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LOW-LEVEL BIOLUMINESCENCE DETECTED IN *MYCENA HAEMATOPUS* BASIDIOCARPS

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Bioluminescence has been reported to occur in approximately 40 species of fungi (see Wassink, **1948**, **1978**, **1979**), nearly two thirds of which are members of *Mycena*. Luminescence, varying among species, occurs in either the basidiocarp, the mycelium, the spores, or in combinations thereof (see O'Kane et al., **1990**). Photometers have long been used to study bioluminescence (see Reynolds, **1972**); however, perception of luminescence in fungi has been largely by eye and/or documented photographically. Use of photometric measurements has generally been restricted to physiological studies of common species and examinations of the possible occurrence of bioluminescence in species suggested to be luminous (e.g., Bermudes et al., **1990**). Knowledge of the distribution of fungal luminescence, therefore, has been generally restricted by the detection limits of the human eye.

Bioluminescence in fungi ranges from readily detectable to dim and difficult to perceive, even to the dark-adapted eye. Variation in human visual acuity and the length of time allowed for dark-adaptation make comparisons of total light output based on visual observation difficult. Luminescence is sometimes described as weak (see Wassink, **1948**, **1978**, and references cited therein) but there are few quantitative measurements of low-level activity with which to make reliable correlations. Petersen and Bermudes (**1992**) have reported that some single spore isolates of *Panellus stypticus* (Bull : Fr.) Karst. emit vary low levels of bioluminescence quantified using a photometer. We report here low levels of bioluminescence in the basidiocarps of *Mycena haematopus* (Pers. : Fr.) Quélet and compare its level of mycelial luminescence with cultures of *P. stypticus* (monokaryotic and dikaryotic), *Lamprotomyces japonicus* (Kawamura) Sing. (monokaryotic and dikaryotic), *Mycena citricolor* (Berk. & Curt.) Sacc. (dikaryotic), *Omphalotus olearius* DC : Fr. (dikaryotic) and *Armillariella mellea* (Vahl : Fr.) Karst. (dikaryotic).

Specimens of *Mycena* collected and examined photometrically were: *Mycena haematopus*, Wisconsin, Milwaukee Co., Grant Park, 4–X–90, coll. D. Bermudes, Milwaukee Public Museum [MIL] catalog 146910; *M. haematopus*, Wisconsin, Green Co., Abraham Woods, 14–X–90, coll. D. Bermudes; *M. leaiana* (Berk.) Sacc., Wisconsin, Milwaukee Co., Grant Park, 4–X–90, coll. D. Bermudes; and *M. leaiana*, Wisconsin, Green Co., Abraham Woods, 14–X–90, coll. D. Bermudes. *Mycena haematopus* was found to possess low levels of luminescence in both young and mature specimens. In *M. leaiana*, also collected in varying stages of development, no luminescence was detected. We used a Pacific Photometrics photomultiplier coupled to an EMI type

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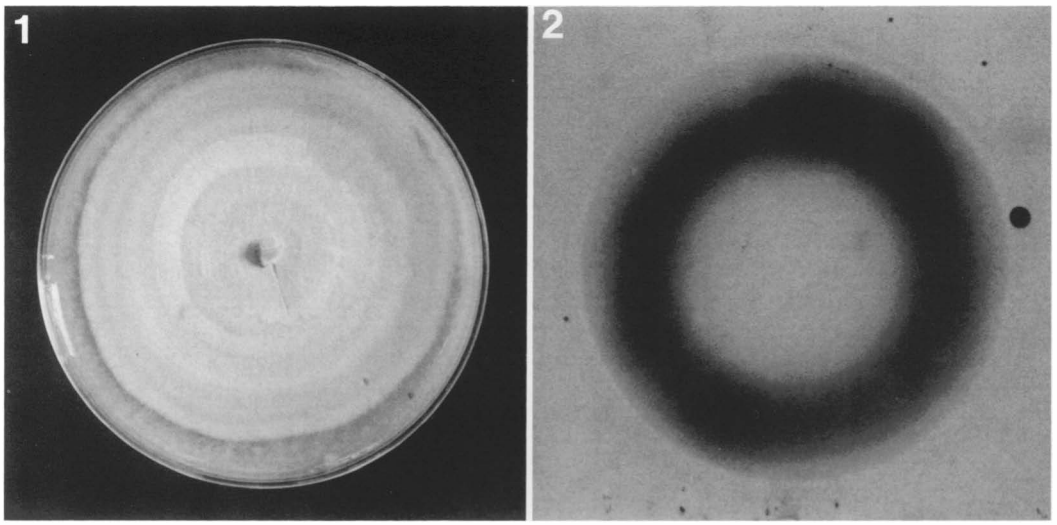

