

REVIEW ARTICLE

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## Testing and evaluation of natural durability of wood in above ground conditions in Europe – an overview

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**Abstract** Natural durability of wood is determined by the European standard EN 252 for specimens in ground contact and EN 113 for basidiomycetes in the laboratory, but no test exists for above ground conditions. For above ground conditions, the European prestandard ENV 12037 and EN 330 are used to determine the durability of treated wood. The most important factors for fungal establishment on the surface and within wood are the moisture content, the surrounding temperature, and the relative humidity. Strength tests are the most sensitive for decay detection, but neither strength tests nor identification of fungi responsible for the decay are included in the standards of above ground durability in field tests. To detect decay, visual examination, pick or splinter tests, and mass loss determination are used. Identifying fungi with traditional methods, e.g., growth on solid medium, is time consuming and complicated. Molecular methods like polymerase chain reaction and sequencing do not require mycological skill for identification to species level, and furthermore the methods do not depend on the subjective judgement like most traditional methods, but are based on the objective information of the target organism (e.g., nucleotide sequences). The next generation of standard field tests will probably consider the drawbacks of standard tests today and be rapid and include both quality tests like molecular identification and nondestructive quantitative tests, e.g., acoustic tests. Laboratory tests can be improved by using fungi identified from field trials and by combining different fungi in the same test and thus simulate degradation in practice.

**Key words** Decay · Fungi · PCR · Standards · Wood testing

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### Natural durability: definition and European standards

The public awareness of environmental issues and the use and impact of chemicals on the environment has increased recently. Wood is considered an environmentally friendly material and it has become more and more controversial to use chemical and poisonous substances as wood preservatives. Do existing European standards sufficiently predict the natural durability of wood used in above ground conditions? Is there a need for development of the standards to suit the demands from the end user in the future? The present article discusses and evaluates test methods for the natural durability of wood in above ground conditions against fungal decay, in both laboratory and field tests.

#### Definition

According to the European standard EN 350-1,<sup>1</sup> natural durability is “the inherent resistance of wood to attack by wood-destroying organisms.” Eaton and Hale<sup>2</sup> defined natural durability or decay resistance as the ability of the heartwood of any wood species to resist decay. For practical purposes sapwood is always regarded as having low natural durability. A more detailed definition by Öqvist<sup>3</sup> considers the durability of wood to be dependent on the interaction between the ability of the wood to keep the moisture content at a low level and the inherited resistance of the wood. The inherited resistance is affected by temperature, amount of nutrients available for microorganisms, and the condition of the cell walls.

#### European standards

Natural durability of wood, exposed above ground, can be evaluated by experience, in above ground field tests, and in laboratory tests.<sup>4,5</sup> Field tests of natural durability became common in the early 1920s, when scientists began to search for alternatives to durable species like chestnut and cedars. The properties of many known wood species, which were

**Table 1.** An overview of the European standard guidance used for principles of testing and classification of natural durability on wood above ground

Method	Test	Used for
EN 350-1:1994 Durability of wood and wood-based products. Natural durability of solid wood. Part 1. Guide to the principles of testing and classification of the natural durability of wood	Guidance	Solid wood
EN 350-2:1994 Durability of wood and wood-based products. Natural durability of solid wood. Part 2. Guide to natural durability and treatability of selected wood species of importance in Europe	Guidance	Solid wood
EN 460:1994 Durability of wood and wood-based products. Natural durability of solid wood. Guide to the durability requirements for wood to be used in hazard classes	Guidance	Solid wood
EN 335-1:1992 Durability of wood and wood-based products. Definition of hazard classes of biological attack. Part 1: General	Guidance	Wood and wood-based products
EN 335-2:1992 Hazard classes of wood and wood-based products against biological attack. Part 2. Guide to the application of hazard classes to solid wood	Guidance	Solid wood
EN 335-3:1995 Durability of wood and wood-based products. Definition of hazard classes of biological attack. Part 3. Application to wood-based panels	Guidance	Particleboard, plywood, fiberboard, oriented strand board, cement-bounded board

considered less durable, were evaluated. The tests reflected a desire to identify timber with properties similar to the known naturally durable species. Various above ground field tests of the natural durability of wood have been carried out to answer specific questions without using a certain standard method.<sup>3,6-11</sup> The first European standard field test of wood in above ground conditions, the L-joint test,<sup>12</sup> was approved 1993, and in 1996 the lap-joint test was published.<sup>13</sup> The first European standard on natural durability was published 1994, as a result of the European committee for standardization (CEN) working group “Natural durability” that started in 1988 (CEN/TC38/WG2). The published standards are EN 350-1,<sup>1</sup> EN 350-2,<sup>14</sup> and EN 460<sup>4,15</sup> (see Table 1 for an overview of guidance and Table 2 for an overview of standard tests). EN 350-1 gives guidance on methods for the determination of the natural durability of untreated solid wood against attack by wood-decaying fungi, insects, and marine organisms. It also shows the principles of classification of the wood species based on the results of the test methods. EN 350-1 classifies natural durability of wood against fungal attack into five classes, 1–5, where 1 is very durable and 5 is perishable. These classes serve both laboratory and field tests, but the evaluation procedures are different. The equation for the field test is based on average life and the laboratory test is based on mass loss. EN 350-2 lists the natural durability of wood species of importance for construction purposes in Europe into durability classes. The list is based on the classification in EN 350-1 and indicates the risk of wood degradation in different service situations (e.g., dry, risk of wetting, not covered). The description comprises relative durability against wood-destroying fungi, dry wood-destroying beetles, termites, and marine organisms. EN 350-1 and EN 350-2 provide guidance on test methods to determine the natural durability of wood against decay. The guidance is based on the laboratory method EN 113<sup>16</sup> (based on mass

