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THE FRUITING AND DEVELOPMENT OF RHODOTUS PALMATUS IN CULTURE

ORSON K. MILLER, JR.

Department of Biology
Virginia Polytechnic Institute & state University
Blacksburg, VA 24061

JOHN G. PALMER

U.S. Department of Agriculture, Forest Products Laboratory
P. 0. Box 5130
Madison, WI 53705

AND

LINNEA S. GILLMAN

Division of State & Private Forestry
U.S. Forest Service, USDA
Denver, CO 80236

SUMMARY

Rhodotus palmatus (Bull. ex Fr.) R. Maire was fruited in culture and the phenotypic responses of the fruiting body to varying light conditions studied. Fruiting occurs in green, yellow or red light above 500 nm but only in the absence of blue light below 500 nm. However, pileus pigment color and size as well as stipe length and size are altered by the light quality and percent transmission through the test filters. A very plastic species, such as this, also exhibits a broad phenotypic response in nature to the changes in light quality and other abiotic factors. The implications of properly evaluating phenotypic variation in systematic mycology are discussed.

INTRODUCTION

Rhodotus palmatus (Bull. ex Fr.) R. Maire is known to occur in Europe, North Africa and in eastern North America.

It is a pleurotoid agaric with creamy pink spores. It grows commonly on the limbs and branches of hardwoods, differs sharply from other agarics by possession of an unusual reticulate ridged cap cuticle, tuberculate warted spores (Miller, 1979) and unusual cultural characters (Miller, 1971). The distinctive network of ridges on the pileus (Miller, 1979, pl. 89) makes it easily identified on sight. Singer (1975) places the monotypic species in Tribus Rhodoteae Imai in the Tricholomataceae Roze of the Agaricales.

Field collecting by the senior author and herbarium collections by others indicate that it fruits sporadically throughout eastern North America during the summer and fall. Over its known range the species varies considerably in its size, pigmentation of the pileus and length of the stipe. In Michigan, for example, fruiting bodies collected in August , 1961, were pinkish orange , "buff pink" , "light salmon orange" to "Grenadine pink" of Ridgway (1912); while in Maryland the pileus color of fruiting bodies collected in mid-October, 1969, was more orange to orange-pink, "orange pink" to "light salmon orange". The Maryland specimens were smaller and had a more variable stipe length than those in Michigan. The host in Maryland was tulip tree (Liriodendron tulipifera L.). Elm (Ulmus americana L.) , basswood (Tilia americana L.) , and red maple (Acer rubrum L.) were hosts in Michigan. In Europe, Aesculus hippocastanum L. the horse-chestnut, is one of the hosts. This species clearly has a wide host range.

In order to explore the range of pigment development and the phenotypic response of the fruiting body, a study of R. palmtus in culture was initiated. The objectives were to explore the effects of light intensity and light quality on the development and maturation of the fruiting body as well as the fungal mycelium.

METHODS

A polysporous isolate of R. palmtus (OKM 8237) was obtained from sporocarps growing on hardwood logs near Chesapeake Bay, Anne Arundel County, MD. The equipment and methods were those used to study Panus fragilis O. K. Miller by Miller and Palmer (1977) including the same temperatures, light and dark periods, and media. All 4 mm diameter inoculum plugs of mycelium and agar were cut from the advancing edge of a one-week-old colony grown at 16°C in the dark. Each plug was inverted so that mycelium was in

TABLE I SPOROCARP DEVELOPEMENT IN *RHODOTUS PALMATUS* EXPOSED TO DIFFERENT LIGHT INTENSITIES AND COLORS (400-750 nm).

