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UNIVERSITE DE NEUCHATEL FACULTE DES SCIENCES

LES CHAMPIGNONS DESTRUCTEURS DU BOIS: CONTRIBUTION A LA CONNAISSANCE TAXONOMIQUE ET BIOLOGIQUE DU GENRE HYMENOCHAETE LEV.

DANIEL J. JOB

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Les champignons destructeurs du bois:
Contribution à la connaissance taxonomique
et biologique du genre Hymenochaete Lév.

de Monsieur Daniel Job

UNIVERSITÉ DE NEUCHÂTEL

FACULTÉ DES SCIENCES

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HYMENOCHAETE CRUENTA (PERS. : FR.) DONK NEW TO SOUTH AMERICA

DANIEL J. JOB

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HYMENOCHAETE CRUENTA (PERS.:FR.) DONK NEW TO SOUTH AMERICA.[1]

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During a taxonomic study of the genus *Hymenochaete* Lév. for Southern South America, a collection was found from Tierra del Fuego that coincides with the description of *H. cruenta* (Pers.:Fr.) Donk, perhaps better known as *H. mougeotii* (Fr.) Cooke. This species does not appear to have been recorded so far from the American continent (Defigio, 1970).

H. cruenta has been considered distinctly Euroasiatic, but was recorded by Cunningham (1963) for Australia and New Zealand. We believe it is of interest to describe this species on the basis of our material.

HYMENOCHAETE CRUENTA (Pers.:Fr.) Donk, Persoonia 1 (1): 51. 1959.

≡ Thelephora cruenta Pers.: Fr., Syst. Mycol. 1: 444. 1821.
= T. mougeotii Fr., Elench. Fung. 1: 188. 1828.
= Corticium mougeotii Fr., Epicr. p. 558. 1838.
= H. mougeotii (Fr.)Cooke, Grevillea 8: 147. 1880.
= H. sphaericola Lloyd, Mycol. Notes 74: 1338. 1925.

Basidiocarp adnate, resupinate, membranous, smooth; hymenial surface purple ("Indian purple", Pl. 47 L 2, according to Maerz & Paul, 1930); margin thin, concolorous.

Fruitbody 250-300 µm thick (Fig. 1 a). Cuticle present, compact, formed by thick-walled, intertwined and cemented hyphae. Abhymenial hairs scant. Context well developed, formed by loosely woven hyphae, 2-3 µm diam., with ascending orientation. Setal stratum seated on context, 80-[illegible]0 µm thick, formed by 2-3 rows of irregularly arranged setae. Setae [illegible]-90 x 8-12 µm, with acuminate apices, naked, lumen scarce, standing outup to 50 µm over the hymenial layer. Hymenium composed of paraphyses, basidia and basidioles. Paraphyses dendriform, originating in the context and slightly projecting over the hymenium; basidia subclaviform, 18-22 x 4-5 µm, with 4 curved sterigmata each; spores suballantoid, 6-8 x 2,5-3,5 µm, smooth, hyaline (Fig. 1 b).

Material studied: ARGENTINA, Tierra del Fuego: Puerto Harberton, leg. Wright & Del Busto, II.1973 (BAFC 29.230).

[1] Research subsidized by the Argentine National Research Council. Publication n°5 of the PRHIDEB.

[2] Fellow of the above Council.

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MAERZ, A. & M. R. PAUL. 1930. Dictionary of Color. McGraw-Hill Co., New York.

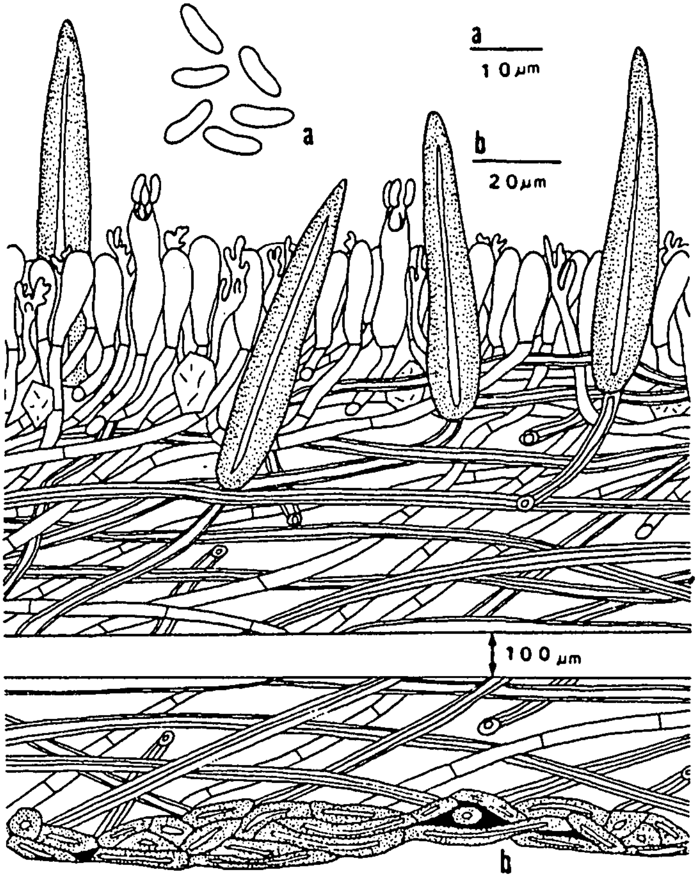

Fig. 1. Cross section through the fruitbody of *Hymenochaete cruenta*. a: spores; b: detail of the basidia, setae and hyphae; in the lower part the amalgamated hyphae can be seen. Arrow in the middle portion indicates thickness.

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CULTURAL AND CYTOLOGICAL STUDIES IN THE GENUS HYMENOCHEATE LEV.

DANIEL J. JOB

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CULTURAL AND CYTOLOGICAL STUDIES IN THE GENUS *HYMENOCHAETE* LEV.[1]

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SUMMARY

The morphology based on the methodology proposed by Nobles (1948, 1958, 1965), and the cytology of polyspermic cultures of *H. cervina* Berk., *H. cinnamomea* (Pers.) Bres., *H. leonina* Berk. & Curt., *H. luteo-badia* (Fr.) Höehn. & Litsch., *H. pinnatifida* Burt and *H. sallei* Berk. & Curt., were studied. Codes and illustrations for those species are supplied, and we propose the utilization of code number 28*d* to describe the presence of dendrophyses in culture.

The very few data available on the behavior of the genus *Hymenochaete* Lév. in culture were mainly the result of studies by Kühner (1950), Nobles (*op. cit.*), Boidin (1958), Stalpers (1978), Léger *et al.* (1983) and more recently Boidin *et al.* (1984).

The study of cultures of *H. cervina* Berk. & Curt., *H. cinnamomea* (Pers.) Bres., *H. leonina* Berk. & Curt., *H.* [illegible]*teo-badia* (Fr.) Höehn. & Litsch., *H. pinnatifida* Burt and [illegible] *sallei* Berk. & Curt., of broad distribution in the Northeast area of Argentina (Job, 1985a) and Southwestern Brazil (Job, 1985b), adds more information on this genus.

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[1] Work carried out as part of the research project on xylophilous fungi of Argentina, subsidized by the Argentine National Research Council (CONICET). Publication N°17 of the PRHIDEB.

[2] Fellow of the above Council.

MATERIAL AND METHODS

The polyspore cultures were obtained from spore-print material. The study of the morphology was carried out based on Nobles' methodology (*op. cit.*) and includes the code using the added numbers mentioned by Boidin & Lanquetin (1983).

The medium employed was:

a) Growth medium: malt agar (Nobles, 1948)
b) Oxidase reaction medium: i- gallic acid agar (Davidson *et. al.*, 1938)
ii- tannic acid agar (idem.)
iii- guaiacol 2% (Boidin, 1958:40)
iv- paracresol (idem. , 1958:41)
v- tyrosine (idem. , 1958:41)

The reaction intensity and the rate of the mycelium growth are given as described by Davidson *et al.* (*op. cit.*). The colors of the mycelium are described based on Maerz & Paul (1930).

For the cytological study the technique mentioned by Boidin (1958) has been followed. The cultures were grown under a collodion film, and were fixed by a saturated solution of Cl_2Hg and glacial acetic acid, finally being colored with slow giemsa.

RESULTS

1- *H. cervina* Berk. & Curt.

Macroscopic characters: growth very slow, reaching 6,5 - 7,5 cm after 6 weeks. Mat subarachnoid, hyaline at first, finally gold brown (pl. 14 F 12) after the 4th. week (Fig. 1). Margin regular, subarachnoid, appressed. Reverse slightly changed to brown near the inoculum. Odor absent.
Reactions:
guaiacol 2%: + +, no growth
gallic ac.: + + +, no growth tyrosine: -, no growth
tannic ac.: + +, no growth paracresol: -, no growth

Microscopic characters: margin formed by generative hyphae of 2 types: 1- twisted, 3 - 3,6 µm diam, afibulate, thin-walled, sparsely septate (Fig. 2) and 2- straight, afibulate 3 - 4,2 µm diam. Aerial hyphae thin-walled generative hyphae 2 - 5,8 µm diam, straight or with zigzag walls (Fig. 3). Hyphae submerged with kidney shaped characteristic protuberances, which begin to be observed after the second week, first in the margin and later in all the mycelium (Fig. 4). Brown hyphae are observed; these have broad walls which form a compact mesh and resulting in a plectenchymatose tissue (Fig. 5).
Cytological studies: cells binucleate, more rarely uni or trinucleate (Fig. 6).
Code: 2*a*, 6, 11, 22, 32, 37, 39, 47, 54, 64.

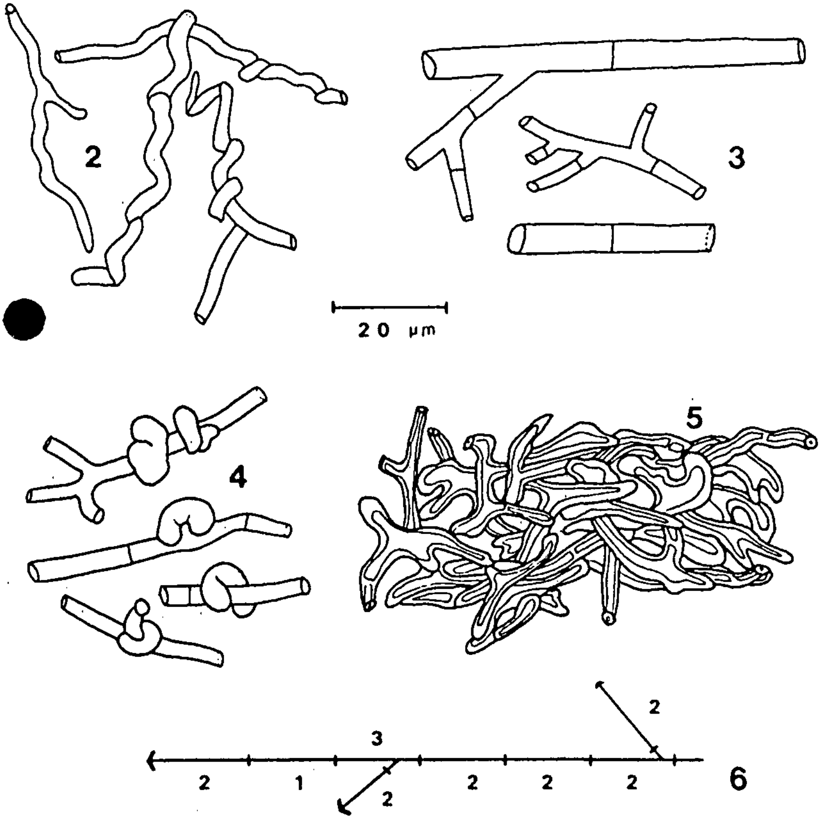

Fig. 2-6: H. cervina 2- advance mycelium generative hyphae 3- mycelial layer hyphae 4- submerged hyphae with kidney s[illegible]ped protuberances 5- plectenchymatic tissue 6- distribut[illegible]n of the nuclei in the polyspermic culture BAFC N°1167.

Culture studied: BAFC N°1167 = Argentina, Misiones, Parque Nacional Iguazú; leg. Job & Rajchenberg; 6-IV-1984 (BAFC N° 30257).*
Remarks: the characteristic of forming generative hyphae with kidney shaped protuberances, that resemble incomplete clamps conections, may be a specific character although not constant, since it has been observed in only one of three

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*The numbers in parentheses are herbarium voucher specimens.

cultures studied of H. berteroi Pat. (Léger, personal communication).

2- H. cinnamomea (Pers.) Bres.

Macroscopic characters: growth slow, covering the plate at the end of the 5th. week. Mycelial layer of texture ranging from felty to cottony, white during the first weeks, later turning yellow ochre (Fox antique pl. 14 K 8). Margin elevated, hyaline, regular, subfelty (Fig. 7). Reverse changed homogeneously to dark brown at the end of the study. Odor sweet.

Reactions:
guaiacol 2%: + + + +, no growth
gallic ac.: + + + +, no growth
tannic ac.: + + +, 0,8 cm
tyrosine: -, 0,5 cm
paracresol: -, 0,5 cm

Microscopic characters: the advance mycelium formed by 1,7 - 3,7 µm diam generative hyphae with few branches, thin walls, afibulate, with numerous constrictions 0,8 - 1,6 µm diam (Fig. 8). Mycelial layer formed by regulary septate, generative hyphae, 2 - 6,8 µm diam, with thin hyaline walls, some of them constricted to 1,2 - 3,4 µm diam, and generative hyphae with amber like oily contents 2,8 - 5,3 um diam (Fig. 9). From the third week on, brown generative hyphae with irregular nodules and short branches form in the central zone of the culture, with slightly thickened walls, that form a scarce plectenchymatic tissue (Fig. 10). Submerged mycelium composed of distorted generative hyphae 2,3 - 3,8 µm diam, hyaline, thin walled, lacking clamps.
Cytological studies: cells coenocytic, specially the terminal ones with variation of 26 to 58 nuclei (Fig. 11).

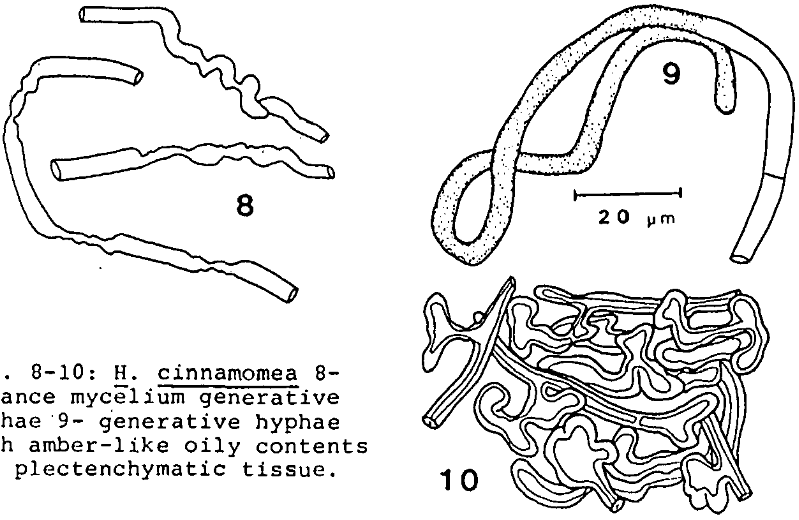

Fig. 8-10: H. cinnamomea 8- advance mycelium generative hyphae 9- generative hyphae with amber-like oily contents 10- plectenchymatic tissue.

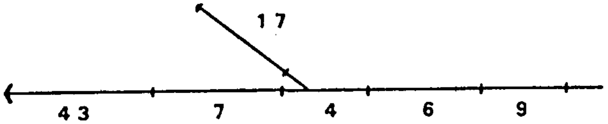

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Fig. 11: *H. cinnamomea* distribution of the nuclei in the polyspermic culture BAFC N°1164.

Code: 2*a*, 6, 7, 11, 32, 37, 39, 45, 50, 54.
Culture studied: BAFC N°1164 = Argentina, Misiones, Parque Nacional Iguazú; leg. Wright, Rajchenberg & Job; 8-IV-1984 (BAFC N°30255).
Remarks: *H. cinnamomea* presents a growth pattern in culture very similar to that of *H. corrugata* (Nobles, 1950) and of *H. sallei*. It differs from these at the macroscopic level, by a slower growth and microscopically by having an inconspicuous plectenchymatic tissue.

Cytologically the studied culture presents coenocytic cells. This differs from the observations of Boidin *et al.* (1984) who found dikaryotic cells.

3- *H. leonina* Berk. & Curt.

Macroscopic characters: growth very slow, reaching 7 - 8 cm after six weeks. Mycelial layer hyaline, subarachnoid, with irregular yellow zones (pl. 12 L 7) near the inoculum (Fig. 12). Margin irregular, subarachnoid, appressed to the substrate. Reverse changed to brown in the area up to 35 mm from the inoculum. Odor of antiseptic.
Reactions:
guaiacol 2%: + + +, no growth
tannic ac.: + + + +, tr. paracresol: -, tr.
gallic ac.: + + + +, tr. tyrosine: -, 0,6 cm

Microscopic characters: advance mycelium formed by regulary branched, afibulate hyphae, 3,5 - 5 µm diam, and branches 1,5 - 2,5 µm diam (Fig. 13). Mycelial layer formed by hyphae similar to the advance mycelium, very few with bulbils. Submerged mycelium characterized by very short branched generative hyphae (Fig. 14). Crystals present in the agar.
Cytological studies: cells binucleate, more rarely with uninucleate ones (Fig. 15).
Code: 2*a*, 6, 7, (22), 32, 37, 39, 47, 50, 54, 64.
Culture studied: BAFC N°1152 = Argentina, Misiones, Parque Nacional Iguazú; leg. Job; 8-IV-1984 (BAFC N°30251).
Remarks: *H. leonina* has distinctive cultural characteristics in the genus. Macroscopically it presents a subarachnoid texture, and a pale mycelial layer. Microscopically it does not form a dense hyphal weft, like other species studied in this genus.

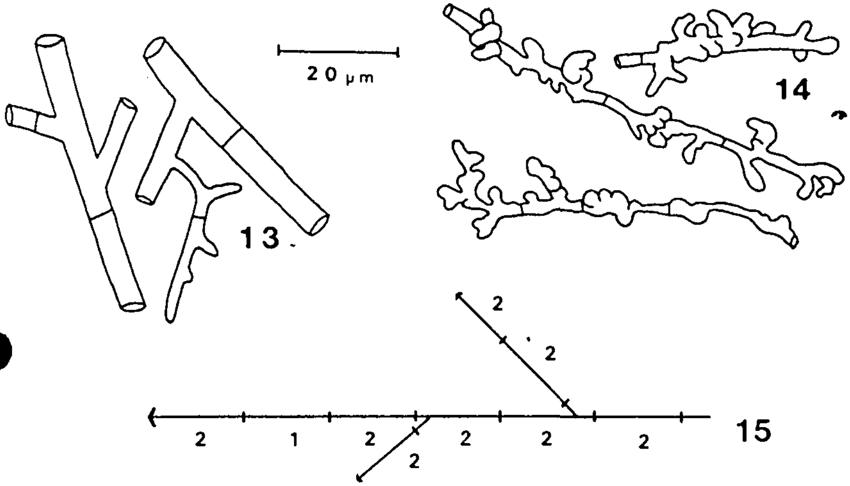


Fig. 13-15: H. *leonina* 13- advance mycelium generative hyphae 14- submerged hyphae with very short branches 15- distribution of the nuclei in the polyspermic culture BAFC N°1152.

4- H. *luteo-badia* (Fr.) Höehn. & Litsch.

Macroscopic characters: growth very slow, reaching 5 - 5,6 cm by the sixth week. Mycelial layer hyaline at the beginning turning brown (Gold Brown pl. 14 F 12) after the fourth week with dark zones (Burnt umber pl. 15 A 12). Texture subfelty. Advance mycelium submerged, irregular, soft, grey (Fig. 16). Culture medium changed to brown in the area around and under the inoculum. Odor absent.
Reactions:

gallic ac.: + + + (+), tr.	tyrosine: -, no growth
tannic ac.: + + (+), tr.	paracresol: -, no growth
guaiacol 2%: + + (+), no growth	

Macroscopic characters: aerial mycelium formed by afibulate, branched, thin walled, generative hyphae of variable diameter, 1,8 - 7 µm (Fig. 17). Mycelial layer formed by hyphae similar to those of the advance mycelium, some with numerous oily contents (Fig. 18). In correlation with the darker zones of the mycelial layer we observed the presence of a plectenchymatic tissue. Numerous crystals present near the inoculum.
Cytological studies: cells binucleate, rarely uni or trinucleate (Fig. 19).
Code: 2a, 6, 11, 32, 37, 39, 47, 54, 64.
Culture studied: BAFC N°438 = Argentina, Misiones, Parque Nacional Iguazú; leg. Job; 22-IX-1984 (BAFC N°30289).
Remarks: H. *luteo-badia* has a growth pattern very similar to that of H. *cervina*, differing from it microscopically by lacking bulbils, and macroscopically by its irregular margin.

