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AN ATTEMPT TO DETECT FULLY-GROWN HOUSE LONGHORN BEETLE LARVAE IN CONIFEROUS WOOD BASED ON ELECTROACOUSTIC SIGNALS

Initial comparative tests of a method for the detection of house longhorn beetle larvae (Hylotrupes bajulus L.) were carried out by means of recording the acoustic effects caused by these insects, as well as another instrumental method based on the use of X-ray pictures. Insofar as the X-ray method brings completely reliable results as regards the detection of the presence of fully-grown house longhorn beetle larvae in wood, the accuracy of situation assessment based on the electro-acoustic method is estimated to be approximately 70-80%, based on the results of the research presented herein. In this research the accuracy of situation assessment is understood as the correct determination of an estimated number of larvae and their condition. The differences in situation assessment accuracies using both methods were statistically verified by means of the Czebyszew method. Since the tests were initial in nature without the use of standard patterns, as these have not been developed yet, the obtained results are considered highly satisfactory.

Keywords: woodworm, wood, longhorn beetle, electroacoustic, instrumental detection, detection of insects, *Hylotrupes bajulus* L.

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Introduction

The answer to the question whether wood contains the living larvae of xylophagous insects which are destroying it, or only abandoned insect galleries, is a basic issue when it comes to taking action concerning the protection of wooden structures and objects. Most often the traditional method for situation assessment is used. Decisions are made based on a series of symptoms, which are often subjectively formulated by the person assessing. According to all handbook publications, amongst these symptoms are the following:

– occurrence of new outlets on the wood surface,
– so-called wood meal spilling out from the outlets, making little mounds or trickles on the wood surface,
– the presence in buildings or at storage yards of living or dead adult forms of a given pest species or its parasites and predators,
– in the case of some insect species the sounds of wood boring by larvae, which is audible to the naked ear.

These symptoms would often be unreliable, therefore attempts to apply instrumental methods to the assessment of wood infestation by insects have been made. The instrumental methods for the detection of xylophagous insects in wood have aroused great interest for many years. However, not all the instrumental methods may be used in practice, especially in field conditions. X-ray photography and CAT scanning, which are examples of effective methods, cannot be classified as field test methods. Video-endoscopy does not enable an insight into the wood inside, but offers only an image of inaccessible structural parts, which must be examined during a traditional examination.

Previous laboratory attempts to apply electro-acoustics to the detection of feeding house longhorn beetle larvae were made in Europe, i.e. in Germany [Kerner et al. 1980; Plinke 1991; Esser et al. 1999] and Poland [Bobiński et al. 2006; Krajewski et al. 2011].

Even in the case of such an unquestionable symptom as the sounds of wood boring by house longhorn beetle larvae, there are some classification issues nonetheless – the insects do not make a sound when the evaluator assessing the feeding grounds would like them to. This may be a result of thermal conditions or periodic inactivity of the larvae due to other reasons. One should also take into consideration that so-called background may overlap with the electro-acoustic record of the vital activity of the larvae. If the house longhorn beetle larvae are large and make a lot of "noise", sometimes they may create effects that may be misinterpreted if heard by the naked ear. The development and perfecting of a method which would enable the recording of some of the vital activities of xylophagous and under-the-bark insects and the interpretation of the number or behaviour of these animals based on the obtained results, would also (besides being of practical use) enable basic research on some aspects of the biology of some species. Based on the promising results of the recording of the electro-acoustic signals of the house longhorn beetle [Bobiński et al. 2006], an assessment of the possibilities of detecting the larvae of this species, using a simplified simulation of conditions in a large-dimensional wooden structure, was carried out at SGGW in Warsaw in the Faculty of Wood Technology in the Wood Protection Department.

Materials and methods

The effects of the movements of house longhorn beetle larvae's maxillae and bodies in the feeding ground were recorded electro-acoustically. Based on the recordings, an attempt to determine the activity of these insects in wood was made together with an attempt to define the presence and approximate number of individual organisms based on the activity determination. The research was carried out in the form of laboratory tests simulating field conditions, taking into account constraints resulting from the lack of developed standards and thus the lack of objectivity showed by the people classifying the presence of the larvae in the galleries.

