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# ON FUNGUS LUMINESCENCE

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# **ON FUNGUS LUMINESCENCE**

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#### I. INTRODUCTION

In 1948 the author published some experience with luminous fungi. Apart from considerations on nutritional and physiological aspects, emphasis was laid on the distribution of luminosity in the fungi which led to a thorough revision and abbreviation of the list of fungi mentioned as luminescent in literature (WASSINK, 1948). For many species luminescence data appeared insufficiently founded, others turned out to be synonyms of species described earlier or elsewhere, and still others had been denoted as luminescent mostly in the tropics at an early date and insufficiently studied. In total, some 17 species turned out to be valid both with respect to species characteristics and to the property of luminescence.

Just before, during and after the war, several species with luminescent fruitbodies were described mainly from the tropics. Especially HANEDA from Japan with mycological assistance of CORNER are largely responsible for this extension of our knowledge. However, also here, confusion arose as to the validity of species names. The author has attempted to sort this out as far as possible and has prepared an extensive documentation on the species now known as luminescent (WASSINK, 1978, in 'Bioluminescence in action', P. J. HERRING, ed.) which will not be repeated here. The outcome of this investigation, however, is condensed in section II, in which a list of species is summed up, now to be considered as valid with respect to species characteristics and luminescence, and their main synonyms. Section III presents a list of more or less easily available pictures of these fungi, including several reproductions. Section IV presents a thorough discussion of new biochemical and related data which could be taken up only in restricted form in 'Bioluminescence in Action', owing to limitation of space. Thus, both articles may serve as complements.

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#### II. LIST OF LUMINOUS SPECIES OF FUNGI AND THEIR SYNONYMS

1. Armillaria mellea VAHL. Mycelium luminous. Europe, Asia, N. America. Armillariella mellea (VAHL, ex Fr.) KARST. Clitocybe mellea WAHL.

2. Armillaria fuscipes PETCH. Mycelium luminous. Ceylon, Java, W. Africa. Tropical species closely related to No 1, may be identical.

Armillariella fuscipes (PETCH) SINGER.

2A Clitocybe tabescens (SCOP.) FR. Non-luminous (probably). Widespread. Closely related to Nos. 1 and 2.

Armillariella tabescens (SCOP. ex Fr.) SINGER.

3. *Pleurotus olearius* D.C. Pileus and mycelium luminous. S. Europe, much rarer in the North. Synonyms suggest a wide distribution over similar climates in various parts of the world.

Omphalotus olearius (D.C. ex FR.) SINGER Clitocybe illudens SCHWEIN. N. America Panus illudens (SCHWEIN.) FR. N. America Pleurotus Lampas BERK. Australia. Pleurotus phosphorus BERK. Tasmania Pleurotus illuminans MÜLL. et BERK. Australia (Queensland). Pleurotus facifer BERK. et CURT. N. America.

Pleurotus candescens MÜLL. et BERK. Australia.

4. Pleurotus japonicus KAWAM. Pileus luminescent. Japan.

Lampteromyces japonicus (KAWAM.) SINGER.

5. Panus stypticus FR. f. luminescens BULLER. Pileus and mycel. luminous. North America.

Panellus stypticus (BULL. ex FR.) KARST. (cf. SINGER). Also spelled: *stipticus*'. The eurasian form non-luminous.

- 6. Mycena polygramma BULL. Mycel. luminescent. Europe.
- 7. Mycena tintinnabulum FR. Mycel. luminescent. Europe.
- 8. Mycena galopus PERS. Mycel. luminescent. Europe.
- 9. Mycena epipterygia Scop. Mycel. luminescent. Europe.
- 10. Mycena sanguinolenta ALB. et SCHWEIN. Myc. lum. Europe.
- 11. Mycena dilatata FR. Mycel. luminescent. Europe.
- Mycena stylobates PERS. Mycel. luminescent. Europe.
   M. clavularis FR. acc. to BOTHE (1931) and KÜHNER (1938). KÜHNER (1938) considers Nos. 11 and 12 as synonyms.
- 13. Mycena zephira FR. Mycel. luminescent. Europe. Also quoted as zephirus FR. (see KÜHNER, 1938).

M. spinipes SWARTZ (acc. to BOTHE and KÜHNER 1.c).

14. Mycena parabolica FR. Mycel. luminescent. Europe.

15. Mycena galericulata (SCOP.) FR., var. calopus FR. Mycel. luminescent. Europe.

Mycena galericulata (SCOP.) FR., var. calopoda FR. Mycena calopus FR.

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Mycena inclinata FR.

Mycena inclinata (Fr.) QUÉLET.

16. Mycena avenacea (FR?) QUÉL., sensu SCROETER, KÜHNER, A. H. SMITH. Mycel. luminous. N. Amer., Europe.

17. Mycena illuminans P. HENNINGS. Pileus luminescent, no data about mycelium. Java.

Mycena bambusa KAWAM. nom. nud. (HANEDA, 1939).

Mycena chlorophos BERK. et CURT. (1887), doubtfully identical. See also No. 21.

18. Omphalia flavida MAUBL. et RANGEL. Pileus non-luminous, mycel. and 'gemmifers' luminous. Trinidad, Porto Rico.

Mycena citricolor (BERK. et CURT.) SACC. (SINGER, 1962, p. 378).

18A. Mycena (Omphalia?) spec. Pileus non-lumínous, small, several pilei on yellow, luminous spot on decaying oak leaf; stipe with disc. Near Utrecht. Neths, 1941. See WASSINK (1948). Found only once.

18B. Omphalia martensii P. HENNINGS. Pileus luminescent, no data about mycelium. Borneo. Cf. also WASSINK (1948), p. 177.

18C. Locellina noctilucens P. HENNINGS. Pileus luminescent, no data about mycelium, New Pommern.

18D. Locellina illuminans P. HENNINGS. Same as previous one. Celebes. Cf. also WASSINK (1948), p. 178. May well be related to tropical luminous *Mycena* (see also Wassink, 1948, 1978).

18E. Fungus igneus RUMPH. Pileus luminescent, no data about mycelium. Amboina. Cf. also WASSINK (1948), p. 170.

19. Polyporus rhipidium BERK. Pileus and mycelium luminescent. Amer., Austral., Bonin isl.

Synonyms, see under No. 35.

The above species, except nos. 2 and 16 have been discussed in WASSINK (1948) and all of them in WASSINK (1978). The following species are founded on material not available to the author in 1948; they are extensively commented on in WASSINK (1978).

20. Pleurotus noctilucens Lev. Pileus luminescent, no data about mycel. Tropics. P. lunaillustris KAWAM.

21. Mycena chlorophos (BERK. et CURT.) SACC. (CORNER 1954, p. 261). Pileus and gills luminous, stem and mycel. less so. Malaya, Ceylon, Japan, Micronesia, etc.

Agaricus (Mycena) cyanophos BERK. et CURT.

Mycena phosphora nom. nud. (cf. HANEDA, 1939, '42).

22. Mycenalux-coeli CORNER sp. nov. Gills and stem bluish phosphorescent, not the spores. Japan, Hachyo Island.

23. Mycena noctilucens CORNER sp. nov. Gills and stem bluely phosphorescent. Micronesia, Yap island. Allied to No. 22.

24. Mycena pruinosa-viscida CORNER sp. nov., var rabaulensis CORNER, var. nov. Spore deposit luminous (HANEDA), Rabaul, Nov. Britt. Close to M. rorida (No. 26).

25. *Mycena sublucens* CORNER sp. nov. Stipe and gills luminous (HANEDA). Amboina. Close to *M. rorida* (CORNER).

26. Mycena rorida (FR.) QUEL., var. lamprospora, var. nov. CORNER. Damp spores luminous (CORNER, 1950, HANEDA, 1955). Ceylon, Malaya, Rabaul, Brazil. Luminescence in the temperate main species doubtful.

27. Mycena manipularis (BERK.) MÉTROD. Hymenium porous. Pileus greenish phosphorescent. Widespread in tropical Asia, Australia, W. Africa, etc.

Poromycena manipularis (BERK.) HEIM (1945).

Poromycena pallescens BOEDIJN.<sup>1</sup>

Poromycena Hanedai KOBAYASI (1951).

Polyporus Hanedai KAWAMURA, nom. nud. (HANEDA, 1939).

Polyporus mycenoides PAT. (1887).

Filoboletus manipularis (BERK.) SINGER (1945).

var. microporus KAWAM. ex CORNER, var. nov. Ponape island.

Polyporus microporus KAWAM., nom. nud. (HANEDA, 1942).

28. Mycena pseudostylobatus Y. KOBAYASI sp. nov. Mycel. luminous, luminosity of fruitbodies unknown. On leaves. Tanao, Miyazaki. Probably very near to the European No. 12.

29. Mycena daisyogunensis Y. KOBAYASI sp. nov. Fruitbodies luminous. Daisyogun cave, Miyazaki, Kayasima. Resemblance to M. capillaris FR.

30. *Mycena spec.* (KOBAYASI). Mycel. greenish luminescent, lum. of fruitbody unknown. Miyazaki prefecture. Not fully identified because of insufficient material.

31. Mycena photogena KOMINAMI. Pileus luminescent, no data about mycel. Formosa.

Nos. 32–34, collected by HANEDA, identified by KAWAMURA but mostly lost during the war and not reidentified by CORNER, and may be open to some doubt. 32. *Mycena microillumina* KAWAM. Pileus luminescent, no observations concerning mycelium. Palao.

33. Mycena yapensis KAWAM. Pileus luminescent, no observations concerning mycelium. Yap Island. Sometimes mentioned as M. vapensis.

<sup>1</sup> In addition to the extensive comments given on *Mycena manipularis* in WASSINK (1978), BOEDUN'S description gives rise to the following remarks.

BOEDIJN (1940, pp. 398–400) describes the species as new: *Poromycena pallescens* BOEDIJN nov. spec.. and illustrates both macroscopic and microscopic features. He found the fungus at Krakatau on wood, in 1933, i.e. 50 years after the entire flora had been destroyed by the famous volcanic eruption.

Referring to the genus *Poromycena* VAN OVEREEM (Icones Fung. Mal. 14–15, p. 4, 1926) BOEDIJN remarks that, except for the hymenium, in other features it is close to *Mycena*, but, as never transitional forms are found, *Poromycena* is a good genus. According to HEIM (1945, p. 34), VAN OVEREEM considered the possibility that his new species *P. decipiens* might simply be a monstrosity of *Mycena pura*, with pores instead of lamellae, but soon became convinced that it was characterized by 'charactères héréditaires fixés'. This did not prevent VAN OVEREEM from stating that 'il est prèsque certain que ces aspects des pores proviennent de formes *pura* à lamelles normales'. This has been discussed in more detail by HEIM (I.c.. see WASSINK (1978). In conclusion we may say that the authors cited above have made plausible that, phylogenetically, pores in *Mycena*-like species are derived from lamellae but that, nowadays, genetically they behave as fixed characters. This would seem to be a reason to retain the genus *Poromycena* aside of *Mycena*.

34. Mycena citrinella var. illumina KAWAM. Pileus luminescent, no observations concerning mycelium. Ponape island.

34A. Mycena phosphora KAWAM. Pileus luminescent, no observations concerning mycelium. Palao.

Mycena chlorophos (BERK. et CURT.) SACC. (No. 21). (CORNER, 1954). 35. Dictyopanus pusillus (LÉV.) SING. comb. nov. (SINGER, 1945, p. 224). Fruitbody and mycel. luminous.

Polyporus pusillus PERS. ex LLOYD, 1924. Polyporus Rhipidium var. pusillus Kobayasi, 1937. Polyporus Rhipidium forma pusillus S. ITO & IMAI, 1940. Polyporus rhipidium BERK., 1846, a variety and sensu lato.(cf. also No. 19).

Dictyopanus Rhipidium PAT., 1900.

The usual, small form in the tropics and subtropics (Amer., Oceania, Austral., S.E. Asia, not known from Africa). The original *Pol. rhipidium* BERK. is much larger (temperate zones of N. and probably S. Amer.) BERKELEY already mentioned the similarity with *Panus stypticus* (No. 5) except for the hymenium. See for details WASSINK (1948, 1978).

Previous identifications of *D. pusillus* from the Eastern tropics may belong to *D. gloeocystidiatus* (No. 37) (Corner, 1954), and *pusillus* may be restricted to the West, var. *sublamellatus*. Venezuela, ca. 1800 m high. Size of the pileus as in the var. *rhipidium* from the temperate zone: var. *pseudorhipidium* SING. (SINGER, 1962, p. 332).

Sublamellatus shows that Dictyopanus is hardly to be separated from Panellus (CORNER, 1954).

36. Dictyopanus luminescens sp. nov. CORNER (1950). Belongs to the luminescent alliance of *Panus stypticus* (CORNER, l.c., p. 423).

37. Dictyopanus gloeocystidiatus sp. nov. CORNER (1954). Pores luminous. Japan, Dictyopanus gloecyst (in some enumerations).

38. Dictyopanus foliicolus Y. KOBAYASI sp. nov. (1951). Mycelia and fruitbodies luminous. Japan.

39. *Marasmius phosphorus* KAWAM. Pileus luminescent, no records about mycel. Palao.

39A. *Marasmius spec.*, cf. HANEDA (1942). Larger than No. 39. Pileus luminescent; no records about mycelium. Ponape(?).