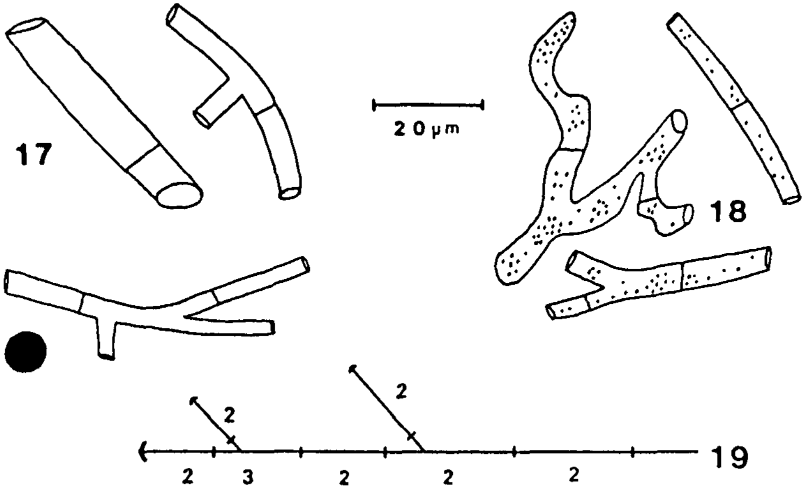


Fig. 17-19: *H. luteo-badia* 17- generative hyphae of the aerial mycelium 18- generative hyphae of the advance mycelium with numerous oily contents 19- distribution of the nuclei in the polyspermic culture BAFC N°438.

5- *H. pinnatifida* Burt

Macroscopic characters: growth very slow, reaching 6 - 7 cm after 6 weeks. Mycelial layer hyaline at the beginning, then golden brown (Cognac pl. 14 J 11 to Soudan pl. 14 L 12). Texture initially felty, turning velvety after the second week. Advance mycelium submerged, forming a wide dark zone around the aerial mycelium (Fig. 20). Culture medium changed intensely to brown in all the plate. Odor lightly sweet.
Reactions:

gallic ac.: + + + +, tr.	tyrosine: -, no growth
[illegible]nic ac.: + + +, tr.	paracresol: -, no growth
guaiacol 2%: + + + +, no growth	

Microscopic characters: aerial mycelium formed by generative hyphae, branched, afibulate, without constrictions 3 - 5,6 µm diam, densely interwoven, numerous more or less branched, pigmented hyphae, with walls regulary thickened, fibrous hyphae 3 - 4,8 µm diam (Fig. 21), which form a dense trama. Brown, thin-walled dendrophyses regulary present in the culture (Fig. 22). Advance mycelium formed by generative hyphae, with enlargements and protuberances. Crystals free in the agar.
Cytological studied: cells coenocytic, with variation of 3 to 7 nuclei (Fig. 23).
Code: 2a, 6, 8, 28d, 32, 37, 39, 47, 50, 54.
Culture studied: BAFC N°640 = Argentina, Misiones, Parque Nacional Iguazú; leg. Job; 23-IX-1984 (BAFC N°30287), and

BAFC N°702 = *ibid*; leg. Job; 8-IV-1984 (BAFC N°30326).
Remarks: Boidin & Lanquetin (1983) proposed the utilization of the code number 28 for the asterophyses in culture. But Burdsall and Nakasone (1981) have used previously this code number 28 for the capitulate spines of *Mycoacia meridionalis*. So we propose now -in agreement with Boidin and Lanquetin- that the code number 28a should be used for the asterophyses and the code number 28d for the dendrophyses.

H. pinnatifida is well characterized by the presence of fibrous hyphae and dendrophyses, structures not found up to the present in cultures of this genus.

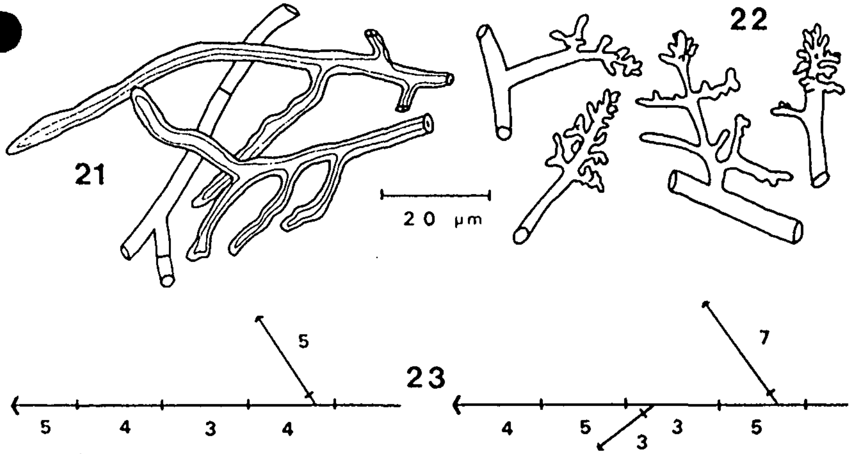


Fig. 21-23: *H. pinnatifida* 21- fibrous and generative hyphae 22- dendrophyses 23- distribution of the nuclei in the polyspermic cultures BAFC N°640 and BAFC N°702.

- *H. sallei* Berk. & Curt.

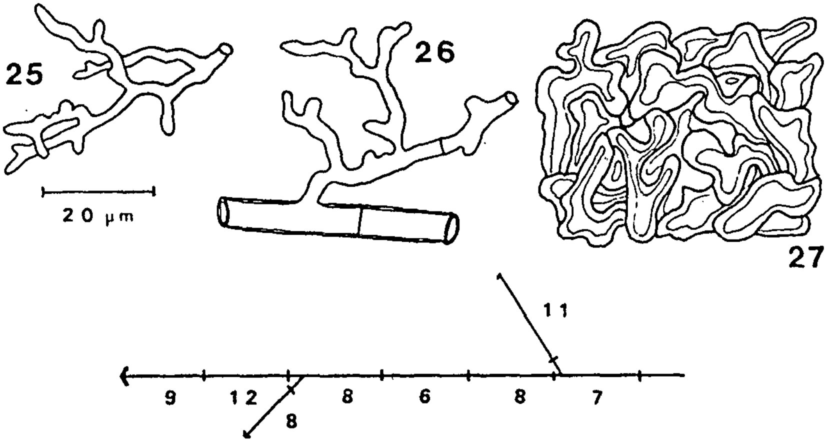
Macroscopic characters: growth rapid, covering the plate during the 3 rd week. Mycelial layer, felted, hyaline at the beginning, turning ochre yellow (pl. 11 L 8) with more densely felty zones slightly orange tinted(pl. 3 B 12). Margin white, raised, felty, regular (Fig. 24). Reverse turning dark brown in the central zone of the culture after the third week. Odor sweet.
Reactions:
gallic ac.: + + + +, 0,6 cm
tannic ac.: + + + +, 0,4 cm
guaiacol 2%: + + +, no growth
tyrosine: -, no growth
paracresol: -, no growth

Microscopic characters: advance margin formed by afibulate, much branched generative hyphae, 2 - 3,6 µm diam (Fig. 25). Felty layer and inoculum formed by afibulate regulary branched generative hyphae, 4,3 - 7,5 µm diam, with walls slightly thickened and branches 2 - 3,2 µm diam (Fig. 26). Crystals free in the agar. Dense zones of the mycelial layer formed by brown hyphae with thickened walls, densely interwoven, which originate a plectenchymatic tissue (Fig. 27). Cytological studies: cells coenocytic, with variation of 6 to 14 nuclei (Fig. 28).

Code: 2a, 6, 11, 32, 37, 39, 43, 50, 54.

Culture studied: BAFC N°599 = Argentina, Misiones, Parque Nacional Iguazú; leg. Job; 23-IX-1984 (BAFC N°30283).

Remarks: *H. sallei* has a rate and type of growth in culture similar to that of *H. corrugata* (Nobles, 1958). Microscopically both species present similar structures requiring t[illegible]r differentiation to be made at the microscopic level s[illegible]e *H. sallei* does not produce a bleaching of the culture medium and has its mycelial layer slightly tinged with orange. Boidin *et al.* (1984) have observed a slower rate of growth for this species.



[illegible] 25-28: *H. sallei* 25-advance mycelium generative hyphae 26- hyphae with thickened axes 27- plectenchymatic tissue 28- distribution of the nuclei in the polyspermic culture BAFC N°599.

KEY FOR THE CULTURAL IDENTIFICATION OF THE *HYMENOCHAETE* SPECIES STUDIED.

2a, 6, 7, 11, 32, 37, 39, 45, 50, 54. . . . *H. cinnamomea*
2a, 6, 7, (22), 32, 37, 39, 47, 50, 54, 64. *H. leonina*
2a, 6, 8, 28d, 32, 37, 39, 47, 50, 54 . . . *H. pinnatifida*
2a, 6, 11, 22, 32, 37, 39, 47, 54, 64 . . . *H. cervina*

2*a*, 6, 11, 32, 37, 39, 43, 50, 54. . . . . *H. sallei*
2*a*, 6, 11, 32, 37, 39, 47, 54, 64. . . . . *H. luteo-badia*

DISCUSSION

From the results obtained in the present study, and from those of Kühner (1950), Nobles (1948, 1958, 1965), Boidin (1958), Stalpers (1978), Léger *et al.* (1983) and Boidin *et al.* (1984)it appears that the species of the genus *Hymenochaete* Lév. studied in culture present evolved characters viz: the presence of laccases and the absence of clamp connections on hyphae. They also share the characteristic of usually forming a plectenchymatic tissue, and not producing asexual reproductive structures, an exception being *H. rubiginosa* (Boidin, *op. cit.*). Macroscopically they present a common pattern from moderate to slow growth; a mycelial layer that turns from ochre to brown; and a variable texture. In all the cases studied there occurred a change in the culture medium to brown. Characteristic structure of the basidiocarps, like setae or dendrophyses have only been observed in *H. cruenta* (Boidin, *op. cit.*) and *H. pinnatifida*, respectively.

In their general macroscopics features they exhibit a similarity with cultures of other Hymenochaetaceae so far studied, particulary species of the genus *Phellinus*. It would thus appear that the culture patterns of the members of this family support its position as a homogeneous taxon.

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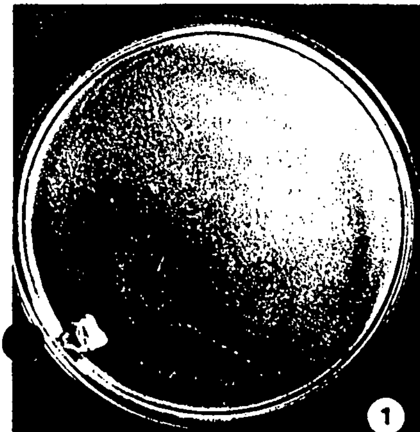
Aspect of six week old cultures of: Fig. 1- *H. cervina* Fig. 7- *H. cinnamomea* Fig. 12- *H. leonina* Fig. 16 *H. luteo-badia* Fig. 20- *H. pinnatifida* Fig. 24- *H. sallei*.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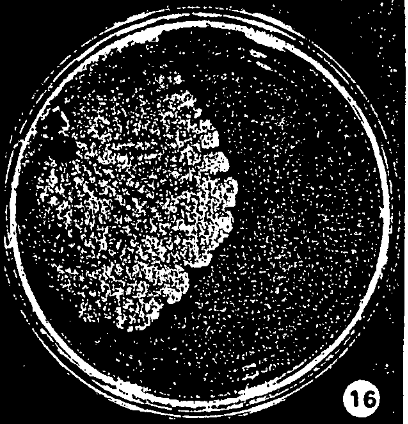
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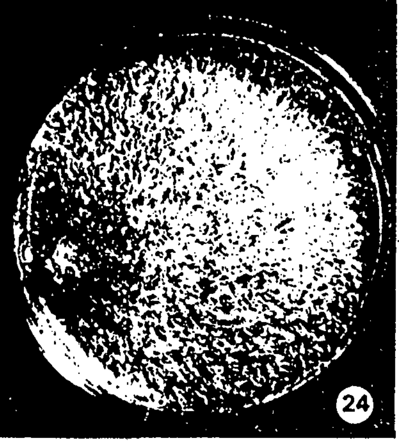
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South African species of *Hymenochaete* (Aphyllophorales)

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Twenty-five South African collections of *Hymenochaete* Lév deposited in the herbarium of the Plant Protection Research Institute, Pretoria, South Africa (PREM), were studied. The collections were distributed in nine taxa, namely: *H. cinnamomea, H. fasciculata, H. flavomarginata, H. leonina, H. luteo-badia, H. ochromarginata, H. pinnatifida, H. semistupposa* and *H. tabacina*. A key for the identification of the species, descriptions and illustrations are given.

Vyf en twintig Suid-Afrikaansversamelde eksemplare van *Hymenochaete* Lév gehuisves in die herbarium van die Plantbeskermingsnavorsingsinstituut, Pretoria, Suid-Afrika (PREM), is bestudeer. Die eksemplare verteenwoordig nege taxa, nl. *H. cinnamomea, H. fasciculata, H. flavomarginata, H. leonina, H. lutea-badia, H. ochromarginata, H. pinnatifida, H. semistupposa* en *H. tabacina*. 'n Sleutel vir die identifikasie van die soorte, beskrywings en illustrasies word gegee.

Keywords: Aphyllophorales, *Hymenochaete*

Introduction

Burt (1918), who studied the genus *Hymenochaete* for North America, the Caribbean and eastern South America, was the first mycologist to introduce new ideas for the study of the species, and he was also the first to use the internal anatomy of the sporocarp to separate the species into three groups.

Later, the genus was studied, in the light of modern criteria, principally by Bourdot & Galzin (1927), Telleria (1980) and Léger (1985) for Europe; Reeves & Welden (1967) for the West Indies; Escobar (1978) for the Neotropics; Rattan (1977) for India; Cunningham (1957) for Australasia; Bononi (1979) and Job (1985a, b, c) for South America; Léger (1980, 1981a, 1982) and Léger & Lanquetin (1983) for equatorial western Africa and Madagascar; and more generally by Defigio (1970).

The data concerning the genus *Hymenochaete* in South Africa are mainly the results of the studies of Wakefield & Talbot (1948), Doidge (1950) and Talbot (1951, 1958). A revision of the collections lodged in the herbarium of the Plant Protection Research Institute, Pretoria (PREM), was carried out with the aim of furnishing more information utilizing modern taxonomic criteria, concerning the South African species known up to the present.

Materials and Methods

Free-hand sections were mounted for microscopical observation in an aqueous solution of phloxine and 5% KOH solution.

The herbaria abbreviations are taken from Holmgren & Keuken (1974). When the sign ! is given after the herbarium abbreviation it indicates the type material therein lodged has been studied personally.

Colours are from Maerz & Paul (1930).

Results

The collections were distributed in nine taxa; of which *H. cinnamomea, H. flavomarginata* and *H. leonina*, proved new for South Africa.

1. **H. cinnamomea** *(Pers.) Bres.*, I.R. Acad. Agiati Atti III. 3: 110. 1897 (Figure 1).

Thelephora cinnamomea Pers., Mycol. Eur. 1: 141. 1822.

Corticium cinnamomeum (Pers.) Fr., Epicr. 561. 1838.

Hymenochaete spreta Peck, N.Y. State Mus. Rept. 30: 47. 1879 (NYS !).

Hymenochaetella arida Karst., Bidr. Nat. Folk 48: 428. 1889 (H !).

Hymenochaetella laxa Karst., Bidr. Nat. Folk 48: 429. 1889 (H !).

Hymenochaete arida (Karst.) Sacc., Syll. Fung. 9: 228. 1891.

Hymenochaete laxa (Karst.) Sacc., Syll. Fung. 9: 222. 1891.

Hymenochaetella rudis Karst. Hedwigia 35: 173. 1896 (H !).

Hymenochaete rudis (Karst.) Sacc. Syll. Fung. 14: 218. 1899.

Isotype: Fries; Herbarium Fries; Europe (K).

Basidiocarp resupinate, adnate, velvety to waxy, cracked when older. Hymenial surface ochre (Bay pl. 7 E 11 to Cocoa pl. 7 E 12), smooth, margin thinning out, concolorous. In section 300 – 1000 μm thick (Figure 1a), stratose, ranging up to 6 strata; each composed of a setigerous layer with 1 – 4 rows of setae, and of a hyphal layer of loosely interwoven hyphae 60 – 100 μm thick, free of setae. Hyphae septate, branched at right angles, 2 – 3 μm diam. Cortex absent. Setae lanceolate to aculeate, naked or ensheathed, 60 – 115 × 5 – 7 μm, projecting up to 60 μm beyond the hymenium. Hymenium composed of basidia and basidioles; basidia subclavate 14 – 18 × 4 – 6,5 μm, with 4 curved sterigmata each. Spores suballantoid 4,5 – 6 × 2,5 – 3,5 μm, smooth, hyaline (Figure 1b).

Material studied: Pietermaritzburg, Natal, leg. W.G. Rump, XII.1943 (PREM 39196); ibid., II.1944 (PREM 35418); Isipingo Beach, Natal, leg. W.G. Rump, 1944 (PREM 36851 as *H.* sp.); Umgeni Bush, Durban, leg. W.G. Rump, 1935 (PREM 41754 as *H.* sp.).

Remarks: The South African collections of *H. cinnamomea* present filiform paraphyses which stand out up to 50 μm over the hymenial surface, as do the European and Australian collections of the species (Cunningham 1957). These paraphyses are fewer or absent in the South American collections of *H. cinnamomea* (Job 1985b).

2. **H. fasciculata** *Talbot apud Wakefield & Talbot* in Bothalia 4: 943. 1948 (PREM !) (Figure 2).

Holotype: W.G. Rump 220; Town Bush, Pietermaritzburg, Natal, South Africa; October 1934 (K); isotype PREM 28297.

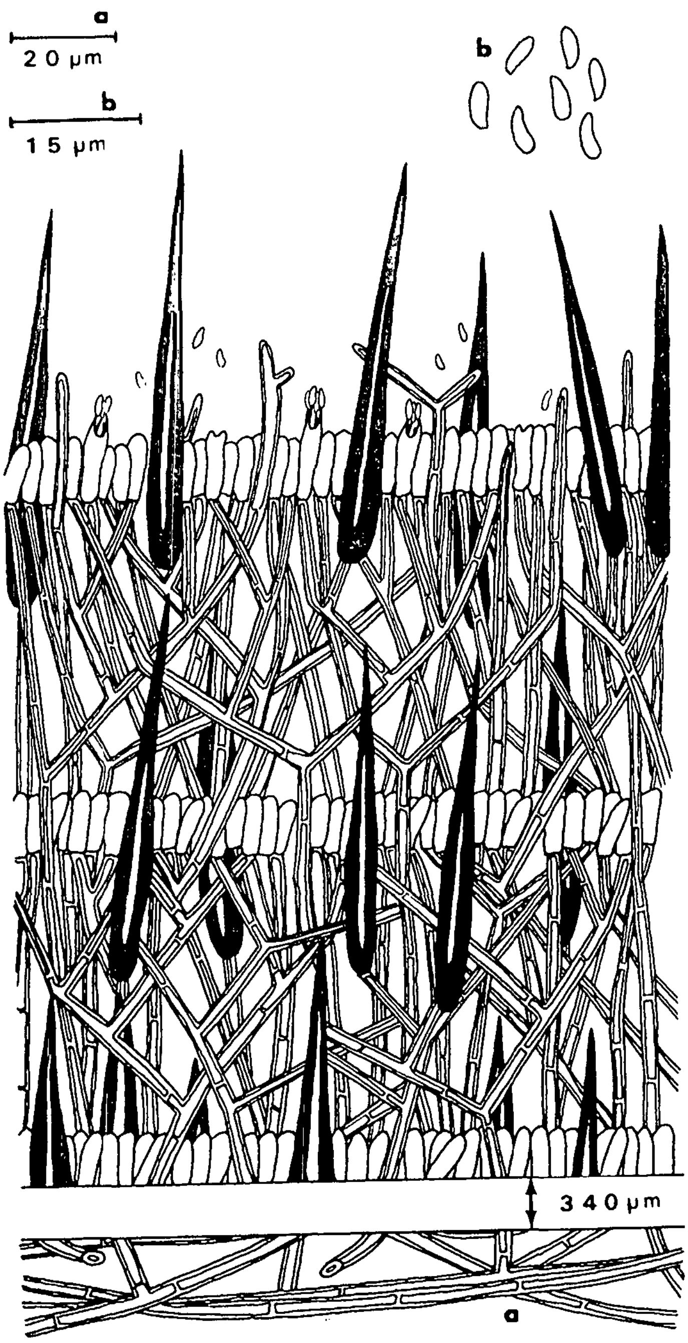

Figure 1 Microscopic characters of *Hymenochaete cinnamomea*; (a) cross section through the basidiocarp (arrow in the middle portion indicates thickness), (b) spores.

