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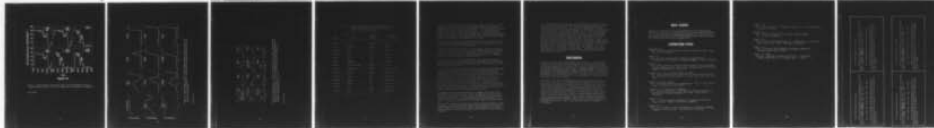
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EFFECT OF LIGHT QUALITY ON DEVELOPMENT OF FRUITING BODIES OF PANUS FRAGILIS.

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10 Orson K. Miller, Jr.
John G. Palmer

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SUMMARY

→ Under a system that permits mass screening of mycelia within bands of the visible spectrum, fruit bodies initiated and developed in two light bands (387-400 nm and 425-430 nm) in axenic culture. Either or both of these light bands will trigger fruitbody initiation at as low an energy level as 0.2 K (1 K = 1,000 microwatts/cm²). Maturation of sporocarp and hymenium requires an energy level of no less than 1.8 K in at least one of these two bands, and consistent production of mature sporocarps requires 3.0 K at one or both peaks. The presence or absence of energy in other wavelengths of the visible spectrum has no detectable effect on either the initiation or maturation of the sporocarp. ↑

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PREFACE

To use wood most efficiently, it is often important that the wood species be accurately identified--to take advantage of the unique properties and capabilities of that species.

Precise identification of the conk- and mushroom-producing fungi that cause decay in the wood is likewise vital if specialists are to combat the decay. But identifying a specific fungus among the many thousands that show up on wood products is a slow, painstaking process, even for experts. Without that accurate identification, control of decay may be only a shot in the dark.

A new technique now promises to speed up the identification of some fungi by a method that involves the reaction of the fungus to particular wavelengths of light. As yet, not too much is known about why or how the technique works. But the importance to the fungus identification expert is that it does--in some instances at least.

The method has apparent application in the identification area as a mass screening technique. If it can separate out 5 percent of the possibilities almost immediately, this can be a giant step forward. And present indications are that it can.

Dear also to the hearts of planners is that not only will the technique work, but it is inexpensive to use, and the elements are easily renewable.

As the fungus identifier now works in the laboratory, he is at a great disadvantage. Seldom does he see a decay conk, mushroom, or other readily identifiable form on a piece of wood. Instead, what arrives at the laboratory for identification is a test tube containing a sample of a cottony mass of mycelium. Even to that expert, many of these samples look alike; so he must note any minor features and compare the results to those of known isolates.

Then, to be certain of the species of fungus, the expert probably has to isolate the material onto a petri dish, grow it for at least 21 days and frequently examine and measure the growth and any specific characteristics. Some situations may require perhaps ten times the 21 days, so the procedure is slow. One shortcut sometimes applies. If a fungus can be forced to fruit--and still look much as it does in nature--then identification from growth on wood can be absolute.

A technique is proposed that will permit screening of large numbers of these fungus mycelia for fruiting by placing mycelia under a determined specific quality (or band) of light. If the particular fungus is affected by the quality of that light, it may initiate the process toward fruiting.

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To test the capability of the procedure to detect a specific fungus functioning in this manner, the authors worked with a known fungus that does not normally fruit. Panus fragilis is a small, white-spored fungus that is a saprophyte on hardwoods in the southern United States. Previously it had been reported to fruit only in the presence of light.

This study established that the technique worked as a screening device, and additional work with other fungi and qualities of light may extend the capabilities apparent now.

Mycologists and plant pathologists can utilize the technique to determine if any in a series of fungi will form a conk or mushroom. Researchers can determine if other characteristics occur, such as formation of pigment. Teachers can also use this relatively simple and inexpensive procedure to demonstrate light effects to students.

John Palmer

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The authors thank M. F. Wesolowski and L. C. Zank, chemists from the Forest Products Laboratory, Madison, Wis., who obtained the monochromatic percent transmission curves from 227 through 50,000 nm.

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EFFECT OF LIGHT QUALITY ON DEVELOPMENT OF FRUITING BODIES OF *PANUS FRAGILIS*

By

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INTRODUCTION

Vegetative mycelia of most Hymenomycetes form neither conidia nor basidiocarps in axenic culture. The procedures developed to identify isolates in culture are elaborate and time-consuming (Davidson, Campbell, and Vaughn 1942, Nobles 1948). The identification pattern for an individual species is prepared by examination of mycelia obtained from an identifiable sporophore obtained in nature or formed in pure culture. Mycelia from a few such species cannot be separated, and many vegetative isolates made from substrates have no matching pattern and therefore cannot be identified. Consequently, systems that will induce fruiting or dependable species-specific mycelial responses are needed. A fungus, originally identified from light-induced fruiting in axenic culture, was studied in a light exposure system designed for exposure of large numbers of these fungi in pure culture.

