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ORIGINAL

Modelling the mortality of *Hylotrupes bajulus* (L.) larvae exposed to anoxic treatment for disinfestation of wooden art objects

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Abstract Experiments were conducted to quantify the effect of several variables on the mortality of insects exposed to an anoxic treatment in order to generate a model linking mortality to these variables. This study aims to explore the possible interest of using such a model to determine the characteristics of treatment (especially duration) needed to guarantee insect mortality with a given level of probability. Trials were performed on *Hylotrupes bajulus* larvae, which is a widespread species known for its high tolerance to anoxic conditions. The studied variables are the initial mass of the larvae, the treatment temperature (21, 30 and 40 °C), the treatment duration (four durations for each temperature tested) and whether the larva is held in wood or in a petri dish (directly exposed to anoxic atmosphere) during the experiment. It was found that, while the last variable is not correlated with mortality, treatment duration and temperature are significantly and positively correlated with it. Larvae with higher body mass were also shown to have a better resistance to the treatment. Based on these results, a model including insect initial mass, treatment temperature and duration, together with the interaction between these two variables, was determined. This relatively simple model appeared to be a useful tool in overcoming the difficulty in defining the modalities for anoxic treatment in order to reach a given level of mortality.

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Introduction

In the last few decades, chemical agents used to disinfest wooden objects have been found to be harmful in several regards (Brokerhof 1989; Reichmuth et al. 1993; Unger et al. 2001; Brandon and Hanlon 2003; Berzolla et al. 2011). Their toxicity for the environment, the curator and the public visiting the museums raises questions from an ethical and a regulatory point of view (Koestler et al. 1993). Furthermore, knowledge of the damage chemical treatments can cause to art objects is increasing (Dawson 1988; Florian 1988; Child 2001; Pinniger 2003; Pinniger et al. 2011). Consequently, alternative methods for insect eradication in art collections have been developed.

The use of controlled atmosphere to eradicate insects is a very ancient practice: for centuries hermetic jars or containers have been buried to preserve stored products over long periods (Davis and Jay 1983). Bailey and Gurjar (1918) and Dendy (1918) published the first studies on the use of controlled atmospheres for the conservation of stored food. They were followed by many studies during the 1950s and even more during the 1980s, after the acceptance by the US Environmental Protection Agency of carbon dioxide, nitrogen and combustion gas for the treatment of raw and processed agricultural products (Williams 1991; Selwitz and Maekawa 1998).

Anoxic treatment is a particular case of atmosphere modification aiming to control pests (Gilberg 1989, 1991; Valentin and Preusser 1990; Rust and Kennedy 1993; Selwitz and Maekawa 1998). This method consists in subjecting insects to a low-oxygen atmosphere (usually <0.1 %) during a certain length of time, in order to eradicate them without causing any harm to the object or leaving any toxic residue.

The first attempts to apply this method to eradicate insects in art objects were made in the 1980s. Gilberg (1989) achieved 100 % mortality for several insect species using nitrogen treatment during 1–3 weeks. He also pointed out that knowledge of stored products treatment with controlled atmospheres cannot be directly applied to art objects, notably because the species involved are not the same. Since then, several studies have been devoted to the application of nitrogen anoxia to large museum objects (e.g. Hanlon et al. 1992; Valentin 2003), to the comparison of the lethal effect of different gases (Valentin 1993) or to the effect of temperature and relative humidity on mortality level inherent to anoxic treatment (Valentin and Preusser 1990). Despite this abundant literature, knowledge of the use of modelling methods to quantify mortality according to treatment duration and other variables has so far been lacking.

According to most authors, mortality inherent to anoxic treatment is due to dehydration. Selwitz and Maekawa (1998) provide three reasons why this hypothesis is the most common one: (1) when submitted to low-oxygen atmospheres, insect respiratory systems display changes which tend to increase the insects' water loss; (2) treatment efficacy is positively correlated with increasing drought and heat conditions; (3) mortality is correlated with rapid mass loss which can only be explained by water loss. However, several authors have suggested that anoxia might have other impacts on insects. Indeed, two main strategies are thought

to be used by animals to cope with anoxia: anaerobic metabolism and metabolic arrest. However, both strategies present important drawbacks such as exhaustion of carbohydrate reserves, accumulation of toxic metabolic end products and the necessity to have low membrane permeability. These drawbacks probably play an important part in anoxia toxicity for animals (Hochachka 1986; Zhou et al. 2000; Harrison et al. 2006).

Despite its efficiency, anoxic treatment presents four main disadvantages. First of all, the treatment has no persistence: it cures an object for the pests which are present, but it cannot protect it against later infestations. Secondly, the treatment duration is long: usually around 21 days (Gunn 2008; Berzolla et al. 2011). Third, low-oxygen concentrations (below 1 %) must be achieved and maintained through the whole treatment (Berzolla et al. 2011). Finally, the treatment efficacy is widely dependent on several environmental variables. However, because of the advantages anoxic treatment presents in comparison with other methods (chemical or heat treatments for instance) it is worth collecting information which can help to optimize its use.