The house longhorn beetle larvae (*Hylotrupes bajulus* L.) used in the research were fully-grown individual organisms of a mass ranging from 30 to 190 mg. A simplified simulation of conditions found within a large-dimensional structure was used. The insects were placed in 200 mm blocks (sections) of structural wood with a cross-section of 60×120 mm. The experiment was carried out on Scots pine (*Pinus sylvestris* L.) and silver fir (*Abies alba* Mill.). A larvae-free Norway spruce (*Picea abies* (L.) Karst.) was also used as the equivalent of a *placebo* used in medical science. All the blocks were randomly marked with letters from A to L.

The larvae were placed in pine and fir wood, 10 individual organisms per block. All the blocks, including the larvae-free ones, had holes bored to place the larvae, holes which were plugged with compacted wood meal left by feeding insects. In the case of larvae-free blocks, wood meal was artificially put in the holes and compacted. The aim of simulating the places seemingly bored by the larvae was to avoid a situation, where evaluators are influenced by the initial number of the test material. The larvae were placed in the holes bored in the blocks' fronts. The holes were twice as deep as a larva's body length, and a diameter approximately 0.5 mm larger than that of a larva's body-width at the widest point. The larvae placed in the wood were left there for 4 weeks in an incubator at a temperature of 28°C, which gave the insects time to bore into the wood.

The research aimed at an objective evaluation of the effectiveness of house longhorn beetle larvae detection in wood. The lack of knowledge about the blocks' contents, which was characteristic of evaluators assessing the recording of electro-acoustic activity of the larvae, was achieved in the following way. Individual blocks with the larvae were subjected to an asphyxiating atmosphere of nitrogen for various periods with a view to causing a different death rate of these insects. The time needed to ensure mortal effects from a low-oxygen atmosphere was determined in separate research devoted to the effectiveness of combating xylophagous insects with nitrogen and argon, the results of which will be published in the future. Due to these actions, the number of living larvae in particular blocks was not known to any of the observers interpreting the results of the experiment. The adopted method for misinforming the observers interpreting the record of the blocks' contents, enabled a reduction in the possibility of them being influenced (while evaluating the larvae's activity) by the knowledge of the number of the larvae placed, as indicated by the number of holes bored in the front of each sample. After gas treatment, the block containing the larvae were mixed with the larvae-free blocks, which also had 10 bored holes in their fronts plugged with wood meal.

The presence of larvae in wood was recorded using a prototype apparatus built at Warsaw University of Technology at the Faculty of Electronics and Information Technology in the Electro-Acoustic Institute. The apparatus was previously used to identify the acoustic characteristics of a single feeding larva of xylophagous insects [Bobiński et al. 2006]. This apparatus processes all and any acoustic waves carried by wood into electric signals. The apparatus is equipped with, among other things, an acceleration sensor, the signal of which was strengthened by a charge preamplifier. Signals were recorded by a sound card and presented in the form of a graphical record on a computer monitor. The signals were recorded as a level of signal amplitude in relation to the maximum amplitude that can be registered by the sound card, i.e. an amplitude, which may be achieved by the maximum regulation of an analogue-digital converter.

The average density of the pine blocks was 440 kg/m^3 and their average ring width was 4.2 mm. The average density of the fir blocks was 448 kg/m^3 and their average ring width was 5.0 mm. To record the electro-acoustic activity of the larvae, the sensor was placed in the middle of a block's front 60×120 mm (situation α), therefore at its cross-section. For comparative reasons, in the case of most blocks a recording of the electro-acoustic effects transversely to the grain was carried out as well, i.e. the sensor was placed in the middle of a sample's surface 120×200 mm (situation β).

The air (ambient) temperature during the recording of the acoustic signals ranged from 20°C to 24°C: for samples A-D approximately 21°C on average and for samples H-L approximately 20°C on average. The acoustic signals were recorded for 24 hours.