FILTER NUMBER	FILTER COLOR	* PERCENT	FILTER TRANSMISSION 400nm 500nm 600nm 700nm	MAXIMUM STAGE OF DEVELOPEMENT
	CLEAR	100		MYCELIUM
ES 849	PALE BLUE	60		MYCELIUM
ES 804	TINTED YELLOW	93		MYCELIUM
	CLEAR	100		MYCELIUM
RH 2424	BLUE	4		MYCELIUM
ES 837	MEDIUM MAGENTA	22		MYCELIUM
ES 866	DARK BLUE	2		PILEUS, MINUTE & STERILE
ES 821	LIGHT RED	16		PILEUS, FERTILE
RH 2092	GREEN	21		PILEUS, FERTILE
ES 809	STRAW	80		PILEUS, FERTILE
RH 2208	YELLOW	85		PILEUS, FERTILE
RH 2422	AMBER	61		PILEUS with long stipe, FER
RH 2423	RED	10		PILEUS, FERTILE
	NO LIGHT	0		LONG INITIALS

* PERCENT TRANSMISSION

= TRANSMISSION < 0.5 K (K=1,000 microwatts/cm^2)

= TRANSMISSION 0.5K OR > 0.5K

contact with the fresh agar in the center of a plastic 90 mm Petri plate, which was immediately placed in the growth chamber. Two runs with two Petri plates each were made under each of eleven filters plus clear plexiglass (Table 1). Temperature was adjusted to 22°C. Transmissions of light within the growth chamber are presented for six filters that are significant (Figs. 1–6). Miller and Palmer (1977, Figs. 2-6) included similar scans of both monochromatic light and light transmitted within the growth chamber for each filter reported in Table 1. To insure constant darkness as a control, Petri plates were covered in three ways: black cloth, aluminum foil, and black cloth plus aluminum foil. Otherwise, conditions were identical to those for uncovered plates. To study the effect of increased nutrient base on the size of the sporocarp and the time required for mature sporocarps to develop, the fungus was grown in 2800 ml wide-bottom flasks each of which contained one liter of the malt-agar medium. Flasks received light through a yellow (RH 2208) filter or were kept in the dark.

RESULTS

Generative mycelium was white and soon became woolly around the inoculum plug. The mycelium grew at the rate of 2.6 cm in diameter per week, and a 90 mm Petri plate was covered in 24 days. A series of orange droplets usually developed on the mycelial mat about 5 to 25 mm from the edge of the plate in about 30 days. Within a day or two, minute white initials formed under the drops. This occurred in the dark or in the absence of high levels of blue light up to 500 nm and usually above 1.0 K. In the presence of light above 500 nm, one initial per plate enlarged, became orange, and expanded into a mature sporocarp with pileus, lamellae, and stipe (Fig. 8). Under most conditions the sporocarp has the orange to orange-pink reticulate or netted pileus, tuberculate spores, and other normal features of the fungus. The entire process from inoculation to sporocarp maturation takes about 45 days.

The stipe does not require light for initiation. Long stalks (initials) are produced in darkness (Fig. 7), but no mature or Immature pilei develop (Table 1). Small amounts of light below 500 nm suppress development when mixed with larger amounts at 600 nm or higher (Table 1). This phenomenon is dramatically illustrated by the use of filter ES 866 (Fig. 3). Sporocarps with minute pilei (Fig, 10) on very short stipes were produced under this filter in very little

light (2% transmission) that was largely in the blue range with some in the red (Table 1). Lamellae, but no basidiospores, were observed on these sporocarps and the same phenomenon occurred on sporocarps grown in low amounts of unfiltered light, In addition, small amounts of light at 600 nm do not offset more intense blue light (410-440 nm) (Table 1, Fig. 3).

Vegetative growth was also reduced in blue light. During both experiments, mycelium exposed under the clear filter to high light intensity and ES 849 (60% transmission) failed to grow over the entire plate. Under the other filters at lower levels of blue light (ES 804, ES 837, RH 2424 and clear low intensity)., however, vegetative growth completely covered the plates. In only one case were initials produced in light from 400 to 450 nm at or above 0.6 K (ES 866, Table 1).

Normal sporocarps and basidia with basidiospores develop when blue light was excluded or reduced to a very low level as in ES 821 (Table 1). Light in the green, yellow, or red range was equally effective for the production of a mature fruiting body, and in fact the spectrum transmitted through RH 2092 does not overlap with that through RH 2423 (Figs. 4 and 6). However, the lifeform and pigmentation of the pileus cuticle vary. Under green light (RH 2092) the pileus was usually about 10 mm broad at maturity, pale orange in color, with well developed ridges and pits (Fig. 8). The stipe was straight and 18-22 mm high. Under amber light (RH 2422) the pileus was also about 5-10 mm broad, with very obscure reticulations and a bright orange color, The stipe, however, was very long (50-65 mm) and flexuous (Fig. 9). Those produced under yellow (RH 2208 and ES 809) filters had orange-pink pilei, 8-15 mm in diameter with well developed ridges and pits. The stipe was straight and ranged between 10-22 mm in length.

