WOODWORM DISINFESTATION OF WOODEN ARTIFACTS BY VACUUM TECHNIQUES

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1. Introduction

Any wood artifact is an ideal incubator for xylophagous insects. They can survive in wood for many biological cycles, involving egg deposition, larval worm development and finally, the transformation of the worms into insects. During the larval stage, the worm uses the cellulose contained in the wood to nourish itself and as a consequence bores deep tunnels through the wooden artifact, destroying the mechanical performance of the material. This problem, in the large majority of conservative interventions, is solved by exposing the artifact to chemicals which, in the case of accidental spillage, can be dangerous for workers performing conservation operations. These methodologies have the further inconvenience of not being very effective in destroying the insects' eggs.

Methods used for woodworm disinfestations can be classified into two major categories: chemical and non-chemical. A detailed comparison of the most widely used methods for controlling woodworm attacks can be found in the works of Lewis and Haverty [1,2]. Chemical products that must be directly applied to the wood, include pyrethrum aerosol and pyrethroid aerosols and liquids (cyluthrin, permethrin, bifenthrin) liquid imidacloprid, liquid nitrogen, and liquid and powder formulations of disodium octaborate tetrahydrate [3].

All these substances have to be carefully handled by trained operators for the reasons mentioned above. As a result, there is growing interest in non-chemical insect control. Non-chemical treatments for wood disinfestation include heat (lethal levels - above 53 ºC- for at least 33 minutes - are used on the wooden artifacts ensuring the death of the parasites [2]; this method however, can damage the wooden artifacts), electrocution (based on high voltage-low current devices) with effectiveness as low as 44% [2] and microwave (MW) irradiations [4].

This paper describes a new technique of disinfestation based on the possibility of producing irreversible damage to worms by creating vacuum conditions around the artifact. The aim of this study therefore was to find a useful protocol for worm disinfestation by vacuum and more precisely, to find the minimum vacuum treatment time for complete disinfestation of walnut wood (*Liquidambar styraciflua*) from *Hylotrupes bajolus* larvae.

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To our knowledge, no experiments have been made regarding this argument. Today, creating a vacuum around an artifact does not seem to be a complex matter and is an interesting way to ascertain the possibility of worm disinfestation as the methodology avoids the use of harmful chemicals, physical heat or electromagnetic radiation treatment, which can produce wood dehydration.

2. Experimental part

The apparatus used to perform the vacuum experiments is illustrated in Figure 1. This was essentially constituted by a spherical dessicator made in Plexiglas, thick enough to withstand a gradient pressure of more than 1.2 bar between the external and internal surfaces, connected to an Edwards rotary vacuum pump able to create a vacuum of 10^{-2} bar, and an appropriate pressure gauge.

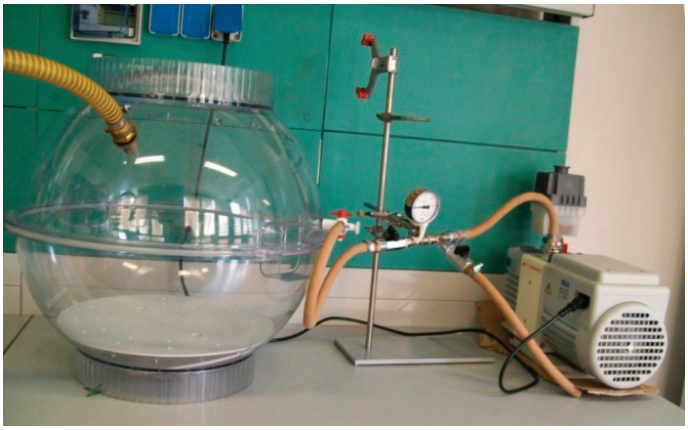

Figure1. Apparatus used to perform vacuum experiments

In order to perform a significant number of experiments it was necessary to breed *Hylotrupes bajolus* larvae by choosing as an incubator chestnut wood already infested with worms. Infested chestnut wood trunks (with an average diameter of 10 cm) were kept for 9 months at a temperature of 20°C with a relative humidity level of 70% in suitable ventilated containers. These environmental parameters allowed optimal growth of a sufficient number of larvae to carry out our experiments. Figure 2 shows a *Hylotrupes Bajolus* larva.

