

## ANATOMICAL CHANGES IN WILLOW WOOD DECAYED BY THE BROWN ROT FUNGUS *CORIOLELLUS MALICOLA* (BASIDIOMYCOTA)

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**Summary:** In Argentina, *Salix* wood is employed mainly in pulp and paper industry. In this country, the brown rotter *Coriolellus malicola* was found in association with willow plantations. The purpose of this work was to study the anatomical changes caused by *C. malicola* in willow wood in order to provide information on the effects of brown rot decay in the yield and quality of pulp. Two willow clones were employed: *Salix nigra* 4 and *Salix babylonica* x *Salix alba* cv I 131-25. Two exposure times were used: 75 and 150 days. The percentages of weight loss produced by this fungus in both clones was ca. 30% at 75 days and ca. 60% at 150 days of decay. *C. malicola* degraded mainly fibre walls. Microscopically, the loss of cell shape, the presence of transwall fractures and the loss of birefringence were the main anatomical modifications observed. According to our observations decayed *Salix* wood by *C. malicola* seems to be inadequate for pulp industry.

**Key words:** brown-rot decay, hardwoods, anatomy, *Salix*, *Coriolellus malicola*.

**Resumen:** Modificaciones anatómicas en madera de sauce por acción *Coriolellus malicola* (Aphylophorales) agente de pudrición castaña. En la República Argentina la madera de *Salix* es empleada principalmente en la industria papelera. En este país el hongo de pudrición castaña *Coriolellus malicola* se encontró asociado a plantaciones comerciales de sauce. El objetivo de este trabajo fue estudiar las modificaciones anatómicas causada por *C. malicola* en la madera de sauce con el fin de aportar información sobre los efectos de la pudrición castaña en los rendimientos y calidad de la pulpa para papel. Se emplearon dos clones: *Salix nigra* 4 y *Salix babylonica* x *Salix alba* cv I. 131-25. Se trabajó con dos tiempos de exposición: 75 y 150 días. Las pérdidas de peso producidas por esta cepa en ambos clones fueron de ca. del 30% a los 75 días y de ca. del 60% a los 150 días de incubación. *C. malicola* degradó principalmente las paredes de las fibras. Microscópicamente, las principales modificaciones anatómicas observadas fueron: deformación del tejido, presencia de fracturas transversales en las paredes celulares y pérdida de birrefringencia. De acuerdo con nuestras observaciones la madera de *Salix* degradada por *C. malicola* sería inadecuada para la industria del pulpado.

**Palabras clave:** pudrición castaña, madera de latifoliadas, anatomía, *Salix*, *Coriolellus malicola*.

### INTRODUCTION

Many microorganisms are likely to degrade wood. The most relevant of them are the xylophagous fungi because they produce important changes on the anatomical, physical and mechanical wood properties

(Wilcox, 1978; Highley *et al.*, 1994).

Wood rotting fungi are known to cause three basic types of degradation: white rot, soft rot and brown rot. Brown rotters remove cellulose and hemicelluloses whereas lignin degradation is limited (Wilcox, 1978; Blanchette, 1995). The modified lignin remaining gives the decayed wood its characteristic colour and consistency (Schwarze, *et al.*, 2003). Brown rot decay involve the penetration of enzymes into cell wall from hyphae growing in the lumen. These enzymes penetrate the entire secondary walls and produce an extensive degradation of cellulose and hemicelluloses (Schwarze *et al.*, 2000).

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Although brown rot decay is typically associated

with softwoods, some brown rotters are reported to attack hardwoods (Wilcox 1968; Schwarze *et al.* 2000).

Wright *et al.* (1973) and Wright & Deschamps (1976) recorded the brown rot fungus *Corirolellus malicola* (Berk. & Curt.) Murr. (Basidiomycetes, Aphyllophorales) on willow plantations in Argentina. As *Salix* wood is employed mainly for paper industry (SAGPyA, 1999) in this country, the study of the decay produced by brown rot fungi is important, because they cause significant reductions in the yield and quality of the pulp (Lindgren, *et al.* 1961).