40. A minute *Nidulariacea*. Fruitbody luminous, no records about mycel. Palao. 40A. *Collybia velutipes*. Culture introduced as luminous by Prof. R. L. AIRTH; this species, however, very probably is non-luminous. See also below, p. 18 and WASSINK (1978). III. ICONOGRAPHY OF LUMINOUS FUNGI

- (a) Pictures of earlier recognized luminous species.
  - 1. Armillaria mellea VAHL.
    - Several popular fungus books, and, e.g.,
    - a) COOKE (1881-83) Vol. I. pl. 32.
    - b) RICKEN (1915), pl. 100, fig. 1 (as Clitocybe mellea WAHL.).
    - c) BRESADOLA (1927) vol. 2, pl. 52.

d) MAUBLANC (1926), pl. 86; (1939), pl. 100, as Armillariella mellea (FR. ex VAHL.) KARSTEN.

- e) WASSINK (1948), pl. II, fig. 10 (as A. putrida, from MURRILL, 1920).
- 2. Armillaria fuscipes PETCH.

a) PETCH (1910), Circ. and Agric. J. Roy. Bot. Gard. Ceylon, 5, no. 10 (quoted after PETCH, 1928).

b) PETCH (1923), p. 176, on Acacia decurrens. PETCH and BISBY (1950), quote A. fuscipes as a synonym of A. mellea (p. 53, no. 380 II. no illustration).

2A. Clitocybe tabescens (SCOP.) BRES. (Armillariella tabescens [SCOP. ex Fr.] SINGER).

a) RHOADS (1925), pl. 6. Probably non-luminescent; DE VRIES reports a luminous culture in CBS, Baarn, Neths. which, however looks more like an *A. mellea*-culture (private communication, see for some more details: WASSINK [1978]).

2B. Agaricus (Collybia) fusipes BULL.

a) COOKE (1881–1883) Vol. II, pl. 141. Non-luminescent, reproduced here in view of the possibility of confusion with *Armillaria fuscipes* **PETCH** when the latter is misspelled *fusipes* and quoted without author's name, as happens sometimes.

3. Pleurotus olearius D.C.

In popular fungus books, and, e.g.,

a) MAUBLANC (1926), pl. 89; (1939), pl. 103 (as *Clitocybe olearia* (Fr. ex D.C.) R. Maire).

- b) KONRAD and MAUBLANC (1924–33), Vol. III, pl. 292, as ibid.
- c) BRESADOLA (1928) vol. 6, pl. 285.
- d) KAVINA and PILAT (1935), Série A, fasc. 11-13, pl. 54.
- e) WASSINK (1948) pl. I, fig. 8, from BRESADOLA (1928).
- f) l.c., pl. I. fig. 9, from SWANENBURG de VEYE (1940).

g) l.c., pl. I, fig. 10 (as *Clitocybe illudens* (SCHW.), SACC., from MURRILL (1915),

h) l.c., pl. I, fig. 11 (ibid.) from BULLER (1924), probably also

i) pl. I. fig. 12 (as Pl. candescens F. v. M., from Mc. ALPINE, 1900).

j) HARVEY (1952), p. 116 (as *P. lampas*).

k) MCILVAINE and MACADAM (1973), pl. XXIXa, p. 96, as *Clitocybe illudens*.

4. Pleurotus japonicus KAWAM.

5.

- a) WASSINK (1948), pl. I, fig. 7, from KAWAMURA (1915).
- b) SINGER (1962), pl. 7, as *Lampteromyces japonicus*, i) in their own light, ii) in daylight from below, iii) in daylight from above.
- Panus stypticus Fr. f. luminescens BULLER.
  - a) RICKEN (1915), pl. 26, fig. 3 (the european, non-luminous form).
    b) WASSINK (1948), pl. II, fig. 11, as *Panellus stipticus* from MURRILL (1920).
    - c) Harvey (1952), p. 104.
- 6. Mycena polygramma BULL.
  - a) COOKE (1881–83), Vol. II, pl. 223.
  - b) RICKEN (1915), pl. 111, fig. 7.
  - c) MAUBLANC (1926), pl. 65, fig. 2; (1939), pl. 79, fig. 2, as *M. poly-gramma* (Fr. ex BULLIARD) QUÉLET.
  - d) BRESADOLA (1928), vol. 5, pl. 237.
- 7. Mycena tintinnabulum FR.
  - a) COOKE (1881-83), Vol. II, pl. 224.
  - b) BRESADOLA (1928), vol. 5, pl. 247, fig. 1.
- 8. Mycena galopus PERS.
  - a) COOKE (1881-83), Vol. II, pl. 207.
  - b) RICKEN (1915), pl. 109, fig. 11.
  - c) BRESADOLA (1928), vol. 5, pl. 248, fig. 1 as M. galopoda PERS.
  - d) MAUBLANC (1939), pl. 80, fig. 1, as *M. galopoda* (not in 1926 edition).
  - e) KONRAD and MAUBLANC (1924-33), Vol. III, pl. 225, as ibid.
- 9. Mycena epipterygia SCOP.
  - a) COOKE (1881-83), Vol. II, pl. 208.
  - b) RICKEN (1915), pl. 109, fig. 12.
  - c) BRESADOLA (1928), vol. 6, pl. 254, fig. 2.
- 10. Mycena sanguinolenta ALB. et SCHWEIN.
  - а) Сооке (1881–83), vol. II, pl. 163.
  - b) RICKEN (1915), pl. 110, fig. 7.
- 11. Mycena dilatata FR.

12.

- a) RICKEN (1915), pl. 109, fig. 10; not in COOKE (see also no. 12).
- Mycena stylobates Fr. ex Pers. sensu Schroeter.
  - а) Сооке (1881-83), Vol. II, pl. 249, А.

No. 12 is considered as synonym of no. 11 by KÜHNER (1938), cf. p. 4.

- 13. Mycena zephira FR.
  - a) COOKE (1881-83), Vol. II, pl. 158.
  - b) RICKEN (1915), pl. 110, fig. 1.
  - c) KONRAD and MAUBLANC (1924-33), Vol. III, pl. 229.
  - d) Bresadola (1928), vol. 5, pl. 228.
- 14. Mycena parabolica FR.
  - a) COOKE (1881-83), Vol. II, pl. 224.
  - b) Bresadola (1928), Vol. 5, pl. 238.

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15. Mycena galericulata (SCOP.) FR. var. calopus FR. (M. calopus FR., M. inclinata FR.).

a) COOKE (1881-83), Vol. II, pl. 223, top figure as Agaricus galericulatus FR., var. calopus FR.; pencil marked 'inclinata'.

b) Moreover, in our copy the top figures of pl. 225 (with red stem) have also been pencil marked '*inclinata*'. (This whole plate labeled M. *alcalina* FR.)

c) RICKEN (1915) as M. calopus FR., pl. 111, fig. 3.

c<sup>1</sup>) RICKEN, l.c., fig. 1, M. galericulata SCOP., the species (non-luminescent).

- d) BRESADOLA (1928), Vol. 5, pl. 234, the species.
- e) l.c., Vol. 5, pl. 235 as *M. calopoda* FR.
- f) l.c., Vol. 5, pl. 236 as M. inclinata.
- g) MAUBLANC (1939); pl. 80, fig. 2, as *M. inclinata* (FR.) QUÉLET (not in 1926 edition).
- h) KONRAD and MAUBLANC (1924–33), vol. IV, pl. 231 as ibid.
- 16. Mycena avenacea (Fr.?) QUÉL. sensu SCHRÖTER, KÜHNER, A. H. SMITH.

a) KONRAD and MAUBLANC (1924–33), pl. 223 II ('very good' according to SMITH, 1947).

- b) LANGE (1914), Flora Agar. Dan. 2, pl. 49B (cf. SMITH, 1947).
- 16A. Mycena pura Pers. (doubtfully luminescent, but worth further investigation).
  - a) COOKE (1881-83), Vol. II, pl. 157.
  - b) RICKEN (1915), pl. 110, fig. 2.
  - c) BRESADOLA (1928), vol. 5, pl. 226
  - d) var. multicolor BRES., vol. 5 pl. 227.
- 17. Mycena illuminans P. HENNINGS
  - a) HANEDA (1939), pl. I, fig. 2, as M. bambusa KAWAM.
  - b) WASSINK (1948), pl. I, fig. 4, from Holtermann (1898).
- 18. Omphalia flavida MAUBL. et RANGEL.
  - a) MAUBLANC et RANGEL (1914), p. 47.
  - b) WASSINK (1948), pl. I. fig. 2, from Buller (1932);
  - c) l.c. pl. II, fig. 1, from MAUBLANC et RANGEL (1914).
  - d) HARVEY (1952), p. 106.
  - e) WASSINK and KUWABARA (1966), p. 258, fig. 4.
- 18A. Mycena(Omphalia?) spec.
  - a) WASSINK (1948), pl. I, fig. 1, original.
- 18B. Omphalia martensii P. HENNINGS.
  - a) WASSINK (1948), pl. I. fig. 3. from Hennings. 1893.
- 18C. Locellina noctilucens P. HENNINGS. No picture available. For more information, cf. WASSINK (1948), p. 178.
- 18D. Locellina illuminans P. HENNINGS.
  a) WASSINK (1948), pl. 1. fig. 5, after HENNINGS (1900). (cf. also under No. 21).

18E. Fungus igneus RUMPH.

- a) WASSINK (1948), pl. I, fig. 6, from RUMPHIUS, 1750.
- 19. Polyporus rhipidium BERK. See Dictyopanus pusillus (no. 35).

(b) Selection of macroscopic pictures of newly described or rediscussed luminous fungi (from several species microscope pictures of mycologically interesting details exist, which are not mentioned here).

- 20. Pleurotus noctilucens LEV.
  - a) HANEDA (1939), as P. lunaillustris KAWAM., Pl. I, fig. 1.
- 21. Mycena chlorophos (BERK. & CURT.) SACC.
  - a) HANEDA (1939) as M. phosphora KAWAM., pl. I, fig. 3.

b) HANEDA (1942) as *M. cyanophos* BERK. et CURT., p. 226 top, right, figs. A, B, C.

c) KOBAYASI (1949), as M. cyanophos BERK. et CURT., fig. 1;

d) l.c. fig. 2 (drawings). The pictures c) and d) show a similarity with those of *Locellina illuminans* P. HENNINGS (WASSINK, 1948, pl. 1, fig. 5, after HENNINGS, 1900).

22. Mycena lux-coeli CORNER sp. nov.
a) CORNER (1954), plate 9, same pictures as in HANEDA (1955), p. 340, in mirror image.

b) A beautiful colour picture by HANEDA in MCELROY and SELIGER (1962) may be of this species or/and the next one. They may well be different species, since in the top picture luminescence is visible also in the upper layer of the pileus (or shines through) whereas in the lower picture, this is not so.

c) HANEDA (1955), p. 340.

23. Mycena noctilucens Corner sp. nov.

a) HANEDA (1939), pl. I. fig. 5 (as *M. noctilucens* KAWAM.. nom. nud. acc. to CORNER, 1954).

24. Mycena pruinosa-viscida CORNER sp. nov.

Mycena pruinosa-viscida var. rabaulensis Corner var. nov.

- a) HANEDA (1942) as Omphalia spec., p. 228 and
- b) p. 229, l.c. acc. to CORNER (the var.). However,
- c) HARVEY (1952) reproduces the picture l.c., p. 228 as Mycenarorida, var. lamprospora, photographed by daylight (see also under no. 26).

25. *Mycena sublucens* CORNER sp. nov. No picture seen, 'much in common with *M. rorida*' (CORNER).

- 26. Mycena rorida (FR.) QUÉLET. var. lamprospora var. nov. CORNER.
  - a) See under no. 24 (c).
  - For the species *M. rorida* FR.: see
  - b) COOKE (1881-83), Vol II, pl. 248, and
  - c) KONRAD and MAUBLANC (1924–33), Vol. III, pl. 228 II.
- 27. Mycena manipularis (BERK.) MÉTROD. Described and pictured many times under various synonyms. The

now most currently used synonym is *Filoboletus manipularis* (BERK.) SINGER.

- a) HANEDA (1939) as Polyporus Hanedai KAWAM. Pl. II, fig. 8, A, B,
- $C_1$  in daylight,  $C_2$  in its own light.
- a<sup>1</sup>) BOEDIJN (1940) as Poromycena pallescens, p. 399, fig. 8.
- b) HANEDA (1942), p. 226, fig. D, as Polyporus Hanedai KAWAM.
- c) HANEDA (1942), p. 226, fig. E;
- d) ibid. p. 227, top, A, B,

e) ibid, p. 227 bottom left, A and B (as *Polyporus microporus* KAWAM.).

f) HEIM (1945) as Poromycena manipularis (BERK.) HEIM, Pl. 3, fig. 1–3.

g) WASSINK (1948) as *Polyporus mycenoides* PATOUILLARD, Pl. II, fig. 2, taken from PATOUILLARD (1887).