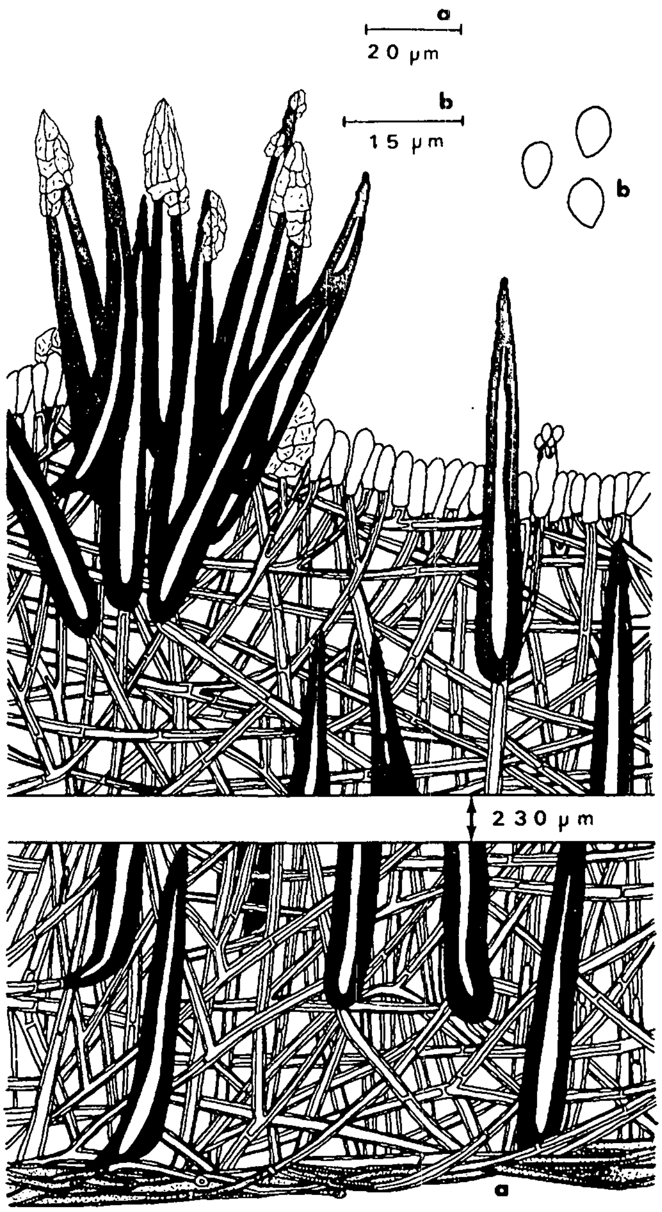

Figure 2 Microscopic characters of *Hymenochaete fasciculata*; (a) cross section through the basidiocarp (arrow in the middle portion indicates thickness), (b) spores.

Basidiocarp resupinate, adnate, membranous, colliculose, cracked, with setae arranged in tuberculate fascicles; hymenial surface umber (Cocoa Brown pl. 15 C 11), margin thinning, adnate, concolorous. In section up to 400 µm thick (Figure 2a), tightly interwoven cortex present. Context interwoven, hyphae branched 1,5 – 3 µm diam. Setigerous layer starting from the cortex and present throughout the context in 3 to 5 rows. Setae aculeate 66 – 98(– 110) × 7 – 9(– 11,5) µm, bending, solitary or in conspicuous fascicles of 6 to 12 (especially at the papillae), with slightly verruculose apices encrusted with crystals, naked or ensheathed, projecting up to 80 µm beyond the hymenium; crystals also present in the context. Basidia urniform 14 – 18 × 5 – 6 µm; basidioles globose 12 – 16 × 3 – 5 µm. Spores ovate to broadly suballantoid 5 – 6 × 3,7 – 5 µm, smooth, hyaline (Figure 2b).

Material studied: Town Bush, Pietermaritzburg, Natal, leg. W.G. Rump, X.1934 (PREM 28297, Isotype); ibid. (PREM 28500); Donnybrook, Natal, leg. M. Doidge, II.1935 (PREM 28932).

Remarks: Talbot (1951) did not find spores and basidia in the type. Defigio (1970) found urniform basidia 14 – 18 × 5 – 6 µm and ovate spores 5 – 6 × 4 – 5 µm in the isotype. I have found both in the isotype and in the collection PREM 28932 ovate to broadly suballantoid spores 5 – 6 × 3,7 – 5 µm. In the three collections studied, the hymenium is in a very poor condition, the basidia are very scarce and weathered. The basidia measurements and morphology are those recorded by Defigio (op. cit.).

Three species with fasciculate setae have been described: *H. fasciculata*, *H. dictator* Cunn. (PDD !) (in Trans. Royal Soc. New Zeal. 85: 32. 1957) and *H. lictor* Petch (in Ann. Roy. Bot. Gard. Perad. 9: 27. 1925). The first differs from *H. dictator* macroscopically by the different colour of the surface (Sudan Brown pl. 14 L 12 in *H. dictator*), and microscopically by having larger setae with apices encrusted (40 – 80 × 6 – 9 µm in *H. dictator*) and by the different structure of the cortex. *H. fasciculata* differs from *H. lictor* in having the setigerous layer starting from the cortex, encrusted setae, crystals in the context and conspicuous fascicles of 6 to 12 setae (whereas *H. lictor* has a smaller number of setae in fascicles, up to six).

3. **H. flavomarginata** Pat. Bull. Soc. Myc. 8: 118. 1892 (FH !) (Figure 3).

Holotype: Patouillard Herbarium No 3021; Rio Mochangaro, Ecuador (FH).

Basidiocarp resupinate, adnate, membranous. Hymenium smooth to slightly colliculose, ochre (Bronze pl. 14 L 8), margin thinning, concolorous. In section up to 120 μm thick (Figure 3a). Hyphae loosely interwoven, 3 – 3,5 μm diam. freely branched and septate. Cortex absent. Setigerous layer resting on the substrate, composed of 1 or 2 rows of setae with acuminate apices. Setae lanceolate to aculeate 70 – 120 (– 130) × 6 – 8(– 9) μm, naked, some of them with the shape of an inverted T, projecting up to 100 μm beyond the hymenium. Crystals present in the context. Hymenium composed of basidia and basidiola. Basidia suburniform 14 – 18 × 4 – 6 μm, sterigmata arquate; basidiola subglobose. Spores oblong-elliptic to broadly suballantoid 6 – 8 × 2,6 – 4 μm, smooth and hyaline (Figure 3b).

Material studied: Eshowe, Zululand, leg. Mrs. Laughton, XI.1941 (PREM 33351 as *H.* sp.).

Remarks: This species is very similar to *H. contiformis* Cunn. (PDD !) (in Trans. Royal Soc. New Zeal. 85: 41. 1957) and *H. pratensis* Viégas (IACM !) (in Bragantia 5: 261. 1945). Their basidiocarps are ochre, adnate, very thin and their spores are characteristically oblong-elliptical and have very large setae seated directly on the substrate. *H. flavomarginata* differs from the others in the size of spores and setae (5 – 6 × 2,8 – 4 μm and 50 – 60 × 6 – 8 μm, respectively for *H. pratensis* and 8 – 9 × 3,5 – 4,5 μm and 95 – 190 × 8 – 12 μm, respectively for *H. contiformis*).

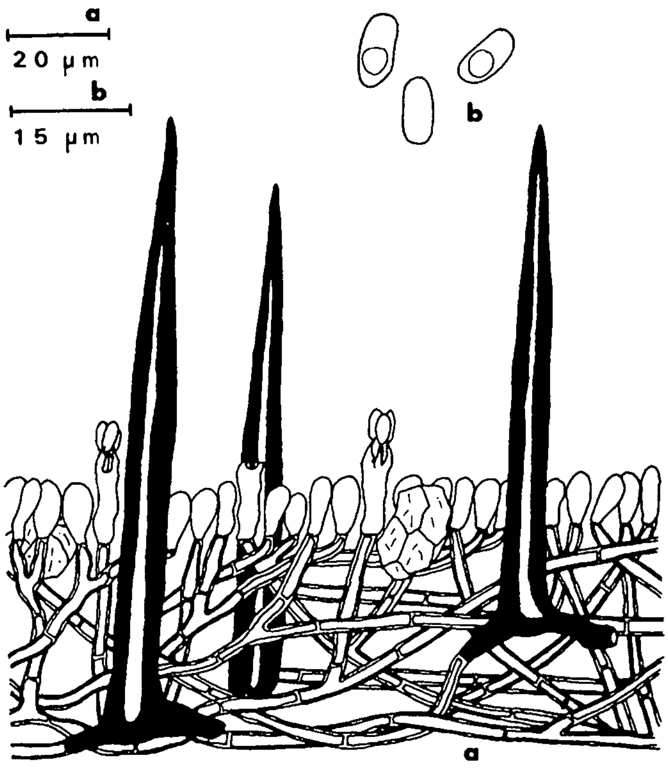

Figure 3 Microscopic characters of *Hymenochaete flavomarginata*; (a) cross section through the basidiocarp, (b) spores.

4. **H. leonina** *Berk. & Curt.*, Linn. Soc. Bot. J. 10: 334. 1868 (FH !) (Figure 4).

Isotype: C.G. Wright 532, Cuba (FH).

Basidiocarp resupinate, velvety, separable from the substrate. Hymenium smooth, brownish-grey (Arizona pl. 13 E 6), margin thinning out, sometimes becoming detached, golden (Antique gold pl. 12 L 8). In section 100 – 700 μm thick (Figure 4a). Cortex present, composed of densely compacted darkened hyphae, mainly inside the context and dividing the latter into 2 layers of more or less the same thickness. Setigerous layer seated on the context, composed of 1 or 2 rows of regulary spaced setae. Setae lanceolate 55 – 85 × 6 – 9 μm, naked or ensheathed, projecting up to 60 μm beyond the hymenium. Hymenium composed of basidia and basidiola. Basidia clavate 14 – 17 × 3 – 5 μm, with 4 curved sterigmata each. Spores allantoid to suballantoid 4 – 6 × 3 – 5 μm, smooth, hyaline (Figure 4b).

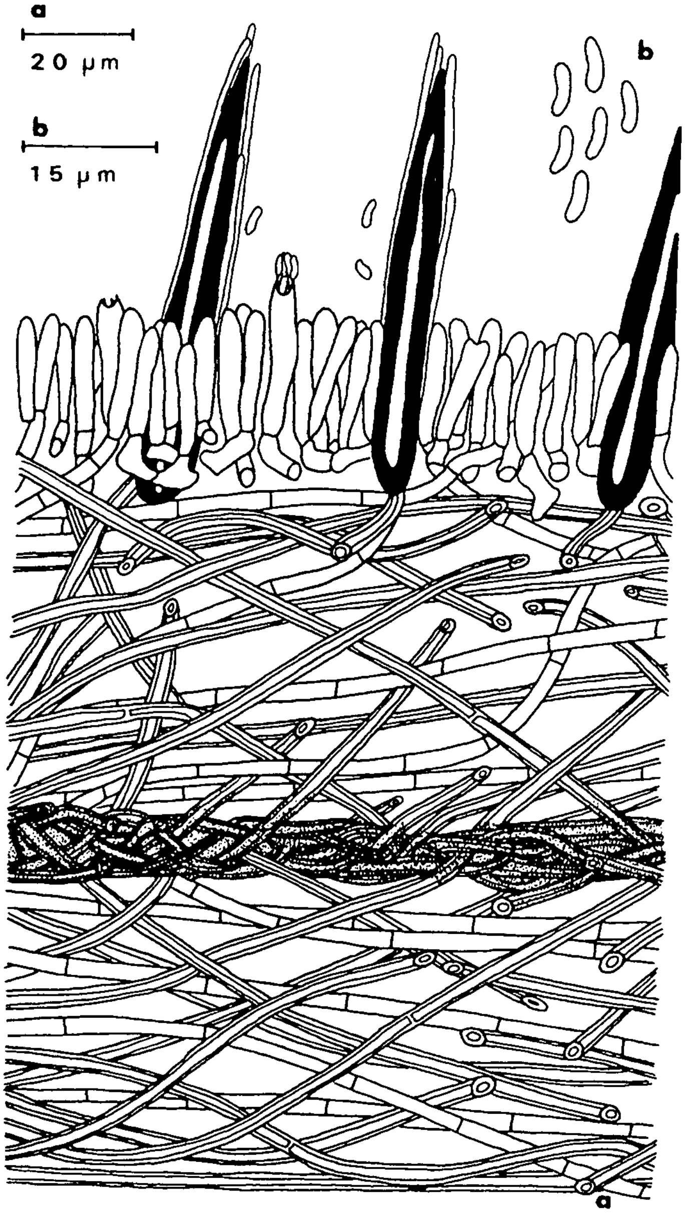

Figure 4 Microscopic characters of *Hymenochaete leonina*; (a) cross section through the basidiocarp, (b) spores.

Material studied: Umlalazi, Nature Reserve, Duna Forest, Natal, leg. J.G. Ferreira, 28.VI.1984 (PREM 47507).

Remarks: The material studied was sterile, but the presence of a median cuticular layer which characterizes *H. leonina* and the coincidence of the remaining features with those corresponding to the species, allowed its identification.

The size and morphology of the spores are according to Job (1985b).

5. H. luteo-badia *(Fr.) von Höhnel & Litschauer,* K. Akad. Wiss. Wien Sitzungesber. 116: 754. 1907 (Figure 5).

Thelephora luteo-badia Fr., Linnaea 5: 526. 1830.

Thelephora kunzei Hooker, Bot. Misc. 2: 163. 1831.

Stereum luteo-badium Fr., Epicr. 547. 1838.

Stereum laetum Berk., Acad. Nat. Sci. Phila. J. 2: 279. 1853 (PC !).

Hymenochaete laeta (Berk.) Berk. Grevillea 8: 146. 1880.

Hymenochaete kunzei (Hooker) Massee, Linn. Soc. Bot. J. 27: 100. 1890.

Hymenochaete pavonia Pat., Philipp. Journ. Sci. 86. 1915 (FH !).

Hymenochaete cubensis Burt, Ann. Mo. Bot. Gard. 5: 337. 1918 (NY !).

Hymenochaete reflexa Burt, Ann. Mo. Bot. Gard. 5: 336. 1918 (NY !).

Paratype: Weigelet MO 5250; Dutch Guiana, under the name *Thelephora luteo-badia* and *Thelephora kunzei* (BPI).

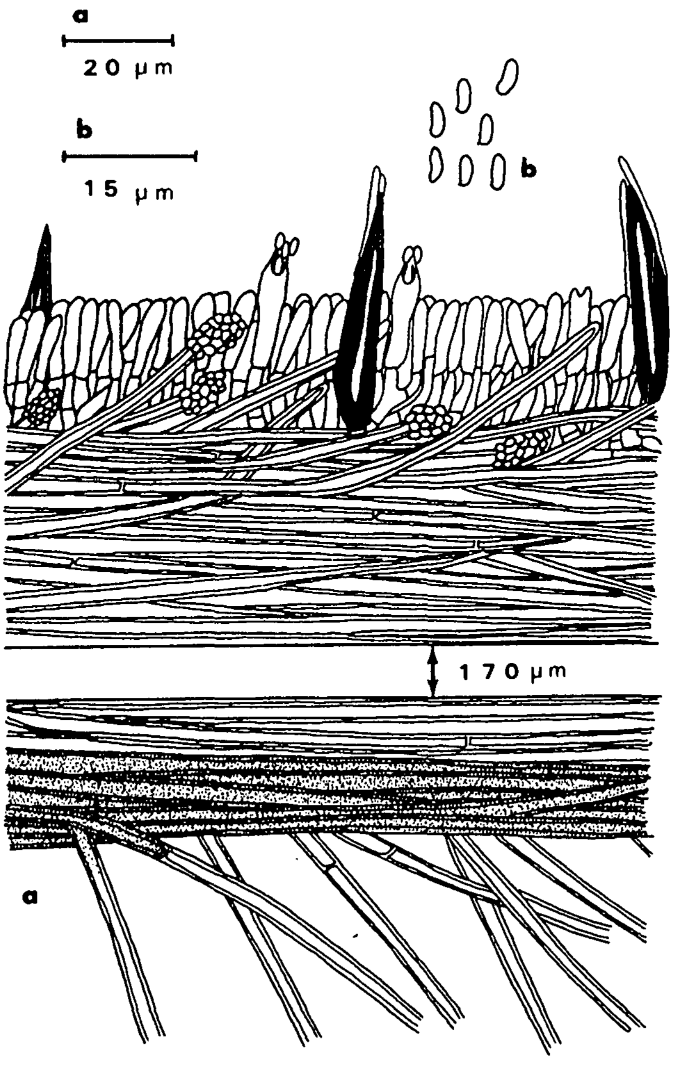

Figure 5 Microscopic characters of *Hymenochaete luteo-badia*; (a) cross section through the basidiocarp (arrow in the middle portion indicates thickness), (b) spores.

Basidiocarp demediate and imbricate, or effuso-reflexed, coriaceous, thin. Pileus up to 4 cm diam., velvety when young, becoming glabrous and fibrillose when older. Adaxial surface brown (Pablo pl. 12 G 7 to Whippet pl. 15 L 10), concentrically sulcate, zonate. Hymenium smooth, greyish-orange (pl. 12 L 6), margin thinning out, concolorous, rarely whitish-orange (Ta Ming pl. 10 L 6). Pileal trama 200 – 400 µm thick (Figure 5a). Cortex present, 30 – 60 µm thick, composed of darkened, densely compacted hyphae, that give rise to a dense layer of abhymenial hairs. Context composed of a well-developed, compact layer of longitudinally arranged hyphae, 2,5 – 3 µm diam. Setigerous layer seated on the context, composed of a single row of widely spaced setae, rarely 2 – 5 rows of setae. Setae very few, lanceolate 30 – 50 × 5 – 7 µm, ensheathed, projecting up to 30 µm beyond the hymenium. Hymenium composed of paraphysate hyphae, basidia and basidioles. Paraphysate hyphae 2 – 3 µm diam., encrusted at the tip with small brown crystals. Basidia clavate 14 – 18 × 4 – 5 µm. Spores suballantoid 4 – 5 × 1,8 – 2,5 µm, smooth, hyaline (Figure 5b).

Material studied: Ngoye, Natal, leg. W. Haygarth, 11.V.1916 [PREM 15558 as *H. nigricans* (Lév.) Bres. (P !)]; Gingindlovu, Natal, leg. W. Haygarth, 25.V.1916 (PREM 15596); Stella Bush, Durban, Natal, leg. P. van der Bijl, 4.IX.1916 (PREM 31687); Nkandla Forest, Natal, leg. R.G. Strey, 7.V.1962 (PREM 42274).

Remarks: *H. luteo-badia* is similar to *H. villosa* (Lév.) Bres. (PC !) (in Ann. Mycol. 8: 588. 1910) and *H. dura* Berk. & Curt. (FH !) (in Linn. Soc. Bot. J. 10: 334. 1868). *H. villosa* is however quite distinct morphologically, since it has submoniliform hyphae and elliptical spores (3 – 4 × 2 – 2,5 µm). *H. dura* also differs morphologically from *H. luteo-badia*. The former is resupinate and has paraphysate dichotomic hyphae and oval to short-elliptical spores (3 – 4,5 × 2 – 2,5 µm).

6. H. ochromarginata *Talbot apud Wakefield & Talbot* in Bothalia 4: 943. 1948 (PREM !) (Figure 6).

Holotype: P.J. Pienaar No 2133, Tenadu, Tembuland, South Africa, 26 February 1912 (K), with isotype PREM 2133.

Basidiocarp effused-reflexed to pileate, velvety, imbricate, hymenial surface brown (Congo pl. 8 H 11), margin thinning, golden brown. Abhymenial surface corrugated, ochraceous (Tan pl. 12 L 11), concentrically sulcate with a few black bands. In section 400 – 1200 µm thick (Figure 6a); context well developed, duplex (Reeves & Welden 1967). Cortex present, compact, formed by thick-walled intertwined and cemented hyphae. Setigerous layer seated on the context, up to 600 µm thick, formed by 5 – 15 rows of densely arranged setae. Setae 26 – 46 × 5 – 7 µm, with acuminate apices, ensheathed, standing out up to 30 µm over the hymenium. Hymenium composed of basidia and basidioles. Basidia suburniform 12 – 16 × 3 – 5 µm, with 4 curved sterigmata each. Spores oblong-elliptical 3 – 4 × 2 – 3 µm, smooth, hyaline (Figure 6b).

Material studied: Tenadu, Tembuland, leg. P.J. Pienaar, 26.II.1912 (PREM 2133, Isotype); Vredehuis, Arcadia, Pretoria, leg. M. Doidge, 14.IV.1913 (PREM 6672); Drakensberg, Natal, leg. Bottomley, 20.VII.1937 (PREM 28879); Donnybrook, Natal, leg. M. Doidge, 1936 [PREM 30260 as *H. rubiginosa* (Fr.) Lév.]; Newswl beach, Natal, leg. W.G. Rump, XII.1946 (PREM 35558).

