THE EFFECT OF LIGHT ON FRUITBODY PRODUCTION IN *PHOLIOTA MARGINATA*

SUTHAPHUN TRIRATANA

Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok.

(Received 1 May 1976)

Summary

Light appears to be necessary for the initiation of primordium, and for the early stages of stipe and pileus differentiation. Continuous light at intensity of 6 ft-cd is sufficient for optimum growth and fruiting. Stipe elongation, pileus expansion, and spore maturation occur in the absence of light.

Introduction

Investigations of the environmental factors affecting basidiocarp formation have been made on relatively few species. In addition, generalizations pertaining to these factors are not clear. Studies of individual species appear to be necessary.

Light is often required for one or more phases in fruitbody production in the Basidiomycetes. Marsh, Taylor, and Bassler[2] presented a review on the effects of light on reproduction of fungi, including many species of the Basidiomycetes. Many Basidiomycetes require light for normal pileus development, such as *Collybia velutipes*[3,4] and *Coprinus myceliocephalus*[5]. Stipe elongation often occurs in the absence of light[6,7]. The illumination time and the intensity of light are both significant variables in the effect of light on fruiting. Low light intensity is sufficient for fruiting in *Coprinus lagopus*[8, 9], while *Pleurotus ostreatus* needs a relatively high light intensity cycle for complete development[10]. Moreover, low light intensity delayed fruiting in *Polyporus brumalis*[3]. Some Basidiomycetes, however, are able to initiate and complete fruitbody development in the absence of light[6, 7, 11].

The aim of this study was to determine the light requirements for fruitbody production and development of *Pholiota marginata* in order to provide the best conditions for further studies of other factors controlling fruiting.

Materials and Methods

The strain of *Pholiota marginata* (Fries) used in experiments is one of the culture collections of the Department of Botany, University of California, Davis, as UCD Botany # 153.

A piece of mycelium from a stock culture was transferred to a Petri dish containing 20 ml of media. This Petri dish culture was incubated at 22°C under

fluorescent light (champion F 30 T 12 Warm White) for one week before being used as inocula. The inocula were cut with a sterilized cork borer 5 mm in diameter. These inoculum discs were then transferred into 250 ml Erlenmeyer flasks with cotton plugs containing 50 ml of nutrient media.

The basal medium was modified from the media described by Aschan[12] and by Snider and Raper[13]. The composition of the basal medium was as follow: α-D-glucose 10 g, KH_2PO_4 1 g, $K_2HPO_4.3H_2O$ 1 g, Mg $SO_4.7H_2O$ 1g, DL-asparagine 1g, ferric citrate solution (1% ferric citrate, O.64% citric acid in water) 0.5 ml, thiamine HCl 50 μg, $MnSO_4.4H_2O$ 20 mg, $ZnSO_4.7H_2O$ 20 mg, NaCl 100mg, $CaCl_2$ 100 mg, purified agar 20 g and glass distilled water 1000 ml.

Fruitbody production was measured by the determination of the dry weight of basidiocarps. The basidiocarp was dried in an oven at 80°C for 48 h, cooled in a desiccator over calcium chloride, and weighed to the nearest milligram.

It is generally accepted that the dry weight of basidiocarps is the most satisfactory value for measuring fruitbody production, since the number and size of fruitbodies often differ in each experiment. The measurement of dry weight alone, however, does not indicate the degree of fruitbody maturation. Aschan-Aberg[4] proposed, therefore, a fruitbody index for the stages of basidiocarp development of *Collybia velutipes*. Leonard and Dick[14], and McLaughlin[15] also employed a fruitbody index for basidiomycetes they studied. In the present study, a fruiting index for *Pholiota marginata* has been established to indicate the developmental stages of the fruitbodies (Fig. 1 and Fig. 2). The time at which, the basidiocarp first developed in each stage was recorded. As usual, primordia of stage–I or I were produced in every replicate at the same time, but subsequent stages often developed asynchronously. The fruitbody index presented was obtained by averaging the initial time required for each stage of development in the total replicates.

Light experiment was performed by exposing cultures growing on the basal media to different conditions of light as follows: continuous light (220 ft-cd); continuous dark; 12 h cycle of light (220 ft-cd) and dark; reduced light (6 ft-cd); and continuous dark after a one-week incubation in light (220 ft-cd). The intensity of light was measured with a Weston Illumination Meter Model 756. The illumination was provided by fluorescent tube lamps suspended above the shelf in the incubator. The temperature was set at 22°C in all treatments.