loss) and field test EN 252<sup>17</sup> (based on visual evaluation and pick or splinter test). Field test EN 252 is a ground contact test that will not be considered here. This means that above ground field tests, which have different conditions when compared with ground-contact tests, do not have proper principles for classification of durability classes, because the lap-joint and L-joint tests are not included in the determination of the natural durability of wood. EN 350-1 and EN 350-2 can be and are used for above ground tests. EN 460, EN 335-1,<sup>18</sup> EN 335-2,<sup>19</sup> and EN 335-3<sup>20</sup> provide guidance on how to use the hazard classes defined in EN 335-1 for wood used in different service situations, above ground, in ground contact, or in fresh or salt water. EN 335-2 applies to the different defined hazard classes of solid wood and EN 335-3 applies to different wood-based panels. EN 460 hazard classes are based on the durability classes in EN-350 and follow the definition given in EN 335-1.

## Fungal infestation

### Infestation

It is generally believed that airborne spores are the main source of the spread of rot fungi in above ground conditions.<sup>21,22</sup> The spores can trigger an infestation of unprotected wood after prolonged moisture exposure.<sup>21</sup> Rot fungi can also be spread by growth of mycelium and mycelial fragments. The establishment of a fungal infestation is crucial for the onset of decay and depends upon the substrate, the temperature, and the moisture supply. The absence of toxic or inhibiting substances from the substrate, e.g., preservative chemicals or heartwood components, also affects fungal survival and spread in the wood. In addition to these factors, the changing nutrient status of the wood during the

**Table 2.** An overview of the European standards tests of natural durability of wood for the laboratory EN 113 and aboveground field tests EN 330 and ENV 12037

Method	Test	Used for	Measure	Fungi	Prospect	Consequence
EN 113:1996 Wood preservatives. Test method for determining the protective effectiveness against wood-destroying basidiomycetes. Determination of the toxic values	Laboratory	Assessing effectiveness of wood preservatives against wood-destroying basidiomycetes	Mass loss	Basidiomycetes	Fast, reproducible, objective results	Unnatural. Use only one fungi at a time
ENV 12037:1996 Wood preservatives. Field test method for determining the relative effectiveness of a wood preservative exposed out-of-ground contact. Horizontal lap-joint method	Field	Assessing effectiveness of wood preservatives and testing natural durability	Visual examination and pick or splinter test	Natural inoculation	Interaction between different fungi	Time consuming, Not reproducible
EN 330:1993 Wood preservatives. Field test method for determining the relative protective effectiveness of a wood preservative for use under a coating and exposed out-of-ground contact. L-joint method	Field	Assessing effectiveness of wood preservatives	Visual examination and pick or splinter test	Natural inoculation	Interaction between different fungi	Time consuming, Not reproducible

**Table 3.** Examples of rot fungi common in temperate regions and the type of rot they cause

Fungi	Type of rot
<i>Antrodia serialis</i> (Fr.) Donk.	Brown
<i>Antrodia sinuosa</i> (Fr.) Karst.	Brown
<i>Antrodia vaillantii</i> (Fr.) Ryv.	Brown
<i>Coniophora puteana</i> (Schum. Ex. Fr.) Karst.	Brown
<i>Gloeophyllum sepiarium</i> (Fr.) Karst.	Brown
<i>Gloeophyllum trabeum</i> (Fr.) Murr.	Brown
<i>Lentinus lepidus</i> (Fr.) Fr.	Brown
<i>Paxillus panuoides</i> (Fr.) Fr.	Brown
<i>Poria</i> spp.	Brown
<i>Serpula lacrymans</i> (Wulfen: Fr.) J. Schröt.	Brown
<i>Bjerkandera adusta</i> (Fr.) Karst.	White
<i>Ceraceomerulius serpens</i> (Tode ex.Fr.) J. Erikss. et Ryvard.	White
<i>Phanerochaete chrysosporium</i> Burds.	White
<i>Phlebiopsis gigantea</i> (Fr.) Jül.	White
<i>Phlebia subseralis</i> ((Schw). In Fr.) Donk.	White
<i>Schizophyllum commune</i> (Fr.) Fr.	White
<i>Trametes versicolor</i> (Fr.) Quéél.	White
<i>Aureobasidium pullulans</i> (deBary.) Arnand.	Soft rot
<i>Phialophora hoffmanii</i> (V. Beyma.) Schol-Schwarz.	Soft rot
<i>Phialophora fastigiata</i> (Lagerb. et Malin.) Conant.	Soft rot
<i>Phoma</i> spp.	Soft rot
<i>Rhinochlaidiella atrovirens</i> (Fr.) Nannf.	Soft rot