Because of these drawbacks, Berzolla et al. (2011) suggest using the influence of environmental factors in order to allow either a reduction in the treatment time or the use of higher oxygen concentrations. Hence, modelling seems to be an interesting approach in order to test systematically different combinations of these variables.

In this context, the first aim of this study is to quantify the impact of ambient temperature, insect body mass, insect living environment and treatment duration on insect mortality during anoxic treatment. Based on this first step, the second objective is to set up mortality models relying on variables that have been shown to influence the treatment efficacy. These models should help determine those characteristics of treatment (especially duration) needed to guarantee insect mortality with a given level of probability.

Materials and methods

Living material

The experiments were performed on the old house borer *Hylotrupes bajulus* (L.) (Coleoptera, Cerambycidae). This species was chosen for several reasons: (1) it is frequent in museums, although it only develops in softwood (National Pest Control Association 1965; Harmon 1993); (2) it is highly resistant to anoxic conditions allowing application of the models to more sensitive species (Valentin 1993); (3) it is one reference species for the assessment of biocide activity (IBN 2004); (4) it is easy to rear, so offering the possibility of obtaining a large controlled population for which age, health and masses are known. This is usually not the case for studies on insect mortality in cultural property in anoxic environments (Berzolla et al. 2011) but is more suited to the use of a precise experimental plan.

Insects were reared in the Laboratory of Wood Technology of the Public Service of Wallonia, in Gembloux (Belgium), where the experiments took place.

The experiments were made on larvae because this is the most damaging life stage of this insect. Moreover, larvae, along with pupae, are the most resistant life stage to this treatment (Valentin 1993) as well as to other treatments such as microwave irradiation (Henin et al. 2014).

In the sample tested, the mean mass of the insects is 176.14 mg (standard deviation = 116.73 mg). Kolmogorov's test did not confirm the fact that the sample is distributed according to the log-normal curve represented in Fig. 1a. However, analysis of the adjustment of the curve on the data (Fig. 1b) reveals that the adjustment underestimates the probability of very high masses. In consequence, in order to obtain a given level of probability (probability for a larvae to have a body mass inferior to a given value), the log-normal distribution would lead to a higher mass value (Fig. 1b). This may be considered as a safety margin, and this log-normal distribution will thus still be used for further analysis.

According to EN 1390, trials must be done on larvae weighing between 50 and 150 mg (IBN 2006). However, in order to take into account insect masses encompassing those which can naturally occur, it was decided not to follow the protocol of EN 1390.

The insects were examined after anoxic treatment, and those that did not react when slightly stimulated with a brush were considered to be dead. However, during the experiments, this method proved to be problematic as some insects considered to be dead showed some movement if observed a few days later. Conversely, insects

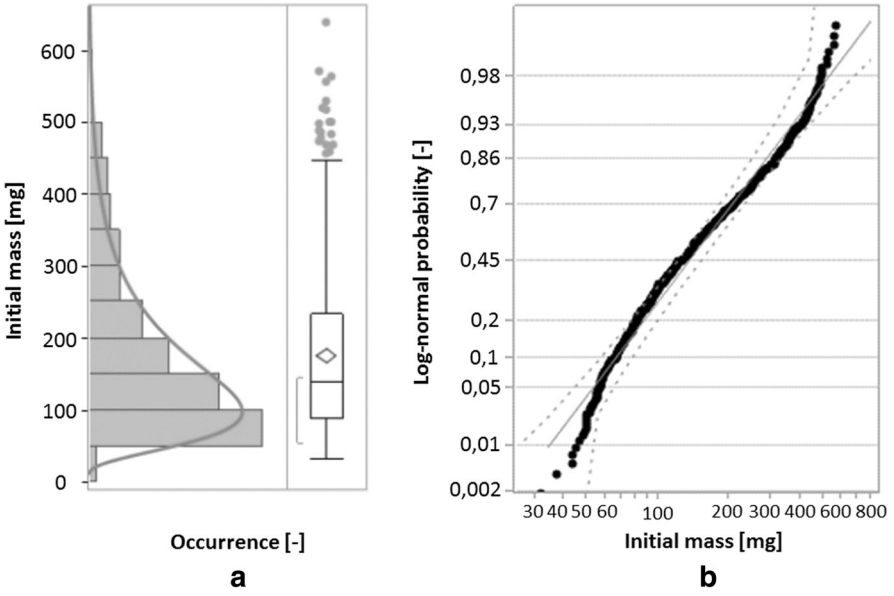

Fig. 1 **a** Distribution of the larvae's mass and log-normal curve adjusted on this distribution. The *box* on the right represents the limits of the first and third quartile. The *horizontal line* inside the box represents the median sample value, and the *diamond* represents the mean and the upper and lower 95 % confidence intervals on the mean. The *bracket* indicates the densest 50 % of the observations. **b** Diagnostic plot of the fitted probability versus the data

that seemed to be alive when regularly observed for 2 weeks after the treatment were not able to bore into the wood when transplanted for two more weeks in boards. Furthermore, some insects showed body movements but had inactive mandibles. Although alive, such insects may not be harmful to art objects. That is why it was decided to place the insects in wooden boards for 2 weeks after the experiments, according to EN 1390 (IBN 2006). Those which bored into the boards were considered to be alive; the others were considered to be dead.