Results

The maximum dry weight of basidiocarps was obtained in reduced light (Table I). Under a high light intensity (220 ft-cd), the cultures fruited poorly and often the basidiocarps had abnormal morphology. Asymmetrical stipes often developed without pilei. The cultures grown under a high light intensity (220 ft-cd) for 12 h followed by a dark period of 12 h produced essentially the same amount of basidiocarps as the cultures grown under reduced light.

Light seemed to be necessary for fruitbody formation. Neither primordia nor basidiocarps were produced in continuous dark, but the cultures that were incubated

Stage			Fruitbody index
0		No growth of somatic mycelium.	0
–I		Somatic mycelium with undifferentiated primordia less than 1 mm in length.	0.5
I		Primordia 1 mm or more in length.	1
II		Primordia with differentiated stipe and pileus.	2
III		Stipes elongating or elongated, tapering or with parallel side, with convex pileus.	3
IV		Basidiocarps expanded with curved margin, basidia immature.	4
V		Basidiocarps mature and expanded, lamella brown, spores released.	5

Fig. 1. Fruitbody developmental stages and index values of *Pholiota marginata*.

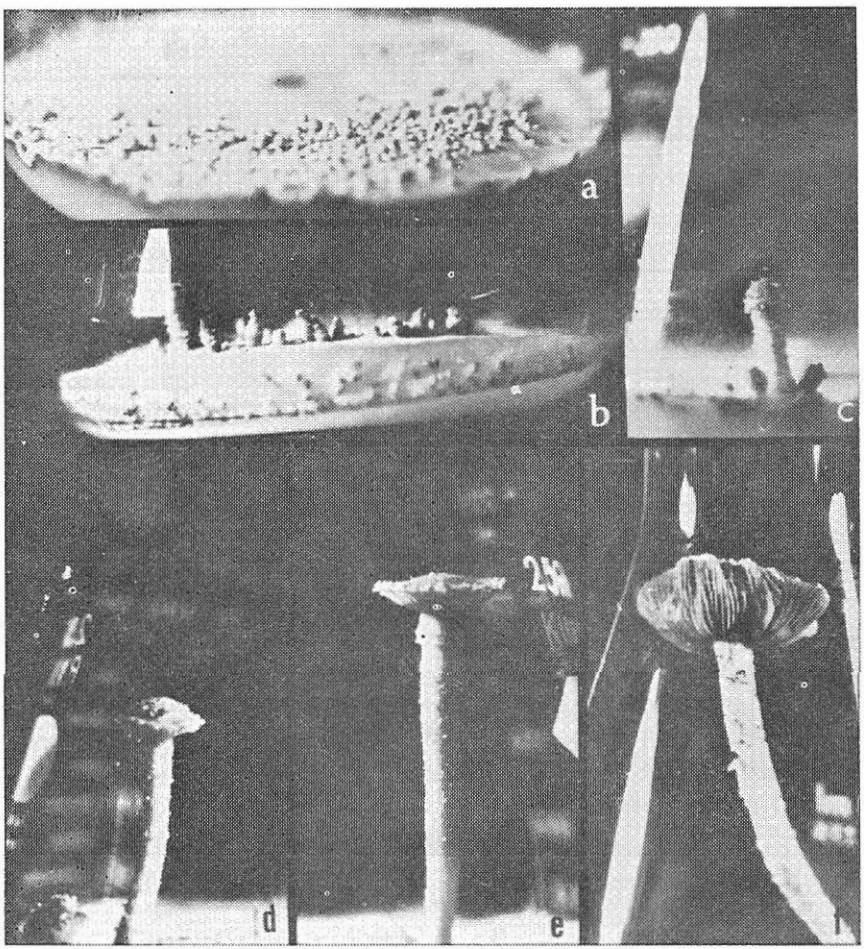

Fig. 2. Basidiocarp developmental stages of *Pholiota marginata:* stage I (a), stage II (b), stage III (c-d), stage IV (e) and stage V (f).

in light before being placed in continuous dark had a tendency to form mature basidiocarps. The period of exposure influenced the fruiting in the dark. The cultures grown in light for two weeks fruited better than the cultures incubated in light for only one week (Table I). The experiment was repeated using the xylose and peptone in place of glucose and asparagine in the basal medium. The results obtained confirmed the concept that the development of the basidiocarps could occur in the absence of light. Primordia of stage II had a greater tendency to develop further in the dark than primordia of stage I. In other words, primordia initiation and the early stages of stipe and pileus differentiation seemed to require light. The primodia of fruiting index 0.5-2 were produced in continuous light later than those in reduced light and alternating light (Fig. 3) but in these conditions they developed in equal rate and reached maturation at almost the same time.