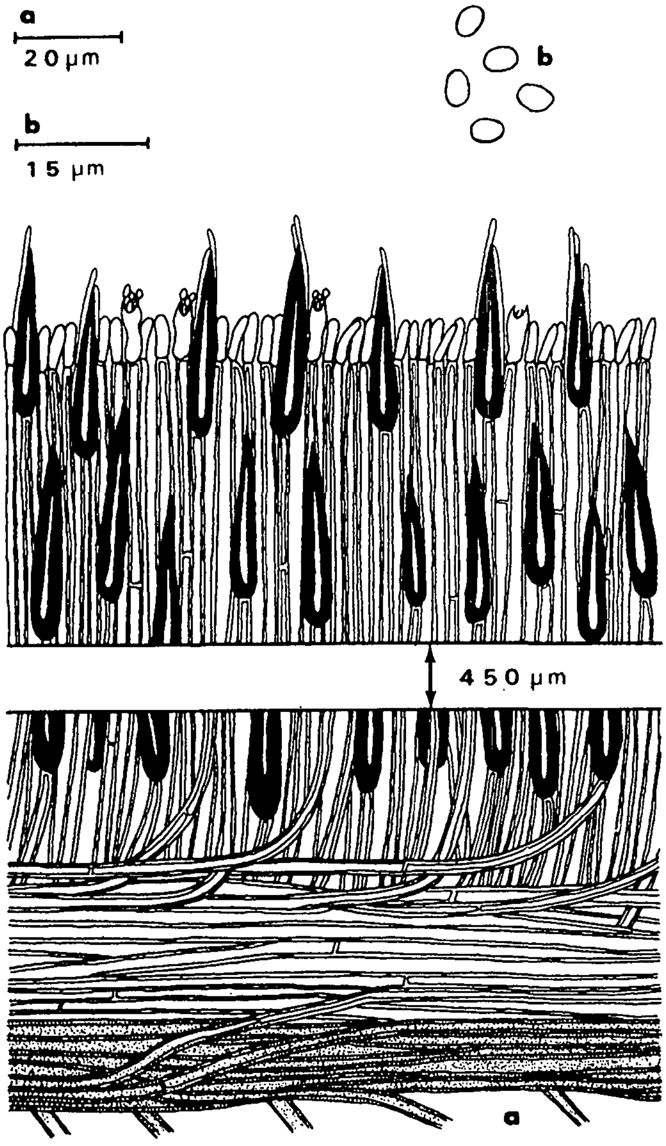

Figure 6 Microscopic characters of *Hymenochaete ochromarginata*; (a) cross section through the basidiocarp (arrow in the middle portion indicates thickness), (b) spores.

Remarks: *H. ochromarginata* is similar to *H. rigidula* Berk. & Curt. (PC !) (in Linn. Soc. Bot. J. 10: 334. 1868) and *H. rubiginosa* (Fr.) Lév. (in Ann. Sci. Nat. III 5: 151. 1846), both having a duplex context, cortex and a densely setigerous layer seated on the context. *H. rubiginosa* is distinguished by its longer setae (30 – 70 µm), and its colliculose, coriaceous, bistre, basidiocarp. *H. rigidula* is similar macro- and microscopically to *H. ochromarginata*, but the former species has larger, naked, setae, (35 –)45 – 60(– 80) × (7 –)9 – 10 µm, with slightly verruculose encrusted apices, and allantoid to suballantoid spores.

Talbot (1951) did not report either basidia or spores in the type specimen, but I have found spores (oblong-elliptical) and basidia (suburniform) in the same material.

7. H. pinnatifida *Burt*, Ann. Mo. Gard. 5: 355. 1918 (NY !) (Figure 7).

Paratype: F.S. Earle and W.A. Murrill No 6 and 31; Havanna Province, Managua, Cuba (NY).

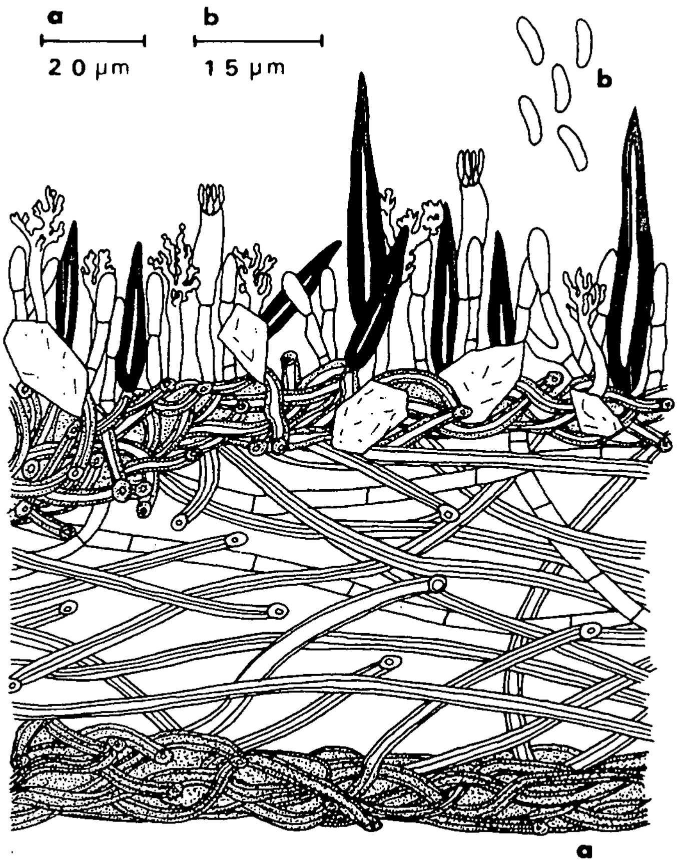

Figure 7 Microscopic characters of *Hymenochaete pinnatifida*; (a) cross section through the basidiocarp, (b) spores.

Basidiocarp resupinate, waxy to velvety, cracked when older. Hymenium smooth, brownish-grey (Hispano pl. 14 D 12 to pl. 3 D 7), margin thinning out, concolorous. In section 100 – 350 µm thick (Figure 7a), rarely stratose (Léger 1981b). Context composed of interwoven, 2,5 – 3 µm diam. hyphae, with parallel orientation. Cortex present, composed of darkened, densely compacted hyphae. Setigerous layer seated on the context 30 – 100 µm thick, rarely up to 250 µm, composed of numerous setae arranged in 8 – 10 rows. Setae lanceolate, 15 – 50 × 3 – 7 µm, naked or ensheathed, projecting up to 40 µm beyond the hymenium. Crystals also present in the subhymenial area. Hymenium composed of dendrophyses, basidia and basidioles. Basidia clavate, 12 – 16 × 3 – 4,5 µm. Spores suballantoid 4 – 5,5 × 1,5 – 2,5 µm, smooth, hyaline (Figure 7b).

Material studied: Table Mountain, Natal, leg. W.G. Rump, VI.1935 [PREM 34356, 34386 both as *H. fulva* Burt (NY !)]; Hluhluwe Game Reserve, Zululand, Natal, leg. W.G. Rump, X.1935 (PREM 35325 as *H. fulva*).

Remarks: Collections 34356 and 34386 have basidia and mature spores; nevertheless, both collections do not have dendrophyses over the hymenial layer, but very few developed dendrophyses were observed in the subhymenial layer. This might be indicative of a young state of growth (J.C. Léger, University C. Bernard, Lyon, personal communication). Since all the other characteristics are in agreement with those of the species, these collections are considered to be *H. pinnatifida* in spite of exhibiting an abnormal development.

8. H. semistupposa *Petch*, Ann. Royal Bot. Gard. Peradeniya 9: 278. 1925 (Figure 8).

Isotype: Herb. Perad. No 5627, Hakgala, Ceylon (K).

Basidiocarp resupinate, loosely attached when moist, cracked when older. Hymenial surface irregulary tuberculate, yellow-

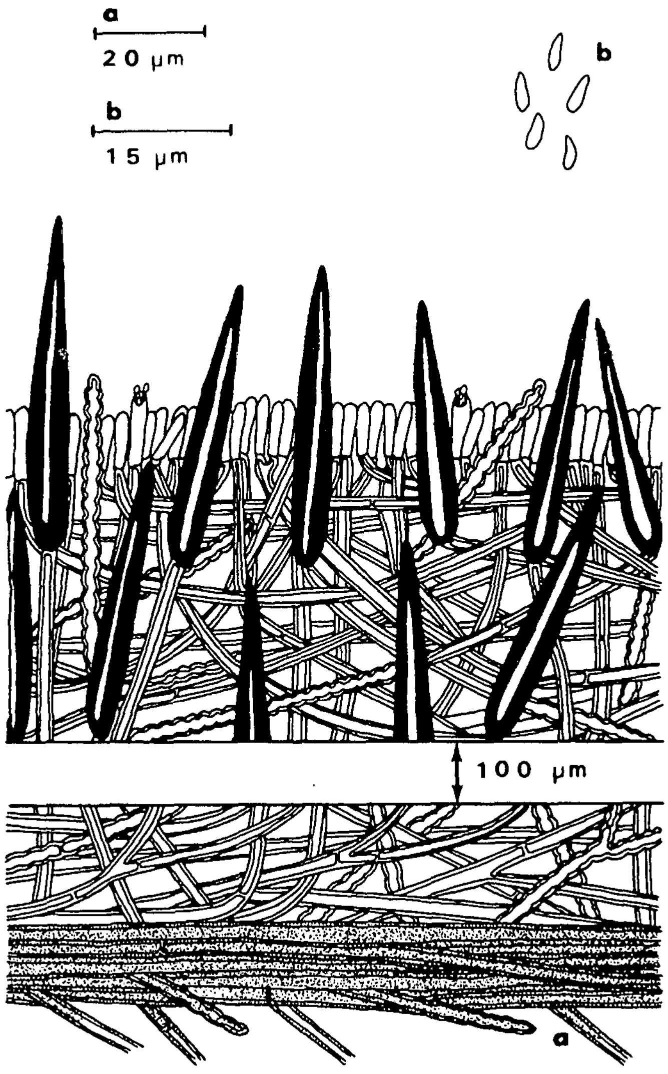

Figure 8 Microscopic characters of *Hymenochaete semistupposa*; (a) cross section through the basidiocarp (arrow in the middle portion indicates thickness), (b) spores.

brown (Mummy pl. 14 J 9 to Bronze pl. 14 L 9), margin thinning out, adnate, cinnamon (Buckthorn pl. 13 L 8). In section 100 – 330 µm thick, (up to 900 µm thick in stratose specimens). Context composed of interwoven, dark yellow, septate, branched hyphae (Figure 9a), many of them submoniliform. Cortex composed of interwoven, deeply coloured, cemented hyphae, and abhymenial hairs. Setigerous layer to 200 µm deep (up to 500 µm in stratose specimens), composed of 2 – 5 rows of setae. Setae fusiform, 40 – 70 × 6 – 9 µm, naked or ensheathed, projecting up to 50 µm beyond the hymenium. Hymenium composed of basidia, basidiola and occasional moniliform paraphysate hyphae. Basidia subclavate, 12 – 16 × 3,5 – 4 µm. Spores suballantoid 3,5 – 5 × 1 – 1,25 µm, smooth, hyaline (Figure 8b).

Material studied: Krantzkop, Natal, leg. W.G. Rump, XI.1935 (PREM 30232); Karkloof, Natal, leg. W.G. Rump, 1944 (PREM 35315); Nkandlha Forest, Natal, leg. R.G. Strey, 7.V.1962 (PREM 42278).

Remarks: In the three species studied I have not seen spores and basidia. The measurements and morphology recorded are those given by Cunningham (1957).

9. H. tabacina *(Sow.:Fr.) Lév.*, Ann. Sci. Nat. III. 5: 152. 1846 (NY !) (Figure 9).

Auricularia tabacina Sow., British Fungi 25. 1797.
Thelephora tabacina (Sow.) Fr., Syst. Myc., 1: 437. 1821.
Thelephora avellana Fr., Syst. Myc., 1: 442. 1821.
Stereum tabacinum (Sow.) Fr., Epicrisis 550. 1838.
Stereum avellanum Fr., Epicrisis 551. 1838.
Hymenochaete avellana (Fr.) Cke., Grev. 8: 146. 1880.
Hymenochaete obesa Cunn., Trans Royal Soc. New Zeal. 85: 15. 1957 (PDD !).

Neotype: W.B. Cooke; White Bird Summit, Mt. Idaho, Idaho; June 13, 1948 (NY), selected by Defigio (1970).

Basidiocarp resupinate, effused-reflexed to pileate. Pileus up to 1 cm diam., rarely imbricate, thin, coriaceous. Abhymenial surface finely tomentose to glabrous, concentrically zonate and sulcate; when dry light to dark brown (Cinnamon pl 12 E 7 to Raw Umber pl 15 L 12). Hymenial surface smooth,

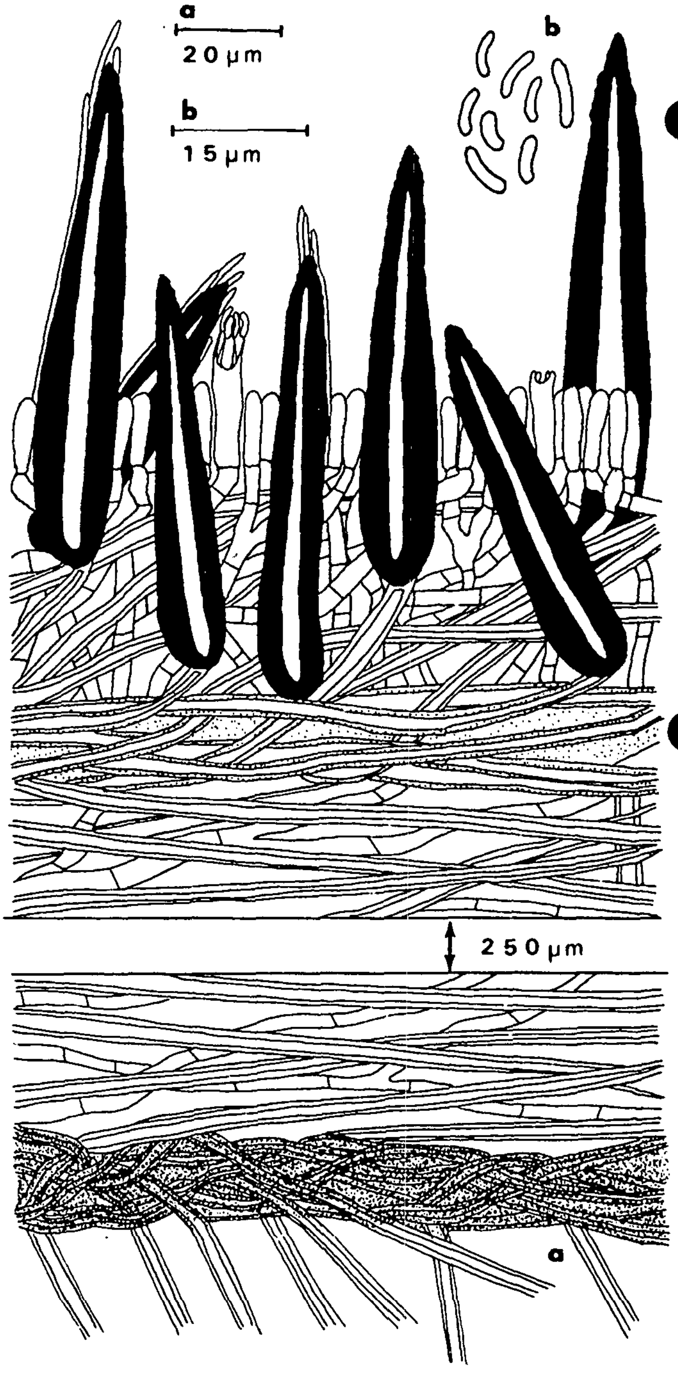

Figure 9 Microscopic characters of *Hymenochaete tabacina*; (a) cross section through the basidiocarp (arrow in the middle portion indicates thickness), (b) spores.

concentrically cracked when older, greyish-brown (Buffalo pl 15 I 11). Margin thinning out, concolorous or pale orange (Chrome 10 K 12). In section 200 – 600 µm thick (Figure 9a). Cortex present 20 – 40 µm thick, composed of cemented, darkened, densely interwoven hyphae. Abhymenial hairs present. Setigerous layer composed of one row of spaced setae, or otherwise attaining 100 – 200 µm deep and composed of 3 – 4 rows of setae. Setae lanceolate (60 –)80 – 120(– 140) × 9 – 16(– 18) µm, naked or ensheathed, with slightly verruculose apices encrusted with crystals, projecting up to 70 µm beyond the hymenium. Hymenium composed of basidia and basidioles. Basidia subclavate 11 – 16 × 4 – 6 µm, with 4 curved sterigmata each. Spores allantoid 5,5 – 7,5 × 1,3 – 2 µm, smooth, hyaline (Figure 9b).

Material studied: Town Bush, Pietermaritzburg, Natal, leg. W.G. Rump, X.1934 (PREM 28287); ibid, XII.1943 (PREM 35320 as *H. pinnatifida*).

Remarks: *H. tabacina* is widespread (Parmasto 1985), and widely distributed in the northern temperate zones of the world, but is seldom found in the Neotropics (Escobar 1978).

I propose to synonymize *H. obesa* with *H. tabacina* since I consider that the features described by Cunningham (1957) to differentiate them, namely '*H. obesa* differs from *H. tabacina* in that setae are less crowded, appear in fewer rows, are shorter, broader (65 – 95 × 10 – 16) and with wide lumina' are within the range of variation of size and disposition of setae in *H. tabacina*.

The nine species of *Hymenochaete* represented in the PREM herbarium can be separated according to the following key:

1. Well-developed context present; setigerous layer seated on context. .... 2.
1'. Well-developed context absent or reduced to a very thin zone of cemented or interwoven hyphae. Setigerous layer seated directly on substrate. .... 8.
2. Cuticule absent, trama generally stratified, formed by 2 or more strata. .... 1. *H. cinnamomea*
2'. Cuticule present. .... 3.
3. Basidiocarp divided into two zones of equal thickness by a cuticule of interwoven hyphae. .... 4. *H. leonina*
3'. Cuticule basal, originating or not abhymenial hairs, but not dividing the basidiocarp in two equal zones. .... 4.
4. Setae up to 70 µm long. Spores suballantoid or oblong-elliptical, up to 5 µm long. .... 5.
4'. Setae 80 – 120 µm long; spores allantoid 5,5 – 7,5 µm long. .... 9. *H. tabacina*
5. Hymenial paraphyses present. Spores suballantoid. Context simple. .... 6.
5'. Hymenial paraphyses absent. Spores oblong-elliptical. Context duplex. .... 6. *H. ochromarginata*
6. Basidiocarp pileate, filiform, encrusted paraphyses present. Context of longitudinally arranged hyphae. .... 5. *H. luteo-badia*
6'. Basidiocarp resupinate, paraphyses moniliform or dendrophysoid. Context interwoven. .... 7.
7. Paraphyses dendrophysoid; setae 15 – 50 µm long. .... 7. *H. pinnatifida*
7'. Paraphyses moniliform; setae 40 – 70 µm long. .... 8. *H. semistupposa*
8. Basidiocarp ochre, up to 120 µm thick; setae solitary. Spores 6 – 8 µm long. .... 3. *H. flavomarginata*
8'. Basidiocarp umber, up to 400 µm thick; setae arranged in conspicuous fascicles of 6 to 12. Spores 5 – 6 µm long. .... 2. *H. fasciculata*

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MYCOLOGIA HELVETICA

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MORPHOLOGY AND CULTURAL STUDIES OF HYMENOCHAETE ATTENUATA

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SUMMARY

The morphology of the basidiocarp, together with the cytology and cultural characteristics of Hymenochaete attenuata, found in Switzerland, have been studied. Code numbers, based on the methodology proposed by Nobles, and SEM photographs for this species are supplied.

RESUME

La morphologie des basidiomes, la cytologie et les cultures d'Hymenochaete attenuata trouvé en Suisse ont été étudiées. Les résultats sont complétés par le code basé sur la méthodologie de Nobles, et par des photographies réalisées au microscope électronique à balayage.

ZUSAMMENFASSUNG

Die Morphologie des Fruchtkörpers, die Zytologie und die Kulturen von in der Schweiz gefundenen Hymenochaete attenuata wurden untersucht. Die Resultate sind mit dem nach Nobles vorgeschlagenen Code ergänzt und mit rasterelektronenmikroskopischen Photographien belegt.

INTRODUCTION

During a taxonomic study of the mycoflora of Switzerland two collections were found that coincide with the description of Hymenochaete attenuata (Lév.) Lév. This species has been considered Indo-asiatic, although also recorded by Cunningham (1957) for Australia and New Zealand.

Hymenochaete attenuata does not appear to have been recorded so far from Europe. We believe it is of interest to redescribe the morphology of this species on the basis of our material and the cultural characteristics.

