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## **THE RELATIONSHIP BETWEEN THE MASS OF OLD HOUSE BORER LARVAE *HYLOTRUPES BAJULUS* (L.) AND THEIR LENGTHS MEASURED USING RADIOGRAPH**

*Old house borer larvae were used in a normative investigation of wood preservatives. X-ray photographs were used to control the procedures of the laboratory tests for toxic substances performed on the old house borer larvae. Such photographs make it possible to determine the location of the larvae inside farm blocks and to measure the lengths of the larvae. In the experiment, wooden blocks on which old house borer larvae were feeding were X-rayed and the lengths of the larvae images on the films were measured. The exact lengths of the larvae extracted from the wood were compared with the lengths of their X-ray images. The mass of the larvae, which ranged from 0.05 g to 0.15 g. were related to the body lengths, ranging from 12 mm to 17.5 mm, as measured on the X-ray image.*

**Keywords:** old house borer, radiographs of the larvae in the wood, larvae used in the tests, relationship between the mass and body length of larvae, wood-damaging insects

### **Introduction**

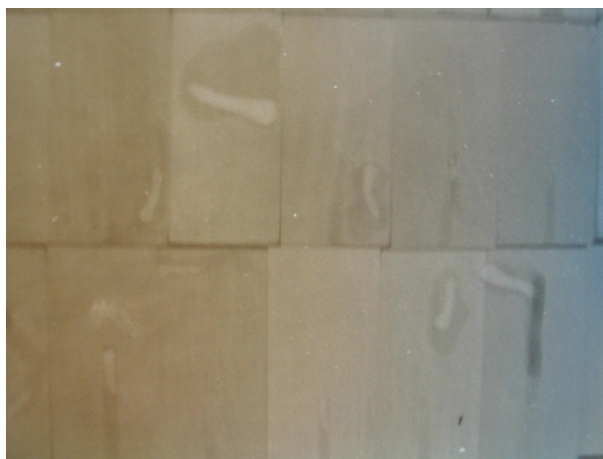
The discovery of X-ray by Wilhelm Roentgen in 1895 enabled information about the interior of various objects to be obtained, which previously had been beyond visual examination. Maloy and Wilsey used X rays in 1930, probably for the first time in history, to detect the decomposition of wood by biodegradation factors [Bletchly and Baldwin 1962]. Further attempts to apply and to improve the method of examining wood-decaying insects were made in the research by Fisher and Tasker [1940], Jacquot [1961], Bletchly and Baldwin [1962], and Amman and Rasmussen [1969]. Examinations executed by Kerner et al. [1980] on the larvae of the old house borer, are also noteworthy. It has been known for

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a long time that the lethal dose of X-rays for old house borer larvae is much higher than the doses used to obtain a radiograph [Schmidt 1961].

The foundations for cultivating the larvae of the most important testing species of insects were also evolved many years ago [Becker 1949; Technau and Behrenz 1958; Cymorek 1975]. Nowadays, these species are used in normative investigations of wood preservatives. Since the 1970s, X-ray photographs have been included in controlling procedures for the laboratory testing of toxic substances, which are performed on old house borer larvae. In laboratory breeding, newly hatched old house borer larvae are harvested from the surface of wooden blocks [EN 47:2005]. As for the old house borer larvae weighing 50-150 mg provided for such tests, they can, with the help of X-ray photographs, easily be extracted from the inside of wooden farm blocks for the examination of wood preservatives [EN 22:1993; EN 47:2005]. Such photographs, however, only make it possible to determine the location of the larvae inside the farm blocks and to measure the lengths of the larvae (fig. 1).



**Fig. 1. Old house borer on X-ray picture of blocks**

The procedure itself is only applicable for the use of larvae weighing 50-150 mg. It is thus necessary to determine the relationship between the mass of the larvae and their length, and to estimate the possible differences between the length measured on the X-ray photographs and the real values under the conditions of laboratory breeding in wooden blocks. The present article was written in order to resolve those issues.

