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PATTERNS OF DECAY CAUSED BY *PYCNOPORUS SANGUINEUS* AND *GANODERMA LUCIDUM* (APHYLLOPHORALES) IN POPLAR WOOD

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SUMMARY

Populus deltoides clones are widely planted in Argentina, in a region called "Paraná River Delta". In this site, *Pycnoporus sanguineus* and *Ganoderma lucidum* (Aphyllophorales) cause white-rot decay in both living and felled poplar trees. The purpose of this work was to estimate, through laboratory decay tests, the ability of both fungi to degrade poplar wood and to describe the patterns of decay using light and scanning electron microscopy. Two exposure times were analyzed: 75 and 150 days. The percent weight loss produced by both fungal strains was similar for both exposure periods (c. 50–60% of wood mass) but microscopic observations showed there were different patterns of decay. Samples inoculated with *P. sanguineus* showed a selective delignification, whereas those inoculated with *G. lucidum* exhibited a combination of simultaneous decay and selective delignification. Separation among cells was the main diagnostic feature for selective decay. By contrast, the presence of erosion troughs, cell wall thinning, bore holes, rounded pit erosion and erosion channels were diagnostic for the simultaneous type of decay.

Key words: *Populus deltoides*, white rot, *Pycnoporus sanguineus*, *Ganoderma lucidum,* simultaneous decay, selective delignification, anatomical characterization.

INTRODUCTION

Many Basidiomycetes are known to cause white-rot decay in hardwoods. These fungi are of interest because they are one of the few groups of microorganisms that can selectively degrade lignin (Otjen & Blanchette 1985). In selectively decayed woods, lignin and hemicelluloses are preferentially removed. Blanchette (1984a) mentioned that fungi that remove lignin selectively without appreciable losses of cellulose are extremely attractive for use in biological pulping processes. Some white rotters can simultaneously degrade all wood components (i.e. lignin, cellulose and hemicellulose)

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(Blanchette & Reid 1986); while some white-rot fungi are capable of both types of decay in the same wood or in different wood species (Blanchette 1984a, b).

Populus species are widely cultivated in Argentina in approximately 123,000 ha of plantations, mainly in the Paraná River Delta area (34° 05' LS; 58° 38' LW) (ASORA 1997). Although many poplar species are grown, *Populus deltoides* clones are the most important in this region. The annual production of poplar species is estimated at approximately 600,000 m^3/year, 70% of which is utilized in the pulp and paper industry and in less proportion for packing cases (SAGPyA 1999). According to Wright and Deschamps (1976, 1976-1977) *Pycnoporus sanguineus* and *Ganoderma lucidum* are among the most important lignivorous fungi that cause white-rot decay in living poplar trees of the Paraná River Delta region, contributing significantly to the loss of quality in the produce.

The damage caused by these fungi on poplar wood as well as the anatomical characterization of their decay have not been investigated. Knowledge of their wood destroying properties would help define more precisely the danger they represent. The purposes of this work are: 1) to employ *in vitro* laboratory decay tests to estimate the ability of *Pycnoporus sanguineus* and *Ganoderma lucidum* in degrading poplar wood, and 2) to describe the patterns of decay produced by both fungi, using light and scanning electron microscopy.

MATERIALS AND METHODS

Sound *Populus deltoides* wood from Paraná River Delta plantations (Buenos Aires Province, Argentina) was employed. Both *Pycnoporus sanguineus* (L. *ex* Fr.) Murr. Bull. Strain 163 (LPSC) and *Ganoderma lucidum* (W. Curt.: Fr.) Karst. Strain 340 (LPSC: Mycologycal Culture Collection of Instituto de Micología Carlos Spegazzini, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata) fungi were used for decay tests. The two separate fungi were grown for two weeks on petri plates containing malt extract-agarose medium prior to inoculating the wood blocks.

For the decay tests, IRAM 9518 (1962) standard with some modifications based on Leutritz technique (1946) was employed. Oven-dried cubic blocks (2 cm side) were weighed to determine the initial weight (IW). Then blocks were introduced in glass bottles with a moistened substrate (sand 166 g, soil 46 g, distilled water 43 cc) and sterilized for 30 minutes at 120 °C. After cooling, the blocks were inoculated with a pure culture of each wood-destroying fungus. Samples were incubated for 75 and 150 days at 27 ± 1 °C and 70% RH. After each incubation period the blocks were removed from the bottles, cleaned to remove mycelia and oven dried for three days until reaching a constant weight, then each was weighed to determine the final weight (FW). The percentages of loss in dry weight due to decay were then calculated. Uninoculated blocks were employed as controls.