- h) KOBAYASI (1951) as Poromycena Hanedai, p. 2, fig. 1A, drawing.
- i) HARVEY (1952) as Polyporus Hanedai, p. 101.
- j) KIET (1975) as Filoboletus manipularis (BERK.) SING., PI VIII, fig. 3. var. microporus CORNER var. nov.
- 28. Mycena pseudostylobates Y. KOBAYASI sp. nov.
  - a) KOBAYASI (1951), p. 2, fig. 1C, drawing.
- 29. Mycena daisyogunensis Y. KOBAYASI sp. nov.
  - a) KOBAYASI (1951), p. 2, fig. 1D, drawing.
- 30. *Mycena spec.* (cf. KOBAYASI 1951).
  - a) KOBAYASI (1951), p. 2, fig. 1E, drawing.
- 31. *Mycena photogena* KOMINAMI No picture seen by the present author.
- 32. Mycena microillumina KAWAM.
  - a) Haneda (1939), pl. II, fig. 7.
- 33. Mycena yapensis KAWAM.
  - a) Haneda (1939), pl. II, fig. 6.

34. Mycena citrinella, var. illuminans KAWAM. No picture seen by the present author, however, for the species (M. citrinella PERS., in pine woods), see
a) COOKE (1881-83), Vol. II, pl. 248.

- 34A. Mycena phosphora KAWAM. See under M. chlorophos (no. 21).
- 35. Dictyopanus pusillus (Lév.) SING. comb. nov.
  a) DENNIS (1952), p. 326, fig. 1, drawing, var. sublamellatus var. nov. (CORNER).
  - b) CORNER (1954), p. 260, fig. 5, drawing.

36. Dictyopanus luminescens sp. nov. CORNER.

- a) CORNER (1950), p. 424, fig. 1, drawing.
- 37. Dyctyopanus gloecystidiatus sp. nov. CORNER.
  - a) CORNER (1954), p. 257, fig. 2, drawing.
  - b) HANEDA (1955), p. 341, as *D. gloecyst*.

- Dictyopanus foliicolus Y. KOBAYASI sp. nov.
   a) KOBAYASI (1951), p. 2, fig. 1B, drawing.
- 39. *Marasmius phosphorus* KAWAM. a) HANEDA (1939). pl. 1, fig. 4.
- 39A. Marasmius spec. (larger than no. 39)
  a) HANEDA (1942), p. 226, bottom, left, figs. A, B.
- 40. A minute, luminous Nidulariacea.a) HANEDA (1955), p. 343.
- 40A. Collybia velutipes (FRIES ex CURTIS) QUÉLET Probably non-luminous. Picture only added as a reference. See also p. 7.
  a) MAUBLANC (1939), pl. 70.

# IV. PHYSIOLOGICAL AND BIOCHEMICAL SUBJECTS

# A. General remarks

We will restrict us mainly in this part to discussing knowledge that has been brought forward after HARVEY's book (1952) appeared, which has rather thoroughly discussed earlier evidence. The main progress appears to be in two directions: 1) The production and study of cell free luminous systems; 2) Advances in culture methods suitable for the study of physiological kinetics. Some early data will be mentioned as introduction, following WASSINK (1948) and HARVEY (1952).

Fungi are among the earliest organisms noticed as luminescent; PLINY mentions an *Agaric*, sitting high up in the trees and producing light. SCHERTEL (1902) quoted PLINIUS' observation as follows: 'In Gallien bringen hauptsächlich die hartschalige Früchte tragenden Bäume den *Agaricus* hervor; es ist dies aber ein weisser, riechender Pilz... Er wächst oben auf den Bäumen und leuchtet zur Nachtzeit'.

SCHERTEL interpretes this as pointing to a *Polyporus* species among which genus there are. according to SCHERTEL, luminous species.

According to HARVEY (1.c.), HENNINGS, 1904, expressed the view that PLINIUS' fungus probably was *Pleurotus olearius*. The location (Gallia, on hard walled fruit bearing trees, thus presumably oaks and walnuts) would speak in favour of this, but other additions, as e.g., a white, odorous fungus, sitting high-up in the trees seems to speak against it. For these reasons Polyporaceae of the vicinity of *P. sulphureus* have been suggested in earlier literature, but luminescence of this species or its allies and synonyms seems doubtful (see WASSINK, 1948, p. 184, 185 and 212); that PLINY indeed observed a luminous fungus can hardly be doubted.

The strongest argument in favour of *Pleurotus olearius* perhaps is that a conspicuously luminous fungus in France or its environment, on the mentioned type of trees could, according to our present knowledge, hardly be anything else, unless the species observed by PLINY would have become extinct since his days. This cannot be fully excluded but it might well be accepted that it, in some wider area, still might be there in that case. One would, however, not know about what species

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one would readily be able to think in this respect!

Early experiments on the physiology of the light emission have been chiefly carried out with luminous wood, and the most famous one is that by BOYLE, in 1667, with an air pump by which he showed that the light from a piece of wood disappeared upon evacuation. BOYLE already observed that luminescence returned upon readmission of air and even that at first the light was stronger than before evacuation. Recently this has been confirmed (see HARVEY l.c., p. 113) as had been shown also for luminous bacteria. Authors in the beginning of this century showed extinction of the light in N<sub>2</sub>, H<sub>2</sub>, CO<sub>2</sub>, and return of light upon readmission of air (ibid.). The extra flash of light after readmission of air lasted about 10 times longer than in bacteria. Low temperature reduced the intensity and prolonged the duration of the flash. The presence of molecular oxygen seems necessary for luminescence in all types of luminous organisms studied so far.

HARVEY mentions a few examples of temperature effects on luminescence intensity. Lower and upper limits in a few species were  $\sim 0^{\circ}$  and  $\sim 37^{\circ}$ C, with an optimum range between  $\sim 10^{\circ}$  and  $25^{\circ}$ C.

The effect of light on luminescence was found negative by MURRILL for *Clito-cybe illudens*, but HARVEY believes more the reports of some other investigators who mentioned no effect. We will see below that more recently a negative effect has again been reported.

HARVEY presents some effects of poisons studied by early and more recent investigators, KRUKENBERG, 1887\*, studied the effect of some alkaloids on 'Agaricus olearius'; HARVEY denotes his results as 'hardly worth recording'.

Effects of some narcotics have been studied e.g. by KRUKENBERG, 1887, KA-WAMURA, 1915, BULLER, 1924, and LUTZ, 1931. In general, narcosis of luminescence was reversible. and LUTZ arrived at the conclusion that fungus luminescence is an autoxidation.

In the beginning of this century, several attempts have been made to produce luminous extracts from fungi, but without success. Crushing the cells always destroyed the light; after HARVEY's book (1952) appeared, enzymatic luminescence has been achieved with fungal systems. This will be discussed in detail lateron. NOBÉCOURT (1926) found an oxidase in the sap from *Armillaria* cultures and thought that it might be concerned in luminescence.

Physical properties of luminescence have already been successfully studied before 1952. Luminous fungi have been photographed in their own light and the results compared with 'normal' photographs. Several examples are in HARVEY'S book (1952) and in HANEDA'S survey (1955).

With the aid of long exposures and a spectrograph, spectral photograms were obtained, showing similarity between spectral emissivity of different species. COBLENTZ and HUGHES, HANEDA, and VAN DER BURG all found the spectra extending from about 470 to 680 nm with a maximum at 520–528 nm. We will come back to this later; it may be remarked here that the emission maximum is

<sup>\*</sup> Authors quoted in this general survey with the year without brackets. mostly are not mentioned in the reference list. They may be looked up, e.g., in WASSINK (1948) or HARVEY (1952).

shifted about 50 nm to the longer wavelengths as compared with the maximum emission of luminous bacteria. Moreover, it provisionally seems that the mutual agreement between the maxima for fungus species is closer than in the bacteria (cf., e.g., HARVEY (l.c.), fig. 24).

Genetic studies using single spore cultures have been of utmost importance for the knowledge of the distribution of luminescence in the fungi (c.f. HARVEY, 1952. p. 108). BOTHE, 1935, thus obtained luminous and non-luminous single spore cultures from *Mycena galopus* and *M. polygramma* and, by fusion, also diploid luminous and dark mycelia. WASSINK (1948) reported the isolation of luminous and non-luminous (mass-spore) cultures of *Mycena polygramma* from different pilei collected in nature (l.c., p. 180); The occurrence of luminous and nonluminous strains of the same species thus does not need a geographical separation as is obvious for *Panus stypticus*. This case and the interbreeding of the two varieties has been extensively studied by MACRAE in HARVEY's laboratory. (HAR-VEY, l.c., p. 109). Luminosity was found to be inherited as a single pair of characters and dominant over non-luminosity.

BOTHE also found weakly luminous strains in his material. He raises the possibility that luminous hyphae may grow mixed with non luminous ones. It is wellknown that in luminous bacteria strains of different luminosity exist. In this respect the fungi up to now have been less extensively studied. The above data may well induce caution in considering a species as definitely luminous or nonluminous. On the other hand, in a species with reported mixed luminosity, one should claim that luminosity is observed in a fair number of cases and on solid grounds (better than, e.g., fruitbodies growing on a luminous piece of wood). However, the degree of luminescence in different specimens of a 'luminous' species is certainly worth studying. It is the author's impression that differences in luminosity in cultures within one species obtained from different specimens in nature may well exist.

#### **B.** Nutritional aspects

Also here, HARVEY has summarized earlier experience. We will first discuss general culture methods (cf., e.g., WASSINK, 1948). The isolation of a luminous fungus from luminous wood is not particularly difficult, but the trouble may be that an indiscriminate, white luminous mycelium is formed, which may be very difficult, if not impossible to identify (e.g., 'mycelium X' of MOLISCH, cf. WASSINK, 1948). The most reliable way for identification is to start from a well determined pileus of a fungus that has luminous mycelia according to previous authors. BOTHE e.g., discovered luminescent mycelia in different European Mycena species, the pilei of which are non-luminescent (cf. sections II and III of this paper).

## (a) Pure cultures

(1) Isolation from luminous wood. Luminous wood sometimes bears the fruit bodies of higher fungi, which, however, may not be the cause of the observed luminescence. By far the most common cause of luminous wood in the Netherlands is *Armillaria mellea* VAHL. A pleasant circumstance is that the outgrowth of

this fungus out of a block of luminescent wood is by building very characteristic rhizomorphs in the agar, which turn brown in their older parts, whereas the tips are white and mostly strongly luminescent. Also aireal mycelium is, in part, brightly luminescent.

In order to arrive at pure cultures from luminous wood, the putrified parts should be carefully removed, using a sterilized knife, until the hard surface of the wood is reached. Immediately below this hard surface some square blocks of wood  $(ca 5 \times 5 \times 5 \text{ mm})$  should be cut apart and transferred onto an oblique agar surface in a culture tube. A good agar for this first transfer is cherry agar. The present author advises against transfer onto petri dishes, since the Basidiomycetes are slow growers, and liable to putrifaction by Imperfects, along the rim of the dish. With some care and experience as to how far the luminous wood should be cut away, one may arrive at pure cultures immediately. After some development has occurred, transfer should be made from the rim of the mycelium, some distance away from the inoculation block, into fresh cherry agar tubes. If purity is sufficiently certain, it is advantageous to make also subsequent transfers to bread agar. on which growth and luminescence for most species are better. Contamination by whatever cause mostly leads to quick loss of luminescence. Owing to the mentioned slow growth, purification of once contaminated cultures is very difficult, and if possible, a new isolation is to be preferred.

(2) Isolation from spores. In order to obtain reliable results, a pileus should be carefully laid on two flamed glass bars (2-4 mm thick), on a thin layer of sterile cherry agar in a petri dish. Care should be taken that no part of the pileus touches the agar. Mostly, at room temperature, a spore picture will be found on the agar surface after 8-12 hours. Then, the pileus is aseptically removed, and small blocks of agar with spores are transferred to cherry agar in the tubes. Sterile cultures will mostly be obtained immediately. In most species the spores are non-luminescent. In this way, of course, mass-spore cultures are obtained which are not suitable for genetic work (see above). Luminescence in the mass-spore mycelia thus obtained may become visible only after some days. Like in the case of isolation from luminous wood, transfers from the outgrowth of the spores can easily be made upon cherry and bread agar.

In large species, e.g. Armillaria mellea VAHL., parts of the pilei can be laid out, instead of entire pilei. Sometimes larvae will have crept out of the exposed fungus parts and mixed with the shed spores. In such cases the spores have to be discarded, and attempts with younger pilei started.

(3) Isolation from parts of the pileus. In principle this goes the same way as e.g. isolation from luminous wood. The pileus is opened up (better torn than cut) so that a sterile inner surface is obtained. Herefrom pieces are cut out with a sterile (flamed) sharp knife and transferred with a sterile needle to cherry agar in tubes. Transfers from the stipe are also possible. Isolation from parts of the pileus or stipe is generally only possible for rather large species.

The most reliable type of isolation is that described under (2), from spores, obtained from well identified pilei.

## (b) Nutrient media

Several media have been described in earlier literature, but the most reliable ones for general use may well be cherry agar and bread agar. The first one is suitable for primary isolations since it is not very liable to bacterial growth; the latter usually gives better growth and stronger luminescence.

WASSINK (1948) gave the following prescriptions:

1 Kg ripe cherries, boiled in 1 l. water; the flesh is rubbed through a metal sieve (pores of about 2 sqmm) and added to the extract. The nutrient agar is made up as follows: Extract as described: 1 part; tapwater: 2 parts; agar 2%. Caution must be taken against too extensive heating; we mostly sterilize the cherry extract and the agar solution separately at double strength and 120° for 20 minutes; both parts are then added together. and immediately filled into sterilized culture tubes. The agar is allowed to solidify in the tubes in oblique position. With due precautions, contamination is readily avoided.