Panus fragilis is a small, white-spored lignicolous agaric that is a saprophyte on some hardwoods and conifers in the southern United States (Miller 1965). Previously the fungus had been known to fruit only in the presence of light in a two-stage process involving induction and maturation of the sporocarp. A low level of a short cycle (e.g., 792-foot candles for 1.5 hours) had been sufficient to induce fruiting body initials, but an increased light intensity or a longer daily period of light was necessary to bring about the maturation of the fruiting body (e.g., 792-foot candles for 12 hours) (Miller 1967).

The following study shows that wave length irradiation in limited regions of the spectrum effect initiation and maturation of the sporocarp.

Table 1.--Scans and observations on visible light and monochromatic light transmissions in ultraviolet (UV), near infrared (near IR), and broad infrared (broad IR) through eleven filter materials used in experimentation

SCANS			
Name	Wave lengths		Measuring instrument (Beckman)
	Begin	End	
UV	227 nm	400 nm	DK-2A
	2,270 A	4,000 A	
Visible	340 nm	750 nm	DK-2A
	3,400 A	7,500 A	
Near IR	700 nm	3,100 nm	DK-2A
	7,000 A	31,000 A	
Broad IR	2,000 nm	40,000 nm	IR-12
	20,000 A	400,000 A	

GENERAL OBSERVATIONS

- UV Scan - Plexiglass filters (RH numbers): Between 227 nm and 350 nm no transmission through acrylic filters.
- Gelatine-coated cellulose acetate filters (ES numbers): Between 227 nm and 290 nm no transmission through gelatin filters.
- Visible Scan - RH and ES filters: Different transmission for each filter.
- Near IR Scan - RH filters: Between 1,100 nm and 3,100 nm transmissions of RH filters similar pairing with curve for transparent acrylic.
- ES filters: Between 850 nm and 3,100 nm transmissions of ES filters similar, pairing apparently with gelatine (different from plexiglass curve).
- Broad IR Scan - RH filters: All curves similar with almost no transmission between 2,000 nm and 40,000 nm, but may be an artifact toward 40,000 nm because of plexiglass thickness.
- ES filters: All curves similar with transmission over much of range between 2,000 nm and 40,000 nm.

METHODS

A pure culture isolate made by C. H. Driver (30694) was used throughout the study and is preserved at the Center for Forest Mycology Research, Forest Products Laboratory, Madison, Wis. and at the Central Bureau voor Schimmelcultures, Baarn, Netherlands.

Because a limited budget did not allow purchase of expensive filters, plexiglass or cellulose acetate films were used to screen various wavelengths of light. Filter materials were purchased from Rohm and Haas (designated RH) and the Edmund Scientific Company (designated ES). RH materials were clear or pigmented plexiglass 1/4-inch-thick. ES materials were pigmented gelatin on thin cellulose acetate films. Eleven filter materials were selected.

Filters were cut to fit over the rim of aluminum pans, and a 1/4-inch-wide strip of black, sponge-rubber weather-stripping was placed along the margin on the underside of the filter. Clamps held the strips tightly against the aluminum pans and excluded side light. During experiments ES filters overlaid 1/4-inch-thick clear RH plexiglass for support. The assembled units are subsequently called exposure chambers.

Percent transmission for each filter material was scanned from 227 nanometers (nm) in the UV to 3,100 nm in the near infrared and from 2,000 to 40,000 nm in the broad infrared (table 1). Cellulose acetate would not transmit at wavelengths shorter than 290 nm and plexiglass at those shorter than 350 nm. Since plexiglass comprised the dividers separating the light emission plenum from the growth plenum, the RH filters, carrier, and the support under the ES filters, no transmissions below 350 nm could be expected. Obviously, additional or other light sources would change transmissions through these filters.

During experiments, exposure chambers were placed in plastic-coated wire baskets that fit on racks on stainless steel movable carts (fig. 1). The racks could be adjusted to successively lower levels at 6-inch intervals beginning with a filter level 11 inches below the growth plenum-light plenum divider. Fluorescent lamps were cool white-reflector bulbs (T-12) with recessed DC base (215 nominal watts), and light was provided for 12 hours at 22° C, followed by dark for 12 hours at 15° C. Spectral curves were measured within exposure chambers with an ISCO Model 5R spectroradiometer, which measures intensity in micro-watts/cm².