For light microscopy studies (LM) decayed material was fixed in formaldehyde-acetic acid-alcohol and embedded in paraffin. Sections (8–12 μm) were double stained with safranin-fast green. 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.

RESULTS

Decay test experiment

Pycnoporus sanguineus and *Ganoderma lucidum* showed a similar aggressive decay ability to degrade poplar wood during the same incubation period (Table 1).

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

Decay fungi	WL (%) 75 days	WL (%) 150 days
Pycnoporus sanguineus	51.85 (± 5.62)	59.05 (± 7.20)
Ganoderma lucidum	52.09 (± 6.15)	58.64 (± 9.18)

Anatomical observations

Decay by Pycnoporus sanguineus

After 75 days of inoculation, a selective delignification occurred (Fig. 1–9). In transverse sections, delignification was easily observed in fibres. Many of them showed a concentric delignification starting from the lumen surface (Fig. 1 & 2). As a result, the secondary walls stained green instead of the compound middle lamellae (ML) and the cell corners stained red with safranin fast-green (Fig. 2). In other fibres adjacent to rays, delignification started from ray cells and then proceeded into the fibre secondary walls (Fig. 3). Consequently, ML appeared partially degraded without a substantial attack of the fibre secondary walls, which stained red.

In longitudinal sections, separation of vessel elements at perforation plates was also observed (Fig. 4).

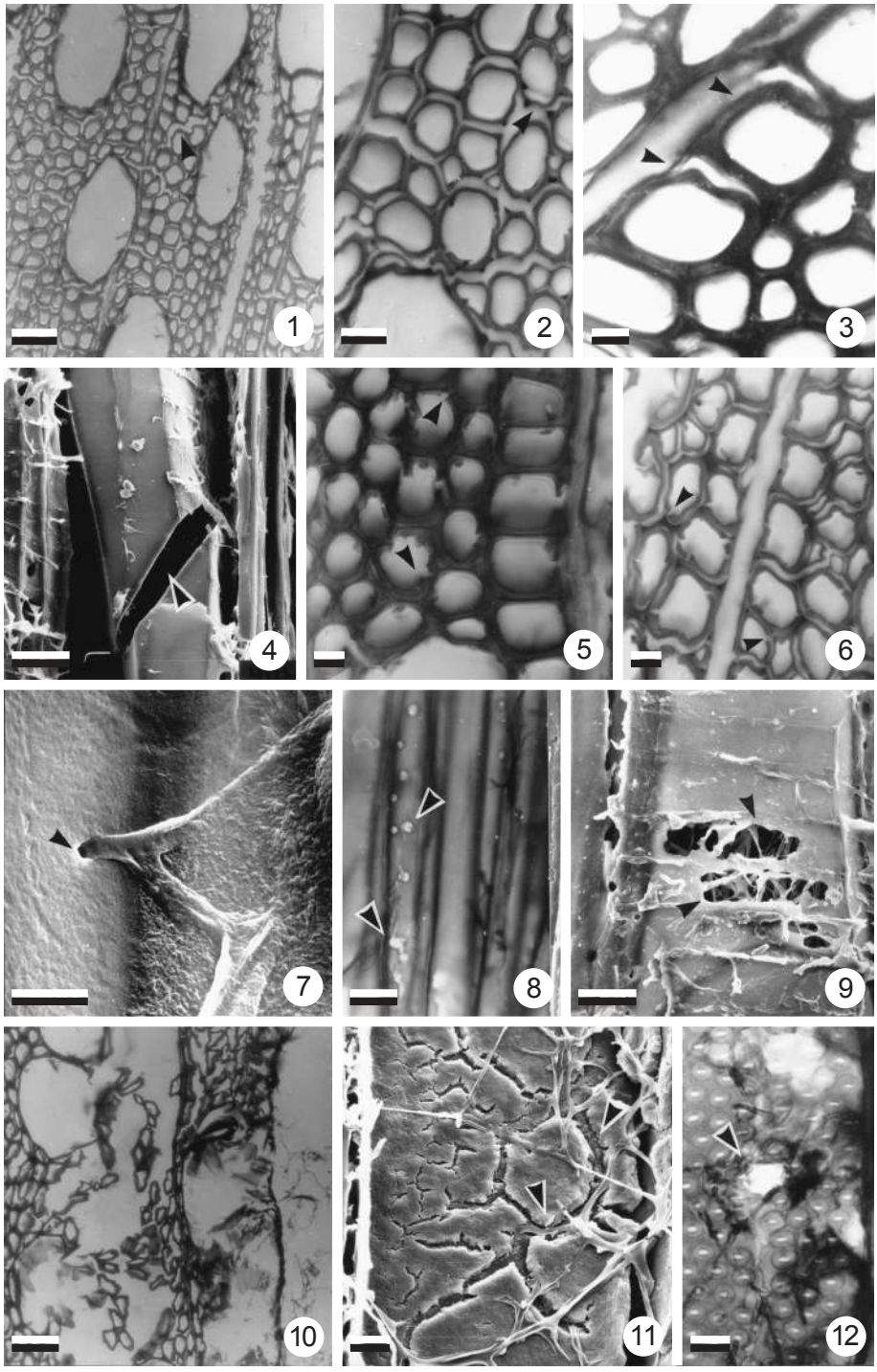
Other signs of decay were detected in these samples. In transverse sections, erosion troughs were observed in some fibres (Fig. 5). The advanced thinning resulted in the localized removal of the cell wall and the ML (Fig. 6). Bore holes were found in fibres and vessel elements (Fig. 7 & 8). In the ray parenchyma cells degradation was so pronounced that they appeared partially, or even completely, disintegrated (Fig. 9).

