SCIENTIFIC REPORT OF THE STSM

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HOST INSTITUTION: CNR-IVALSA, Preservation and Biodegradation Laboratory, Via Madonna del Piano 10, Sesto Fiorentino 50019 FLORENCE (Italy).

WORK TITLE:
"Evaluation of fungal effectiveness of wood treated through a polyphenol enzymatic grafting"

DURATION: 3/02/15-12/02/15

1. PURPOSE OF THE STSM

This STSM represents the continuation of the work done by the applicant in the CNR-IVALSA from 15/09/14 to 15/12/14 in the framework of her doctoral thesis. During the mentioned period, extracts from different tree specimens were obtained and their biocidal activity was assesed through *in vitro* assays. From these preliminary studies, the extracts of the following species were selected in order to develope the grafting:

1. *Pinus pinaster*
2. *Pinus radiata*
3. *Japanese cedar*
4. *Eucalyptus globulus*
5. *Sequoia sempervirens*
6. *Pawlonia tomentosa*

Among a huge variety of compounds, these extracts are supposed to contain phenolic compounds. The purpose of this STSM is to carry out the grafting of these compounds onto real wood samples and, finally, to assess if the treatment is efficacy against funghi attack by means of a modified EN113 standard methodology.

2. DESCRIPTION OF THE WORK CARRIED OUT DURING THE STSM

The durability test were done only with the fungus *Coniophora puteana*, as it has been seen that has been the most sensible to these extracts in the previous screening plate tests.The duration of the acelerated durability tests was 8 weeks (instead of 16 weeks in the EN 113).

First, agar plates of malt extract were inoculated with the fungus and left to growth in an environmental chamber.

Secondly, mini-blocks of a non-durable specie (*P. sylvestris*) were impregnated with the extracts and the enzyme laccase (so that grafting of the target compounds could be achieved) according to the EN113 standard. In this treatment, laccase acts as a catalyzer and also bonds covalently the target compounds to the hydroxyls groups of lignin in wood. After impregnation, mini-blocks were dried at room temperatura under ventilation cabinet for

18 hours, and finally put in the owen , at 103ºC for 18 h. Figure 1 shows the different colors of the Wood mini-blocks after grafting treatment.

Figure 1. Mini-blocks after grafting.

Mini-blocks were kept under 20ºC and 65% humidity and submitted to tyndallisation prior to fungi contact.

Finally, mini blocks were put into contact with the fungi (Figure 2) in order to start the mini-block accelerated test.

Figure 2. Mini-blocks accelerated test.

3. DESCRIPTION OF THE MAIN RESULTS OBTAINED

The impregnation process was characterized through the determination of Weight Percent Gain (WPG1) referred to the anhydrous mass of untreated wood.

$$WPG1\ (\%) = (M_t - M_0)/ M_0$$

where M_t is oven-dry weight of treated wood, and

M_0 is oven-dry weight of untreated wood

Results are reflected in Table 1. Ethanolic extracts grafted with laccase have shown a higher gain in comparision with toluene extracts.

Table 1. WPG1 of the impregnated mini-blocks.

Sample Description	Dried weight (g)	Dried weight after impregnation(g)	WPG1 (%)	WPG1 mean (%)
CONTROL BUFFER	0,702	0,703	0,14	0,16
	0,813	0,813	0,00	
	0,698	0,694	-0,57	
	0,786	0,787	0,13	
	0,691	0,688	-0,43	
	0,75	0,748	-0,27	
	0,713	0,728	2,10	
CONTROL TOLUENE	0,661	0,676	2,27	1,85
	0,525	0,539	2,67	
	0,619	0,633	2,26	
	0,797	0,808	1,38	
	0,901	0,909	0,89	
	0,706	0,719	1,84	
	0,849	0,863	1,65	
CONTROL TOLUENE + ENZYME	0,724	0,747	3,18	2,92
	0,75	0,773	3,07	
	0,793	0,815	2,77	
	0,668	0,69	3,29	
	0,726	0,736	1,38	
	0,725	0,744	2,62	
	0,602	0,627	4,15	
TOLUENE EXTRACT PINUS PINASTER	0,751	0,778	3,60	3,51
	0,733	0,758	3,41	
	0,617	0,645	4,54	
	0,735	0,76	3,40	
	0,618	0,643	4,05	
	0,781	0,794	1,66	
	0,689	0,716	3,92	
TOLUENE EXTRACT	0,7	0,73	4,29	3,77
	0,807	0,834	3,35	