To standardize as nearly as possible the quantity of light to which the fungus would be exposed within each of the exposure chambers, filters with lower transmissions were located nearer the light source. Light intensity within the growth room was routinely checked for change during and between experiments with a GE Model 23-foot candle meter. To achieve

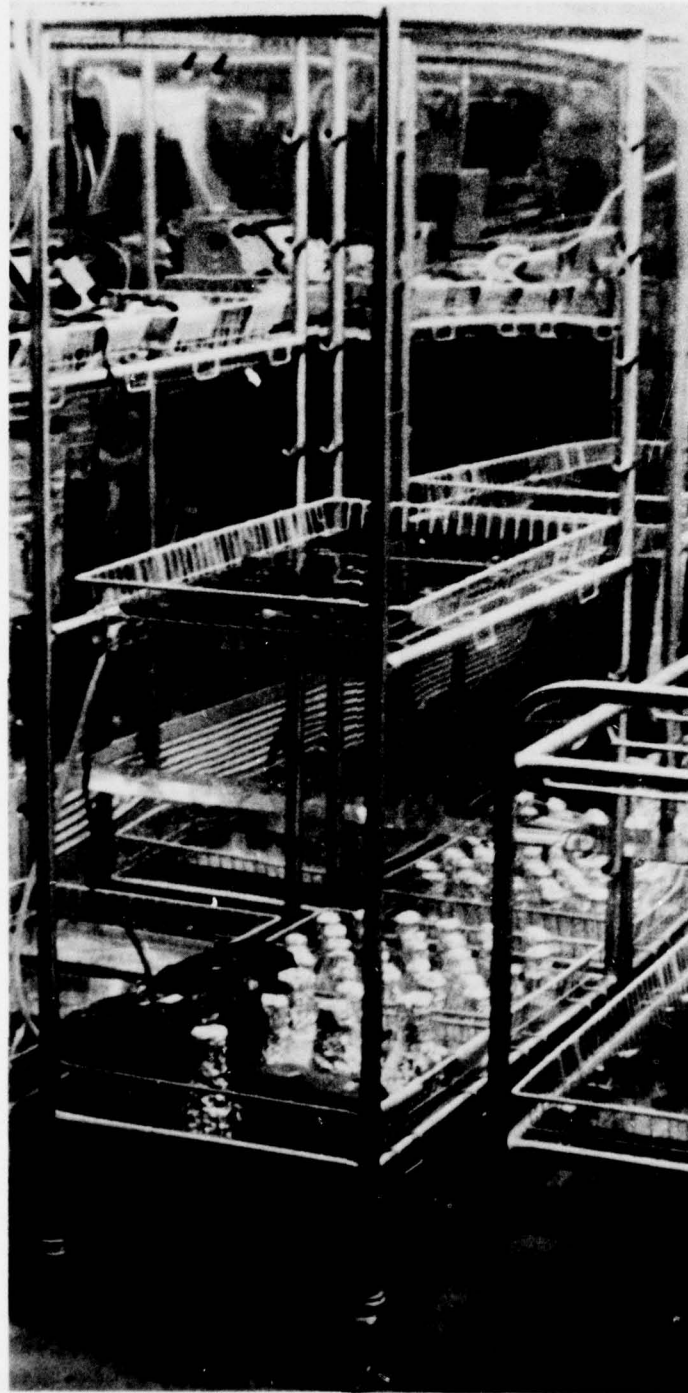


Figure 1.--The light exposure system in the growth chamber during experiments.

darkness (no measurable light over the scan of the spectroradiometer), petri dishes were wrapped in each of three ways: Several layers of aluminum foil, thick black cloth, and thick black cloth over an aluminum foil wrapper.

When the lights were on, the temperatures rose higher in exposure chambers nearer the light source. At the same level the temperature rose higher in those with transmissions at and above 650 nm. During experiments air was pulled through each chamber by a vacuum pump, which was connected by hose to a metal tube in a light-tight port. Air entered the chamber through two similar ports at the opposite end of each chamber. Pumps began 1 hour after lights were turned on and operated until 1 hour after they were turned off. This circulation kept air temperatures within the exposure chambers at or below 26° C during the light cycle and reduced accumulation of metabolic gases.

Temperature probes within the six exposure chambers plus one in the growth chamber with chamber air constantly monitored chamber air temperatures on a meter outside the growth room. In the three exposure chambers nearest the light source, readings as high as 26° C were occasionally attained after 10 or more hours of light, especially with filter RH-2423, but the isolate of *Panus fragilis* tolerates temperatures in excess of 30° C. Temperatures in the low chambers were constantly at or near 22° C during the light cycle. The night temperature quickly dropped to 15° C in all exposure chambers at the onset of the dark cycle and subsequently varied no more than 0.6° C in any chamber.

When environmental control was lost because of equipment malfunction, the experiment was terminated. Four replicate experiments were successfully completed and are reported here.

