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EFFECT OF LIGHT AND AERATION ON FRUITING OF *LENTINULA EDODES*

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Adequate light and aeration are essential for the fruiting of *Lentinula edodes* (syn. *Lentinus edodes*). The stimulatory wavelengths were dependent on the composition of the growth medium. Red wavelengths (620–680nm) stimulated and blue wavelengths (400–500nm) inhibited fruiting on low calcium (< 40 p.p.m.) media. Blue wavelengths stimulated fruiting on high calcium media (> 130 p.p.m.). Addition of oak bark extract to the medium permitted otherwise suboptimal light intensities to stimulate fruiting. Cultures were receptive to light stimulation during vegetative growth and showed memory of the exposure, fruiting later in the dark. Exposure, especially to blue light, elicited pigmentation of primordia. Only pigmented primordia were capable of expansion. Inadequate aeration (probably resulting in CO_2 accumulation) near the time of fruiting resulted in failure of the primordia to expand. The practical implications of these findings are discussed.

Traditionally the shiitake mushroom (*Lentinula* (= *Lentinus*) *edodes*) has been cultivated in forests on hardwood logs, e.g. oak (San Antonio, 1981; Leatham, 1982). Attempts are currently underway to produce this important edible basidiomycete more rapidly and efficiently in environmentally controlled chambers on lignocellulosic particles (Han *et al.*, 1981). To achieve optimal production in chambers, it is essential to know about its requirements for light and aeration.

Light regulates reproductive development in a wide range of fungi. The effective wavelengths are generally in the near ultraviolet, blue, red or far-red spectral regions (Tan, 1977). Light effects are often complex and can be influenced by other environmental or nutritional factors. Depending on the fungus and developmental stage, light may stimulate or inhibit development (Barnett & Lilly, 1950; Madelin, 1956; Ingold & Nawaz, 1967; Eger, Gottwald & Von Netzer, 1974; Kamada, Kurita & Takemaru, 1978; Kamada & Tsuji, 1979; Durand, 1982, 1983). Fungi with both light-stimulated and -inhibited stages may develop optimally only in a light/dark cycle (Timnick, Lilly & Barnett, 1951 ; Manachere, 1977). Certain wavelengths can reverse some light effects (Lukens, 1965; Tan, 1974).

Mechanisms exist in fungi which allow 'memory' of prior light exposure. Even a brief exposure of a receptive culture may elicit later development in the dark (Bisby, 1925; Badham, 1980). Phenoloxidase activity or pigmentation are frequently associated with both light responses (Gadd, 1982) and development (Leatham & Stahmann, 1981). Phenoloxidase function may possibly both allow memory of exposure and mediate the later response (Lynch & Geoghegan, 1979; ROSS, 1982). Oxidized regulatory metabolites (Zafar & Colotelo, 1969,1978) or other compounds may elicit the response (Gruen, 1963; Trione & Leach, 1969; Uno, Yamaguchi & Ishikawa, 1974).

Lentinula edodes has been reported to fruit on logs in the dark (Nisikado & Miyawaki, 1943; Tokimoto & Komatsu, 1978). However, the fruit bodies were less pigmented than normal, misshapen, and sporulated poorly. More information has been gained with flask-grown cultures. After the vegetative mycelium is established in such cultures, primordia buried within the mycelium begin to exude liquid droplets (Leatham, 1985). With the correct light treatment, the primordia expand upward, and the aerial primordia finally form fruit bodies. Based on work with such cultures, Ishikawa (1967) reported that : blue wavelengths were the most stimulatory, the optimum intensity was 10 1x, the minimum intensity was 10^{-2} to 10^{-4} 1x, at least 24 h light exposure was required to initiate fruiting, localized exposure resulted only in localized fruiting, and following exposure, dark periods increased the number of fruit bodies. Ando (1974) reported that, depending on the growth medium, blue and/or 'pink' wavelengths were necessary for fruiting (spectral information not given).

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Aeration and certain nutrients are important for the fruiting of many basidiomycetes. Adequate aeration and a calcium-rich casing layer (Delmas, 1978) are essential to fruit the commercial mushroom *Agaricus bisporus.* Air exchange removes excess metabolically generated CO_2 (Long & Jacobs, 1969; Nair & Hayes, 1975) which inhibits primordia expansion into mushrooms (Lambert, 1933; Nair, 1972). Although CO_2 is generally accepted to be the volatile inhibitor for basidiomycetes (Plunkett, 1956; Niederptuem, 1963; Taber, 1966; Zadrazil, 1974), other compounds have often not been ruled out (Mader, 1943). Little has been reported about the effect of aeration or nutrients such as calcium on the fruiting of *L. edodes.* However, addition of oak bark extract (Leatham, 1983; Leatham & Stahmann, 1964) can stimulate the fruiting of this important mushroom.

We report here that light and aeration are essential for the fruiting of L. edodes. The nature of the light and aeration requirements, the receptive stages of the life-cycle, and the marked effects of medium composition are discussed.

MATERIALS AND METHODS

Unless otherwise stated, cultures of *L. edodes* heterokaryon strain ATCC 48085 were grown at 21 ± 2 °C in 250 ml cotton-stoppered Erlenrnyer flasks on 25 ml of three media (see figures and legends for usage): (I) 40 g l^{-1} rolled oats (sterilized like the other media), (II) a semi-defined medium (Leatham & Stahmann, 1984), or (III) the previously described chemically defined medium (Leatham & Stahmann, 1981) - with or without supplementation with 40 g l^{-1} rolled oats. High-calcium media were produced by adding 0·5 g l^{-1} $CaCl_2 . 2H_2O$. Oak (*Quercus rubra* E.) bark extract, and fermenter-grown or homogenate inoculum (produced from cultures grown on the chemically defined medium) were used as described previously (Leatham & Stahmann, 1981). Relative culture growth was determined as oven-dry weight (60") of duplicate distilled water-washed cultures harvested 41 d after inoculation. The average error in growth determinations was 4% with a range of 1-9%. Formation of dense small structures on the surface of cultures was scored as successful production of aerial primordia. Formation of button-stage fruit bodies was scored as successful fruiting.

