**Comparing visual inspection of trees and molecular analysis of internal wood tissues for the diagnosis of wood decay fungi**

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**Summary**

The extent to which the presence of wood decay fungi in standing trees is underestimated when diagnosis is based on the visual inspection of trees was studied and whether the rate of underestimation may vary depending on the environmental context (urban vs. forest sites) and the fungal species was tested. A total of 903 broadleaf and conifer standing trees were inspected for the presence of fruiting bodies or other signs, and sampled through a drill-based technique. Multiplex PCRs were used to analyse wood samples. Trees with emerging fruiting bodies ranged from 0% to 11.6% depending on site. However, when analysed through molecular methods, the rate of infected trees raised to 15.7% to 58.0%. On average, visual inspection of trees underestimated more than 90% of infected trees compared to molecular methods in both environmental contexts. Higher rates of underestimation were observed for *Armillaria* spp., *Heterobasidion* spp. and *Phaeolus schweinitzii*, while lower rates (<80%) for *Ganoderma* spp. and *Perenniporia fraxinea*. The range of variation of the underestimation rate was limited; therefore, 90% underestimation may be used for estimating trees infected by wood decay fungi based on the frequency of fruiting bodies and other signs.

## Introduction

In forest ecosystems wood decay fungi play an important role for biodiversity, $CO_2$ dynamics, nutrient cycling and forest regeneration and as indicators of forest health (Rayner and Boddy, 1988; Blanchette, 1991; Stenlid *et al*., 2008). As a result, in Scandinavia for instance, foresters use management techniques to maximize fungal biodiversity and maintain "red lists" of threatened and endangered fungi (Glaeser and Lindner, 2011). Furthermore, standing and fallen decaying trees are species-rich environments, and deadwood has been recognized as providing resources for a variety of living organisms (Lonsdale *et al*., 2008). However, wood decay fungi significantly reduce the quality of timber in production forests (Oliva *et al.*, 2010) and they may reduce the protection function of trees against avalanches, rock falls and debris flow in protection forests (Giordano *et al*., 2012). The loss of wood mechanical properties caused by wood decay fungi can predispose trees to the risk of windthrows or limb failures (Lonsdale, 2000; Hickman *et al*., 2011). In urban landscapes, tree or limb failures triggered by wood decay can lead hazardous situations, resulting in significant damages of property and/or tragic injuries.

The assessment of the presence of wood decay fungi may be required in pristine, production and protection forests, as well as in urban settings for the timely detection of potentially hazardous situations. Since different fungi may differ in their ecology, aggressiveness and ability to colonize wood tissues, an accurate diagnosis of the fungal species involved is usually preferable as it may allow a prediction on how the wood decay will progress and of the probability of failure or decline (Lonsdale, 1999; Guglielmo *et al*., 2007; Glaeser and Lindner, 2011). During tree hazard assessment, conventional diagnostic methods to detect wood decay fungi are mainly based on visually inspecting trees for the occurrence of external indicators of decay including fruiting bodies (Rayner and Boddy, 1988; Renvall *et al*., 1991; Hintikka, 1993; Renvall, 1995; Nicolotti *et al*., 2010) and of any other evidence of structural