Plastic petri dishes (20 mm depth, 100 mm diameter) were used in all experiments since the commercially available types transmit at 80 percent of incoming light, beginning at 350 nm through the visible spectrum.

After inoculation, starter plates were kept for 6 days at 16° C to obtain a colony diameter of 18 to 20 mm. Inoculum plugs 4 mm in diameter were cut from peripheral mycelium and were inverted in the center of plates to be used in experimental runs. In two runs, the four plates for each of the 14 exposure chambers were kept in total darkness for 1 week and in two were placed directly in the exposure chambers. Growth was observed daily during the light cycle.

Since thermal shock may induce responses, one replicate of petri dishes was grown in the dark over the same periods of time for 12 hours at 10° C followed by 12 hours at 20° C, and a second replicate at 10° C and 25° C.

RESULTS

Irradiation available to affect growth in an exposure chamber is a function of two factors: Wave lengths emitted by the one or more light sources, and wave lengths transmitted by the filter materials.

Wave length emission charts for the many types of light sources are available from manufacturers. The wave lengths and relative transmission by each of these filters are presented in figures 2 through 5, presuming radiation at all wave lengths between 340 and 400,000 nm. In general, transmissions through RH filters matched that through clear plexiglass, which was intermittent between 2,000 nm and 40,000 nm. Clear plexiglass transmitted continuously from 350 nm in the ultraviolet (UV) to 2,230 nm in the near IR. Between 1,100 and 40,000 nm, all RH filter curves were the same with almost no transmissions. Thus pigments in both ES and RH filters were principally functional only in the visible spectrum (fig. 6). Plexiglass determined transmission in other parts of the spectrum.

Under the cool white fluorescent lamps of the growth chamber, a light transmission curve was prepared for each of the 11 pigmented filters, as well as for the clear plexiglass within the exposure chamber (fig. 6). Low emission plus radiation absorption and reflection effectively eliminated transmissions into exposure chambers below 385 nm even for clear plexiglass (fig. 6). Clear plexiglass transmits at 68-70 percent through the visible spectrum, which can be defined as 400-700 nm (Weast 1972-1973). Thus RH-2423, which transmits in a normal probability curve between 350 nm and 400 nm with a maximum of 10 percent, and between 600 nm and 750 nm with a maximum of 82 percent at 700 nm, did not transmit into exposure chambers below 600 nm. For experimental purposes the RH-2423 was a single band filter (fig. 6). ES filters transmitted in similar fashion over much of the range between 850 and 40,000 nm.

During the experimental period, no measurable pigment deterioration occurred, even at high light intensities. Curves prepared before, during, and after the experimentation periods for utilized and unused filters possessed similar percent transmissions at similar wavelengths. The radiation measurements graphed in figure 6 were those recorded within each exposure chamber located in the position on the cart listed in table 2.

At shorter wavelengths (280-500 nm) our curve corresponds to that presented by Leach (1971) for plastic petri dishes. Fruiting could not be induced on the semisynthetic Vogel's (1956) medium plus 10 mg of yeast extract per liter. Therefore, 30 ml of malt agar (12.5 g of malt extract and 20 g of agar diluted to 1,000 ml) were added to each petri dish in all experiments.

