INFLUENCE OF LIGHT ON THE VEGETATIVE GROWTH AND FRUITBODY FORMATION OF PLEUROTUS SAJOR-CAJU (FR.) SINGER

IKECHUKWU A. OKWUJIAKO

(Received corrected version 15 December 2000)

ABSTRACT

The effects of light on the vegetative growth and fructification of *Pleurotus sajor-caju* are reported in this paper. Although light was shown to inhibit vegetative growth, it was found necessary for the production of friutbodies in vitro and under semi-field conditions. However, the light stimulus for fruitbody initiation could not be received by mycelium younger than five days. The implications of these findings on commercial mushroom farming are highlighted.

KEYWORDS: Light growth friutbody Pleurotus sajor-caju.

INTRODUCTION

Mushroom cultivation is an important way of recycling agricultural wastes such as straw, leaves, banana pseudostems, and other plant products. Those mushroom species such as *Stropharia rugoso-annulata* which grow on unsupplemented straw (Okwu and Hayes 1984) are of particular interest.

Pleurotus sajor-caju, a primary saprophyte, able to initiate the decomposition of plant lignocellulose, can also grow and fructify on uncompos ted and unsupplemented straw (Jandaik 1974). Jandaik and Kapoor (1974) obtained the greatest yield of this mushroom on banana psuedostems followed by rice straw, wheat straw and wheat straw compost. People in the tropical and sub-tropical regions including Nigeria, use edible mushroom including Pleurotus species as an ingredient in their food (Okwujiako 1990).

P. sajor-caju grows and produces fruitbodies at 25-30 °C without the necessity of a

casing layer (Jandaik and Kapoor, 1974; Okwu, 1981). Light requirement has been demonstrated for *P. ostreatus* (Eger et al., 1974; Zadrazil, 1974); for *Volvariella volvacea* (Chang, 1972) and *Coprinus lagopus* (Chapman and Fergus, 1973).

Jandaik and Kapoor (1974) observed that light did not affect the production of fritbodies in *P. sajor-caju*. However, during preliminary investigations, the present author observed that plate cultures of *P. sajor-caju* either failed to fructify at all or produced initials, which could not develop further into fruitbodies in dark incubators. On the contrary they produced better-developed fruitbodies when exposed to light. It was in view of these rather contrasting observations that the present work was undertaken to study the influence of light on the growth and fructification of this macrofungus.

MATERIALS AND METHODS

(a) Effect of light on the vegetative

Editor-In-Chief's Note: This paper was initially published in Global J. Pure & Appli. Sci. 2(1): 1-7 (1996). It is been republished because of fundamental errors in the earlier printing. This is regretted.

growth and fruitbody formation of *P. sajor-caju* on culture plates.

Thirty-five sterile disposable polystyrene Petri plates (8.5 cm diameter) of 2% malt extract agar (MEA) were prepared and inoculated with 6mm mycelial discs taken from the peripheral zone of young colonies. 25 of these plates were kept in continuous darkness (set A); 5 in alternating light (12 hours of 460 lux) and dark (12 hours) periods (set B) and the last 5 in continuous light of 460 lux (set C). Incubation took place at 25 ±1°C. After 5 days, 5 plates were withdrawn from set A for colony diameter measurement and examining for primordium formation. Subsequent examinations were carried out at 2 day intervals. Set B and C plates (in alternating light and dark conditions; and in continuous light respectively) were also measured and examined on the 5th and 7th days and left for further development.

(b) To determine the sensitivity of *P. sajor-caju* mycelium to illumination for friutbody formation after incubation in different durations of continuous darkness:

Forty-five plates of 2% MEA were prepared and inoculated as described above and incubated at 25 ± 1 °C. Five plates were exposed to continuous light from the beginning of the experiment. The remaining 40 plates were, at 2-day intervals, successively exposed to continuous light after measuring the colony diameter and examining for primordium formation. The first set of 5 plates was exposed after 3 days of dark incubation.