According to Wilcox (1993) microscopical examination remains the most accurate dependable means of diagnosing and evaluating wood decay. Many authors consider that the first signs of brown rot decay are the presence of hyphae in the cell lumen and the loss of birefringence in the cell walls (Wilcox 1968, 1993; Anagnost 1998, Schwarze *et al.*, 2000).

The purpose of this work was to study the anatomical changes caused by *C. malicola* in willow wood in order to provide information on the effects of brown rot decay in the yield and quality of pulp.

## MATERIALS AND METHODS

### *Sample preparation*

Sound sapwoods of *Salix nigra* 4 and *S. babylonica* x *S. alba* cv. I 131-25 from Paraná River Delta plantations, Buenos Aires Province, Argentina (34° 05' LS; 58° 38' LW) were used. Both clones are the most widely planted in this area, where climatic and soil conditions are optimal for this species (Blanco, 1977; Alonso, 1980). Five trees of each clone were randomly selected. A 2-meter-long log immediately above the stump was extracted from each specimen. Logs were sawn into 5 x 5 cm pieces and air-dried until constant weight was reached. Once dry, small cubic blocks (2 cm x 2 cm x 2 cm) to be submitted to decay in vitro were cut.

### *Decay test experiment*

*Corirolellus malicola* (Berk. & Curt.) Murr. (BAFC Strain 472) was grown for two weeks in Petri plates with malt extract-agarose medium.

ASTM D-2017 (78) standard with some modifications based on IRAM 9518 (1962) was employed. Twenty glass bottles (cylindrical, capacity nominal 450 cm<sup>3</sup>) were filled with 43 cc distilled water

and 200 g of a substrate (sand 160g, soil 40 g) previously oven dried (105± 2 °C) and sifted. Then two feeder strips were introduced in each bottle. Culture bottles were sterilized for 30 minutes at 120 °C and inoculated with *C. malicola*. After 20 days blocks were placed over feeder strips.

Twenty cubic blocks (2 cm side) of each clone were used for decay test. Blocks were oven dried (70 °C) and weighed to determine the initial weight (IW); then moistened by immersion in distilled water and sterilized for 20 minutes at 100° C.

The assay was incubated for 75 and 150 days at 27±1°C and 70% RH. After each incubation period 10 blocks per clone were withdrawn from the bottles, cleaned to remove mycelia, and oven dried at 70°C for three days until constant weight, considered as final weight (FW). Percentages of weight loss (WL %) due to decay were then calculated as:

$$WL \% = \frac{IW - FW}{IW} \times 100$$

Twenty uninoculated blocks (10 of each clone) were introduced in the culture bottles, as was previously described, and employed as controls.

### *Anatomy of decay*

Wood samples at different stages of decay were selected. For light microscopy studies (LM) of cell wall degradation and hyphal growth, pieces of decayed material were fixed in formaldehyde-acetic acid-alcohol and embedded in paraffin (D'Ambrogio de Argüeso, 1986). Sections (8-12 µm) were double stained with safranin-fast green.

To detect cellulolysis some sections were left unstained and viewed between crossed polarizing filters. Loss of birefringence was considered as an indicator of cellulose depletion and, as a consequence, of brown rot decay (Wilcox, 1968, 1993; Schwarze *et al.* 2003).

Blocks were also examined in a Jeol JSMT-100 scanning electron microscope (SEM). Specimens were mounted on stubs without pretreatment and covered with gold-palladium. Sound wood was used as control.

## RESULTS AND DISCUSSION

### *Weight loss due to decay*

The percentages of weight loss (% WL) after each incubation period are given in table 1. No significant differences of % WL were found between *Salix* clones at both incubation times.