Light treatments, sources, and measurement

Unless otherwise stated, light treatments were on a 9 h light/15 h dark cycle. Light treatments were carried out in shelving covered with black air-permeable cloth. Gradients or cross-gradients were produced by placing the light source(s) in one end or opposite ends of the shelf respectively. Light sources included tungsten-filament incandescent bulbs (Westinghouse Electric Corp., Bloomfield, N.J., U.S.A.) and fluorescent bulbs: Agro-Light (Westinghouse Electric Corp.), Cool-White (International Telephone and Telegraph Corp., Lynn, Mass., U.S.A.), and Gro-Lux (Sylvania, Danvers, Mass., U.S.A). Where filtered light sources were used, the filters were made with the appropriate layers of blue, green, orange, or red Polythane® Brand plastic sheeting (Dennison, Inc., Framingham, N.J., U.S.A.). Light emission spectra were taken by an Isco model SR spectroradiometer and an Isco model SRR Programmed Scanning Recorder (Instrumentation Specialties Co., Lincoln, Neb., U.S.A.) and values are expressed as the relative spectral energy intensity (μ Watt cm^2 nm^1) versus wavelength (nm). Measurements of incident light intensity (1x) were taken with a silicon photocell photographic light meter (Minolta Auto Meter II, Minolta Camera Co. Ltd, Japan). Dark treatments were carried out in boxes made lightproof with the black cloth. Dark grown cultures were viewed and scored for development only after the termination of the experiments.

Aeration treatments and O_2 measurement

Aeration was reduced either by enclosing cultures in 25 x 38 cm, 0.04 mm (1·5 ml) thick polypropylene bags (Clavies 13182, Bel-Art Products, Peguannock, N.J., U.S.A.), or by capping the cotton-stoppered culture flasks with one layer of the same membrane and sealing with rubber bands. Trapping experiments were with membrane-capped cultures into which a io mm x 13 cm test tube loaded with a trapping agent was placed prior to sterilization. The agents were either 2 g of a suitable trapping chemical plus 2 ml H_2O or 5 g of activated charcoal with no H_2O. Oxygen measurements were made in membrane-capped 500 ml filter flasks loaded with twice the normal volume of medium and inoculum with a 6 mm O.D. x 10 cm oxygen-dependent probe (Borkowski & Johnson, 1967; Johnson, Borkowski & Engblom, 1964). The probe was suspended 3 cm above the colony surface by anchoring the connecting wires in the flask side port with RTV 154 silicon rubber sealant (General Electric Co., Waterford, N.Y., U.S.A.). Probes were calibrated both before and after the experiment in air and in a vessel made anaerobic with sodium dithionite. The oxygen concentration within triplicate cultures was recorded for 40 d using a Honeywell 3-channel recorder (Minneapolis- Honeywell Register Co., Philadelphia, Pa,, U.S.A.).

Table 1. *Effect of different light sources, light intensities, and media on fruiting*

(Sets of two replicate cultures each were grown in four different light gradients at I 2 different light intensities on three different low-calcium media. The incident light intensities (Io, 1x) tested are given for each light source (Spectra: Fig. 1). The following media and fermenter-grown inoculum were used: (A) defined medium with oats, (B) defined medium minus glucose with oats, and (C) and oat medium with 0·8 11.$^{-1}$ bark extract (M_3). The final calcium concentrations were 28, 28 and 36 p.p.m. respectively. The number of days after inoculation that fruiting first occurred in each culture is given with (–) denoting no fruiting. Asterisks denote that the button stage fruit bodies matured to normal fruit bodies showing typical wild-type morphology. The total number of cultures successfully forming button stage or normal fruit bodies due to each light source, and medium are summarized.)

Number of days for the first fruiting

Medium and replicate no....													Media totals	
	Cool-white fluorescent light intensity (lx)													
	22	24	27	32	43	54	65	110	170	270	650	1350	Button	Normal
A1	—	—	—	—	—	—	—	—	44*	—	—	—		
A2	—	—	—	—	—	—	—	—	—	—	—	—	1	1
B1	—	—	—	—	—	35	38	—	—	—	—	—		
B2	—	—	—	—	—	—	—	—	—	—	—	—	2	0
C1	35	—	—	—	—	35*	—	—	—	—	38	—		
C2	—	—	35*	—	—	—	35*	—	—	—	—	—	5	3
											Light source totals:		8	4
	Agro-lite fluorescent light intensity (lx)													
	22	24	27	32	43	48	54	97	150	190	380	1290		
A1	39	—	—	—	—	—	36	39*	36	—	—	—		
A2	—	—	—	—	37	—	—	—	51	—	—	51	7	1
B1	—	—	—	—	—	40	—	48	36	—	—	36		
B2	—	—	—	—	—	—	36	—	36	—	—	57	7	0
C1	—	—	—	—	—	40*	—	39	32*	35*	35*	35*		
C2	36	—	—	36	32*	—	34*	39	32*	—	39	32	14	8
											Light source totals:		28	9
	Gro-Lux fluorescent light intensity (lx)													
	22	24	27	32	38	43	54	65	97	170	340	1290		
A1	—	36*	—	—	—	—	—	—	—	—	—	—		
A2	—	—	—	—	—	—	—	—	—	—	—	36*	2	2
B1	—	—	—	—	—	—	35*	—	—	36	35*	36		
B2	—	—	—	—	—	—	—	—	36*	35*	36	36	8	4
C1	—	36*	36*	—	—	35*	—	35*	35*	35*	35*	35*		
C2	—	—	—	36	32*	35*	—	—	35	35*	35*	35*	15	13
											Light source totals:		25	19
	Incandescent light intensity (lx)													
	11	11	12	13	15	19	22	24	43	65	170	810		
A1	—	—	—	—	—	—	—	—	—	—	—	—		
A2	—	—	—	—	—	—	—	—	—	—	—	—	0	0
B1	—	—	—	—	—	27	30	—	—	—	30	27		
B2	—	—	—	—	—	—	—	—	27*	—	30	27	7	1
C1	—	—	27	—	31*	—	—	—	—	—	29*	—		
C2	—	—	—	—	—	—	—	—	27	—	—	30*	5	3
											Light source totals:		12	4

RESULTS

Responses to light

Necessity of light for fruiting. At least 10 cultures were testing for fruiting in the absence of light using each medium/inoculum combination used in the light experiments that follow. These dark-grown cultures often produced numerous 1–2mm diam primordia buried within the mycelium. Although they exuded liquid droplets as normal, neither significant pigmentation nor aerial expansion of the primordia occurred in dark-grown cultures.

