BS EN 46-2:2016

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Wood preservatives — Determination of the preventive action against recently hatched larvae of Hylotrupes bajulus (Linnaeus)

Part 2: Ovicidal effect (laboratory method)

bsi.

National foreword

This British Standard is the UK implementation of EN 46-2:2016. It supersedes BS EN 46-2:2009 which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee B/515, Wood preservation.

A list of organizations represented on this committee can be obtained on request to its secretary.

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Produits de préservation du bois - Détermination de l'action préventive contre les larves récemment écloses d'Hylotrupes bajulus (Linnaeus) - Partie 2: Effet ovicide (Méthode de laboratoire)

Holzschutzmittel - Bestimmung der vorbeugenden Wirkung gegenüber frisch geschlüpften Larven von Hylotrupes bajulus (Linnaeus) - Teil 2: Ovizide Wirkung (Laboratoriumsverfahren)

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European foreword

This document (EN 46-2:2016) has been prepared by Technical Committee CEN/TC 38 "Durability of wood and wood-based products", the secretariat of which is held by AFNOR.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by December 2016, and conflicting national standards shall be withdrawn at the latest by December 2016.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN 46-2:2009.

Significant technical differences between this document and EN 46-2:2009 are as follows:

a) introduction of new harmonised specifications for wood quality;

b) option to omit control test specimens treated with the solvent or diluents only when the solvent or diluents is water of drinking quality.

The standard EN 46 is composed of two parts:

— EN 46-1, *Wood preservatives — Determination of the preventive action against recently hatched larvae of Hylotrupes bajulus (Linnaeus) — Part 1: Application by surface treatment (laboratory method)*

— EN 46-2, *Wood preservatives — Determination of the preventive action against recently hatched larvae of Hylotrupes bajulus (Linnaeus) — Part 2: Ovicidal effect (laboratory method)*

EN 46 consists of two parts to enable preventive action of wood preservatives, against recently hatched larvae of *Hylotrupes bajulus*, which are intended to be applied by surface treatment; Part 1 is required to determine the larvicidal effect of preservatives and Part 2 is required to determine the ovicidal action of the preservatives after egg-laying of young females.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Introduction

This test method describes a laboratory method of test which gives a basis for the assessment of the preventive action of a wood preservative, when applied as a surface treatment for timber, against eggs of *Hylotrupes bajulus.*

In combination with EN 46-1 it provides a means of checking whether larvae may hatch from eggs laid on the treated wood surface and whether they are capable of boring through the treated surface and of surviving in the untreated part of the wood.

This standard provides information for the sealing of all but one lateral face when specimens are to be treated by dipping.

When products which are very active at very low concentration are used, it is very important to take suitable precautions to isolate and separate, as far as possible, operations involving chemical products, other products, treated wood, laboratory apparatus and clothing. Suitable precautions should include the use of separate rooms, areas within rooms, extraction facilities, conditioning chambers and special training for personnel (see also Annex D for environmental, health and safety precautions).

1 Scope

This European Standard specifies a method for the determination of the preventive action of a wood preservative against eggs of *Hylotrupes bajulus* (Linnaeus) when the preservative is applied as a surface treatment to wood.

This method is applicable to:

— water-insoluble chemicals which are being studied as active insecticides;

— organic formulations, as supplied or as prepared in the laboratory by dilution of concentrates;

— organic water-dispersible formulations as supplied or as prepared in the laboratory by dilution of concentrates; or

— water-soluble materials, for example salts.

The method is applicable whether or not the test specimens have been subjected to appropriate ageing procedures.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 46-1, *Wood preservatives — Determination of the preventive action against recently hatched larvae of Hylotrupes bajulus (Linnaeus) — Part 1: Application by surface treatment (laboratory method)*

EN 73, *Wood preservatives — Accelerated ageing of treated wood prior to biological testing — Evaporative ageing procedure*

EN 84, *Wood preservatives — Accelerated ageing of treated wood prior to biological testing — Leaching procedure*

EN ISO 3696, *Water for analytical laboratory use — Specification and test methods (ISO 3696)*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
representative sample
sample having its physical and/or chemical characteristics identical to the volumetric average characteristics of the total volume being sampled

[SOURCE: EN 1001-2:2005, 4.71]

3.2
supplier
sponsor of the test (person or company providing the sample of wood preservative to be tested)

Note 1 to entry: Adapted from EN 1001–2:2005, 4.83.

