was 24° C ± 6° and a relative humidity of 30% ± 20%. The details of this experiment (Table 3) show that poorly aerated cultures do not initiate as readily as those which are aerated; and cultures in which the CO_2 has been removed or the ethylene oxidized show approximately the same amount of initiation as cultures which are aerated by allowing the caps of the tubes to be loosened.

Experiment 2 (broadband filter experiments, qualitative effect of light). This experiment was conducted in order to determine what general wavelengths of light were necessary for the initiation of fruitbody initials. Cultures were prepared as described above. The cultures were then inserted through the ends of cardboard boxes (11 x 11 x

TABLE 3

EXPERIMENT 1 (EFFECT OF ATMOSPHERIC COMPOSITION ON INITIATIVE POTENTIAL)

Replicate	Number of Cultures	Day Scored	Treatment	Purpose	Success of Initiation
1a	5	11	Tightly closed caps	No aeration	0[a]
	10	11	Cotton replaced caps	Limited aeration	10[a]
	6	11	NaOH, loose caps	Absorb CO_2, aerate	66[a]
	10	11	NaOH, closed caps	Absorb CO_2, no aeration	30[a]
	4	11	Loose caps	Aerate	75[a]
1b	6	9	NaOH, closed caps	Absorb CO_2, no aeration	50[a]
	7	9	NaOH, loose caps	Absorb CO_2, aerate	100[a]
	7	9	Closed caps	No aeration	14[a]
	7	9	Loose caps	Aerate	85[a]
1c	10	22	Loose caps	Aerate	90[a]
	10	22	NaOH + $KmnO_4$	Absorb CO_2, oxidize volatiles	0[a]
1d*	2	9	NaOH, $KMnO_4$	Absorb CO_2, oxidize volatiles	1[b]
	2	9	$KMnO_4$	Oxidize volatiles	2[b]
	2	9	NaOH	Absorb CO_2	3[b]
	2	9	Not modified	Control	4[b]
	2	9	NaOH, $KMnO_4$ after 1 week of growth	Absorb CO_2, oxidize volatiles after vegetative maturity	5[b]

TABLE 3 (continued)

Replicate	Number of Cultures	Day Scored	Treatment	Purpose	Success of Initiation
	2	9	Taped lids	Limit aeration	6[b]
1e**	1	50	Sealed glass flask	No aeration	Did not initiate

[a]Percent of cultures initiating.

[b]Most to least initiation.

*These cultures were grown in small Petri plates (20 x 60 mm) after being placed in sealed glass jars (6cm diam. 12cm height) along with (in some cases) 2.5% NaOH added to filter paper or 5% $KMnO_4$ added to vermiculite.

**Inoculated into 1 liter flask with 150ml of media slanted lengthwise and sealed with a rubber stopper.

8cm) which had one of four broadband filters (18-A, blue, green, red) mounted in the top. The transmission of the 18-A (Eastman Kodak, 1970), blue, green, and red filters (Carolina Biological Supply Company) is given in Figure 3, page 16. The ends of the tubes extended out the sides of the boxes. After the dark period they were subjected to illumination at room temperature (24° C ± 6°) and relative humidity of 30% ± 20%. Table 4 provides the experimental details.

Experiment 3 (quantitative effect of light). This experiment was conducted in order to obtain a rough estimate of the amount of light which was necessary to initiate fruitbodies. Cultures were prepared as described above and the details are given in Table 5. The results of this experiment showed that only 0.5 milli second xenon flash was necessary to induce the formation of fruitbody initials.

Experiment 4 (effect of temperature on fruitbody initiation). This experiment was conducted to determine if high or low temperature at the time of illumination would affect light treatment effectiveness for fruitbody initiation. Cultures were prepared as described above. Illumination was provided by a xenon arc lamp (see light sources) at a distance of 6cm. One flash was given per day. Six cultures were chilled at 4° C for 30 minutes prior to being illuminated after being wrapped in aluminum foil. Six

TABLE 4

EXPERIMENT 2 (BROADBAND FILTER EXPERIMENTS, QUALITATIVE EFFECT OF LIGHT)

Replicate	Number of Cultures	Day Scored	Treatment	Purpose	Formation of Fruitbody Initials
2a*	3	12	18-A filter	Illuminate with UV light	+
	4	12	Blue filter	Illuminate with blue light	+
	4	12	Green filter	Illuminate with green light	-
	4	12	Red filter	Illuminate with red light	-
2b**	3	10	18-A filter	Illuminate with UV light	+
	4	10	Blue filter	Illuminate with blue light	+
	4	10	Green filter	Illuminate with green light	+ (slight)
	4	10	Red filter	Illuminate with red light	-

*The 18-A, blue, and green filter illuminated with "cool white" fluorescent lamp at 8cm. The red filter was illuminated with 100 watt incandescent lamp at a distance of 8cm. Boxes covered with aluminum foil.

**Illuminated with 100ft-c. of "cool white" fluorescent light. Boxes covered with black felt cloth.

TABLE 5

EXPERIMENT 3 (QUANTITATIVE EFFECT OF LIGHT)

Replicate	Number of Cultures	Day Scored	Treatment	% of Cultures Initiating
3a	10	7	1 flash/day for 6 days (xenon arc)	80

cultures were warmed at 38° C for 30 minutes prior to illumination after being wrapped in aluminum foil. Six cultures were not modified. For the details of this experiment, see Table 6. This experiment showed that photoinitiation could not be inhibited by varying the temperature within a certain range.

Cultural Morphology

Two cultural morphologies are easily recognized and are referred to as "cottony" and "stringy." These morphologies are thought to be environmentally influenced, since in Experiment 1 it was observed that tightly capped cultures exhibited a cottony morphology, whereas, loosely capped cultures exhibited a stringy morphology. Cottony morphology is exhibited when hyphae grow vertically off the substrate and are diffuse; their appearance is like a wad of cotton. Stringy morphology (Figure 4) refers to hyphae that are organized into rhizomorphs, i.e., strands of hyphae. These rhizomorphs develop flat on the substrate and are frequently dichotomously branched, and range in size from 0.4 to 1.0 mm in width. Cultural morphology is of extreme importance because cultures with stringy morphology initiate much more readily than cottony morphology cultures.

When true basidiocarp initials become visible to the naked eye, they appear as diffuse knots of white hyphae 200-300 µm in diameter and 30-50 µm in height (Figure 5).

TABLE 6

EXPERIMENT 4 (EFFECT OF TEMPERATURE ON FRUITBODY INITIATION)

Replicate	Number of Cultures	Day Scored	Treatment	Formation of Initials
4a	6	6	Illuminated at 21° C	+
	6	6	Illuminated at 4° C	+
	6	6	Illuminated at 38° C	+

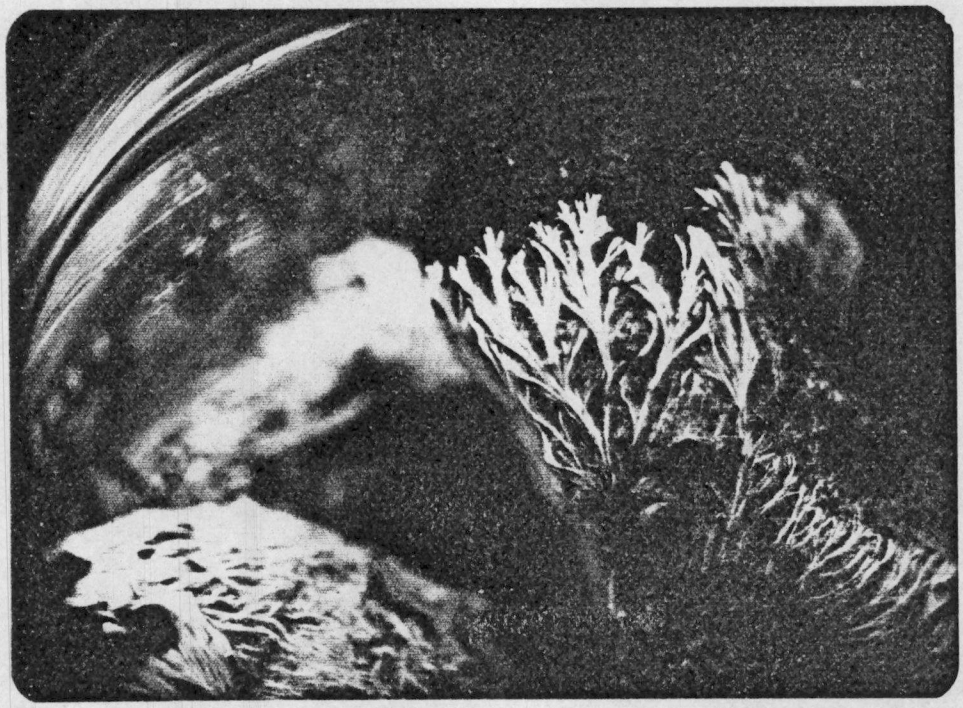
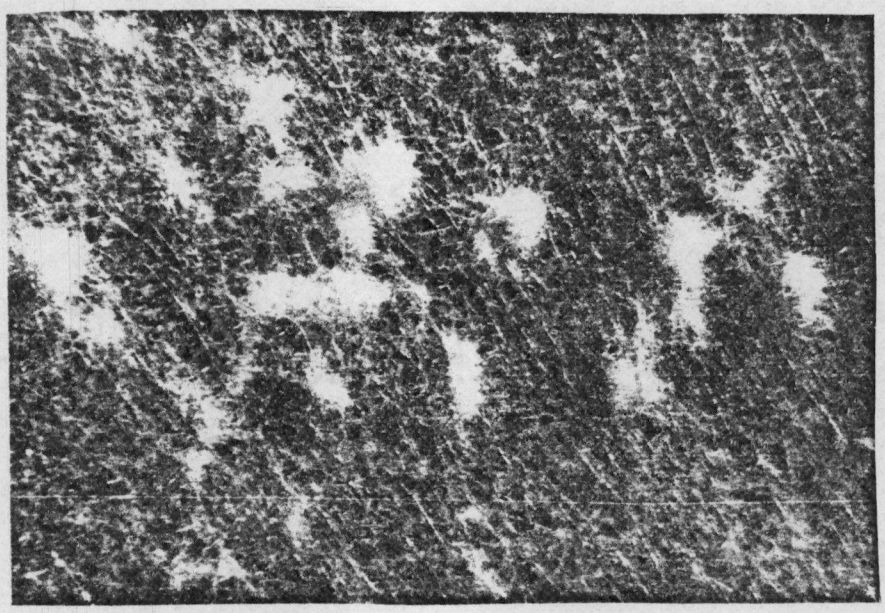
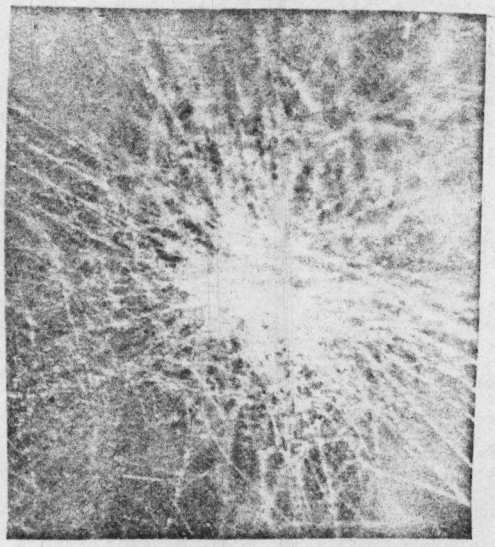