86 weakness or decay (e.g. wounds from pruning or other injuries, dead bark, deterioration in
87 crowns, etc.), often followed by instrumental analyses aimed at assessing the localization and
88 the extent of the decay (Mattheck and Breloer, 1992; Nicolotti *et al*., 2003). In the absence of
89 such external signs and/or structural defects, trees tend to be categorized as not significantly
90 hazardous (Lonsdale, 1999). Nonetheless, it must be emphasized that fruiting bodies
91 distribution on a tree can give misleading information, since it does not always directly reflect
92 mycelial distribution and activity in the wood (Rayner and Boddy, 1988). Furthermore, in
93 general, fruiting bodies of wood decay fungi may be sporadically visible and they usually
94 emerge at advanced stages of the infection (Terho *et al*., 2007; Vasaitis, 2013).
95 This may represent a serious problem as in the early and intermediate stages of infection their
96 presence could be overlooked. The extent to which the presence of wood decay fungi inside
97 trees is overlooked when the diagnosis is based on the visual inspection of fruiting bodies is
98 largely unknown, though it is expected to vary depending on the age of infection and possibly
99 on the environmental context and on the fungal species.
100 The accurate diagnosis of wood decay fungi in standing trees may require the extraction of
101 wood tissues followed by a fungal isolation step (Nicolotti *et al*., 2010). However, the
102 efficiency of the culture-based methods is limited by constraints arising during fungal
103 isolation from wood tissues (Nicolotti *et al*., 2010). It is worth noting that not all wood decay
104 fungi can be easily cultured *in vitro*. Moreover, diagnostic methods based on pure culture
105 analysis and tests (e.g., growth rates at different temperatures, oxidative and colorimetric
106 reactions on different culture media, biochemical and immunological traits as reported in
107 Nobles, 1965; Stalpers, 1978; Anselmi and Bragaloni, 1992; Jellison and Jasalavich, 2000;
108 Clausen, 2003) are time-consuming and often unsuited to distinguish between closely related
109 species (Schmidt, 2007).

Nevertheless, rapid and sensitive diagnostic methods, such as PCR-based approaches (e.g., multiplex PCR assays), have been developed for detection of the presence of the most important and widespread wood decay fungi of both broadleaves and conifers in the northern Hemisphere (Guglielmo *et al*., 2007, 2008; Nicolotti *et al*., 2009; Parfitt *et al*., 2010; Gonthier *et al*., 2015). These methods, deemed reliable especially if used in combination with appropriate sampling protocols (Guglielmo *et al*., 2010), are becoming routine associated with instrumental techniques for the decay detection (Glaeser and Lindner, 2011) during Visual Tree Assessment (VTA) analysis.

Wood decay fungi have been traditionally regarded as microbes decomposing wood tissues. While most of them may infect standing living trees thus behaving as parasites (Rayner and Boddy, 1988), there is an increasing body of literature indicating that latent phases in symptomless tissues are possible (Vasaitis, 2013).

In this work we compared the frequency of trees with emerging fruiting bodies or other signs of wood decay fungal presence in standing trees with that of infected trees as determined by multiplex PCR-based methods applied on internal wood tissues, with the following aims: i) to assess the extent to which the presence of wood decay fungi is underestimated when the diagnosis is based on the visual inspection of trees, ii) to test whether the rate of underestimation may vary depending on the environmental context (urban sites vs. forest sites) and iii) the fungal species involved.

## Materials and methods

During autumn of 2010, stems of a total of 903 randomly selected standing and living trees were inspected from the base to about 2 m aboveground for the presence of fungal fruiting bodies. Surveys were conducted in autumn to maximize the probability of observing fungi (e.g. *Armillaria* spp., *Phaeolus schweinitzii* (Fr.) Pat.) whose fruiting bodies develop in that

season. Only trees showing exudations, cankers or cracks at the root collar, that are reported as symptoms of *Armillaria* spp. infection (Guillaumin and Legrand, 2013), were inspected for the presence of subcortical rhizomorphs and/or white mycelial fans by debarking the collar and roots with a knife. In addition, trees were sampled at the root collar (about 5 cm aboveground) by collecting wood chips through a previously described and successfully tested drill-based technique (Guglielmo *et al*., 2010). Each tree was drilled four times (drillings at 90° from one another) and wood chips generated from different drillings were pooled together in a 9-cm diameter Petri dish and used as substrate for the DNA-based assay (Guglielmo *et al*., 2010). Samplings were carried out in both urban sites, including parks and streets, and forest sites, including protection forests in western Italian Alps. In detail, 165 trees were located in 22 urban areas of the city of Genoa (Liguria, Italy), 468 trees in 8 urban areas of the city of Turin (Piedmont, Italy) and 270 trees in 3 forest sites (40 trees in Claviere – Piedmont, Italy; 142 trees in Aymavilles and 88 trees in Champoluc – Aosta Valley, Italy) (Table 1). Sampled trees included both broadleaf species, i.e. *Aesculus hippocastanum* L., *Acer* spp. and *Platanus* spp., and conifer species, i.e. *Cedrus* spp., *Cedrus deodara* (Roxb.) G. Don f., *Cupressus* spp., *Larix decidua* L., *Picea abies* (L.) H. Karst., *Pinus* spp., *Pinus halepensis* Mill., *Pinus pinea* L., *Pinus pinaster* Aiton, *Pinus uncinata* Mill. and *Taxus* spp.