4 Principle

In this laboratory method treated wood panels are offered to freshly mated *Hylotrupes bajulus* females. The hatching ability of the larvae on the treated timber is examined. When the ovicidal action is insufficient, the mortality of the hatched larvae on and/or in wood treated with the same formulation is also established according to EN 46-1.

5 Test materials

5.1 Biological material

5.1.1 ***Hylotrupes bajulus*** **(Linnaeus)** females.

5.1.2 Source of females

The insects shall preferably be obtained from cultures reared as e.g. described in Annex B.

Use only sound and lively insects.

5.2 Products and reagents

5.2.1 **Paraffin wax**, for fixing the glass plate in all cases and for sealing the end faces of test specimens to be treated with solutions in all cases in which water is the continuous phase.

NOTE Paraffin wax with a setting point of 52 °C to 54 °C has been found to be suitable.

5.2.2 **Gelatine**, for sealing the end faces of test specimens to be treated with solutions in which an organic solvent is the continuous phase.

5.2.3 **Water**, complying with grade 3 of EN ISO 3696.

5.2.4 **Solvent or diluent**, a volatile liquid that will dissolve or dilute the preservative but does not leave a residue in the wood at the end of the post-treatment conditioning period that has a toxic effect on the insects.

CAUTION — Do not use benzene or other solvents which pose a health risk.

5.3 Apparatus

5.3.1 **Culturing chamber**, with air circulation, and controlled at (28 ± 2) °C and at relative humidity (70 ± 5) %.

5.3.2 **Conditioning chamber**, well ventilated and controlled at (20 ± 2) °C and at relative humidity (65 ± 5) %.

The conditioning of test specimens may be carried out in the laboratory work area (see 5.3.3) provided that this has the conditions specified for the conditioning chamber (see 5.3.2).

5.3.3 Laboratory work area, well ventilated, where treatment of the test specimens is carried out.

CAUTION — It is essential to follow safety procedures for handling flammable and toxic materials. Avoid excessive exposure of operators to solvents or their vapours.

5.3.4 Testing chamber, ventilated and air-conditioned, controlled at (22 ± 2) °C and at relative humidity between (70 ± 5) %.

5.3.5 Petri dishes of glass or polyvinylchloride (PVC), diameter ca. 9 cm for mating the insects and for egg-laying.

5.3.6 Weights, to provide ballast for the test specimens.

The weights shall not react with any materials with which they come into contact during the test.

5.3.7 Safety equipment, protective clothing, appropriate for the test product and the test solvent, to ensure the safety of the operator.

5.3.8 Glass plates, (48 ± 1) mm long and (25 ± 1) mm wide, intended to provide a lateral slit on the test specimens.

5.3.9 Ordinary laboratory equipment, including a balance capable of weighing to an accuracy of 0,01 g and equipment for applying a liquid product by brushing or by pipette.

5.3.10 Protective gloves

6 Sampling

The sample of preservative shall be representative of the product to be tested. Samples shall be stored and handled in accordance with any written recommendations from the supplier.

For the sampling of preservatives from bulk supplies, the procedure given in EN 212 should be used.

7 Test specimens

7.1 Species of wood

The reference species is Scots pine (*Pinus sylvestris* Linnaeus)[1)].

Additional tests may be carried out using other species but, if so, this shall be stated in the test report.