Only cultures exposed to light were capable of

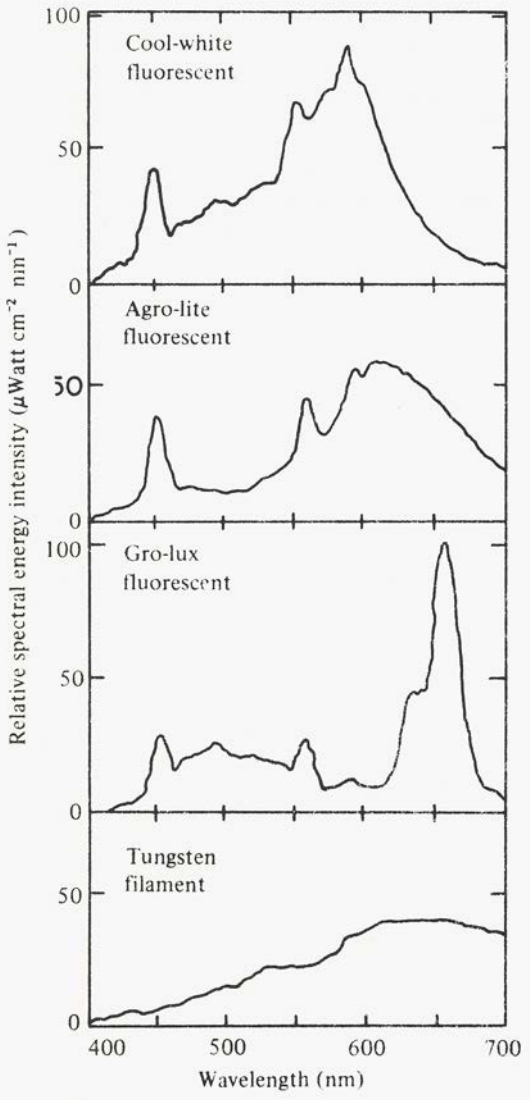

Fig. 1. Emission spectra of light sources used in the light gradient experiments.

fruiting. Exposure activated both the pigmentation of primordia and primordia expansion. Upon exposure, the top surface and upper one-third of the tissue pigmented on only a few of the many primordia buried in each culture. These primordia produced pigmented exudation droplets and expansion then followed. During expansion, the pigmented surface was carried upward, finally forming the darkly pigmented upper pileus surface characteristic of *L. edodes*.

Effects of light wavelengths, intensities and growth medium composition. Ability to fruit was dependent on the combination of light source and intensity, and on growth medium composition (Table 1). On low-calcium media ($\leqslant$ 40 p.p.m.), light sources such as Gro-Lux bulbs, with both high red and blue light output (Fig. 1), gave the most consistent fruiting and the most frequent production of fruit bodies with wild-type morphology. Cool-white bulbs, with low red-light output, gave the lowest frequency of fruiting. Higher light intensities generally increased both the frequency of fruiting and the density of fruit-body pigmentation. A medium containing oak hark extract promoted fruiting at lower light intensities than other media. Agro-Lite fluorescent bulbs were the only light source that allowed significant fruiting on a medium which contained glucose as an additional carbon source to oats.

The age at which cultures fruited was also markedly dependent on the light source. On day 35–36, strong, nearly synchronous, fruiting occurred with Gro-Lux bulbs. However, fruiting occurred nearly one week earlier with incandescent bulbs. The incandescent bulbs uniquely have high red and far-red light output, but poor blue-light output (Fig. 1).

Use of filtered light sources and different media showed that red and/or blue light had strong

Table 2. *Effect of light wavelengths on development usinga low-calcium medium*

(Sets of five replicate cultures each were grown in four different light gradients at each of to different light intensities. Spectra are shown in Fig. 2. The oat medium with 0·811⁻¹ bark extract and homogenized inoculum were used. The final calcium concentration was 36 p.p.m. Because no intensity effects were evident? values are expressed independent of light intensity as the total cultures developing out of 50.)

Wavelength(s) present	Intensity (lx)	Number developing	
		Primordia	Fruit bodies
Red	22–340	49	49
Blue	5–170	16	2
Red plus blue	10–258	6	3
Red *v.* blue (cross-gradient)	22–340 10–258	9	4

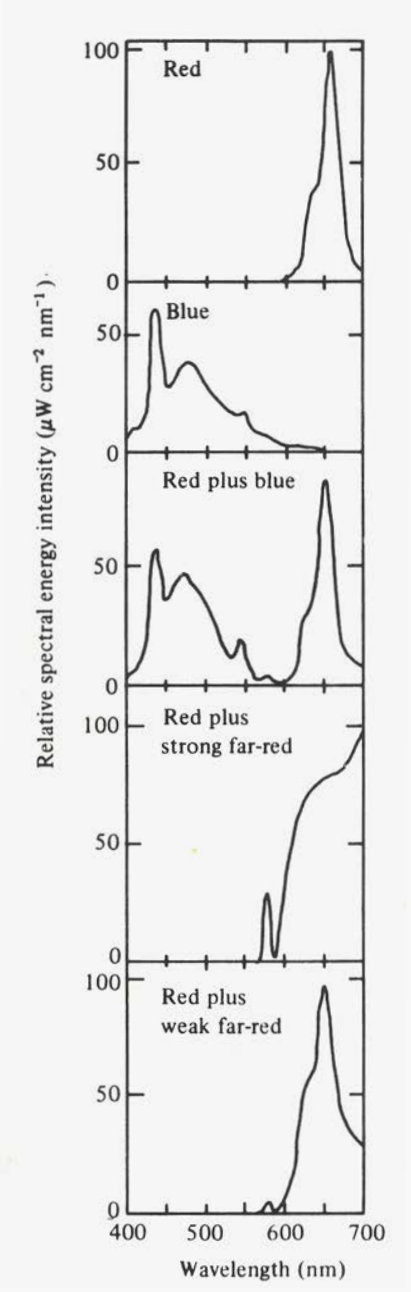

Fig. 2. Emission spectra of filtered light sources used in the light gradient experiments. The five lighting sources were constructed as follows: red = one 20 W Gro-Lux bulb filtered with four layers of red plastic; blue = two 20 W cool-white bulbs filtered with four layers of blue plastic; red plus blue = two 20 W Gro-Lux bulbs filtered with four layers of partially faded blue plastic; red plus strong far-red = three 60 W incandescent bulbs filtered with two layers of red, and one layer of each of orange and green plastic; and red plus weak far-red = one 20 W Gro-Lux bulb, one 40 W and two 25 W incandescent bulbs filtered with four layers of red plastic.