Temperature, treatment duration and environment (petri wood)

In order to assess the influence of temperature on insect mortality in anoxic conditions, three experimental sets were designed. The first one was held at 21 °C [the mean temperature of 21.2 ± 0.6 °C was recorded by a data logger (EBRO® type EBI20)]. This set aimed to simulate the treatment in "standard conditions". The second set was held at 30 °C and the third one at 40 °C in an oven. The choice of these temperatures was based on the facts that: (1) the lower temperature has to correspond to the mean temperature which can be found in places where such treatments are usually performed; (2) this protocol is meant to be applied to art objects which are, by nature, fragile: high temperatures are thus unsuitable; (3) beyond ca. 50–55 °C, insects die because of heat instead of anoxia. Therefore, the latter temperatures could not be used in this experiment. For each experiment, the nitrogen gas flow had a relative humidity of 40 %. This value is in accordance with the environmental guidelines of the International Council of Museums–Committee for conservation (ICOM–CC) and the International Institute for Conservation of Historic and Artistic Works (IIC) (ICOM-CC 2014).

Mortality was assessed after different treatment durations. The timing of these evaluations is shown in Table 1. For each combination of temperature and treatment duration, there were two repetitions, each one consisting in an anoxic bag containing two petri dishes and two wooden boards (see Experimental set-up).

In addition, sets of two petri dishes and two wooden boards were used as control groups and were held in normal air atmosphere both in the room and in the oven during the experiment. These control groups have been submitted to the same manipulations as the treated insects.

Table 1 Treatment duration according to temperature

21 °C	30 °C	40 °C
		12 h
		1 day
		2 days
	5 days	5 days
10 days	10 days	(10 days)
15 days	15 days	(15 days)
20 days	20 days	
25 days		

Evaluations between brackets were initially planned but were not completed because shorter durations enabled to achieve 100 % mortality

The effect of the living environment of the insects on their mortality was studied by placing some larvae in wooden boards and others in petri dishes. Each petri dish contained two larvae: a small one (<90 mg) and a large one (>180 mg). Each wooden board contained six larvae: two small ones, two large ones and two intermediate ones (between 90 and 180 mg). The $10 \times 15 \times 2$ cm^3 boards were made of uncoated Scots pine sapwood.

This set-up aimed to test the effect of the environment on treatment efficiency. Indeed, in addition to numerous studies on anoxic treatment directly using art objects (see, e.g. Hanlon et al. 1992; Rust and Kennedy 1993; Daniel et al. 1993; Maekawa 1999), numerous studies have used petri dishes, vials or cages (see e.g. Birkenmeyer and Dame 1970; Jay and Cuff 1981; Soderstrom et al. 1986; Gilberg 1989; Valentin and Preusser 1990; Rust and Kennedy 1993) and some have used wood boards or similar in-material set-ups (see e.g. Rust and Kennedy 1993; Valentin 1993; Gunn 2008). To the authors' knowledge, however, very few studies have focused on insects both in and outside the material. The use of both set-ups allowed to (1) compare these results with those found in the literature and (2) study the effect of the environment on treatment efficiency.

Globally, for each set of treatment conditions (temperature/treatment duration), there were two anoxic bags containing twelve larvae in wooden boards each and four larvae in petri dishes each. Therefore, the number of replicates was 4: two bags containing two replicates each. The duplication of the bags aimed to provide a backup in the event that one of them was punctured, torn or lost its airtightness.

The only exception was the combination 40 °C/5 days, which was composed of six anoxic bags.

In the models, these variables are abbreviated as follows:

- Treatment duration: D;
- Temperature: T;
- Initial mass of the insect: M;
- Living environment of the insect during treatment (wood board/petri dish): E.

Experimental set-up

Anoxic conditions were achieved through the use of VELOXY® manufactured by RGI bioSteryl Tech (Genova, Italy). The device is fitted with a molecular sieve that extracts the oxygen from the airflow. The remaining flow is composed mainly of nitrogen. The experimental set-up is presented in Fig. 2.

Nitrogen flow was sent to plastic bags each containing two wooden boards and two petri dishes. The plastic bags were connected in parallel.

For the first 3 days of each experiment, the machine was kept running from 8 a.m. to 5 p.m. in order to entirely extract the oxygen contained in the wood. This should have been sufficient to keep the oxygen level very low (around 0.1 %). But as a precaution, after the first 3 days and for the rest of the experiments, the machine was kept running between 8 and 11 a.m. and between 2 and 5 p.m. Previous tests, conducted by the authors before the experiment, have shown that this schedule