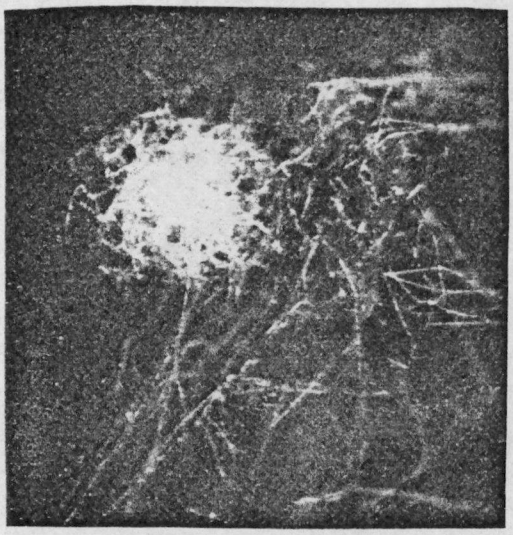
Figure 4. Psilocybe cubensis growing on the surface of dung. The hyphae are organized into rhizomorphs ("stringy"). 1.5 X.



1500 µm

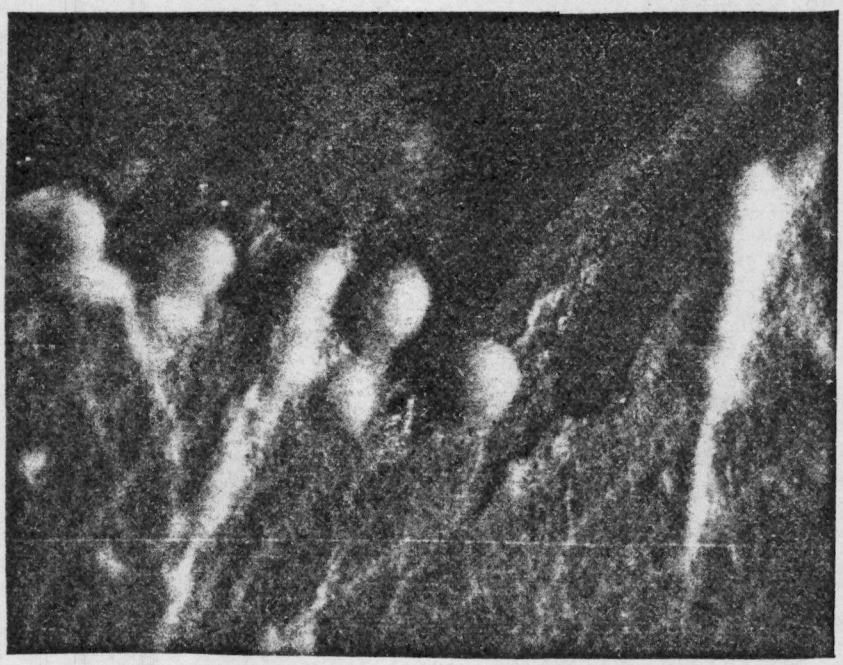


300 µm

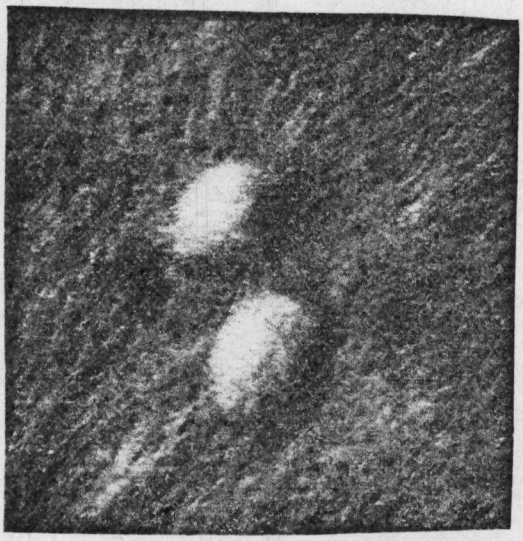


200 µm

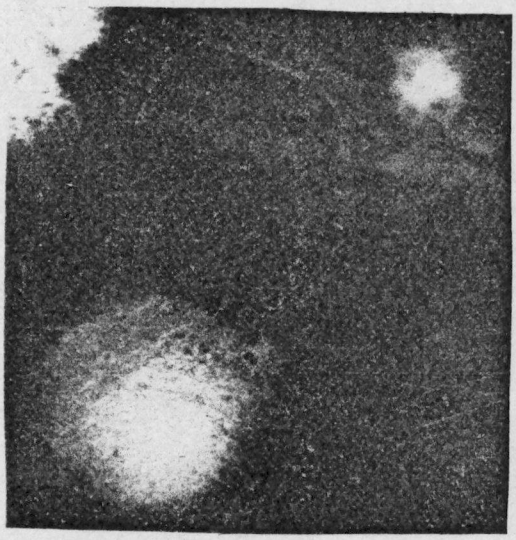
Figure 5. Basidiocarp initials grown in culture.



2500 µm



2500 µm



600 µm

Figure 5 (continued)

The second day they enlarge to 300-1000 μm and become compact and spherical. By the third day they have grown to 1-2 mm in diameter, 2-4 mm in height and have become generally pyramidal in shape. On the fourth day the covering to the cap breaks and the brown pigment of the pileus is visible. Further development involves elongation of the stipe and later expansion of the pileus.

Scoring

The scoring of extent of fruitbody initiation can be accomplished in many ways. The simplest way is to observe the presence or absence of fruitbody initials. (For preliminary experiments 1, 2, 3, and 4, this method was used.) A more quantitative estimate involves viewing success of fruitbody initiation in two ways: (1) the time it takes for fruitbody initials to form and (2) the number of fruitbody initials per total surface area of substrate in the slanted tube. The time factor was measured by developmental stages (see Table 7). The visually estimated average of each culture's developmental stage is a measure of how rapidly the culture was initiated. Intermediate categories were used when appropriate. Measurement of the density factor involved an estimate of the number of fruitbody initials on the total surface (approximately $3cm^2$) of the slanted tube (see Table 7). Intermediate categories were also used here.

TABLE 7

DESCRIPTION OF THE CATEGORIES USED TO EVALUATE THE STAGE OF FRUITBODY DEVELOPMENT AND THE DENSITY OF FRUITBODY INITIALS*

Assigned Numerical Value	Descriptive Observations
	Observed Stage of Fruitbody Development
0	Mycelial cords (pro-initials)
1	Flat aggregations of hyphae
2	Spherical small initials (600 µm diam.)
3	Spherical large initials (1000 µm diam.)
4	Initials pyramidal in shape
5	Brown pigment of the pileus visible
	Observed Density of Initials in Tubes
1	Very light (1-3 initials)
2	Light (4-8 initials)
3	Medium (9-30 initials)
4	Heavy (31-60 initials)
5	Very heavy (61+ initials)

*Degree of initiation = stage of development + density of initials.

As can be seen in Figure 6, scores obtained using either the density factor or the stage factor did not differ markedly. I feel, however, that a more conservative estimate (one which shows less fluctuation) may be obtained by averaging the values from the two methods. A plot of this average falls between the factors plotted separately. The sum of the scores of these two factors was called the "degree of initiation" and was a useful quantitative index to the experimental induction of fruitbody initiation. This method was used to estimate the success of fruitbody initiation in Experiments 5 and 6.

Dose-Effect Experiments

Experiment 5 (the quantitative effect of light).

This experiment was designed to determine the quantity of light which was necessary to initiate fruitbodies. Monochromatic light (460nm) at 8 different dosages was used to initiate fruitbodies. For treatments requiring dosages greater than $.345 \times 10^4$ ergs/cm^2 (23 ergs/sec/cm^2 for 30 sec per day for 5 days), an increase in the time of illumination was used to increase the dose. For treatments of less than $.345 \times 10^4$ ergs/cm^2 neutral density filters (Eastman Kodak, 1970) were used to decrease the dose. For the lowest dose, both a decrease in the time of illumination and neutral filters were used to decrease the dose.