(c) Effect of light on the vegetative growth and fruitbidy formation of *P. sajor-caju* on rice straw

Preparation of grain spawn and cultivation on rice straw:

Millet grains were washed in continuous stream

of tap water for one hour to remove chaff and dust, and left in a bucket of tap water to soak overnight. After draining, the grains were distributed into one-liter, wide-mouthed, flat-bottomed pyrex flasks up to half-litre mark and autoclaved at 121 °C for one hour each day for 3 consecutive days. The final moisture level was 50-55% and pH 5-6.

The flasks were then inoculated with 1cm mycelial discs taken from MEA culture and incubated at 25 ± 1 °C for 2 weeks. The flasks were shaken at 2 – day intervals to disperse colonized grains and discourage tissue formation. The resulting spawn was used as 'seed' to inoculate rice straw cultures in cultivation cabinets.

To prepare the rice straw cultures, clean dry rice straw was cut up into less than 10cm pieces and soaked for 24 hours in a barrel of tap water. The wet straw was packed into 13 x 13 x 12cm polypropylene pots perforated at the base and allowed to drain until hand-squeezed straw just allowed drops of water to form between the fingers (Szudyga, 1978). The water content of straw so treated was determined by drying overnight at 85°c to constant weight.

Five hundred grams of such prepared straw were put into each of 35 pots. These pots were then covered with aluminum foil and autoclaved at 121 °C for one hour daily for 3 consecutive days. Each sterilized pot was aseptically inoculated with 5% spawn and incubated in specially constructed cultivation cabinets with a thermostat to maintain the temperature at 25 ± 1 °C. Humidity was maintained at 85 - 90% as measured with a hair-type hygrometer (Philip Harris). 25 of such culture pots were left in continuous darkness (Set D); 5 in alternating light (460lux) and dark conditions (Set E) and 5 in continuous light of 460 lux (Set F). After 5 days, 5 pots were withdrawn from Set D for visual estimation of mycelial growth and examining primordium formation. Subsequent examinations took place at 2-day intervals. Set E and F were examined everyday.

RESULTS

In experiment (a), vegetative growth after 5 days was highest in Set A (continuous darkness) and least in Set C, (continuous light Table 1). The effect of light on primordium formation was in reverse order to that on vegetative growth. After 7 days no primordium was formed in Set A while 55 and 330 primordia were produced in B and C respectively (plate 1).

In experiment (b) the set of plates in continuous light from the start had not yet initiated fruiting and just 1.4cm in colony diameter after 3 days. At the exposure of the first set from darkness after 3 days, the mean colony diameter was 2.6cm and on the 5th day, numerous primordia were observed. The 2nd set of plates were exposed on the 5th day and had a mean colony diameter of 6.3cm. All the plates in subsequent sets were completely covered with mycelium before exposure.

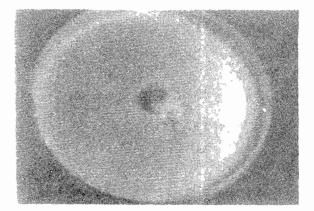
The result shown in figure 1 indicates that the greatest number of primordia and fruitbodies were produced in the set exposed after 7 days of dark incubation. The number of primordia produced in the plates exposed to continuous

Table 1: Effect of light on the vegetative growth and fruitbody formation of Pleurotus sajor-caju on MEA

| | Continuous darkness | Alternating | light | and | Continuous light |
|--|---------------------|-------------|-------|-----|------------------|
| Mean mycelial growth rate in 5 days (mm/day) | 15,8 | 12.3 | | | 7.0 |
| Mean no. of primordia after 7 | 0 | 55 | | | 330 |

Table 2: Effect of light on vegetative growth and fruitbody formation on rice straw.

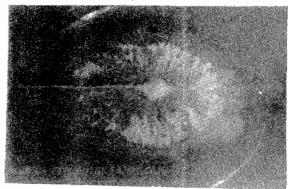
| | Weight (g) of fresh mushrooms | Yield/kg substrate | Time taken to produce primordia |
|----------------------------------|----------------------------------|--------------------|---------------------------------|
| Continuous darkness (D) | 0 | 0 | 00 |
| Alternating light & darkness (E) | 32.4 ± 8.3 | 64.8 | 15 days |
| Continuous light (F) | 53.9 ± 8.3 | 107.8 | 12 days |



a. continuous darkness



b. alternating light and darkness.



c. continuous light.