## **Materials and methods**

In the first stage of the experiment, 171 old house borer *Hylotrupes bajulus* (L.) larvae were extracted from laboratory breeding. Their body lengths ranged from

3.5 to 22.0 mm. The lengths were measured with an accuracy of 0.5 mm. Each larva was weighed with an accuracy of 0.001 g. The mass of the larvae ranged from 0.001-0.245 g. The lengths ( $x$ ) and mass ( $y$ ) of the larvae were compared and the relationship between them was calculated.

In the next stage of the experiment, 27 blocks made of Scots pine *Pinus sylvestris* (L.) sapwood, measuring  $15 \times 20 \times 50$  mm, were taken from laboratory breeding. Each block contained 1 old house borer larva carving the wood. Adding 27 wooden blocks with no larvae, a bundle was created in the form of an array, measuring  $225 \times 300 \times 15$  mm. The blocks with larvae were separated using the empty blocks in order to prevent two larvae carving the wood from meeting. In this way, the possibility of injury and cannibalism between the larvae was excluded.

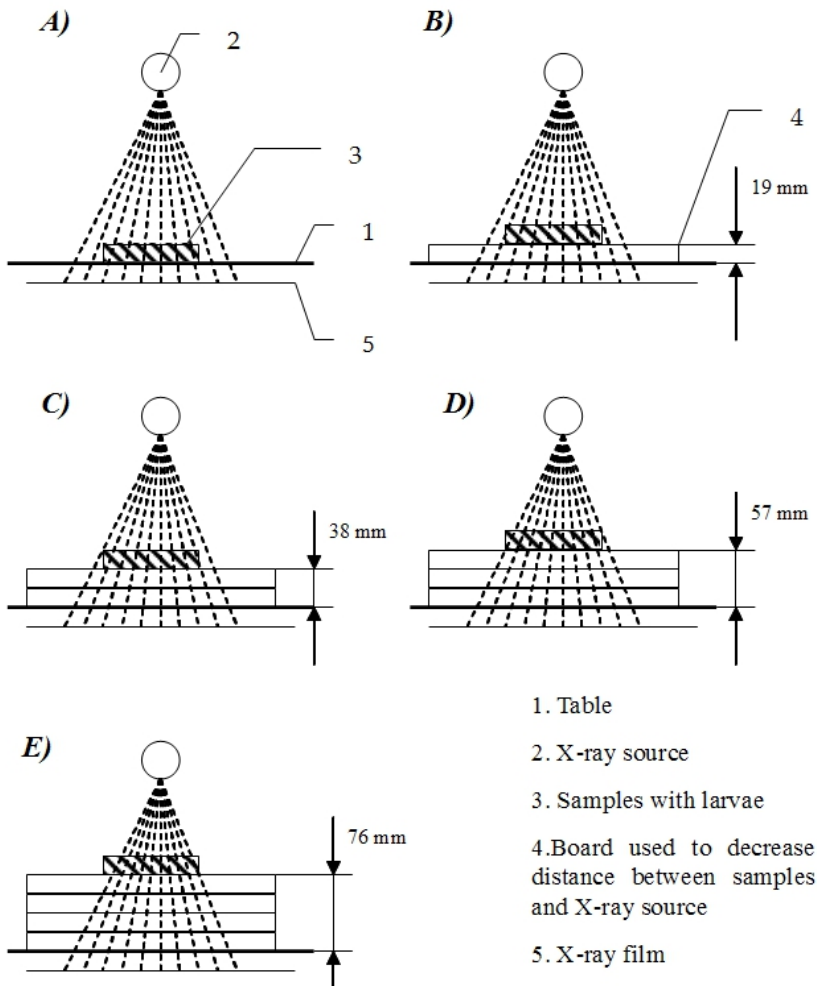


Fig. 2. Section of bundle of wood blocks tested in second stage of experiment

Next, the array with the old house borer larvae feeding on the wood was X-rayed. 5 X-ray pictures of the bundle were taken, each time adding a 19 mm Scots pine wood plank (fig. 2). The planks were added in order to simulate an increasingly unfavourable location of the larvae relative to the detector (in this case, the X-ray film).