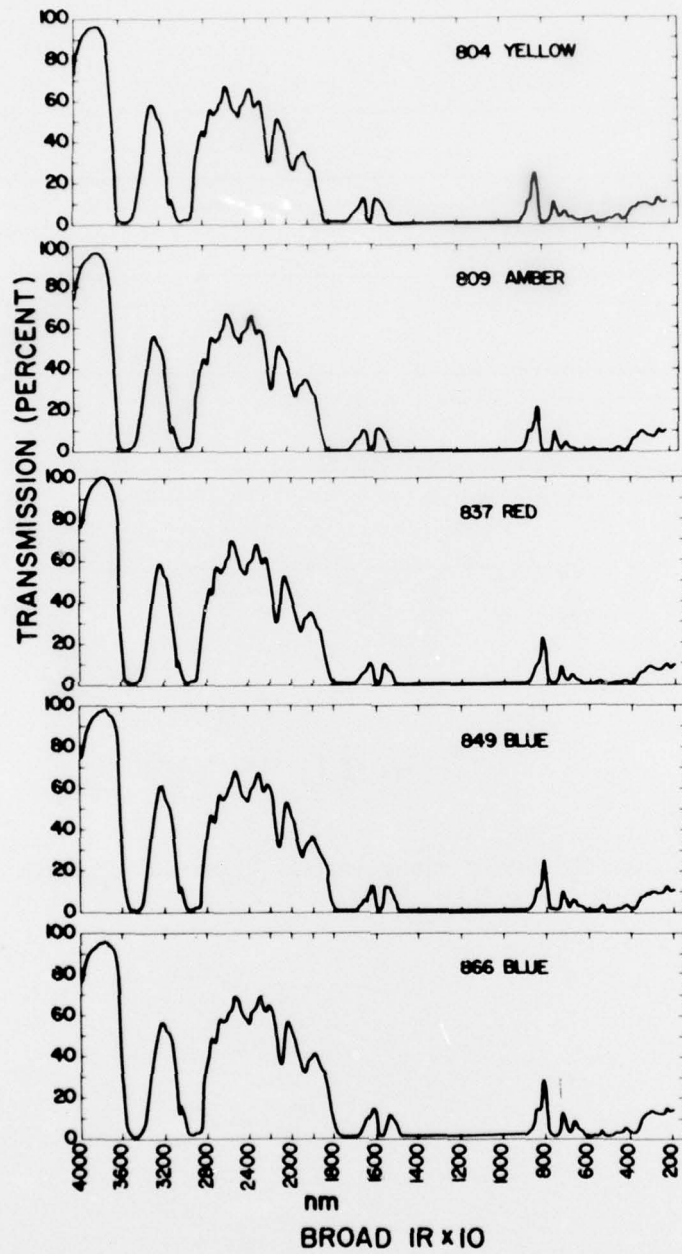


Figure 2.--Monochromatic light scans (broad infrared) through filters of gelatin-coated cellulose acetate.

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TRANSMISSION (PERCENT)

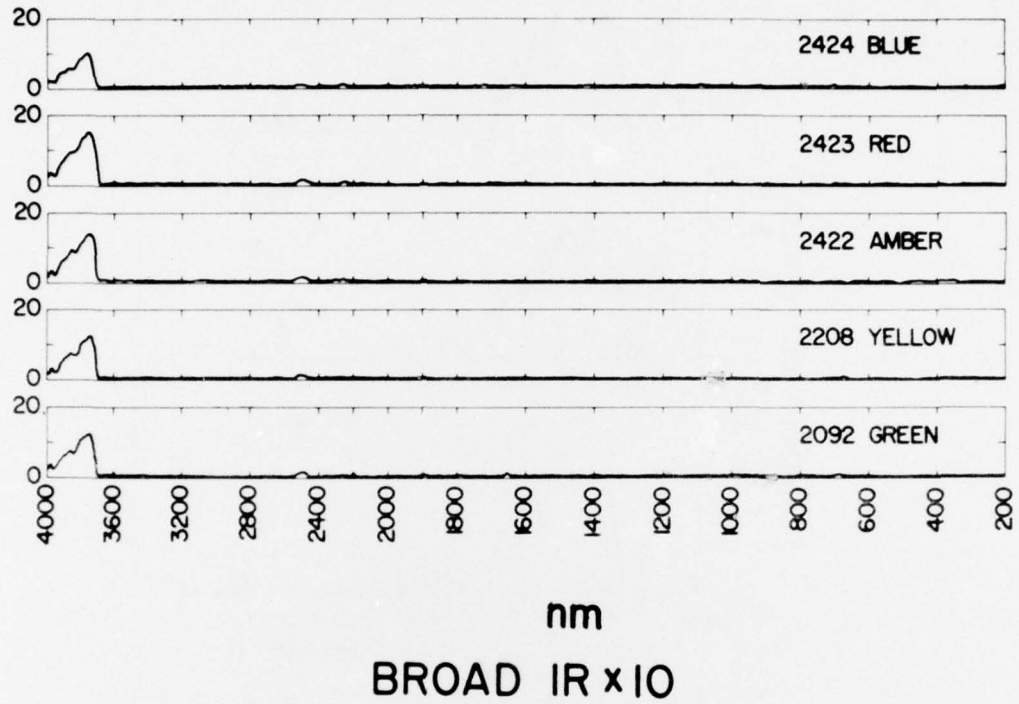


Figure 3.--Monochromatic light scans (broad infrared) through filters of acrylic plastic (plexiglass).