Bread agar: This is preferred to bread media as such. White bread is sliced, the crusts removed, and dried at room temperature, then ground into fine crumb in a morter. The medium is composed as follows: Dried bread crumb: 10%, agar 1.8%, in tap water.

#### (c) Submerged cultures

Notwithstanding the fact that in the late 50's and the 60's important studies on the physiology and biochemistry of luminescence in fungi have been made with the traditional surface cultures (see below), especially in view of biochemical work it seemed attractive to try to grow larger mycelial masses with the aid of submerged cultures on a shaking device.

The first attempts in this direction were made in our group in 1945 when *Armillaria mellea*, *Mycena polygramma* and *Omphalia flavida* were successfully grown in submerged culture. Spherical mycelia balls developed, which were luminous. except for *Armillaria mellea* which was non-luminous (unpublished, see, however, also WASSINK and KUWABARA, 1966). The same observation was reported later by AIRTH (1961) and again confirmed in our laboratory. Aside of *Mycena polygramma* and *Omphalia flavida*, luminous mycelial balls were also obtained from *Panus stypticus luminescens*, isolated in 1952 from fruiting bodies, collected near Washington, D.C.

To obtain primary submerged cultures, we proceeded as described lateron (see p. 29).

As a further aim of our work we have tried to develop a culture method in which a fully defined carbon source should be used for comparative data on growth, respiration and luminescence.

Initial trials, on glycerin basis, were unsuccessful (WASSINK and KUWABARA, 1966). Good results were obtained in the same way in which the author successfully grew the mould *Phycomyces Blakesleeanus*, viz. by adding some vitamincomponent prepared from yeast extract (WASSINK, 1934, 1974). Thiamine has been claimed to replace the yeast extract in the case of *Phycomyces* (SCHOPFER, 1949) but with *Omphalia flavida* it yielded only half the growth obtained with yeast

extract addition prepared according to the prescription used for *Phycomyces*, or obtained with the addition of Difco yeast preparation (WASSINK, 1974). Neither the 'complete' mineral-sugar medium, nor the vitamin addition alone yielded any appreciable growth, only the combination did. With this completely clear medium with a well-defined energy source (glucose), observations on growth, respiration and luminescence were made (see section IV, D, c).

## C. In vitro-luminescence and enzymological data

The first successful attempt to obtain light emission from extracts of luminous fungi was reported by AIRTH and MCELROY (1959). By that time, luminous extracts from fireflies, *Cypridina* and bacteria had already been obtained. Essentially, the technique is identical with that applied by DUBOIS in 1885: cell-free luminescence can be obtained by the combination of a cold water extract (containing luciferase) and a hot water extract (luciferin). AIRTH and MCELROY ascribe the failure to realize this in fungi up to then to the following factors: a) in crude aqueous extracts the luciferase is too diluted or inhibited; b) the luciferin in the hot water extract is very labile; c) they observed that the presence of reduced pyridine nucleotides is essential for light emission. These conclusions were supported by extraction experiments. Herefor mycelial mats from *Collybia velutipes\** or *Armillaria mellea* were dried over  $P_2O_5$  under reduced pressure, and an acetone powder was prepared from them. The authors describe in detail the preparation of a hot water extract and a cold water extract.

The result was that DPNH (reduced diphosphopyridine nucleotide) and luciferase and luciferin were required for significant light emission. TPNH (reduced

\* It should be observed that AIRTH, also in his further papers refers to *Collybia velutipes* as a luminous fungus, at least with luminous mycelia, and even mentions to have obtained his culture from the present author's laboratory. As far as I remember I have never observed luminescence in this species and have never found it mentioned by authors on the distribution of luminescence in fungi (WASSINK, 1948 and section II of this paper). I am afraid this confusion will never be removed, since Dr. AIRTH died and his group dissolved several years ago (private communication from Dr. T. J. Mabry).

It may still be remarked that, according to HARVEY (1952), BOTHE grew a number of Collybia's among which C. velutipes, and found them non-luminous (l.c., p. 107).

AIRTH and coworkers refer to this fungus – which they extensively used as a source of enzymes – as *Collybia velutipes* in all their subsequent papers. Two possibilities may be considered: 1) AIRTH indeed has come across a luminous strain of *C. velutipes* arisen out of a strain from our laboratory or from elsewhere; 2) Somewhere between the source of this strain and the use of it in AIRTH's laboratory, an error in the labeling has taken place.

In our further discussion of the work of AIRTH and his collaborators we will refer to the strain as 'C. velutipes'.

Subsequent papers from AIRTH's group reveal some further particuliarities:

FOERSTER et al. (1965) mention that the strain 'obtained from the laboratory of Plant Physiology at Wageningen, Netherlands' was 'judged dikaryotic in view of the presence of clamp connections' (p. 487). AIRTH and FOERSTER (1965) studied also some strains of *C. velutipes* obtained from Dr. Ashan ÅBEY i.e. some monokarystic and dikaryotic strains and found them non-luminous (l.c., p. 498).

Enzymes of *Panus stypticus luminescens* were completely interchangeable with those of 'C. velutipes' in reactions with electron acceptor from *Armillaria mellea* (AIRTHet al. 1966, p. 218). 'C. velitipes' grew non-luminous in submerged culture, which, however, contained a certain amount of enzymes (l.c., p. 219, see also later).

triphosphopyridine nucleotide) also stimulates light emission. Molecular oxygen is also required.

The situation of the emission peak for fungal luminescence  $\sim$  530 nm suggests the possible involvement of a flavin, addition of flavin adenine di- and mononucleotide in place of or in addition to the hot water extract did not stimulate luminescence. The same holds e.g., for folic and ascorbic acid, cytochrome c. and yeast extract.

Since the bacterial luminous system responds to either of the reduced pyridine nucleotides, the possibility existed that the fungus and bacterial systems were identical. However, neither aldehydes, nor flavins, nor bacterial luciferase stimulated fungal luminescence. Neither did luminescent reactants from fireflies or *Cypridina*.

It is remarkable that this introductory paper on fungus luminous extracts, of little over 1 page, contains already so many basically important facts about the behaviour of these extracts. Not all facts even have been mentioned in the above discussion.

In subsequent years, AIRTH and co-workers gave valuable additions to the basic facts. They found, e.g. (AIRTH, 1961) that crystalline, bovine plasma albumin, added to the reaction mixture, stimulated luminescence (l.c., fig. 2). It is suggested that the bovine plasma albumin adsorbed an inhibitor from the reaction mixture. This could be in the hot water extract or in the enzyme preparation or in both.

Additionally, in the same paper, AIRTH mentions some effects of poisons and specific substances on the maximum intensity of luminescence of the fungal luminous system in vitro. In all the in vitro studies, '*Collybia velutipes*' was used as an enzyme source and *Armillaria mellea* as a source of substrate. Inhibition of luminescence was found e.g. by potassium cyanide (concentrations  $\sim 10^{-4}-10^{-3}$ ), ortho-phenantroline and 8-hydroxyquinoline ( $\sim 10^{-3}$ ), and also by FMN, FAD, FMNH<sub>2</sub> and FADH<sub>2</sub> ( $\sim 10^{-6}-10^{-5}$ ). Remarkably, no inhibition was observed by NaN<sub>3</sub> and arsenites ( $\sim 10^{-3}$ ).

In 1962 AIRTH, and AIRTH and FOERSTER report the isolation of two proteinaceous fractions by high speed centrifugation, both of which are necessary for light emission. For instance, the supernatant of a 198,000 g centrifugation is heat labile, non-dialyzable and ammonium sulfate precipitable, which 'may' indicate a proteinaceous nature. This fraction is supposed to catalyze a reaction in which reduced diphosphopyridine nucleotide (DPNH or NADH) acts as an electron donor to an unknown substance which is subsequently used in the actual light reaction.

The second fraction is in the pellet of the high speed centrifugation and is also enzymic in nature. It acts directly in the light reaction and confirms to a classical 'luciferase'. Data suggest that it catalyzes the oxidation of the, so far unknown, substance resulting from the action of the supernatant enzyme.

In their last contribution, AIRTH and coworkers (1970) formulate their concept as follows:

(1) NADH + H<sup>+</sup> + X  $\xrightarrow{\text{sol. enz.}} XH_2 + NAD^+$ (NADPH) (NADP)

(2)  $XH_2 + \frac{1}{2}O_2 \xrightarrow{\text{luciferase}} X + H_2O + \text{light}$ 

The soluble enzyme is denoted as reduced pyridine nucleotide oxidase (RPNoxidase). For these studies AIRTH et al. mostly derived their hot water extract from *Armillaria mellea* and the two enzymes from 'Collybia velutipes'.

The essentially similar earlier formulation of the above reaction complex (AIRTH and FOERSTER, 1962) was:

 $A + DPNH \xrightarrow{E_s} AH_2 + DPN$  and

 $AH_2 + O_2 \stackrel{Ep}{=} A + HOH + light$ 

A very interesting observation, contributing to this picture was the following: When (material from) the pellet is added to the reaction mixture prior to the supernatant, there is a slow gradual increase in light intensity until a final steady state maximum is reached. If the order is reversed, the initial rate of the light emission is strongly increased while the maximum is about the same.

It should be observed that between the various additions of compounds incubation periods of the order of  $2 \times 1-1^{1}/_{2}$  minutes are included, for further details the reader should consult the original paper (1.c., legend fig. 1).

Lateron, AIRTH and coworkers (1966) have gone in some more detail into the question of the incubation period, again using supernatant and pellet from 'C. velutipes' preparations and a hot water extract from A. mellea. Only preincubation for increased length of time led to an increase in the initial rate of the reaction, thus suggesting the accumulation of  $XH_2$  during the pre-incubation with the soluble enzyme. Incubation times from 20–600 sec. showed increasing initial rates (l.c., Table 3, and fig. 3).

Another important observation was that, in the presence of the hot water extract and the soluble enzyme, DPNH oxidation is stronger than without the hot water extract; this indicates a substance in the hot water extract required for DPNH-oxidation (i.e. A or X respectively in the above formulae). The nature of this substance still seems unknown.

The bioluminescent reactions now (partly) elucidated have been categorized by various authors in some 6 or 7 classes (see, e.g. CORMIER and TOTTER, 1968, Table I; AIRTH et al., 1970, Table I, being much the same). Bacteria and fungi have been brought under the same heading, viz. *pyridine nucleotide linked* reaction systems. There are. furtheron, considerable differences in the reactive compounds: Bacteria:

1) NADH + H<sup>+</sup> + FMN + reductase  $\rightarrow$  FMNH<sub>2</sub> + NAD<sup>+</sup>

2)  $FMNH_2 + RCHO + O_2 + LH_2ase \rightarrow light$ 

Fungi:

1) NADH + X + reductase  $\rightarrow$  XH<sub>2</sub> + NAD<sup>+</sup>

2)  $XH_2 + O_2 + LH_2ase \rightarrow light$ 

As already mentioned, the bacterial reactants FMN and long chain aldehyde are inactive in the fungal system. In my opinion  $XH_2$  might have been denoted as  $LH_2$  (luciferin) whatever its composition may be.

Another interesting question, studied by AIRTH and coworkers (1964, 1966) was

the relation between enzyme production in luminous and non-luminous forms of *Panus stypticus*. A luminescent strain was found to contain both enzymes (soluble and particulate); these enzymes were completely interchangeable with those of 'C. *velutipes*'. Both enzymes were absent or inactive in the non-luminescent European form of *P. stypticus*. No reliable evidence was obtained as to presence or absence of the electron acceptor in either *Panus* form.

Did Panus stypticus produce luminescent and non-luminescent strains owing to genetic differences (MACRAE, 1937, '42), AIRTH et al. (1966) mention that 'C. velutipes' produces mycelia which are non-luminous to the dark-adapted eye by submerged culturing (and aeration by shaking and aseptic air supply). Tests on luminescence by combining supernatant enzyme and particulate enzyme from surface and liquid cultures and vice versa led to the conclusion that a submerged culture produces both enzymes, the soluble enzyme being about as active as in surface-grown cultures, and the particulate enzyme being  $\frac{1}{3}$  as active.

The above may summarize the results obtained by AIRTH's group with respect to in-vitro luminescence.

# D. In vivo luminescence, biophysical and physiological data

## (a). The work of AIRTH and coworkers

AIRTH and FOERSTER (1960) with Armillaria mellea measured the emission spectrum and found the maximum at 530 nm, practically identical with the spectra obtained by VAN DER BURG (1943) for A. mellea, Omphalia flavida, and Mycena polygramma which the latter author found mutually very much alike (cf. also WASSINK and KUWABARA, 1966).

The effect of temperature was also measured with surface-grown Armillaria mellea. An ARRHENIUS plot (log light intensity against 1/absol. temp.) showed a fairly exponential relationship with temperature from  $\sim 10-25^{\circ}$ C.

Ultraviolet light of 366 nm causes a sharp decrease of luminescence intensity in *Armillaria mellea*, followed by a gradual increase to a new level. This increase is speeded up by removal of the u.v. source. The new level (reached after about 80 minutes) may be higher or lower than the original stationary level (before irradition), dependent on various circumstances. The authors additionally quote that *in vitro* ultra-violet light readily destroys the luminescent activity of a hot water extract, but does not affect or even stimulates the activity of an enzyme preparation.