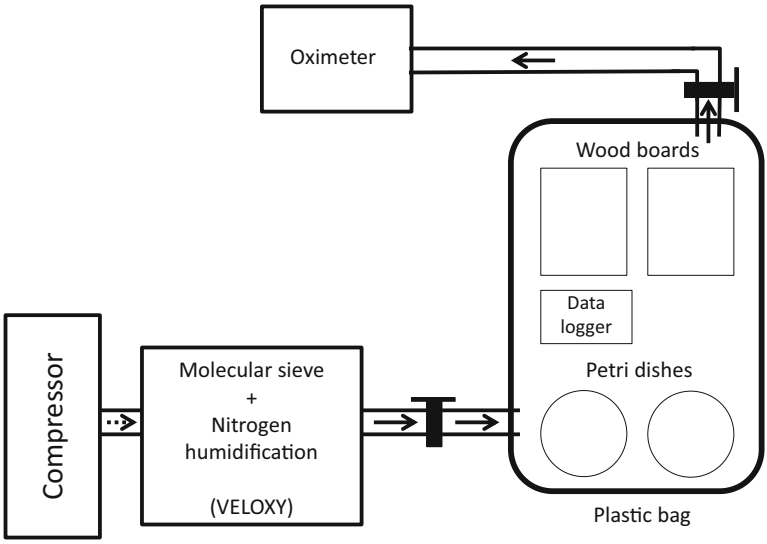

Fig. 2 Diagram of the experimental set-up. *Dashed arrow* represents air flow; *plain arrows* represent deoxygenated air (nitrogen)

enables to maintain the oxygen level below 0.1 % in the plastic bags. During the whole experiment, oxygen level was monitored thanks to an oximeter, confirming that the oxygen concentration never exceeded 0.1 %.

Statistical analysis

The models established in this study aim to forecast the effects of anoxic treatment on larvae mortality. In this particular case, logistic regression is the most adequate approach as it relates the probability of occurrence of a dichotomous variable (i.e. death or survival) to explicative variables influencing the latter. The logistic regression has the following expressions:

$$\ln \frac{p(1|X)}{1-p(1|X)} = a_0 + a_1 x_1 + \cdots + a_j x_j \quad (1)$$

where $p(1|X)$ is the mortality probability for a given set of explanatory variables X, a are the parameters, and x the explanatory variables.

The target mortality level was set at 99.9968 %. This level corresponds to probit 9 efficacy level, which is required in International Standards for Phytosanitary Measures ISPM No 15 "Guidelines for Regulating Wood Packaging Material in International Trade" (FAO 2011). The probit method was first proposed by Bliss (1934) as a method for minimizing errors in comparing the effects of toxic agents on an organism. This methodology aims to transform mortality percentage and express it as probability units also known as probits (Haack et al. 2011).

Baker (1939) selected probit 9 as the efficacy level required to avoid accidental dissemination of organisms (Robertson et al. 2007; Haack et al. 2011). During the 1960s, this threshold was adopted by the US Department of Agriculture and many countries for quarantine security (CPM 2013).

In recent times some authors have questioned the relevance of probit 9 as a threshold for pest eradication (Haack 2011; Schortemeyer et al. 2011). They point out that a very large amount of insects must be tested to achieve this level of statistical security: 93,613 insects treated without survivor (Couey and Chew 1986). The arbitrary nature of this threshold also raises concerns along with the fact that it is too strict for rarely infected products. Despite these criticisms, probit 9 is still the norm for some international standards and was accordingly chosen here as the target mortality threshold.

Results

The results of the experiments are presented in Tables 2 and 3. These tables also display the difference in the results according to the moment of the observation of the larvae (at the opening of the bags or after 2 weeks spent in wooden boards after the treatment).

An exploratory analysis aims to investigate the impact of each variable (ambient temperature, treatment duration, environment and insect initial mass) separately on insect mortality. This step does not enable the establishment of mortality models, but it allows evidencing the most influent variables. It appears that the location of the insect in a wooden block versus in a petri dish has only a slight effect on mortality (results not presented). These observations are consistent with previously published ones (Rust and Kennedy 1993). Ambient temperature, treatment duration and insect body mass have a stronger influence on the mortality rate. It has also been shown that mortality is negatively correlated with insect initial mass and positively correlated with temperature (results not presented).

The positive correlation between temperature and mortality has been known for a long time (Valentin 1993), but the effect of insect mass has, to the best of the authors' knowledge, never been studied in the specific context of anoxic treatment of art objects. However, studies in other contexts evidenced negative correlation between insect mass and sensitivity to oxygen depletion (Matheson and Parsons 1973; Upitis et al. 1973; Greenlee and Harrison 2004; Navarro 2006).

In a second step, a full model including each variable x_i (Eq. 1) and every combination between variables is proposed. This model points out that seven variables or variable combinations might significantly influence mortality. Reduced models are then built using these variables. Even if the initial mass is not a significant variable of the full model, results from the first step and information from the literature lead to think that this is a key variable for the physiological processes taking place during anoxic treatment (Valentin and Preusser 1990; Valentin 1993, 1998; Reierson et al. 1996; Selwitz and Maekawa 1998). Thus, it will be included in the reduced models (as a "forced" variable). The full model and two options for

Table 2 Percentage of living and dead larvae in wooden boards for each experimental set-up

	20 °C		30 °C		40 °C	
	Opening	Opening + 2 weeks	Opening	Opening + 2 weeks	Opening	Opening + 2 weeks
0.5 day						
Living					83.3	8.3
Dead					16.7	91.7
1 day						
Living					8.3	0
Dead					91.7	100
2 days						
Living					0	0
Dead					100	100
5 days						
Living			70.8	58.3	0	0
Dead			29.2	41.7	100	100
10 days						
Living	66.7	50	8.3	4.2		
Dead	33.3	50	91.7	95.8		
15 days						
Living	29.2	8.3	4.2	0		
Dead	70.8	91.7	95.8	100		
20 days						
Living	4.2	0	0	0		
Dead	95.8	100	100	100		
25 days						
Living	0	0				
Dead	100	100				

Mortality/survival ratios are given for two different timings of observation: at the opening of the anoxic bags (immediately at the end of the treatment) and after having transplanted the larvae for 2 weeks in wooden boards

reduced models are presented in Table 4. Each of these models has the same structure as Eq. 1.