MATERIAL AND METHODS

Light microscope: Free-hand sections were mounted for observation in a solution of cotton blue-lactophenol, or in a solution of congo-red.

Scanning electron microscope: The material was fixed according to the method used previously by Keller (1985); in which samples of the basidiocarp were fixed in $KMnO_4$, dehydrated in acetone series and then subjected to critical point drying. The samples were coated with gold and observed using a Philips 500.

Cultural and cytological studies: The polysperme cultures were obtained from spore-prints. The study of the morphology was carried out based on Nobles's method (1948, 1958, 1965), with her code, and furthermore it includes the added numbers mentioned by Boidin & Lanquetin (1983).

The medium employed was:

a) growth medium: malt agar (Nobles, 1948)

b) oxidase reaction medium:
- 1: gallic acid agar (Davidson et al., 1938)
- 2: tannic acid agar (idem.)
- 3: guaiacol 2% (Boidin, 1958)
- 4: paracresol (idem.)
- 5: tyrosine (idem.)

The reaction intensity and the rate of the mycelial growth are given as described by Davidson et al. (op. cit.).

For the cytological study we followed the technique mentioned by Boidin (1958). Cultures were grown under collodion film, and fixed by a saturated solution of HgCl2 and glacial acetic acid,and finally were stained with slow giemsa.

The colour of the mycelium culture and basidiocarp are described on the basis of Seguy's colour chart (1936).

RESULTS

a- Description

Hymenochaete attenuata (Lév.) Lév., Ann. Sci. Nat., Bot, sér. III, 5, 152. 1846. (= *Stereum attenuatum* Lév., Ann. Sci. Nat., Bot. sér. III, 2, 212. 1844.)

Basidiocarp thin, coriaceous to papery, resupinate with slightly elevated margins, effuso-reflexed or pileate-sessile. At first orbicular (1-2 mm diam) with reflexed margins, soon confluent. Hymenial surface warm sepia (Pl. VIII, 116), slowly colliculose, sometimes creviced in small areas. Reflexed portions 0,5 - 2 cm diam, rarely imbricate, with the adaxial surface brown (Pl. VIII, 117), banded with concentric zones of different shades of brown. Margin thinning out, fibrillose, brown clair (Pl. IX, 131).

Context well developed 150 - 250 um thick, parallel with the substrate, formed of loosely interwoven radially arranged hyphae. Hyphae thick-walled 1,7 - 3,6 um diam, golden yellow, septate and usually branched at right angles. Cuticle absent. Abhymenial hairs arising directly from the context. Setal layer seated on the context, formed by one or two rows of irregulary arranged setae. Setae (55) - 70 - 105 x 6 - 8 - (9) um, aculeate, with acute apices (Fig.3a), ensheathed or naked, a few with slightly (Fig.3b) or more strongly (Fig.3c) twisted axes; and some setae (40) - 50 - 90 x 6 - 9 um, with dome-shaped apices (Fig.2a,b,c).

Hymenium composed of basidia and basidioles. Basidia subclavate 18 - 26 x 4 - 5 um, with 4 curved sterigmata. Spores cylindrical to elliptical 4,5 - 6 x 2,5 - 3,5 um (Fig.1b and 3d, e), smooth, hyaline, thin-walled, neither amyloid nor dextrinoid.

Material examined: Switzerland, Jura, Develier; 10 october 1987; leg. J. Keller (NEU 4212a); Id.; 18 october 1987; leg. J. Keller & D. Job (NEU 4212b).

Substratum: Effused on bark or decorticated dead branches of Alnus; associated with a white pocket rot.

b- Cultural and cytological studies:

Macroscopic characters: growth very slow, reaching 6,8 - 7,2 cm after 6 weeks. Mycelial layer felted, white at the beginning, turning ocre yellow (Pl. IX, 131) after the 4th week, and brown (Pl. VIII, 172), banded with concentric zones of dark brown (Pl. VIII, 116) near the margin, after the 5th week. Margin regular, cottony, white, and advanced mycelium submerged (Fig.1a).

Reverse changed to brown in the area up to 4,5 - 6 cm from the inoculum. Odour absent.

Reactions: guaiacol 2%: + + + (+); no growth paracresol: - ; tr.
tannic acid: + + + ; no growth tyrosine : - ; tr.
gallic acid: + + + + ; no growth

Microscopic characters: Margin formed by 1,5 - 2,8 µm diam generative hyphae, straight or more rarely twisted, afibulate, with few branches and thin walls. Submerged mycelium composed of generative hyphae, afibulate, irregulary submoniliform after the second week, with swellings (6 - 14 µm diam) and few kidney-shaped protuberances. Mycelial layer formed, at the beginning, by regular septate, afibulate, generative hyphae, 1,8 - 4 µm diam, with thin hyaline walls. From the second week, afibulate, generative hyphae with golden thickened walls form in the culture,a few with amber, oily contents. Bipyramid crystals, probably of calcium oxalate monohydrate (Keller, 1985) are present after the 3rd week in culture. From the 4th week in correlation with the dark concentric zones of the mycelial layer we observed brown generative hyphae, with irregular short branches, and slightly thickened walls, which form a compact mesh and resulting in a plectenchymatous tissue.

Cytological studies: cells binucleate, rarely uni- or trinucleate.

Code: 2a, 6, 11, (22), 26, 32, 37, 39, 47, 54.

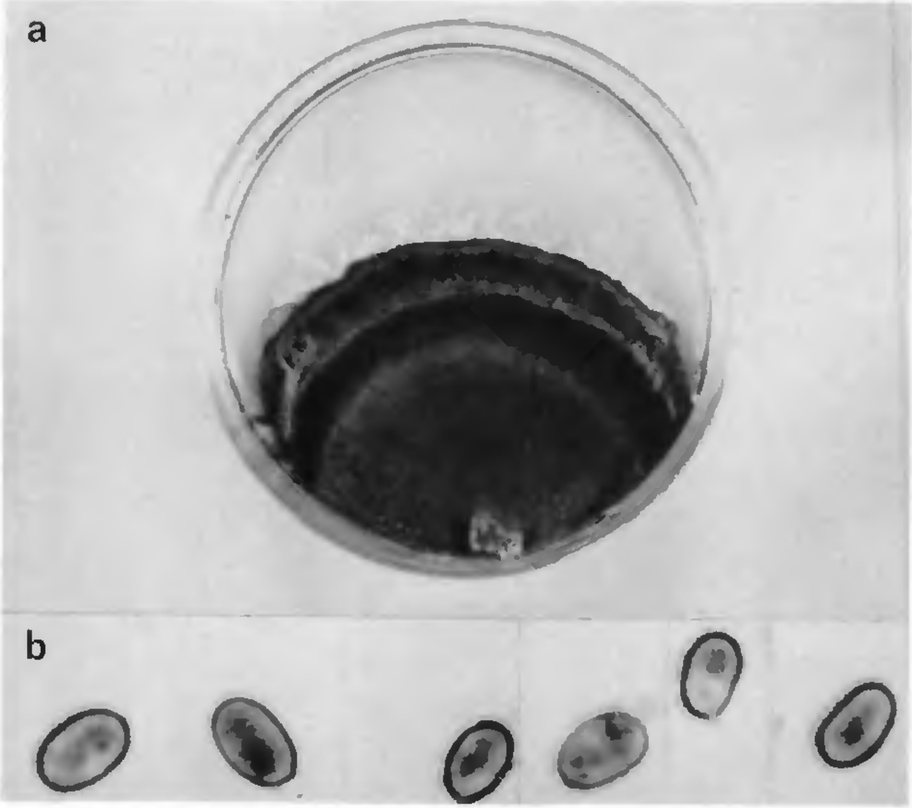

Fig.1a: Aspect of six-week-old culture (x 1).

Fig.1b: Elliptical spores (light microscopy, x 1000).

DISCUSSION

Both Swiss collections agree in the macro- and microscopical features with the New Zealand material of H. attenuata studied by Cunningham. Nevertheless the European collections seem to show more variety in spore form.

Hymenochaete attenuata can be recognized by the absence of a cuticle and the unusual characteristic in the genus of possessing two types of setae: (a-) setae aculeate, twisted or not with acute apices (Fig.3a). (b-) setae shorter with rounded, domed apices (Fig.2a,b,c). The young stage of the aculeate setae differs from the former in having a thinner, golden yellow wall (light microscope) and acute apices (Fig.2a).

The same dimorphism in the setae was found by Cunningham (1957) in an authentic Léveillé specimen from Java, deposited at the Kew Herbarium.

In SEM study, spore form is generally cylindrical (Fig.3d). Nevertheless in the study with the light microscope of the spore-print and hymenium, the spores are more variable and many are elliptical (Fig.1b), as in H. attenuata material. This change may result from desiccation of the material and the elasticity of the spore walls.

Hymenochaete attenuata seems to be closely related to H. sallei Berk. & Curt. Both have effuso-reflexed to pileate fructifications, medium-sized setae and spores, and the absence of a cuticle, but H. sallei has allantoid to suballantoid spores (Job, 1985) and setae only with acute apices. In the cultural characteristics the species have a different code number (Job, 1986) and the former present plurinucleate cells in the polysperme culture (Boidin, 1984).

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FIGURES

Fig.2a: Evidence of the dimorphism of setae: young aculeate setae (top left arrow) and mature setae with domed apices (bottom right arrow) x 1680.

Fig.2b: Mature aculeate setae (on the left) and mature domed setae (on the right) x 2200.

Fig.2c: Detail of domed setae, ensheathed, x 3650.

Fig.3a: Setae naked with acute apices, x 2300.

Fig.3b: Setae slightly twisted, x 2900.

Fig.3c: Setae strongly twisted, x 2700.

Fig.3d: Cylindrical spore, x 20400.

Fig.3e: Elliptical spore, x 20900.

Fig. 2

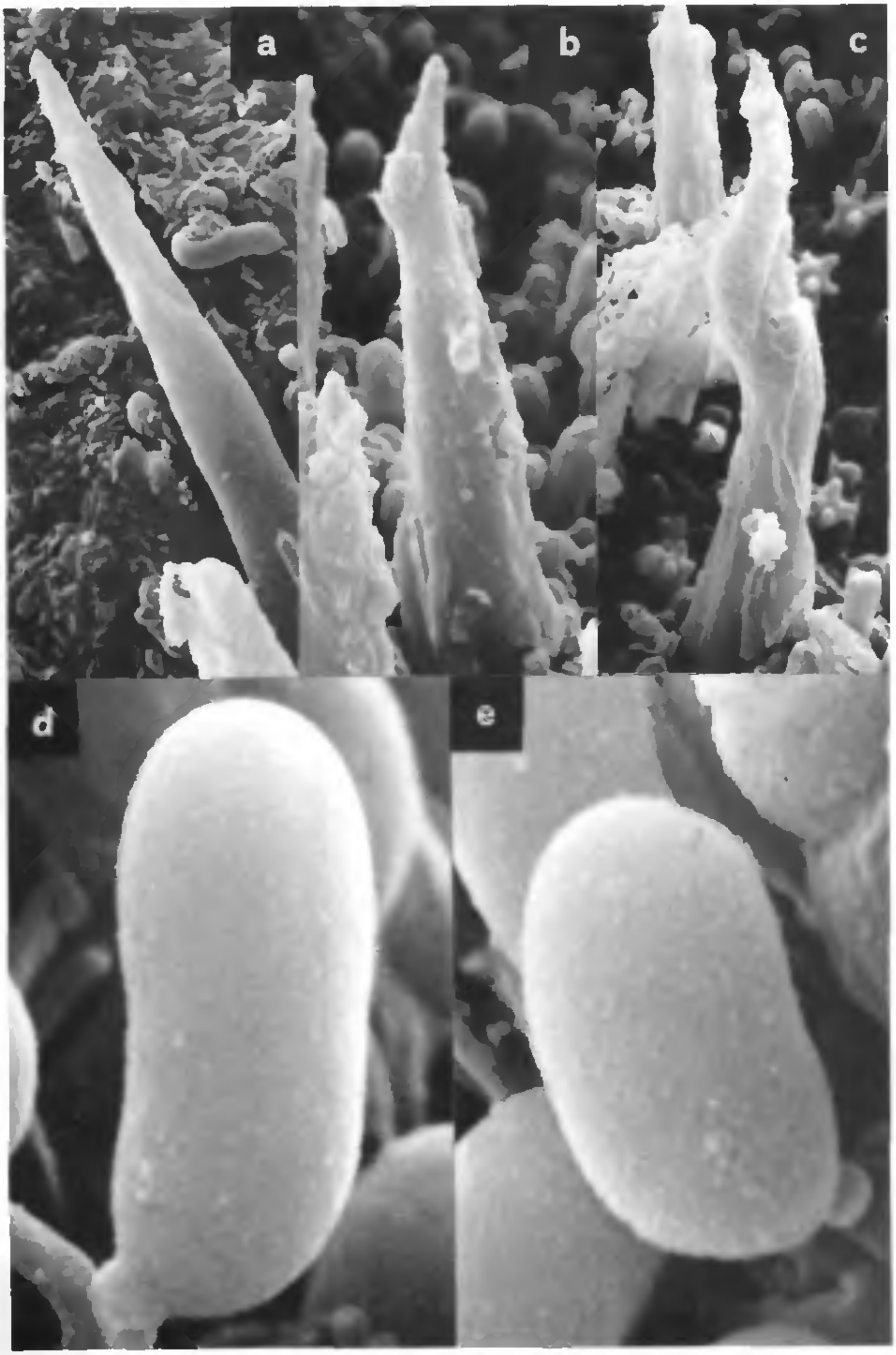

Fig. 3

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ETUDES MICROSTRUCTURALES DE LA DEGRADATION DE *PICEA ABIES* PAR *HYMENOCHAETE* SPEC. (APHYLLOPHORALES)

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SUMMARY

The mechanisms of penetration, weight loss and changes of structure and ultrastructure of blocks of *Picea abies* wood inoculated in vitro with polyspermic cultures of *Hymenochaete corrugata*, *H. pinnatifida*, *H. sallei* and *H. tabacina* were studied. In all the species studied four mechanisms of penetration and both selective and simultaneous white wood-decay were found. The different macro- and microscopical wood-rot patterns at the specific level are described and SEM photographs are supplied.

RESUME

Des blocs de Picea abies ont été inoculés par des cultures polyspermes de Hymenochaete corrugata, H. pinnatifida, H. sallei et H. tabacina afin d'étudier les mécanismes de pénétration des hyphes ainsi que les pertes de poids, les modifications de structure et d'ultrastructure du bois. Toutes les espèces étudiées attaquent le bois selon 4 mécanismes différents et provoquent deux types de pourritures blanches, la simultanée et la sélective. Les caractéristiques macroscopiques et microscopiques des pourritures sont décrites pour chaque espèce et illustrées par des photographies réalisées au MEB.

ZUSAMMENFASSUNG

Holzblöcke von Picea abies wurden mit polyspermen Kulturen von Hymenochaete corrugata, H. pinnatifida, H. sallei und H. tabacina infiziert, um die Eindringungsmechanismen der Hyphen sowie den Gewichtsverlust und die strukturellen und ultrastrukturellen Aenderungen des kolonisierten Holzes zu verstehen. Alle vier Arten zeigen 4 verschiedene Eindringungsmechanismen und weisen sowohl die simultane als auch die selektive Weissfäuleauf. Die macro- und mikroskopischen Eigenschaften der Holzfäulen sind für alle 4 Arten beschrieben und mit REM-Bildern illustriert.

INTRODUCTION

Dès le 19e siècle, la dégradation du bois par les champignons lignivores a fait l'objet de nombreux travaux, tant du point de vue morphologique que biochimique. Hartig (1878) a séparé les champignons lignivores en deux groupes, en se basant sur la couleur du bois dégradé: Les champignons produisant de la pourriture blanche et les champignons produisant de la pourriture brune. Puis, Falck (1926) a mis en évidence les différences d'activités chimiques dans les deux groupes: Les espèces responsables de la pourriture brune altèrent la cellulose, les espèces responsables de la pourriture blanche dégradent la lignine et les hémicelluloses en plus.

Les modifications structurales du bois dans les deux groupes de pourritures ont été étudiées en détail, par exemple par Bjorkman et al. (1949), Wilcox (1968) et Liese (1970). Ces auteurs ont observé que les mécanismes de dégradation de la pourriture blanche n'étaient pas homogènes, mais que deux types de comportements existent :(a-) destruction simultanée de tous les composants de la paroi cellulaire (pourriture blanche simultanée); (b-) dégradation de la lignine et de l'hémicellulose surtout et dégradation très faible ou nulle de la cellulose (pourriture blanche sélective).

Ces dernières années, la dégradation du bois par les espèces xylophages a suscité un regain d'intérêt dû à la grande importance de ces microorganismes dans le recyclage des sources de carbone du bois, à l'utilité des espèces produisant la pourriture blanche sélective dans la dégradation biologique de la lignine, à l'application possible au prétraitement des copeaux papetiers et des pâtes à papier (Erikson et al., 1980), à la biodégradation des résidus lignolitiques et à son utilisation dans l'alimentation du bétail (Chesson et al., 1980).

A cet égard, peu d'informations existent à propos des espèces du genre Hymenochaete (Job & Wright, 1986), genre type d'une famille comportant d'importants destructeurs du bois.
Dans le présent travail, nous avons examiné l'activité destructrice de cultures polyspermes d'H. corrugata (F.) Lév., H. pinnatifida Burt, H. sallei Berk. & Curt. et H. tabacina(Sow.:Fr.) Lév. in vitro, sur blocs de Picea abies (L.) Karsten.

Les paramètres analysés ont été:

- les pertes de poids des blocs inoculés;
- les modifications structurales du bois infecté;
- les mécanismes d'invasion et les modifications microstructurales dans le bois colonisé.

MATERIEL ET METHODES

Cultures: H. corrugata = FP 125019. H. tabacina = FP 125071.
H. pinnatifida = BAFC 640. H. sallei = BAFC 599.

Blocs de bois: ils sont coupés en forme de parallélipipèdes de 30 mm de long, 10 mm de large et 3 mm de haut, l'axe principal étant parallèle aux trachéides.

Milieu de culture et techniques d'inoculation: pour chaque espèce, des inoculums sont ensemencés dans 12 boîtes de Petri de 10 cm de diam., avec 30 ml de milieu au malt-agar (Nobles, 1965). Lorsque le milieu est recouvert par le mycélium, des blocs de bois stériles, préalablement pesés, sont déposés aseptiquement à la surface de ce milieu, à raison de 10 par boîte. Les boîtes sont ensuite incubées à 25° C (± 1° C) et recouvertes de parafilm pour éviter la dessication du milieu de culture (Garcia Sola, 1974). Toutes les 2 semaines, 2 boîtes de chaque espèce sont prélevées, soit 20 blocs; l'opération se répète pendant 12 semaines. A chaque prélèvement, 16 blocs sur les 20 disponibles par espèce sont pesés (poids sec), les 4 derniers sont conservés pour l'étude ultrastructurale au MEB.

Microscopie électronique à balayage: le matériel a été fixé selon la méthode utilisée par Keller (1985); les blocs sont fixés au $KMnO_4$, déshydratés dans des bains d'acétone de concentration croissante, séchés par la méthode du point critique, recouverts d'or et observés sur un Philips 500.

RESULTATS

Perte de poids:

Le tableau I présente les résultats obtenus pendant les 12 semaines d'incubation.

Semaines	Espèces			
	H. corrugata	H. pinnatifida	H. sallei	H. tabacina
2	4.61±0.13	1.34±0.09	0.97±0.11	3.42±0.18
4	6.97±0.35	3.36±0.37	2.60±0.35	5.69±0.15
6	11.41±0.62	6.10±0.81	3.80±0.53	9.30±0.20
8	14.64±0.35	6.70±0.43	4.62±0.53	13.29±0.47
10	18.71±0.61	7.47±0.43	6.03±0.51	15.70±0.44
12	20.54±0.69	8.31±0.48	6.78±0.59	19.16±0.53

±Erreur standard

Tableau I: perte de poids (en pourcentage du poids sec initial).

La figure I précise la perte de poids pendant la période d'incubation. La perte de poids peut être interprétée comme mesure de la quantité de bois transformé en CO2, H2O ou autres produits volatils.

Modifications structurales:

Les différences morphologiques des pourritures observées sur les blocs sont telles, à la fin de l'expérience, qu'elles permettent une détermination macroscopique pour chacune des espèces; leurs caractéristiques les plus remarquables sont:

H. corrugata: Après 2 semaines, les modifications se présentent sous forme de surfaces irrégulières, faiblement décolorées, entourées par une ligne brunâtre clair. Après 4 semaines, ces zones pâlissent fortement et les lignes brunâtres deviennent plus foncées. Puis, dans les semaines qui suivent, les surfaces décolorées confluent et couvrent toute la surface du bloc (Fig II,a). Cette décoloration provient de la dégradation de la lignine dans les zones affectées; elles sont facilement mises en évidence par la coloration à la phloroglucine-HCL (Jensen, 1962).