7.2 Wood quality

The wood shall be free from visible cracks, stain, decay, insect damage and other defects. The wood shall not have been water-stored, floated, chemically treated or steamed. The wood shall originate from trees preferably felled in winter. The trees shall be cut immediately after felling

1) In southern European countries the pine species most frequently infested by *Hylotrupes bajulus* may be used as an alternative, provided that the suitability of the species for use in the tests specified in this standard has been demonstrated in all aspects (development of larvae, resistance of impregnation, etc.).

and the timber rapidly air dried or kiln dried at temperatures below 60 °C. The wood shall not have been stored for more than five years.

The wood shall be exclusively sapwood containing little resin and having between 2,5 annual rings per 10 mm and eight annual rings per 10 mm. The proportion of latewood in the annual rings shall not exceed 30 % of the whole.

It is recommended to use test specimens of similar growth rate within a single test.

7.3 Provision of test specimens[2)]

Prepare planed strips having a cross-section of (25 ± 0,5) mm × (15 ± 0,5) mm removing a minimum of 2 mm from any surfaces exposed during drying. The longitudinal faces shall be parallel to the direction of the grain. The annual rings shall have a contact angle of 45° ± 15° to the broad faces. Make transverse cuts, neatly to give sharp edges and a fine-sawn finish to the end-grain surfaces, to give test specimens (50 ± 0,5) mm long.

The test specimens shall originate from a minimum of three trees or shall be taken at random from a stock originally of more than 500 test specimens.

7.4 Dimensions of test specimens

The dimensions of each test specimen after reaching equilibrium in the conditioning chamber (5.3.2) shall be (50 ± 0,5) mm × (25 ± 0,5) mm × (15 ± 0,5) mm.

Mark each test specimen so that it can be identified throughout the test.

7.5 Number of test specimens

7.5.1 Test specimens for egg-laying

a) Six treated test specimens (no more than two originating from the same tree unless taken at random from a stock of more than 500) for each preservative, each concentration and each duration of treatment;

b) three untreated control test specimens (each originating from a different tree unless taken at random from a stock of more than 500) for a complete test of any given preservative;

c) three control test specimens treated with the solvent or diluent (5.2.3 or 5.2.4) (each originating from a different tree unless taken at random from a stock of more than 500) if a solvent or diluent (including water) is used.

Control test specimens under c) may be omitted if the solvent or diluents is water of drinking quality

When dipping is to be used (8.1.3.3) it is advisable to treat more than the specified number of test specimens so that, after weighing, any test specimens with abnormally high or low retentions can be rejected from the batch.

NOTE To gain further information on a formulation, the manufacturer may find it useful to test a version of the preservative where the active ingredient(s) has been removed.

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2) For special tests, test specimens may be obtained according to a given series. As a result, it may be preferable to take test specimens from pretreated strips. Where pretreated strips are used details should be included in the test report.

7.5.2 Test specimens for checking the tunnelling ability and the mortality of the larvae

In addition to the test specimens for egg-laying at least six test specimens shall be prepared for each preservative concentration and retention for checking the tunnelling ability and the mortality of the newly hatched larvae.

8 Procedure

8.1 Preparation of the test specimens

8.1.1 Conditioning of the test specimens prior to sealing

Allow the test specimens to condition in the conditioning chamber (5.3.2) for a minimum of two weeks.

8.1.2 Sealing of block faces

8.1.2.1 General

When treatment is to be by brushing or by pipette then only the transverse faces of test specimens shall be sealed. When treatment is to be by dipping then all faces, except one 25 mm x 50 mm face, shall be sealed. The material used for sealing shall be resistant to the penetration of wood preservatives under test. The sealings specified in 8.1.2.2 and 8.1.2.3 have been proven as suitable.

8.1.2.2 For tests with preservative solutions in which water is the continuous phase, apply three coats of the paraffin wax (5.2.1) at about 90 °C so that the first coat adheres closely to the wood and the successive coatings bond to one another. Condition the sealed test specimens in the conditioning chamber (5.3.2) for at least one day.