The authors extensively studied the effect of the withdrawal of oxygen from *Armillaria mellea* cultures. As in luminous bacteria, after floating with nitrogen, readmission of air causes a light flash. This reached about 600% of the previous intensity, which is about twice the flash in bacteria. The duration of anaerobiosis had no great effect between 15 and 120 seconds, both in fungi and bacteria. Longer durations caused somewhat irregular effects. The authors extensively discussed models and calculations to explain the observed effects, in parts comparing them with presentations of previous authors. The general outcome was that during anaerobiosis luciferin accumulates, whereas a sometimes observed secondary rise

of luminescence intensity may be due to a solid binding between oxyluciferin and luciferase which only gradually liberates the enzyme again. It may be mentioned that at the time of this article the authors had not yet observed that 2 enzymes cooperate in luminescence of fungi.

They also published results obtained with in-vivo material of 'C. velutipes' regarding the relation between bioluminescence and some other characteristics. For the painstaking and detailed description of techniques used, the reader is referred to the original paper (FOERSTER et al., 1965). Surface culturing was used throughout.

The extension of the rim of the mycelial mat was linear for about 20 days or more. A gradual increase in dry weight of mycelial disks of the same size was found up to about 14 days (i.e. a denser mycelial growth per unit surface). Electron microscope and chemical tests revealed the formation of glycogen, increasing with mycelial age. No evidence for re-use of glycogen in metabolism was found.

Light emission, both as specific activity (light units/mg dry weight) and as total light units/sample, are considered by the authors to be maximal in mycelia between 2 and 6 days of age. It seems to me that this conclusion somewhat overestimates the high point in both curves at 6 days (cf. the original paper). Reducing somewhat this effect yields the picture that total light emission remains highest between 2 and 6 days, but specific activity gradually declines from the youngest stages onward. Interestingly, it then would come more in a line with specific activity for endogenous respiration which is highest after about 2 days (they conclude that maximal specific activity for bioluminescence occurs somewhat after that for  $O_2$ -uptake). Total respiration per sample was not mentioned.

ATP-concentration in the tissue/mg dry wt. and total per sample parallel each other during the entire period of observation (20 days). Both show a high, sharp peak in mycelia of about 7 days old, i.e. in a period of decreased respiration and decreased luminescence. The authors suggest that the ATP-peak may be due to decreased ATP-consumption.

AIRTH and FOERSTER (1965) moreover discussed the effect of nutritional factors on light emission of 'C. velutipes'. Using various data from literature on growth of C. velutipes, AIRTH and FOERSTER grew their isolate in a medium of known chemical composition. It contained a rather extensive list of inorganic salts, a carbon source, a nitrogen source and a vitamin addition. All media were solidified with agar for surface growth. Light emission occurred when the fungus grew at pH 5–7 with an optimum at pH 6.0. In liquid media the fungus tended to shift pH to a higher hydrogen concentration. Glucose was found to be the best carbon source, while ammonium nitrogen or aspartic acid were the best nitrogen sources. No growth was found with complete absence of light emission.

Various investigators had already studied the growth factor requirement of C. *velutipes*, and found that thiamine is required for growth 'and the present results suggest the same is true of this isolate'. A large number of possible factors was tested but only thiamine proved to be effective. It was also established that the thiamine effect on luminescence was entirely produced by the 'thiazole' moiety of the molecule, while the 'pyrimidine' moiety had no effect. Thus, the requirement

for thiamine may be attributed to the inability of the organism to synthesize the thiazole ring. Remarkably, ethanol, at a substrate level, could replace thiamine for luminescence and growth. The authors tentatively suppose that ethanol via acetal-dehyde acts in acetyl-CoA synthesis. The thiamine analogues pyrithiamine and oxythiamine inhibit the thiamine effect on luminescence, the first mentioned one was more active than the latter.

We will see lateron that thiamine and other yeast-extract factors are growth requirements for different fungi, in chemically defined media, e.g. for *Phycomyces* and the luminous *Omphalia flavida* (WASSINK, 1934, 1974). Thus, the fact that a similar requirement existed both for *Collybia velutipes* from different origin and the 'C. velutipes' isolate used by AIRTH c.s. cannot be considered as additional proof for their identity. On the other hand it should be remarked that the very important observations by AIRTH et al., discussed above, specifically those on enzyme isolations, are by no means invalidated by the fact that uncertainty seems to remain as to the specific identity of their isolate, and the qualification of '*Collybia velutipes*' as a luminous fungus.

Additionally it may be observed that after the outstanding results mentioned in the first paper (1959) not much further progress has been achieved in the chemical characterization of the compounds involved ('luciferin' and 'luciferase').

## (b). The work of BERLINER and coworkers and related observations

A second group who did important work in the biophysics and physiology of fungal luminescence was that of BERLINER. BERLINER dicovered the diurnal periodicity in the light intensity in some luminous fungi and furthermore, with some coworkers, studied the effects of a number of physical agents on luminescence intensities, and developed methods to detect the luminous intensity in various parts of the hyphae.

BERLINER (1961a) studied the question whether there was a daily rhythm in luminescence intensity in the following luminous fungi: Armillaria mellea, A. fusipes, Clitocybe illudens, Mycena galopus, M. polygramma, Panus stipticus, and Omphalia flavida (nomenclature and spelling as used by BERLINER). A daily rhythm was found in surface cultures of A. mellea, M. polygramma, and P. stipticus, in the others it was not observable. The effect was independent of growth of the cultures either in total darkness, continuous light or 12:12 or 14:10 hrs daylight and darkness. Total darkness was used in most experiments, a photomultiplier was placed near above the culture in a petri dish. Most observations started at the age of 14 days, the cultures then had 4 cm diameter, the rhythm was observed for 5 days in which the diameter reached 9 cm; the light intensity did not increase very much since the central part got dimmer. Maximum daily luminescence occurred between 6 and 9 p.m., lowest between 6 and 9 a.m.; the peaks lasted for about 2 hours. The rhythm was already detectable 48 hours after inoculation; it may be maintained until lack of nutrient and possible other factors stop luminescence which may wait until after 7 weeks. The daily luminescence amplitude may reach up to 35%. Readings of luminous intensities were taken every 3 hours, and every 30 minutes during the peaks.

In a subsequent paper, BERLINER (1961b) reported the used species as follows:(dikaryotic cultures of) Armillaria mellea (VAHL. ex FR.) KUMMER, Armillaria fusipes PETCH, Mycena galopus (PERS. ex FR.) QUÉL., M. polygramma (BULL. ex FR.) S. F. GRAY, Panus stipticus (BULL. ex FR.) Fr., Clitocybe illudens (SCHW.) SACC., and Omphalia flavida (CKE.) MAUBL. et RANGEL. The cultures were maintained in the dark at 22°C, on 10% bread crumb agar which provided 'maximum sustained luminescence'. (See also WASSINK, 1948, HARVEY, 1952).

Light intensities were again determined from agar surface cultures in petri dishes with the aid of a sensitive photomultiplier in the range of 300–700 nm which comprises the emission range of 470–640 nm with a maximum of about 530 nm as reported by VAN DER BURG (1943) for Armillaria mellea, Mycena polygramma and Omphalia flavida. Luminous intensity versus time showed a bellshaped figure with a total duration of about 9 weeks and a maximum intensity of about 800 millimicrolumens in the 4th week for 3 different strains of Panus stipticus.

Temperature effects were also studied in *Panus stipticus*. Full recovery was found after 2 weeks at  $-10^{\circ}$ C. The optimum range was 18° to 26°C. High temperatures (above 30°) caused rapid luminescence decline but recovery after return to 25°C. Above 40°C the damage quickly became irreversible. At temperatures above optimum there was an initial rise in luminescence followed by a sharp decline. The higher the temperature, the sooner the decline started. At 60°C the decline started immediately; the extinction was irreversible after 10 minutes. The supraoptimal effects remind very much of those observed by J. KUIPER (1910) for respiration of pea seedlings at supraoptimal temperatures.

Effects of X-radiation (*P. stipticus*). In view of the daily periodicity of luminescence, the cultures were exposed to X-ray treatment between 11 a.m. and 1 p.m. Treatment with 3 R to 20 R yielded a 2–4 fold increase in luminescence intensity within 2–3 hours, this increase was maintained for > 5 days. Growth increased to about 3 times that of the controls for 24–48 hrs after exposure.

X-ray treatment with 100 R to 3000 R neither killed the cultures nor extinguished the luminescence. There was an immediate sharp rise (1/2-3 min.) in intensity with more drops and rises in the next 3 hrs. Within the first 6-8 hrs., increases of 20-75% of the original intensity were reached and maintained. Readjustment to the diurnal pattern was fairly rapid, as it was also at the lower Xray exposures, nor was there any change in the colour of the light. At the higher Xray exposures, there was no visible difference in growth with unexposed cultures.

Since the survival of *Panus stipticus* was not affected by X-ray doses up to 5000 R and light emission was increased, the author provisionally concludes that a) a radiation-labile component normally partially inhibits light emission, or b) Xirradiation stimulates, either directly or through the formation of byproducts, a radiation stable component essential for luminescence. Moreover, she concludes that the availability of accurate photometric techniques allows long-term studies of the physiology of light emission.

Subsequently, the effect of monochromatic ultraviolet light on the luminescence of *Panus stipticus* was studied (BERLINER and BRAND, 1962). Culture and measurement technique was much as used before; for details see the original paper; temperature was 23–23.5°C. The irradiation time was adjusted to a minimum causing 50% luminescence intensity, and the recovery effects thereafter were studied. The mentioned minimum time varied from 15–30 minutes for the different wavelengths.

At the wavelengths 220, 245, 255, and 265 nm there was a quick recovery of luminescence intensity to more than 100%, then a renewed drop, in some cases again to 50%, followed by a renewed recovery in which the daily periodicity is gradually more expressed. At 366 nm a fairly similar picture was found.

Contrarily, at 280 nm after the initial dip to 50%, a quick recovery to only about 75% occurred, followed by a long and lasting decrease to about 5%, and after about 5 days some recovery to about 20%; after about 5–7 days recovery to over 100% might occur accompanied by restoration of daily periodicity.

The authors provisionally suggest that the above effects may be due to a photolabile compound essential for light emission with absorption at 280 nm, and a photolabile compound absorbing at 245–265 and at 366 nm which normally inhibits luminescence.

BERLINER (1965) studied the effects of various antibiotics, of 2.4-dinitrophenol, Armillaria mellea to shock vibration, and acceleration tests 'as anticipated by sending and re-entry of a space probe', and also to ultrasonic vibration. P. stipticus cultures were unaffected by all treatments; A. mellea reacted to all treatments with temporary stimulation from 40-400%. Light intensity returned to the level before the treatments within 5 hours. Visible damage or change in growth rate were not observed. The authors assume that the stimulation of light emission is due to release of enzymes and substrates into the medium. This explanation does not seem very likely to me, since extra-cellular luminescence in cultures, as far as I know, has never been observed (except for specific preparations, as made, e.g., by AIRTH et al., which, however, require specific precautions).

BERLINER (1965) studied the effects of various antibiotics, of 2.4-dinitrophenol, and of  $CO_2$  on the luminescence of A. mellea, A. fusipes and Panus stipticus.

The antibiotic effects were studied in different concentrations. The substances used were: actidione (cycloheximide), penicillin G, bacitracin, chloromycetin, viomycin, streptomycin sulfate, neomycin sulfate, and mycostatin. Neomycin sulfate and penicillin G affected neither the growth nor the luminescence in *Panus stipticus*, cultured as above. The antibiotics were added to the bread crumb agar medium.

The effects of the various antibiotics in a large range of concentrations was mutually different. Some had hardly an effect on luminescence intensity in low concentrations, while luminescence gradually decreased at higher ones (e.g., mycostatin and viomycin). Relative ineffectiveness over a large range of concentrations. followed by a rather strong stimulation at higher concentrations, and a sharp decline at still higher concentrations was found with bacitracin. Actidione showed strong stimulation (up to 160% of the control) followed by a sharp decline at higher concentrations; a similar picture, extended to higher concentrations occurred with streptomycin sulfate. The strongest effect was shown by chloromycetin which was by far the most inhibitive of all; already at 5

p.p.m. luminescence was fully inhibited while all the others showed either no effect or (strong) stimulation.

The effect of 2,4-dinitrophenol in *P. stipticus* after 3 days caused a very strong increase in light emission which was anticipated since it also increased  $O_2$ -uptake; 6 p.p. 100 m. after 3 days caused 800% increase in luminescence intensity. Higher concentrations reduced the stimulation; after 5 and 7 days the stimulation by low concentrations was less whereas that of high concentrations (up to 1600 p.p. 100 m.) was relatively greater. For more details, see the original.

The effect of  $CO_2$  was tested by growing cultures of *P. stipticus*, *A. mellea*, and *A. fusipes*, in anaerobic jars in 'atmospheres of 5% and 10%  $CO_2$ ', for 2 weeks. It is not quite clear whether any other gas was present in these atmospheres. At any rate, an expected extra light flash after readmitting air after 2 weeks did not occur. The *Armillaria* species showed morphogenetic effects of the treatment.