In order to determine whether enough information is brought by the variables included in a model or whether additional variables are needed, a lack of fit test was performed. The aim of this test is to evaluate whether the terms included in the model are sufficient for a proper fitting of the results (Weisberg 1980; Neter et al. 1990). This is done by comparing the variability of repeated measurements with the variability of the residuals of the model, as one expects that both should compare well if there is no misspecification for the model. The results of this test are presented in Table 5.

As can be seen from Table 5, simplifying the model induces a slight diminution of the fitting of this model (diminution of the negative log-likelihood). However,

Table 3 Percentage of living and dead larvae in petri dishes for each experimental set-up

	20 °C		30 °C		40 °C	
	Opening	Opening + 2 weeks	Opening	Opening + 2 weeks	Opening	Opening + 2 weeks
0.5 day						
Living					75	0
Dead					25	100
1 day						
Living					25	0
Dead					75	100
2 days						
Living					0	0
Dead					100	100
5 days						
Living			62.5	25	0	0
Dead			37.5	75	100	100
10 days						
Living	100	37.5	12.5	0		
Dead	0	62.5	87.5	100		
15 days						
Living	37.5	0	0	0		
Dead	62.5	100	100	100		
20 days						
Living	0	0	0	0		
Dead	100	100	100	100		
25 days						
Living	0	0				
Dead	100	100				

Mortality/survival ratios are given for two different timings of observation: at the opening of the anoxic bags (immediate at the end of the treatment) and after having transplanted the larvae for 2 weeks in wooden boards

even the simplest one (model 2) does not display a lack of fit (Chi-square probability for the tree models indicates that none of them is significantly different from a saturated one) indicating that the variance of the residuals is not significantly different from the variance of the repetition of results for a same set of variables' condition.

In order to compare the three models, performance criteria are presented in Table 6.

The AIC (Akaike information criterion) measures the quality of the models taking into account their complexity (applying a penalty proportional to the number of variables). If no other indicators are used, the model with the lowest AIC value is preferred. Table 6 shows an increase in AIC from the full model to model 2. This indicates that, while model 2 is simpler, it does not fit the data as well as the others.

Table 4 Three model options to simulate mortality according to the specific parameters or combination of parameters considered

	Full model	Reduced model 1	Reduced model 2
M	×	×	×
D	×	×	×
T	×	×	×
E	×	×	
M × D	×		
M × T	×		
D × T	×	×	×
M × E	×		
D × E	×	×	
T × E	×	×	
M × D × T	×		
M × D × E	×		
M × T × E	×		
D × T × E	×	×	
M × D × T × E	×		

M initial mass of the insect, *D* treatment duration, *T* temperature, *E* living environment (wooden board/petri dish)

Crosses (×) indicate which parameters are included in each model

Table 5 Lack of fit test results for the three models tested

Model	Source	Degrees of freedom	Negative log-likelihood	Chi-square	Prob. > Chi-square
Full model	Lack of fit	452	102.45	204.89	1,0000
	Saturated	467	6.07		
	Fitted	15	108.52		
Model 1	Lack of fit	459	116.55	233.094	1,0000
	Saturated	467	6.07		
	Fitted	8	122.61		
Model 2	Lack of fit	461	127.69	255.39	1,0000
	Saturated	465	6.07		
	Fitted	4	133.76		

However, the differences between AIC values are small, indicating close performances.

The similar values of misclassification rates for the three models also indicate close performances.

The significance of the effect of each variable on the response is assessed by means of a likelihood ratio test. Results are presented in Table 7.

Table 6 Performance criteria of the models

	AIC	Misclassification rate
Full model	250.12	0.0793
Model 1	263.59	0.1122
Model 2	277.64	0.1083

Table 7 Results of the likelihood ratio test

	Source	Likelihood ratio Chi-square	Prob. > Chi-square
Full model	M	0.43	0.5105
	D	277.49	<0.0001*
	T	190.40	<0.0001*
	E	16.88	<0.0001*
	M × D	0.51	0.4757
	M × T	0.48	0.4905
	D × T	20.62	<0.0001*
	M × E	0.49	0.48
	D × E	19.60	<0.0001*
	T × E	20.95	<0.0001*
	M × D × T	0.59	0.4408
	M × D × E	0.53	0.4672
	M × T × E	0.46	0.4959
	D × T × E	14.20	0.0002*
	M × D × T × E	0.61	0.4346
Model 1	M	16.07	<0.0001*
	D	284.52	<0.0001*
	T	222.93	<0.0001*
	E	16.23	<0.0001*
	D × T	31.81	<0.0001*
	D × E	19.85	<0.0001*
	T × E	20.48	<0.0001*
	D × T × E	15.27	<0.0001*
Model 2	M	16.03	<0.0001*
	D	288.58	<0.0001*
	T	230.85	<0.0001*
	D × T	18.68	<0.0001*