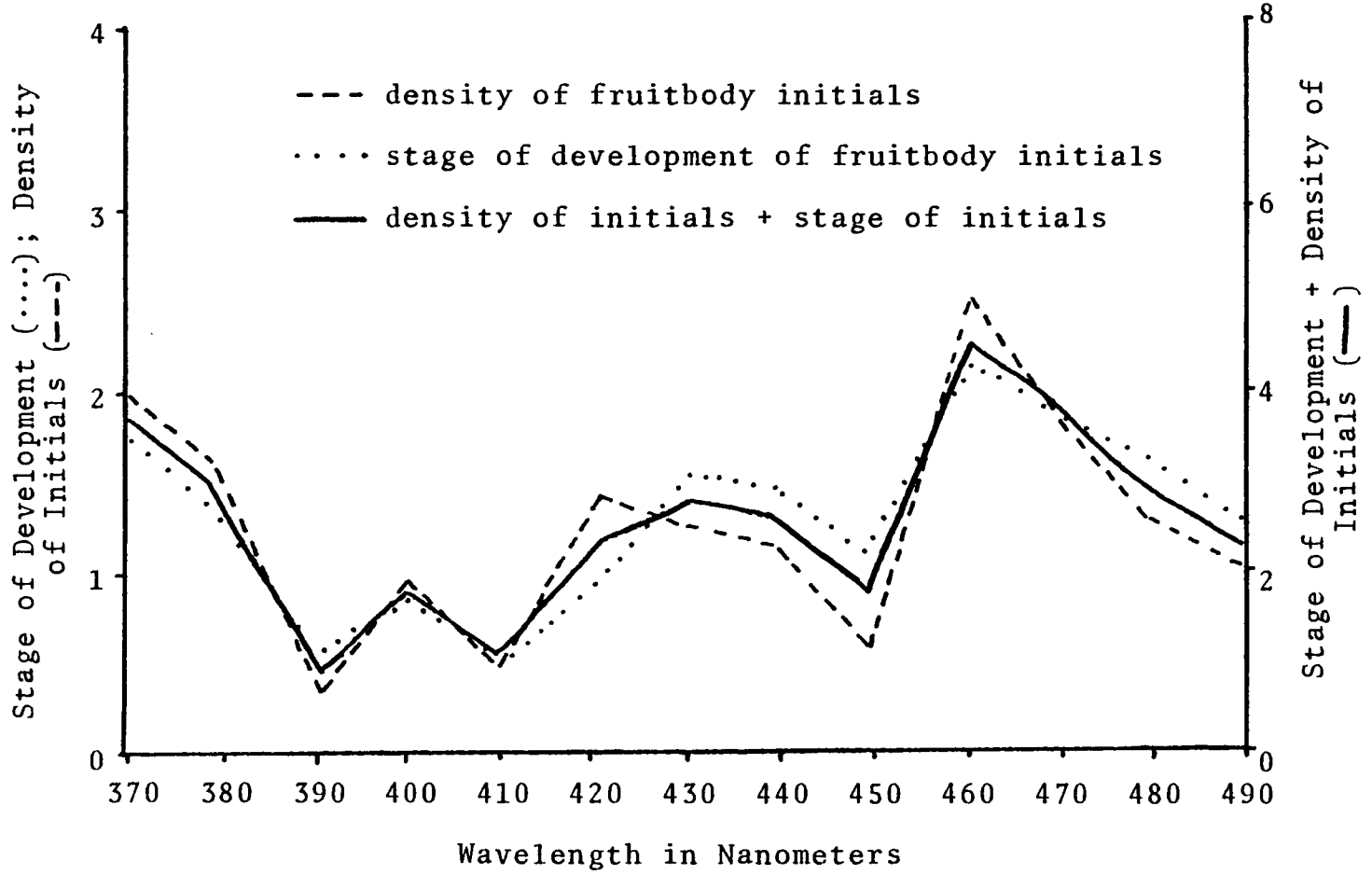


Figure 6. Comparison of three methods of evaluating degree of initiation in Experiment 6e.

As can be seen by Figure 7, the degree of initiation is approximately equal for dosages greater than .345 x 10^4 ergs/cm^2. Also Figure 7 shows that a nearly linear relationship exists between dose and effect between doses of .345 x 10^4 and .086 x 10^4 ergs/cm^2. Table 8 shows that the ratio of ergs cm^{-2} to the average degree of initiation is approximately the same over a range of times and intensities when the total dose remains constant.

Spectral Sensitivity Experiments

Experiment 6 (wavelength effectiveness on fruitbody initiation). This experiment was designed to determine the qualitative effect of light on fruitbody initiation. Cultures were prepared as usual and the monochromat r was used (see Chapter II). Cultures were illuminated each day for 30 seconds at 23 ergs/sec/cm^2 at wavelengths from 370-510nm and scored (see Scoring, page 30) on days 5, 6, 7, and 8. Wavelengths below 370nm were not studied because of insufficient energy of the light source. Since the results from Experiment 2 showed that wavelengths greater than 500nm caused very little fruitbody initiation, no further experiments using wavelengths greater than 510nm were attempted with the monochromator.

Although most of the manipulation of cultures was made in the dark, it was possible to obtain a rough estimate of the degree of initiation visible in the light of the

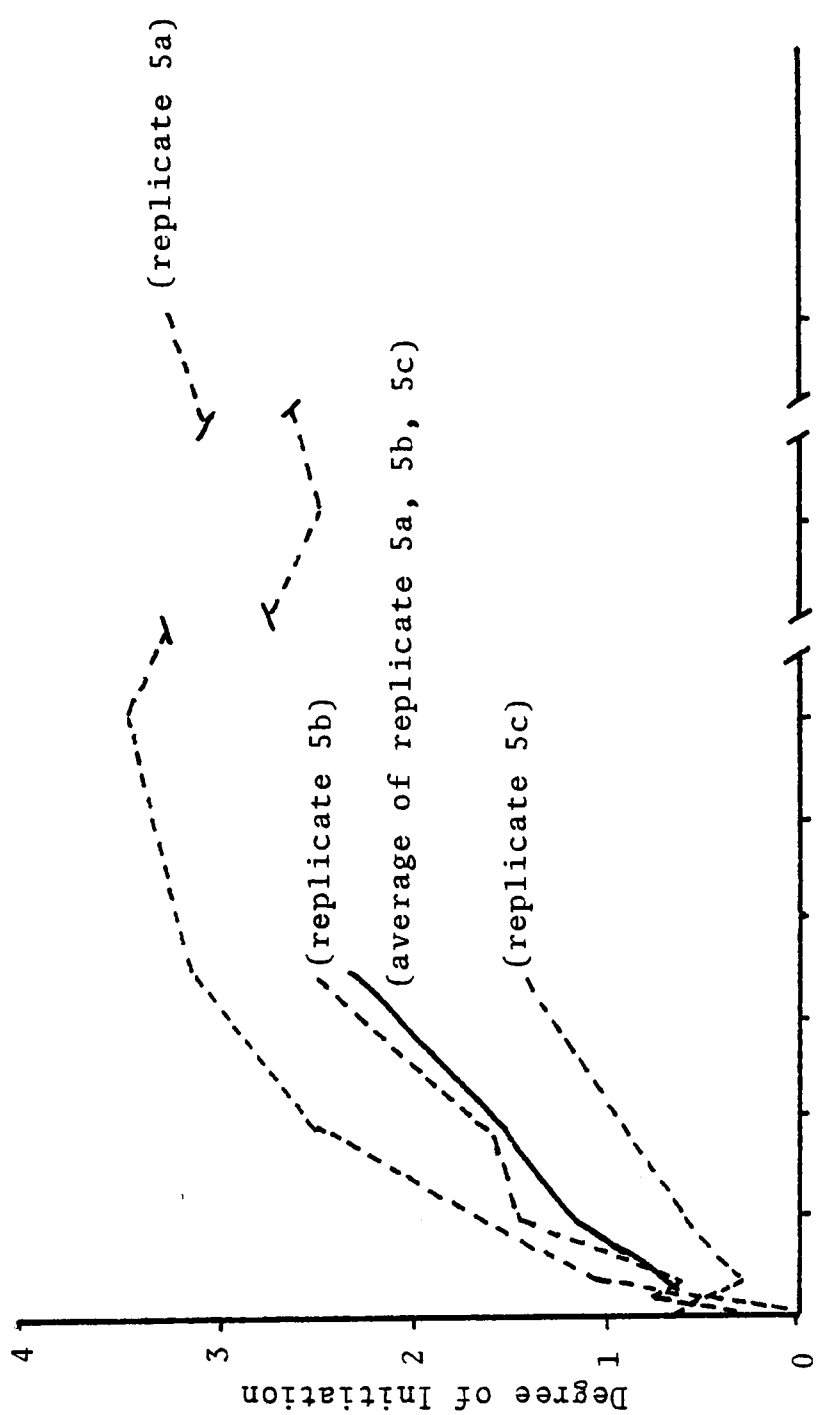


Figure 7. Experiment 5, dose-effect experiment, the quantitative effect of light.

TABLE 8

DOSE-EFFECT EXPERIMENTS: EXPERIMENT 5 (THE QUANTITATIVE EFFECT OF LIGHT AT 460NM)

Replicate	Number of Cultures	Treatment*	Average Degree of Initiation	Ergs cm^{-2}/ Average Degree of Initiation
5a**	12	2.7	3.2	0.840
	12	1.3	2.4	0.542
	12	.6	3.4	0.176
	12	.345	3.2	0.107
	12	.172	2.5	0.069
	12	.086	1.6	0.054
	12	.034	1.2	0.028
5b**	8	.345	2.5	0.138
	8	.172	1.6	0.107
	8	.086	1.5	0.057
	8	.034	.6	0.056
	8	.017	.8	0.021
5c**	10	.345	1.4	0.246
	10	.172	.9	0.191
	10	.086	.6	0.143
	10	.034	.3	0.113

*x 10^4 ergs/cm^2.

**Scored on day 6.

monochromator. Cultures were scored when 50% of the cultures exhibited initiation which typically occurred on the sixth day. Due to variability (see Chapter IV) between replicates, it was necessary to score some replicates on days 7 or 8 in order to obtain a level of initiation that was significantly different for dark grown controls. Although in dark grown controls fruitbodies rarely initiated, it was frequently difficult to distinguish small rhizomorphs from initials; this can explain, in part, the degree of initiation measured in dark grown controls. Because of the variability, likewise, it was best to score one replicate on the fifth day. This was because it was deemed inadvisable to score replicates exhibiting greater than average fruitbody developmental stage of 3 because of the possibility that other photobiological events (and other active wavelengths) might be operative. It is not known if the wavelengths which are necessary for initiation of fruitbodies are the same ones necessary for fruitbody development after initiation.

