Biological Resistance of Eucalyptus camaldulensis Wood to the Decay Fungus, Coniophora puteana

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ABSTRACT

The objective of this study was to evaluate the biological resistance of *Eucalyptus camaldulensis* wood to the decay fungus *Coniophora puteana*. Many aspects of the interactions between host wood structure and fungal activity can be revealed by high resolution light microscopy. Microscopic examination showed the cell wall decay pattern produced by the brown-rot fungus *Coniophora puteana* to be different from the degradation pattern known to be typical for brown-rot fungi in other woods. Results showed *Eucalyptus camaldulensis* wood is very durable to decay. Detailed knowledge of decomposition processes does not only aid prognosis of decay development in living trees for hazard assessment but also allows the identification of resistance woods that can be used for biotechnology processes in the wood industry.

Key words: Brown rot, Decay resistance, Anatomical assay, *Coniophora puteana*, Micro-morphological study, late-wood, *Eucalyptus camaldulensis*.

Introduction

Wood degradation is caused by white-rot and/or brown-rot and soft-rot fungi [28] which are widely distributed in both tropical and temperate environments.

In spite of the white-rot basidiomycetes have received particular attention in industrial processes such as wood pretreatment for paper pulping [14,4]. Brown rot fungi degrading wood as well as white rot fungi.

The wood-inhabiting fungi as well as their colonization and damaging of wood are influenced by various physical/chemical and biological influences. Abiotic factors such as temperature, humidity, aeration, pH and nutrients affect mycelium growth and thus wood decay [28,6]. Aeration is important also; the absence of air results in a high concentration of carbon dioxide, which is toxic and reduces decay. Reduced water availability also affects wood decay, since water is necessary for nutrient absorption, extracellular enzyme activity and maintenance of its integrity.

Elements present in forest and other soils can also be a nutrient source for fungi, enhancing fungal capacity to degrade wood. The wood nitrogen content can be increased by ground contact or by means of translocation through the mycelium. The characterization of the wood is important not only for its correct utilization but also for the market promotion that is necessary for wood derived from plantations. For the Genus *Eucalyptus* the characterization is even more important because there is a great variability in the natural durability of heartwood among different species and also within the same species.

Anatomical and non-anatomical characters are important in this case.

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Changes in pore size and volume resulting from wood decay have not been studied [8] but there is few and therefore further study have been topic in this research.

Brown-rot decay of wood is characterized by rapid and extensive decomposition of cellulose in the initial stage of wood decay. Softwood species are most susceptible to brown rot attack than hardwood species. Brown-rot decay can be easily distinguished under microscopy from other fungal attacks from a combination of features, including loss of birefringence, absence of erosion troughs and cavities and near normal morphological appearance of the degraded wood cells [5]. However, the micro-morphology is variable depending upon host and wood cell wall types attacked and in some situations the cavity formations reminiscent of soft rot attack.

Microscopic studies showed that the S2 layer of tracheids is preferentially degraded whereas the S3 layer and lignin-rich middle lamella remain intact [15]. The structural polysaccharides in the S2 layer are degraded easily at a distance from the hyphae of brown-rot fungi. It has been suggested that brown-rot fungi secrete extracellular degradative agents of low molecular weight, which are capable of penetrating the S3 layer from the cell lumen and diffusing into the S2 layer in the initial stage of fungal attack. Schmitt observed the soft rot-like decay pattern in hardwood produced by C puteana. These modes of degradation are different from the pattern known to be typical for brown-rot decay, and this suggests that some brown-rot fungi can also substantially degrade lignin [15,5].

As part of a larger study of the microscopic studies, an opportunity was created to state the features diagnostic of brown rot and ability of light microscopy to detect the early stages of image [26] such as clamp connections. Features diagnostic for decay fungi in wood, were easily viewed in pervious research in commercial woods in forestry of Iran [16].