Stipe elongation and pileus expansion apparently do not require light. The structure of basidiocarps grown in the absence of light differed slightly from those grown in light. The basidiocarps grown in the dark were slimmer and taller than those exposed to light during development. The stipes of basidiocarps incubated in the absence of light were distinctly longer (Table II) and straight. In reduced light, the length of the stipes was also greater when compared to those grown under continuous light.

TABLE I INFLUENCE OF LIGHT ON FRUITBODY PRODUCTION BY *PHOLIOTA MAGINATA*.

Condition of light	No. of replicates forming basidiocarps	Total no. of basidiocarps	Mean dry wt (mg)
Continuous light[a]	3 (9)[c]	4	50.4
Continuous dark	—	—	—
12 h cycle of light[a]-dark	9 (9)	45	155.0
Reduced light[b]	9 (9)	34	160.9
One week light[a]-dark	4 (9)	8	38.9
Two week light[a]-dark	5 (5)	16	97.7

Incubation time for all cultures was 55 days.

a. The light intensity was 220 ft-cd

b. The light intensity was 6 ft-cd

c. The total number of replicates is indicated in parentheses.

TABLE II INFLUENCE OF LIGHT ON THE ELONGATION OF THE STIPE OF *PHOLIOTA MARGINATA*.

Light condition	Mean stipe length (mm)
Continuous light (220 ft-cd)	28.5 (22)[a]
12 h light (220 ft-cd)-12 h dark cycle	38.1 (22)
Reduced light (6 ft-cd)	43.3 (34)
Dark[b]	60.0 (8)

a. The number of basidiocarps is indicated in parentheses.

b. The cultures were incubated in the light for one week before being placed in the dark.

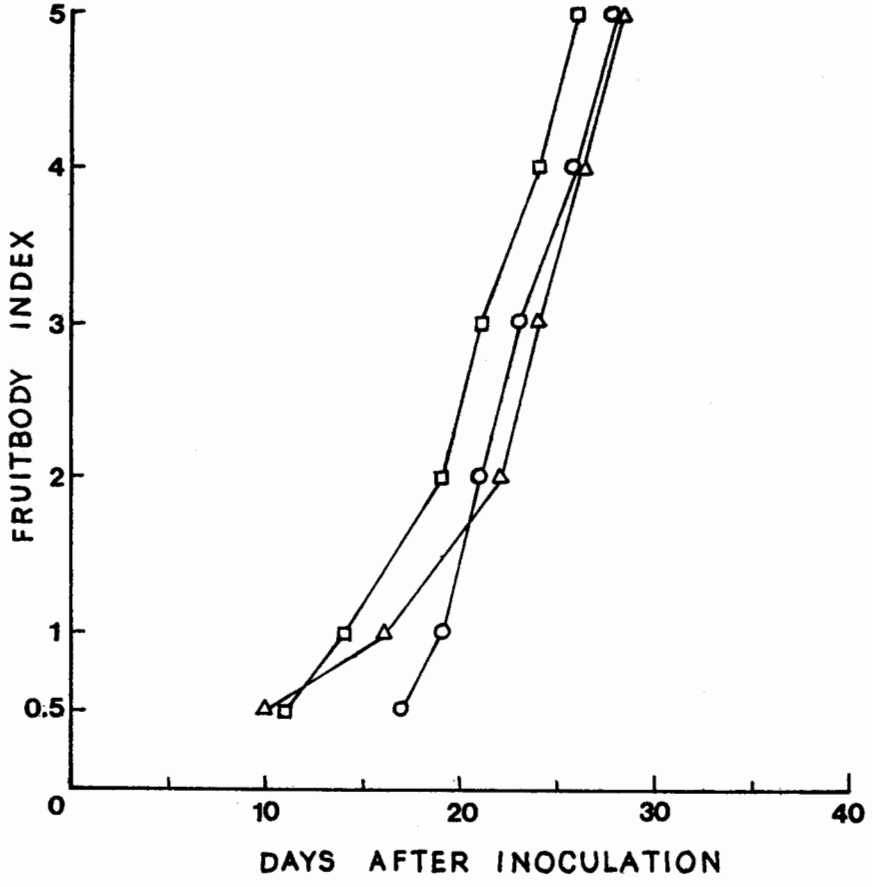

Fig. 3. Rate of fruitbody development by *Pholiota marginata* in different conditions of light: continuous light of 220 ft-cd (0), 12 h light of 220 ft-cd–12 h dark cycle (□), and reduced light of 6 ft-cd (△), at 22°C. Average of nine of replicates per point.