FIGS. 1, 2. *Mycena haematopus*. 1. *M. haematopus*, ATCC 62052, grown on bread crumb agar (3% bread crumbs, 1.5% agar) under normal laboratory lighting conditions. 2. X-ray film exposed to the same culture as FIG. 1 showing maximum light emission occurs at the periphery of the culture. Cultures in petri plates with the lids on were inverted over the X-ray film.

9781 A phototube as previously described (Bermudes et al., **1990**). In one collection of *M. haematopus*, luminescence from a single cap 17 mm in diameter measured 1.6×10^7 quanta sec^{-1} from above the cap and 3.7×10^7 quanta sec^{-1} from below. Luminescence from the stipe measured only 6.5×10^5 quanta sec^{-1}. In the second collection, luminescence from a single cap 11 mm in diameter measured 1.7×10^7 quanta sec^{-1} from above the cap and 1.5×10^7 quanta sec^{-1} from below while the stipe did not exhibit detectable luminescence. In contrast, a basidiocarp of *Panellus stypticus* approximately 10 mm average diameter that was collected at the Green Co. location measured 2×10^9 quanta sec^{-1} from above the cap and 4.6×10^9 quanta sec^{-1} from below. Basidiocarps of *P. stypticus* were visible to the dark-adapted eye while those of *M. haematopus* were not. However, light-emission from *M. haematopus* was detectable on X-ray film after 20 h exposure.

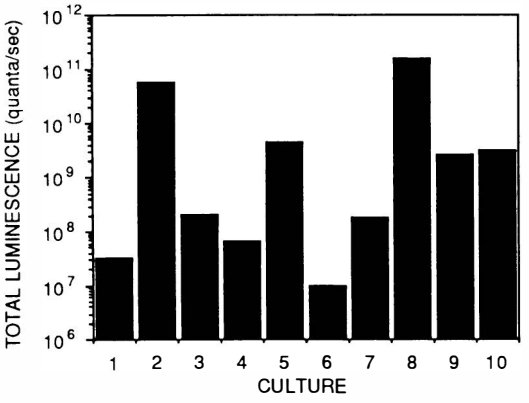

FIG. 3. Relative maximum levels of total bioluminescence observed in cultures grown on bread crumb agar. 1) *Mycena haematopus*, ATCC 62052; 2) *Panellus stypticus*, ATCC 66462 (dikaryotic); 3) *P. stypticus*, R. H. Petersen 2416-3 (monokaryotic); 4) *P. stypticus*, R. H. Petersen 2416-19 (monokaryotic); 5) *Lamptero-myces japonicus*, R. H. Petersen 2305, polyspore-2; 6) *L. japonicus*, R. H. Petersen 2305-4 (monokaryotic); 7) *L. japonicus*, R. H. Petersen 2305-13 (monokaryotic); 8) *Mycena citricolor*, ATCC 34884; 9) *Omphalotus olearius*, University of Wisconsin Center for Forest Mycology Research HHB 2268-s; and 10) *Armillariella mellea*, University of Wisconsin Center for Forest Mycology Research GB 795-s.

The mycelium of *M. haematopus* from Germany has recently been reported to be dimly bioluminescent (Treu and Agerer, **1990**), although no quantitative measurements were made. Attempts to culture *M. haematopus* from the Wisconsin specimens either by spores or by tissue culture (Molina and Palmer, **1982**) using YM (0.4% yeast extract, 1% malt extract, 0.4% sterile-filtered glucose and 1.5% w/v agar) or MsY (2.5% w/v molasses, 0.5% w/v yeast extract, and 1.5% w/v agar) media were unsuccessful. Two cultures of *M. haematopus* were obtained, strain 62052 (ATCC) and one cultured from the tissue of a