Various studies have emphasized the resistance of vessel cell walls to decomposition by white rot fungi. The resistance of vessels to decomposition appears to be related to their high lignin: carbohydrate ratio, lignin monomer composition and cell wall morphology. Earlier studies provide sound evidence that the resistance of parenchyma cells to decomposition by brown rot fungi is not associated with the lignin composition or the total lignin content within parenchyma cells. Thus, it appears that the cell wall morphology rather than the lignin composition or total amount of lignin is responsible for the higher resistance of parenchyma cells to decomposition by brown rot fungi. Interestingly, weight losses from different wood species appeared to correlate with the content of parenchyma cells found in the xylem. fibre tracheids surrounding vessels and xylem ray parenchyma were resistant to decomposition. Brown rot were examined microscopically in wood blocks in some woods by Schwarze et al. [22]. Although all the host/fungus combinations showed similar modes of cell wall degradation, they differed in intensity within various parts of the annual ring [22].

The results obtained in this work, together with other data reported in literature on the mechanical and physical properties may contribute to the characterization and appreciation of this wood species.

Materials and methods

Systematic and Description Eucalyptus camaldulensis:

Family: Myrtaceae

Distribution: Woodland and forests of all mainland states, particularly along watercourses.

Common Name: River red gum

Habitat: The typical growth of this group of trees is for the trunk to grow more or less straight up for about two metres followed by a right-angled bend. It is apparently a response to the local environmental conditions but the habit appears to be genetically fixed

Conservation Status: Not considered to be at risk in the wild.

Characteristic: That leaf extract and essential oil of Eucalyptus spp. have antifungal and antibacterial activites.

General Description:

The river red gum is one of the best known of all eucalypts. It is common in much of semi-arid Australia. It is a medium sized tree usually branching not far above the ground. It may reach 30 - 40 meters in height .the timber is termite resistant and is used in many applications where contact with the ground is needed.

Some studies demonstrated that leaf extract and essential oil of Eucalyptus spp. have antifungal and antibacterial activities [2].

Wood Samples:

Wood samples of approximately 50×20×20 mm were obtained from 6-year old debarked stems of E. camaldulensis from northern forest of Iran.
Fungal Strain and Inoculation:

Pure culture had been already prepared for Coniophora puteana [17] and it was used to inoculate Petri dishes (9.0 cm) containing 20 ml malt extract agar (MEA), which were incubated at 28±1 °C for 7 days. Mycelial discs (5 mm diameter) were then removed from the peripheral growth zone of the mycelium and used to inoculate koller dishes containing 60 ml malt extract agar. Sterile wood samples set in koller dishes. After 16 week incubation in the dark at 27 °C and 80% constant humidity wood blocks were collected, the superficial mycelium removed and the weight loss determined. Wood blocks from uninoculated chambers served as control.

Specific Gravity:

Specific gravity was determined on the basis of oven-dry weight and green volume

Anatomical Characteristics:

A radial strip was divided into 1 × 1 ×1cm blocks from the pith to the bark. Blocks were softened with boiling water and transverse sections 20–3 0 µm in thickness were cut using a sliding microtome. Sections were stained with counter stains [17] and mounted for permanent slides with Canada balsam.

To obtain clear cell boundaries, monochromic photographs were taken through a light microscope at a magnification of 10 and 40.

To measure vessel diameter, vessel area and vessel density by means of an image analyzing system

Fiber diameter and fiber length To determine the fiber length, wood specimens (T × R × L) 2 × 2 × 10 mm were sampled at intervals of 1 cm from the pith to the bark. Fibers were macerated using a mixture of 30% hydrogen peroxide:glacial acetic acid at a ratio of 1:1 at 55-60 °C Macerated fibers were thoroughly mixed and were spread on a glass slide, and 100 unbroken fibers were selected for measurement.

As basic density is a common measure of wood density and is a very important pulpwood parameter, influencing many aspects of the pulp and paper industry including freight costs, chipping properties, pulp yield per unit mass of wood, and paper quality and therefore it is an integral part of tree breeding programs. Density and fiber length are used to evaluate the suitability of a wood for a particular application. Properties such as basic density, fiber length, cell wall thickness, whiteness, and quality of extractives are used by the pulping industry as indicators of wood quality for different industrial processes and final paper products.

Eucalyptus Camaldulensis Properties:

Density and fiber length are important parameters used by the pulping industry as indicators of wood quality for different industrial processes and final paper products.

Natural Durability Against Wood Destroying Fungi:

Natural durability, as used in this study, is the resistance of untreated wood to fungal decay

Results and discussion

The biological durability of wood is one of the most important properties of this building material. The characterization of this property is of great importance in appropriate material selection; both to avoid unnecessary spending caused by the need to replace damaged parts and to reduce the impacts on remaining natural forests.