The results of data scored on day 6 (Exp. 6e-6j) are in the Appendix. The average degree of initiation at each wavelength for 6 day data is given in Figure 8. The data for replicates scored on days 5, 7, and 8 (Exp. 6a-6d) are in the Appendix. The average degree of initiation for all wavelengths scored on all days (Exp. 6a-6j) is given in Figure 8. Data scored on days 5, 7, and 8 were normalized

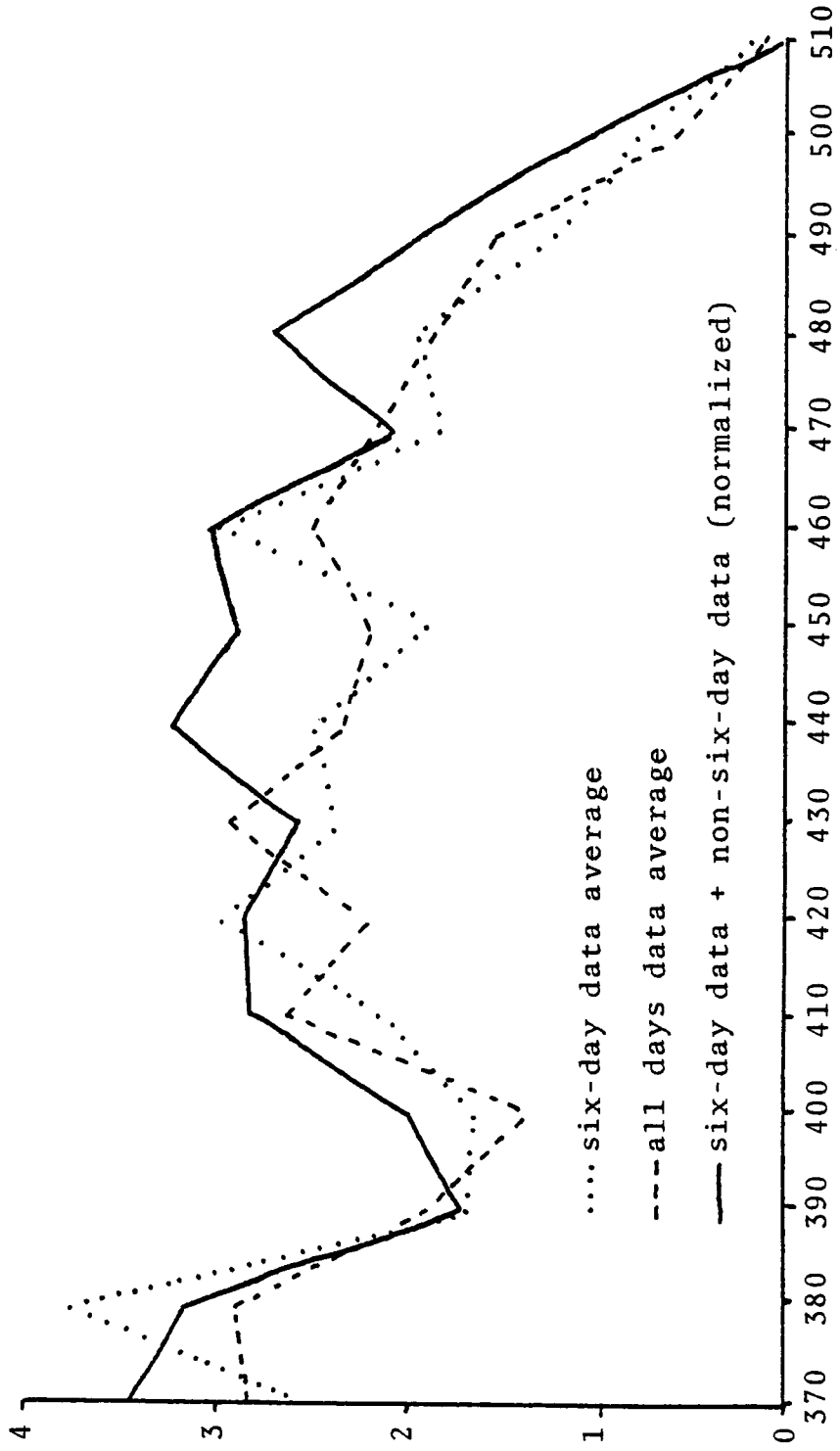


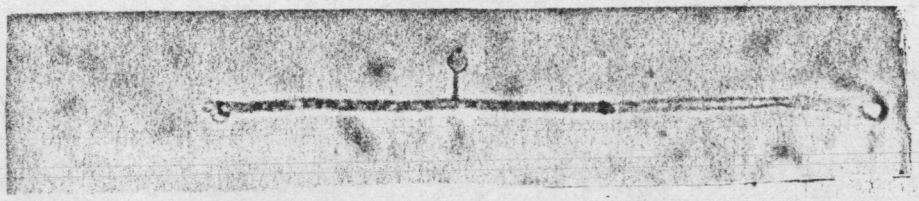
Figure 8. Experiment 6, spectral sensitivity of photo-initiation.

with data scored on day 6. Replicates 6a and 6d were normalized to the average degree of initiation at 460nm of 6 day data. This was accomplished by multiplying the degree of initiation at each wavelength by a factor (the factor was the number that, when multiplied by the degree of initiation at 460nm for a replicate, makes the result equal to the average 6 day data degree of initiation). Since replicates 6b and 6c did not have data for 460nm, they were normalized similarly for 6 day 430nm data.

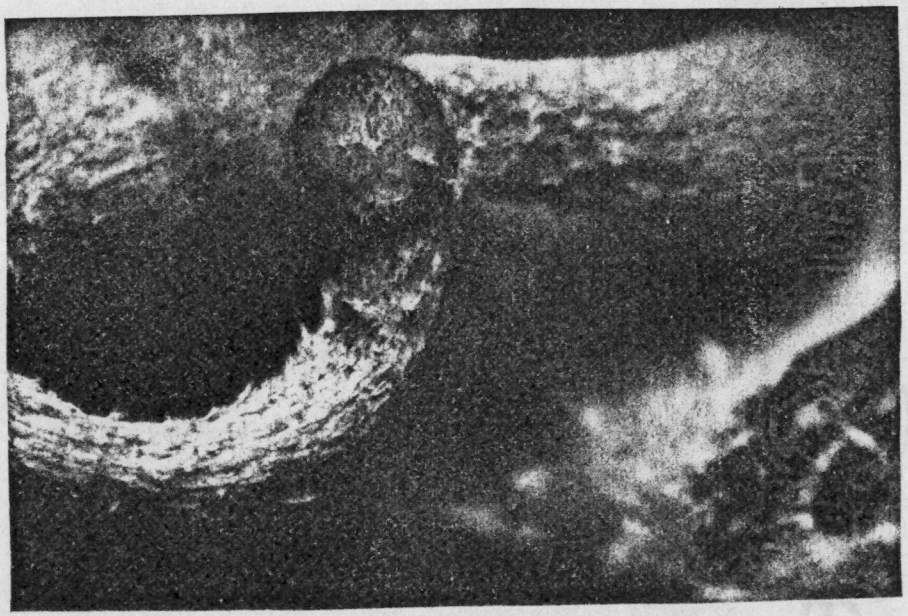
In order to account for different numbers of cultures in the various replicates, the average degree of initiation per treatment per replicate was multiplied by the number of cultures in that replicate. These numbers were used for 6 day data. For data collected on days other than day 6, the normalized degree of initiation was multiplied by the number of cultures in each replicate in each treatment. These numbers were averaged with the average value for 6 day data to obtain an average degree of initiation for each treatment. This is represented by the normalized data in Figure 8.

Unusual Forms

Cailleux (cited in Heim, 1958) described cultural characteristics of P. cubensis and concluded that all Psilocybes which he studied formed "ramifications acremoni-formes" except P. cubensis. I have seen these structures (Figure 9A) in this species in Experiment 1e. They appear



A. _____ 15 µm



B.

C.

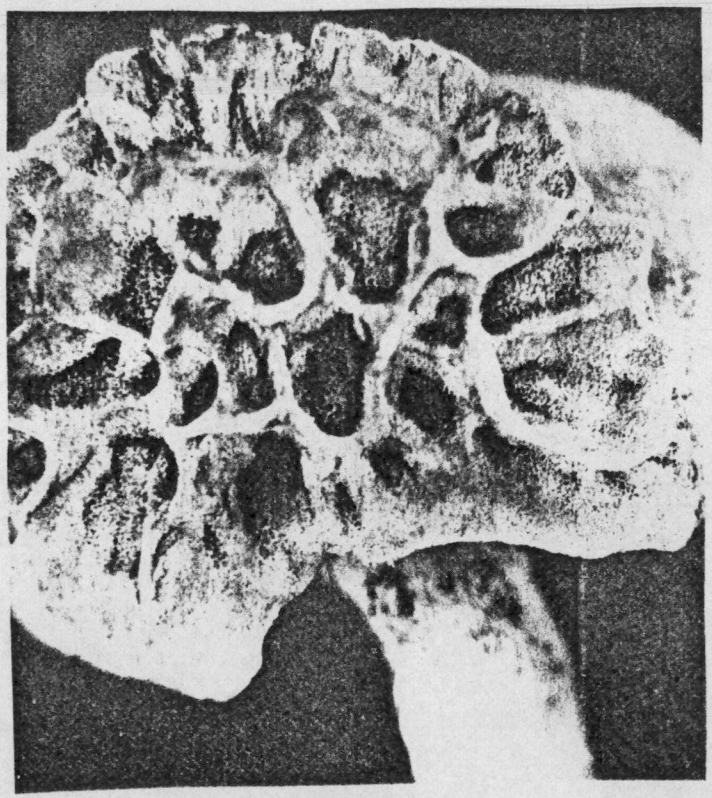


Figure 9. Unusual forms.