M initial mass of the larvae, *D* treatment duration, *T* temperature, *E* living environment

Asterisks (*) indicate parameters that are significant for a *p* values threshold of 0.05

Statistics presented in Tables 4, 5, 6 and 7 make it possible to choose the best model. It appears that model's performances are quite similar, but, because of its lower complexity, model 2 seems to be more suitable for practical use.

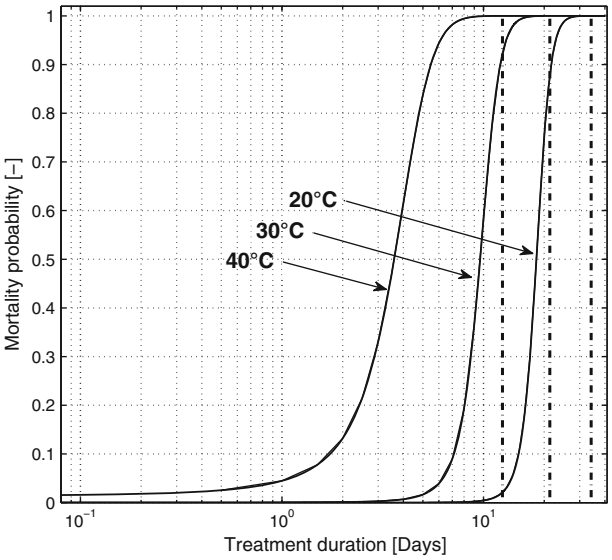

Fig. 3 Graphical representation of the mortality model (model 2). The *thick vertical dashed* lines represent the time needed to reach probit 9 level of mortality probability

The selected model predicts insect mortality along treatment using initial mass of insects, treatment duration, temperature and the combination of treatment duration and temperature. Graphical representation of this model is presented in Fig. 3, and analytical expression is given in Eq. 2.

Concerning the graphical representation of the model (Fig. 3), initial mass has been fixed at 900 mg. This value is extremely high for the old house borer and has in fact never been observed in the present sample. However, using the statistical distribution of the mass of the insects, it was determined as the threshold assuring that <1 % of the theoretical population has a higher body mass and is liable to resist the treatment.

The mortality model has the following expression:

$$\ln\left(\frac{\pi}{1-\pi}\right) = \exp(-24.703 - 0.005 \times M + 0.935 \times D + 0.676 \times T + 0.029 \times (D - 8.553) \times (T - 31.722)) \quad (2)$$

where π is the mortality probability, M is the initial mass of the larvae expressed in milligrams, D is the treatment duration expressed in days, and T is the temperature expressed in °C.

The ROC curve (receiver operating characteristic) (Fig. 4) is a representation of the model sensitivity (proportion of larvae predicted to be dead compared to the number of larvae actually dead) versus 1—the model specificity (proportion of larvae predicted to be alive compared to the number of larvae actually alive). A useless model would be a model with a diagonal ROC curve, while a perfect model would have a ROC curve passing through the upper left corner of the graph. In this

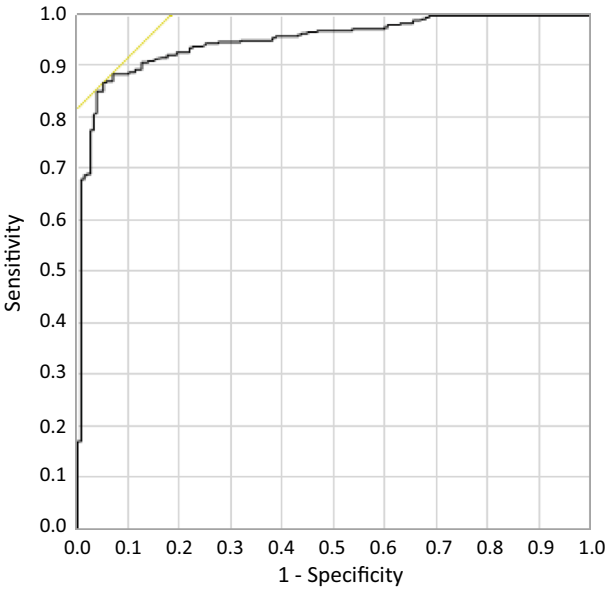

Fig. 4 ROC curve of model 3

latter case, the classification based on the model would always be right. A high area under the curve thus indicates the model's classification ability.

The shape of the curve and the high value of the area under it (0.953) indicate the high performance of the model in classing the treated larvae into each category (dead or alive).

The use of a new data set for validation should, however, give more information about the model performances.

In Table 8, the outcomes of reduced model 2 are compared to those found in the literature.