Plate 1:

Effect of light on the vegetative growth and primordium production in P. sajor - calu on MEA

light from the beginning of the experiment was relatively low.

The result of experiment (c) followed the same trend as experiment (a). In continuous darkness (D) the mycelium had completely permeated and covered the substrate before the 12th day, with a luxuriant over-growth covering the rim of the pots 2cm on the outside (plate 2.i). However, up to the 12th day of dark incubation

Values are means of yield from 5 replicate pots followed by standard deviation.

there were no primordia. The pots in alternating light and darkness (E) had less vegetative growth but primordia were observed on the 15th day. These developed into mature fruitbodies (plate 2,ii). The pots in continuous light (F) showed the least mycelial growth. The mycelium was scarcely visible on the top layers of the substrate but numerous primordia had developed before the 12th day. These matured into friutbodies (plate 2,iii). At the end of the experiment on the 28th day, the yield of fruitbodies was as summarized in table 2.

DISCUSSION:

The results clearly show that light inhibits mycelial growth but is absolutely necessary for the formation of primordia of *P. sajor-caju*. The development of fruitbodies from primordial stage also requires light. For when a plate culture containing developing fruitbody initials was put in the dark, the initials gradually reverted to mycelium. This observation was also made by Eger et al. (1974) for *P. ostreatus*.

Any factor favouring sustained profuse mycelial growth tends to suppress reproduction (Ross 1979). The corollary is also held as a fact that factors which stimulate reproduction may inhibit mycelial growth.

It is interesting to note that in experiment (b), no plate cultures produced premordia before 5 days from the time of

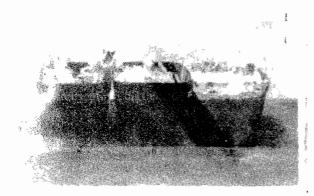


Plate 2: Effect of light on the vegetative growth and fruitbody production in P. sajor - caju on rice straw.

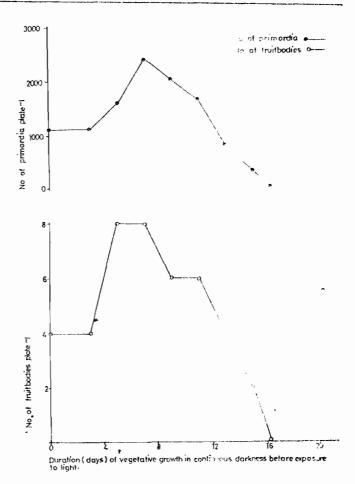


Figure 4 Sensitivity of <u>Pisajor-cajumy</u>celium toilli — milor for fruitbody för my after incubation for delibrent durations of arthusus darkness

inoculation. *P. sajor-caju* has an obligate requirement for light for fruitbody production, but this light stimulus cannot be received by a very young mycelium. The first 5 days of incubation seem to be the period of incompetence when the mycelium is intrinsically incapable of fructification. Similar observations were made for *Coprinus congregatus* (Ross, 1979) whose competent period starts after 4 days of incubation. This could be also equivalent to the duration of vegetative growth needed to attain minimum biomass enabling the culture to accumulate enough storage materials required for fruitbody formation.

In this investigation, the mycelium was found to be most sensitive to light after 7 days of dark incubation when the greatest numbers of primordia and fruitbodies were produced. While it is probable that after this period, the culture

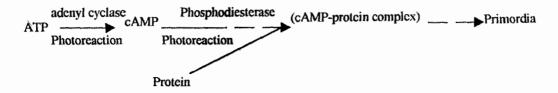


Figure 2: A scheme illustrating the mechanism of primordium formation in *Coprinus macrorhizus*. Source: Uno et al. 1974.

had accumulated enough reserve materials for maximum fructification, the absence of primordia and fruitbodies in cultures incubated for more than 17 days in darkness, suggests that sensitivity to light rather than accumulation of food reserve may be the critical factor.