medium-dependent effects. With a low calcium medium ($\leqslant$ 40 p.p.m.), low intensities of red light (Fig. 2) were necessary to stimulate fruiting (Table 2). But development advanced to only subnormally pigmented button-stage fruit bodies. On this low-calcium medium, low intensities of blue light even in the presence of red light (Fig. 2), inhibited development. Increased pigmentation occurred with blue light alone or with high blue/red light ratios. In contrast, on three high calcium (> 135 p.p.m.) media, red (Fig. 2) or red plus far-red light (Fig. 2) were not sufficient to stimulate fruiting (Table 3). However, fruiting did occur in a red plus blue light gradient. But, the fruit bodies matured only to the button-stage and were much more densely pigmented than normal.

Light sensitive period and memory of light exposure. Fruiting was stimulated by light exposure only at specific ages. The sensitive ages were detected by exposing sets of dark-grown cultures to the daily light cycle at progressively later ages. If exposed later than midway in the life cycle, fruiting did not occur (Fig. 3). Progressively later exposures gave progressively later formation of aerial primordia.

By exposing sets of cultures to the daily light cycle at specific ages it was possible to both pinpoint the ages at which cultures were light sensitive, and to demonstrate that a memory mechanism was functional (Table 4). Cultures were not light sensitive at the time of inoculation. However, even though fruiting did not occur until near day 35, stimulation was possible as soon as growth was underway (by day 3) through approximately day 12 or 15. Relatively short exposures on day 12, e.g. 17 min, 4370 1x-min light, were fully effective at stimulating subsequent fruiting in the dark. The fruit bodies produced in the dark were less pigmented than normal and often did not mature past the button stage. Even though the cultures had not been illuminated on the upper-side during their expansion, the button-stage mushrooms were correctly oriented with the cap uppermost.

Certain light treatments were capable of increasing development on a salicylic acid containing medium giving poor development with a standard light cycle (Table 5). The most effective treatments tested were 4 d exposure to the light cycle at any time between day 4 and 16. Cultures receiving additional exposure earlier than this period, e.g. day o to 8, produced aerial primordia, but failed to fruit. Cultures exposed late in this period and then afterwards, e.g. day io to 40, even failed to develop aerial primordia.

Table 3. *Effect of light wavelengths on fruiting using high-calcium media*

(Sets of two replicate cultures each were grown in four different light gradients at each of eight different light intensities on three different high-calcium media. Spectra are shown in Fig. 2. The following calcium supplemented media and fermenter-grown inoculum were used: oat medium with 8 ml l^{-1} bark extract (A), semi-defined medium (B), and semi-defined medium with 8 ml l^{-1} bark extract (C). The final calcium concentrations were 161, 138 and 138 p.p.m., respectively. Because no intensity effects were evident, values are expressed independent of light intensity as the number fruiting out of 16 cultures on each medium.)

Wavelength(s) present	Intensity (lx)	Number fruiting on medium A	B	C
Red	22–340	0	0	0
Red plus blue	10–258	7	14	10
Red plus strong far-red	32–340	0	0	0
Red plus weak far-red	22–340	0	0	0

Response to aeration

Necessity of adequate aeration for fruiting. Restricting aeration by capping cultures with polypropylene membranes did not prevent formation of pigmented buried primordia or exudation (Fig. 4). However, it decreased their aerial expansion and thus, prevented fruiting. Increasing the area of membrane available for gas exchange markedly increased the frequency and extent of development.

In contrast to their effect on development, membrane caps had little effect on the total colony growth. Based on dry weight, membrane-capped cultures showed no more than a 10% decrease in total growth after the entire 41 d incubation period (Fig 5).

CO_2 is the probable fruiting regulator. Measurement of ambient O_2 concentrations and the use of chemical traps in membrane-capped cultures showed that CO_2 is likely to be a fruiting inhibitor. The use of oxygen-sensitive electrodes in membrane-capped cultures showed that the O_2 concentration above the colonies (initially at 20·9% v/v) gradually decreased during growth but never fell below 10·5% v/v. When placed within the culture flasks, potassium hydroxide traps but not sodium carbonate loaded traps overcame the inhibition caused by insufficient aeration (Table 6).

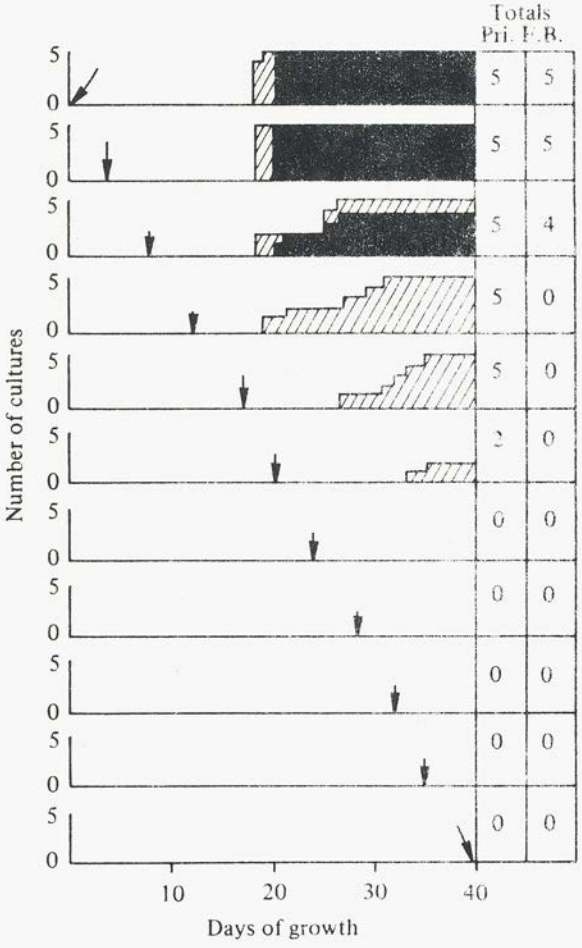

Fig. 3. Effect of progressively later light exposure on development. Sets of five replicate dark-grown cultures were placed into the light cycle at four-day intervals. Light was from Gro-Lux bulbs (258 1x, spectrum: Fig. 1). The oat medium supplemented with 2 ml l^{-1} bark extract and homogenized inoculum were used. Arrows denote the day of placement into the light cycle for each set of cultures. Cross-hatched areas denote the number of cultures producing primordia. Solid areas denote the number of cultures with primordia further expanding into fruit bodies. The total number of cultures successfully forming primordia (Pri.) and fruiting bodies (F.B.) are summarized in the adjoining boxes.