#### *Anatomy of decay*

*Corirolellus malicola* caused the same pattern of decay in both clones. Macroscopically decayed material showed a brown discoloration. Blocks with higher weight losses (ca. 60% WL) showed in addition shrinkage and a cubical pattern of cracks. The characteristic shrinkage of brown rotted wood is attributed to the extensive removal of carbohydrates. Thus, when the wood is dried, the lignin residue and carbohydrate depolymerization products lack sufficient strength to maintain the integrity of the cells (Wilcox, 1968).

Microscopically sound *Salix* wood showed the typical shape and cell wall thickness of vessels and fibres (Fig. 1 A-B).

At 30% WL decayed wood exhibited a slight loss of cell shape. Transwall fractures were frequent in fibres and vessels (Fig. 2 A).

Separation among fibres was occasionally observed in some specimens (Fig. 2 B). Cell separation occurred between the S1 layer and the compound middle lamella (ML), which appeared intact at this stage of decay. Wilcox (1968) observed separation among cells in brown rotted *Liquidambar styraciflua*. However, in a later paper, the author considered that this feature was not diagnostic of brown rot decay because its appearance was not regular (Wilcox, 1993). As in *Salix* wood separation among cells was rarely observed, we don't consider this as diagnostic feature of brown rot decay, in agreement with Wilcox (1993).

In longitudinal sections ray parenchyma cells appeared unaltered (Fig. 2 C), as occurred in sound wood (Fig. 1 C). Wilcox (1968) and Worrall *et al.* (1997) observed the same feature in brown rot decayed *Liquidambar styraciflua* and *Betula alleghaniensis* at similar weight losses (near 30% WL). Moreover, Anagnost (1998) mentioned that brown rot fungi do not attack rays in hardwoods.

In decayed *Salix* wood bore holes were rarely found. In contrast, hyphal penetration through pits was frequently observed (Fig. 2 D). Shwarze *et al.* (2000) also noted that in *Robinia* sp., the brown rotter *Laetiporus sulphureus* advanced to neighboring cells via pit connections. Cell wall erosion and mycelia were not registered in sound *Salix* wood (Fig. 1 D).

Examination under polarized illumination revealed loss of birefringence in fibre walls in some portions of the samples (Fig. 2 E). By contrast, vessels appeared relatively intact and birefringent during decay. Comparable patterns were observed by Wilcox (1968) and Schwarze *et al.* (2000) in brown rotted *Liquidambar styraciflua* and *Robinia pseudoacacia* woods, respectively. In sound wood samples both, fibres and vessels, appeared birefringent (Fig. 1 E).

Longitudinal sections of sound willow wood showed birefringence in vessel, fibre and ray parenchyma cell walls (Fig. 1 F). However, a weak birefringence was observed in ray parenchyma cells at 30% WL (Fig. 2 F). Schwarze *et al.* (2003) observed in brown rotted *Quercus robur*, *Acer pseudoplatanus*, *Betula pendula* and *Robinia pseudoacacia* woods that rays could be seen bright at lower weight losses (5.51-12.11% WL). They related the absence of radial fibril orientations in the secondary walls of vessels and parenchyma cell walls with a presumably increase in the resistance to degradation by these fungi. However, at higher weight losses (30%) they could probably also be degraded, as occurred in willow wood.

At ca. 60% WL the diagnostic features of brown rot decay were more pronounced. Loss of cell shape and cell wall thinning were evident (Fig. 3 A-B). In some samples fibres appeared completely collapsed (Fig. 3 B). As a result of shrinkage, vessels and rays acquired a sinuous run (Fig. 3 C). Cracks into cubical fragments were also registered (Fig. 3 D-E). Under polarized light, mainly vessel elements displayed birefringence (Fig. 3 F).