The obtained results were classified independently by three evaluators. The evaluators were marked with numbers from 1 to 3. The following recording times were analysed: 24 hours, 2 hours and 20 minutes of recording time. In order to present multi-hour records graphically, the recording effects were compressed by computer. To assess the activity and contents of the feeding grounds, the records of electro-acoustic activity of the larvae, recorded when the sensor was placed in the middle of a block's front 60×120 mm (situation α), were used. The records taken when the sensor was placed in the middle of a sample 120×200 mm (situation β) did not substantially differ from the first variant. The assessments of particular evaluators are given in table 1. It was assumed that the recording of electro-acoustic activity of the "entomological contents" of the feeding grounds characterised the presence and number of the larvae in the wood. The following scale of the electro-acoustic assessment of the feeding grounds' activity, which should stem from the following number of living larvae in a block, was applied:

– no record of activity – lack of larvae in the wood or all larvae are dead,
– low activity recorded – the equivalent of the presence of a small number of living larvae in the wood (1–4 individual organisms),
– high activity recorded – the equivalent of the presence of a large number of living larvae in the wood (5–10 individual organisms).

Table 1. Evaluation of electroacoustic signals as a result of the presence, activity and abundance of old house borer larvae (marked in bold are accurate assessment)
Tabela 1. Ocena elektroakustycznej aktywności, będącej wynikiem obecności, czynności i liczebności żywych larw spuszczela pospolitego w drewnie iglastym (pogrubionym drukiem zaznaczono trafne oceny)

Oznaczenie klocka, gatunek drewna i teoretycznie zakładana aktywność larw (na podstawie zawartości klocka) *Determination of sample, wood species and theoretically expected activity of the larvae (based on the content of the sample)*	Liczba żywych larw w momencie prowadzenia nasłuchu *The number of live larvae at the time of listening*	Aktywność larw w drewnie według obserwatora nr 1 *Activity of larvae in the wood according to observer 1*	Aktywność larw w drewnie według obserwatora nr 2 *Activity of larvae in the wood according to observer 2*	Aktywność larw w drewnie według obserwatora nr 3 *Activity of larvae in the wood according to observer 3*
1	2	3	4	5
A, sosna, aktywność mała *A, pine, little activity*	2 żywe larwy *2 live larvae*	brak no	**mała** ***little***	**mała** ***little***
B, sosna, aktywność duża *B, pine, substantial activity*	10 żywych larw *10 live larvae*	**duża** ***substantial***	**duża** ***substantial***	**duża** ***substantial***
C, jodła, aktywność mała *C, fir, little activity*	1 żywa larwa *1 live larva*	brak *no*	brak *no*	brak *no*

Table 1. Continued
Tabela 1. Ciąg dalszy

1	2	3	4	5
D, jodła, aktywność duża *D, fir, substantial activity*	10 żywych larw *10 live larvae*	**duża *substantial***	**duża *substantial***	**duża *substantial***
E, świerk, brak aktywności *E, spruce, no activity*	nie obsadzono larw *larvae not inserted*	**brak *no***	**brak *no***	**brak *no***
F, jodła, aktywność duża *F, fir, substantial activity*	8 żywych larw *8 live larvae*	**duża *substantial***	**duża *substantial***	**duża *substantial***
G, sosna, aktywność duża *G, pine, substantial activity*	6 żywych larw *6 live larvae*	mała *little*	**duża *substantial***	**duża *substantial***
H, świerk, brak aktywności *H, spruce, no activity*	nie obsadzono larw *larvae ot inserted*	**brak *no***	**brak *no***	**brak *no***
I, świerk, brak aktywności *I, spruce, no activity*	nie obsadzono larw *larvae not inserted*	**brak *no***	**brak *no***	**brak *no***
J, sosna, aktywność mała *J, pine, little activity*	2 żywe larwy *2 live larvae*	**brak *no***	**brak *no***	**brak *no***
K, jodła, brak aktywności *K, fir, no activity*	brak żywych larw w wyniku duszącego działania azotu *no live larvae as a result of choking action of nitrogen*	**brak *no***	**brak *no***	**brak *no***
L, świerk, brak aktywności *L, spruce, no activity*	nie obsadzono larw *larvae not inserted*	**brak *no***	**brak *no***	**brak *no***
Udział prawidłowych ocen sytuacji *The share of the correct assessment of the situation*	→	67%	83%	83%

Following this, X-ray photographs of the samples were taken in order to see the positions of the larvae, and then the wood was chipped. The X-ray photographs were verified by the assessment of the larvae's condition by means of direct observation after the blocks were split into very small pieces of wood in which no larva could hide. The movement was considered an attribute of a living larva – the insects brought out from the wood were regarded as alive if they showed liveliness, either spontaneously or when mechanically irritated.