8.1.2.3 For tests with preservative solutions in which the continuous phase is an organic solvent that dissolves paraffin wax, use the gelatine (5.2.2): apply the first coat as an aqueous solution of 200 g/l at 40 °C, then after a minimum of 8 h of drying, apply two further coats of an aqueous solution of 300 g/l at 50 °C. Condition the sealed test specimens in the conditioning chamber (5.3.2) for at least one day.

8.1.3 Treatment of the test specimens

8.1.3.1 Preparation of the treatment solutions

8.1.3.1.1 Solid preservatives

— Water-soluble preservatives:

Dissolve the preservative in the water (5.2.3) to the concentration recommended by the manufacturer.

— Non-water-soluble preservatives:

Dissolve the preservative in an appropriate solvent (5.2.4) to the concentration recommended by the manufacturer.

All treatment solutions shall be freshly prepared.

8.1.3.1.2 Liquid preservatives

If appropriate, use the preservative without further preparation other than any necessary stirring. If it is a concentrate, dilute the preservative with the diluent specified by the manufacturer to the required working concentration.

A formulation of the preservative to be tested without active ingredients shall also be included in the test.

All treatment solutions shall be freshly prepared.

8.1.3.2 Treatment by brushing or by pipette

In the laboratory work area (5.3.3) apply the preservative either by brushing or by pipette to the 50 mm x 25 mm lateral face that would have been furthest from the centre of the tree.

Depending on the type of treatment, the volume (application by pipette) or mass (application by brushing) of the treatment solution shall be determined to obtain the surface application specified by the manufacturer. Place the specimens such that the 50 mm x 25 mm lateral face that is to be treated is uppermost and apply the appropriate fluid uniformly to that face.

When the preservative is applied by brush, place the test specimens on a balance while being brushed to determine the amount of preservative applied to the nearest 0,01 g.

When the preservative is applied by pipette, move the pipette across the fibre direction and the amount of preservative applied shall be determined to the nearest 0,01 ml.

Several applications can be necessary to apply the required amount. In this case the coats should be applied sufficiently quickly to avoid any solidification of certain substances which can impede the penetration of further coats.

Care shall be taken to avoid fluid running off the lateral face being treated.

8.1.3.3 Treatment by dipping

When the treatment of specimens is by dipping all faces, except one 25 mm x 50 mm face, shall be sealed (see 8.1.2).

Weigh to the nearest 0,01 g any sealed test specimen, to obtain its initial mass.

Immerse one test specimen after the other in the treatment solution, moving it during dipping. The dipping time to be used shall be one of the following, agreed beforehand according to the purpose of the test:

— Either one 10 s period and/or two periods of 10 s at an interval of 24 h.

If the rate of solidification of some constituents of a preservative formulation would have the effect of retarding its penetration during the second dipping, this interval has to be reduced. The interval employed shall be mentioned in the test report.

— Or a period sufficient for a determined quantity to be retained by the test specimen[3)].

Using forceps, remove each test specimen from the preservative fluid and sponge off fluid from all the sealed faces of the specimen. Keeping the face that has not been sealed upper most, immediately weigh to the nearest 0,01 g.

In the case of water-soluble chemicals, for example salts, and water-insoluble chemicals which are being studied as active ingredients, calculate the mass of chemical retained for each test specimen from the mass of solution absorbed and its concentration.

In the case of organic formulations or organic water-dispersible formulations, the retention is expressed for each test specimen in terms of the corresponding mass of the formulation ready for use, but if a concentrate is supplied the retention is expressed in terms of the dilution applied.

Calculate the mass of preservative retained in grams per square meter of timber surface.

8.1.4 Drying and conditioning of the test specimens after treatment

If the end-sealing has been damaged before or after treatment, reject the test specimens concerned from the tests.

After treatment, condition the test specimens for four weeks in the environment specified for the conditioning chamber (5.3.2). Arrange the test specimens on their lateral narrow faces, resting on glass rods, not touching one another. Invert the test specimens twice a week.

NOTE The drying and conditioning of the test specimens depend on the nature of the product under test and on the solvent or diluent used. For slow drying products it may be necessary to extend the conditioning process.