A. "Ramifications acremoniformes."

B. Basidiocarp with secondary basidiocarp initiated from the pileus (8X).

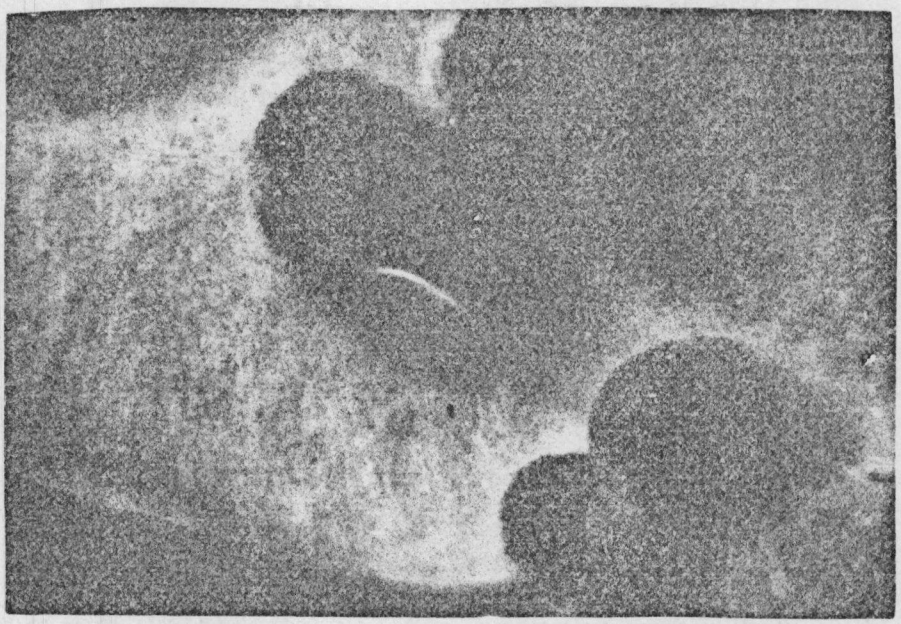
C. Morchelloid form (8X).

to be one celled conidia borne on unbranched conidiophores which develop perpendicularly to unspecialized hyphae.

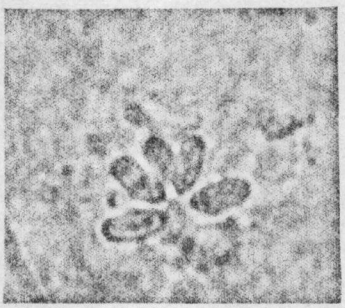
Although no systematic study of fruitbody development at various temperatures was made, certain aberrations were observed which may be associated with high temperature (approximately 28° C). In one instance secondary basidiocarps were initiated from the interior of the pilei and these grew out with the base of the stipe exerted first (Figure 9B). Other effects which were possibly related to high temperature were: excessive lengthening of the stipe, lack of turgor, and the tendency for the veil to remain vertically appressed to the stipe. Sometimes the stipe and pileus exhibited positive geotropism. In one instance a mature mushroom was formed totally inverted.

Certain unknown conditions sometimes cause abnormal basidiocarp formation, i.e. "poriod" of "morcheloid" forms (Figure 9C). Similar forms have been noted for related taxa by Watling (1971), McKnight (1971), and Plunkett (1956).

At times, unusual initials were formed. When grown at 10° C brown initials (Figure 10A) have been seen which never become pyramidial in shape, but rather increase in size as spheres up to 5 mm in diameter. Spores (Figure 10B) have been noted in these structures.



A. ——— 2.5 mm



B. ——— 5 µm

Figure 10. Gastromycetous initials grown at 10° C in closed slant tubes and illuminated with diffuse sunlight.

A. Initials.

B. Spores.

CHAPTER IV

DISCUSSION

Light is necessary for fruitbody initiation of Psilocybe cubensis. The degree of initiation in dark grown controls in Experiment 6 was very slight. This value was not subtracted from other values obtained under light treatments, and was approximately equal to the value of the degree of initiation of cultures with a 510nm light treatment. In some instances this value for dark grown controls was due to my inability to distinguish small rhizomorphs from initials. In other instances it may have been due to light leakage into the storage boxes. Also, although no empirical evidence was obtained, it was observed that cultures inoculated from initiated cultures could show some initiation as a residual effect transferred by the inoculant plug. Additionally it was observed that this initiative stimulus was not translocated to newly formed hyphae.

Evidence was obtained in preliminary experiments that showed a lack of aeration inhibited the ability of cultures to form fruitbody initials. Since NaOH (a CO_2 absorber) and $KMnO_4$ (an oxidizer) partially reversed the inhibition of the formation of fruitbody initials, it is believed that gaseous products of metabolism (possibly CO_2

and ethylene) are involved in the inhibition. Any attempt to grow cultures for light studies should include proper aeration. Further preliminary experiments concerning the qualitative effect of light on fruitbody initiation showed that photo-initiation was wavelength dependent. Near UV and blue wavelengths were the most active. Little or no fruitbody initiation occurred at wavelengths greater than 500nm. Additionally, it was found that exceedingly small amounts of light were necessary to initiate fruitbodies, i.e., 0.5 millisecond of a xenon arc lamp flash per day for 5 days. Also within a certain range (4°-38° C) temperature did not inhibit photo-initiation.

Dose-effect experiments conducted at 460nm showed that a maximum effect was obtained when cultures were illuminated with greater than $.345 \times 10^4$ ergs/cm^2 (extended over a 5 day period) dosages of light; these cultures exhibited approximately equal degree of initiation. A nearly linear relationship between dose and effect was found to exist (evidence to suggest that the Bunsen-Roscoe law of reciprocity holds) between the dosages of $.086 \times 10^4$ ergs/cm^2 and $.345 \times 10^4$ ergs/cm^2 at 460nm. Results from the spectral sensitivity studies are valid only if the dose-effect relationships are similar at other wavelengths.

Spectral sensitivity experiments show that at least two peaks exist for fruitbody initiation--one in the UV and one in the blue. Good evidence was obtained to show that

lower (than 440 and 460nm) activity exists at 450nm. Data from all experiments supported these contentions. Further qualification of the action spectrum was met with some difficulty (which can be seen from the graph of the standard deviation range in Figure 11) which was caused by the variance in the ability of groups of cultures (replicates) to form fruitbodies. This may have been caused by the variable CO_2 content of the room or the red light which was used for the inoculations (see below). Short wavelengths (370nm) were extremely effective in initiating fruitbodies. Blue wavelengths of 440nm and 460nm are also extremely effective in the initiation of fruitbodies. Wavelengths near 480nm may represent a shoulder on the graph of the action spectrum of fruitbody initiation.

One of the consequences of any spectral sensitivity study is a comparison of the action spectrum to the absorption of pigments. Such a comparison can provide tentative evidence of the likelihood that such pigments are the photobiological receptors. A long-standing controversy exists as to the identity of the receptor compound in blue light reactions. Some researchers favor carotenoids and other researchers favor flavins (or flavoproteins). The "in vitro" absorption of these two compounds is similar, but the carotenoids (and not the flavins) show the fine detail in the blue region, characteristic of many action spectra, while the flavins (and not the carotenoids) show the

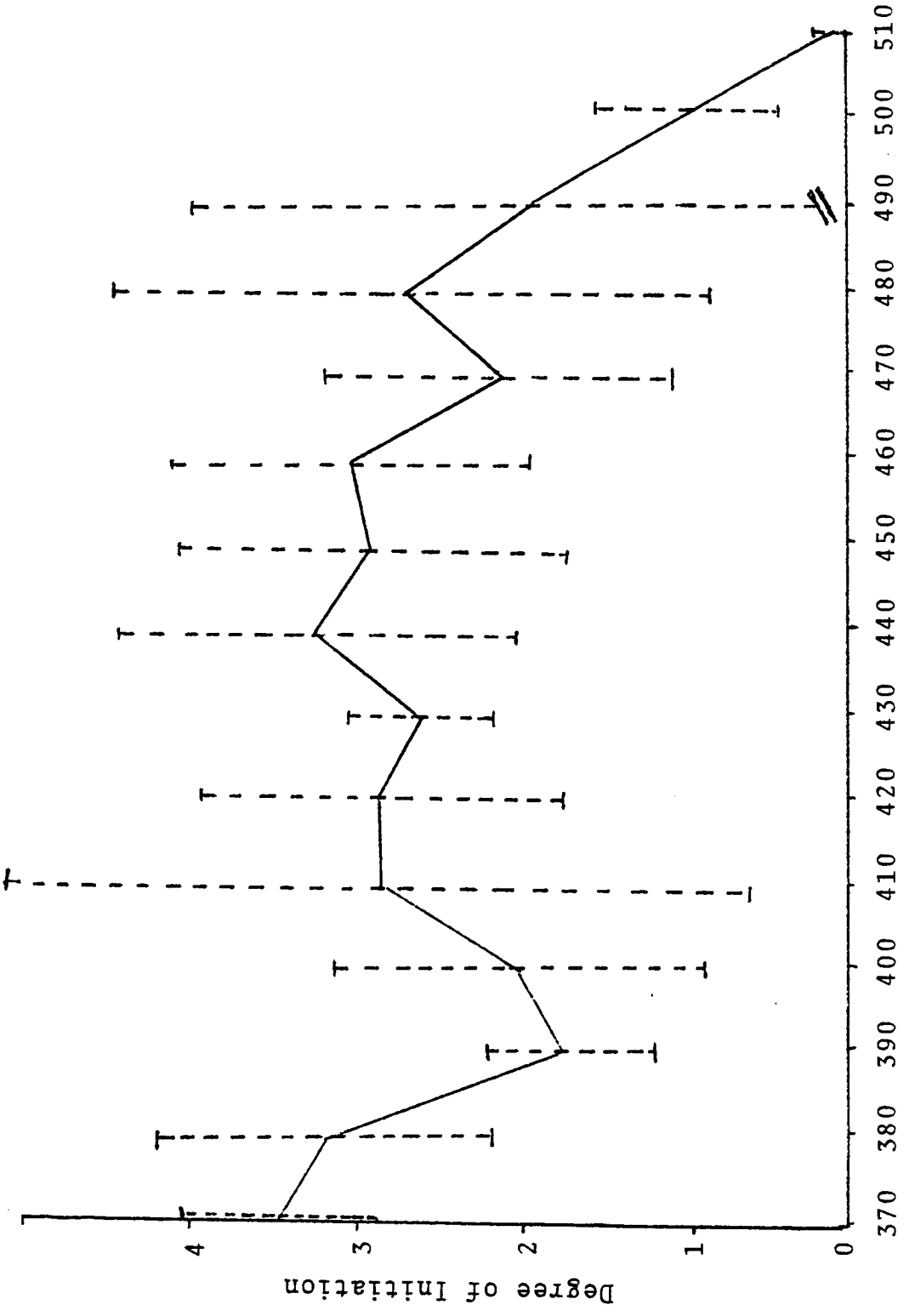


Figure 11. Spectral sensitivity of photo-initiation showing standard deviation range of all data in Experiment 6.

characteristic absorption in the UV. However, UV absorption can be explained by the "cis-carotenoids" (cited in Carlile, 1970), and the fine detail in the blue region can be demonstrated in some flavoproteins (cited in Carlile, 1965), or by the manipulation of solvents (Carlile, 1965). It is unlikely, therefore, that action spectrum determinations will provide unequivocal evidence for either of the possible candidates as the receptor.