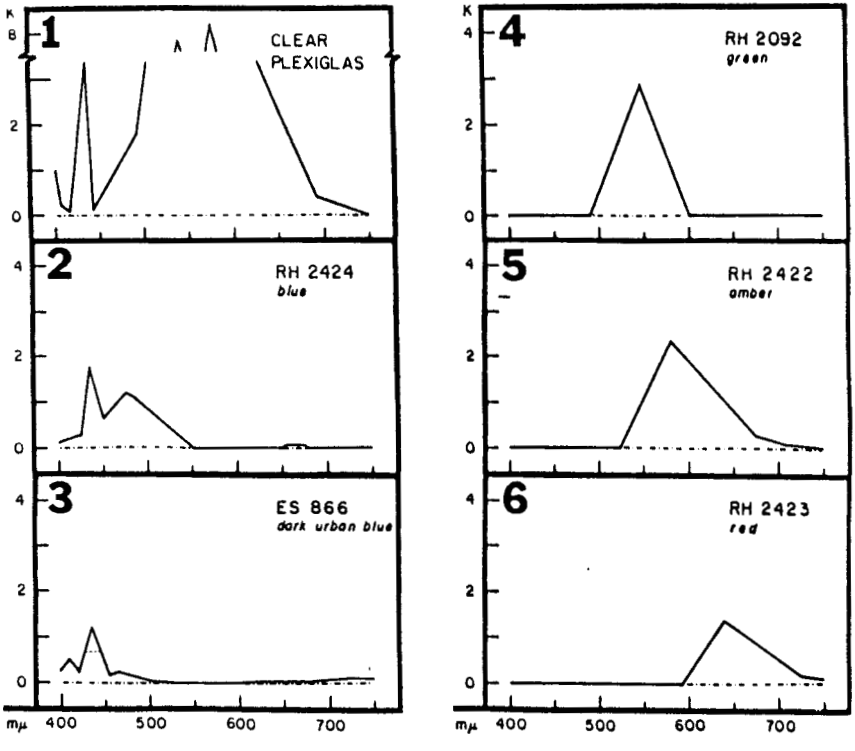
Sporocarps grown in the large flasks under yellow light (RH 2208) resemble in size, statue, form, and color those encountered in nature. The stipe was longer (60-90 mm), but the diameter about the same as those found in nature, 5-7 (-10) mm. Mature caps were consistently 18-22 mm in diameter which is within the lower size range of those found in nature.

DISCUSSION

Phenotypic responses of *Rhodotus palmatus* to different wavelengths of visible light result in substantial modifications of form and color of the fruiting body. For example, amber light (Filter RH 2422, Table 1) resulted in the development of a fruiting body with an elongated, flexuous stipe (Fig. 3) and a bright orange, small pileus with obscure reticulations. Under green light (Filter RH 2092, Table 1) on the other hand, the fruiting body had a short, straight stipe (Fig. 2), and a pale orange, large pileus with well developed ridges and pits. Under both conditions, nomal basidia and spores were produced. The Michigan fruiting bodies, noted earlier, collected under a canopy of green leaves, had soft pinkish-orange colors in the pileus similar to those associated with the green filter while those fruiting in October in Maryland, after the leaves had fallen, were more orange to orange-pink in color. Since relatively high light intensities prevailed at the field sites, no particular differences in stipe length were noted.

Light at the blue end of the spectrum has been commonly associated with light-induced phenotypic responses in Basidiomycetes. Bulat (1954) investigated the production of pigment in cultures of *Dacrymyces ellisii* Coker. Coloration in sporocarps under field conditions varied from yellow to deep orange. A color range from white to buff to orange-buff and finally deep orange was produced in mycelium in culture at both 50 and 100 foot candles, It required at least 3 to 4 days to reach the maximum color intensity under both light intensities tested. The pigment did not form in the dark but once formed in the light it was not lost or modified by darkness. Both *Polyporus brumalis* Pers. ex Fr. and *Collybia (Flammulina) velutipes* (Fr.) Kummer "require light for normal cap development" (Plunkett, 1958). However, *P. brumalis* attained larger cap diameters as light intensities increased but the stipes were often considerably longer than usual in low light levels. Of interest is the fact

Figs. 1-6. Quantities of light (K = 1,000 microwatts/cm^2) between 390 and 750 nm radiated from cool white fluorescent lamps and transmitted through six pigmented filters (RH = pigment in plexiglass; ES = pigment in cellulose acetate supported by clear plexiglass).