Source: Henningsson and Käärrik,<sup>6</sup> and Milberg<sup>33</sup>

successive stages of decay must be considered.<sup>2</sup> Among the nutritional factors, the nitrogen content of the wood has been found to play the most important role. Mature wood contains little nitrogen (e.g., 0.03%–0.1% by dry weight) compared with plants (1%–5% by dry weight). Decaying fungi are able to utilize large amounts of carbohydrates and lignin in the presence of relatively small amounts of nitrogen. These fungi have an extremely economic use of nitrogen in their metabolism. Experiments have shown that decaying fungi re-use nitrogen in their own mycelium, or by lysis of other fungi present in the wood during the decay.<sup>23</sup> Studies of spore germination are complex because fungi can produce several different types of spores. There are, for example, basidiospores, chlamydospores, and conidia. These different spores may have varying requirements for germination, and therefore experiments on one type of spore may not apply to the others.<sup>24</sup> Examples of rot fungi in the temperate region are listed in Table 3.

Wälchli and Raschle<sup>25</sup> found the infestation by airborne spores to be of minor importance in their study about the occurrence of *Serpula lacrymans* (Wulfen: Fr.) J. Schröt. in Switzerland. More often the causes of infestation were waste wood stored in basements, containers made of wood infested with the fungus, or carrying parts of mycelia, and even transmission by means of contaminated sacks, footwear, or tools were thought to have occurred. This is a special case and applies to the spread of *S. lacrymans*, and might not be valid for other species.

Dietz and Wilcox<sup>26,27</sup> found that the fungi primarily responsible for above ground decay in structures in California were the same species already present in the green timber when the structure was built. The role of spores and airborne hyphal fragments in fungal infestation in California

and also in regions with low Scheffer climate index, an indicator of the amount of rainfall and the temperature in a region, was questioned. It was concluded that preinfestation of fungi in wood would be more likely than infestation by airborne hyphal fragments or spores. According to Viitanen<sup>28</sup> and Carll and Highley<sup>29</sup> fungi can survive in a dried state, which makes preinfestation of untreated wood possible. For kiln-dried or hot-pressed wood, preinfestation should not be a problem due to the high temperatures, which are lethal for the fungi, but not necessarily for spores. Choi et al.<sup>30</sup> found *Gloeophyllum sepiarium* to be the major aboveground wood decayer in North America in copper chrome arsenate-treated wood. This contradicts the preinfestation theory because *G. sepiarium* is not common in standing trees.<sup>31</sup>

## Colonization

The fungus that is successful in establishing itself first on the wood depends on environment (e.g., rainfall and temperature) and may determine the subsequent succession of fungi that colonize it.<sup>21,31</sup> This means that wood exposed in close proximity, but in different environments are subject to different decaying successions.<sup>21</sup> The process of colonization is dynamic where the nature of the microenvironment continually changes. There is also a difference between colonization and detection of visible decay.<sup>21</sup> The invasion of secondary fungi largely destroys evidence of the primary colonizers.<sup>31</sup> The degradation of wood is a complex process involving interactions between microorganisms and wood and also interactions between microorganisms themselves. Ecological investigations tracking succession from initial infestation to final decomposition are rare.<sup>23</sup> Choi et al.<sup>30</sup> reported the colonization of CCA-treated decking.

The fungal flora able to grow in heartwood and sapwood are different, which is a clear indication of the influence of naturally occurring antifungal substances in heartwood. It is therefore of interest to identify which species are able to grow in heartwood and sapwood. Only those species that can tolerate the concentration of tannins or other polyphenols will be found in heartwood and have the chance of becoming established there.<sup>32</sup>

Mycologists generally recognize three types of interactions between fungi: competition, antagonism, and mutualism. Still, little is known about the interactions between fungi growing in the same piece of wood. While it is quite normal to find several species of basidiomycetes growing on the same log, it is rare to isolate more than one basidiomycete species from the same area in a piece of wood. This means that mycelium from different fungi seldom becomes intermingled. The cause could be some sort of antagonism of a chemical nature. Another interaction is hyphal interference (e.g., one hypha type may have a negative effect on the other), which seems to be a highly efficient mechanism for inactivating other hyphae that are potential competitors for the same substrate.<sup>21</sup> Generally, a colonization sequence of fungi in wood is initiated by fungi living on cell contents like

sugar and starch (e.g., moulds), followed by fungi decomposing cellulose and lignin. The last stage fungi are living on partially decomposed cell wall material and residues of the early colonizer.<sup>32</sup> The succession order for many decaying fungi is still an unknown field.

