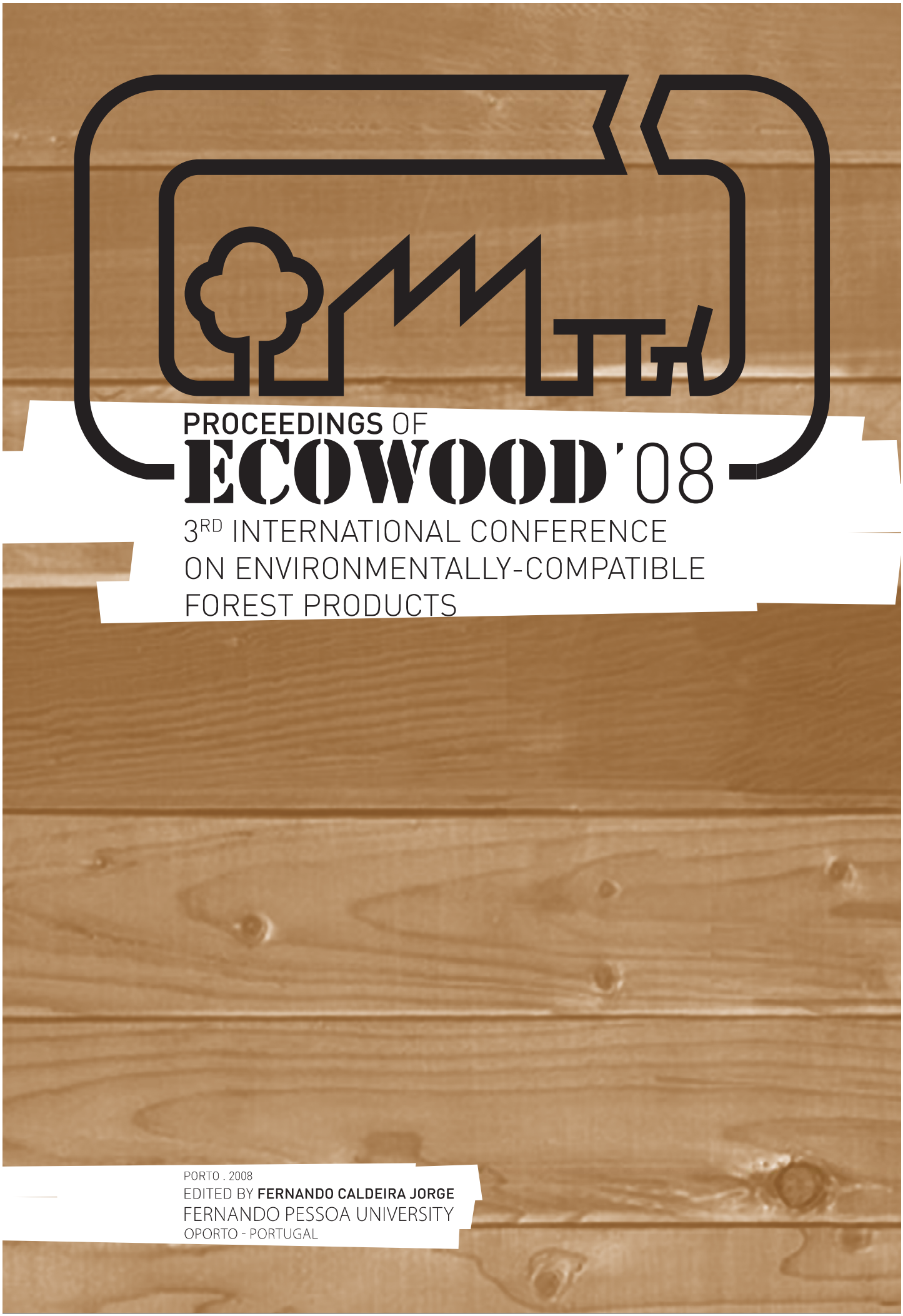

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KNOWLEDGE OF THE POTENTIAL OF LIMONENE AS AGENT TO PRESERVE THE *PINUS PINASTER* WOOD

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SUMMARY

Pinus pinaster wood is very susceptible to fungal degradation and that could be a great problem for its commercialization. In this work it was tested the capacity of limonene to prevent fungal development of two degradative fungi, *Trametes versicolor* and *Trichoderma citrinoviride,* on *Pinus pinaster* wood.Wood samples were subjected to two treatments: i) impregnation with limonene from *Pinus pinea* (stone pine) or distilled water; ii) autoclave sterilization process or non sterilization. The assessment of fungal growth was done by image analyses after 29 days of incubation under laboratorial conditions and the results were expressed as growth area. The results showed that limonene impregnation significantly reduces fungal development independently of the application of autoclave sterilization process.