Table 4. ***Period of light sensitivity and effect of short light exposure***

(Sets of five replicate cultures each were exposed to the standard light cycle during the specified days. Light was from Gro-Lux fluorescent bulbs (258 1x, spectrum shown in Fig. 1). The oat medium with 2 ml l^{-1} bark extract and fermenter-grown inoculum were used. Cultures were scored for development on day 41. The entries show the number of cultures developing primordia or fruit bodies out of each set of five.)

Exposure (days)			Number developing	
Start	End	Total	Primordia	Fruit bodies
0	40	40	5	2
3	40	37	5	4
6	40	34	5	2
9	40	31	3	2
12	40	28	5	4
15	40	25	2	0
18	40	22	2	0
21	40	19	0	0
24	40	16	0	0
0	0	0	0	0
0	3	3	4	4
0	6	6	5	5
0	9	9	5	5
0	12	12	5	5
0	15	15	5	5
0	18	18	5	5
0	21	21	5	5
0	24	24	5	5
0	4	4	5	5
3	7	4	5	5
6	10	4	5	5
9	13	4	5	5
12	16	4	4	4
15	19	4	4	2
18	22	4	0	0
21	25	4	0	0
24	28	4	I	1
8	16	8	4	4
10	14	4	5	5
11·5	13·5	2	5	5
12	12	I	2	2
12	l	1/2	5	5
12	*	1/4	5	5
12	l	1/8	2	2
12	l	1/16	4	4
12	*	1/32	4	4

* Less than one day of exposure given on day 12.

Aeration sensitive period. Development in membrane-capped cultures was sensitive to insufficient aeration only when buried primordia normally began expansion (Fig. 5). Failure to remove membrane caps from cultures by the time primordia had begun growth inhibited many cultures from producing aerial primordia and prevented all cultures from fruiting. Progressively later membrane removal resulted in progressively later formation of aerial primordia.

DISCUSSION

These studies have demonstrated or confirmed several aspects concerning the effects of light on the development of *L. edodes:* (1) light is essential for fruiting; (2) red light stimulates and blue light can inhibit fruiting on low-calcium media; (3) red light is not sufficient and blue light is required to stimulate fruiting on high-calcium media; (4) the spectra of light used can affect how early fruiting

Table 5. ***Ability of certain light treatments to improve frequency of development with a salicylic acid containing medium normally giving poor fruit body development***

(Sets of five replicate cultures were exposed to the standard light cycle during the specified days. Illumination was as for Table 4. The defined medium with 2 ml l^{-1} bark extract, 5 μM salicylic acid, and fermenter-grown inoculum were used. Cultures were scored for development on day 41. Entries show the number of cultures developing primordia or fruit bodies out of each set of five.)

Exposure (days)			Number developing	
Start	End	Total	Primordia	Fruit bodies
0	40	40	5	0
2	40	38	4	0
4	40	36	3	1
6	40	34	4	3
8	40	32	3	1
10	40	30	1	0
12	40	28	1	0
14	40	26	0	0
16	40	24	0	0
0	0	0	0	0
0	2	2	0	0
0	4	4	0	0
0	6	6	3	2
0	8	8	5	1
0	10	10	4	0
0	12	12	5	0
0	14	14	5	2
0	16	16	5	1
0	4	4	I	0
2	6	4	4	0
4	8	4	5	4
6	10	4	4	3
8	12	4	5	4
10	14	4	5	4
12	16	4	5	5
14	18	4	5	0
16	20	4	5	1

occurs; (5) more than one colour of light may be required to obtain fruit bodies with normal morphology and pigmentation; (6) relatively short exposures to low-light intensities are sufficient to effect development; (7) addition of oak bark extract may allow otherwise suboptimal light intensities to be stimulatory; (8) the period of light sensitivity for stimulation is during the vegetative growth period (long before fruiting occurs); (9) cultures have a mechanism which allows memory of light exposure and later fruiting in the dark; (IO) even a relatively short light exposure may stimulate fruiting; (11) light exposure activates primordia for expansion; (12) only pigmented primordia expand; (13) light (especially blue) promotes pigmentation of primordia and fruit bodies; (14) for normal pigmentation, light is required after the stimulation of fruiting has occurred, e.g. perhaps at the time of fruit body expansion; (15) specialized light treatments may be used to increase the extent of development on otherwise suboptimal media; (16) in light-activated cultures fruiting in the dark, factors other than light, e.g. gravity (Schwalb & Shandler, 1974) must control final fruit body shape and orientation.

A wide variety of specific photomorphogenetic responses have been documented both in higher plants (Salisbury & Ross, 1978) and in fungi (Tan, 1977). Generally the responses in higher plants are both dependent on red, far-red or blue light and also the combination/order in which the plant tissue was exposed. The plant light receptors are generally believed to be phytochrome (Briggs & Rice, 1972) for red, far-red effects and either phytochrome or flavoproteins (Briggs, 1976) for blue light effects. Light-dependent pigmentation