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## Moisture content of wood and temperature

### Moisture content

Experience with wood in its many uses indicates that dry wood in protected environments or water-saturated wood seldom decay. The important questions to many users of wood have been to know the critical wood moisture limits when decay begins or stops and how varying the amount of water in wood affects the rate of decay development. These questions are difficult because moisture gradients also exist in wood from the outer to the inner zones.<sup>2</sup> Moisture is usually measured as moisture content (based on dry weight) and is generally expressed as percentage. Below the fiber saturation point ( $\approx 30\%$ ) the water is tightly bound to polymers in the cell wall and unavailable for most fungi.<sup>22</sup>

The main water source for above ground field tests is rainwater. Rot fungi cannot be established if the wood moisture content is below 30%, but they can withstand longer or shorter periods of dryness when established in the wood.<sup>33</sup> Some species like *Lentinus lepideus* (Fr.) Fr., *Antrodia sinuosa* (Fr.) Karst., *Gloeophyllum sepiarium* (Fr.) Karst., and *G. trabeum* (Fr.) Murr. can survive for 6–9 years in wood at a moisture content of around 12%. The optimum moisture content for decay for most rot fungi is between 30% and 80%.<sup>2,24,33</sup> One exception is *S. lacrymans*, which has its optimum at 20%–55%.<sup>34</sup> When the moisture content rises above the optimum, the decay becomes slower because of the reduced oxygen concentration (oxygen has a lower solubility in water than in air) and an almost anaerobic condition develops in saturated wood. The optimum moisture content for any fungus depends on the cell wall/air space ratio of the wood in which it is growing. It will be higher in very light wood and lower in very dense ones.<sup>32</sup> Brown rots in general are sensitive to the reduced air supply, whereas soft rots can grow easily in soaked wood.<sup>22</sup>

Rapp et al.<sup>35</sup> suggested the inclusion of a Moisture-induced Risk Index (MRI) as one parameter in the European standard ENV 12037 and EN 330 when assessing durability of wood above ground. The MRI is a linear relation based on moisture content and time, and is closely related to the number of days when the wood moisture content exceeds 25%. Morrell<sup>36</sup> called for development of moisture–temperature relationships for primary fungi that attack buildings. He considers a model to be most useful when it predicts losses in bending strength or other critical engineering properties. Engineers could then use the model to predict rates of decay under varying environmental conditions.



**Table 4.** An overview of test methods used for evaluating durability

	Subjective	Objective	Fast	Time consuming	Quality	Quantitative	Consider fungi flora
Visual evaluation	x		x			x	x
Macroscopic evaluation	x			x	x		x
Pick or splinter test	x		x			x	
Density loss		x	x			x	
Weight loss		x	x			x	
Strength test		x		x		x	
Acoustic test		x	x			x	

## Temperature

Temperature affects the metabolic activities of fungi like digestion, assimilation, respiration, translocation, and synthesis that are mediated by enzymes. Metabolic reaction rates increase with increasing temperature until some reaction becomes rate limiting, or the heat denatures the enzymes.<sup>22</sup> The optimum temperature for the common Nordic rot fungi is between 22° and 36°C,<sup>33</sup> but there are exceptions like *S. lacrymans*. The optimum temperature for *S. lacrymans* is around 18°–20°C; the lethal temperature is 35°–37°C. Most rot fungi can withstand long periods of freezing and periods of repeated freezing and thawing.<sup>37</sup>

## Methods to evaluate durability

The most-used methods to evaluate durability today are visual evaluation, image analysis, microscopic evaluation, pick or splinter test, density and mass loss, and various strength tests. The methods detect the extent of decay, but only the visual and microscopic evaluation may consider which fungi might be responsible for the decay. An overview of the different test methods is presented in Table 4.

### Visual evaluation

Visual evaluation includes discolorations, cracks, mycelium or fruit bodies, and signs of insect attack that can be observed by the naked eye. The visual evaluation rates the fungal infestation on a scale,<sup>38</sup> for example, a four-grade scale where 0 means no growth and 3 means very abundant growth, with a surface coverage of more than 75%. Image analysis is a tool facilitating objective measurements of wood discoloration caused by the presence of mould and stain fungi. Results are similar to those developed by experienced evaluators. Image analysis has the potential to improve the reliability and reproducibility of laboratory trials.<sup>39</sup> Microscopic detection of wood decay is not possible until the mass loss is at least 5%–10%.<sup>29,40</sup>

### Pick or splinter test

The pick test is a simple method for detecting surface decay in poles and timber. In practice, a sharp screwdriver or knife

is driven into the wood at an acute angle and bent back in order to snap a small piece of wood from the surface. The break characteristics of the splinter removed are then examined. A brash break reflects reduced strength and the possible presence of decay, whereas a splintery break reflects sound wood. The pick test measures toughness and is fairly sensitive to early decay.<sup>41,42</sup> The drawbacks of the pick test is the destructive evaluation, a quite large sample is removed, the inability to accurately assess the internal conditions of the wood,<sup>22</sup> and the subjectivity of the test. The accuracy and reproducibility may vary with factors like experience of the performer, latewood content, and fiber orientation.<sup>41</sup> Decay could be detected as early as with 5%–10% mass loss by the pick test.<sup>41</sup> This is close to the level when decay first becomes detectable under the microscope. Considerable wood strength is lost in the early stages of decay, and therefore high sensitivity of tests is desirable.