Keywords: limonene, fungal biodegradation, *Trametes versicolor*, *Trichoderma citrinoviride*, wood preservation.

INTRODUCTION

Wood is composed by cellulose (40-50%), hemicelluloses (25-40%), lignin (20-35%) and other structural components as terpene, phenol, alkaloids and tannins (Sjostrom, 1993). Wood decay fungi can cause important economic lost. Depending on wood components attacked during decomposition process wood decay fungi can be split up in several groups: blue-staining, soft-rots, white rots and brown-rots (Knapp, 1985). Many fungi digest only one of the components of wood leaving the others behind. A lignin-digesting fungus leaves the white cellulose behind; this is called a white-rot. Brown-rot fungi are the most destructive ones primarily causing a rapid despolymerization of the cellulose and hemicellulose components of wood but shortly removing the lignin (Chang *et al.,* 1981).

Trametes versicolor (L.) Lloyd is a frequent white-rot fungus known for its ability to completely degrade wood stumps (Cabral *et al.,* 1985, 1987).

The genera *Trichoderma* include species with different behaviours from endophytic to plant pathogenic fungi (Samuels, 1996). The species *Trichoderma citrinoviride* Bissett can be considered a cellulolytic fungus as it shows more capability to degrade cellulose and hemicelluloses than lignins (Henriques, 2007).

Limonene is a natural hydrocarbon, cyclical, unsaturated compound and belongs to the terpene group. It occurs naturally in certain trees and shrubs, being the largest constituent in several essential oils, as pine trees and citrus fruit. It's used as a solvent, in rubber industries, fragrance and food (Derfer and Traynor, 1989). Its antiseptic proprieties made it a good choice as a natural wood preserver.

The aim of this work was to obtain a first evaluation of the capacity of limonene to prevent the development of *T. versicolor* and *T. citrinoviride* on pinewood.

MATERIALS AND METHODS

Dry pinewood samples (2 x 2 x 1 cm), cut in radial section, were impregnated with limonene or distilled water and stabilised during 24 hours at room temperature.

Two degradative fungi were tested: *Trametes versicolor* TR489 obtained from fungal fructification on eucalyptus wood and *Trichoderma citrinoviride* B2 isolated from *Platypus cylindrus* Fab. (Coleoptera: Platypodidae) galleries on cork-oak, both cultures from collection of Unidade de Protecção de Plantas/INRB, Oeiras. Cultures were maintained on PDA medium (potato dextrose agar) at 25ºC in the dark.

Polypropylene MAGENTA® vessels (GA7, Sigma-Aldrich 77 × 77 × 97 mm) filled with 10 ml glass beads (5 mm diameter) and 10 ml of distilled water were prepared. All the materials were sterilized in autoclave during 20 minutes at 121ºC and 1 bar

pressure. Wood samples were deposed over the glass beads surface and inoculated with fungal plugs (l6 mm diameter) collected from actively growing colonies. Boxes were sealed with PARAFILM® and maintained at 25ºC in the dark during 29 days. Moisture level was verified regularly.

Experimental design was factorial (2 x 2 x 3 x 5) with two impregnation treatments (limonene/distilled water), two wood sterilization treatments (autoclave sterilized wood/unsterilized wood), three fungal treatments (*T. versicolor* TR 489/*T. citrinoviride* B2/control without fungi) and 5 repetitions.

The fungi development was assessed with an image analysis system using a digital camera with 6Mega Pixel and software COGNEX VISION PRO4 CR(2). Data were subjected to variance analysis (ANOVA), multiple comparing test and least significant difference with programme STATISTICA® version 6.0.

When fungi contaminants were detected isolation was attempted by transferring wood samples to PDA medium. Isolations were incubated at 25ºC in the dark during 15 days or until fungi development. Observation under microscope permits to identify some genera (Barnett and Hunter, 1988; Halin, 1997).

RESULTS AND DISCUSSION

The results of a first ANOVA for all factor involved in this study determine that 60% of the total variance observed can be attributed to differences in behaviour of the two fungi tested. So, for better analysis of other factors we present the results of fungi development individually (Figures 1 and 2).