Samples exposed to fungi for 150 days showed the same patterns of decay. Due to the advanced delignification, cells appeared almost completely detached from each other (Fig. 10) and many cells were deformed or destroyed due to the loss of rigidity of their walls. In vessels, signs such as erosion channels (Fig. 11) or large holes (Fig. 12) were also observed.

Under SEM hyphae were observed within all cell types (i.e. vessel elements, fibres and parenchyma).

Decay by Ganoderma lucidum

After 75 days of inoculation, selective and simultaneous white-rot decay was found in the same sample (Fig. 13–21). In transverse sections, selective delignification was observed in fibres. As previously mentioned, two directions of delignification were identified. In the concentric delignification, secondary walls stained green with safranin fast-



green whereas cell corners and ML with remains of lignin stained red (Fig. 13). In some portions of the samples, separation among cells due to the complete degradation of the ML was pronounced (Fig. 14). In other areas, lignin was first removed from the rays and then the walls of the fibres (Fig. 15). As a result, secondary fibre walls stained red, while the ML appeared partially degraded. Simultaneous decay was shown by general cell-wall thinning and by presence of erosion troughs in fibre walls (Fig. 13 & 16). In some instances, the erosion reached the ML completely removing the cell wall in a localized area (Fig. 16).

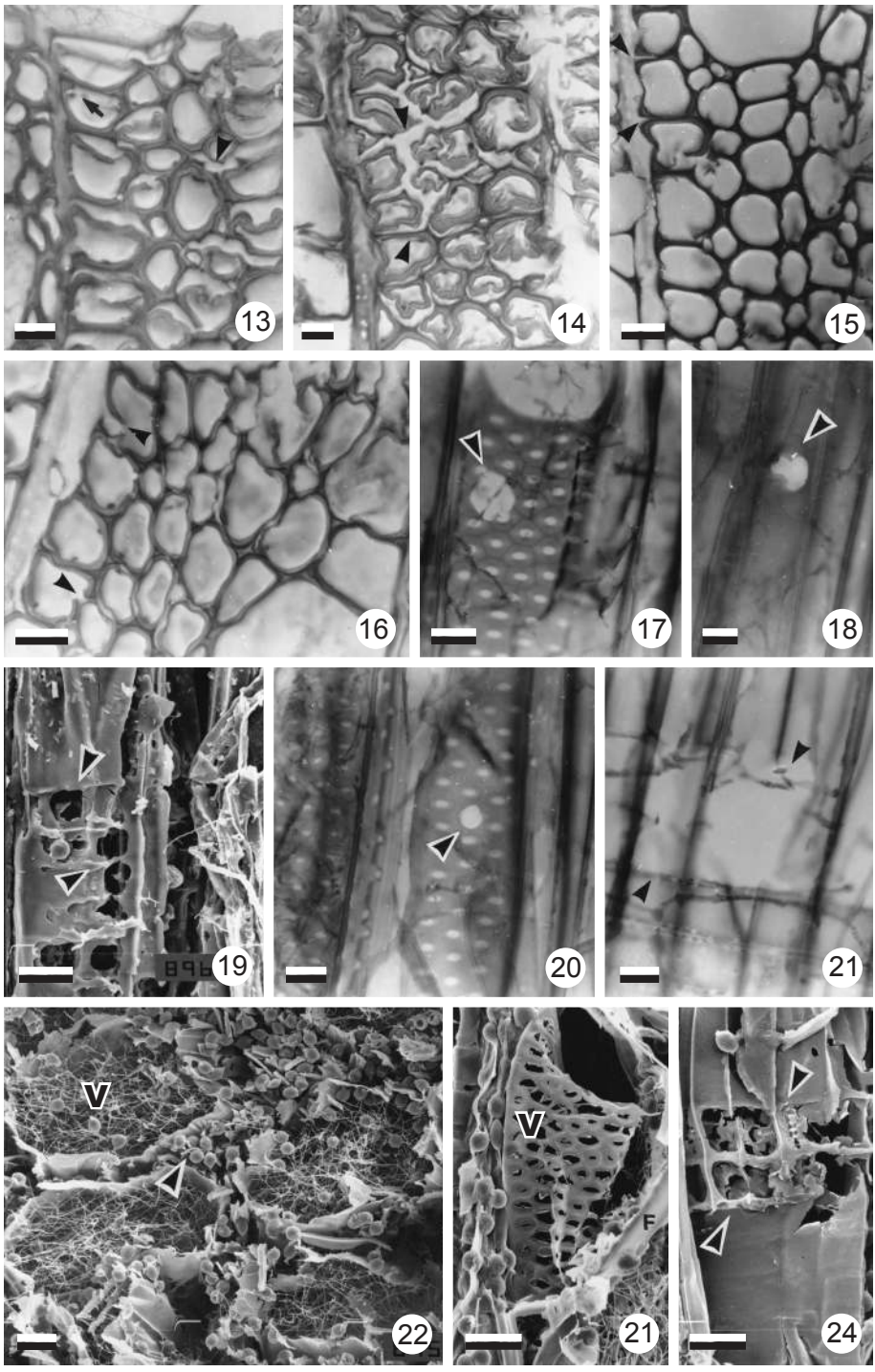
In longitudinal sections, other signs of simultaneous decay, such as large holes or rounded pit erosion, were noticed (Fig. 17–20). Large holes were observed in vessels (Fig. 17), fibres (Fig. 18) and ray parenchyma cells (Fig. 19); rounded pit erosion also was present in vessels (Fig. 20). Rays were less resistant to degradation, so many of them appeared almost completely destroyed (Fig. 21).

After 150 days of inoculation, only a simultaneous type of decay prevailed (Fig. 22–24). As a consequence of the pronounced erosion, only remains of vessels and cell corners were evident in transverse sections (Fig. 22). Abundant chlamydospores were also distributed throughout the tissue. In longitudinal sections, remnants of fibres (Fig. 23) and rays (Fig. 24) could also be detected. Under SEM hyphae were observed mainly in the vessels' lumina.