### Density and mass loss

Density loss is a rough decay indicator used by timber graders, and is useful because density is closely correlated with strength properties. Density loss is not comparable between decay caused by white rot and brown rot fungi. White rot fungi causes a substantial mass loss but little change in volume, whereas brown rot fungi causes substantial volume and weight reductions.<sup>22</sup>

Mass loss is commonly used in laboratories to assess the natural durability of wood. One reason for this is the availability of balances in the laboratory and that the variation between samples is low compared with strength tests (see below). Blocks are conditioned by oven drying (e.g., 103°C) or at constant temperature and relative humidity (RH), for example, 20°C and 65% RH, and their weights are measured before and after test. Mass loss is expressed as a percentage of the original dry weight.<sup>2</sup> Earlier, mass loss was considered to probably be the best basis upon which to compare results in different experiments involving wood decay. The main drawback of mass loss is its inability to detect the early stages of decay. Strength toughness and impact bending strength (see below) are the most sensitive measures for the early stages of decay.<sup>40</sup>

## Strength tests

Strength tests involve irreversible destructive testing of specimens to failure. Even with a uniform set of specimens considerable variations in results are obtained. Strength test results are usually expressed as the energy applied per unit area or volume. There are many factors that need to be considered when strength is assessed, e.g., density, grain angle, uniformity (clear specimens or specimens with defects, particularly knots and splits), moisture content, temperature, rate of loading, age of wood, and previous histories of load. All these factors interrelate and should be considered in strength evaluation.<sup>2,22</sup> The decrease of toughness or resistance to impact loading caused by fungi is the most sensitive property for detecting the early stages of decay, followed by static bending properties.<sup>40</sup> In laboratory tests, strength loss may be rapid. Appreciable strength losses may be detected after only 2 weeks exposure to a fungus. In a study by Henningsson<sup>43</sup> on birch wood and the brown rot fungus *Polyporus marginatus* (Swartz ex Fr.) Karst, there was a 47% loss in impact bending strength after only 2 weeks incubation, while a 7% mass loss was reported. Ruddick<sup>44</sup> and Nicholas and Crawford<sup>24</sup> also found the strength test to be more sensitive than weight loss. Early studies of the effects of fungi on the strength properties of timber established that decay by basidiomycetes (brown and white rot decay types) has negative effects on strength properties.<sup>2</sup> Wilcox<sup>40</sup> concluded that in the initial stages of wood decay there are small differences in strength loss caused by brown or white rot, or if the decay appears in softwood or hardwood. When mass loss reaches 5%–10%, one should expect a loss in strength properties of at least 60%–80%. A sensitive strength property is static bending, where losses of 50%–70% can be expected at 5%–10% mass loss.<sup>40</sup> Reinprecht and Tiralova<sup>45</sup> confirmed that strength loss is more sensitive than mass loss in detecting early decay of wood in their study of three brown rot fungi and found an exponential relationship when correlating the modulus of rupture (MOR) with mass loss. Curling et al.<sup>46</sup> supported this finding with their study of the relationship between mass loss, strength loss, and the hemicellulose composition for degradation by brown and white rot fungi. They used the four-point bending test described by Winandy and Morrell<sup>47</sup> to determine MOR. A four-point test produces a constant bending moment and stress between the inner loading points and accurately evaluates strength in the weakest area of the decayed specimens. A relationship between hemicellulose composition and the strength properties of wood was also found, which support earlier work of Winandy and Morrell.<sup>47</sup>

## Acoustic tests

Wood is an excellent transmitter of sound waves and produces characteristic acoustic emissions when it is stressed mechanically. Wood colonized by microbial agents obtains an altered ability to transmit or emit sound. This alteration in acoustic properties can be exploited to detect various

stages of decay. When sound waves move through wood they will pass around decay pockets or voids, which slows down the rate of the sound transmitted through the wood. The increased transmission time of a sound wave can be used to detect decay. This technique is promising for the nondestructive monitoring of changes in wood over the course of decay. However, changes caused by microorganisms have been difficult to distinguish from normal wood characteristics and from changes associated with wood heterogeneity. In general, acoustic techniques have improved and are still developing.<sup>22,48</sup>

Ross et al.<sup>49</sup> found a relationship between the stress wave transmission time and the bending strength (MOR) of oriented strand boards subjected to the brown rot fungus *G. trabeum*. It also demonstrated that stress wave transmission is more sensitive for detecting strength loss than mass loss. Noguchi et al.<sup>50</sup> found acoustic emission to be a sensitive indicator of the early stages of decay, but it is unclear how to apply acoustic emission in field tests.