In global, after 29 days of incubation both fungi showed a reduction on development in wood impregnated with limonene.

This reduction is especially evident with *T. versicolor* (F=5.55, P<0.05) in unsterilized wood (Figure 1). This fungus also presents a great dependence on the autoclaving treatment (explaining 44% of the total variance) with a significant reduction on development in autoclave sterilized wood (Figure 1). Interaction between autoclave sterilization and limonene impregnation is highly significant (F=16.2, P<0.001). The strong negative effect observed with the autoclave sterilization of the wood suggests that heat could transform some of the wood constituents in an unfavourable way to this particular species.

T. citrinoviride presents a significant reduction on development in wood impregnated with limonene in both sterilized and unsterilized wood (F=12.03, P<0.01) (Figure 2). The autoclaving treatment present also significant differences (F=13.39, P<0.01) but contrarily to *T. versicolor* wood sterilization increase fungus development. Although some *Trichoderma* species are also known for their antagonistic abilities in soil (Cox *et al*. 2001) the species tested in this study didn't showed capability to compete with fungi present in wood.

Development of contaminant fungi from *Trichoderma* genus were observed on control treatment (no inoculated) not subjected to autoclave sterilization.

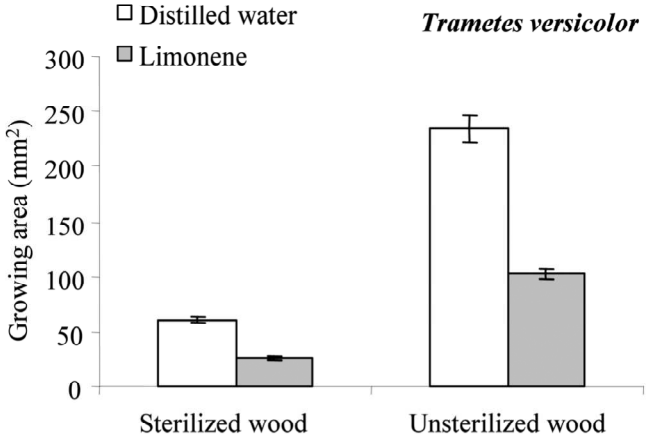

Figure 1: Growing area of ***Trametes versicolor*** in wood with two impregnation treatments (limonene or distilled water) and two sterilisation treatments (sterilized/unsterilized). Bars represent standard deviation of the mean (n=5).

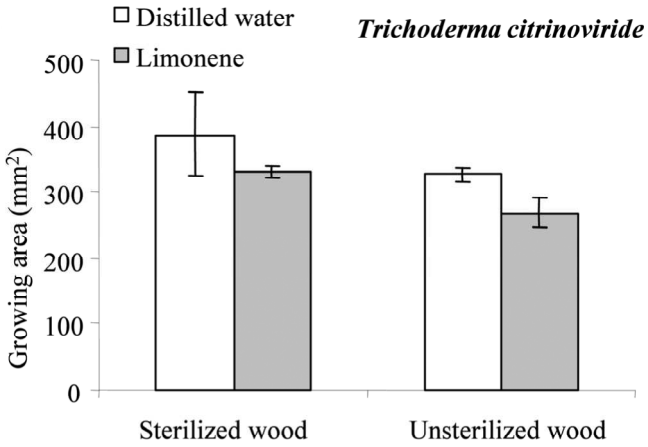

Figure 2: Growing area of *Trichoderma citrinoviride* in wood with two impregnation treatments (limonene or distilled water) and two sterilisation treatments (sterilized/unsterilized). Bars represent standard deviation of the mean (n=5).

CONCLUSIONS

Wood impregnation with limonene can increase its durability preventing the development of wood decay fungi.

The autoclaving treatment was introduced in this experiment to reduce endophytic contamination with other fungi species. Depending on the species inoculated the response to autoclave sterilization of the wood was opposite suggesting that some wood constituents could suffer a transformation caused by heat that decreases the development of certain fungus.

Although some *Trichoderma* species are also known for their antagonistic abilities in soil (Cox *et al*. 2001) the species tested in this study didn't showed capability to compete with endophytic fungi present in wood.

The results show that the limonene could be a good wood preserver product with an additional advantage of its non toxicity for the human health.

Future research should focus on mechanical behaviour of the wood treated with limonene and on the study of its durability.

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