If the assumption is made that the blue light receptor in higher green plants and fungi is the same (see Briggs, 1976), some interesting observations can be made between the present study and the work that has been done with phototropism of grass coleoptiles. The first observation concerns the dose-effect relationship which has been shown to be exceedingly complex (Curry, 1969). For example, at relatively low light energies, there is a positive curvature of coleoptiles, then a decrease in response to increased levels of light. Furthermore, this is followed by a negative response (a curvature away from light) when light energies are increased still further. Next, a second positive response occurs with increasing light intensities. This second positive response (unlike the first positive and the first negative) does not obey the reciprocity law. Whereas the amount of light energy used in my study would probably fall in the range of energy effective for the first positive response, this would not likely be the case

in nature. Furthermore, based on preliminary experiments, fruitbody development after initiation reminds one of the second positive response in which a time factor becomes important in the dose-effect relationship, but not necessarily an increase in total light dosage. It is not known whether the wavelengths of light necessary for fruitbody development after initiation are the same as the wavelengths necessary for initiation.

A second observation concerns the use of red light as a "safe" light. Because red light was inactive in causing initiation, it was considered safe to use for the manipulation of cultures. However, Curry (1969) and Chon and Briggs (1966) have shown that pretreatment with red light decreases sensitivity to the first positive response and increases sensitivity to the second positive response. Cohn and Briggs (1966) have suggested that this effect may be reversed by far red light, thus suggesting a connection to phytochrome which has not yet been demonstrated in fungi. This remains to be seen.

In the present study it was found that light was necessary for fruitbody initiation. The effect of light could be inhibited by certain physiological conditions such as atmospheric CO_2 content or vegetative maturity. Although the amount of light necessary for fruitbody initiation was small, only blue and UV wavelengths were active. The crude action spectrum for fruitbody initiation which was

determined agrees well with other blue light reactions in fungi and, interestingly enough, green plants as well.

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APPENDIX

APPENDIX

DATA OF EXPERIMENTS 5 AND 6

TABLE 9

DATA FOR EXPERIMENT 5A*

Treatment (Ergs/cm^2)	Stage of Development (Degree of Initiation) / Density of Initials	Average
2.7 x 10^4	2/.5 (2.5) 2/3 1.5/2 (3.5) 3.5/3 (6.5) 2/2 (4) 0/0	
	2/1 (3) 2/1 (3) 0/0 2/.5 (2.5) 2/2 (4) 2/2.5 (4.5)	3.2
1.3 x 10^4	0/0 (0) 1.5/3 (4.5) 3/.5 (3.5) .5/.5 (1) 0/0 (0) 2/2.5 (4.5)	
	1.5/2.5 (4) 2/1.5 (3.5) 1.5/1 (2.5) 1/.5 (1.5) .5/.5 (1) 1.5/2 (3.5)	2.4
.6 x 10^4	2/1.5 (3.5) 1.5/2.5 (4) 1.5/2 (3.5) 2/.5 (2.5) 2.5/2.5 (5) 2.5/2.5 (5)	
	1.5/2 (3.5) 2.5/.5 (3) 0/0 (0) 1.5/1.5 (3) 2/3 (5) 1/2.5 (3.5)	3.4
.34 x 10^4	2/3 (5) 2/2.5 (4.5) 2/2.5 (4.5) 2/2 (4) 1.5/.5 (2) 1/.5 (1.5	
	1.5/2.5 (4) 0/0 (0) 1.5/2 (3.5) 2/2 (4) 2/.5 (2.5) 2/.5 (2.5)	3.2
.17 x 10^4	.5/.5 (1) 2/1 (3) 3/2.5 (5.5) 1/.5 (1.5) 1/3 (4) .5/.5 (1) 0/0 (0)	
	1.5/1 (2.5) 2/3 (5) 2/2.5 (4.5) 0/0 (0) 1.5/1 (2.5)	2.5
.08 x 10^4	2/1 (3) .5/.5 (1) 2/.5 (2.5) 0/0 (0) 2/.5 (2.5) 1.5/2 (3.5)	

TABLE 9 (continued)

Treatment (Ergs/cm^2)	Stage of Development (Degree of Initiation) Density of Initials	Average
	1.5(3) 1.5(2.5) 0(0) 0(0) 0(0) .5(1) / 1.5 1 0 0 0 .5	1.6
.03 x 10^4	0(0) .5(1) 0(0) 0(0) 1.5(3.5) 2 (2.5) / 0 .5 0 0 2 .5	
	.5(2.5) 0(0) / 2 0	1.2
control	0(0) 0(0) 0(0) 0(0) 0(0) 0(0) / 0 0 0 0 0 0	0.0

*Scored on day six.

TABLE 10

DATA FOR EXPERIMENT 5B*

Treatment (Ergs/cm^2)	Stage of Development (Degree of Initiation) Density of Initials	Average
.34 x 10^4	$\frac{2(3)}{1}$ $\frac{0(0)}{0}$ $\frac{1(4)}{3}$ $\frac{2(4)}{2}$ $\frac{1.5(3.5)}{2}$ $\frac{0(0)}{0}$	
	$\frac{1(3)}{2}$ $\frac{1.5(2.5)}{1}$	2.5
.17 x 10^4	$\frac{0(0)}{0}$ $\frac{1.5(2.5)}{1}$ $\frac{0(0)}{0}$ $\frac{2(3)}{1}$ $\frac{1(2)}{1}$ $\frac{2(4)}{2}$	
	$\frac{0(0)}{0}$ $\frac{1(1.5)}{.5}$	1.6
.08 x 10^4	$\frac{1(2)}{1}$ $\frac{1(3)}{2}$ $\frac{1(2)}{1}$ $\frac{0(0)}{0}$ $\frac{0(0)}{0}$ $\frac{0(0)}{0}$ $\frac{.5(1)}{.5}$	
	$\frac{2(4)}{2}$	1.5
.03 x 10^4	$\frac{0(0)}{0}$ $\frac{0(0)}{0}$ $\frac{0(0)}{0}$ $\frac{0(0)}{0}$ $\frac{0(0)}{0}$ $\frac{0(0)}{0}$ $\frac{1(2)}{1}$	
	$\frac{2(3)}{1}$	.6
.01 x 10^4	$\frac{1(2)}{1}$ $\frac{.5(1)}{.5}$ $\frac{0(0)}{0}$ $\frac{1(2)}{1}$ $\frac{0(0)}{0}$ $\frac{0(0)}{0}$	
	$\frac{1(2)}{1}$ $\frac{0(0)}{0}$	.8
control	$\frac{0(0)}{0}$ $\frac{0(0)}{0}$ $\frac{0(0)}{0}$ $\frac{0(0)}{0}$ $\frac{.5(1)}{.5}$ $\frac{.5(1)}{.5}$	.3

*Scored on day six.

TABLE 11

DATA FOR EXPERIMENT 5C

Treatment (Ergs/cm^2)	Stage of Development (Degree of Initiation) Density of Initials	Average
.34 x 10^4	2/1.5 (3.5) 1/1 (2) 0/0 (0) 2/2 (4) .5/.5 (1) 0/0 (0)	
	0/0 (0) 2/1 (3) .5/.5 (1) 0/0 (0)	1.4
.17 x 10^4	0/0 (0) 2/1 (3) 2/2 (4) 0/0 (0) 0/0 (0) 0/0 (0) 1/1 (2)	
	0/0 (0) 0/0 (0) 0/0 (0)	.9
.08 x 10^4	0/0 (0) .5/.5 (1) 0/0 (0) 0/0 (0) .5/.5 (1) 2/2 (4)	
	0/0 (0) 0/0 (0) 0/0 (0) 0/0 (0)	.6
.03 x 10^4	1/.5 (1.5) 0/0 (0) 1/1 (2) 0/0 (0) 0/0 (0) 0/0 (0)	
	0/0 (0) 0/0 (0) 0/0 (0) _/_ (_)	.3
control	0/0 (0) 0/0 (0) 0/0 (0) 2/1 (3)	.7

TABLE 12

AVERAGE DATA FOR EXPERIMENT 6

Treatment	Six Day Data Exp. 6e-6j	All Day Data Exp. 6a-6j	Six Day Data + All Day Data (Normalized)	Stan. Dev. Six Day Data + All Day Data (Normalized)
370nm	2.5	2.8	3.5	.6
380nm	3.7	2.9	3.2	1.0
390nm	1.5	1.9	1.7	.5
400nm	1.6	1.5	2.0	1.2
410nm	2.1	2.7	2.8	2.3
420nm	3.0	2.2	2.8	1.1
430nm	2.4	2.9	2.6	.5
440nm	2.5	2.4	3.3	1.3
450nm	1.9	2.2	2.9	1.3
460nm	3.0	2.5	3.0	1.1
470nm	1.8	2.2	2.1	1.1
480nm	1.9	1.9	2.7	1.9
490nm	1.2	1.5	1.9	2.0
500nm	.7	.6	1.1	.6
510nm	.2	.2	.2	.1
control	.07	.19		