Caffeine and nicotine had no strong effects on light emission in A. mellea and P. stipticus. Especially in the latter, appreciable effects were noticed only at concentrations of the order of  $10^{-4}$  in the media; the submerged hyphae of A. mellea were not sensitive.

BOTAN (1963) investigated the effects of ethyl urethene and sodium amytal on luminescence intensity of *Panus stipticus*, *Armillaria mellea*, *A. fusipes*, *M. polygramma*, *Clitocybe illudens* and *Collybia velutipes* (names and spelling of the names according to the author). Ethyl urethene was applied in concentrations of 0.3 and 0.78 molar, amytal of 0.003 molar. Discs of mycelial mats (55 mm.) were freed from agar and applied on filterpaper in petri dishes and moistened from below with the required solutions. Amytal reduced the light output by 30–80%, except in *A. fusipes* where 30% stimulation occurred. The higher urethane concentration reduced light intensity by about 90–95%, the lower by 30–80%, however, *Mycena polygramma* showed a stimulation of 50%.

BOTAN and GRAFF (1964) studied the effects of various nucleotides and combinations hereof on luminescence of Armillaria mellea, A. fusipes, and Panus stipticus, with the same method as used by BOTAN (1963). Several of them, or certain combinations, yielded strong stimulation of luminescence, especially in the Armillaria's.

In *Panus stipticus*, the highest stimulation was 74% in a TPNH and FMN mixture  $(3 \times 10^{-5} \text{M})$ . But, *A. fusipes* showed 399% increase upon the FAD and TPNH mixture  $(3 \times 10^{-5} \text{M})$ . In *A. mellea*, the highest increase, 268%, occurred, using DPNH  $(3 \times 10^{-5} \text{M})$ , the next highest, 227%, using FMN and DPNH  $(3 \times 10^{-5} \text{M})$ . The authors suggest that in luminescence of *A. fusipes* FAD and TPNH are involved, in *A. mellea* FMN and DPNH. They remark that the 'poor response' of *P. stipticus* may be due to the fact that the test compounds either are not necessary for luminescence in this species, or are badly absorbed.

I would like to remark that absorption of this type of compounds from outside by living cells may appear somewhat improbable and the high degrees of stimulation recorded are remarkable and interesting. As far as I have been able to ascertain, these results have not been published in a more extensive article, nor have they been corroborated elsewhere. Neither have I seen them quoted by other authors, not even by BERLINER, who worked in the same laboratory. We have seen above that NADH (DPNH), according to AIRTH et al. (1962, 1970), plays a rôle in the preparatory reaction with the soluble enzyme, and that FMNcompounds are supposed to play a rôle in bacterial but not in fungal bioluminescence. Thus. the stimulatory effects observed by BOTAN and GRAFF are of interest. (The nucleotide compounds referred to above are quoted in the same way as used by the authors).

BERLINER and HOVNANIAN (1963) determined that the light output of single dikaryotic hyphae is sufficient to obtain an autophotograph. Such autophotographs were obtained by placing strips of sensitive film directly over the edge of a culture of *Armillaria* or *Panus*. After processing, the films were examined microscopically. For the present, it appeared that light emission occurred throughout the cell, but finer structural details could not be seen owing to the fact that the grains of the photographic emulsions available were too uneven and too course.

BERLINER and DUFF (1965) studied the ultrastructure of Armillaria mellea hyphae in ultrathin sections examined under the electron microscope. The most interesting observation for the study of bioluminescence seems to be that electrondense granules ranging in size from  $0.025 \,\mu$ m to  $0.075 \,\mu$ m of unknown origin and function were found in all sections of luminescent hyphae, which granules were not present in non-luminescent material. There was no way, however, to correlate their presence with active light emission. Their occurrence was far more regular than that of any other reported cytoplasmic inclusions. The authors suggest that the function of these granules may be studied by isolation methods, e.g., ultra centrifuging, and may contribute to understand details of the mechanism of the light reaction. We have seen above that AIRTH et al. were successful in isolating a 'luminous' substrate and two enzymes from luminous fungi. The granules described by BERLINER might well be the site of e.g., the particulate enzyme.

CALLEJA and REYNOLDS (1970a) studied increase in diameter of circular mycelial inoculates on agar and the amount of light emitted by a colony in Armillaria mellea and Panus stipticus, originally obtained from Dr. R. L. AIRTH. They observed total light with a sensitive photomultiplier from above and below at a fixed hour per day (in view of periodicity), and every 2 days recorded the diameter of the colony, for about 120 days. In about 5 weeks of linear growth, the colonies reached their maximum diameter (about 60 mm). In Armillaria mellea luminescence roughly increased together with linear increase in diameter up to a maximum at the end of the linear growth phase, then strongly declined and remained more or less at a lower level thereafter. First, light from above appeared much stronger than from below, lateron both were much the same. Since, in a way, 'total light' was measured, in my opinion it may be asked in how far the parallelism between growth and luminescence increase in the beginning merely reflects the increase in the amount of luminous hyphae rather than an increase in luminosity per sé.

Cultures of *Panus stipticus* grew more rapidly, but sooner reached a stationary level. As in *A. mellea*, maximum luminescence was obtained when growth started to deviate from linearity, but the intensity was much lower than in *A. mellea*.

Formation of growth rings, reflected in luminescence, was more evident in P. *stipticus*. Apart of the phenomena already discussed, the authors confirmed BULLER's (1924) and BERLINER's (1961) observations as to a circadian rhythm in luminously in both species used, with minima at 9 a.m., and maxima at 9 p.m.

Moreover, the authors observed remarkable oscillations in luminosity after transfer of a circular inoculum from a culture of A. mellea to a fresh agar plate, both in the inoculum and in the old culture. Chiefly, 100 minutes after inoculation luminescence was about 2–3 times as high as in the beginning. The increase was, however, interrupted by two minima, within 10 minutes, and after about 50 mins from the beginning. After the maximum at ca 100 mins, light intensity gradually decreased to about its initial value.

As a whole, the luminescence-time curve after inoculation reminds very much of the chlorophyll-fluorescence time curve in cultures of *Chlorella*, occurring after the start of an illumination, however, on a different time scale. What happens here in 100 mins. happens in *Chlorella*-fluorescence in about 20 seconds. Since the rise of luminescence also occurs in the mother-mycelium, the authors conclude that the increase in luminescence is not a reaction to fresh medium, but to injury. One may perhaps suppose that the 'injury' essentially means increased admissibility of oxygen. This would increase the comparability with the mentioned chlorophyll fluorescence case. Also here observations indicated that the ups and downs, and the general shape of the time-curve reflect the oxygen or oxido-reduction level around the chlorophyll. High values of fluorescence reflected relatively reduced states, low levels relatively oxidized states. In fungus luminescence this then would be the reverse which seems very plausible.

CALLEJA and REYNOLDS (1970b), like BERLINER and HOVNANIAN (1963) aimed at studying the spatial distribution of luminescence in single hyphae. They used a different method, viz., image intensification (REYNOLDS, 1968). The main conclusion seems to be that luminescence is a function of the age of a hyphal segment. At any instant of time during growth, light emission was confined to a region removed from the growing point. The latter was always dark as long as it remained a growing point. 'The luminescent region of the primary hyphae moved with the hyphal tip away from the center of the colony and caught up when the tip ceased to be a growing point. It was at this time that luminescence was concentrated in the hyphal tips'. Whereas light was not uniformly distributed along a single segment of a hypha, the authors could not relate 'hot spots' with morphological structures at the level of resolution they worked at.

These interesting observations are probably related to the often observed phenomenon that cultures, e.g., from spores start to give light only after some days. This is also very obvious in submerged cultures of e.g., *Omphalia flavida* started from fragmented, luminescent cultures, which resume luminescence only after some days (personal observation of the present author).

However, there seems to be more to the timely distribution of luminescence. In *Omphalia flavida* submerged cultures, sometimes old cultures luminesce again or conspicuously increase their intensity, and may remain strongly luminous for several weeks. This is so far unexplained.

## (c). The work of WASSINK and KUWABARA and related subjects

A cooperation between WASSINK and KUWABARA in 1964–65 aimed at attempts to separate and identify active principles from luminous fungus cultures. *Omphalia flavida* was chosen as the only object on the basis of earlier experience. In order to obtain sufficiently large amounts of mycelium for chemical work, semilarge scale submerged shake cultures were attempted. In the combined effort it was mainly WASSINK's responsibility to develop the culture method (WASSINK and KUWABARA, 1966), and KUWABARA's responsibility to deal with extraction of compounds involved in the luminescence (KUWABARA and WASSINK, 1966). As remarked before (p. 20), around 1945 WASSINK grew some species (viz., *Armillaria mellea*, *Mycena polygramma* and *Omphalia flavida*) in submerged culture; *Armillaria mellea* under these conditions was non-luminescent, the two others were. An isolation of the American *Panus stipticus*, made in 1952, also produced luminescent submerged cultures (cf. WASSINK and KUWABARA, 1966).

For arriving at semi-large scale submerged cultures, the procedure was briefly as follows. A surface culture on cherry-agar in a tube is aseptically taken out and carefully subdivided with a sterilized knife in a sterilized petri dish. The pieces are precultured by shaking them under submerged conditions in erlenmeyer flasks of 200-250 ccm capacity with 40-50 ccm liquid culture medium (cherry extract or bread extract). The pieces of the inoculum thus readily develop into mycelial balls. These balls may be fragmented again in the same way as above, and subcultured, but we found it easier to fragment them under aseptic conditions with the aid of a WARING blender (for details see WASSINK and KUWABARA, l.c.). Half speed of the blender resulted in sufficient fragmentation in about 15 seconds. If the procedure is run in darkness, fragmentation can be observed by the gradual dimming of the light emitted by the culture. Even cultures fragmented to darkness (according to the adapted eye) may still grow out if brought into culture medium. The contents of the blender jar are routinely divided over a series of 5-litre flasks with 1.5 litre culture medium each. The blending procedure is mostly performed in the culture medium of the culture to be fragmented; the impression exists that addition of some phosphate buffer may increase the resistance of luminescence against the blending.

The semi-large scale culturing was so far only carried out with *Omphalia flavida*. Luminescence is mostly visible immediately after inoculation; after ca. 1 day it fades to invisibility, and becomes evident again after ca. 1 week (at 23–25°C) when the culture is about full-grown. It then increases rapidly and remains at a high level for about 2 weeks or more (l.c. fig. 2).

The 5-litre flasks with 1.5 l. 5% bread extract medium are suitable for harvesting after about 3 weeks from inoculation. Flasks then each yield about 250 g. fresh weight (pressed mycelium) corresponding to 21 g. dry weight of mycelium. The solid material in the bread crumb extract is solubilized by the action of the fungus and does not appreciably affect dry weight determination at harvest. Twenty one g. dry weight appear to arise from ca 56 g. dry wt. of bread introduced, yielding an 'economic coefficient' (fungus wt./food wt.) slightly below 0.4 (l.c., Table 1).

Material so cultivated formed the starting point for KUWABARA's attempt to

enrich and purify compounds involved in luminescence (KUWABARA and WAS-SINK, 1966). Routinely, 15 kg of fresh mycelial mass (dry wt. ca 1.3 kg) was subjected to an extensive extraction, purification and concentration procedure (for details, l.c.), and ultimately yielded 12 mg of a microcrystalline brownish orange substance. This substance, according to the authors is active as a luciferin in combination with the enzyme system as described by AIRTH and FOERSTER (1962). Luminescence measurements were made with a photomultiplier/amplifier combination that is not described in the paper. Visual estimations of luminescence were not made. KUWABARA observed, i.a., the following properties of the active, probably luciferin-like components. The pure active substance had absorption maxima at 320 nm and (a smaller one) at 270 nm: It is suggested that the latter may be due to a C = O absorption. In solution, the active substance, on absorption of radiation at 320 nm, shows a fluorescence peak at 490 nm (at pH  $\sim$  6.5); at pH 12, the excitation peak shifts from 345 to 370 nm, but fluorescence does not change. The oxidized form was obtained by leaving the alcohol-water solution of the active substance at room temperature in the light for several hours. The absorption spectrum of the oxidized form was shifted to slightly longer wavelengths, viz., with maxima at 333 and 272 nm, and a fluorescence peak at about 500 nm. An aqueous solution of the active substance gives a bright chemiluminescent flash on addition of NaOH and  $H_2O_2$ . This can be seen by the dark-adapted eye. Renewed addition of H<sub>2</sub>O<sub>2</sub> reincreased the light intensity after (rapid) decay of the first flash. The quantum yield of chemiluminescence was estimated  $> 10^{-2}$ . Finally, the emission spectra of in-vitro enzymatic luminescence and non-enzymatic chemiluminescence were determined; for the enzymatic reaction Dr. AIRTH's fungal luciferase system was used. The peak of non-enzymatic luminescence was at 542 nm, and that of enzymatic light emission at 524 nm (at pH 6.5). The authors still leave open the question whether the 'active substance' is true fungal luciferin.

AIRTH et al. (1970) report to have obtained a sample of the crystalline compound sent by KUWABARA from England. The substance proved to be inactive in the cell-free system from *Collybia velutipes* both in the presence and absence of NADH or NADPH. The authors do not exclude that the substance has been inactivated during transport, but, on the other hand, do not feel sure that KU-WABARA and WASSINK (1966) indeed have crystallized fungal luciferin.