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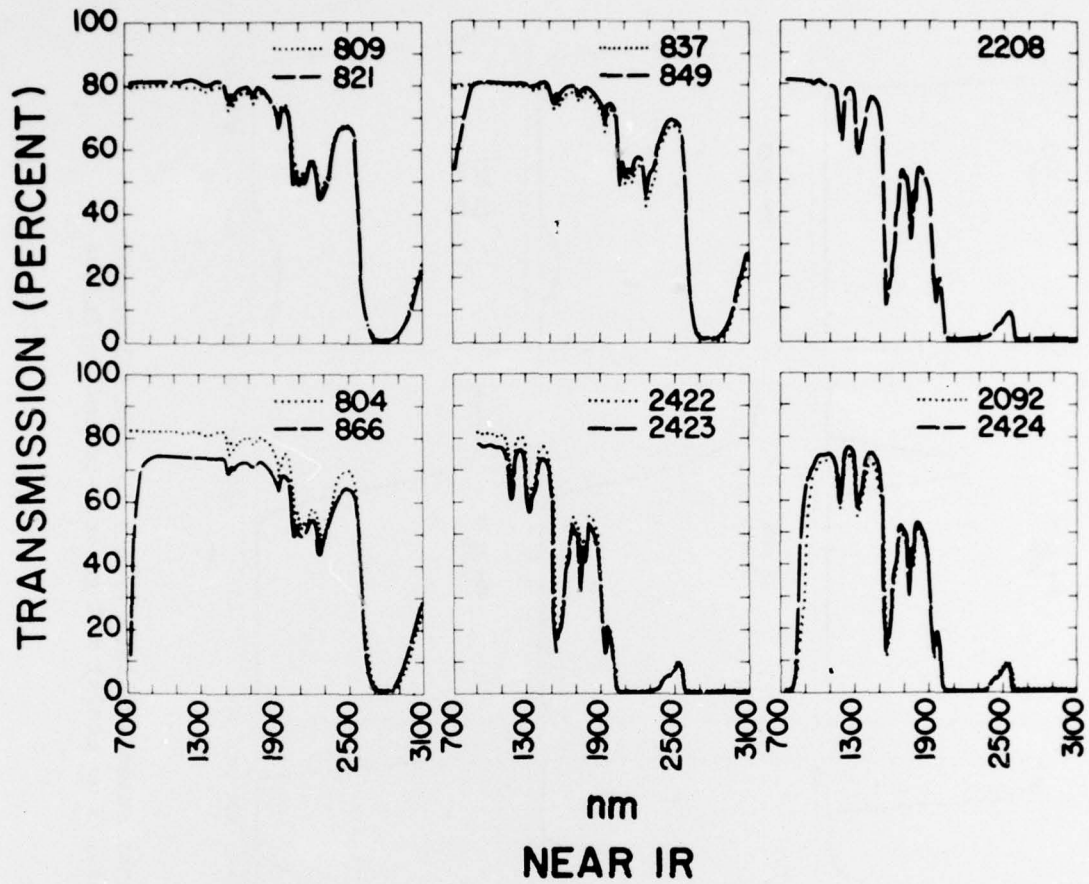


Figure 4.--Monochromatic light scans (near infrared) through filters of gelatin-coated cellulose acetate (3 numerals) or plexiglass (4 numerals).

(M 145 406)