Discussion

Light appears to be necessary for fruitbody formation of *P. marginata*. A low light intensity of 6 ft-cd is sufficient to initiate primordia production and for full development of the basidiocarps. Vorderberg[8] found that a continuous light of low intensity was sufficient for fruitbody development of *Coprinus lagopus*. A short exposure time of one second of 25 ft-cd was found to be effective for initiation of basidiocarp development in *C. lagopus* by Madelin.[9]

Light seems to be a trigger for the biochemical processes leading to fruiting. The cultures of *P. marginata* that had been treated with light for a period of time were able to fruit in the dark. It is possible that the necessary substances required for fruiting were produced during the light treatment period and these unknown substances were responsible for the development of fruitbodies.

Primordia of stage I to stage II that were grown in light seemed to have the essential substances initiated by light; therefore, they were able to continue development in the dark. The cultures on the basal medium incubated in the light for two weeks had a greater tendency to fruit in the dark than those incubated in the light for one week. This was probably because some of primordia of stage II have already been produced on the cultures before the dark period. Lu[16] stated that basidiocarp formation in cultures of *Cyathus stercoreus* is a process in which photochemical reactions are involed, and the amount of light energy required is constant. He also proposed that a hypothetical "photoreceptive precursor" developed in the mycelium. Leach and Trione[17] studies the action spectra for light induced sporulation in several species of Ascomycetes and Fungi Imperfecti, and they proposed that P_{310} sporogens, resulting from a direct or indirect photochemical reaction, is the key substance for sporulation. It is possible that the light sensitive species in Ascomycetes, Fungi Imperfecti and Basidiomycetes might have the same or similar basic light-induced mechanism involved in sporulation and fruiting. Additional studies, however, are needed to clarify the mechanisms of these effects.

The development of the basidiocarps of *P. marginata* is independent of light. Pileus expansion, basidia maturation and releasing of spores occurred in the dark. The size of caps, however, was slightly reduced if light was absent during basidiocarp maturation. Light is required for normal pileus formation in a number of Basidiomycetes[3, 6, 7]. Lange[5] found that *Coprinus myceliocephalus* grown in the dark developed longer stipes with smaller pilei that did not expand but collapsed before the basidiospores were released. In *Schizophyllum commune* light is also required for normal basidiocarp formation[18].

The elongation of the stipe in darkness is a common phenomenon in the Basidiomycetes as reported by Brefeld[6] and Lange[5]. The stipe of species of *Coprinus* elongated in the dark[5, 6]. Chakrabarty[7] studied *Agariceus polyparus* and found that the length of the stipe increased in complete darkness. The stipe of the *Polyporus brumalis* also elongates in a low light intensity[3]. In the present study of *Pholiota marginata* the stipes of the basidiocraps incubated in the dark are longer and more straight than in the basidiocarps grown in continuous light.

The elongation of the stipe and the lack of curvature in the dark could possibly be due to the photoresponse of the hymenium in the stipe or the interaction of light with growth substances. The existence of a hormone has been reported in the fruitbodies of *Agaricus bisporus*[19, 20]. Hagimoto[4] domonstrated the relation between the height of the fruitbody, types of stipe curvature, and the hormone in the gills of *Agaricus bisporus*. Gruen[22] studied *Agaricus bisporus* and demonstrated the existence of an internal growth regulating mechanism located in the lamellae which controlled both stipe elongation and cap expansion.