If, in the case of slow drying products, the conditioning period is extended, the extended conditioning period shall be stated in the test report.

If the test specimens are to be subject to an ageing procedure (according to EN 73 or EN 84), this shall be carried out after this drying procedure.

8.2 Exposure of the test specimens to the insects (ovicidal action)

Mate young *Hylotrupes* females in Petri dishes (5.3.5). After mating transfer the females singly to other Petri dishes (5.3.5) with centrally placed test specimens (7.5.1). Place the treated face of the test specimen on the bottom of the Petri dish leaving a gap for egg-laying. The test specimens are now ready for egg-laying by the house longhorn beetle.

Egg-laying extends over seven days under the conditions of the testing chamber (5.3.4). After the first egg-laying remove the females.

In case less than ten eggs have been deposited on a test specimen, repeat the egg-laying procedure with a freshly mated female. In case no eggs have been laid within one week, repeat the procedure with up to three freshly mated females. In case no eggs have been laid and/or the insects have died state it in the test report.

When no eggs have been laid on treated test specimens, transfer those females to untreated test specimens to check their fertility. If eggs are now deposited, this is an indication of a repellent action of the preservative. State it in the test report.

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3) The dipping time depends upon the type of preservative and may extend to several hours for water-soluble preservatives. The progress of absorption is monitored by successive weightings of the treated test specimens. For this long period dipping the treated specimens are immersed together and kept submerged by the weights (5.3.6).

After egg-laying lift the test specimen carefully and very slowly from the bottom of the Petri dish and transfer it to another Petri dish by turning it by 90° (egg deposit on the lateral face). Place the test specimens with their long, narrow faces on 2 mm thick spacers to prevent hatching larvae from boring into the wood.

Determine the ovicidal action on at least six treated test specimens for each preservative and concentration. In case the number of test specimens with egg deposits is smaller, state it in the test report.

Examine daily the eggs laid on the test specimens for freshly hatched larvae in the testing chamber (5.3.4) and register their number. The larvae shall have hatched completely from the egg membranes and shall be mobile. Count the number of hatched and of dead larvae .

If a sufficient number of larvae have hatched, with each ten larvae of each clutch of eggs test their tunnelling ability into treated test specimens according to EN 46-1.

Proceed the same way with the control specimens.

8.3 Validity of the test

The test shall be considered valid if at least 70 % of the larvae introduced to all of the untreated control test specimens survive and, if applicable, at least 70 % of those introduced to all of the control test specimens treated with the solvent or the diluent alone, survive.

9 Expression of results

9.1 Ovicidal test

For each egg deposit the following results are registered:

— number of females used in the tests;

— number of eggs laid on the test specimens;

— number of hatched larvae;

— egg mortality (%).

If, subsequent to the tests to EN 46-2, a tunnelling ability of the larvae according to EN 46-1 is tested, the data will be recorded as described in 9.2 and the over-all mortality as calculated in 9.3.

9.2 Tunnelling control

The following results are registered:

— number of larvae placed on each test specimen;

— number of dead larvae which have not tunnelled;

— number of dead larvae which have tunnelled;

— total larval mortality (%);

— number of live larvae and the state of these larvae;

— number of larvae not retrieved;

— test duration: 4 or 12 weeks.

9.3 Total mortality

From the data recorded under 9.1 and 9.2, the total mortality (T (%)) is calculated:

$$T(\%) = e(\%) + \frac{[100 - e(\%)] \times l(\%)}{100}$$

where

e(%) is the egg mortality;

l(%) is the total larvae mortality.