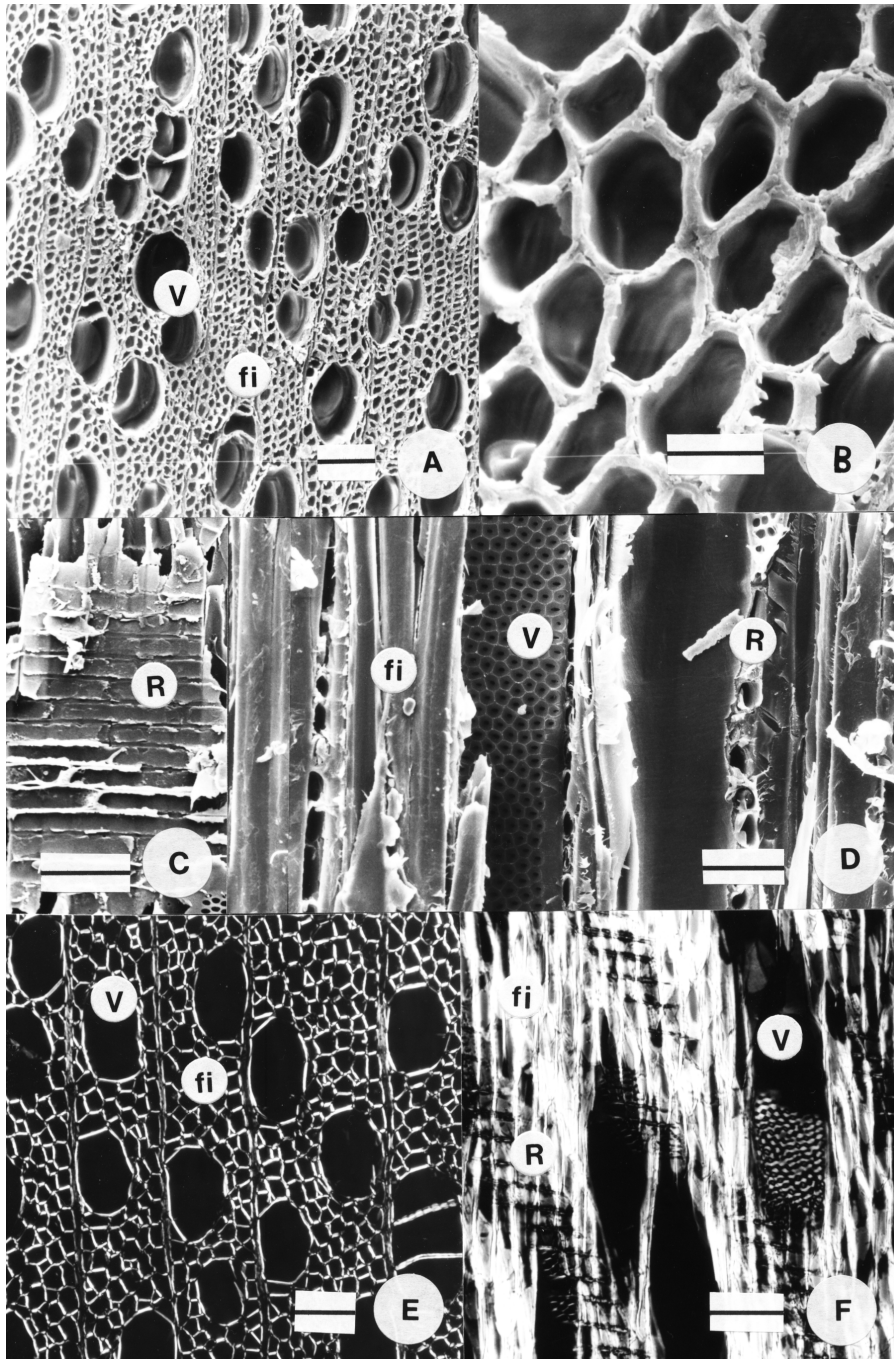
Cellulolysis by brown rot fungi involve the radial penetration of enzymes into cell wall from hyphae growing in the lumen. The S2 layer of the secondary wall is the first to be attacked and decomposed towards the middle lamella, which remains unaltered together with the S3 layer (Schwarze *et al.* 1997). In the present study the ML was resistant to attack and appeared birefringent during decay.