CORMIER and TOTTER (1966) describe a rather long-living phosphorescence (half-life about 0.8 sec.) in mycelial suspensions of *Omphalia flavida* upon exposure to ultraviolet light. The authors mention that mycelial suspensions of nonluminous species do not exhibit this phenomenon, but they mention only testing of suspensions of *Aspergillus niger* and *Neurospora crassa*. These however, being Imperfects, stand rather apart from *Omphalia*, and it would have been more conclusive when, e.g., mycelial suspensions from non-luminous *Mycena* species did not show this phosphorescence. A partial purification of the phosphorescent substance was achieved. Suspended in water-free acetone. it shows the same phosphorescence more brilliantly.

The energy distribution and the position of the emission peak (530 nm) are similar for bioluminescence and phosphorescence of the purified substance in

acetone; phosphorescence in suspensions of mycelium in distilled water is broadened with a peak shift to 540 nm. Sufficient homogenization of mycelia destroys their bioluminescence, but not their ability to phosphorescence.

The (purified) material responsible for the phosphorescence would phosphorescence only when suspended in organic solvents with most of the water removed from the suspension.

The authors are tempted to speculate that this phosphorescence and bioluminescence of *O. flavida* are related through the excitation of a common molecular species.

Very similar results have been reported about a similar compound, again in acetone solution, enriched from mycelium of *Pleurotus olearius*, by LOVELLE, DUROSAY et MICHELSON (1972). The long time luminescence (phosphorescence) upon ultra-violet irradiation has a half life of 0.2 sec. and its emission peak at 510 nm. The authors call these values 'sensiblement différentes' of those reported by CORMIER and TOTTER for *Omphalia flavida*.

Above 1% of water added to the acetone solution strongly decreases the phosphorescence, which is totally abolished at 2% water. In aqueous solution, however, there is still a fluorescence, excitable at 375 nm and emitting at 480 nm. The authors found the same properties in all luminous fungi cultivated in their laboratory (no species names mentioned), and suggest that the relation of the phosphorescent substance with compounds active in bioluminescence in vitro as studied by AIRTH et al. may be due to a certain structural analogy (indicated by the absorption maximum, 260 nm, and the fluorescence), and also by a molecular weight around 500 measured by calibration on the Sephadex column. The relation may be closest with the principal product of luciferin oxidation.

So far for studies about biochemical and biophysical aspects.

WASSINK (1974) made some coordinated observations on the kinetics of growth, respiration, luminescence and energetics in *Omphalia flavida*. For this purpose the fungus was grown in a simple inorganic salt – glucose solution with a small vitamin addition, viz., either purified yeast extract, a small amount of 'Difco' yeast preparation or thiamin.

Ultimate dry wt. production and growth rate with thiamin were about half that obtained with the yeast preparations. It was ascertained that these preparations did not results in any appreciable growth without the energy source (sugar). Cultures were mostly followed for about 3 weeks, and determinations made every 2 days.

In a glucose-yeast extract culture increase in dry wt. ceased rather abruptly after 9-10 days, dw. remained constant for about 7 days and then slightly decreased. Glucose decrease in the medium at first parallels dw. production, and lateron continues in a similar way until exhaustion. Sugar consumption in the later phase obviously can only be accounted for by respiration of the fungus. Change in pH was also followed, and converted into change in  $[H^+]$ . Increase in  $[H^+]$  also paralleled fairly well dw. production, but continued somewhat longer, then came to a standstill and started to decrease practically at the moment glucose was exhausted. Rates/day were estimated from the slopes of the curves discussed

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above. As expected, the rates of dw. production, glucose consumption, and change in  $[H^+]$  show obvious parallelism before maximum dw. production is reached, and certain deviations afterwards following from those discussed above.

Glucose-thiamin cultures showed somewhat different features. As already remarked, dw. increase and the maximum reached are lower than in glucose-yeast extract cultures. Increase in  $[H^+]$  very nearly parallels glucose decrease, and in the ascending part of the curve, also dw. increase. Different from the situation in glucose-yeast extract cultures, dw. reaches its maximum considerably earlier, long before glucose is exhausted. Glucose consumption then continues at the same rate as before, until exhaustion, accompanied by increase in  $[H^+]$  at more or less the same rate as before. Rate determinations showed that there was a dip in the rates of  $[H^+]$  increase about the time that dw. reaches its maximum (rates ~0), after which the rate of  $[H^+]$  increase again becomes greater.

One may suggest that full-grown cultures continue to consume sugar and produce organic acid(s). Both for the glucose-yeast extract cultures and those on glucose-thiamin, part of the acidification may be due to, e.g., accumulation of  $SO_4^-$ -ions owing to the consumption of  $NH_4^+$  from  $(NH_4)_2SO_4$ . However, the changes in acidity observed in the glucose-yeast extract cultures in the later parts of the culture period, do not seem to support this possibility. The impression had been gained in previous experiments that the fungus starts vigorous growth only after it has 'succeeded' in bringing pH down to around 4.0. Experiments started in phosphate buffers of different initial pH strongly supported this idea. Especially the start of vigorous growth after  $\log[H^+]$  was brought to -4.0 in the most alkaline solution (initial pH 7.17) was striking. Titration of a culture solution (glucosethiamin) in which Omphalia flavida had grown for  $3^{1/2}$  weeks, compared with that of fresh medium showed a fairly constant difference of ca 14 ml 0.1n NaOH requirement per 100 ml solution. Interesting was the rather flat range between pH 3.8 and 5.2 indicating that the acids produced have their pK somewhere in this region. All this requires further investigation.

In order to obtain reliable data concerning the relation between dw. production, respiration rate and luminescent intensity a reliable dosage of mycelium balls per WARBURG vessel (as used for the measurements) had to be worked out. At successive harvest dates the culture was sieved with increasing mess-width, so that 4 fractions with increasing size of mycelial balls were obtained. Preliminary measurements showed that the rate of O<sub>2</sub>-uptake increases with decreasing ball size (or increasing number of balls/mgdw.) pointing to limitation of the rate of O<sub>2</sub>-uptake by a diffusion process (either of oxygen or of, e.g., glucose).

In a combined experiment with a yeast extract glucose culture (150 ml) accumulated dw., rate of O<sub>2</sub>-uptake (mm/mg dw/h), accumulation of dw., and light intensity increased in a similar way, only light intensity probably continues to increase after dw. accumulation has reached its maximum. In the course of development of the culture, rate of O<sub>2</sub>-uptake decreases smoothly and continuously. Rate of dw. accumulation (g/day) first increases, and then smoothly decreases, much like the rate of O<sub>2</sub>-uptake, but without irregularities, passing on to negative values in the end. Features were less clear in the glucose-thiamin cultures, e.g., owing to low values, especially of luminous intensity. For more details, see the original paper.

Furthermore, some preliminary data on caloric value and the efficiency of energy conversion in the process of producing fungus material from glucose have been obtained, using the glucose-yeast medium. The efficiency of glucose conversion into mycelium (cal/cal) was very near to 40% over the culture period. Earlier experiments with large-scale bread extract cultures yielded fairly the same figure. This conversion factor is lower than the one usually found and suggested for bacteria and fungi (~0.6).

The kinetic experiments on comparison of dry wt. increase and glucose consumption suggest much higher yield especially during the period of vigorous growth when the curves for dw. increase and sugar consumption run closely parallel and near to each other. Computations using these data in connection with the caloric values indeed show temporary yields up to 0.9. Low values at the beginning of the growth period and (of course) during the period when dw. increase had fairly stopped, may explain the much lower overall yields during longer periods. A further evaluation of the rates of dw. accumulation, sugar consumption, respiration and acidification seems very much worthwhile.

The physiological features discussed were obtained from submerged growth in shake cultures. Ball size proved important, and their outer ranges represent early stages of mycelial growth. Surface growth may occur when cultures grow very dense and shaking becomes less effective. From experience, obtained by the author with surface growth of *Phycomyces* (WASSINK, 1934) it may be expected that the rate of respiration will continually decrease with ageing. The effect of oxygen tension in submerged cultures on luminescence in fungi still has to be explored. Early observations indicate that certain poisons, like cyanide, affect luminescence much less than oxygen consumption (WASSINK and VAN DER BURG, unpublished).

The intensity of fungus luminescence is low, and more or less so for all bioluminescence phenomena. Thus, bioluminescence proves to be an improbable phenomenon; it is tempting to bring this into connection with the circumstance that heterotrophs have to build up the luminescent emitter molecule out of energy packages of the order of 10 kcal/g.mol (ATP and similars), which emitter must be able to release at once an energy jump of the order of 50 kcal/g.mol.

HASTINGS (1975) remarked that in fungi luminescence, where no biological use of the light is evident, 'the system may function at the biochemical level by providing chemically generated excited states'. It should be noticed that the above suggestion of ATP as a general energy transporter in heterotrophic growth does not imply that ATP should play a rôle in the process of fungal bioluminescence sensu stricto which it, according to evidence available so far, (see above, pp. 19–20) does not.

#### (d). Recent development in mitogenetic radiation

Some remarks may be made about a subject that has not been considered in the field of bioluminescence for many years, viz., very weak emissions in the ultra-

violet and in the visible region, but invisible to the human eye and only recordable by biological or quantum counting detectors. This subject came into literature around 1920 by studies of A. GURWITCH who claimed that these radiations are emitted by tissues in active cell division and on their turn promote cell division in other tissues (mitogenetic radiation). Interest in western literature died out around 1936, seems to have been persued in Russian literature and has been revived recently also by studies of QUICKENDEN and QUE HEE (1974, 1976).

In 1974 they reported the use of photon counting equipment to detect two distinct periods of luminescence during the growth of liquid cultures of the yeast *Saccharomyces cerevisiae* (an Ascomycete). In their 1976 paper the authors aimed at determining the spectral distribution, and reported that during the logarithmic phase of growth, emission was observed as a broad uv-band between 200 and 425 nm, and as a visible-region band between 525 and 700 nm. During the stationary phase, there were two narrow bands centred at 250 and 650 nm, and a broad band extending from 325 to 525 nm. The authors have compared the uv-components with GURWITCH's mitogenetic radiation, and possible chemical and radiolytic sources of the luminescence are discussed.

The authors quote from their previous work (1974) that the luminescence then observed 'bears some relation to mitogenetic radiation insofar as it is emitted during the stage of most rapid proliferation with a quantum efficiency in the vicinity of 1 photon per cell division'. To the present author this efficiency appears unbelievably low; it would be hard to visualize how a complicated process like a cell division could produce an average of 1 photon. Perhaps a strong absorption of radiation produced in surrounding tissues might explain the low efficiency as a 'rest yield'.

A different suggestion of QUICKENDEN and QUE HEE, however, starts from the observation that the bombardment of pure water by cosmic and other environmental ionising radiation, leads to the excitation of uv and visible emission in addition to ČERENKOV radiation. 'It is possible that mitogenetic radiation arises by a similar mechanism and is simply a fluorescence induced in susceptible biological molecules either by cosmic radiation or by the far uv ČERENKOV radiation excited by the cosmic rays'. The authors moreover quote BARENBOIM and DOMANSKI for reporting excitation of bacterial and yeast suspensions by the ČERENKOV radiation from added <sup>32</sup>P. The spectral distribution of the fluorescence thus excited, was similar to that of mitogenetic radiation. Biologically important molecules like tryptophan, DNA, RNA were similarly excited to produce their characteristic fluorescence emission peaks.

The authors consider it as clear that mitogenetic emission occurs only at certain stages of the cell growth cycle. This does not rule out, however, the cosmic ray hypothesis since fluorescence of cell metabolites will be a function of their concentration and thus depend on the stage of growth.

The authors stress the importance to determine which portion of the cellular luminescence arises from cosmic rays and which from the more commonly proposed chemiluminescent reactions. Moreover, more work appears required to establish if certain emitted wavelengths are 'truly mitogenetic and can stimulate cell division'.

Life of a cell is bound to a continuous flow of energy. Ultimately the origin of this is the solar radiation. The organism uses the energy available to it (in heterotrophes as chemical energy from its food, ultimately produced from solar energy by photo-autotrophic organisms or from chemical energy by some chemoautotrophs) in complicated metabolic processes.

Part of this energy is used for synthetic processes, part for producing kinetic energy for transport processes in the cell or in the entire organism, and part is 'dissipated' as radiation. The majority of the latter part is long wave length radiation or 'heat'. Rather famous is the flower system of certain large Araceae that produces so much heat in a certain stage of development that it may be felt by the hand. However a (for thermodynamical reasons small) part of the radiation may be (or probably always should be) in shorter wave length regions, i.e. 'visible' or even uv. In this range then come bioluminescence and probably 'mitogenetic rays'.

HARVEY once remarked that bioluminescence is so seemingly arbitrarily spread over the natural system that it looks as if someone had thrown a handful of sand over the system and that where a sandcorn fell, a luminous form came into being.

Trying to connect results of the mitogenetic ray people with bioluminescence, it rather would appear that bioluminescent forms are not so much peculiar in that they emit radiation but that they do this at a level surpassing the threshold of human visibility.

