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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 mycéliums 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	Percent loss KL	%KL/%CHC
Control	25	0.36 + 0.23	--- (63.60)	-- (27.59)	0.433
H. attenuata	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		
H. corrugata	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
H. pinnatifida	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
H. rubiginosa	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
H. sallei	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
H. tabacina	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).

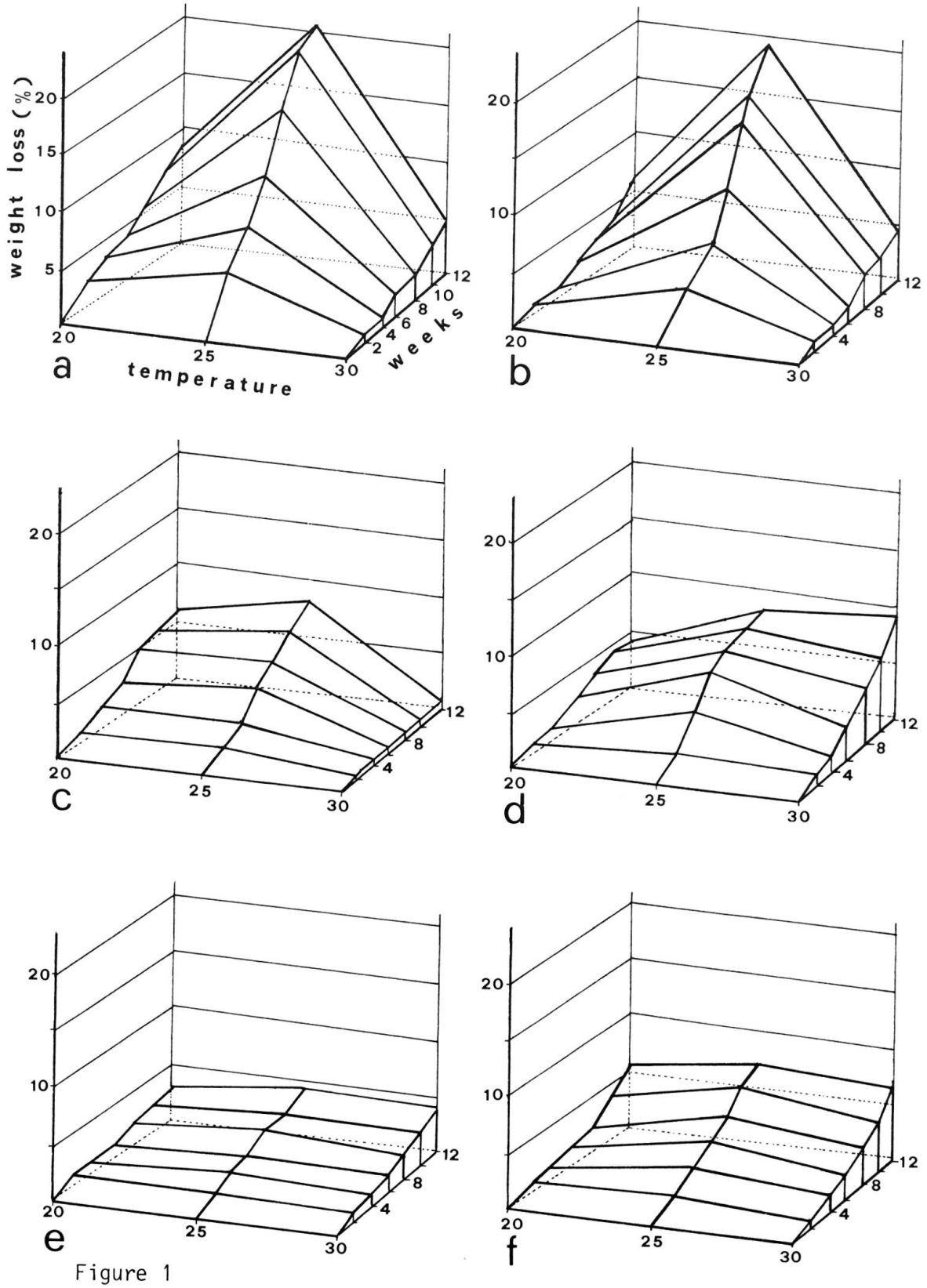
weight loss (%)
20
15
10
5
20
25
30
temperature
2
4
6
8
10
12
weeks
a
b
c
d
e
f