H. pinnatifida: Au début, nous avons observé 1 à 3 lignes brunâtres, irrégulièrement disposées en surface. Puis, le nombre de lignes restant stable, il y a une augmentation évidente de la pigmentation du bois qui devient uniformément foncé à la fin de l'expérience (Fig. II, b).

H. sallei: Pendant la 2ème semaine, 2 à 5 lignes brun clair traversent la surface affectée. Dès la 6ème semaine, il y a augmentation de leur nombre et de leur intensité. En même temps, la coloration du bois apparaît plus foncée, des zones lysées deviennent visibles pour confluer et occuper finalement le 50% de la surface après 12 semaines (Fig.II, c).

H. tabacina: Dans les 2 premières semaines, aucun signe de dégradation n'est visible. Dès la 4ème semaine, apparaissent des zones lysées recouvertes de mycélium ainsi qu'une grande quantité de petites taches noires de 0.2-0.5 mm diam.; le volume du bloc de bois est légèrement réduit, réduction qui s'amplifie les semaines suivantes pour atteindre des valeurs élevées en fin d'expérience (Fig. II, d).

Les lignes noires observées en surface des blocs affectés par H. corrugata, H. sallei et H. pinnatifida correspondent au tissu plectenchymatique du mycélium aérien (Fig. III, a,b). Dans le bois, ce tissu oblitère les lumières des trachéides et les rayons parenchymatiques (Fig. III, d) en adoptant une disposition diffuse à l'exception de H. corrugata chez qui il limite nettement la zone d'attaque (Fig. III, e,f).

Mécanismes de pénétration et de propagation des hyphes:
Dès la 2ème semaine à partir de l'inoculation, tous les blocs sont complètement envahis par le mycélium, même dans les zones profondes.

Dans chacune de nos espèces examinées, nous avons trouvé les quatre mécanismes de pénétration et de propagation mentionnés par Radtke et al. (1981) chez les champignons lignivores: 1° pénétration et propagation par les lumières cellulaires, 2° propagation par les ponctuations, 3° perforations des parois, 4° destructions du complexe de la lamelle mitoyenne. Cependant, il n'y a pas de limite temporelle définie entre la phase d'invasion passive (Greaves & Levy, 1965) dans laquelle le mycélium pénètre par les lumières cellulaires et les ponctuations et la phase d'invasion active, dans laquelle une émission enzymatique est nécessaire pour percer les parois cellulaires.

Pendant les 4 premières semaines, il y a prépondérance de l'invasion passive; les hyphes avancent principalement par les lumières cellulaires des rayons parenchymatiques (Fig.IV, a) et, dans une moindre mesure, par les ponctuations (Fig. IV, b). Par ailleurs, nous avons observé la présence de microhyphes (Liese & Schmid, 1962) qui sont importantes non seulement dans la phase active comme l'avait indiqué Dirol (1980), mais aussi dans la phase passive (Fig. IV, c).

Dans la 10ème semaine, l'invasion active s'intensifie; les hyphes perforent une grande quantité de cellules (Fig. IV, e,f), ou, dans une plus faible proportion, celles-ci progressent entre les cellules par le complexe de la lamelle mitoyenne (Fig. IV, d).

Altérations microstructurales:
Les 4 espèces étudiées in vitro produisent, en même temps, les deux types de pourritures blanches dans tous les types cellulaires: la sélective et la simultanée. Ce résultat diffère de l'observation faite par Blanchette (1984b) chez Ganoderma tsugae, où les 2 types de pourritures coexistent, mais sur des types cellulaires différents.

Dans les premiers stades de la pourriture blanche sélective, il y a une relation évidente entre la destruction du complexe de la lamelle mitoyenne et la présence des hyphes dans les zones incriminées (Fig. V, a). Pour les 4 espèces, les rayons parenchymatiques sont les premiers éléments altérés par ce type de dégradation (Fig. V, b) et ils sont finalement collapsés à cause de la diminution de leur résistance (Fig. V, c). Par la suite, les effets de cette pourriture commencent à être visibles dans les trachéides qui sont détruites en quantité variable selon les blocs (Fig. V, d,e); toutes présentent la destruction de la lamelle mitoyenne et une absence d'altération de la couche S2 (Fig V, f).

Au SEM, nous avons observé la présence de substances granulaires, dans le cas de la pourriture blanche simultanée; elles semblent être en relation avec la destruction complète des constituants des parois cellulaires (Fig. VI, a,b,c). Ces substances sont agglutinées à intervalles plus ou moins réguliers le long des hyphes (Fig. VI, d).

Ces amas irréguliers expliquent probablement les perforations successives observées dans les stades précoces de la pourriture blanche simultanée (Fig. VI, e,f). La production des lésions successives semble être un phénomène assez répandu chez les basidiomycètes lignivores, et a été mise en évidence aussi chez les ascomycètes et les deutéromycètes (Hale & Eaton, 1985). Dans les premiers stades de cette pourriture les rayons parenchymatiques et les zones des trachéides en contact avec eux sont aussi les premiers éléments altérés (Fig. VII, a,b). Par la suite, toutes les trachéides sont affectées (Fig. VII, d), mais les pourtours de leurs ponctuations aréolées résistent remarquablement bien à ce type de dégradation (Fig. VII, e,f); cette résistance est vraisemblablement due à leur forte teneur en lignine (Gunning & Steer, 1975).

DISCUSSION

Le Tableau I montre une nette différence des pertes de poids entre H. sallei et H. pinnatifida d'une part et H. tabacina et H. corrugata d'autre part.

Sachant que les mécanismes d'invasion et les altérations microstructurales sont semblables dans les 4 espèces, cette différence pourrait être expliquée par une plus grande activité enzymatique des 2 dernières espèces mentionnées. Cette forte capacité de dégradation entraînerait, comme l'a postulé Blanchette (1980) pour Phellinus pini, la très large distribution géographique de H. corrugata et H. tabacina.

La figure I montre que la rapidité de dégradation provoquée par H. pinnatifida diminue dès la 6ème semaine. Macroscopiquement, cette espèce produit un très fort obscurcissememt de la zone attaquée (Fig. II, b); celle-ci révèle, vue au MEB, une grande quantité de cellules obturées par des substances amorphes (Fig. III, c). La libération et l'accumulation de ces substances pendant la dégradation peuvent probablement réduire l'activité destructrice des myccéliums. Un tel phénomène a été observé par Blanchette (1984c) chez Xylobolus frustulosa où la présence de substances occlusives n'empêche pas la colonisation par les hyphes, mais arrête le processus de dégradation.

Macroscopiquement, la dégradation des blocs de bois de Salix humboldtiana provoquée par les 2 espèces H. sallei et H. pinnatifida dans les mêmes conditions expérimentales (Job & Wright, 1986), est identique à la dégradation de Picea abies, quand bien même la structure anatomique du bois est nettement différente; cependant Blanchette (1984a) a observé qu'en conditions naturelles le bois de différentes essences peut être dégradé de façon différente par le même champignon. En tenant compte des travaux de Lopez Real (1975 a) et Lopez Real & Swift (1975 b; 1977) qui stipulent que la rapidité d'apparition et la distribution des lignes noires dans le bois dégradé par les champignons xylophages dépend des facteurs du milieu, les variations observées dans la nature seraient donc plus influencées par les conditions changeantes du milieu que par les différences de structure des substrats.

Les espèces du genre Hymenochaete ont la capacité de produire les deux types de pourritures blanches, caractéristique mise en évidence également chez d'autres espèces de la famille des Hymenochaetaceae (Blanchette, 1980; Otjen & Blanchette, 1982); dans ce dernier cas cependant il y a des différences anatomiques ou des occlusions de substances chimiques délimitant la zone affectée par la pourriture blanche sélective, ce qui ne semble pas être le cas chez les espèces du genre Hymenochaete. D'ailleurs, on a trouvé que la dégradation enzymatique se produit dans la zone de contact avec les hyphes (Fig. V, a), aux premiers stades de la pourriture blanche sélective, mais que, dans un stade plus avancé, la dégradation de la lignine se fait à distance considérable des hyphes; ceci suggère la présence d'un système enzymatique hautement diffusible. Ce système agissant à distance a été trouvé aussi chez d'autres aphyllophorales par Blanchette et al.(1985). Néanmoins, dans le cas de la pourriture blanche simultanée, la formation de trous exclusivement au voisinage immédiat des hyphes fait penser à un sytème enzymatique à diffusion restreinte, dans lequel les substances granulaires trouvées (Fig. VI, a,b,c,d) interviennent probablement dans la rétention du complexe enzymatique.

Notre travail met en évidence l'importance des espèces du genre Hymenochaete comme destructeur du bois, non seulement parce que les 4 mécanismes de pénétration sont maîtrisés, expliquant ainsi la rapidité de leur attaque, mais aussi parce qu'ils ont la capacité de produire les 2 types de pourritures blanches. Puisque ces pourritures sont indépendantes du substrat, il est indispensable d'approfondir ces études afin de connaître mieux les facteurs influençant les différents types de dégradation.

Les études des pertes de poids, complétées par des analyses microstructurales au MEB, ont de nets avantages sur les autres méthodologies pour préciser le pouvoir et les types de dégradations produites par les espèces xylophages. Elles sont supérieures aux analyses chimiques du bois dégradé, par exemple, dans lesquelles la délignification peut être masquée par une forte proportion de pourriture blanche simultanée (Blanchette, 1986), ou aux analyses de l'activité enzymatique, faiblement corrélée à l'activité destructrice du bois; cette dernière méthode ne doit donc pas être utilisée comme indicateur de la capacité de dégradation du bois (Hegarty et al., 1987).

REMERCIEMENTS

Nous tenons à remercier les Professeurs A. Aeschlimann et M. Aragno pour la mise à notre disposition des appareils nécessaires à ces recherches, ainsi que Y. Delamadeleine pour sa collaboration.

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Fig. I: **Pertes de poids en %**; ■= H. corrugata □= H. tabacina

▲= H. pinnatifida △= H. sallei

○= témoin

Fig. II: **Modifications structurales**; blocs inoculés avec a- H. corrugata; b- H. pinnatifida; c- H. sallei; d- H. tabacina; périodes d'incubation: 2, 4, 6, 8, 10 et 12 semaines.

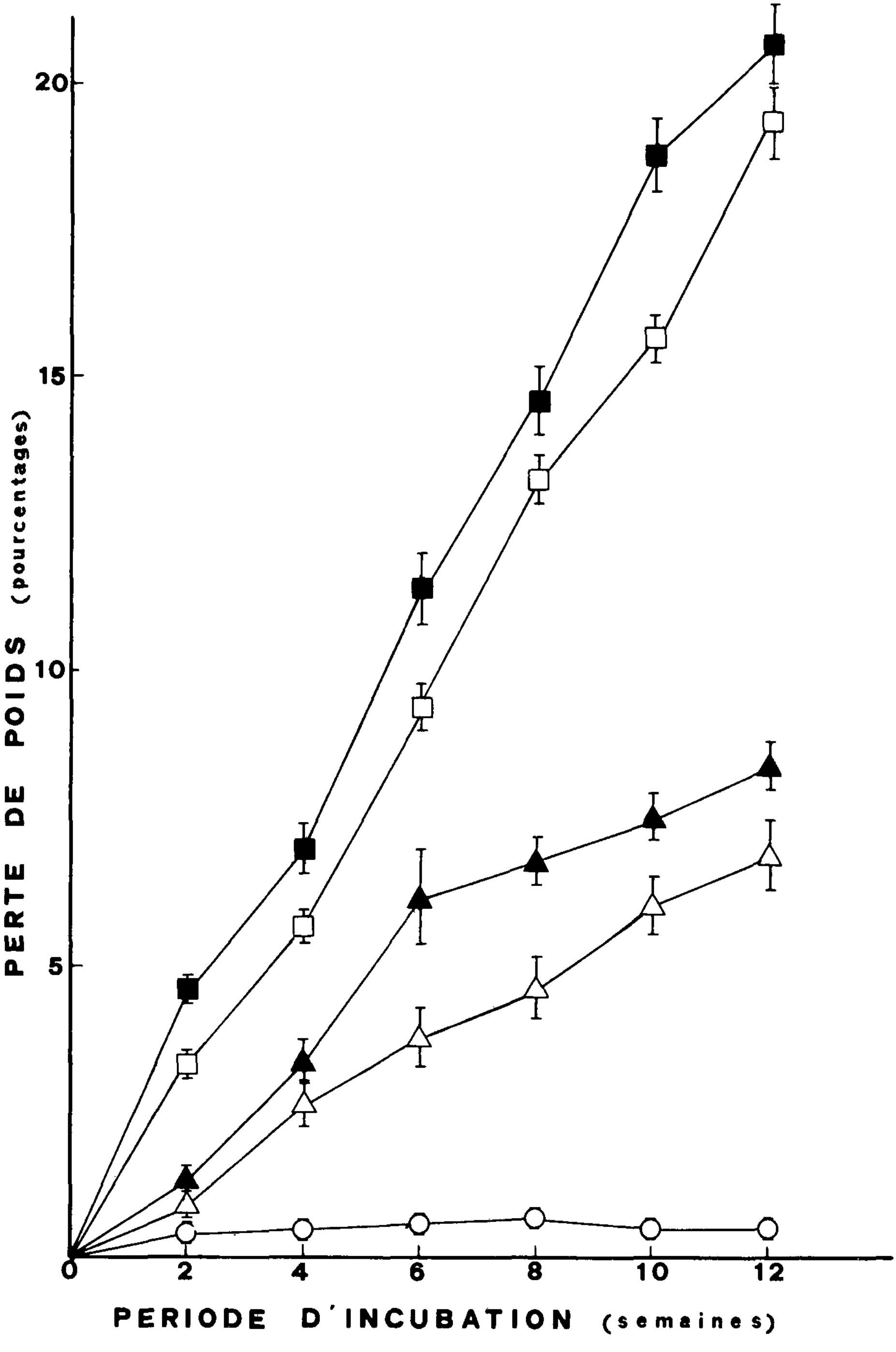
Fig. III: **Lignes noires**; a-H. pinnatifida: formation de plectenchyme au sein du mycélium aérien (1 x). b- Id.: localisation des lignes noires après élimination du mycélium aérien (1 x). c-Id.: occlusion des trachéides par des substances amorphes (550 x). d- H. sallei: occlusion des trachéides par le plectenchyme (360 x). e- H. corrugata: front d'attaque délimité par le plectenchyme (50 x). f- Id.: détail (250 x).

Fig. IV: **Mécanismes d'invasion**; a- H. corrugata: invasion des hyphes dans les lumières cellulaires (240 x). b- H. tabacina: hyphe traversant la ponctuation (1750 x). c- H. pinnatifida: microhyphe (1300 x). d- H. corrugata: hyphe dans le complexe de la lamelle mitoyenne (flèche, 1000 x). e- H. tabacina: hyphe perçant la paroi cellulaire d'une trachéide (flèche, 1700 x). f- H. sallei: hyphe dans la paroi cellulaire (flèche, 1700 x).

Fig. V: **Pourriture blanche sélective**; a- H. pinnatifida: début de la dégradation sélective; absence de lamelles mitoyennes dans les zones affectées (flèche, 550 x). b- H. sallei: destruction des rayons parenchymatiques (220 x). c- H. pinnatifida: rayon parenchymatique collapsé (300 x). d- Id.: trachéides sans lamelle mitoyenne (140 x). e- H. tabacina: trachéides libres (210 x). f- H. sallei: détail de trachéides sans lamelle mitoyenne (430 x).

Fig. VI: **Pourriture blanche simultanée**; a- H. sallei: début de la formation des trous (4000 x). b- Id.: stade plus avancé de la dégradation (4300 x). c- Id.: présence de substances granulaires dans les trous (flèche, 1800 x). d- Id.: hyphe sur le paroi d'une trachéide avec amas de substances granulaires (1000 x). e- H. pinnatifida: pourriture blanche simultanée discontinue provoquant des séries des trous (180 x). f- ld: détail (370 x).

Fig. VII: **Pourriture blanche simultanée**; a- H. corrugata: début de la destruction des rayons parenchymatiques et des trachéides par la pourriture blanche simultanée (430 x). b- H. tabacina: stade plus avancé de la pourriture blanche simultanée, destruction complète des rayons (210 x). c- H. sallei: accumulation d'hyphes dans les trachéides en contact avec les rayons (600 x). d- H. tabacina: stade avancé de la dégradation simultanée, destruction complète des hyphes, mais sans atteinte du complexe de la lamelle mitoyenne (220 x). e- H. corrugata: résistance à la dégradation des bords des ponctuations (430 x). f- H. pinnatifida: Id. (360 x).


20
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10
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PERTE DE POIDS (pourcentages)
PERIODE D'INCUBATION (semaines)

