Light Inhibition of Thallus Growth of Armillaria mellea

James E. Doty and P. C. Cheo

Research Division, Los Angeles State and County Arboretum, Arcadia, California 91006.

ABSTRACT

Potato-dextrose agar (PDA), when pre-exposed to a light source for 21 days, does not support growth of *Armillaria mellea*. On fresh PDA, 2 h exposure to light every 24 h during culture, inhibits thallus growth 50%. Different isolates of *A. mellea* on PDA vary in their sensitivity to light inhibition. Light was less inhibitory to less vigorous isolates and was most inhibitory to isolates normally producing abundant rhizomorphs. Replenishing cultures in light and dark incubators with light and dark-treated media resulted in little inhibition of thallus development under dark incubation, but significant inhibition under light incubation. These results are interpreted as indicating light may directly inhibit thallus development.

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Raabe (1) reported in 1953 that growth of *Armillaria mellea* (Vahl ex Fr.) Kummer. in culture is inhibited by light. Subsequent work by Weinhold and Hendrix (3) showed that *A. mellea* and *Rhizoctonia solani* Kuhn are sensitive to substances produced when media were illuminated with 40 W incandescent bulbs. Strong inhibition of the growth of an *A. mellea* isolate obtained from R. D. Raabe (Raabe 152) was noted in our laboratory on potato-dextrose agar (PDA) pre-exposed to light (5,400 lux, G.E.-fluorescent lamps, 14 W, cool-white) or exposed during culture. When plates of PDA had been illuminated for 21 days they became toxic to the fungus. Further transfer of the inoculum plugs to fresh PDA and subsequent incubation in the dark, produced no growth. On PDA medium not pre-exposed to the light, the initiation of growth from the inoculum plug took place either in the dark or under illumination. In the light, however, growth rate and rhizomorph formation was inhibited when compared to dark controls. In another experiment, cultures were illuminated for 0, 2, 4, 8, and 24 h/day. Illumination for 2 h/day resulted in a 50% reduction in growth.

Variations in the degree of growth inhibition among individual culture plates was correlated with initial strength of the inoculum. The initial growth rates of individual inocula were found to be greatly influenced by the physiological state of the parent thallus which was influenced by age, environmental conditions, and the condition of the medium. Variation in light inhibition among replicate plates could be greatly reduced by careful attention to the age of the parent thallus and the zone within each thallus from which the inocula were cut.

Variation in the degree of growth inhibition was also correlated with the inherent vigor of the isolate being tested. When seven isolates of differing cultural growth habits were tested for light sensitivity, all grew less in light than in the dark. Light was less inhibitory to less vigorous isolates (slow growth, no rhizomorphs) and was most inhibitory to isolates which normally grew rapidly and produced abundant rhizomorphs (Table 1).

The above observations, which corroborate the work of previous authors (1, 2, 3) do not, however, indicate whether or not the thallus of *A. mellea* itself is directly affected by light. To determine this, an experiment was designed wherein evidence of direct inhibition might be obtained.

Forty *A. mellea* cultures (Raabe 152) were prepared using inocula cut with a No. 1 cork borer from the edge of a thallus growing on water agar as in previous experiments. However, each inoculum was then placed in a 125-ml Erlenmeyer flask containing 10 ml of sterile potato-dextrose broth (PDB) and agitated on a rotary shaker (100 cpm) for 1 wk to produce uniform growth. The cultures were then allowed to stand for 5 days without agitation to allow differentiation of aerial and subaerial growth.

Forty-eight 250-ml Erlenmeyer flasks were filled with 220 ml PDB and autoclaved at 121 C for 15 min. These flasks were then divided into two sets. One set of 24 flasks was placed in light-tight boxes and placed in a dark incubator. The second set of 24 flasks were placed in an incubator which was illuminated 12 h/day. The forty *A. mellea* cultures were similarly divided into two groups such that twenty cultures received no illumination and twenty cultures received 12 h of illumination per day. Both incubators were adjusted to 24 C.

Each day at the beginning of the 12-h light period for the illuminated cultures, the cultures were removed from the incubators. The old medium in each culture flask was

TABLE 1. Growth of seven isolates of *Armillaria mellea* cultured on PDA in constant darkness or with 5,400 lux constant illumination

Isolate Number	Rhizomorphs in culture	Dry Weight (g)		Inhibition (%)
		Cultured in dark	Cultured in light	
764	abundant	194.7 ± 51	38.3 ± 4[a]	80
776	abundant	190.4 ± 20	59.9 ± 4	69
781	none	50.3 ± 21	20.7 ± 14	59
794	few	95.4 ± 6	27.1 ± 7	72
795	none	34.5 ± 12	21.0 ± 6	39
796	abundant	274.6 ± 40	81.2 ± 9	70
804	abundant	222.4 ± 15	39.2 ± 10	82

[a]The values given are the averages of five replicates for each treatment and their standard deviations.

TABLE 2. Growth of *Armillaria mellea* cultures illuminated 12 h/day or 1 h/day (handling time only) and provided daily with new portions of potato-dextrose broth previously stored in dark or illuminated 12 h/day

Illumination (hours/day)			
Cultures	Media	Growth (mg dry weight)	Number of replicates
1	0	258 ± 35[a]	9
1	12	232 ± 65	8
12	0	91 ± 36	10
12	12	100 ± 24	10

[a]Data shown are the means and standard deviations of eight to ten replicates as indicated.

discarded and replaced with medium which had been stored with the cultures in the incubators. One-half, or 10, of the cultures incubated in the dark received medium that had been stored in the dark and the remaining half of the cultures received medium that had been illuminated 12 h/day during storage. Similarly, one-half of the cultures incubated with 12 h of illumination per day received illuminated medium and one-half of the cultures received medium stored in the dark. After a total handling time of 55 min, the cultures were returned to their respective incubators. All the culture vessels were marked so that the treatment they received was consistent throughout the experiment.

After being cultured in this manner for 19 days, the thalli were each removed from the flasks, rinsed with distilled water, and their dry weights determined. Growth of thalli exposed to 12 h of illumination per day was inhibited approximately 60% (Table 2). This inhibition was not significantly diminished by daily replacement of the medium with dark-stored medium. The growth of thalli exposed to 1 h of illumination per day was only slightly influenced by pre-exposure of the medium. The lack of sensitivity of the thalli to light-exposed media in this experiment may be explained by the observation of Weinhold and Hendrix (3) that *A. mellea* is less sensitive to exposed media after growth is initiated.

These data indicate that direct inhibition of the thallus by light is a major factor in the growth of *A. mellea* in culture. In established cultures such as those in our studies, direct inhibition may be greater than indirect inhibition caused by light-medium interactions. Further work is required to determine whether the spectral responses reported by Raabe (1) are the results of medial or thallal sensitivities.

LITERATURE CITED

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