Figure 1

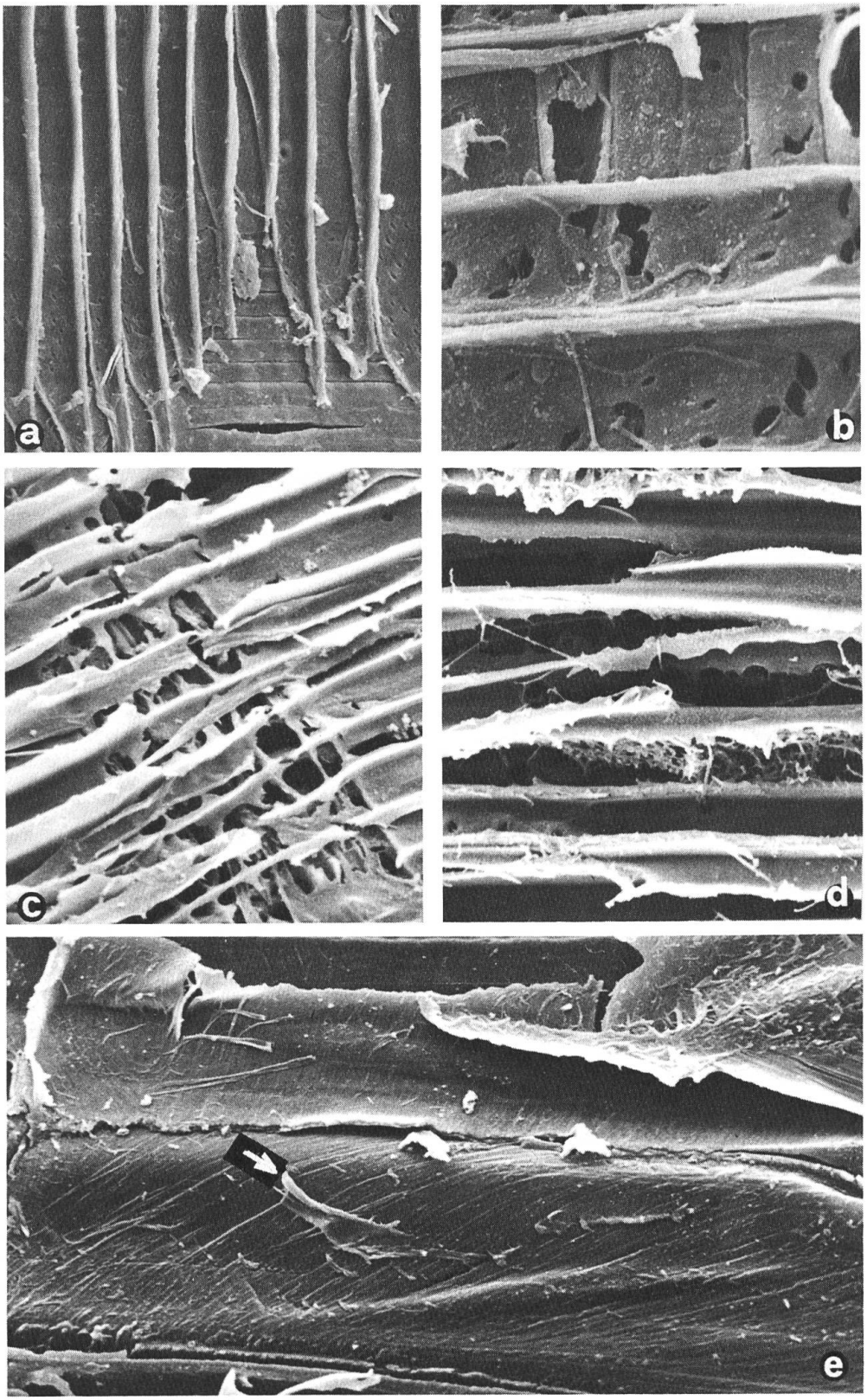

Figure 2