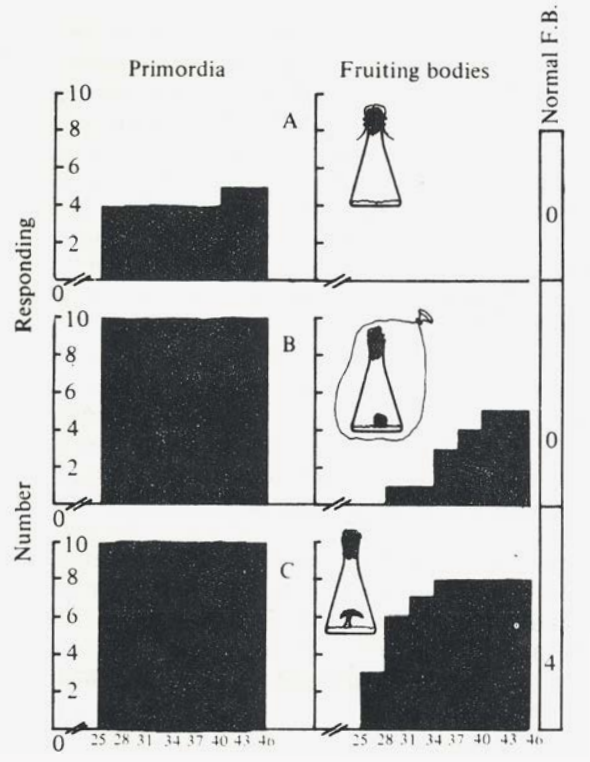

Fig. 4. Effect of restricted aeration on development. Sets of io replicate cotton-stoppered cultures were (A) capped with polypropylene membranes; (B) sealed in polypropylene plastic bags, or (C) left as they were (no restriction). Light was from Gro-Lux bulbs (258 1x, spectrum: Fig. 1). The oat medium supplemented with 2 ml l^{-1} bark extract and fermenter-grown inoculum were used. The total number of cultures forming primordia or fruit bodies is shown as solid areas. The total number of cultures successfully forming mature fruit bodies showing typical wild-type morphology are summarized in the adjoining boxes.

responses generally resulting from flavonoid deposition also occur in higher plants, e.g. Mancinelli et al. (1976). Light exposure in higher plants is known to induce several enzymes (Hahlbrock & Grisebach, 1975) including phenylalanine lyase (Smith, 1972), a key enzyme required for the synthesis of flavonoids and other aromatic secondary metabolites.

Photomorphogenetic responses in fungi are most commonly dependent on blue, near-ultraviolet and ultraviolet wavelengths (Tan, 1977). Likely flavoprotein receptors for these wavelengths have been demonstrated (Briggs, 1976). Phytochrome-like red, far-red and blue light responses (Muñoz & Butler, 1975) and also yellow light responses have also been documented in fungi (Tan, 1977). However, phytochrome-like or other receptors have not yet been conclusively demonstrated in fungi. In the current study, it is likely that at least red, blue and possibly far-red light, e.g. see the tungsten light treatment in Table 1, may have biological effects in *L. edodes.* However, before meaningful work on the identification of the light receptor(s) can be carried out, accurate action spectra must be determined for the fruiting stimulatory, fruiting inhibitory and pigmentation promoting effects of light on *L. edodes.*

Little is known about how light absorption by receptors activates basidiomycete primordia nor why certain chemicals or nutrients can modify the responses to light. Here light-induced pigmentation of primordia correlated with the ability to expand, i.e. the asymmetric deposition of pigment appeared to clearly mark the axis for future upward expansion. Further research is needed to determine if pigment formation or if phenoloxidase function promotes primordium expansion and how chemicals/nutrients such as calcium or salicylic acid modify the sensitivity or response to light.

Recently it was discovered that after L. edodes depletes its extracellular nitrogen source, it enters a period preparatory for fruiting (Leatham, 1985). This period is characterized by extensive turnover of soluble protein, and by the accumulation of protease-resistant enzymes. The timing for light stimulation could be explained if later expansion is possible only for those primordia that were activated before the metabolism shifts from vegetative growth to development.

The studies summarized here have also demonstrated several key aspects concerning the effects of

Table 6. ***Ability of chemical traps to stimulate fruiting in polypropylene membrane-capped cultures***

Chemical trap	Compounds absorbed/inactivated	Number fruiting (10 replicates)
Potassium hydroxide	CO_2 and acids	9
Activated charcoal	Ethylene and miscellaneous organics	2
Sodium carbonate	Acids	1
Sodium metaperiodate	Reductants and strong acids	1
Ascorbic acid	Oxidants and bases	0
Citric acid	Bases	0

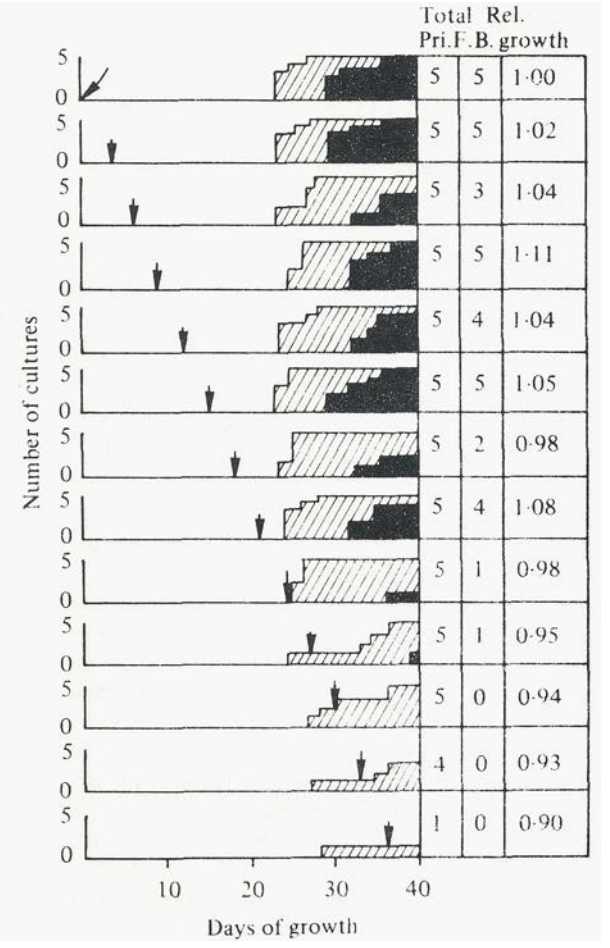

Fig. 5. Effect of progressively later exposure to increased aeration on growth and development. Sets of five replicate polypropylene membrane-capped cultures were uncapped at three-day intervals. Light was from Grow-Lux bulbs (258 1x, spectrum: Fig. 1). The defined medium with 2 ml l^{-1} bark extract, 5 μM salicylic acid, and fermenter-grown inoculum were used. Arrows denote the day when the membranes were removed from each set of cultures. Cross-hatched areas denote the number of cultures producing primordia. Solid areas denote the number of cultures with primordia expanding into fruit bodies. The total number of cultures successfully forming primordia (Pri.) and fruit bodies (F.B.) and relative colony growth (dry weight; 1·00 = 317 mg) are summarized in the adjoining boxes.

aeration on development : (1) inadequate aeration inhibits fruiting much more strongly than it does vegetative growth; (2) aeration does not stimulate fruiting simply by providing a significant increase in O_2; (3) aeration removes a volatile inhibitory metabolite; (4) CO_2 is the likely inhibitory metabolite; and (5) the period of sensitivity to inadequate aeration (CO_2) is when the primordia normally begin expansion.