Brown-rot fungi colonize the wood via the rays and spread in the longitudinal tissue through pits and by means of micro-hyphae. They grow inside the cell lumina and there in close contact with the tertiary wall.

Typically, brown-rot fungi do not cause lysis zones around their hyphae, while this is characteristic of many white-rot fungi.

Results for each tested blocks and test fungus are summarized in Table 1, where the most significant data for the classification of natural durability are reported the durability index (DI), Although wood blocks were strongly colonized by hyphae of all brown rot fungi after six and twelve week’s incubation, C. puteana even caused a slight increase in dry weight due to the large amount of mycelium within the wood .in other hand difference in fibre efficiency( %) were little (Table2)

The heartwood of a species is usually much more resistant to fungal attack in service than the sapwood. As decay resistance is often correlated with the content of fungicidal compounds (inhibitory phenols and terpenes that accumulate during the transition of sapwood into heartwood). In study of Sabrina etals in [19] durability is also affected by variation within species. also Scheffer et al. [20] concluded that use of white oak will provide superior decay resistance in general, but some individual trees of this species may have only moderate resistance. Furthermore, they reported variability in decay resistance with locality, tree size, and position of the wood in the tree.

Variation in durability within a species or individual tree can also be due to different stages of the decay process, prior to visible changes in the wood.
Wood tissues are known to vary in the composition of their cell walls, the variability being greater and more complex in hardwood species as compared to softwood species. It is not therefore surprising that the extent of cell wall degradation depending upon the type and content of lignin and the presence, abundance, composition and location of extractives within wood cells, high lignin content of these structures has been attributed to be the main reason for their resistance.

Generally, it applies to wood fungi: The minimum for wood decay is near the fiber saturation point of about 30% u, however, commonly slightly above this range because only then there is free water in the lumen void space. Some rot fungi, however, could colonize wood in laboratory culture, whose moisture was significantly below fiber saturation (minimum 18% ) before the mycelium contacts the woody substrate, because these fungi transported water from a moisture source by means of mycelium [28]. The optimum moisture for Coniophora puteana were 3 0–70%

Wood fungi are inhibited as the cellular spaces of wood become fully saturated with water. The maximum wood moisture content allowing fungal growth is determined by the minimum air content within the wood cell. cellulose, hemicellulose, and lignin are as macromolecules too large to be taken up into the hypha. for see these microstructure we studied qualitative and quantitative characters by a light microscope. Vessels were observed using transverse section at × 40 magnifications whereas rays and its structure and perforation plate were viewed using tangential longitudinal sections at × 40 or × 100 and perforation plate were viewed using radial section.

Fig. 1: Transverse section. exclusively solitary vessel. Vessel outline rounded.
Fig. 2: Radial section. vessels elements in multi-sectioned form.
Fig. 3: Tangential section. Homocellular ray cells procumbent. Eucalyptus camaldulensis-not decay.

Fig. 4-6: Transverse and Radial section of decayed wood of Eucalyptus camaldulensis. vessel elements with multi sectioned parts.

Fig. 7-8: Tangential section of E. camaldulensis. 1-2 serrate rays.
Table 1: Mass loss in Eucalyptus camaldulensis after 16 week incubation at 27°C in dark condition