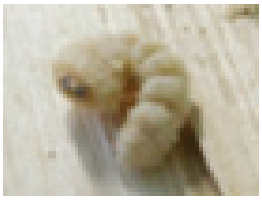

Figure 2. Hylotrupes Bajolus larva

Preliminary experiments were conducted on larvae extracted from their natural environment (i.e. the wood where they were bred) and placed in isolation in the apparatus shown in Figure 1. They were then exposed to a vacuum of 10^{-2} bar. The experiment showed that the larvae are able to maintain their viability for a few hours in these vacuum conditions. Only after more than 10 hours treatment, the worms, in their larval state, lost their mobility and appeared to be smaller in diameter. We therefore concluded that under vacuum conditions, worms gradually lose water and become progressively dehydrated. Worms that were kept isolated moreover, were no longer able to rehydrate themselves to regain their viability. At this stage we were unable to answer the following question: did worms that were kept in their natural habitat and subjected to vacuum conditions inside their tunnels behave in the same way as isolated worms, or could they rehydrate themselves by retrieving water from the wood? In other words, we were unsure whether the conclusion regarding the worms extracted from their natural environment could be assumed valid for the worms left in their habitat, as is the case for larvae-infested artifacts, without any further evidence. Worms inside the wood could indeed rehydrate themselves by taking moisture from the wood by mechanisms of diffusion or simply feeding on the cellulose.

Another problem to solve was to find measurable parameters which would indicate worm death.

Our conclusion was that in order to solve these problems, experiments would need to be conducted leaving the larvae inside their natural habitat and measuring a number of parameters linked to their activity to ascertain their viability. To this end, the larvae contained in the pieces of chestnut wood where they had been naturally bred were subjected to vacuum conditions. All chestnut wood testing samples were of a cylindrical shape measuring 5 cm in height and with a diameter of about 10 cm. Each of them contained a single worm nested in the area between the bark and the dead wood. In order to follow the viability of the larvae in the course of the vacuum treatment we opted to measure the quantity of waste powder produced by the worm during its eating activity. This waste material can be easily collected by simply tapping the cortex of the wood cylinder next to the open cavity created by the worm, placing the sample with the cavity face down. A sample used in the experiments (sample N.7), as well as the waste powder produced by the worm at certain stages of the experimentation is shown in Figure 3.

Figure 3. One of the samples used in the experiments

The cavity produced by the larva is clearly visible on the left side of the sample. The waste powder extracted by the procedure described above is also shown.

Experiments were carried out on control samples, where larvae were left undisturbed, and samples submitted to 8, 24, 120 and 140 hours of vacuum treatment at a pressure of 10^{-2} bar. For each of these conditions three samples were tested in order to reduce experimental error. The waste powder was collected and weighed at given time intervals. Overall research time was 35 days for each sample. From now on, the waste powder produced by the worms will be referred to as WP for brevity.

3. Results and discussion

WP produced by the worms' feeding activity during the vacuum experiments are reported in Figure 4. At the beginning of the experiments, each of the samples was cleaned by removing all the WP produced until then by the hosted worm. On each of the days corresponding to the experimental points reported in the graphs in Figure 4, the accumulated WP was removed from the samples, then weighed. The result corresponds to the WP produced in the interval between the actual and previous measurement of the day before. The weight of this WP was then added to the previous one. The vacuum treatment started on the 9th day of experimentation in all of the cases, as indicated by the black arrow in Figure 4.

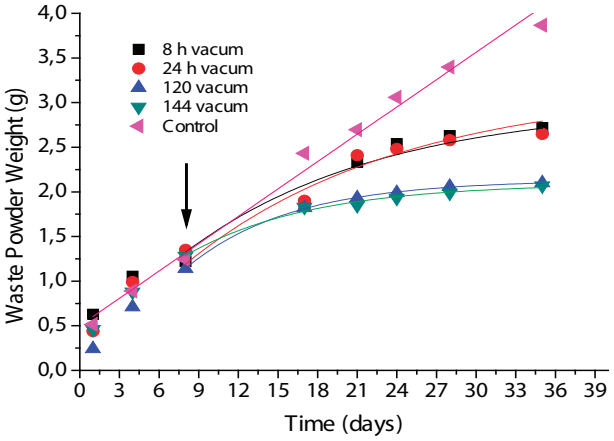

Figure4. WP produced by the worms as a function of days, for four different vacuum times (hours) treatments and for the control sample that was not vacuum treated. Points corresponding to each of the trends represents the average WP quantities measured from the activity of three different worms (see text). The vacuum treatment started on the 9th day, indicated by the black arrow.