Discussion

Model outputs

Treatment durations calculated from this model are longer than those found in the literature. It is hypothesized that this difference originates from the fact that, contrary to previous studies, the present results do not represent the treatment duration needed to eliminate a particular sample of insects. Rather, they represent the treatment duration needed to eradicate any insect population, provided that the population used in this study is representative of those found in museums. In other words, the treatments modelled here are particularly long probably because they take into account the possibility of having to treat insects with a very high body

Table 8 Comparison of the results obtained in this study to those of baseline studies

	Temperature (°C)	Treatment duration (days)
Valentin 1998	20	19
	30	9
	40	1
Gunn 2008	25	14
Gialdi and Ratto 2002	25	21
	20	28/35
Model 2	21	36
	30	21
	40	12

For Model 2, the durations are the ones needed to comply with probit 9 mortality level

mass. The very high mortality probability (probit 9 level) targeted here also explains these particularly long treatment durations.

The model calibrated on old house borer has been used to estimate the time needed to eradicate other species. Two considerations have to be taken into account to extrapolate the model to other species. First of all, the old house borer is the most resistant insect to anoxic treatment among the insect pests commonly found in museums, archives and herbaria (Valentin 1993). Secondly, other species have a lower sensitivity to heat than old house borer (Valentin 1998). As a result, the application of this model to other species will lead to conservative predictions at low temperatures and more precise ones at high temperatures.

Considering initial masses ranging between 200 and 400 mg, which is probably more realistic for several insect species commonly found in museums, the model provides treatment times of 33–35 days, 20.5–21.5 and 12–12.5 days for 20, 30 and 40 °C, respectively. These results are similar to those of the SAVE ART project, whose objective was to study the possibility of using the VELOXY device to eradicate insects from art collections. The authors of this study propose 3 weeks at 22 to 24 °C and up to 5 weeks at 20 °C (Gialdi and Ratto 2002).

This observation confirms the hypothesis previously stated that the difference between the here presented results and those of previous research is mainly due to the insect mass security margin that must be taken into account in order to ensure statistical liability of the predictions.

As mentioned earlier, the main limitation of this model is the assumption that the distribution of M observed in these experiments here is representative of those found in art collections. This hypothesis is difficult to verify. However, the most important values for an accurate estimate of the treatment duration required for a probit 9 level of efficacy are the extreme values (tail of the statistical distribution). Consequently, the values based on the true distribution would probably be close to those used here.

As a third step, the model is used to draw a map of the probabilities of mortality according to the conditions to which the treatment is applied. This map (Fig. 5)

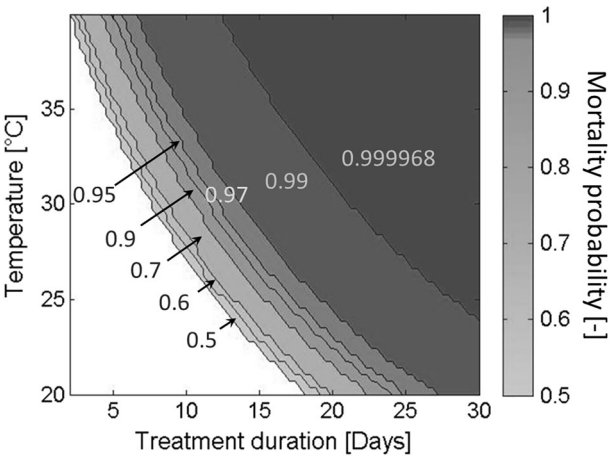

Fig. 5 Map of mortality probability according to temperature and treatment duration. Probability levels (*in per cent*) are indicated in the figure. These areas have a maximum error margin of 5 %

makes it easy to determine the treatment duration needed to reach a certain level of efficacy at a given temperature.

This map illustrates the potential of modelling mortality due to anoxic treatment. Contrarily to most existing data, this map proposes treatment duration for a continuous range of temperature—instead of a limited set of discrete values—and relies on a model and not merely on an insect sample. Consequently, these results can be generalized to other situations.

Effect of the mortality assessment protocol on the predicted mortality

As previously discussed, determining whether an insect is dead or alive can be difficult and classical methods may induce some bias.

Therefore, a new protocol was tested in which the insects were placed in small wooden boards for several days or weeks after the treatment. After this period, each larva (even alive) which did not bore into the wood was considered dead because it could not damage the object anymore and would probably not be able to complete pupation.

Comparing the mortality before and after placing the larvae in wooden boards, it appeared that the classical method underestimates the treatment efficacy. Indeed, observing insect mortality immediately after the opening of the bags leads to consider as alive insects which do not present any danger for the object anymore. Hence, an intermediate state called "mortally affected" was defined to designate such insects. Practically, for the rest of the analyses, mortally affected and dead larvae are considered without distinction. The new model is presented in Fig. 6(b) and is compared to the previous model Fig. 6(a).