From a pulp and papermaker's point of view wood characteristics such as basic density, fibre length, chemical composition and whiteness are of importance for the paper industry (Senisterra *et al.* 2000). Discolored decayed wood tend to darken pulps and to increase the consumption of chemical. In addition, the pulp from decayed wood contains many short fibres, with low tensile strength, low tear and low bursting strength (Shema, 1955; Lindgren *et al.*, 1961).

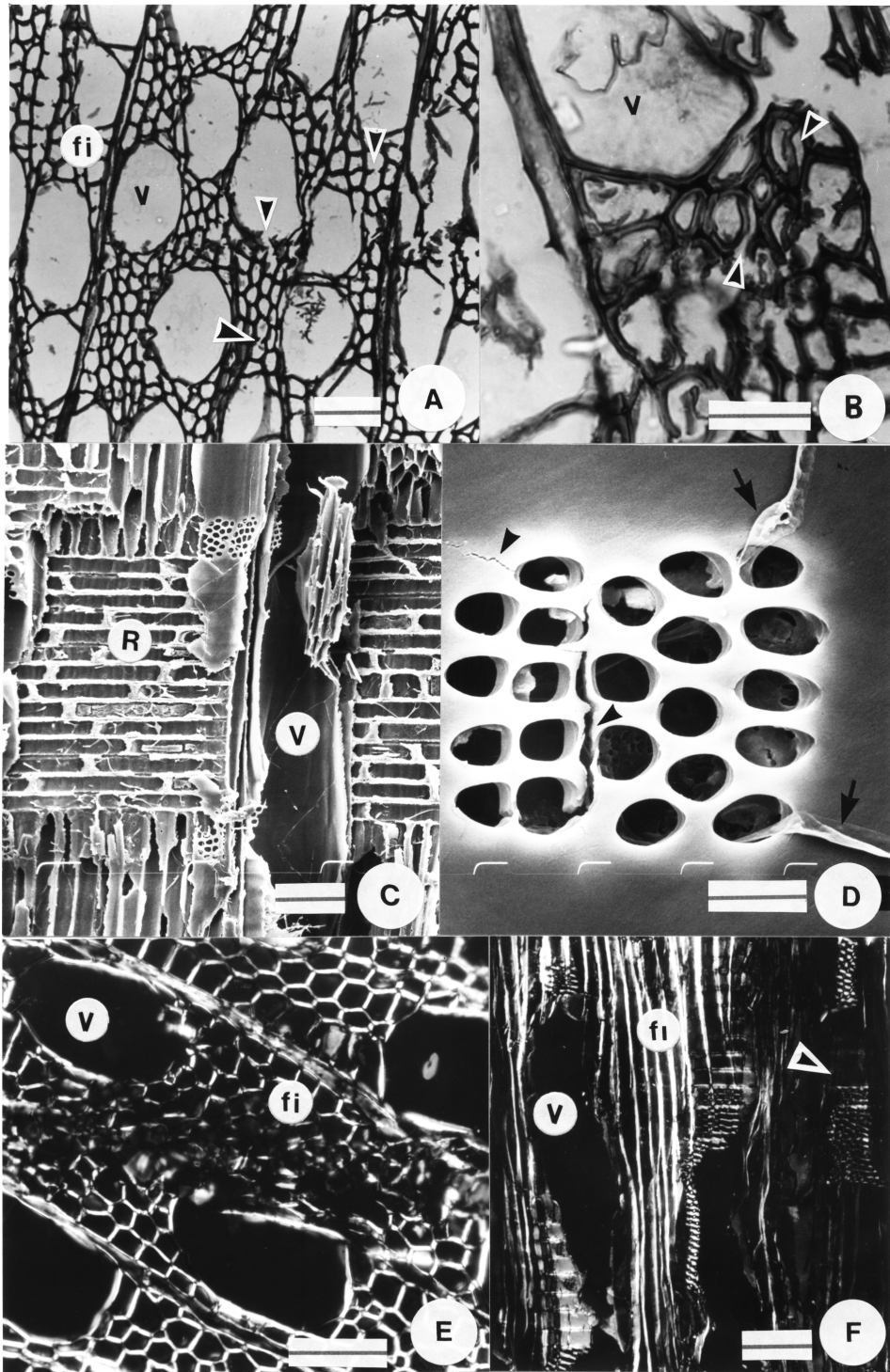
In the present study brown rotted willow wood