DISCUSSION AND CONCLUSION

The white-rot fungi *Pycnoporus sanguineus* and *Ganoderma lucidum* produced similar weight losses in *Populus deltoides* wood under laboratory conditions (50–60% of wood mass in 2–5 months). The elevated percentages obtained let us consider them as extremely destructive under favorable temperature and humidity, causing a serious deterioration in the quality of the timber. Although *in vitro* wood-decaying tests cannot be taken as an absolute evidence of the behavior of the xilophagous fungi, they are useful to determine their wood-destroying properties.

Fig. 1–12. Anatomical features of decay by *Pycnoporus sanguineus* in *Populus deltoides* wood 75 (Fig. 1–9) and 150 (Fig. 10–12) days after inoculation showing wood cells with selective delignification (Fig. 1–4 & 10) and simultaneous decay (Fig. 6–9, 11 & 12). – 1: General view in transverse section. Note separation among cells due to the degradation of the ML (arrowhead). – 2: Detail of delignification in a centrifugal direction with remnants of lignin only in cell corners between cells (arrowhead). – 3: Transverse section showing delignification in a centripetal direction (arrowheads). – 4: Separation of vessel elements at the perforation plates (arrowhead). – 5: Erosion troughs in fibre walls (arrowheads). – 6: Interruptions in fibre walls due to the advanced erosion from the lumen surface to the ML (arrowhead). – 7: Minute bore hole in fibre wall (c. 0.9 μm) (arrowhead). – 8: Enlarged bore holes in fibre walls (c. 7 μm) (arrowheads). – 9: Large holes in ray parenchyma cells (arrowhead). – 10: Transverse section showing an advanced selective delignification. Cells appear deformed or collapsed due to the loss of rigidity of their walls. – 11: Erosion channels in vessel wall (arrowhead). – 12: Large holes in vessel wall (arrowhead). — Scale bars in 1 = 50 μm; in 2, 3, 5, 6, 10, 11 = 10 μm; in 4, 8, 9 = 25 μm; in 7 = 5 μm; in 12 = 15 μm.