Uno and Ishikawa (1973 a,b and 1974) and Uno, et al. (1974), have put up a scheme to explain the stimulatory effect of light and the repressive effect of excess glucose in the fructification of Coprinus macrorhizus through the metabolism of adenosine 3' 5'cyclic monophosphate (cyclic AMP or cAMP). According to their postulate, (fig. 2), the enzyme adenyl cycle synthesizes cAMP from ATP in illuminated mycelia of C. macrorhizus. The accumulated cAMP combines with cAMPbinding protein to form cAMP- protein complex said to be an intermediate complex in phogenesis. These reactions are primordiu light mediated.

CONCLUSION

It is seen from this work that although light suppresses mycelial growth of *P. sajorcaju*, this fungus obligately requires light for primordium formation and development of fruitbodies. It is also seen that this light stimulus cannot be received by a very young mycelium. Harnessing these features is important in successful commercial farming of *P. sajor-caju*. Spawn production and spawn running of this fungus should take place in the dark for the production of abundant mycelium which is then exposed to light at the most sensitive period for maximum fructification.

ACKNOWGEMENTS

The author immensely thanks Dr. W.A. Hayes for providing the initial culture for this work and for his suggestions.

The author is also grateful to the University of Calabar for supporting part of this work through a senate research grant.

REFERENCES

Chang, S. T., 1972. The Chinese Mushroom.

The Chinese University of Hong Kong,
99pp.

Chapman, E.S. and Fergus, C.L. 1973.

An investigation of the effect of light on basidiocarp formation of *Coprinus domesticus*. Mycopathol et Mycol. Appl, 51:315-326.

Eger, G., Gottwald, H.D. and Von Netzer, U., 1974. The action of light and other factors, on sporophore initiation in Pleurotus ostreatus. Mushr. Sci. ix (Part 1), 575 - 583.

Jandaik, C.L., 1974. Artificial cultivation of Pleurotus sajor-caju. Mushr. J. 22: 405.

Jandaik, C.L., and Kapoor, J.N. 1974. Studies on cultivation of *Pleurotus sajor-caju* (Fr.) singer. Mushr. Sci. ix (Part 1): 667-672.

Okwu, C.I.S., 1981. Ph.D Thesis Univ. of Aston Birmingham, 208pp. (Unpubl.)

Okwu, C.I.S., and Hayes, W.A., 1984. Casing layer in cultivation of *Stropharia* rugoso-annulata; Biologia Africana1:39

- 46

- Okwujiako, I.A., 1990. The effect of vitamins on the vegetative growth and fruitbody formation of *Pleurotus sajor-caju*.

 Mushr. J. Tropics 10: 35 39.
- Ross, I.K. 1979. Biology of the Fungi. McGraw-Hill, New York, 499pp.
- Szudyga, K., 1978. Stropharia rugoso-annulata.
 In: Chang S.T. and Hayes, W.A. (Eds.).
 the Biology of Cultivation of Edible
 Fungi. Academic Press, New York, pp.
 559-571.
- Uno, I. And Ishikawa, T., 1973a. Purification and identification of the fruitinginducing substance in Coprinus macrorhizus. J. Bacteriol, 113: 1240-1248.
- Uno, I. and Ishikawa, T., 1973b. Metabolism of adenosine 3' 5' cyclic monophosphate and induction of fruiting bodies in Coprinus macrorhizus. J. Bacteriol, 113:1249-1255.

- Uno, I. And Ishikawa, T., 1974. Effect of glucose on the fruiting body formation and adenosine 3'5' cyclic-monophosphate levels in *Coprinus macrorhizus*. J. Bacteriol. 120:96-100.
- Uno, I., Yamaguchi, M. and Ishikawa, T., 1974.

 The effect of light on fruiting body formation and adenosine 3'5' cyclic-monophosphate metabolism in Coprinus macrorhizus. Proc. Nat. Acad. Sci. U.S.A., 71 (2): 479 483.
- Zadrazil, F., 1974. The ecology and industrial production of *Pleurotus ostreatus*, *Pleurotus florida*, *Pleurotus cornucopiae* and *Pleurotus eryngii*.

 Mushr, Sci. ix: 621 652.