The results of the actual "entomological contents" of the 12 blocks, accurately imaged in the X-ray photographs, were compared with the assessments carried out by each of the 3 evaluators drawing conclusions based on the recorded electro--acoustic signals. If the evaluator's assessment of the feeding ground conditions (the presence of living larvae and their number within the assumed limits), carried out separately for each block based on the electro-acoustic signals, was correct, then it was marked with the number 1, if the assessment was wrong, then it was marked with the number 0. In the case of the X-ray photographs, all 12 results of the interpretations were correct and their assessment number was 1. Hence, these results became an element of the verification of the accuracy of the assessment carried out by means of classifying the electro-acoustic recordings as particular categories. For the 12 cases of assessment carried out by each evaluator separately, an arithmetic mean was calculated. Using Czebyszew inequality, the significance of the difference between an average from a given evaluator's assessments of the blocks' contents, carried out by means of recording the electro-acoustic activity of the larvae, and the average from classifications made based on the X-ray photographs, which corresponded with the visual assessment of the blocks' contents carried out after they were split, was determined.

Using this method, the absolute difference between the arithmetic mean from the assessments carried out separately by each evaluator and the mean from classifications based on the X-ray photographs were compared, with a triple standard error in the difference of these arithmetic means:

$$|\bar{x}R - \bar{x}N| \geq 3 \cdot \varepsilon\,(\bar{x}R - \bar{x}N) \qquad (1)$$

while:

$$\varepsilon\,(\bar{x}R - \bar{x}N) = \sqrt{}\,[\Sigma\,(\bar{x}R - xR_1)^2 + \Sigma\,(\bar{x}N - xN_1)^2] : n\,(n-1) \qquad (2)$$

where: $\bar{x}R$ – an average assessment based on the X-ray photographs,

xR – classification of the "contents" of block no. "i" based on the X-ray photograph,

$\bar{x}N$ – an average assessment based on the interpretation of the electro-acoustic effects carried out by evaluator N,

xN_i – classification of the assessment of evaluator N concerning block no. "i",

n – number of compared assessments (corresponding with the number of blocks).

In the case that the first inequality (or equation) is satisfied, the difference between the means is significant, otherwise the difference may be incidental. In order for the method to hold promise for practical use, the difference between the average assessment carried out by each evaluator and the average assessment resulting from the X-ray photographs (which corresponds with the actual situation evaluated after the blocks are split) should be insignificant.

Results and discussion

The levels of amplitudes of the recorded electro-acoustic impulse range from approximately -20 to 0 dB on average in relation to a maximum amplitude, which may be recorded by the sound card. These are levels recordable by the sound card. The 20-minute recordings, cut from a multi-hour recording, proved to be the most useful for an assessment of the presence of the larvae in the wood and determination of their number. The examples of the recordings of the electro-acoustic activity of the numerous larvae in the wood and of the lack of larvae, but with background interference, are presented in fig. 1.

The results of the assessment of the presence and number of the larvae in the wood are given in table 1.

Comparing the absolute difference between the arithmetic means obtained from the assessment carried out using the recording of electro-acoustic effects and X-ray photographs with a triple value of the standard error between them, it was observed that there was no statistically significant difference between the assessment of the condition of the blocks' "contents" carried out using the recording of the electro-acoustic activity of the larvae and the actual condition.

In the case of evaluator no. 1 it was $0.33 < 0.43$, and in the cases of evaluators no. 2 and no. 3 it was $0.17 < 0.34$.