In this connection a note of EBERHARD (1975) may be of interest, viz., that anaerobic cultures of luminous bacteria are non-luminous, but, even after repeated anaerobic transfer, produce a lot of light upon aeration. EBERHARD suggests that luciferase which is obviously synthesized during anaerobiosis, is involved also in processes other than bioluminescence. Such an observation would also tend to decrease the peculiarity of bioluminescence.

## V. SOME RECENT REVIEWS AND BOOKS

In recent and subrecent surveys on bioluminescence, the briefness of the sections on fungus luminescence reflects the degree of neglect this field has suffered from as compared with the progress made in the study of bioluminescence in other groups. Attention may be drawn to the following reviews and books containing (mostly, brief) sections on fungus luminescence.

R e vi e w s: MCELROY and SELIGER, in Scientific American 207 (1962), CORMIER and TOTTER, in Ann. Rev. Biochem. 33 (1964), CORMIER and TOTTER, in Photophysiology vol. 4 (1968), HASTINGS in Ann. Rev. Biochem. 37 (1968), AIRTH et al. in Photobiol. of Microorganisms, ed. HALLDAL (1970), ROSALES in Acta manilana A12 (1974).

The last article discusses fungus luminescence only and is the most recent survey available. It is divided into two parts; before and after 1960. It contains useful information, but is rather fragmentary and has a fair number of printing errors and other inaccuracies.

B o o k s: Light and life, ed. MCELROY and GLASS (1961); Bioluminescence in progress, ed. JOHNSON and HANEDA (1966); The luminescence of biological systems, ed. JOHNSON (1955), as far as fungi is concerned important for HANEDA's survey of new luminous species from the Far East (cf. section II of this paper). For more complete bibliographic data about these publications, consult the reference list (section IX). – Aside of the above, several review articles and symposium volumes appeared in which fungus luminescence is not referred to at all; these will not be mentioned here.

Most recent book: Bioluminescence in action (P. J. HERRING, ed.), Acad. Press, London, N.York (1978).

# VI. CONCLUSION AND SUMMARY

This paper serves as a complement to WASSINK (1978), and together with this it acts as a continuation and extension of WASSINK (1948).

It appears that the extension of knowledge since 1948 was mainly in extension of the number of tropical species distinguished as luminescent and secondly in extension of knowledge of enzymological and physiological processes leading to luminescence.

After a brief introduction (section I), an enumeration of luminous species is given (section II). The number of taxonomically valid and doubtlessly luminescent (either in the fruitbody or in the mycelium, or in both) species has increased from 17 in 1948 to around 40 now. Especially the genus *Mycena* has been shown to contain many luminous species, and the number of cases of luminescence in species of this genus may still be expected to increase further. So, e.g. recently in *M. avenacea*, a long-known European species, luminescence was discovered. Also remarkable are luminous species with porous hymenium as close allies to species of the genera *Mycena* and *Panus*. An extensive discussion of taxonomic data and synonyms of the luminous species mentioned is in WASSINK (1978).

Section III is an iconographic list, accompanied with reproductions of pictures, as far as possible.

In section IV an extensive survey of biochemical and physiological data is given. It appears that, so far, only AIRTH and MCELROY (1959) and AIRTH et al. have successfully managed to obtain fungal luminescence *in vitro*. As bacterial luminescence, fungal luminescence appears to belong to a reaction type including pyridine nucleotides (cf. p. 20).

Attention has been drawn to recently renewed interest in very weak ultraviolet and visible radiation, seemingly accompanying the development of cultures of certain microorganisms. e.g. *Saccharomyces cerevisiae*. The improbability of bioluminescence in a thermodynamical sence thus may come into a more general frame of consideration. A condensed survey of data is given in WASSINK (1978).

Section V lists a few surveys and books in which fungus luminescence is discussed mostly in the frame of other bioluminescent processes which is useful for comparison of reaction types. In several groups, in the last 20 years analysis has proceeded further than in fungi.

## VII. OUTLOOK ON FURTHER RESEARCH

It seems worthwile to spend a few words on this subject. and we will consider some different items.

1). The distribution of luminescence in fungi. The survey presented in section II and more extensively in WASSINK (1978) shows that especially in the tropics many additions to the number of luminous fungi have been given, also in such a way that species have been recognized as luminescent which had been described already long ago, without the authors having noticed luminescence. Probably, in many cases, the authors were different from the observers and, moreover, collected by day. In this respect, and also as far as 'real' new species are concerned, attention to tropical species still appears very much worthwile. Especially indicated appear observations on the genera Mycena, Panus, and Pleurotus. The first two genera, moreover, have porous alliances (e.g. Poromycena and Dictyopanus) under which luminous species have been described under various names by different authors. As far as *Pleurotus* is concerned, several tropical species have been recognized as synonyms of P. olearius D.C., but this does not, up to now, hold for all of them, and the extension of luminosity in the genus Pleurotus seems worth further consideration. The existence of a tropical luminescent cospecies of Armillaria mellea Vahl, viz., A. fuscipes Petch seems to have been dropped in favour of full identity of both, but still seems worth further studies, and also their relationship with the probably non-luminescent Clitocybe tabescens SCOP.

The temperate zones of North America and Eurasia may well contribute further to the extension of the number of luminescent fungal species. Not many new discoveries have been reported since the explosive addition BOTHE gave nearly 50 years ago to luminosity in European species of *Mycena*, but recently luminosity has been observed in a culture of *M. avenacea* (Fr?) QuéL. sensu SCHRÖTER, KÜHNER, A. H. SMITH (cf. WASSINK, 1978) a species which was known for a long time. This justifies to expect that luminosity may well be observed in still more species in the temperate regions of the Northern hemisphere, especially among the less common ones, but moreover it seems worth while to look for the species indicated by BOTHE with respect to geographical distribution and luminescence.

2) Biochemistry. This field appears very promising for further research especially since not much progress seems to have been made after the papers of AIRTH et al. around 1960. Especially the chemistry of the 'luciferin' and 'luciferase' compounds involved requires further exploration, and the reason for the requirement of additional compounds, as e.g., reduced pyridine nucleotides. The requirement for a soluble and a particulate proteinaceous compound is also very interesting and is much worth further study. Likewise is the influence of possible inhibitors to which experience with bovine plasma addition gave suggestions. It seems that progress in the chemistry and biochemistry of luminescence in the fungi will benefit in the future from the further development of massive submerged culture methods.

Close relation with bacterial luminescence does not seem to obtain since long chain aldehydes and flavin mononucleotides were found to be inactive in the
fungal system so that the latter one seems to be simpler (cf. section IV C for further details and suggestions).

3). Methods for and physiology of submerged mass cultures. There is some belief in literature that various luminous fungi do not produce luminescent mycelia in submerged culture. The present author so far confirmed this only for Armillaria mellea. but grew perfectly luminous suspensions of, e.g., Mycena polygramma, Panus stypticus luminescens, and Omphalia flavida in bread extract media (cf. sections IV Bc and IV Dc). With the latter species methods for mass culturing were further developed (section IV Dc and WASSINK, 1974), so that completely clear media were achieved, containing glucose, a simple salt solution, and a small amount of either thiamin or purified yeast extract, producing abundant growth, However, thiamin addition yielded only about half the growth obtained with yeast extract. Preliminary attempts to increase growth with thiamin by addition of small amounts of various amino acids so far failed, but further research in this direction certainly is very much worth while. Some of the compounds indicated as being present in the 'Difco' yeast preparation so far also failed to increase further the growth with thiamin, but this can as yet not be considered conclusive and further attempts should be made. It needs hardly to be observed that a clear medium with a well-defined simple energy source (e.g. glucose) is essential for comparative studies of growth (dry wt. production), respiration and luminescence. Preliminary data on these subjects are, e.g., in WASSINK (1974). Selective effects of poisons, of antibiotics, of photoperiodicity on luminescence, and of agents, selectively influencing growth, respiration and luminescence are certainly worth being studied further. The same holds for the 'economic coefficient', i.e. the proportion between synthesized fungal substance and used substrate, and its possible variation with the age of the culture or with agents as mentioned above. For this subject see also WASSINK (1966, 1974). AIRTH and FOERSTER (1965) concluded that in thiamin, the thiazole moiety of the molecule contained the active part. Similar investigations would appear useful for so far unknown compounds increasing growth beyond the level reached with thiamin alone. Data obtained by BERLINER and coworkers on effects of poisons, and X-rays, on surface cultures of various luminous fungi certainly deserve repetition with submerged cultures especially from the viewpoint of differentiation between effects on growth(rate). rate of respiration and luminescent intensity. BERLINER'S work contains much stimuli for the study of time effects of the various agents, and segregation of stimulation to inhibition and vice versa. In so far as studies on submerged cultures are concerned, the size of the mycelial balls is of importance for, e.g., the rate of respiration per mg. dry wt. (WASSINK, 1974). This fact, in connection with age effects, diffusion of respirable substrate, oxygen, etc. certainly also deserve further study. Acid production probably occurs on a large scale in submerged cultures of Omphalia flavida. Another remarkable phenomenon was that this fungus tends to bring down pH to something around 4 before vigorous growth seems able to start. These phenomena certainly are worth further study, also as far as the nature of the acid production is concerned (c.f. WASSINK, 1974).

4). The relation between histology and luminescence. Closely related to this

subject may, in part, also be the time course of luminescence. In this respect interesting observations have already been made by BERLINER et al. and by CALLEJA and REYNOLDS who also developed sensitive methods enabling to study luminescence along separate hyphae. Moreover, the relation between certain 'granules' and luminescence, requires further investigation, for which perhaps methods of the mentioned authors may be combined with those applied by AIRTH et al. for in-vitro studies. So far, the above observations have been restricted to surface cultures. In submerged cultures also, distinct time lags in the development of luminescence, may be observed which may be related to certain zones of the hyphae being more luminescent than the very youngest parts (cf. WASSINK, 1974). The fact also that certain species fail to produce luminescence in submerged culture is worth further investigation in various directions (see further in text).

5). Different luminosity in different strains of the same fungus. Certain species have been observed to produce luminescence in connection with the genetic patron of different strains. The most well-known example is *Panus stipticus* with luminescent strains in America, and non-luminescent ones in Eurasia for which the geographic separation has prevented interbreeding. But it appears probable that in (various?) other species the intensity of luminescence produced in various strains (also arisen from multisporous isolations) is different. The present author long ago possessed a culture of Mycena polygramma that belonged to the best ones in the collection, and another one that, to the naked eye was completely dark (unpublished). A study of luminosity with respect to different isolations within a species seems very much worthwhile, especially with a view to physiological work on the comparison of, e.g.growth, respiration and luminescence. In a further phase of knowledge, it may be attempted to further increase luminescence in a strongly luminous culture by changing the culture method, either in the liquid or the gaseous phase, and by the addition of specific nutrients (e.g. amino acids, vitamins, etc.).

6). Mitogenetic radiation. The study of this subject has recently been revived (see text), and it seems of great interest to investigate luminous fungi in this respect. Reports are that in the main object used by QUICKENDEN and QUE HEE (Saccharomyces cerevisiae) the 'mitogenetic' emission in its strength and spectral composition depends on the stage of development of the culture. It appears of interest to see in how far this type of radiation can be detected also in surface and submerged cultures of luminous fungi and whether it is spectrally separated from the visible emission or whether there is an overlap. Non-luminescent strains, or culture methods leading to non-luminescence may, in this respect, prove useful. Selective filtering or sensitive spectroscopy will prove indispensable.

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## X. PLATES

For the origin and the numbering of the figures see Section III (pp. 8–13), where all the relevant information may be found. The numbers and characters are inserted on the plates in **bold face type**. In some cases other characters or numbers, coming from the original figures have not been removed; they have no reference to the reproductions on the plates but in some cases they give additional information, especially where (part of) the original legends were retained.

## Notes

Fig. 1b. The tissue overcrossing the stipe of the largest specimen is from another picture on the same plate and should be discarded.

Fig. 9b. The stipe of the left specimen on the original was interrupted by the pileus of another species on the same plate.

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but differs from it in its darker, blackish-brown stalk. The



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17,18A,18B,18D,18E



18**A**a

18Ba







Omphalia flavida growing on Borreria ocymoides in **Puerto Ri**co. Photo by J. van Overbeek.



 Portion de feuille d'Eriobolrya japonica portant un exemplaire d'Omphalia flavida (un peu grossi). — 2. Chapeaux jeunes d'Omphalue flavida.— 3. Un exemplaire adulte.

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Figure 4. a,b. Fruiting bodies of Omphalia flavida, which appeared on a densely grown submerged culture.
c. Detail from b, enlarged.

d. Fruiting body, showing size (in centimeters). e. Pileus, showing hymenium, enlarged (actual cross section ca 5 mm).

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Mynume auditations KAWAM.

23a





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第三朝 – Polyporus microporous KAWAM. コアミヒカリダア

A. 自然光にて撮影 ×15

B. 暗葉中にて茸の光にて撮影 1時間数出 (F.35, ライカ、パンド フキバル)

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Text-lig. 2. D. glossystidiatio, a diagram of the structure of the piletis to the occurrence of the glosseystidia.

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第 二 闘 *Marasmius* sp. チャクチバヒカリガキ A. 自然大 B. ×1.5

39Aa



39, 39A, 40

Fue. 3. Minute humitous fungt of the family Nidularitheae from Palao, Micronesia (x 54).

40a



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