Fig.I

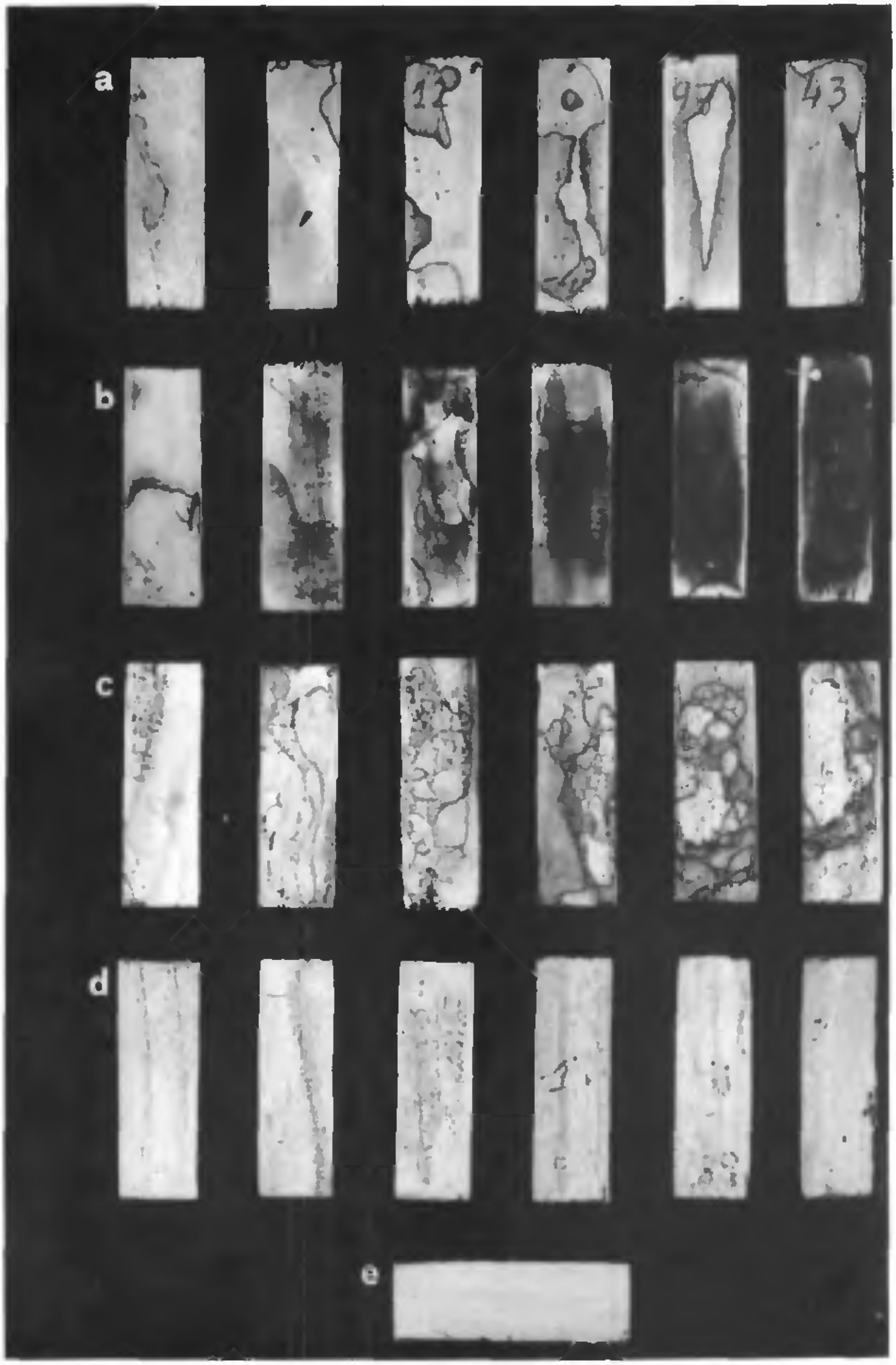

Fig.II

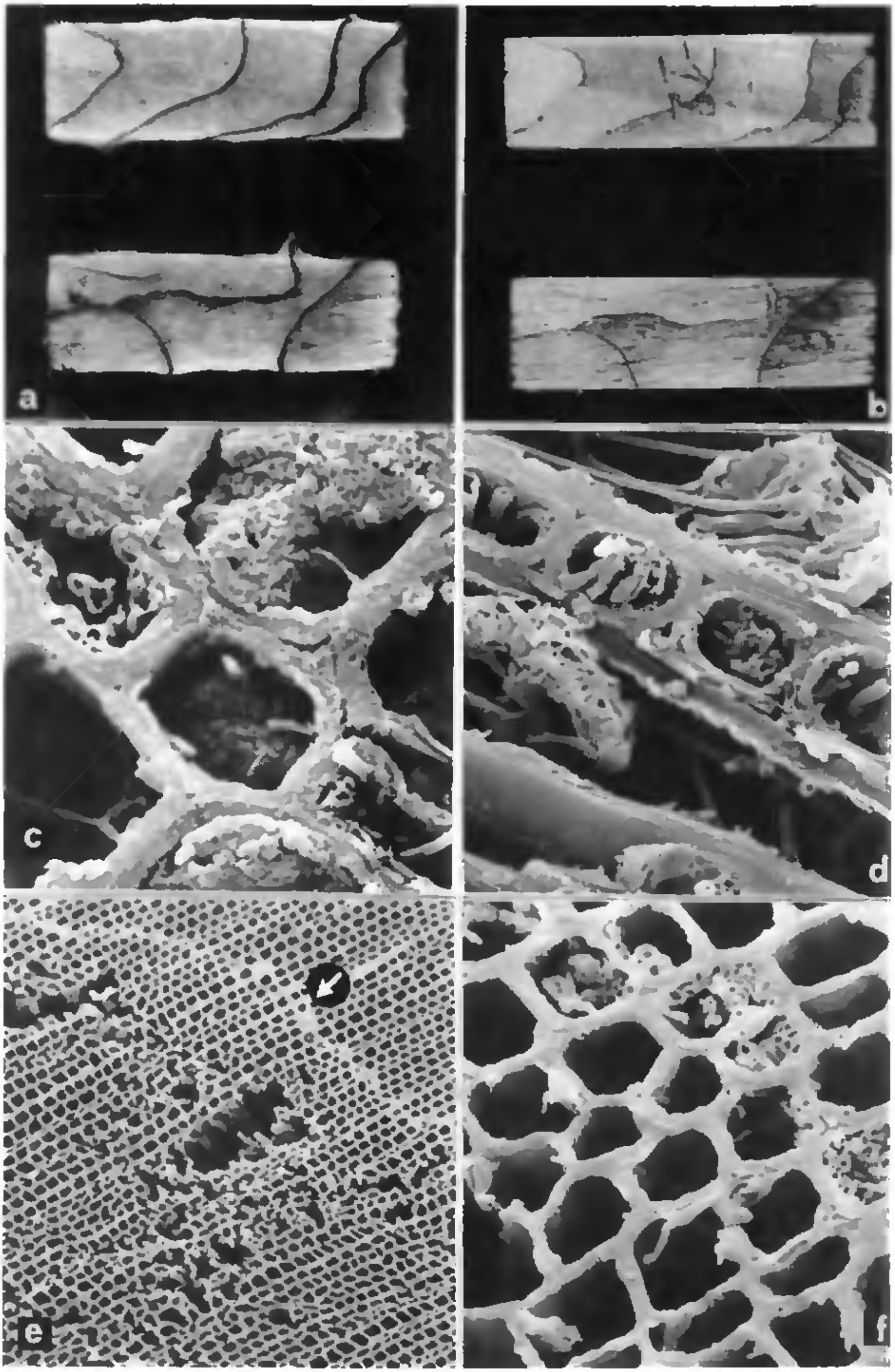

Fig.III

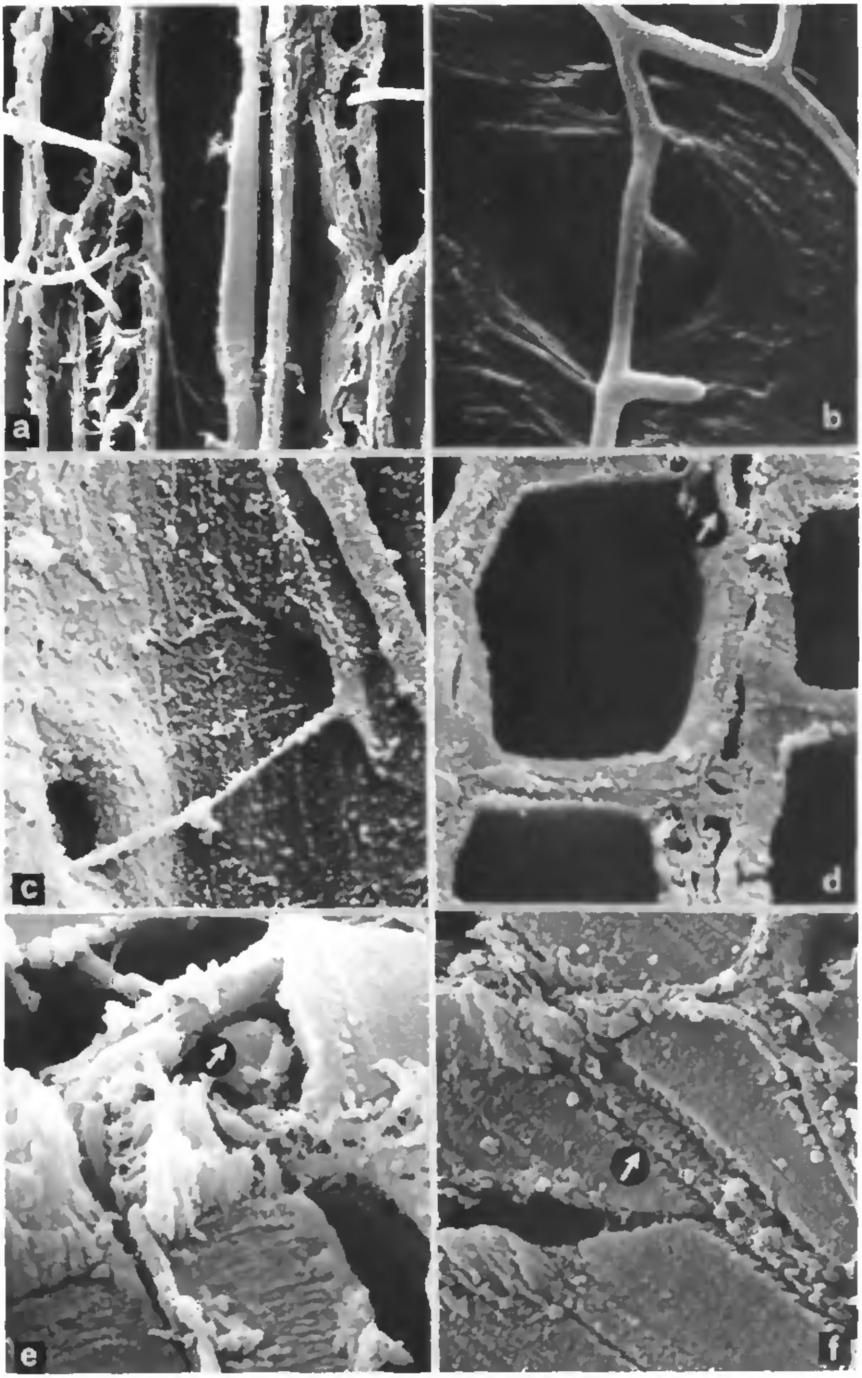

Fig.IV

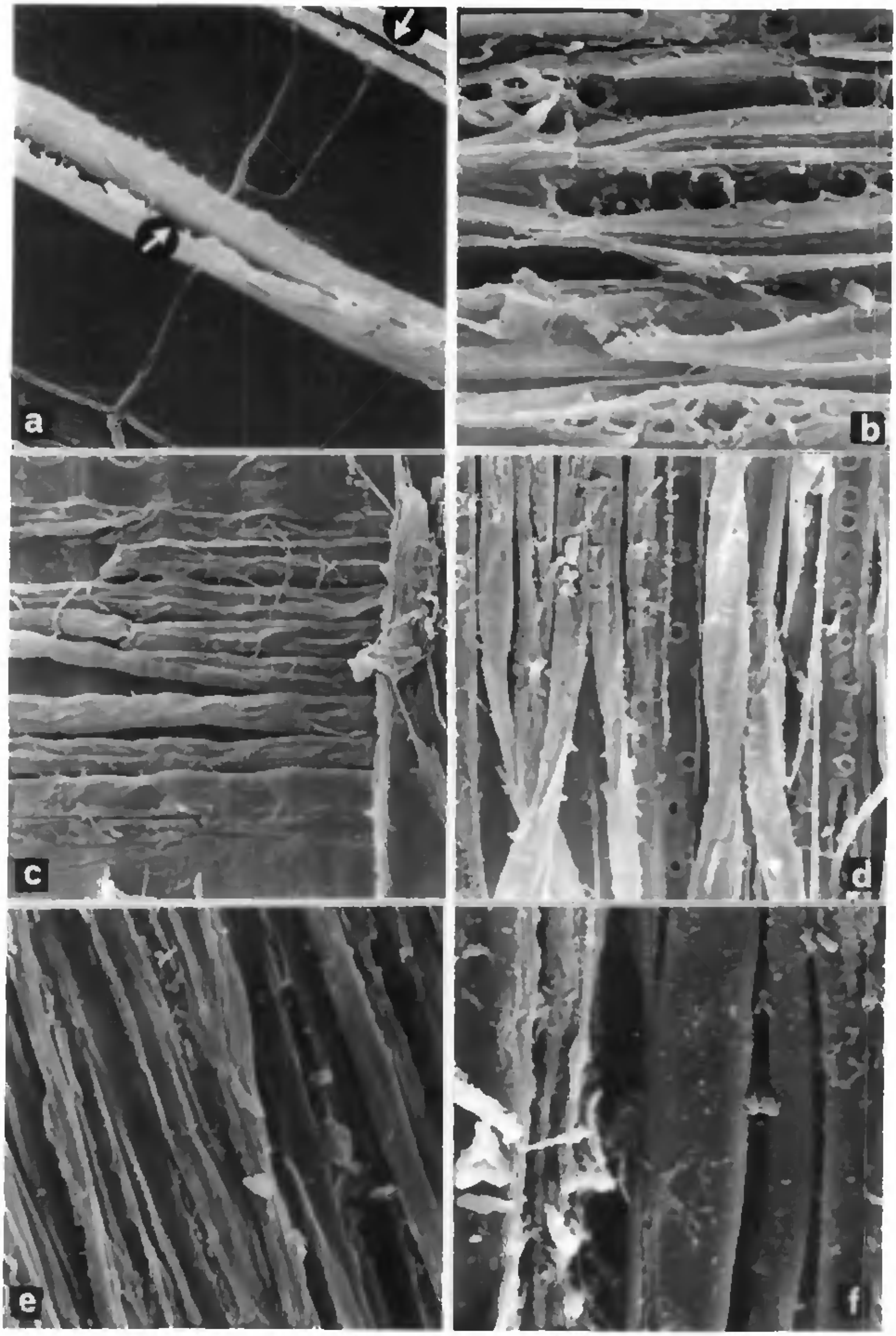

Fig.V

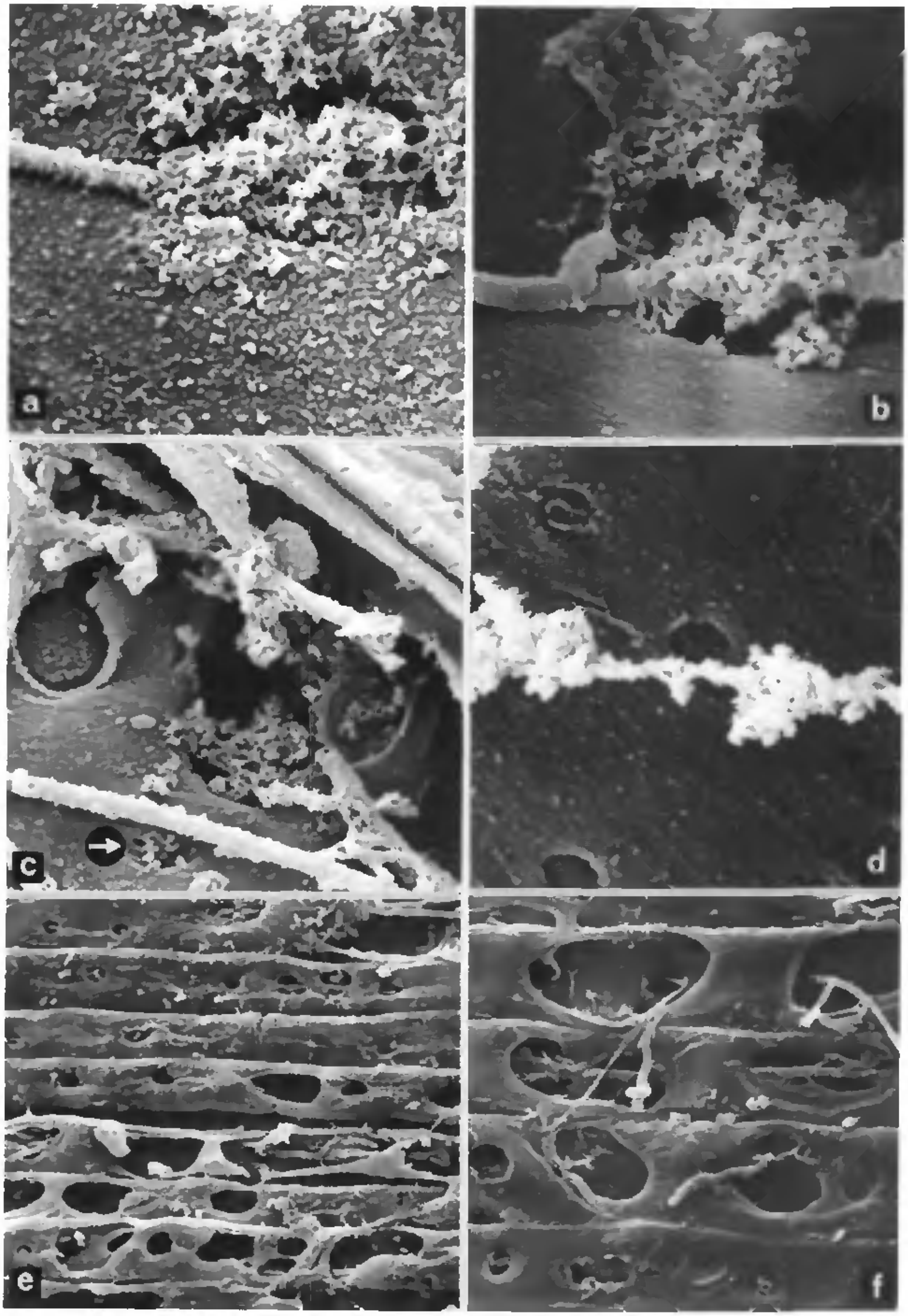

Fig.VI

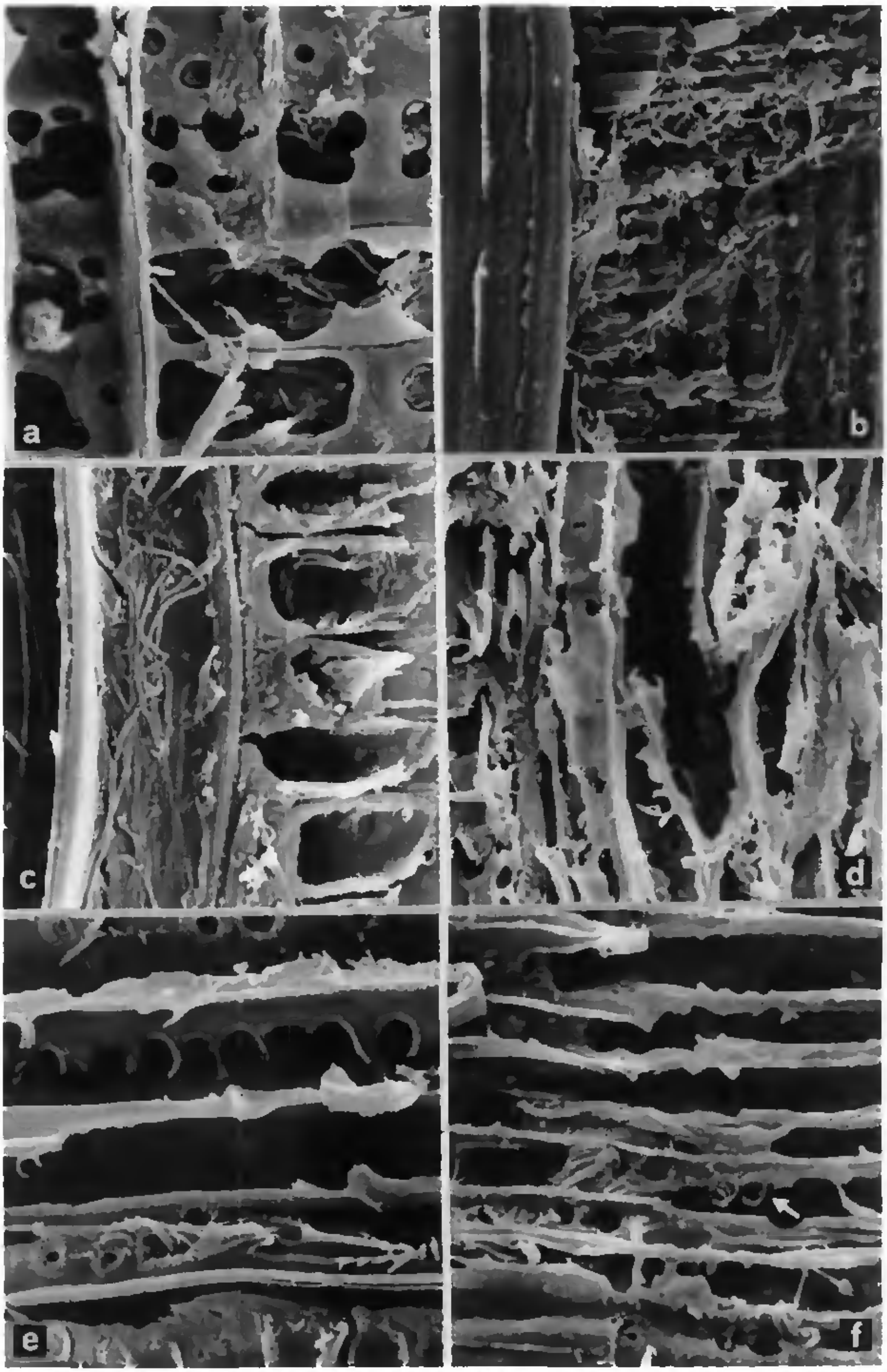

Fig.VII

MYCOLOGIA HELVETICA

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DEGRADATION OF WOOD BY SPECIES OF HYMENOCHAETE: DIFFERENCES IN RESPONSE TO TEMPERATURE.

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SUMMARY

Weight loss and micromorphological and chemical changes in wood blocks of *Picea abies* decayed by *Hymenochaete attenuata*, *H. corrugata*, *H. pinnatifida*, *H. rubiginosa*, *H. sallei* and *H. tabacina* at different temperatures are described. *H. corrugata* and *H. tabacina* were the most active fungi and decayed the wood at levels comparable with the strong decay fungi. Although weight loss and lignolytic activity of some of the isolates were modified by temperature, the ultrastructural pattern of decay and the mycelial mechanisms of invasion were not influenced by that factor.

RESUME

Des blocs de Picea abies attaqués par Hymenochaete attenuata, H. corrugata, H. pinnatifida, H. rubiginosa, H. sallei et H. tabacina présentent des pertes de poids et des altérations tant microstructurales que chimiques. H. corrugata et H. tabacina sont les espèces les plus actives et leur pouvoir destructeur égale celui des champignons intensément lignivores. La perte de poids des blocs et l'activité lignolytique de certaines souches dépendent de la température; à l'inverse les mécanismes de pénétration des myceliums et le mode de destruction observé à l'échelle ultrastructurale ne sont pas influencés par ce facteur.

ZUSAMMENFASSUNG

Gewichtsverluste sowie mikromorphologische und chemische Aenderungen kennzeichnen Picea abies-Holzblöcke, die von Hymenochaete attenuata, H. corrugata, H. pinnatifida, H. rubiginosa, H. sallei und H. tabacina als Substrat verwendet wurden. H. corrugata und H. tabacina weisen die grösste Zersetzungskraft auf und entsprechen in dieser Hinsicht den stärksten Holzabbauern. Der Gewichtsverlust der Blöcke und die Zersetzungskraft einiger Arten sind temperaturabhängig; dagegen werden die Eindringungsmechanismen der Hyphen und die ultrastrukturelle Zersetzungsform durch diesen Faktor nicht beeinträchtigt.

INTRODUCTION

It is well documented that white-rot fungi can produce, either in field or in laboratory studies, simultaneous or selective wood-decay (Liese, 1970; Blanchette et al., 1985; Blanchette & Reid, 1986). These two types of decay differ in micromorphological and chemical characteristics and can be evaluated with chemical analyses and SEM techniques. The simultaneous decay is characterized by the elimination of all wood components at approximately the same rates relative to the original amounts present, whereas in selective decay, lignin is removed preferentially and decomposed more quickly (Adaskaveg and Gilbertson, 1985).