Previously only the recordings of the electro-acoustic activity of a single house longhorn beetle larva were published, without any classification concerning the effectiveness of the determination of the larvae number in wood [Kerner et al. 1980; Plinke 1991; Bobiński et al. 2006; Krajewski et al. 2011]. It was observed that the sound signals emitted by house longhorn beetle larvae during feeding encompass the frequency range hovering at 10 kHz, while in the case of house furniture beetle larvae (*Anobium punctatum* De Geer) this range hovers at approximately 20 kHz [Esser et al. 1999].

Despite the lack of a previously developed reference scale, in the case of the test results described herein, in most cases the three evaluators correctly classified the approximate number of larvae in the wood. However, none of the evaluators achieved 100% classification accuracy, although the "eavesdropped" larvae quite often may be audible to the naked ear. Difficulties in the interpretation of the records might have been caused by the very limited activity of some larvae in the blocks, larvae which beforehand were subjected to the asphyxiating atmosphere of nitrogen for a longer time. The insects probably needed a relatively long time to get over the modified atmosphere with the reduced content of oxygen in favour of nitrogen. In such cases, the presence of a living larva might not be revealed as a recording of electro-acoustic activity corresponding with a healthy insect.

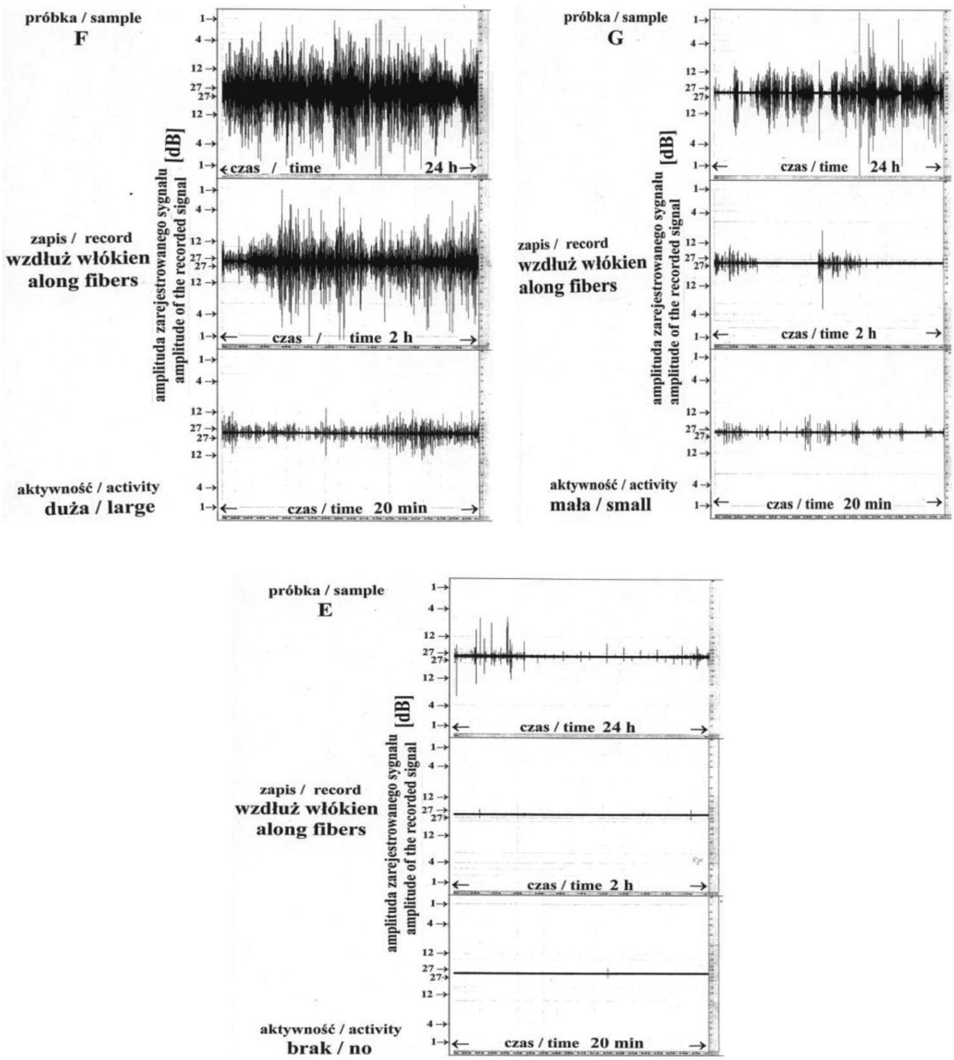

Fig. 1. Varying examples of electroacoustic activity of old borer house larvae in wood: F – large, G – small and E – zero, when 24-hour recordings are compressed and 2-hour and 20-minute fragments are isolated from them

Rys. 1. Przykład zapisu różnej intensywności elektroakustycznej aktywności larw spuszczela pospolitego w drewnie: F – dużej, G – małej i E – zerowej przy skompresowaniu wyników odsłuchiwania w czasie 24 h oraz wyjęciu z nich okresów 2 h i 20 min

Research on the electro-acoustic method for the detection of insects in wood are continuing in the Wood Protection Institute in co-operation with the Electro--Acoustic Institute, with a view to applying it in practice and in discovering some aspects of the biology and behaviours of both xylophagous and under-bark species.