10 Test report

The test report shall include at least the following information (see also Annex A for an example):

a) number and date of this European Standard;

b) name of the supplier of the preservative under test;

c) specific and unique name or code of the preservative tested, with an indication of whether or not the composition has been declared;

d) name and concentration of active ingredient;

e) solvent or diluent used;

f) species of wood used;

g) method of application and, if applicable, the concentration or concentrations of preservatives tested, expressed as mass fraction;

h) date of the application of the preservative;

i) for each test specimen treated:

   1) if dipping:

      i) mass, in g, of solution absorbed;

      ii) corresponding mass of test product per unit surface area, in g/m^2;

   2) if treating by pipette:

      i) volume of solution applied to the treated surface of the test specimen (or, where necessary), the volume of solvent or diluent in ml;

      ii) corresponding amount of the product under test in g/m^2 or ml/m^2;

   3) if brushing:

i) mass, in g, of solution applied to the treated surface of the test specimen (or, where necessary), the volume of solvent or diluent in ml;

ii) corresponding amount of the product under test in g/m^2 or ml/m^2;

j) method of drying the test specimens;

k) any ageing procedures carried out, specifying the type, conditions and duration, with possible reference to a standard;

l) date of exposure of the test specimens to the insects;

m) date of the insertion of the larvae into the test specimens;

n) date(s) of examination of the test specimens;

o) results obtained;

p) name of organization responsible for the test report and date of completion of the test;

q) name and signature of the officer(s) in charge of testing;

r) following note:

"The interpretation and the practical conclusions that can be drawn from this test report demand a specialized knowledge of the subject of wood preservation and, for this reason, this test report cannot of itself constitute an approval certificate."

s) any variation from the described test method and any factors that may have influenced the results.

Annex A
(informative)

Example of a test report

Number and date of this European Standard	EN 46-2:2016
Name of supplier	company S
Name and type of preservative	"Y" preservative in the form of an organic solvent, ready for use, formulation declared
Name and concentration of active ingredient in mass fraction	W 0,25 %
Density of the wood preservative	0,92 g/ml
Solvent or diluent used	none
Species of wood used	Scots pine (*Pinus sylvestris*) Linneaus)
Concentration of the preservative tested	preservative used undiluted
Type and number of treatments	applied by two brush coats
Date of application of preservative	2015-07-14
Quantity of preservative applied	100 g/m^2
Method of drying	As specified in EN 46-2
Method of ageing	by evaporation for 12 weeks according to EN 73
Date of exposure to insects	2015-10-07
Date of insertion of larvae	2015-10-21
Date of examination of the test specimens	2016-01-06
Results	See Table A.1.
This report has been prepared by the institute	ABC
Location and date	Z 2011-01-20
Name(s) and signature(s) of the officer(s) in charge	NN

NOTE The interpretation and the practical conclusions that can be drawn from this test report demand a specialized knowledge of the subject of wood preservation and, for this reason, this test report cannot of itself constitute an approval certificate.

Table A.1 — Determination of the preventive action against eggs and newly hatched larvae of the house longhorn beetle (*Hylotrupes bajulus* L.)

Type and number of test specimens		Concen-trations tested	Quantity of preservative applied	Hatching ability				Tunnelling ability					Total mortality
				Eggs per batch	Egg mortality per batch			Larvae recovered			Larvae not recovered	Larvae mortality	
							e(%)	Not having tunnelled	Having tunnelled	Live after tunnelling		l(%)	T(%)
		Mass fraction %	g/m²	n	n	%	mean					mean	mean
Control	1	-	-	95	9	9,5	8,9	0	0	9	1	3,3	12,0
	2	-	-	125	10	8,0		0	0	10	0		
	3	-	-	64	6	9,4		1	0	9	0		
Product Y	4	100	120	256	187	73,0	62,7	6	1	3	0	73,5	90,1
	5	100	120	88	55	62,5		3	2	4	1		
	6	100	120	64	20	31,3		5	2	3	0		
	7	100	120	145	98	67,6		8	0	2	0		
	8	100	120	98	75	76,5		6	1	2	1		
	9	100	120	100	65	65,0		5	2	1	2		
Product Y	10	100	150	65	60	92,3	97,9	5	5	0	0	100	100
	11	100	150	120	116	96,7		4	6	0	0		
Product Y	12	100	150	111	109	98,2		2	7	0	1		
	13	100	150	91	91	100	97,9	-	-	-	-		
	14	100	150	93	93	100		-	-	-	-		
	15	100	150	46	46	100		-	-	-	-		

Annex B
(informative)

Technique for culturing *Hylotrupes bajulus* (Linnaeus)

B.1 General

Before undertaking culturing of *Hylotrupes bajulus,* a basic knowledge of the biology of this insect should be acquired from literature and from official organizations conducting research into wood preservation.