When traditional X-ray imaging and digital techniques were compared, X-ray film pictures were chosen as more practical, considering the conditions of the insectarium where a lot of dust is created when the wooden farm blocks are cracked. In the examination, a MOBILAX 201 (Farum, Poland) X-ray camera was used (typically utilised in veterinary clinics). The X-ray pictures were taken using KODAK Medical X-ray Film (General Purpose Blue).

When the photographs had been taken, the lengths of the larvae images on the films were measured. In the case of bent individuals, the measurement was based on the length of an elastic thread, arranged on the image along the body axis of the larva. Of course, some of the larvae may have been bent non-parallel to the axis of the sample, which may have influenced the results. However, the small thickness of the samples limited the risk to a degree. Next, the blocks were cracked, the larvae were extracted and their exact lengths were measured with an accuracy of 0.5 mm. The exact lengths of the larvae were compared with the lengths of their X-ray images and the percentage differences were calculated.

## Results

The results of the first stage of the experiment are shown in figure 3.

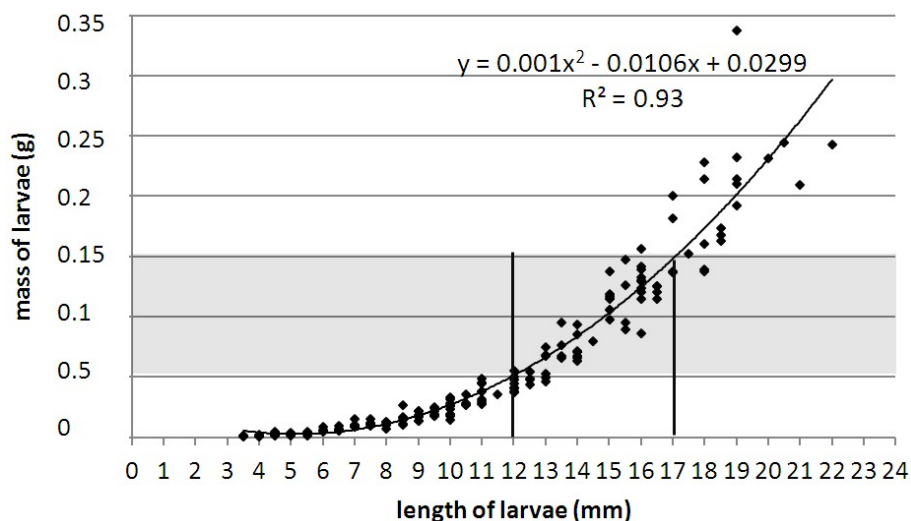


Fig. 3. Relationship between the mass of the old house borer larvae and their body lengths

The relationship between the mass of the larvae and their lengths measured on the X-ray images can easily be described using the following formula:

$$y = 0.001 x^2 - 0.0106 x + 0.0299$$

The coefficient of determination for this relationship is  $R^2=0.93$ .

According to the determined relationship, it can be assumed that mass ranging from 0.05 g to 0.15 g correspond to body lengths ranging from 12 mm to 17.5 mm, as measured on the X-ray image.

The results of the second stage of the experiment are shown in the table 1.

**Table 1. Impact of distance between wood layer with larvae and X-ray film on accuracy of measurement of larvae body lengths on X-ray image**

Percentage difference between real body length and measured length of the X-ray image	Number of wood layers decreasing the distance between larvae and X-ray source				
	0	1	2	3	4
	Number of accurate measurements of body lengths within given percentage ranges				
up to 10%	15	12	6	13	13
11-20%	4	7	12	4	7
above 20%	5	5	5	6	3

In the table, the numbers of the results are given from each measuring group (i.e. measured on X-ray pictures with 0, 1, 2, 3 and 4 additional wood layers), differing from the real body lengths within the given percentage ranges: 0-10%, 10-20%, and above 20%. The blurriness of some of the images of the larvae on the X-ray films made it impossible to measure their lengths. Such cases amounted to approximately 4 cases (3 to 5) on each photograph, i.e. almost 15% of all individuals. Thus, the total numbers of measured individuals shown in the table differ from 27.