TABLE 13

DATA FOR EXPERIMENT 6A*

Treatment (Ergs/cm^2)	Stage of Development (Degree of Initiation) Density of Initials	Average
370nm	2/.5 (2.5) 0/0 (0) 0/0 (0) 2/I (3) 2/2 (4) 1/I (2) 0/0 (0)	
	1/2 (3) 1/I (2) 1/.5 (1.5) 0/0 (0) _/_ (_)	1.6
400nm	0/0 (0) 2/.5 (2.5) .5/.5 (1) 1.5/.5 (2) 0/0 (0) 1/.5 (1.5)	
	2/.5 (2.5) 0/0 (0) 0/0 (0) 0/0 (0) 0/0 (0) 0/0 (0)	.8
420nm	0/0 (0) 0/0 (0) 0/0 (0) 0/0 (0) 2/I (3) 2/I (3) 0/0 (0)	
	2/2 (4) 0/0 (0) .5/.5 (1) .5/.5 (1)	1.0
440nm	2/I (3) 0/0 (0) 0/0 (0) 2/I (3) 2/2 (4) 0/0 (0) 2/2 (4)	
	2/2 (4) 2/2 (4) 0/0 (0) 0/0 (0) 1/.5 (1.5)	1.9
450nm	1/I (2) 1.5/2 (3.5) .5/.5 (1) 0/0 (0) 1/.5 (1.5) 2/1.5 (3.5)	
	0/0 (0) 0/0 (0) 0/0 (0) 2.5/1.5 (4) 0/0 (0) 2/1.5 (3.5)	1.6
460nm	1.5/1.5 (3) 0/0 (0) .5/.5 (1) 1/I (2) 0/0 (0) .5/.5 (1) 1/.5 (1.5)	
	2/I (3) 1.5/1.5 (3) .5/.5 (1) 0/0 (0) 0/0 (0)	1.3
480nm	2/2 (4) 1.5/1.5 (3) 1.5/I (2.5) .5/.5 (1) 0/0 (0) 2/.5 (2.5)	
	0/0 (0) 0/0 (0) 0/0 (0) 1/I (2) 0/0 (0) 0/0 (0)	1.4

TABLE 13 (continued)

Treatment (Ergs/cm^2)	Stage of Development (Degree of Initiation) Density of Initials	Average
500nm	0(0) 0(0) 0(0) 1(2) 0(0) 0(0) 2(3) 0 0 0 I 0 0 I	
	0(0) 0(0) 0(0) 0(0) 0(0) 0 0 0 0 0	.5
control	0(0) 0(0) 0(0) 2(3) 0 0 0 I	

*Scored on day seven.

TABLE 14

DATA FOR EXPERIMENT 6B*

Treatment (Ergs/cm^2)	Stage of Development (Degree of Initiation) / Density of Initials	Average
370nm	$\frac{2}{1.5}$(3.5) $\frac{3}{3}$(6) $\frac{3}{3}$(6) $\frac{2}{2.5}$(4.5) $\frac{2}{4}$(6) $\frac{2}{3.5}$(5.5)	5.2
390nm	$\frac{2}{3}$(5) $\frac{2}{2}$(4) $\frac{2}{3}$(5) $\frac{0}{0}$(0) $\frac{1}{1.5}$(2.5) $\frac{2}{2.5}$(4.5)	3.5
410nm	$\frac{2}{3.5}$(5.5) $\frac{2}{2}$(4) $\frac{3}{1.5}$(4.5) $\frac{2}{3.5}$(5.5) $\frac{1}{.5}$(1.5) $\frac{2}{1.5}$(3.5)	4.0
430nm	$\frac{2}{2}$(4) $\frac{2}{4}$(6) $\frac{2}{4}$(6) $\frac{2}{2}$(4) $\frac{0}{0}$(0) $\frac{3}{4}$(7)	4.5
450nm	$\frac{3}{2.5}$(5.5) $\frac{2}{2}$(4) $\frac{2}{2.5}$(4.5) $\frac{2}{3.5}$(5.5) $\frac{2}{3}$(5) $\frac{.5}{.5}$(1)	4.2
470nm	$\frac{3}{4.5}$(7.5) $\frac{2}{3}$(5) $\frac{2}{3}$(5) $\frac{2}{3.5}$(5.5) $\frac{2}{2.5}$(4.5) $\frac{2}{3}$(5)	5.4
controls	$\frac{0}{0}$(0) $\frac{0}{0}$(0) $\frac{0}{0}$(0) $\frac{0}{0}$(0)	

*Scored on day seven.

TABLE 15

DATA FOR EXPERIMENT 6C*

Treatment (Ergs/cm^2)	Stage of Development (Degree of Initiation) Density of Initials	Average
410nm	.5/.5(1) 2/1(3) 2/1(3) 1/.5(1.5) 1/.5(1.5) 2/1(3)	2.2
420nm	0/0(0) 0/0(0) 2/2(4) 1/2(3) 1.5/1(2.5) 1.5/1.5(3)	2.0
430nm	2/2(4) 2/2(4) 0/0(0) 3/2(5) 2/3(5) 2/2(4)	3.7
control	0/0(0) 0/0(0) 0/0(0) 2/1(3)	

*Scored on day eight.

TABLE 16

DATA FOR EXPERIMENT 6D*

Treatment (Ergs/cm^2)	Stage of Development (Degree of Initiation) / Density of Initials	Average
370nm	2(4) 1(2) 2(3) 0(0) 0(0) 2 (3.5)	2.0
	2 1 1 0 0 1.5	
380nm	2(3) 0(0) 0(0) 2 (3.5) 0(0) 0(0)	1.0
	1 0 0 1.5 0 0	
390nm	0(0) 0(0) 0(0) 0(0) 2(3) 1(2)	.8
	0 0 0 0 1 1	
400nm	.5(1.5) 2(5) 0(0) 2(3) 2(3) 0(0)	2.0
	1 3 0 1 1 0	
410nm	.5(1.5) 1 (2.5) 2(4) 2 (4.5) 2(4) 2(4)	3.4
	1 1.5 2 2.5 2 2	
420nm	1(2) 1 (2.5) 0(0) 1(2) 2(4) 2(4)	2.4
	1 1.5 0 1 2 2	
430nm	0(0) 1(2) 1(2) 2 (3.5) 0(0) 1 (2.5)	1.6
	0 1 1 1.5 0 1.5	
440nm	.5(1.5) 2(4) 1(3) 2(4) 2(4) 0(0)	2.7
	1 2 2 2 2 0	
450nm	3 (5.5) 2 (4.5) 1(2) 1(2) 0(0) .5 (1.5)	2.6
	2.5 1.5 1 1 0 1.5	
460nm	1(2) 2(4) 1(3) 0(0) 0(0) 0(0)	1.5
	1 2 2 0 0 0	
470nm	1(3) 0(0) 2(3) 0(0) 1.5(5.5) 1(2)	2.0
	2 0 1 0 4 1	
480nm	1(3) 1(2) 2(5) 1(4) 1 (3.5) 2(4)	3.6
	2 1 3 3 2.5 2	
490nm	2 (3.5) 0(0) 1.5(3.5) 2(3) 2(3)	2.6
	1.5 0 2 1 1	

TABLE 16 (continued)

Treatment (Ergs/cm^2)	Stage of Development (Degree of Initiation) Density of Initials							Average
500nm	2(3) / I	0(0) / 0	2 (2.5) / .5	0(0) / 0	0(0) / 0	0(0) / 0		.9
control	0(0) / 0	0(0) / 0	0(0) / 0	0(0) / 0	0(0) / 0	0(0) / 0	.5(1) / .5	

*Scored on day five.

TABLE 17

DATA FOR EXPERIMENT 6E*

Treatment (Ergs/cm^2)	Stage of Development (Degree of Initiation) Density of Initials	Average
370nm	2.5(5) 2.5 2(5) 3 0(0) 0 2.5(5) 2.5 2.5(4.5) 2 1.5(3.5) 2	3.8
380nm	2(2.5) .5 1.5(3.5) 2 1.5(3.5) 2 0(0) 0 2(5) 3 1.5(4.5) 3	3.1
390nm	0(0) 0 1(1.5) .5 0(0) 0 0(0) 0 0(0) 0 2(3.5) 1.5	.83
400nm	1(3) 2 1(1.5) .5 0(0) 0 1.5(4) 2.5 1(2) 1 1(1.5) .5	2.0
410nm	0(0) 0 0(0) 0 0(0) 0 1(1.5) .5 1.5(2.5) 1 1.5(3.5) 2	1.2
420nm	1.5(4.5) 3 1.5(4) 2.5 0(0) 0 0(0) 0 1.5(3.5) 2 1.5(2.5) 1	2.4
430nm	2(4) 2 2(4.5) 2.5 2(3) 1 1(2) 1 1(1.5) .5 1(2) 1	2.8
440nm	2.5(5.5) 3 .5(1) .5 2(4.5) 2.5 1(2) 1 3(3.5) .5 0(0) 0	2.7
450nm	0(0) 0 2(3) 1 1.5(2) .5 1.5(3.5) 2 2(2.5) .5 0(0) 0	1.8
460nm	2(4.5) 2.5 1.5(4) 2.5 3(5.5) 2.5 2(5) 3 2(4.5) 2.5 (_) _	4.7
470nm	.5(1) .5 1.5(3.5) 2 1(2) 1 1(1.5) .5 0(0) 0 1(2) 1	1.6
480nm	2(3.5) 1.5 2(3) 1 2(4) 2 2(4) 2 1.5(3) 1.5 0(0) 0	2.9
490nm	1.5(2.5) 1 1(1.5) .5 1.5(3.5) 2 1.5(3.5) 2 2(2.5) .5 0(0) 0	2.2
control	0(0) 0 0(0) 0 0(0) 0 0(0) 0	

*Scored on day six.

TABLE 18

DATA FOR EXPERIMENT 6F*

Treatment (Ergs/cm^2)	Stage of Development (Degree of Initiation) Density of Initials	Average
380nm	2/4(6) 2/1(3) 2/2(4) 2/1.5(3.5) 3/3(6) 2/1.5(3.5)	4.3
400nm	2/2(4) 0/0(0) 2/2(4) 0/0(0) 0/0(0) 2/1(3)	1.8
420nm	2/1(3) 3/2(5) 2/2.5(4.5) 1/.5(1.5) 2/2(4) 0/0(0)	3.0
440nm	2/3(5) 2/2(4) 2/3(5) 2/2(4) 1/.5(1.5) 1/.5(1.5)	3.5
460nm	3/3(6) 2/2(4) 2/2(4) 2/2(4) 2/3(5) 2/3(5)	4.6
480nm	2/.5(2.5) 0/0(0) 2/.5(2.5) 1/1.5(2.5) 2/2(4) 2/2.5(4.5)	3.0
control	0/0(0) 0/0(0) 0/0(0) 0/0(0)	

*Scored on day six.