[Table 1]

Fungal identifications were performed through conventional techniques based on the observation of macroscopic and microscopic features of fruiting bodies using taxonomic keys to species (Breitenbach and Kränzlin, 1986; Hjortstam *et al*., 1987; Bernicchia, 2005; Gonthier e Nicolotti, 2007). In the presence of old fruiting bodies and/or with uncertain morphology, identifications were confirmed through DNA sequencing of Internal Transcribed

Spacers (ITS) region comparing sequences with those of known fungi through basic local alignment search tool (BLAST; http://www.ncbi.nlm.nih.gov/BLAST), as previously described (Guglielmo *et al*., 2007).

Molecular analyses of both urban and forest samples were performed directly on wood chips as described by Guglielmo *et al.* (2010). Fungal DNA was extracted using the E.Z.N.A.™ Stool DNA Isolation Kit (Omega) on wood samples lyophilized and homogenized through a FastPrep FP120 Cell Disrupter (Qbiogene, Irvine, CA). Either the multiplex PCR-based assay described by Nicolotti *et al.* (2009) or that described by Gonthier *et al.* (2015) were used depending on the fact the sample was collected from a broadleaf or a conifer tree, respectively. In both multiplex PCRs, specific amplicons of different fungal taxa were easily identified based on their size in gels containing 1% (w/v) of high-resolution MetaPhor (Cambrex) and 1% (w/v) of standard agarose, after electrophoretic migration.

Comparisons between frequencies of trees with emerging fruiting bodies or other signs of fungal wood decay presence and infected trees as determined by multiplex PCR-based methods were performed through the Chi-squared test for trees in urban sites, trees in forest sites and for the whole dataset. The frequencies of trees detected and not detected as infected by any wood decay fungal taxon by using the two methods (visual inspection of fruiting bodies or other signs of wood decay fungi vs. multiplex PCR-based methods) were cross-tabulated and compared with the Chi-squared test or the Fisher exact test for expected frequencies lower than 5 in at least one cell of the contingency table. The rate of underestimation (*U*) of the visual inspection of trees compared to multiplex PCR-based diagnosis was computed as follows:

$$U\% = \frac{\text{infected trees MP} - \text{infected trees VI}}{\text{infected trees MP}} \cdot 100$$

where MP and VI were the number of infected trees detected through multiplex PCR-based methods and visual inspection of fruiting bodies or other signs of wood decay fungi, respectively. The rate of underestimation between urban and forest sites were compared by using the Chi-squared test on absolute frequencies. For all tests a 0.05 threshold was used as cut-off value. Statistical analyses were carried out on R programming language (R Core Team, 2013).

**Results and Discussion**

Emerging fruiting bodies and subcortical rhizomorphs and/or white mycelial fans were observed during visual inspection in 1.2%, 1.5% and 11.6% of trees in the city of Genoa, city of Turin and in the Champoluc forest, respectively; in the two forest sites of Claviere and Aymavilles no fruiting bodies and subcortical rhizomorphs and/or white mycelial fans were observed.

However, when trees were analysed through multiplex PCR-based methods, the rate of trees infected by at least one target wood decay fungal species raised to 15.7%, 17.9%, 58.0%, 45.0% and 45.1% in the city of Genoa, city of Turin and in the Champoluc, Claviere and Aymavilles forests, respectively.