<table>
<thead>
<tr>
<th>N</th>
<th>M1</th>
<th>M2</th>
<th>M1-M2</th>
<th>M1-M2, % (M1) classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17.595</td>
<td>17.283</td>
<td>0.312</td>
<td>1.7 D1</td>
</tr>
<tr>
<td>2</td>
<td>15.016</td>
<td>14.445</td>
<td>0.571</td>
<td>3.8 D1</td>
</tr>
<tr>
<td>3</td>
<td>16.547</td>
<td>16.193</td>
<td>0.354</td>
<td>2.1 D1</td>
</tr>
<tr>
<td>4</td>
<td>18.239</td>
<td>17.8</td>
<td>0.439</td>
<td>2.4 D1</td>
</tr>
<tr>
<td>5</td>
<td>20.052</td>
<td>19.647</td>
<td>0.405</td>
<td>2.0 D1</td>
</tr>
<tr>
<td>6</td>
<td>18.403</td>
<td>18.093</td>
<td>0.31</td>
<td>1.7 D1</td>
</tr>
<tr>
<td>7</td>
<td>16.919</td>
<td>16.538</td>
<td>0.381</td>
<td>2.2 D1</td>
</tr>
<tr>
<td>8</td>
<td>15.255</td>
<td>14.925</td>
<td>0.33</td>
<td>2.2 D1</td>
</tr>
<tr>
<td>9</td>
<td>17.639</td>
<td>17.267</td>
<td>0.372</td>
<td>2.1 D1</td>
</tr>
<tr>
<td>10</td>
<td>18.256</td>
<td>17.848</td>
<td>0.405</td>
<td>2.6 D1</td>
</tr>
<tr>
<td>11</td>
<td>17.201</td>
<td>16.765</td>
<td>0.436</td>
<td>2.5 D1</td>
</tr>
<tr>
<td>12</td>
<td>14.088</td>
<td>13.767</td>
<td>0.321</td>
<td>2.3 D1</td>
</tr>
<tr>
<td>13</td>
<td>18.09</td>
<td>17.66</td>
<td>0.43</td>
<td>2.4 D1</td>
</tr>
<tr>
<td>14</td>
<td>19.258</td>
<td>18.903</td>
<td>0.382</td>
<td>2.0 D1</td>
</tr>
<tr>
<td>15</td>
<td>18.09</td>
<td>17.66</td>
<td>0.43</td>
<td>2.4 D1</td>
</tr>
</tbody>
</table>

Where m1 = initial oven dry mass and m2 = final dry mass with significant difference at p<0.05.

Table 2: Fibre efficiency in before and after incubation.

<table>
<thead>
<tr>
<th></th>
<th>before decay</th>
<th>after decay</th>
<th>fibre efficiency%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.856</td>
<td>1.513</td>
<td>53%</td>
</tr>
<tr>
<td>Treatment</td>
<td>1.795</td>
<td>0.825</td>
<td>46%</td>
</tr>
</tbody>
</table>

Figs.4-6 show the presence of little hyphae in vessel. Transverse section of Eucalyptus camaldulensis wood showing several vented pit regions (1,4). The vessels are intact their remnants (arrows) can be seen in the lumen and the degraded parts of the vessel wall. Cited that the same kinds of woods do not necessarily possess the same class of resistance across all decay fungi types.

The xylem rays of eucalypts are almost exclusively 1-3-seriate (3,7,8) but may reach 5 cells wide in rare specimens. They are homo-cellular or slightly hetero-cellular with 1-3 marginal rows of upright cells.

In Eucalyptus sp, large multi-serrate rays from 5 to 25 cells wide are described. These rays are homo-cellular or hetero-cellular with occasional upright cells interspersed amongst the procumbent cells. The rays are very irregular in shape, often appearing to be conglomerates with several bi- or tri-serrate tails at each end. Aggregate rays, involving obliquely oriented fibres as well as irregular areas of ray parenchyma 40-50 cells wide, are also present. Such aggregate rays are up to 20 mm or more in height and up to 3 mm wide. The size of the ray parenchyma cells in the abnormal rays is more or less the same as in normal rays except for occasional larger cells.

The large rays are associated with prominent dimples in the outer tangential surface of the wood.

Fink investigated the formation of abnormally large xylem rays in both hardwoods and softwoods and found that these structures were associated with adventitious root primordia. In the two species examined in the present report, the morphology of the abnormal rays is distinctly different, even though these species are closely related and their normal wood anatomy is similar.

The results obtained in this work, together with other data reported in literature on the mechanical and physical properties may contribute to the characterization and application of this wood species. Further research using different wood tree species and systems is required in order to provide further information about wood decay by this species. Optimizing parameters of our methodology may enable later standardization of this technique as a quantitative measurement, more sensitive than weight loss, of basidiomycete decay of untreated wood.

Further studies are required to determine the extent of the relationship between strength loss, weight loss, and fibre efficiency (Table 2) and hemicellulose composition [24]. Durability is also affected by variation within species. For example, Scheffer et al. [20] concluded that use of white oak will provide superior decay resistance in general, but some individual trees of this species may have only moderate resistance. Furthermore, they reported variability in decay resistance with locality, tree size, and position of the wood in the tree. Variation in durability within a species or individual tree can also be due to different stages of the decay process, prior to visible changes in the wood.

References

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