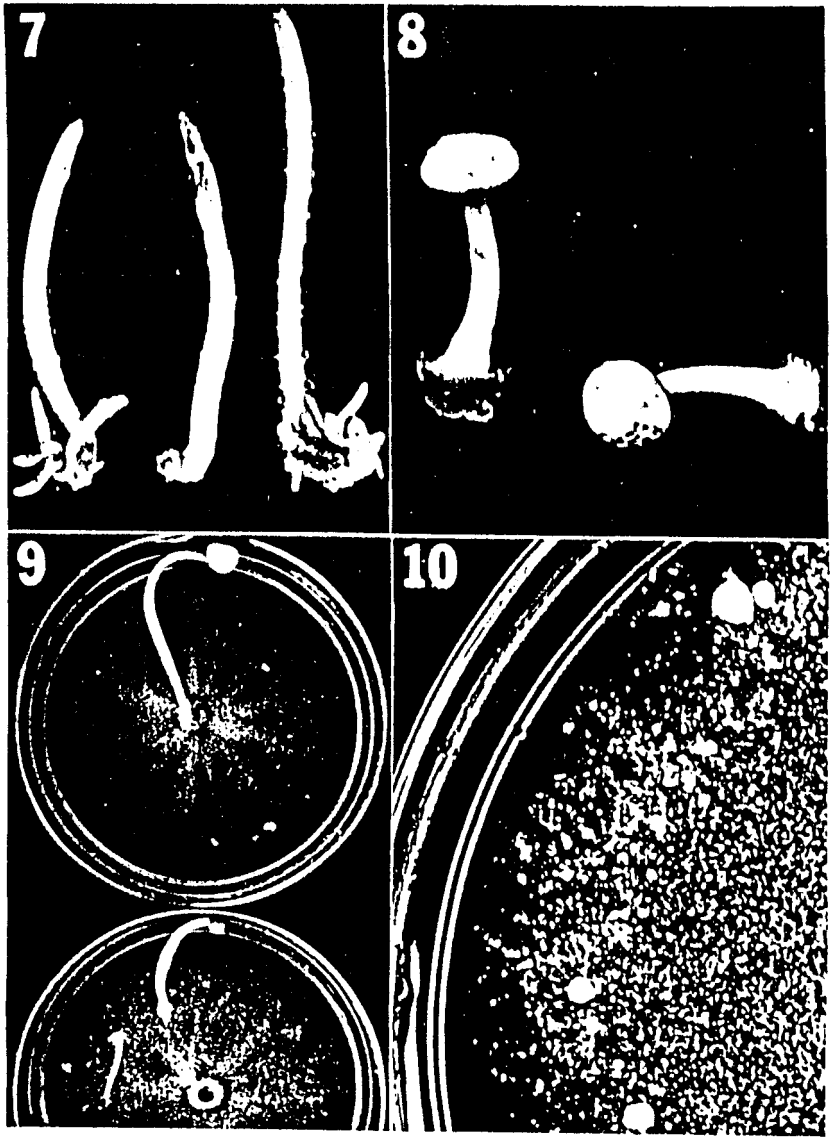

K
8
1
CLEAR
PLEXIGLAS
2
RH 2424
blue
3
ES 866
dark urban blue
4
RH 2092
green
5
RH 2422
amber
6
RH 2423
red
0
2
4
mμ 400 500 600 700

that similar responses may be induced by other factors, e.g. *Collybia velutipes* had elongated stipes and reduced cap sizes in response not to light but to "as little as 1.8% of carbon dioxide". Sporocarp initiation and/or development, subsequent maturation of the hymenium, and the production of fertile spores are other responses generally occurring in the presence of blue light (Alasoadura, 1963; Borriss, 1934; Aschan-Aberg, 1960; Ingold and Nawaz, 1967; Barnett and Lilly, 1952: and Miller and Palmer, 1977). Blue light, according to Borriss (1934), induces phototropism in *Coprinus lagopus* (Fr.) Fr.