The frequency of trees with emerging fruiting bodies or with other signs (subcortical rhizomorphs and/or white mycelial fans) was significantly lower ($P < 0.05$) than the frequency of infected trees as detected by using the multiplex PCR-based methods, both considering the whole dataset and data from single sites (Figure 1).

[Figure 1]

Our results suggest that diagnosis based on visual inspection of fruiting bodies or other signs of wood decay fungi underestimated on average more than 90% of infected trees (from 80.4% to 100% underestimation depending on site), with a similar situation in both environmental contexts (91.8% in urban sites vs. 92.4% in forest sites). The number of sampled sites is limited, however, the similar rate of underestimation observed in urban and forest sites ($P > 0.05$) and in general in all sites is noteworthy, and may be used to roughly estimate the number of trees infected by wood decay fungi in a site based on the frequency of fruiting bodies or other signs.

While multiplex PCR-based methods may be helpful in detecting fungi at an incipient stage of wood colonization (Guglielmo *et al.*, 2007; Nicolotti *et al.*, 2009; Gonthier *et al.*, 2015), at any time of the year and in the absence of external indicators of decay (e.g. fruiting bodies), the visual inspection of fruiting bodies or other signs does not seem to be a reliable diagnostic method. According to Vollbrecht and Agestam (1995), it is difficult to classify infected trees based on external signs and symptoms of decay. This observation is also supported by previous reports on wood-inhabiting fungal communities (Johannesson and Stenlid, 1999; Rajala *et al.*, 2010; Gonthier *et al.*, 2012; Schmidt *et al.*, 2012; Ovaskainen *et al.*, 2013). Although inventories of wood decay fungi have traditionally been based on inspecting trees for the occurrence of fruiting bodies (Renvall *et al.*, 1991; Hintikka, 1993; Renvall, 1995; Nicolotti *et al.*, 2010), it should be emphasized that fruiting body development is highly variable and affected by environmental factors as well as by the fungal species (Rayner and Boddy, 1988). Therefore, such inventories should span over several years in order to be representative of the fungal diversity (Rayner and Boddy, 1988; Johannesson and Stenlid, 1999; Vainio and Hantula, 2000). Furthermore, the absence of fruiting bodies is not necessarily indicative of the absence of wood decay fungi (Rayner and Boddy, 1988).

Currently, the evidence is increasingly accumulating on the latent presence (in more general terms 'endophytic colonization' *sensu* Hendry *et al*., 2002) of wood decay fungi in the functional sapwood of intact trees (Parfitt *et al*., 2010; Vasaitis, 2013) in which they rarely if ever develop overtly to produce decay columns or fruiting bodies. For instance, some fungal species, such as *Daldinia concentrica* (Bolton) Ces. & De Not. (Griffith and Boddy, 1990), *Fomes fomentarius* (L.) Fr. and *Fomitopsis pinicola* (Sw.) P. Karst. (Vasaitis, 2013), were consistently detected in sound wood in living trees, suggesting that a period of latent establishment precedes the development of active decay. Nevertheless, the infection venues in intact trees, the mechanisms of their symptomless persistence and the factors triggering decay development are mostly unknown to date (Griffith and Boddy, 1990; Parfitt *et al.*, 2010; Vasaitis, 2013).

Multiplex PCR-based methods cannot allow to discriminate between the latent presence and the active presence (decay development) of wood decay fungi in standing trees. However, the identification of a serious wood decay fungal species, regardless if active or not, should be regarded as an important alarm bell for monitoring purposes. While the diagnosis of wood decay fungi in standing trees based on multiplex PCR does not allow to infer the localization and the extent of the decay column, other instrumental analyses may be used for such purposes, and these may include both non-destructive techniques (e.g. tomography and infrared thermography) and destructive techniques (e.g. Resistograph, Densitomat and Fractometer). Most of these techniques are generally used during tree hazard assessment in urban landscapes (Ouis, 2003), though new devices based on electrical conductivity (i.e. Rotfinder) were developed and are available for the detection of decayed trees in the forest (Oliva *et al.*, 2011).