However, light and electron microscopy shows that *P. sanguineus* and *G. lucidum* caused different patterns of decay. This was proved by the presence of distinctive features and by the staining technique. According to our findings, *P. sanguineus* produced a selective delignification of the tissue, manifested by cell separation. Anagnost (1998) considered this feature as the best indicator of the selective type of decay. The staining technique contributed also to separate the selective delignification from the simultaneous decay, as proposed by Srebotnik and Messner (1994).

In the present work, the completely delignified tissue stained green with safranin fast-green. This suggests that little lignin remained after 150 days of decay. Although *P. sanguineus* produced a selective delignification of the tissue, other signs of degradation, such as bore holes, large holes and erosion channels, were detected. This agrees with Anagnost (1998), who mentioned that fungi which selectively delignify wood can produce anatomical features similar to simultaneous decayers. However, a uniform cell-wall thinning typical of simultaneous decay was not observed.

The presence of hyphae within all cell types and not exclusively in vessels coincided with Wilcox's observations in sweetgum wood decayed by *Polyporus versicolor* (Wilcox 1968).

On the other hand, *G. lucidum* caused a combination of selective delignification and simultaneous decay in the same sample, in agreement with Blanchette (1984 a), who expressed that some white-rot fungi are capable of producing both types of decay in the same wood. Blanchette (1984 b) mentioned that *G. lucidum* causes a simultaneous removal of all cell wall components in *Quercus niger*. In a later paper, the author included *G. lucidum* in a list of the most serious decayers that selectively delignify hardwoods (Blanchette 1991). Adaskaveg *et al*. (1990) revealed that some isolates of *G. lucidum* can remove both lignin and polysaccharides whereas others can produce an extensive delignification. Many factors influence degradation patterns. Furthermore, Kirk and Moore (1972) found that the rate of lignin/carbohydrates removal by fungi varied depending on the wood used as a substrate. According to our findings, it seems that both fungal isolate and the wood species influence the type of decay produced by *G. lucidum*. In this instance, there was a combination of selective delignification and simultaneous degradation.

Fig. 13–24. Anatomical features of decay by *Ganoderma lucidum* in *Populus deltoides* wood 75 (Fig. 13–21) and 150 (Fig. 22–24) days after inoculation with selective delignification (Fig. 13 & 14) and simultaneous decay (Fig. 13 & 15–24). – 13: Centrifugal direction of delignification with remnants of lignin in cell corners and portions of ML (arrowhead) and erosion trough in fibre wall (arrow). – 14: Separation among cells due to the complete degradation of the ML (arrowheads). – 15: Centripetal direction of delignification in fibres adjacent to rays (arrowheads). – 16: Advanced cell wall thinning and cell wall interruptions in fibres (arrowheads). – 17: Large hole in vessel wall (arrowhead). – 18: Large hole in fibre wall (arrowhead). –19: Large holes in ray cells (arrowheads). – 20: Rounded pit erosion in vessel wall (arrowhead). – 21: Complete destruction of ray parenchyma cells (arrowheads). – 22: Vessel remnants (V) with abundant hyphae and chlamydospores (arrowhead). – 23: Portions of vessel (V) and fibre (F) walls. – 24: Remnants of ray parenchyma cells (arrowheads). — Scale bars in 13–16 = 20 μm; in 17, 18, 20, 21 = 15 μm; in 19, 22–24 = 25 μm.

Vessel elements apparently were the most resistant to degradation by both fungi, in agreement with Levin and Castro (1998), who observed the same results for poplar wood decayed by *Trametes trogii*. Blanchette (1988) mentioned various possible reasons for the persistence of the vessel elements in wood degraded by white rot, as larger S_1 and S_3 versus the smaller S_2 layers in their walls, high lignin content and high relative concentration of guaiacyl versus syringyl lignin. Faix *et al*. (1985) stated that white-rot fungi that attack hardwoods more rapidly degrade the syringil units of the lignin than the guayacil units. The increased concentration of guayacil lignin in the vessel walls could explain the vessels' resistance to decay observed in *Populus deltoides*, although a chemical analysis should be done to confirm this hypothesis.

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