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## Laboratory methods for testing wood durability

### Traditional laboratory methods

Laboratory evaluation of natural durability began in the 1940s as an attempt to further explain the nature of durability and to identify compounds toxic to fungi in the wood.<sup>22</sup> In most cases, warm water and organic solvents were used to remove extractives from the wood. The extractives were then tested for activity against a variety of decay and nondecay fungi. Most tests were performed in petri dishes or decay chambers using malt agar. Although such tests provided a relative guide to chemical toxicity, they could not evaluate more subtle effects such as variation in deposition of extractives in the wood or interactions between different extractives, which also contribute to natural wood durability. Many chemicals responsible for natural wood durability are as toxic or are more toxic than existing wood preservatives.<sup>22</sup>

Laboratory testing creates a situation that may be defined as artificial and therefore the results should be used comparatively. The duration of the standard basidiomycete test EN 113 is 16 weeks. Treated specimens and one untreated specimen are placed into a culture vessel on sterilized supports. When the specimens are inserted the culture vessel is already inoculated with a fungus. At the end of the test the specimens are withdrawn from the culture vessel and the mass loss is determined.<sup>16</sup> The EN 113 trial is carried out in small vessels where only one fungus at a time is used as the test organism under sterile conditions. This means that there is no interaction between fungal species and other types of microorganisms, which occur in field trials and other situations where wood is used in practice.<sup>51</sup> Laboratory tests give more objective results and are reproducible whereas field testing is time consuming and subject to human assessment errors. Although the laboratory tests are artificial and only use one fungus at a time in most cases,

there is, according to Eaton and Hale,<sup>2</sup> close agreement between field and laboratory data. Van Acker et al.<sup>51</sup> on the other hand found that to be able to distinguish between durability classes 1–3 in EN 350-1, field tests like the L-joint and lap-joint tests (described below) are needed. Van Acker et al.<sup>52</sup> also found that the classifications in EN 350-2 do not correspond with results from laboratory test EN 113. The result of the laboratory test rates the specimens as more durable than the list in EN 350-2 and they suggested that this might indicate that the conditions in the laboratory test are not appropriate. This is supported by Rapp and Augusta.<sup>53</sup> McNamara<sup>54</sup> stated that laboratory tests have little meaning in a wood preservative standardization process. Instead, field tests at sites known to be aggressive to preservative-treated wood are strongly recommended. Nilsson and Edlund<sup>55</sup> considered this view as extreme and suggested that neither field nor laboratory tests should be excluded. The most difficult problem for both field and laboratory tests is to deal with all wood-decaying organisms and hazards to be able to predict service life. EN 113 measures mass loss as a mean of decay instead of the more sensitive strength loss, which would be possible to measure in laboratory (Table 2). A central aspect in testing wood durability is the species identification of decaying fungi, because different fungi cause different kinds of damage. To make laboratory tests reliable it is valuable to identify which fungi are responsible for decay in the field and under different exposures. This could be difficult, because all fungi do not develop fruiting bodies and mycelial identification is arduous.

#### Molecular methods for detection of fungi

Determining which fungus is the most likely to be associated with a specific wooden part of a building might allow for specifications that are more closely tailored to the organisms likely to colonize the wood. In these situations, there is tremendous potential for using molecular methods for rapid identification of the flora colonizing the wood. Studies of species associated with various building components have been performed earlier, e.g., by isolating the fungus on a selective media.<sup>36</sup> The isolation of a fungus is a more time-consuming method and allows only the fungus, which is favoured by the selected media, to grow. There might also be a possibility that the fungi, that develop fruit bodies are not the ones with the most aggressive decay. This means that the observed fungi (fruit body) might not be the actual decayers; instead fungi growing inside the wood as mycelium are the aggressive decayers. For identification of mycelium inside the wood or on the surface, molecular methods can be used.

Polymerase Chain Reaction (PCR) can amplify extracted DNA from complex environmental samples like soil and plants.<sup>56,57</sup> PCR amplifies the specific DNA fragment exponentially, but does not identify the fungus. To identify the fungus further analysis is required, and the amplification is usually done to get enough DNA. Since its development in 1985,<sup>58,59</sup> the specificity, sensitivity, and speed of PCR-

based technologies have led to application in a wide range of biological research areas and for all classes of organisms.<sup>60</sup> The most used application in wood science has been species-specific primers,<sup>61</sup> fingerprinting<sup>56,57,62–70</sup> and sequencing.<sup>71,72</sup> Using species-specific primers is a fast way to identify if a species is present or not. In this analysis, only a certain chosen species will be amplified, which means that if a PCR product is received the fungus is present; otherwise it is not. It could be useful when information about a specific fungus presence or absence is needed. Fingerprinting is based on PCR amplification of genomic DNA with selected primers. These primers could be 9–13 bases long with a guanine–cytosine (G + C) content of 50% as in Random Amplified Polymorphic DNA (RAPD).<sup>73</sup> In Amplified Fragment Length Polymorphism (AFLP) the genomic DNA is cut by restriction enzymes before the amplification and in Restriction Fragment Length Polymorphism (RFLP), the amplified DNA fragment is cut by specific restriction enzymes. All these fingerprinting techniques create a genetic fingerprint, which usually is viewed as several bands on a gel. To be able to identify the fungus in the sample there needs to be a reference sample to compare the band pattern on the gel. Using these fingerprinting methods only allows one fungus in each sample. If there are several fungi in the original sample they either need to be cloned or another method could be used, like T-RFLP (described below). Sequencing the DNA means that all the nucleotides in the region concerned are identified and translated to the letters T (thymine), A (adenine), C (cytosine), or G (guanine). These can then be compared with other known sequences in GenBank, or a sequence of known fungi. The Basic Local Alignment Search Tool (BLAST) is one method for rapid searching in nucleotide databases, like the NCBI's GenBank <http://www.ncbi.nlm.nih.gov/>.