B.2 Obtaining parent beetles

A culture can be begun by taking larvae from naturally infested wood and inserting them head first into suitably sized drilled holes in pine sapwood blocks as described under B.6 and allowing them to pupate in order to obtain reproductive adults.

It is necessary to ensure that the insects have not been in contact with toxic products or with treated wood at any stage in their development.

Adult beetles of the brown variety should be removed.

B.3 Mating

Place together a male and female adult on a face, cover them with a Petri dish lid and leave them in daylight, as *Hylotrupes bajulus* is a diurnal insect.

Quite soon. the male will approach the female and mating will take place with the male uppermost. Then separate the two insects as they will bite and damage one another if left confined together.

A male can fertilize two or three females a day.

B.4 Egg-laying

B.4.1 Isolate the females after fertilization and place them for egg-laying, using either of the techniques given in B.4.2 and B.4.3.

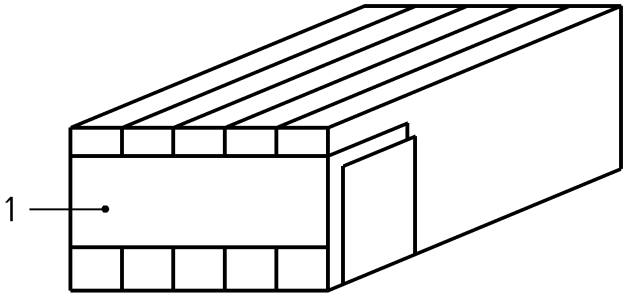
B.4.2 Place the females in glass bottles containing small blocks of pine sapwood, resting on a filter paper disc. The egg-laying will take place between the block and the filter paper on which they can be seen easily.

B.4.3 Place the females on the surface of pine sapwood blocks introduced in an appropriate vessel and which have been previously split into several pieces and then reassembled with adhesive tape placed at one end, as shown in Figure B.1, so that open cracks remain which diminish in width from the free end to the end bound with the tape. By removal of the tape, the different pieces of the block can be separated easily and any eggs laid in the cracks can be seen. Place the females singly on each composite block and cover with a glass lid.

It should be noted that mating repeated every day or every two days stimulates egg-laying.

Once a day check if eggs have been laid. If so, remove the filter paper or the block which carries them and give the female fresh blocks for further egg-laying, allowing continued mating in order to activate egg-Iaying.

A favourable temperature for egg-Iaying is about 25 °C.



Key

1 adhesive tape

Figure B.1 — Block with adhesive tape

B.5 Hatching of eggs

Place the surfaces (discs of filter paper or split blocks) on which eggs have been laid in such a manner that, on hatching, the young larvae fall into a glass vessel from which they cannot escape.

The optimum conditions for hatching are as follows:

— Temperature: about 28 °C;

— Relative humidity: about 80 %.

B.6 Larval development

Place the newly hatched larvae in culturing blocks, as described below. They should not have been deprived of food for more than three days before being inserted in the block. Handle the larvae carefully using a soft brush or vacuum tweezers.

As the larvae *Hylotrupes bajulus* eat one another, they should be kept apart during culturing by having one larva per block. Insert each larva into a hole, pierced with a bradawl at right angles to the grain of the wood, to a depth of 4 mm to 6 mm.

Culturing blocks shall be of pine sapwood. Prior to insertion of the larvae, impregnate the blocks under vacuum with an aqueous solution of 10 g/l peptone and 5 g/l yeast (yeast can be replaced with 0,01 g/l lactoflavine), then dry them. In this way, the duration of larval development can be shortened to about one-tenth of its natural length. The size of the blocks is not critical but they may conveniently be of dimensions 50 mm × 25 mm × 15 mm, the length being parallel to the grain of the wood.