showed discoloration, cell wall fractures and loss of birefringence indicating cellulose depolymerization.

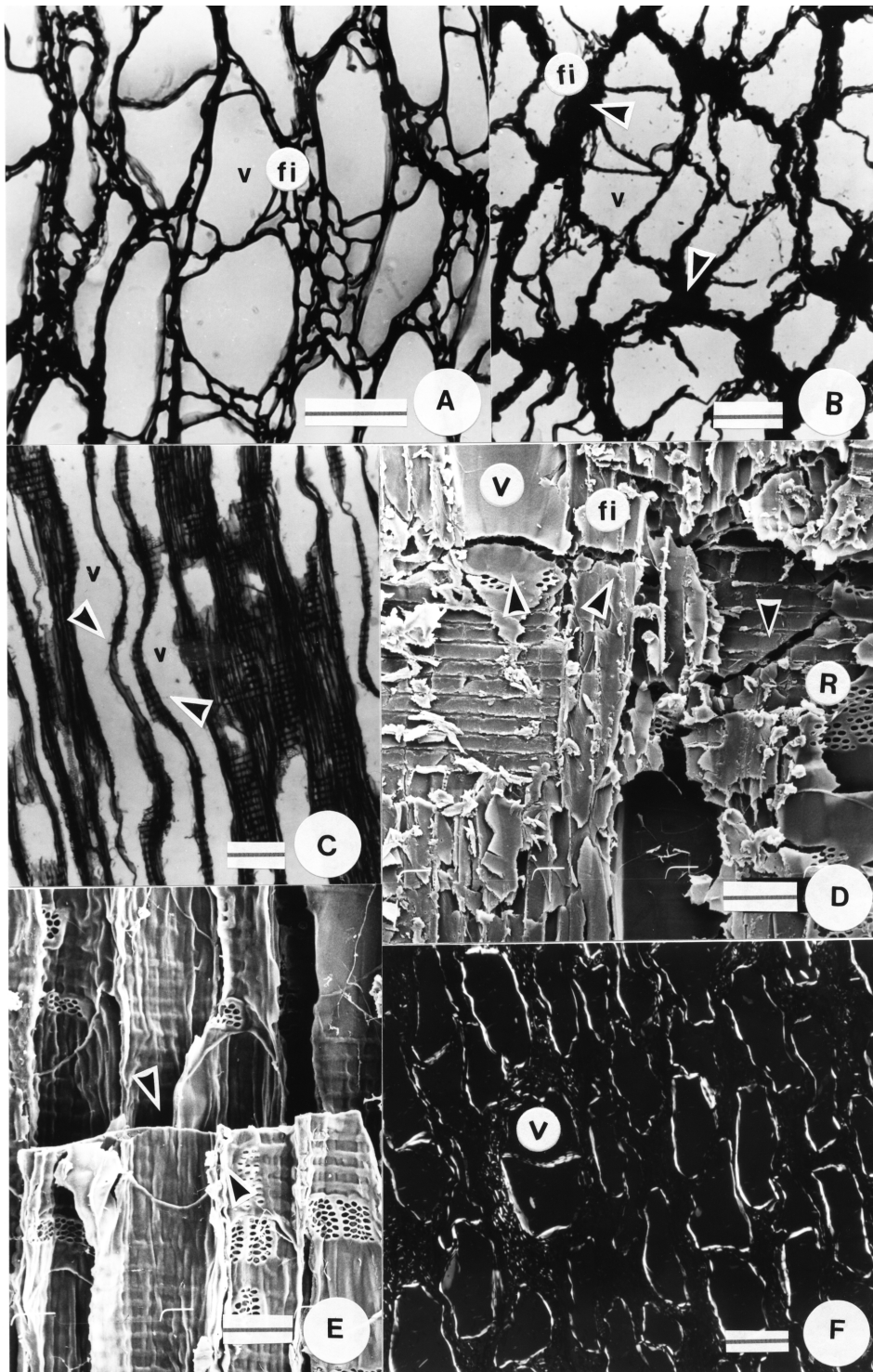
These characteristics produce negative effects on the yield and quality of pulp.