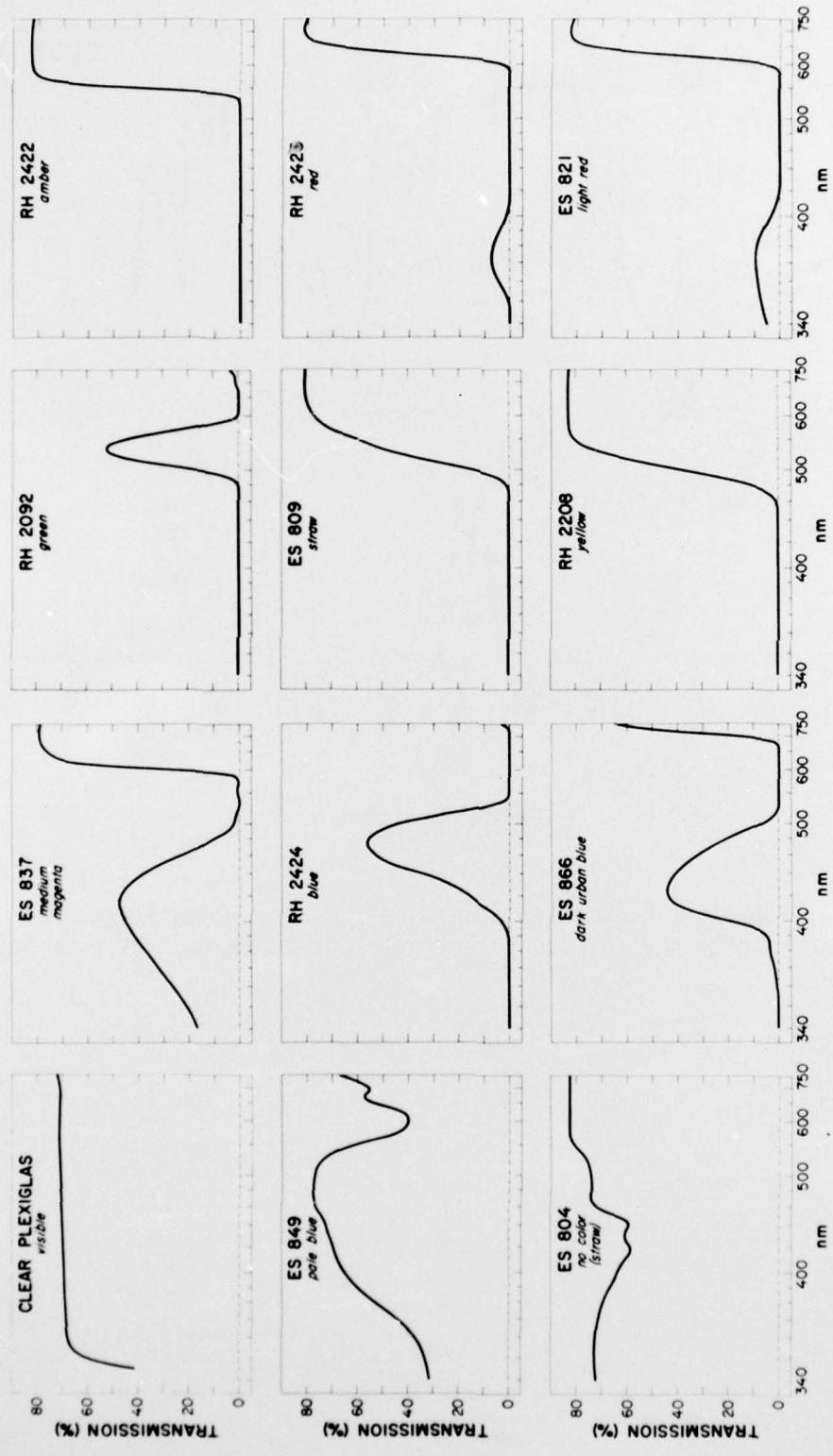


Figure 5.--Monochromatic light scans (visible light) through filters of acrylic plastic (plexiglass, 3 numerals) and gelatin-coated cellulose acetate (4 numerals).

(M 145 417)

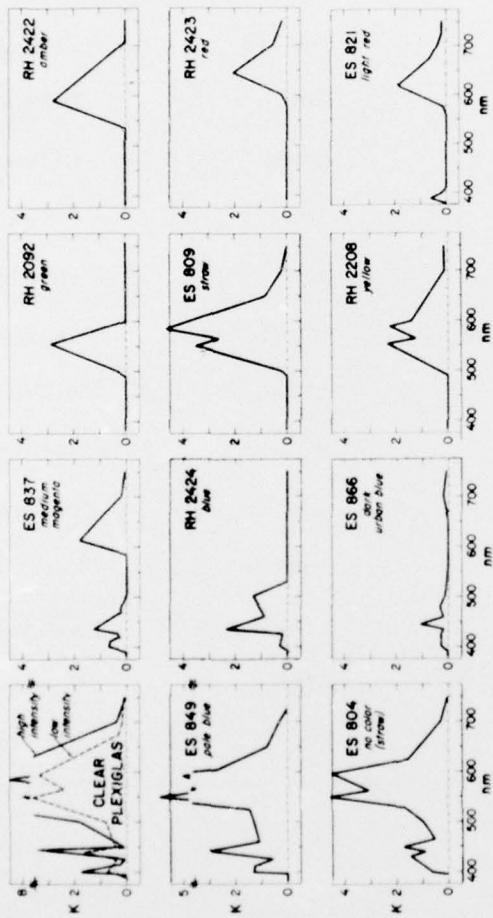


Figure 6.--Quantities ($K = 1,000$ microwatts/cm²) of light radiated from cool white fluorescent lamps and transmitted through eleven pigmented filters (RH = plexiglass; ES = cellulose acetate + plexiglass support) between 390 and 750 nm.

(M 145 418)

Table 2.--Fungus development in light transmitted through
 11 filters compared to unfiltered fluorescent
 light, filtered, and no light

Number	FILTER		Shelf position on cart	Fungus development
		Color		
Clear	none		middle	Pileus
ES-849	pale blue		middle	Pileus
ES-804	straw		low	Pileus
RH-2424	blue		high	Pileus
Clear	none		low	Pileus
ES-837	magenta		low	Initials
ES-866	dark urban blue		middle	Initials
ES-821	light red		high	Minute Initials
RH-2092	green		middle	Vegetative
ES-809	straw		low	Vegetative
RH-2208	yellow		low	Vegetative
RH-2422	amber		low	Vegetative
RH-2423	red		high	Vegetative
Covered	none		low	Vegetative

Fruiting responses were the same for mycelia immediately exposed to light treatments and those exposed after an initial week of total darkness. Thermal shock studies did not induce fruiting. In light the white mycelium covered a petri dish in approximately 8 days. Small pale pink areas developed within 9 to 18 days after inoculation. Sporophore initials appeared 3 to 8 days later in the pigmented areas of appropriate treatments (table 2), followed by elongation and pileus production from one or nearly two initials per plate in an additional 3 to 7 days.