The growth of *Hylotrupes bajulus* is most rapid between 29 °C and 30 °C with an optimum relative humidity of 97 % to 98 %, but very high humidities favour the development of moulds. It is therefore preferable to use a relative humidity of (70 ± 5) %.

When conditions of microclimate and nutrition are perfect, the male adult insects can appear at about six months. However, as soon as the larvae reach a size such that the volume of the blocks is insufficient to permit normal growth, it is preferable to transfer them into blocks of larger dimensions that are not impregnated with peptone and yeast, in which larval growth can be continued until pupation.

Larvae of *Hylotrupes bajulus* exhibit a long diapause but pupate more rapidly when they are exposed to low temperature. It is therefore advantageous to place the large blocks outdoors in winter at a temperature of 5 °C to 10 °C. In this way, a mass emergence of insects can be obtained, with a high proportion emerging within a short space of time. This is particularly suitable for establishing new cultures.

B.7 Enemies and parasites

Take care to avoid infestation by hymenopterous parasites and coleopterous predators by closing the rearing vessels with fine mesh grilles.

The insects likely to cause the greatest damage to *Hylotrupes bajulus* cultures are:

— *Rhoptrocentrus piceus* Marshall (*Braconidae*);

— *Scleroderma domesticum* Latreille (*Bethylidae*).

Experience has shown that special precautions against mites are unnecessary.

Annex C
(informative)

Differentiation of heartwood and sapwood in *Pinus* species

C.1 Principle

The phenolic compounds in the heartwood of *Pinus* species are detected by the formation of a coloured complex with o-dianisidine.

C.2 Reagents

o-dianisidine diazonium chloride solution, 20 g/l.

Dissolve 2 g of o-dianisidine diazonium chloride in 100 ml of water. Prepare freshly as required.

NOTE o-dianisidine diazonium chloride stabilized with zinc chloride may be purchased as 'Fast blue B salt'.

C.3 Apparatus

C.3.1 Spray equipment, capable of producing a fine even spray.

Both gas-powered aerosol units and small hand-powered units have been found to be suitable.

C.3.2 Soft paint brush, 5 mm to 50 mm wide, depending on the size of the timber section to be tested.

C.4 Procedure

Prepare the wood sample to give a clean surface and remove any loose sawdust. Brush or spray the solution (C.2) onto the timber section.

CAUTION — It is advisable to wear protective goggles and to work under a fume hood when carrying out spray tests.

The heartwood of most *Pinus* species is coloured magenta and the sapwood is coloured yellow.

Annex D
(informative)

Environmental, health and safety precautions within chemical/biological laboratory

When preparing this standard, consideration was given to the minimisation of environmental impacts caused by the use of the methods of analysis.

It is the users' responsibility to use safe and proper techniques in handling materials in the methods of analysis specified in this standard.

The following list is not exhaustive but users of this standard may use it as a guide to the use of safe and proper techniques. They should:

— investigate if European Directives, transposed European legislation and national laws, regulations and administrative provisions apply;

— consult manufacturers/suppliers for specific details such as material safety data sheets and other recommendations;

— use safety equipment and wear protective clothing, usually goggles and coats, appropriate for the test product and the test chemicals, in all laboratory areas, to ensure the safety of the operator;

— be careful about flammable materials and substances that are toxic and/or human carcinogens and generally take care during transportation, decanting, diluting and dealing with spillages;

— use a fume cupboard during preparation of organic solvent solutions;

— store, handle and dispose of chemicals in a safe and environmentally satisfactory manner: including chemicals for laboratory test, test specimens, unused solvents and reagents that have to be disposed of.

Bibliography

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[3] EN 1001-2:2005, *Durability of wood and wood based products — Terminology — Part 2: Vocabulary*

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