Little is known about how aeration controls the expansion of primordia. During the remarkably rapid expansion of a primordium, abundant energy (ATP) is undoubtably needed to synthesize essential components such as UDP-glucose and UDP-*N*-acetyl-glucosamine (precursors for cell wall formation). Perhaps accumulation of CO,, an end-product of energy metabolism, simply prevents an adequate rate of ATP regeneration during this critical period. Further research is needed to test this hypothesis.

Survival and proliferation of fungi like *L. edodes* require that they produce mushrooms on a site optimal for spore dispersal. In nature, the presence of light, good aeration, and bark are all uniquely present at the surface of a log. The data presented here suggest that *L. edodes* may fruit most reliably with an optimal combination of these factors. These studies also suggest that environmental factors like light and aeration can be manipulated within commercial production chambers to encourage optimal production of fruit bodies with the desired density of pigmentation (colour) and normal morphology.

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REFERENCES

ANDO, M. (1974). Fruit-body formation of *Lentinus edodes* (Berk.) Sing. on the artificial media. *Mushroom Science* **9,** 415–422.

BADHAM, E. R., (1980). The effect of light upon basidiocarp initiation in *Psilocybe cubensis. Mycologia* **72,** 136–142.

BARNETT, H. L. & LILLY, V. G. (1950). Influence of nutritional and environmental factors upon asexual reproduction of *Choanephora cucurbitarum* in culture. *Phytopathology* **40,** 80–89.

BISBY, G. R. (1925). Zonation in cultures of *Fusarium discolor sulphurem. Mycologia* **17,** 89–97.

BORKOWSKI, J. D. & JOHNSON, M. J. (1967). Long-lived steam-sterilizable membrane probes for dissolved oxygen measurement. *Biotechnology and Bioengineering* **9,** 635–639.

BRIGGS, W. R. (1976). The nature of the blue light photoreceptor in higher plants and fungi. In *Light and Plant Development* (ed. H. Smith), pp. 7-18. London: Butterworth.

BRIGGS, W. R. & RICE, H. V. (1972). Phytochrome: chemical and physical properties and mechanisms of action. *Annual Review of Plant Physiology* **23,** 293–334.

DELMAS, J. (1978). Cultivation in western countries: growing in caves. In *The Biology and Cultivation of Edible Mushrooms* (ed. S. T. Chang & W. A. Hayes), pp. 251-298. New York and London: Academic Press.

DURAND, R. **(1982).** Photoperiodic response of *Coprinus congregatus:* effects of light breaks on fruiting. *Physiologia Plantarum* **55,** 226–230.

DURAND, R. (1983). Light breaks and fruit-body maturation in *Coprinus congregatus:* dark inhibitory and dark recovery process. *Plant and Cell Physiology* **24,** 899–905.

EGER, G., GOTTWALD, H. -D. & VON NETZER, U. (1974). The action of light and other factors on sporophore initiation in *Pleurotus ostreatus. Mushroom Science* **9,** 575–583.

GADD, G. M. **(1982).** Effects of media composition and light on colony differentiation and melanin synthesis in *Microdochium bolleyi. Transactions of the British Mycological Society* **78,** 115–122.

GRUEN, H. E. (1963). Endogenous growth regulation in carpophores of *Agaricus bisporus. Plant Physiology* **38,** 652–666.

HAHLBROCK, K. & GRISEBACH, H. (1975). Biosynthesis of flavonoids. In *The Flavonoids* (ed. J. R. Harborne, T. J. Mabry & H. Mabry), pp. 866–915. New York: Academic Press.

HAN, Y. H., UENG, W. T., CHEN, L. C. & CHENG, S. (1981). Physiology and ecology of *Lentinus edodes* (Berk.) Sing. *Mushroom Science* **11,** 623–658.

INGOLD, C. T. & NAWAZ, M. (1967). Sporophore development in *Sphaerobolus:* effect of blue and red light. *Annuls of Botany (New Series)* **31,** 469-477,

ISHIKAWA, H. (1967). Physiological and ecological studies on *Lentinus edodes* (Berk.) Sing *Journal of the Agricultural Laboratory (Abiko, Japan)* **8,** 1--57.

JOHNSON, M. J., BORKOWSKI, J. & ENGBLOM, C. (1964). Steam sterilizable probes for dissolved oxygen measurement. *Biotechnology and Bioengineering* **6,** 457–468.

KAMADA, T., KURITA, R. & TAKEMARU, T. (1978). Effects of light on basidiocarp maturation in *Coprinus macrorhizus. Plant and Cell Physiology* **19,** 263-275.

KAMADA, T. & TSUJI, M. **(1979).** Darkness-induced factor affecting basidiocarp maturation in *Coprinus macrorhizus PIant and Cell Physiology* **20,** 1445-1448.

LAMBERT, E. B. (1933). Effect of excess carbon dioxide on growing mushrooms. *Journal of Agricultural Research* (USDA) **47,** 599–608.

LEATHAM, G. F. (1982). Cultivation of shiitake, the Japanese forest mushroom on logs: a potential industry for the United States. *Forest Products Journa1* **32;** 29–35.

LEATHAM, G. F. (1983). A chemically defined medium for the fruiting of *Lentinus edodes. Mycologia* **75,** 905-908.

LEATHAM, G. F. (1985). Growth and development of *Lentinus edodes* on a chemically defined medium. In *Developmental Biology of the Higher Fungi.* The British Mycological Society Symposium Series **10** (ed. D. Moore, L. A. Casselton, D. A. Wood & J. C. Frankland), pp. 403–427. Cambridge: Cambridge University Press.

LEATHAM, G. F. & STAHMANN, M. A. (1981). Studies on the laccase of *Lentinus edodes.* Specificity, localization and association with the development of fruiting bodies. *Journal of General Microbiology* **125,** 147–157.