**Fig. 1.** Sound willow wood. **A-D**, SEM micrographs. **A**, Transverse section showing the characteristic cell shape of sound vessels and fibres. Note absence of mycelia and fractures. **B**, Typical fibre walls in detail. **C-D**, Longitudinal sections. **C**, Typical aspect of sound rays. **D**, Typical aspect of sound fibre and vessel walls. Note absence of mycelia and fractures. **E-F**, Polarized light micrographs. **E**, Transverse section showing brightness of vessel and fibre walls. **F**, Longitudinal section showing birefringent ray, fibre and vessel walls. References: v: vessel; fi: fibre; r: parenchyma ray. Bars: **A**, **E-F**= 50  $\mu$ m; **B**= 10  $\mu$ m; **C-D**= 100  $\mu$ m.



**Fig. 2.** Anatomical features of brown rot decay in willow wood at ca. 30 % WL. **A-B**, LM micrographs. **A**, Slight loss of cell shape and transwall fractures in vessels and fibres (arrowheads). **B**, Separation among fibres (arrowheads). **C-D**, SEM micrographs. **C**, Unaltered ray parenchyma cells. **D**, Cracks (arrowheads) and hyphal penetration via pits (arrows). **E-F**, Polarized light micrographs. **E**, Transverse section showing loss of birefringence in fibres. **F**, Longitudinal section. Loss of birefringence in ray parenchyma cells in the right half of the micrograph where degradation occurred (arrowhead). References: v: vessel; fi: fibre; r: parenchyma ray. Bars: **A**, **E-F**= 50  $\mu$ m; **B**= 25  $\mu$ m; **C**= 100  $\mu$ m; **D**= 10  $\mu$ m.



**Fig. 3.** Anatomical features of brown rot decay in willow wood at ca. 60 % WL. A-C, LM micrographs. A, Marked loss of cell shape and cell wall thinning. B, Portion of the sample showing collapse of fibre walls (arrowheads). C, Sinuous run of vessel walls (arrowheads). D-E, SEM micrographs. D, Cracks extended throughout the tissue (arrowheads). E, Cubical pattern of cracks (arrowheads). F, Polarized light micrograph showing birefringence mainly in vessels. References: v: vessel; fi: fibre; r: parenchyma ray. Bars: A-B, D-F= 50  $\mu$ m; C= 100  $\mu$ m.

**Table 1.** Average percent weight loss (WL) during each incubation time

Clone	WL (%) 75 days	WL (%) 150 days
<i>S. 131-25</i>	29.39 a (28.3)	65.72 a (10.5)
<i>S. nigra 4</i>	27.54 a (36.7)	67.54 a (9.04)

\* Different letters represent statistical differences with Tukey at  $p < 0.05$ . Coefficient of variation (CV %) is given between brackets.

## CONCLUSION

The anatomical changes observed in brown rotted *Salix* wood were: transwall fractures, loss of birefringence mainly in fibre walls, loss of cell shape, cell wall thinning and a typical brown discoloration. *C. malicola* attacked predominantly fibre walls, which are employed in paper industry. According to our observations *Salix* wood decayed by *C. malicola* is not appropriate for pulp and paper industry.

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