First of all, it is interesting to note that the total amount of WP produced by the control worm samples follows an almost linear trend from the beginning to the end of the experiments. The slope is 0.10 grams/day with a margin of error of about 4%. This means that the eating rate of the control worms remains constant and is not affected by manipulation of the samples during the WP collecting procedure. In the other cases, before vacuum treatment, the slope of the WP accumulation remains very close to that

of the control, while after treatment, the WP accumulation loses the linear trend to assume an exponential one. This is clearly seen in Figure 4 where the linear fit for the control and the exponential fit for the vacuum treated samples are reported as continuous lines. The exponential fit is described using the following equation:

$$WP = WP_{\infty} + Ae^{-kt}$$

where, *WP* is the cumulative waste powder produced at the time t, WP_{∞} is the cumulative waste that would be produced at infinite time, *k* is the kinetic constant of worm degradation expressed as $days^{-1}$ and A is a pre-exponential factor which is related to the starting WP accumulated at the time of vacuum treatment.

The fitting results are reported in Table 1.

Table 1. Exponential Fitting results of the WP data of vacuum treated worms shown in Figure 4

Sample	WP_{∞}	k	R^2
8 h	3,17274 (0,43887)	0.06 (0.03)	0,94954
24 h	2,98241 (0,39273)	0.07 (0.03)	0,92817
120 h	2,09386 (0,05906)	0.106 (0.022)	0,98029
144 h	2,13252 (0,01226)	0.125 (0.005)	0,99948
Values in parenthesis are standard deviations of the data			

It is interesting to note that the metabolic activity of the worms does not stop abruptly with the beginning of the vacuum treatment (Figure 4), but gradually decreases to zero in an exponential trend. The kinetic constant of this trend gradually increases as the vacuum time increases, as shown in Table 1. We can assume that this constant represents the rate of worm degradation induced by the vacuum treatment. Therefore, the longer the treatment, the faster the degradation. This is also reflected in the rate at which the WP reached infinite value. This was indeed reached after about 15 days from treatment in the cases of the 120 h and 144 h samples (5 and 6 days of vacuum treatment, respectively) while, only after about 25 days in the other cases (0.3 and 1 day of vacuum treatment).

In addition to the waste production analysis, at the end of the experiment, we observed each of the treated worms and found that all of the worms examined had died as a result of the significant dehydration caused by the vacuum treatment.

Summarizing the results, we have shown that for a vacuum time of the order of 8 hours, worms stop their activity completely after about 25 days post treatment. For the longer vacuum time treatments of 120 and 144 hours (5 and 6 days) worm activity stops after about 10 days from treatment. Moreover, visual inspection of the worms at the end of the experiments, enabled us to verify that their death had been brought about by dehydration induced by vacuum.

Therefore, we can conclude that 8 hours of vacuum treatment at 10^{-2} bar are enough to disinfest wood artifacts from *Hylotrupes bajolus.* Although worms will continue their activity for some time after they have been exposed to the vacuum, the biological damage caused to them during the treatment is irreversible and ultimately leads to the worms' death.

This appears to be a very promising technique, since it does not expose operators to any chemical or physical risk.

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Biographical notes

Giuseppe Chidichimo is full professor of physical chemistry at the University of Calabria (UNICAL), Italy. He has been head of the Department of Chemistry at UNICAL (1987-1996), President of the Scientific and Technological Park of Calabria (CALPARK) (1996-2000), President of the Course of Studies in Chemistry at UNICAL (1998-2003) and President of the Italian Chemical Society - “Calabria” section for two periods of three years. In 1997 he received the “*Laurea Honoris Causa*” at the University of Niznj Novgorod (Russian Federation). He has founded three active Spin Off companies: TEBAID (for technology transfer in the field of innovative materials); DIARCO RESTAURO (for technology transfer in the field of Cultural Heritage Conservation and Restoration; SIRIA (for technology transfer in the field of environmental studies). He is author of more than 200 scientific papers and 20 international patents, concerning basic and applied research in the field of innovative materials, physical chemistry, Cutural Heritage conservation. He has been Invited Speaker at many national and international scientific meetings.

Amerigo Beneduci, completed his MS (cum laude) in Chemistry at the University of Calabria (UNICAL), Italy, in 1998 and obtained his Ph.D. degree in Chemistry from UNICAL in 2002. From 2003 to 2008 he worked as research fellow at the Department of Chemistry-UNICAL. He also served as adjunct professor of analytical chemistry from 2003 to 2008 and as professor of thermodynamics and chemical kinetics from 2012 to 2015. Dr. Beneduci is author of more than 30 peer-reviewed papers and has attended several international and national meetings as speaker. He is the co-founder of the Spin Off company SIRIA for which he is supervisor of the chemistry area.

Francesco Dalena has an MS in Chemistry with a specialization in “Chemistry of advanced materials”, from the Chemistry Department of the University of Calabria (Italy). He is a Ph.D. student at UNICAL working on a project on the restoration of wooden artifacts and ancient papers infested by biological agents.