To follow the fungal colonization of wood community studies is useful. This has been done for fungi in soil using PCR-based technologies like Denaturing Gradient Gel Electrophoresis (DGGE)<sup>60,69</sup> and Terminal Restriction Fragment Length Polymorphism (T-RFLP).<sup>69,74–76</sup> These methods could bring forward useful information about the fungal successions for wood exposed in different above ground environments. The advantage of using molecular methods for these studies is, besides the speed of the analysis, the objectivity. All fungi in a complex sample will be detected; there is no cultivating step that could favour certain species.

Using molecular techniques makes it possible to identify fungal species directly from mycelium. There is no need for fruit bodies or cultivation, which makes it a rapid and exact method and it is even possible to identify species directly from wood samples.<sup>67,71</sup> When fungi are grown on laboratory media it can be difficult to observe isolate variation, which is possible with sequencing techniques. When the entire sequence information is available for identification the isolate variation becomes evident.<sup>77</sup>



**Fig. 1.** The body of the L-joint tilted back 10° and the joint between the two specimens

### Field test of wood durability for above ground conditions

#### Standard field tests

In 1981 it was decided at the International Research Group on Wood Preservation (IRG) meeting in Yugoslavia that interested laboratories should cooperate with field trials based on L-joints (EN 330), as a way to achieve controlled and comparative tests within the CEN countries and also to allow greater international comparison.<sup>78,79</sup> An L-joint<sup>12</sup> consists of two members attached to each other forming an L shape (Fig. 1). Each member is 203 mm long and has a cross section of 38 × 38 mm. L-joints are placed on racks facing south and are tilted back 10° to the horizon. The L-joint is a test for painted wood as opposed to the lap-joint which is a test for unpainted wood. The extent of fungal attack on the external surfaces and in the joint area is rated according to a specific rating system 0–4 (0 is sound, 1 slight attack, 2 moderate attack, 3 severe attack, and 4 failure) and compared with a reference. The rating is based on visual evaluations and the pick or splinter test. The tests compare different preservatives. The cross-sectional dimensions are smaller than those for lap-joint testing (described below) enabling the production of selected high-quality samples in a simpler way. The duration of the test is for a minimum period of 5 years or until the notional mean rating for the untreated control replicates for nondestructive inspection is equal to or greater than 2.0.<sup>12</sup> Comparable extensive testing has used similar systems outside Europe as well.<sup>80</sup>

Carey<sup>81,82</sup> examined the progress of visible decay in both treated and untreated L-joints, the reproducibility between trials, and the possibilities for predicting long-term perfor-



**Fig. 2.** The body of the lap-joint and the joint in the middle of the unit

mance from the early stages of visible decay. It was found that the mean life of replicates for untreated L-joints varied between 8.0 and 10.7 years. The difference was caused by the variation both in the time to the first visible decay and the time for decay to progress until failure of the actual replicate. The onset of decay in a particular replicate did not result in the early failure of that replicate. The variation between trials was not dependent upon the time of year the trial was performed.

The lap-joint test<sup>13</sup> consists of two overlapping parts held together mechanically and placed horizontally at 1.2 m above the ground (Fig. 2). The lap-joint dimension is 38 × 86 × 300 mm and the close fitting part in the middle is 60 mm. The extent of fungal attack on the external surfaces and in the joint area is rated according to a specific rating system 0–4 (0 is sound, 1 slight attack, 2 moderate attack, 3 severe attack, and 4 failure) and compared with a reference. The rating is based on visual evaluations and the pick or splinter test. Molnar et al.<sup>83</sup> found that visual examination of the lap-joint test might not be adequate to ascertain the state of decay. Discoloration of the sample can confuse the assessment and can increase the rating of the test object, which still might be fully internally sound. Destructive sampling may be essential to obtain meaningful and comparative results. The duration of the lap-joint test is for a minimum period of 5 years. If the median for the rating of joint surfaces of the untreated control replicates is less than 3.0 after 5 years, the test continues until a minimum value of 3.0 is achieved. It is recommended to continue the test until all replicates have failed.<sup>13</sup> An overview of the field tests is shown in Table 4.

After 5 years of lap-joint exposure Johansson et al.<sup>10</sup> obtained the following results: no treated samples exposed above the ground had yet been decayed, and very few untreated samples had been severely attacked by wood-destroying fungi. This leaves some doubt whether the lap-joint method is suitable for aboveground testing in temperate climates. Changes have been made to the ENV 12037 standard and it is now acceptable to place the samples in shade to accelerate decay.