LEATHAM, G. F. & STAHMANN, M. A. (1984). Stimulatory effect of nickel or tin on the fruiting of *Lentinus edodes. Transactions of the British Myological Society* **83,** 513–517

LONG, P. E. & JACOBS, L. (1969). Some observations on carbon dioxide and sporophore initiation in the cultivated mushroom *Agaricus bisporus* (Lange) Singer. *Mushroom Science* **7,** 373–384.

LUKENS, R. J. (1965). Reversal by red light of blue light inhibition of sporulation in *Alternaria solani. Phytopathology* **55,** 1032.

LYNCH, F. J. & GEOGHEGAN, M. J. (1979). Regulation of growth and cercosporin photoinduction in *Cercospora beticola. Transactions of the British Mycological Society* **73,** 311–327.

MADELIN, M. F. (1956). The influence of light and temperature on fruiting of *Coprinus lagopus* Fr. in pure culture. Annals of Botany *(New Series)* **20,** 467–480.

MADER, E. O. (1943). Some factors inhibiting the fructification and production of the cultivated mushroom, *Agaricus campestris* L. *Phytoplathology* **33,** 1134-1145.

MANACHÉRE, G. (1977). Formation of basidiocarps. In *Biotechnology and Fungal Differentiation.* FEMS Symposium No. 4 (ed. J. Meyrath & J. Bu'lock), pp. 43–65. London; Academic Press.

MANCINELLI, A. L., YANG, C. H., RABINO, I. & RUZMANOFF, K. M. (1976). Photoreceptor pigments for blue light in *Neurospora crassa. Plant Physiology* **58,** 214-217.

MUÑOZ, V. & BUTLER, W. L,. (1975). Photoreceptor pigments for blue light in *Neurospora crassa. Plant Physiology* **55,** 421-426.

NAIR, N. G. (1972). Observations on virus disease of the cultivated mushroom *Agaricus bisporus. Mushroom Science* **8,** 155-170.

NAIR, N. G. & HAYES, W. A. (1975). Some effects of casing soil amendments on mushroom cropping. *Australian Journul of Agricultural Research* **26,** 181–188.

NIEDERPRUEM, D. J. (1963). Role of carbon dioxide in the control of fruiting of *Schizophyllum commune .Journal of Bacteriology* **85,** 1300–1308.

NISIKADO, Y. & MIYAWAKI, Y. (1964). On the relation of temperature and light to the development of sporophores in *Cortinellus berkeleyanus. Berichte des Ohara Instituts für Landwirtschaftliche Forschungen in Kurashiki (Japan)* **9,** 230-237.

PLUNKETT, B. E. (1956). The influence of factors of the aeration complex and light upon fruit-body form in pure cultures of an agaric and a polypore. *Annals of Botany (New Series)* **20,** 563–586.

Ross, I. K. (1982). The role of laccase in carpophore initiation in *Coprinus congregatus. Journal of General Microbiology* **128,** 2763–2770.

SALISBURY, F. B. & ROSS, C. W. (1978). Photomorphogenesis. In *Plant Physiology* (2nd edn), pp. 290–303. Belmont, CA.: Wadsworth Pub. Co.

SAN ANTONIO, J. P. (1981). Cultivation of the shiitake mushroom. *Horticulture Science* **16,** 151-156.

SCHWALB. M. N. & SHANDLER, A. (1974). Phototropic and geotropic responses during the development of normal and mutant fruit bodies of the basidiomycete *Schizophyllum commune*. *Journal of General Microbiology* **82,** 209–212.

SMITH, H. (1972). The phytocontrol of flavonoid biosynthesis. In *Phytochrome* (ed. K. Mitrakos & W. Shropshine, Jr), pp. 433–481 New York: Academic Press.

TABER, W. A. (1966). Morphogenesis in basidiomycetes. In *The Fungi* **II,** *The Fungal Organism* (ed. G. C. Ainsworth & A. S. Sussman), pp. 387–412. New York: Academic Press.

TAN, K. K. (1974) Red-far-red reversible photoreaction in the recovery from blue light inhibition or sporulation in *Botrytis cinerea*. *Journal of General Microbiology* **82,** 201-202.

TAN, K. R. (1977). Light-induced fungal development. In *The Filamentous Fungi* ill (ed. J. E. Smith & D. R, Berry), pp. 334–357. New York: John Wiley.

TIMNICK, M. B., LILLY, V. G. & BARNETT, H. I.. (1951). The influence of light and other factors upon sporulation of *Diaporthe phaseolorum* var. *batatitis* from soya bean. *Phytopathology* **41,** 327–336,

TOKIMOTO, K. & KOMATSU, M. (1978). Biological nature of *Lentinus edodes*. In *The Biology and Cultivation of Edible Mushrooms* (ed. S. T. Chang & W. A. Hayes), pp. 445-459. New York and London: Academic Press.

TRIONE, E. J. & LEACH, C. M. (1969). Light induced sporulation and sporogenic substances in fungi. *Phytopathology*, **59,** 1077–1083.

UNO, I., YAMAGUCHI, M. & ISHIKAWA, T. (1974). The effect of light on fruiting body formation and adenosine 3':5'-cyclic monophosphate metabolism in *Coprinus macrorhizus*. *Proceedings of the National Academy of Scienccs of the United States of America* **71,** 479–483.

ZADRAZIL, F. (1974). The ecology and industrial production of *Pleurotus ostreatus*, *Pleurotus florida*, *Pleurotus cornucopiae*, and *Pleurotus eryngii*. *Mushroom Science* **9,** 621–652.

ZAFAR, S. I. & COLOTELO, N. (1969). Influence of peroxidase and catalases on mycelial growth and pycnidium production by *Plenodomus meliloti* in light and darkness. *Canadian Journal of Botany* **47,** 505–512.

ZAFAR, S. I. & COLOTELO, N. (1978). Influence of various inorganic oxidants and organic compounds on mycelial growth and pycnidial production of *Plenodomus meliloti* in light ana darkness. *Canadian Journal of Botany* **56,** 1588–1593.

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Errata

The data in Figure 4 were obtained using the defined medium with 2 $ml\,l^{-1}$ bark extract, 5 µM salicylic acid, and fermenter-grown inoculum, not the oat medium as stated.

The data in Figure 5 were obtained using the oat medium supplemented with 2 $ml\,l^{-1}$ bark extract and fermenter-grown inoculum, not the defined medium as stated.

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