Figure 6 highlights that, at 40 °C, mortality model 2b presents high mortality levels for short treatment durations and even for larvae in the absence of treatment

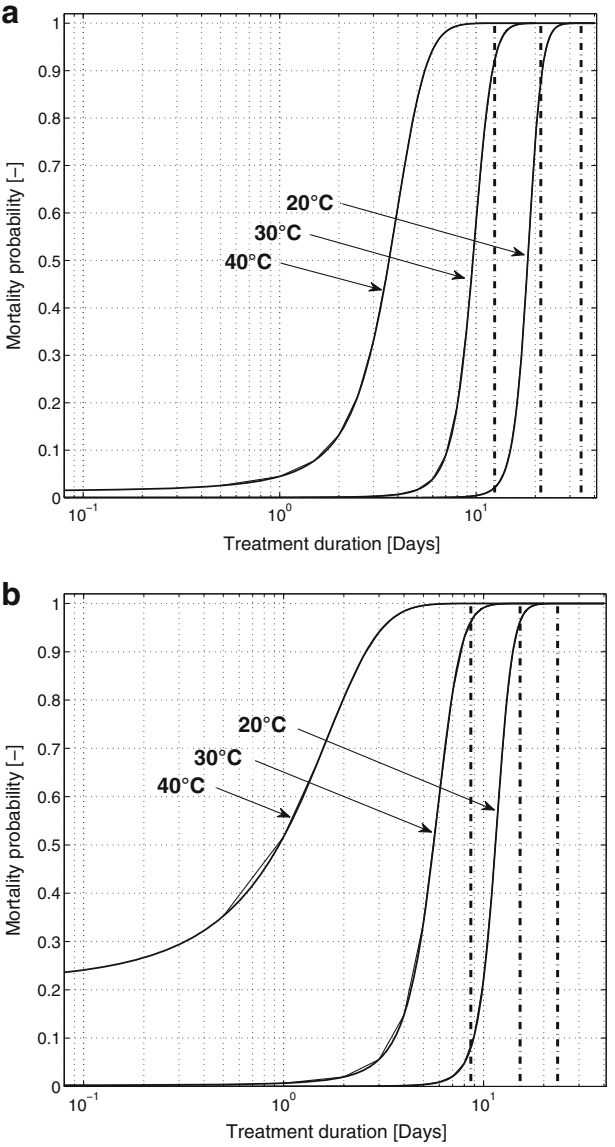

Fig. 6 Comparison of the models based on the mortality determined before (model 2, **a**, same as Fig. 3) and after transplanting the larvae in wooden boards (model 2b, **b**)

(treatment duration = 0 days). However, the very low mortality level observed on the control group at 40 °C (one dead larva out of 42) contradicts the hypothesis that mortality is due to temperature or manipulations. Instead, it seems to indicate that the model is unable to take into account the rapid variation of mortality at this temperature. This observation leads to the conclusion that the area of validity should

Table 9 Treatment duration theoretically needed to achieve 99.9968 % mortality for insect mass up to 900 mg, according to ambient temperature and to the timing applied to measure insects' mortality

Temperature (°C)	Treatment duration calculated from the model "before transplanting" (days)	Treatment duration calculated from the model "after transplanting" (days)
21	36	24.5
30	21	15.5
40	12	9

be limited to durations corresponding to high mortality probabilities. Cross-plotting of data and the model have shown that at 40 °C, the model overestimates mortality for the first day and a half of treatment. After this period, it represents more accurately the evolution of mortality with the safety margin described earlier. This is a minor drawback as the interesting parts of the model considering the application are the treatment durations corresponding to high mortality probability, which correctly fit the data.

Figure 6 also shows that treatment durations are significantly lower for the model "after transplanting" than for the model "before transplanting". This illustrates the fact that some insects still alive at the opening of the plastic bags are not able to damage the objects. Unable to feed themselves, they will ultimately die.

The outcomes of both models are compared in Table 9.

Comparing the results provided by both models points out a difference in the treatment duration that might be chosen by a conservator.

Conclusion

This study confirmed the influence of temperature and treatment duration on insect mortality during anoxic treatment; it evidenced for the first time the effect of insect body mass. Conversely, the environment (wooden boards vs petri dishes) in which the insect is held during the experiment did not exhibit any effect on anoxic treatment efficacy. However, this study, like the one by Rust and Kennedy (1993), uses only small wooden boards, uncoated and of simple geometry (simple parallelepiped). Additional studies should be conducted in order to assess whether more complex objects influence treatment efficacy or not.

This study also evidenced the possibility of the use of modelling as a way of overcoming the difficulty in defining the modalities for anoxic treatment. It appears to be a promising approach. Simple models with good performances have been established and can be used to define treatment conditions.

It has also been shown that assessing larvae mortality after transplanting them for 2 weeks into wooden boards reduces the predicted treatment duration. This is due to the existence of a "mortally affected" state that in practice can be assimilated to a "dead" state. While this study gives new insights into this matter, the analysis of the "mortally affected" state through a protocol dedicated to this question should lead to a better understanding of insect reaction to anoxia treatment. Further additional

studies are thus required in order to assess the specific impact of accounting or not for this state in the modelling process itself.

Although the use of modelling is of great interest for a better mastery of anoxic treatment, more studies should be conducted, testing, for instance, additional variables (humidity of the gas flow for example) or other insect species commonly found in works of art (such as *Anobium punctatum* (DeGeer) or *Lyctus* spp.) which might influence treatment efficacy.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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