By contrast, *Rhodotus palmatus* responds at the red end of the spectrum. The maturation of the sporocarp and development of the spores take place when there is light only at the red end of the spectrum, i.e. above 490 nm (Table 1). In addition, the phenotypic characteristics, e.g. color, stipe length, and pileus size, vary considerably with changes in the red end of the spectrum (Table 1). Consequently, when screening isolates for the induction of fruiting bodies with fertile hymenia but unknown requirements, it is necessary to use the red as well as the blue end of the spectrum. There also appears to be a necessity to have a rather high level of light over extended periods of each day. No attempt was made to define the necessary quantity of light nor the amount required per day. However, it can be seen from Table 1 that successful fruiting of *Rhodotus palmatus* was attained using a day length of 12 hours with 1.2 to 3.5 K (1000 microwatts per cm^2) of light.

In summary, in order to properly interpret the variation in the characteristics of a given fungus species, the range in phenotypic responses must be studied. In the absence of this type of investigation, the change in the shape, size, and pileus color of *Rhodotus palmatus* might be assumed to be species specific differences. In fact,

Figs. 7-10. Fruiting responses of *Rhodotus palmatus* in dark and light: Fig. 7, Initials formed in dark, Fig. 8. Mature sporocarps having lamellae with basidiospore-bearing hymenia produced in green light (Filter RH 2092). Fig. 9. Fruiting bodies with elongated stipes developed in amber light (Filter RH 2422). Fig. 10. Infertile sporocarps with small pilei and short stipes produced at low quantities of blue and red light (Filter ES 866).


7
8
9
10

however, very plastic species under different abiotic influences will exhibit wide variation in their phenotype as reported by Miller (1971) for Lentinellus cochleatus (Pers. ex Fr.) Karst,, in contrast to more conservative responses of other taxa, Lastly, any attempt to induce Homobasidiomycetes to fruit in culture must take into account the fruiting response reported here in the presence of red light rather than the usual fruiting requirement for blue light,

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LITERATURE CITED

Alasoadura, S. O. 1963. Fruiting in *Sphaerobolus* with special reference to light. Ann. Bot., NS 27:123-145.

Aschan-Aberg, K. 1960. The production of fruit bodies in *Collybia velutipes* III. Influence of quality of light. Physiologia Plantarum 13:276-279.

Barnett, H. L. and V. G. Lilly. 1952. The effect of color of light on sporulation of certain fungi. Proc. West Virginia Academy Science 24:60-64.

Borriss, H. 1934. Uber den Einfluss ausserer Faktoren auf Wachstum und Entwicklung der Fructkorper von *Coprinus lagopus*. Planta 22:644-684.

Bulat, T. J. 1954. Effect of light on color in *Dacrymyces*. Mycologia 46:32-36.

Ingold, C. T. and M. Nawaz. 1967. Sporophore development in *Sphaerobolus*: effect of blue and red light. Ann. Bot., NS 31:469-477.

Miller, O. K., Jr. 1971. The relationship of cultural characters to the taxonomy of the agarics. p. 197-215. IN: Evolution of the higher BAsidiomycetes. R. H. Petersen, ed. University of Tennessee Press, Knoxville Tennessee. 562 p.

Miller, O. K., Jr. 1979. Mushrooms of North America. 3rd softback edition, E. P. Dutton, Inc. New York. 368 p.

_____ and J. G. Palmer. 1977. Effect of light quality on development of fruiting bodies of *Panus fragilis*. USDA, Forest Service Research Paper, FPL 300. 15 p.

Plunkett, B. E. 1958. The effects of aeration and light on toadstools. Mushroom Growers' Association Bull. 99:76-79.

Ridgway, R. 1912. Color standards and color nomenclature. Published by the author. Washington, D. C. 43 p., 53 pl.

Singer, R. 1975. The Agaricales in Modern Taxonomy. J. Cramer, West GErmany 912 p., 84 pl.