PINUS RADIATA	0,694	0,717	3,31	
	0,801	0,825	3,00	
	0,732	0,759	3,69	
	0,663	0,689	3,92	
	0,619	0,649	4,85	
TOLUENE EXTRACT JAPANESE CEDAR	0,749	0,772	3,07	3,31
	0,742	0,764	2,96	
	0,724	0,748	3,31	
	0,687	0,713	3,78	
	0,7	0,724	3,43	
	0,722	0,747	3,46	
	0,728	0,751	3,16	
TOLUENE EXTRACT EUCALYPTUS GLOBULUS	0,65	0,677	4,15	2,83
	0,616	0,636	3,25	
	0,773	0,791	2,33	
	0,807	0,823	1,98	
	0,741	0,76	2,56	
	0,759	0,778	2,50	
	0,705	0,726	2,98	
	0,697	0,717	2,87	
CONTROL ETHANOL	0,736	0,746	1,36	1,40
	0,652	0,662	1,53	
	0,828	0,835	0,85	
	0,608	0,617	1,48	
	0,795	0,803	1,01	
	0,601	0,61	1,50	
	0,627	0,638	1,75	
	0,64	0,651	1,72	
CONTROL ETHANOL + ENZYME	0,724	0,739	2,07	3,05
	0,736	0,752	2,17	
	0,579	0,594	2,59	
	0,703	0,728	3,56	
	0,624	0,647	3,69	
	0,653	0,678	3,83	
	0,686	0,71	3,50	
	0,661	0,681	3,03	
ETHANOLIC EXTRACT OF PINUS PINASTER	0,703	0,737	4,84	5,20
	0,614	0,659	7,33	
	0,717	0,755	5,30	
	0,697	0,731	4,88	
	0,654	0,687	5,05	
	0,738	0,772	4,61	
	0,75	0,783	4,40	
ETHANOLIC EXTRACT OF PINUS RADIATA	0,734	0,763	3,95	4,44
	0,759	0,791	4,22	
	0,658	0,699	6,23	
	0,748	0,778	4,01	
	0,676	0,707	4,59	
	0,783	0,812	3,70	
	0,712	0,743	4,35	
ETHANOLIC EXTRACT OF	0,65	0,684	5,23	4,97
	0,765	0,799	4,44	

JAPANESE CEDAR	0,788	0,822	4,31	
	0,73	0,764	4,66	
	0,715	0,751	5,03	
	0,635	0,672	5,83	
	0,703	0,74	5,26	
ETHANOLIC EXTRAT OF EUCALYPTUS GLOBULIUS	0,783	0,805	2,81	3,18
	0,734	0,758	3,27	
	0,672	0,693	3,12	
	0,727	0,751	3,30	
	0,746	0,769	3,08	
	0,578	0,601	3,98	
	0,849	0,872	2,71	
ETHANOLIC EXTRACT OF SEQUOIA SEMPERVIRENS	0,782	0,829	6,01	6,43
	0,704	0,749	6,39	
	0,704	0,752	6,82	
	0,694	0,742	6,92	
	0,76	0,804	5,79	
	0,746	0,791	6,03	
	0,636	0,684	7,55	
	0,712	0,754	5,90	
ETHANOLIC EXTRACT OF PAWLONIA TOMENTOSA	0,691	0,722	4,49	4,14
	0,807	0,839	3,97	
	0,757	0,786	3,83	
	0,78	0,807	3,46	
	0,901	0,928	3,00	
	0,633	0,667	5,37	
	0,604	0,631	4,47	
	0,7	0,732	4,57	

After 8 weeks, probes should be taken off and measurement of the weight loss will be evaluated in order to evaluate the mass loss of the probes and, consequently, the efficency of the grafting treatment. For this reason, there will be no results of mass loss until the end of the experiment (which will be finished the 12 of april of 2015).

4. FUTURE COLLABORATION WITH THE HOST INSTITUTION

When the experiment is finished, the host institution will be the responsible of measuring the weight loss. Then, future collaboration wiht the host institution is required, as it would be necessary to analyse the final results.

5. FORESEEN PUBLICATIONS/ARTICLES RESULTING FROM THE STSM

Once the results of the durability assays are obtained, if they are of scientific interest, a publication in a journal of the Science Citation Index is desired. Consequently, a future collaboration between the two institutions is inititiated right now.

6. CONFIRMATION BY THE HOST INSTITUTION OF THE SUCCESSFUL EXECUTION OF THE STSM

The letter of the Host Insitution is the annex 1.

7. OTHER COMMENTS