It should be added that research on the use of this method for the detection of the presence of the larvae of a dangerous species of Asian longhorn beetle (*Anaplophora glabripennis*) (carried to Europe) in the wood of living and dying trees [Becker 2000] and also in the wood of pallets and crates [Fleming et al. 2005], as well as for the detection of various species of earth termites, arouses great interest all over the world. Hitherto the research on termites was carried out as laboratory [Lemaster et al. 1997] and field tests [Fujii et al. 1999; Mankin et. al. 2002; Dunegan 2005; Mankin, Benshemesh 2006].

Conclusions

Although the tests were relatively little advanced, the results obtained using the method of electro-acoustic recording of the activity of the grown larvae of the house longhorn beetle, were satisfying in terms of the assessment of the contents of the feeding grounds of this insect. It can be assumed that this method holds great promise of success, provided it is further improved, and may probably be applied to the detection of wood pests and to the entomology used in wood science and forestry when some aspects of the biology of xylophagous (and probably also cambium-feeding) insects are researched. The average accuracy of the assessments of 3 observers evaluating the activity of the house longhorn beetle larvae hovered at approximately 70-80%, and the average results of the assessment of the feeding ground contents, obtained by this method, were not statistically different from the actual contents of the tested wood samples determined by examination of the X-ray photographs and by evaluation.

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PRÓBA WYKRYWANIA WYROŚNIĘTYCH LARW SPUSZCZELA POSPOLITEGO W DREWNIE IGLASTYM NA PODSTAWIE SYGNAŁÓW ELEKTROAKUSTYCZNYCH

Streszczenie

Przeprowadzono wstępne badania porównawcze metody wykrywania larw spuszczela pospolitego (*Hylotrupes bajulus* L.) za pomocą rejestracji efektów akustycznych powodowanych przez te owady z inną metodą instrumentalną, opartą o wykorzystanie zdjęć rentgenowskich. O ile metoda rentgenowska daje całkowicie pewne wyniki w stosunku do wykrywania obecności wyrośniętych larw spuszczela pospolitego w drewnie, to poziom trafności oceny sytuacji przy użyciu metody elektroakustycznej szacuje się na podstawie rezultatów niniejszych badań na ok. 70-80%. Jako trafność oceny sytuacji rozumiano tu prawidłowe określenie szacunkowej liczby larw i ich stanu. Różnice w trafności oceny sytuacji za pomocą obu metod poddano weryfikacji statystycznej przy użyciu metody Czebyszewa. Ponieważ były to wstępne badania bez użycia standardowych wzorców, które nie zostały jeszcze wypracowane, uznaje się uzyskane wyniki za bardzo zadowalające.

Słowa kluczowe: drewno, spuszczel pospolity, owady, elektroakustyka, metody instrumentalne, wykrywanie owadów, *Hylotrupes bajulus* L.