In recent years laboratory research was done in order to determine the type and capacity of degradation of white-rot species of the family Hymenochaetaceae (Job & Wright, 1986; Otjen et al., 1987; Job & Rajchenberg, 1988), considered of particular relevance because it includes some of the most important heart-rots in living trees (Gilbertson, 1980). Specifically, in a previous ultrastructural study (Job & Keller, 1988) we have shown that some species of the genus *Hymenochaete* are capable of producing both simultaneous and selective decay in *Picea abies* blocks and show four mechanisms of wood invasion. In order to obtain a better understanding of the decay process caused by *Hymenochaete* species and to determine how the patterns of degradation previously found in the genus (Job & Keller, 1988) are modified by environmental factors, we studied and compared the growth-rate of the mycelium, the weight loss and the micromorphological and chemical changes that occur in wood blocks of *Picea abies* (L.) Karsten inoculated with isolates of *H. attenuata* Lév., *H. corrugata* (Fr.) Lév., *H. pinnatifida* Burt, *H. rubiginosa*(Dick.:Fr.) Lév., *H. sallei* Berk. & Curt., and *H. tabacina* (Sow.:Fr.) Lév., incubated under different temperatures.

MATERIAL AND METHODS

The following cultures were studied

H. attenuata = NEUF 4212   *H. corrugata* = FP 125019
H. pinnatifida = BAFC 640   *H. rubiginosa* = NEUF 400
H. sallei = BAFC 599   *H. tabacina* = FP 125071

Wood decay studies: "in vitro" weight loss was analysed using previously described techniques (Job & Keller, 1983). Once sterilized and inoculated, the blocks were incubated in Petri dishes with malt-extract agar (Nobles, 1965) 12 weeks at 20, 25 and 30 °C + 1 °C. Every two weeks 20 blocks, inoculated with each fungus species at each temperature, were withdrawn and 16 of them placed in a desiccator and reduced to dry weight, whereas the remaining ones were kept for SEM studies.

Chemical analyses: control blocks and decayed blocks incubated 12 weeks with each fungus, with weight losses aproximately equal to the mean of the 16 replications, were chosen for chemical analysis. The blocks were ground to pass through a 40-mesh screen and extracted 20 cycles in a Soxhlet, first with ethanol-benzene (1:2 v-v) and then with ethanol 95 % (Tappi, 1975). Acid insoluble lignin (Klason lignin) was preformed using 200 mg samples from the extracted wood as described by Effland (1977). Chlorite holocellulose was determined using also 200 mg samples from the extracted wood as described by Seifert (1983). Both analyses were replicated three times for each treatment. Data were subjected to statistical comparison of means by Student-t test ($P = 0.05$). Changes in the concentration of acid-soluble lignin were measured from the Klason filtrate by ultraviolet spectrophotometry (Cowling, 1960).

Mycelium growth rate: for growth rate determination, each isolate was grown for nearly a month in malt extract agar (Nobles, 1965) before inoculating triplicates were made on 10 cm Petri dishes containing the same medium and incubated at 20, 25 and 30 °C + 1 °C for three weeks. Growth rates were measured and the maximum for each triplicate was considered.

Scanning electron microscope studies: the material was fixed according to the method used previously by Keller (1985), subjected to critical point drying and then coated with gold and observed with a Philips 500 microscope.

RESULTS

1. Weight loss:

The results obtained for each species, at each temperature during the 12 week incubation period are shown in figure 1.

The optimal temperature for the degradation of blocks was 25 °C, and the greatest weight losses were caused by H. corrugata and H. tabacina (20.54% and 19.16% respectively). However, the fungi tested caused a wide range of weight losses with differences occurring both between species and at different temperatures in the same species. Losses of dry weight, at the end of the experiment, were ranged from 3.26% (H. rubiginosa) to 20.54% in the inoculated blocks, and was insignificant in the control (0.34%).

Figure 1 shows that the species tested presented a different sensitivity to the incubation temperature. Whilst in the case of H. rubiginosa and H. sallei (Fig. 1e,f) no clear influence of the temperature was noted, in the other species, particularly H. corrugata and H. tabacina (Fig. 1a,b), marked differences in the weight losses occurring at the different temperatures were observed. The capacity of degradation of H. pinnatifida increased slightly with the increase of temperature (Fig. 1d), whereas the H. attenuata isolates died and the decay stopped at 30 °C (Fig. 1c).

2. Chemical analyses:

The average percentage of weight loss at the end of the experiments with the blocks used for the chemical analyses, and the average percentage loss of Klason lignin and chlorite holocellulose for wood decayed by each culture at different temperatures are presented in Table 1. The ratio of the Klason lignin (%KL) and the chlorite holocellulose (%CHC) percentages are shown in Table 1. Decreases in the %KL/%CHC are indicative of selective delignification.

Table 1 shows that, except in the case of H. attenuata at 30 °C, both lignin and holocellulose were degraded by all fungi at each temperature. In almost every species approximately equal amounts of lignin and holocellulose, relative to the original amounts present, were removed from the decayed wood. Nevertheless, in the blocks decayed by H. tabacina at 25°C the %KL/%CHC ratio indicated a clear increase of selective degradation. Although the change of the incubation temperature of H. pinnatifida (25 to 30 °C) did not influence the percentage of weight loss (8.23% and 8.78% respectively), its capacity of lignin degradation was clearly modified by that change (17.80% and 7.39% loss, respectively). The apparent acid-soluble lignin for each treatment showed no significant differences between the control and decayed wood.

3. Macro- and micromorphological changes in decayed-wood:

The macromorphological alterations produced in the decayed wood (blacks lines, stains and bleaching) differed with the different species studied. Nevertheless, in the same species, at the different temperatures of incubation, all samples appeared to follow some trend in the decaying process, and it could be assumed that each sample represents a different stage in a common decay process.

A steady darkening of the zone attacked was evident in the pine-blocks decayed by H. attenuata, H. sallei and especially by H. pinnatifida.

Despite the different species examined and the difference in the macroscopic appearance of the decayed wood, the ultrastructural gross patterns of decay appeared similar in the various samples investigated, and the four mechanisms of invasion mentioned by Job & Keller (1988) for the genus were found.

The Picea wood-blocks decayed by each species studied presented evidence of simultaneous decay typical of white rots. Bore holes were present in the ray parenchyme and tracheid cells, and large voids resulted from the enzymatic erosion of the cell walls and the gradual coalition of degraded areas.

As could be expected, an evident relationship exists between weight loss and the size of the affected area. In the blocks with low weight loss the areas affected were smaller and generally coalition of bore holes was not observed (Fig. 2b). However when the weight loss increase (>7%) ray parenchyme cells were completely destroyed (Fig. 2c) and large voids were observed in the tracheid cells (Fig 2d).

We also observed smaller areas, not uniformly distributed throughout the decayed wood of selective delignification, in which the altered tracheids had the middle layer removed and exposed the spirally oriented macrofibrils of the secondary wall (Fig. 2e). No decomposition was apparent in any of the control blocks at any temperature (Fig. 2a).

Species	T °C	Percent weight loss	Percent loss CHC	KL	%KL/%CHC
Control	25	0.36 + 0.23	--- (63.60)	-- (27.59)	0.433
<u>H. attenuata</u>	20	6.26 + 0.40	- 4.99 (64.46)	- 6.44 (27.61)	0.428
	25	7.86 + 0.30	- 6.44 (64.58)	- 8.86 (27.29)	0.422
	30	0.63 + 0.12	not determined		
<u>H. corrugata</u>	20	8.39 + 0.19	- 9.29 (63.06)	-12.50 (26.35)	0.417
	25	21.02 + 0.30	-22.16 (62.68)	-23.51 (26.72)	0.426
	30	4.90 + 0.17	- 5.44 (63.28)	- 8.34 (26.59)	0.420
<u>H. pinnatifida</u>	20	4.90 + 0.17	- 6.23 (62.71)	- 7.79 (26.75)	0.426
	25	8.23 + 0.09	- 4.66 (66.07)*	-17.80 (24.71)*	0.373*
	30	8.78 + 0.24	-10.37 (62.49)	- 7.39 (28.01)	0.448
<u>H. rubiginosa</u>	20	3.16 + 0.03	- 2.80 (63.63)	- 3.30 (27.55)	0.433
	25	3.93 + 0.18	- 4.59 (63.16)	- 4.03 (27.56)	0.436
	30	3.48 + 0.10	- 2.94 (63.95)	- 5.26 (27.08)	0.423
<u>H. sallei</u>	20	5.24 + 0.22	- 5.71 (63.28)	- 5.61 (27.48)	0.434
	25	6.66 + 0.28	- 9.92 (61.38)	- 4.09 (28.33)	0.461
	30	6.57 + 0.23	- 8.14 (62.19)	- 5.35 (27.95)	0.449
<u>H. tabacina</u>	20	6.10 + 0.42	- 6.31 (63.40)	- 9.74 (26.52)	0.418
	25	19.13 + 0.19	-10.38 (70.48)*	-37.24 (21.41)*	0.303*
	30	4.29 + 0.23	- 4.57 (63.41)	- 6.96 (26.82)	0.423

Table 1: Percentage weight loss and percentage loss of Klason lignin (KL) and chlorite holocellulose (CHC) relative to the original amounts present. Values in parentheses are not corrected with respect to the original percentage (* value significantly different from the others of the same species P=0.05).

4. Rate growth of mycelium:

Table 2 shows the growth values at different temperatures of all the isolates studied.

Species	radial growth in mm at		
	20 °C	25 °C	30 °C
H. attenuata	2.6	2.8	--
H. corrugata	3.7	8.4	3.2
H. pinnatifida	2.8	4.1	2.6
H. rheicolor	4.2	5.2	3.8
H. rubiginosa	2.0	2.8	1.6
H. tabacina	2.1	8.0	2.4

Table 2: Radial growth of the mycelia (21 days).

DISCUSSION

Degradation studies under controlled laboratory conditions are useful for comparison of the degradative activity of several species of fungi (Ander & Eriksson, 1977; Otjen & Blanchette, 1985), although they cannot be taken as absolute evidence of the behaviour of wood-rotting fungi under natural conditions (Blanchette, 1984; Blumenfeld, 1984). We observed that all the isolates tested are capable of decaying Picea wood. Nevertheless only H. corrugata and H. pinnatifida have been reported to grow on coniferous wood. This indicates that the host specificity observed in nature does not depend on the type of lignin present in gymnosperms (guaiacyl lignin) and angiosperms (guaiacyl and syringil lignin, Janshekar & Fiechter, 1983), but on host resistence, competition or other ecological requirements.

Both H. corrugata and H. tabacina decay Picea wood at levels comparable with those of many Homobasidiomycetes classically considered as strong decay fungi (Cowling, 1961; Elliot et al., 1970). However, we observed a difference in the degradative activity of those species when the temperatures of incubation were different. Part of this variation could be explained by the rapid growth rate of H. corrugata and H. tabacina at 25 °C (2 to 4 times) when compared to the other temperatures selected for this study. In the other species tested, the percentage of weight loss at different temperatures was not proportional to their growth rate.

Otjen et al. (1987) have postulated that since the most selective lignin-degrading fungi are also capable of causing a simultaneous rot, the environmental factors which initiate or suppress cellulase activity may ultimately be responsible for their overall selectivity for lignin. We observed in H. pinnatifida that the use of different cultural conditions may change the specificity of fungi for lignin. At 25 °C, this species presented a high capacity of lignin degradation (Table 2), but, since the change of incubation temperature (25 to 30 °C) neither influenced the general degradative activity (weight loss) nor the mechanism of penetration and invasion of the mycelium. At 30 °C the degradation of cellulose is favoured whereas the ability to selectively degrade lignin is reduced. These results suggest that the selective or simultaneous decay of wood components are influenced by temperature and an increase of this factor may repress ligninase activity. Although the weight losses at the different temperatures in the case of H. tabacina are not comparable, the differences observed in the capacity of lignin degradation at 25 °C may be due to a different behaviour of the isolates and may reflect a predominance of simultaneous rot in the first stage of decay and a change to a selective decay in the later stages.

The macroscopic appearance of decayed wood differed among the species studied; micromorphological characteristics, however, were similar in all fungi tested. The ultrastructural analysis showed that the blocks decayed by Hymenochaete species can be selectively delignified. However, when the entire decay wood was chemically analysed, even if only a small portion of wood was degraded selectively, the isolates appeared non-selective for lignin and their delignification capacity was masked.

The results obtained showed that the mechanisms of penetration and the gross ultastructural changes in the wood decayed were not modified by the change of the incubation temperature. On the other hand the growth rate, weight loss and the ligninolytic activity of some of the isolates tested may be clearly modified by that change. We observed that each species presented a different sensitivity to temperature, and the findings may not be generalized at the genus level.

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FIGURES

Fig. 1. Weight loss at different temperatures due to decay during the incubation periods: a- H. corrugata, b- H. tabacina, c- H. attenuata, d- H. pinnatifida, e- H. rubiginosa f- H. sallei.

Fig. 2. a- Radial face of Picea abies wood with undecayed tracheids and ray cells (300 x), b- H. rubiginosa, bore holes in tracheids and ray cells (1160 x), c- H. tabacina, coalesced bore holes in ray parenchyme cells (420), d- H. pinnatifida, tracheids completely destroyed (480 x), e- H. tabacina, delignified tracheid with primary wall (arrowhead) partially removed exposing spiral macrofibrils of the secondary wall (1900 x).

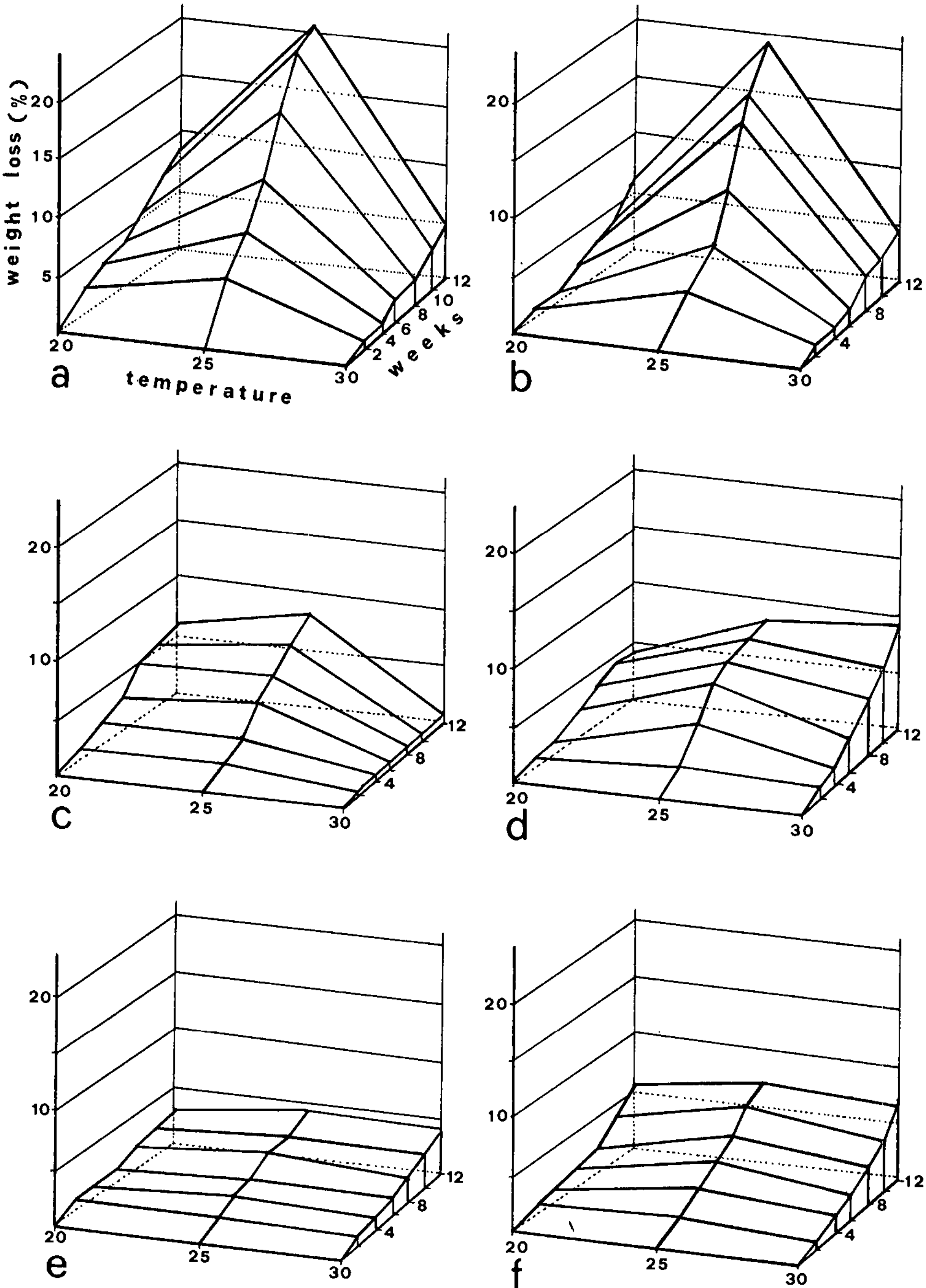
weight loss (%)
5
10
15
20
20
25
30
temperature
2
4
6
8
10
12
weeks
a
b
c
d
e
f

Figure 1

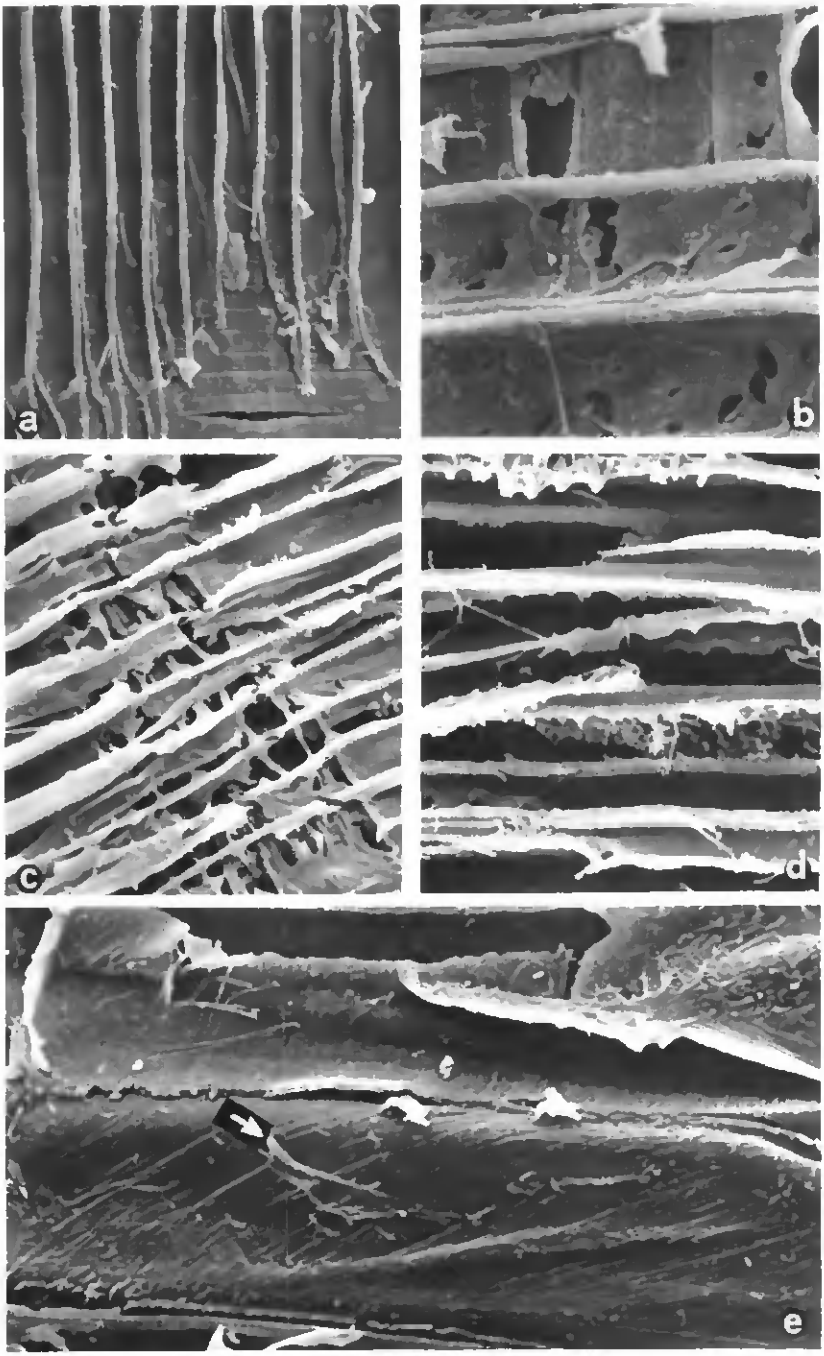

Figure 2

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