## Discussion

It has been known for a long time that the old house borer is very widespread and, on a global scale, the damage caused by its larvae is considerable [Becker 1949; Becker 1970]. Although during the last fifty years the total number of buildings attacked by the old house borer has reportedly decreased due to the smaller number of entirely wooden buildings [Krajewski 1995, Dominik 2005], interest in research on this species as a pest has not waned [Chiappini and Nicolialdini 2011; Reddy 2011]. This fact justifies pursuing the large-scale laboratory breeding of the species for the normative investigation of wood

preservatives and other experimental aims, as well as for developing the techniques of extracting larvae for testing.

Due to the small thickness of the wooden blocks used in the examination, some of the problems identified by Fisher and Tasker [1940] and Bletchly and Baldwin [1962] did not arise. The mentioned above researchers stated, among other things, that wood-destroying larvae are difficult to detect in blocks of wood of big thickness. Old house borer larvae are usually bred in small wooden blocks (e.g.  $15 \times 23 \times 50$ mm). The quality of the images of old house borer larvae on an X-ray picture depends on the quality of the X-ray technology applied and on the expertise of the personnel operating the X-ray equipment. This fact is affirmed, for example, by the excellent results obtained by Bletchly and Baldwin [1962], who were able to detect on film even small parasitic mites *Pediculoides ventricosus* (Newp.) and quite small parasitoidal wood-boring insects, e.g. *Theocolax formiciformis* (Westw.). However, such good results have not always been obtained. In the case of X-ray films, at present the choice ranges between only a few companies, although as a rule, it is more common to apply the digital recording of X-ray pictures. It has been ascertained that on the Kodak films used in the experiment adults and pupae of old house borer are not easily distinguished. Fisher and Tasker [1940] also claimed that the adults of wood-boring beetles are not easy objects to detect in wood. Their claim may have applied, however, to dead individuals, and relating to their mummification, as stated by Krajewski and Witomski [2004]. It is difficult to conduct a comprehensive discussion of image quality as related to older publications on X-ray imaging due to considerable changes in the X-ray film market since the mid-twentieth century, as well as the use nowadays of the digital recording of X-ray pictures. The digital recording of X-ray photographs results in the necessity to print the images in order to use them in the insectarium conditions, where a significant amount of dust can appear during wood cracking. As initially established, the digital recording of X-ray pictures does not eliminate the above-mentioned problems with interpreting the wood-boring insects tested [Krajewski and Witomski 2004]. For this reason, in this study, X-ray films made by Kodak were used. In the case of small laboratories, the entomologist performing the examination on wood borer larvae does not take the X-ray photographs himself but is forced to cooperate with independent personnel operating the X-ray equipment, e.g. in veterinary clinics, who are commissioned to execute the task.

According to some opinions, the larvae of old house borer exhibit a linear growth in body mass up to 200 mg [Rasmussen 1961]. Based on the results of the authors of the present article, it seems that the relationship between the mass of the larvae and their body lengths can be described by the means of a quadratic polynomial. Glaring measurement errors of larvae lengths in the X-ray picture (more than 20%) can be associated with the non-parallel position of individuals against the surface of the film, or detector, in the case of the digital recording of

the image. No serious measurement errors were noted for the measurement of body lengths due to the bigger distance between an individual and the film as a result of simulating the bigger thickness of the X-rayed wood by adding more layers of wooden planks.

## Conclusions

The executed experiments show that X-ray pictures may not always faithfully illustrate the real lengths of larvae. However, the accuracy of this measurement is high enough to serve the purpose of extracting biological testing material containing individuals with mass ranging from 0.05 g to 0.15 g. The mass of the larvae from this range is related to the body lengths ranging from 12 mm to 17.5 mm, as measured on the X-ray image.

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### List of standards

- EN 22:1993** Wood preservatives. Determination of eradicant action against *Hylotrupes bajulus* (Linnaeus) larvae (laboratory method)
- EN 47:2005** Wood preservatives. Determination of the toxic values against larvae of *Hylotrupes bajulus* (Linnaeus). Laboratory method

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