**Fig. 3.** Bodies of **A** the Johansson method, **B** the double layer, and **C** the staple bed



Both L-joints and lap-joints include some sort of joint to effectively trap rainwater. The units provide a realistic evaluation of the performance of wood but are dependent on rainfall and temperature at the test site. The visual evaluation and pick and splinter test make it difficult to detect incipient decay and the rating often depends on the moisture content of the sample at the time of evaluation.

#### Accelerated methods

Various accelerated methods have been suggested<sup>38,80,84–90</sup> to overtake the drawback of the long duration of field testing. There are accelerating tests using the standard dimensions,<sup>80,85,91</sup> like the L-joint and various test designs at different distances from the ground to effectively trap moisture. Some examples of the designs are the Johansson method, the double layer, and the staple bed, which are described below (Fig. 3).

#### Accelerated test using standard dimensions

Accelerating tests above the ground include, among others, the L-joint test where infested wood blocks are joined to the corner of L-joints. Here a water reservoir slowly releases enough moisture to infest the L-joints.<sup>85</sup> Similar methods using tests of window frames have been conducted by Fougereousse<sup>84</sup> and artificial infestation of window frames was reported by Deon and Trong.<sup>87</sup> There are also some accelerating tests that are not conducted in a fungus cellar or use artificial infestation.<sup>10,35,92</sup> The construction in the L-joint test traps moisture and spores effectively during natural weathering and temperatures. Testing wood in above ground conditions mainly focuses on trapping rainwater by using joint members or by the arrangement of the specimen.

#### The Johansson method

Wood specimens ( $22 \times 95 \times 500$  mm) are put together and exposed at an angle of  $60^\circ$  facing south at 0.5 m above the ground (Fig. 3). The wood specimens can be evaluated separately or all together, as the evaluation is visual. Visual judgment is conducted for discoloration (0–2, where 0 is no discoloration, 1 some discoloration, and 2 severe discoloration) and for rot attack (0–3, where 0 is sound, 1 is slight to moderate attack, 2 is severe attack, and 3 failed).<sup>10</sup> The rot

attack is judged by the pick and splinter test. Johansson et al.<sup>10</sup> found that the Johansson method is more effective than the lap-joint test regarding attack by rot fungi. After 5 years of exposure, moderate to severe rot in the overlapping areas was achieved. The advantages of the Johansson method are the faster decay than the standard lap-joint and L-joint tests, and the simple preparation of samples. The more rapid decay for the Johansson method can be caused by penetration of rainwater in the end cut of the specimens, which are exposed at a favorable angle for penetration.

#### Double layer

The double layer is an above ground test using natural factors of exposure. The double layer consists of specimens ( $25 \times 50 \times 500$  mm) arranged in a tight horizontal double layer, supported at the end cuts by beams of untreated Norway spruce (*Picea abies* (L) Karst.) ( $100 \times 100$  mm) (Fig. 3). The samples are only 100 mm above the ground. The upper layer is shifted 25 mm lateral to the lower layer. In this arrangement the rainwater is effectively trapped between the two layers. The double layer arrangement has shown faster decay than the standard lap-joint and L-joint tests. It is possible to detect decay after only 6 months of exposure.<sup>90</sup> The advantage of the double layer is the simple construction with no screws or built-up racks and this makes the setup very fast and easy. The double layer method is faster than both the standard methods (EN 330 and ENV 12037) in causing decay because of the close proximity to the ground, thus trapping the moisture more effectively. The double layer test has been exposed in five test sites with different climates in Germany to test the natural durability of wood. After 3 years of exposure, the double layer reveals higher durabilities for larch, Douglas fir, and pine than those obtained with EN 350.<sup>91</sup>

#### Staple-bed test

The staple bed consists of specimens ( $98 \times 250$  mm) stapled above each other, with the bottom layer placed on the ground (Fig. 3). Each layer is then placed perpendicular to the one below and builds up a staple with five rounds. The upper layer is oriented in the north–south direction. The staple bed is easy to set up and the specimens are uncomplicated to prepare. This method was developed as an attempt to get the material exposed to different kinds of attack and

hazards in the same test. The first time the staple-bed test was performed the moisture contents were measured in treated wood in order to determine moisture conditions in the different layers.<sup>93</sup> Specimens in the bottom layer are exposed to the same rot hazards as specimens in ground contact whereas specimens in the top layer are in above ground conditions. This method is therefore not completely comparable with the L-joint or lap-joint tests. The staple-bed test was expected to give accelerated results concerning decay. After 36 months in the field, no clear rot attack or differences in moisture content could be detected.<sup>93</sup> This was expected because of the use of preservatives in the setup.

## Recommendations

Accelerated tests like the double layer should be used as a complement to long-term field tests. Laboratory tests are good as screening tests to obtain a fast first opinion about a new species or treatment. The first fast screening would be an encouragement for the wood industry to try different more environmentally friendly treatments and get a fast response if the treatment is acceptable.

There is also a need for more information and a better understanding concerning the process of microbial colonization and succession of wood. In addition, the interactions between different microorganisms involved in the decay process are largely unknown and further research is needed.

The use of new techniques, such as PCR and sequencing, will substantially improve the possibility for developing testing methods for prediction of the behavior of wood and wooden constructions, in the future.

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