TABLE 19

DATA FOR EXPERIMENT 6G*

Treatment ($Ergs/cm^2$)	Stage of Development (Degree of Initiation) Density of Initials	Average
370nm	0(0) 2.5(4.5) 2(3) 3(5) 0(0) 0(0) 0 2 1 2 0 0	2.0
400nm	2.5(4.5) 0(0) 0(0) 2(3) 0(0) 0(0) 2 0 0 1 0 0	1.2
420nm	3(6) 0(0) 2.5(4.5) 3(4) 2 (2.5) 2(4) 3 0 2 1 .5 2	3.5
430nm	0(0) 0(0) 0(0) 2 (3.5) 2(4) 2(4) 0 0 0 1.5 2 2	1.9
440nm	0(0) 2 (4.5) 0(0) 2(5) 1(3) 1(3) 0 2.5 0 3 2 2	2.6
450nm	0(0) 0(0) 3(5) 1(3) 2(4) 2(4) 0 0 2 2 2 2	2.6
460nm	2.5(5.5) .5(1) 1(2) 2(4) 1.5(3.5) 3(6) 3 .5 1 2 2 3	3.6
470nm	1.5(4.5) 0(0) 2(4) 0(0) 3(5) 0(0) 3 0 2 0 2 0	2.2
480nm	0(0) 0(0) 0(0) 0(0) 0(0) 2(4) 0 0 0 0 0 2	.6
control	0(0) 0(0) 0(0) 0(0) 0 0 0 0	

*Scored on day six.

TABLE 20

DATA FOR EXPERIMENT 6H*

Treatment (Ergs/cm^2)	Stage of Development (Degree of Initiation) Density of Initials	Average
460nm	1(2)/1 2(3)/1 2(5)/3 2(4)/2 2/2.5 (4.5) 2/1.5 (3.5)	3.7
470nm	0(0)/0 2(4)/2 0(0)/0 .5(1)/.5 .5(1)/.5 0(0)/0	1.0
480nm	3(6)/3 2(4)/2 1(2)/1 .5(1)/.5 0(0)/0 0(0)/0	2.1
490nm	2(4)/2 0(0)/0 .5(1)/.5 0(0)/0 0(0)/0 .5(1)/.5	1.0
500nm	1(2)/1 1/.5 (1.5) 0(0)/0 .5(1)/.5 0(0)/0 0(0)/0	.7
510nm	0(0)/0 0(0)/0 0(0)/0 .5(1)/.5 0(0)/0 .5(1)/.5	.3
control	0(0)/0 0(0)/0 0(0)/0 0(0)/0	

*Scored on day six.

TABLE 21

DATA FOR EXPERIMENT 6I*

Treatment (Ergs/cm^2)	Stage of Development (Degree of Initiation) Density of Initials	Average
440nm	$\frac{2}{1}(3)$ $\frac{0}{0}(0)$ $\frac{0}{0}(0)$ $\frac{0}{0}(0)$ $\frac{2}{1}(3)$ $\frac{0}{0}(0)$ $\frac{2}{1.5}(3.5)$	
	$\frac{2}{1.5}(3.5)$ $\frac{1.5}{1}(2.5)$ $\frac{.5}{.5}(1)$	1.6
450nm	$\frac{0}{0}(0)$ $\frac{0}{0}(0)$ $\frac{2}{2}(4)$ $\frac{2}{2}(4)$ $\frac{1.5}{1}(2.5)$ $\frac{2}{2}(4)$ $\frac{0}{0}(0)$	
	$\frac{0}{0}(0)$ $\frac{0}{0}(0)$ $\frac{0}{0}(0)$	1.4
460nm	$\frac{0}{0}(0)$ $\frac{1}{1}(2)$ $\frac{.5}{.5}(1)$ $\frac{2}{2}(4)$ $\frac{1}{.5}(1.5)$ $\frac{0}{0}(0)$ $\frac{0}{0}(0)$	
	$\frac{1}{2}(3)$ $\frac{1}{2}(3)$ $\frac{1}{1}(2)$	1.6
470nm	$\frac{2}{1}(3)$ $\frac{1}{.5}(1.5)$ $\frac{0}{0}(0)$ $\frac{1}{1}(2)$ $\frac{1}{1}(2)$ $\frac{0}{0}(0)$ $\frac{1}{1}(2)$	
	$\frac{0}{0}(0)$ $\frac{0}{0}(0)$ $\frac{1}{1}(2)$	1.2
480nm	$\frac{1}{1}(2)$ $\frac{2}{1.5}(3.5)$ $\frac{0}{0}(0)$ $\frac{0}{0}(0)$ $\frac{0}{0}(0)$ $\frac{0}{0}(0)$ $\frac{2}{2}(4)$	
	$\frac{0}{0}(0)$ $\frac{0}{0}(0)$ $\frac{0}{0}(0)$	.9
490nm	$\frac{0}{0}(0)$ $\frac{0}{0}(0)$ $\frac{1}{1}(2)$ $\frac{0}{0}(0)$ $\frac{.5}{.5}(1)$ $\frac{1}{1}(2)$ $\frac{0}{0}(0)$	
	$\frac{0}{0}(0)$ $\frac{0}{0}(0)$ $\frac{1}{1}(2)$	.7
510nm	$\frac{0}{0}(0)$ $\frac{0}{0}(0)$ $\frac{0}{0}(0)$ $\frac{.5}{.5}(1)$ $\frac{0}{0}(0)$ $\frac{0}{0}(0)$ $\frac{0}{0}(0)$	

TABLE 21 (continued)

Treatment (Ergs/cm^2)	Stage of Development (Degree of Initiation) Density of Initials						Average
	$\frac{0(0)}{0}$	$\frac{0(0)}{0}$	$\frac{0(0)}{0}$				.1
controls	$\frac{0(0)}{0}$	$\frac{0(0)}{0}$	$\frac{0(0)}{0}$	$\frac{0(0)}{0}$	$\frac{.5(1)}{.5}$	$\frac{.5(1)}{.5}$	

*Scored on day six.

TABLE 22

DATA FOR EXPERIMENT 6J*

Treatment (Ergs/cm^2)	Stage of Development (Degree of Initiation) Density of Initials	Average
390nm	$\frac{3}{1}(4)$ $\frac{0}{0}(0)$ $\frac{.5}{.5}(1)$ $\frac{.5}{.5}(1)$ $\frac{2}{1}(3)$ $\frac{2}{2}(4)$ $\frac{2.5}{2}(4.5)$	
	$\frac{0}{0}(0)$ $\frac{0}{0}(0)$ $\frac{1.5}{2}(3.5)$ $\frac{.5}{.5}(1)$ $\frac{2}{2}(4)$	2.1
400nm	$\frac{0}{0}(0)$ $\frac{2.5}{2}(4.5)$ $\frac{2}{1}(3)$ $\frac{2}{1}(3)$ $\frac{0}{0}(0)$ $\frac{0}{0}(0)$	
	$\frac{3}{2.5}(5.5)$ $\frac{0}{0}(0)$ $\frac{0}{0}(0)$ $\frac{0}{0}(0)$ $\frac{2}{1}(3)$ $\frac{0}{0}(0)$	1.6
410nm	$\frac{0}{0}(0)$ $\frac{2.5}{1.5}(4)$ $\frac{2.5}{1.5}(4)$ $\frac{0}{0}(0)$ $\frac{2}{.5}(2.5)$ $\frac{.5}{.5}(1)$ $\frac{1.5}{1}(2.5)$	
	$\frac{2}{1}(3)$ $\frac{3}{3.5}(6.5)$ $\frac{0}{0}(0)$ $\frac{2}{2.5}(4.5)$ $\frac{2}{.5}(2.5)$	2.5
460nm	$\frac{0}{0}(0)$ $\frac{0}{0}(0)$ $\frac{0}{0}(0)$ $\frac{2}{2.5}(4.5)$ $\frac{3}{3.5}(6.5)$ $\frac{3}{4}(7)$ $\frac{\_}{\_}(\_)$	
	$\frac{0}{0}(0)$ $\frac{0}{0}(0)$ $\frac{0}{0}(0)$	1.8
470nm	$\frac{3}{3}(6)$ $\frac{0}{0}(0)$ $\frac{0}{0}(0)$ $\frac{2}{2}(4)$ $\frac{4}{3}(7)$ $\frac{3}{3}(6)$ $\frac{3}{4}(7)$	
	$\frac{0}{0}(0)$ $\frac{0}{0}(0)$	3.0
480nm	$\frac{0}{0}(0)$ $\frac{0}{0}(0)$ $\frac{2}{1}(3)$ $\frac{3}{3}(6)$ $\frac{0}{0}(0)$ $\frac{0}{0}(0)$ $\frac{1}{.5}(1.5)$	
	$\frac{2.5}{2.5}(5)$ $\frac{3}{3}(6)$ $\frac{3}{3}(6)$	2.7
controls	$\frac{0}{0}(0)$ $\frac{0}{0}(0)$ $\frac{0}{0}(0)$ $\frac{0}{0}(0)$ $\frac{0}{0}(0)$ $\frac{0}{0}(0)$ $\frac{0}{0}(0)$ $\frac{0}{0}(0)$	
	$\frac{0}{0}(0)$ $\frac{.5}{.5}(1)$	

*Scored on day six.

VITA

Edmond Ragland Badham was born October 17, 1952 in Raleigh, North Carolina. He received his high school education at Virginia Episcopal School in Lynchburg, Virginia. After graduation he attended the University of North Carolina at Chapel Hill. After two years he transferred to North Carolina State University at Raleigh where, in 1974, he received his B.S. degree in Agriculture. After graduation he accepted a job with the Agricultural Extension Service as a county agent in Whiteville, North Carolina. After a year at this job he returned to Graduate School at The University of Tennessee, Knoxville. In March 1978 he received the M.S. degree in Botany.

The author plans to spend approximately one year abroad prior to returning to Graduate School to work toward his Doctoral Degree in Botany with emphasis in Mycology.