specimen from North Carolina, Macon Co., Blue Valley, Forest Service rd. 79, 23–V–89, coll. S. A. Gordon, field no. 1958. Both cultures were also found to emit low levels of light, but only the brighter of the two, ATCC 62052, was capable of exposing X-ray film after 48 h (FIGS. 1, 2). Maximum light emission occurred at the periphery of the culture when grown under normal laboratory light conditions. Similar results were reported by Bermudes et al. (**1990**) for *Panellus stypticus*. Comparison of the light emission levels of some bioluminescent cultures is presented in FIG. 3. The level of luminescence emitted by *M. haematopus* is similar to some single-spore isolates of *P. stypticus* (Petersen and Bermudes, **1991**) and *Lampteromyces japonicus* and significantly lower than dikaryotic cultures of *L. japonicus, P. stypticus, Omphalotus olearius, Mycena citricolor* or *Armillariella mellea*. Culture origins and the media used are given in the legend to FIGS. 1, 3.

Luminescence was not reported for the basidiocarps of *M. haematopus* in the compilations by Wassink (**1948, 1978, 1979**) or O'Kane et al., (**1990**), nor is *M. haematopus* synonymous with any of the species names listed as luminescent based upon the taxonomy of Singer (**1986**). Luminescence has also been reported in the mycelium of morphologically similar species *M. sanguinolenta* (Alb. & Schw.: Fr.) Quélet by Bothe (**1931**), but not in the basidiocarp. Photometric measurements of these basidiocarps may also reveal low-level bioluminescence.

The occurrence of low-level luminescence raises questions both new and old. There are a number of nonexclusive hypotheses for the occurrence of bioluminescence of fungi discussed by Sivinski (**1981**). One hypothesis suggests that luminescence occurs for the attraction of dispersal agents (McAlpine, **1900**; Ewart, **1906**; Johnson, **1919**; Lloyd, **1974**), although this has been doubted by some (e.g., Murrill, **1915**; Buller, **1924**). However, the presence of luminescence most strongly in the gills (e.g., *P. stypticus*; Buller, **1924**, Watling, **1981**; O'Kane et al., **1990**) or spores (e.g., *Mycena rorida* var. *lamprospora* Corner or *M. pruinosoviscida* var. *rabaulensis* Corner; see Wassink, **1978, 1979**) and some attraction of invertebrates to luminous basidiocarps (Sivinski, **1981**) would seem to support this view. Some specimens of *M. haematopus* we examined also emitted light most strongly from the gill-bearing side of the cap. However, the visual acuity and attraction of invertebrates by the appropriate wavelengths and intensity have not been established. Further studies along the lines of those performed by Sivinski (**1981**) as well as determinations of visual detection limits of the organisms hypothesized to be affected would be required in order to establish an effect. Another hypothesis suggests that light emission is a by-product of some other biochemical function (Buller, **1924**). The presence of luminescence solely in the mycelium and base of the stipe of *A. mellea* (Buller, **1924**) might support this view. Fungal luminescence is oxygen-dependent (Buller, **1924**; Hastings, **1952**) and in cell-free systems (Airth and Foerster, **1962, 1964**) is stimulated by hydrogen peroxide (D. Bermudes, unpubl. results). After chemical activation, the substance panel isolated from *P. stypticus* is chemiluminescent in the presence of hydrogen peroxide (Nakamura et al., **1988**; Shimomura, **1989**), while the presence of other chemicals from crude extracts of *P. stypticus* stimulated by superoxide anion have also been indicated (Shimomura, **1991**). A relationship of the bioluminescence reaction to lignin degradation has been suggested (Lingle, **1989**) and may act as a detoxification method for peroxides, such as those formed in the process. Many of the luminous fungi engage in wood and leaf litter decay, including *M. haematopus*. A third hypothesis for fungal luminescence, accounting for its presence in the mycelium and/or the basidiocarps, is the repulsion of heterotrophs which might otherwise ingest either the fungus or its substratum (Sivinski, **1981**). However, low-level bioluminescence poses the same difficulties to hypotheses involving an aposematic function as it does to ones involving attraction.

This study suggests that fungal luminescence may occur more commonly than human visual perception has detected. Further attention to the occurrence of luminescence may help reveal its taxonomic distribution and importance to those fungi which possess it.

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Key Words: basidiocarps, bioluminescence, *Mycena haematopus*

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