References

1. Carlile, M.J. (1965) The phytobiology of fungi. *Annu. Rev. Plant Physiol.* **16,** 175-202.
2. Marsh, P.B., Taylor, E.E., and Bassler, L.M. (1959) A guide to the literature on certain effects of light on fungi. The Plant Disease Reporter Supplementary No. 261, pp. 251-312.
3. Plunkett, B.E. (1956) The influence of factors of the aeration complex aud light upon fruit-body form in pure cultures of an agaric and a polypore. *Ann. Bot.* **20,** 563-586.
4. Aschan-Aberg, K. (1960) The production of fruit bodies in *Collybia velutipes*. III. Influence of the quality of light. *Physiol. Plant.* **13,** 276-279.
5. Lange, M. (1948) Two species of *Coprinus* with notes on their culture characters. *Mycologia* **40,** 739-749.
6. Brefeld, (1877) Botanische Untersuchungen Uber Schimmelpilxe. III. Basidiomyceten. I. Arthur Felix, Leipzig, Germany, p. 266.
7. Chakrabarty, M. (1941) Production of fruit-bodies of *Agariceus polyporus* Berk. in artificial culture. *Curr. Sci. (Bangalore)* **10,** 26-28.
8. Vorderberg, K. (1950) Die Abhangigkeit der Fructkorperentwicklung bei *Coprinus lagopus* von inneren und ausseren Faktoren. *Planta* **37,** 612-625.
9. Madelin, M.F. (1956) Studies on the nutrition of *Coprinus lagopus* Fr., especially as affecting fruiting. *Am. J. Bot.* **20,** 307-330.
10. Koch, W. (1958) Untersuchungen über Mycelwachstum und Fruchtkorper bildung bei einigen Basidiomyceten. *Arch. Mikrobiol* **30,** 409-432.
11. Campbell, A.H. (1938) Contribution to the biology of *Collybia radicata* (Relh.) Berk. *Trans. Brit. Mycol. Soc.* **38,** 202-212.
12. Aschan, K. (1954) The production of fruit bodies in *Collybia velutipes*. I. Influence of different culture conditions. *Physiol. Plant.* **7,** 571-591.
13. Snider, P.J. and Raper, T.R. (1958) Nuclear migration in the Basidiomycete *Schizophyllum commune*. *Am. J. Bot.* **45,** 538-546
14. Leonard, T.J. and Dick, S (1968) Chemical induction of haploid fruiting bodies in *Schizophyllum commune*. *Proc. Nat. Acad. Sci. U.S.* **59,** 745-751.
15. McLaughlin, D.J. (1970) Environmental control of fruitbody development in *Boletus rubinellus* in axenic cultures. *Mycologia* **62,** 307-331.
16. Lu, B.C. (1965) The role of light in fructification of the Basiciomycete *Cyathus stercoreus*. *Am. J. Bot.* **59,** 432-437.
17. Leach, C.M. and Trione, E.J. (1966) Action spectra for light-induced sporulation of the fungi *Pleospora herbarum* and *Alternaria danci*. *Phytochem. Phytobiol* **59,** 621-630.
18. Raper, J. and G.S. Krongelb (1958) Genetics and environmental aspects of fruiting in *Schizophyllum commune Fr. Mycologia* **50,** 707-740.

19. Urayama, T. (1956) Das wuchshormon des fruchtkorpers von *Agaricus campestris L. Bot. Mag., (Tokyo)* **69**, 298-299.

20. Hagimoto, H. and M. Konishi (1960) Studies on the growth of fruitbody of fungi II. Activity and stability of the growth hormone in the fruit body of *Agaricus bisporus* (Lange) Sing. *Bot. Mag. (Tokyo)* **73**, 283-287.

21. Hagimoto, H. (1963) Studies on the growth of fruit body of fungi. IV. The growth of the fruit body of *Agaricus bisporus* and the economy of the mushroom growth hormone. *Bot. Mag. (Tokyo)* **76**, 256-263.

22. Gruen, H.E. (1967) Growth regulation in fruit bodies of *Agaricus bisyorus. Mushroom Sci.* **VI**, 103120.

บทคัดย่อ

แสงมีความจำเป็นต่อการเริ่มสร้างดอกเห็ดระยะที่เป็นตุ่ม (primordium) และจำเป็นต่อ differentiation ของก้านและหมวกในระยะแรก แสงสว่างต่อเนื่องซึ่งมีความเข้ม 6 ft-cd เพียงพอต่อการเจริญเติบโตและการสร้างดอกเห็ด การยืดตัวของก้าน การขยายตัวของหมวก ตลอดจนการที่สปอร์แก่เต็มที่สามารถเกิดขึ้นได้ในที่ไม่มีแสง