In our study, ten widespread fungal species or genera inhabiting urban and forest standing trees were identified on the basis of visual inspection of fruiting bodies or other signs and/or

on the basis of multiplex PCR-based methods (Table 2). Data obtained by analysing fungal DNA directly from wood samples showed that the number of fungal taxa infecting standing trees is higher than that inferable on the basis of fruiting bodies or other signs observed on the same trees. This is especially noticeable in the urban sites, where only fruiting bodies of *Fuscoporia torulosa* (Pers.) T. Wagner & M. Fisch., *Ganoderma* spp., *Kretzschmaria deusta* (Hoffm.) P.M.D. Martin, *Inonotus/Phellinus* spp. and *Perenniporia fraxinea* (Bull.) Ryvarden were observed, while multiplex PCR also detected *Armillaria* spp., *Fomitopsis pinicola*, *Phaeolus schweinitzii* and *Porodaedalea* spp. in addition to the above cited species. Only *Armillaria* spp. and *Heterobasidion* spp. were observed/detected in forest sites; the high incidence of these two fungal taxa confirms that they are very widespread root and butt rot fungi in protection forests of western Italian Alps (Giordano *et al.*, 2010).

With the exception of *Porodaedalea* spp. and *F. pinicola*, all differences in term of frequencies of infected trees detected through the two approaches (visual inspection of fruiting bodies or other signs vs. multiplex PCR-based methods) were significant (Table 2). However, it is worth noting that in these latter two cases lack of significance may be due to the limited number of positive samples.

The rates of underestimation (Table 2) of the visual inspection compared to multiplex PCR-based methods were in agreement with the ecological observations concerning the persistence of fruiting bodies of different fungal taxa in the field. In fact, *Armillaria* spp. and *P. schweinitzii* form annual fruiting bodies that persist only for a short period of the year (usually in autumn) (Lonsdale, 1999), resulting sporadically visible (underestimation rate: 100%); in our study *Armillaria* spp. was observed only in the form of rhizomorphs and/or white mycelial fans. Although *Heterobasidion* spp. form perennial fruiting bodies, they develop almost solely in hidden places: under moss cover at the stem base or on roots of dead standing trees, under root systems of fallen trees, under fallen trunks in moist places or in hollowed

283 stumps (Korhonen and Stenlid, 1998; Garbelotto and Gonthier, 2013). It should be noted that
284 in our study no dead trees or stumps were sampled and this may justify an underestimation
285 rate of 100%. *Inonotus/Phellinus* spp. include both species with perennial and annual fruiting
286 bodies (underestimation rate: 94.7%). For instance, *Inonotus hispidus* (Bull.) P. Karst. forms
287 annual fruiting bodies that can appear from May to February and sometimes persist for some
288 months in a black desiccated or putrescent state after death (Lonsdale, 1999); on the contrary,
289 *Fomitiporia punctata* (P. Karst.) Murrill ( =*Phellinus punctatus*) and *Fuscoporia torulosa*
290 (=*Phellinus torulosus*) (underestimation rate: 81.8%) form fruiting bodies that can persist for
291 several years (Bernicchia, 2005). In addition, it should be noted that in our study *F. torulosa*,
292 commonly associated with broadleaf species (Campanile *et al*., 2008) including *Quercus* spp.
293 (its preferential host), was unexpectedly detected only in conifer tree species. *Kretzschmaria*
294 *deusta*, which is reported as hardly detectable during visual tree inspection (Guglielmo *et al*.,
295 2012), was observed in one of the seven infected trees (underestimation rate > 80%). The only
296 two fungal taxa that showed an underestimation rate below 80% were *P. fraxinea* and
297 *Ganoderma* spp., species that typically form perennial fruiting bodies on the main stem at the
298 base of the tree. Fruiting bodies of these fungi are reported as easily detectable during visual
299 tree inspections (Schwarze and Ferner, 2003).
300
301 [Table 2]
302
303 In conclusion, visual inspection of trees for the presence of fruiting bodies or other signs of
304 wood decay fungi underestimated on average and irrespective of the environmental context
305 (i.e. urban sites and forest sites) more than 90% of infected trees compared to the more
306 sophisticated PCR-based assays, which are thus preferable for diagnostic purposes within tree
307 hazard assessment programmes, especially if used in combination with instrumental analyses