Normally only one complete sporophore developed in one plate within 21 to 31 days: If two initials matured, they located on opposite sides of the plate as previously reported (Miller 1967).

In exposure chambers covered with clear plexiglass, fruiting bodies were produced in one or more plates but a larger number developed in chambers closest to the light source. In darkness only wooly white mycelium was produced. Effects of filters on growth and development of P. fragilis (table 2) could be classified in three groups:

1. Vegetative growth without development of initials occurred under five filters: Red RH 2423, yellow RH 2208, amber RH 2422, straw ES 809, and green RH 2092. Transmittance peaked between 533 and 575 nm with very little to none below 533 nm.

2. Initials and rare pilei but no mature sporocarps were produced under four filters: Medium magenta ES 837, straw color ES 804, dark urban blue ES 866, and light red ES 821. Each of the first three filters peaked twice in a continuous transmission band between 387 and 400 nm and 425 and 430 nm with intensities between 1.0 and 1.5 K. The fourth (ES 821) peaked once at 388 nm at a maximum intensity of 0.2 K over a very narrow band. The total area under the curve in the UV-blue for ES 821 was much less than any of the other three. The many initials were minute (between 1.0 and 2.0 mm in height) compared with those under ES 866 which measured 8 to 18 mm in height and also had a greater diameter. Both of these filters transmitted in the red with an area of no transmission between the UV-blue and red transmission zones.

The pigment associated with fruiting in P. fragilis was usually pink, darkening to purple with passage of time, and was most pronounced over the top of the initials. After a period of time some initials developed small apical branches. This phenomenon was noted previously when low levels of light were provided on a short daily light cycle (Miller 1967).

Under ES 804 (straw color), which has a spectral pattern similar to clear plexiglass, one pileus developed during the four runs, but initials were formed abundantly in all plates. Under clear plexiglass at low light intensity many initials were produced in every plate, but only one pileus developed.

3. A mature pileus with gills and a fertile hymenium was produced under the two filters (ES 849 and ES 804) which had two peaks between 387 nm in the UV and 430 nm in the blue. Under ES 804 the amplitudes exceeded 1.8 K, and under ES 849 one peak exceeded 3 K. Mature sporocarps developed under these filters. RH 2424 had a single peak at 425 nm (1.9 K) and no visible light transmission between 550 and 750 nm. Large well-developed sporocarps with caps ranging from 5 to 11 mm in diameter were produced. However, mature sporophores developed in only three out of four test plates in one run and in one out of four plates in another: In two runs large well-developed initials did not mature. The variations under RH 2424 may have resulted from high temperatures since the exposure chamber had to be close to the light source to obtain the 1.9 K intensity. It is also probable that air flow within a chamber kept the temperature in some individual petri dishes lower than in others. The chambers with ES 849 and ES 804 were on the middle and lower shelves (table 1), where over-heating was not a factor.

DISCUSSION

Regions of the UV-visible spectrum which are effective in the production of fruiting body initials and the maturation of the fruiting body would place Panus fragilis in the group of blue-sensitive organisms, including such Homobasidiomycetes as some isolates of Coprinus lagopus (Borriss 1934, Madelin 1956) and Sphaerobolus stellatus (Alasoadura 1963). Recent reports for Favolus arcularius (Kitamoto, Suzuki, and Furukawa 1972) and Nectria haematococca var. cucurbitae (Curtis 1973) indicate that more than one peak in the blue seems to be effective for the stimulation of initials and the maturation of the fruiting body.

Our study did not employ monochromatic light sources, and light was provided at one intensity for the duration of the experiment. In previous experiments short doses of light had failed to stimulate fruit body production, but the intensity of light over time of exposure appeared to be a key variable (Miller 1967). It had been decided, therefore, to use filters with rather broad transmission in various parts of the visible spectrum to initially assess the effect of light quality. Having established that the UV-blue (387 to 430 nm) is active, monochromatic light sources at effective intensities can now further quantify the specific light responses of Panus fragilis. They can also establish the specific wavelength intensities in the UV, visible, and near IR that initiate and determine maturation of initials and subsequent stages of mushroom development.

NEXT STEPS?

Intensity requirements at active wavelengths also need investigation. The UV-blue light intensities decrease with successive stages in development of the basidiocarp of Sphaerobolus stellatus, and the succession of the terminal stages appears to be speeded up by red as compared with blue light (Alasoadura 1963, Ingold and Nawaz 1967).

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