aimed at assessing the localization and the extent of the decay. Although the rate of underestimation varied depending on the fungal species, the average rate of underestimation we observed may be used for roughly estimates of the number of trees infected by wood decay fungi in a site based on the frequency of fruiting bodies or other signs.

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**Conflict of interest statement**

None declared.

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## References

Anselmi, N. and Bragaloni, G. 1992 A method to identify wood decay basidiomycetes by using enzymatic comparisons. *Micologia Italiana* **2**, 15–20.

Bernicchia, A. 2005 *Polyporaceae s.l.* Candusso, 594 pp.

Blanchette, R.A. 1991 Delignification by wood-decay fungi. *Annu. Rev. Phytopathol*. **29**, 381–398.

Breitenbach, J. and Kränzlin, F. 1986 *Champignons de Suisse. Tome II Champignons Sans Lames*. Mykologia, 412 pp.

Campanile, G., Schena, L. and Luisi, N. 2008 Real-time PCR identification and detection of *Fuscoporia torulosa* in *Quercus ilex*. *Plant Pathol.* **57**, 76–83.

Clausen, C.A. 2003 Detecting decay fungi with antibody-based tests and immunoassays. *Wood Deterior. Preserv.* **845**, 325–336.

Garbelotto, M. and Gonthier, P. 2013 Biology, epidemiology and control of *Heterobasidion* species worldwide. *Annu. Rev. Phytopathol.* **51**, 39–59.

Giordano, L., Nicolotti, G. and Gonthier, P. 2010 Presence and abundance of root rot, butt rot and stem rot fungi in protection forests of the western Alps. *J. Plant Pathol.* **92** (Supplement 4), S4.84.

Giordano, L., Lione, G., Nicolotti, G. and Gonthier, P. 2012 Effect of *Heterobasidion annosum s.l.* root and butt rots on the stability of Norway spruce: an uprooting test. In *Proceeding of the XIII International Conference on Root and Butt Root of Forest Trees*. P. Capretti, C. Comparini, M. Garbelotto, N. La Porta and A. Santini (eds). University Press, Firenze, pp. 247–250.

Glaeser, J.A. and Lindner, D.L. 2011 Use of fungal biosystematics and molecular genetics in detection and identification of wood-decay fungi for improved forest management. *For. Path.* **41**, 341–348.

Gonthier, P. and Nicolotti, G. 2007 A field key to identify common wood decay fungal species on standing trees. *Arboric. Urban. For.* **33**, 410–420.

Gonthier, P., Brun, F., Lione, G. and Nicolotti, G. 2012 Modelling the incidence of *Heterobasidion annosum* butt rots and related economic losses in alpine mixed naturally regenerated forests of northern Italy. *For. Path*. **42**, 57–68.

Gonthier, P., Guglielmo, F., Sillo, F., Giordano, L. and Garbelotto, M. 2015 A molecular diagnostic assay for the detection and identification of wood decay fungi of conifers. *For. Path*. **45**, 89–101.

Griffith, G.S. and Boddy, L. 1990 Fungal decomposition of attached angiosperms twigs. I. Decay community development in ash, beech and oak. *New Phytol.* **116**, 407–415.

Guglielmo, F., Bergemann, S.E., Gonthier, P., Nicolotti, G. and Garbelotto, M. 2007 A multiplex PCR-based method for the detection and early identification of wood rotting fungi in standing trees. *J. Appl. Microbiol.* **103**, 1490–1507.

Guglielmo, F., Gonthier, P., Garbelotto, M. and Nicolotti, G. 2008 A PCR-based method for the identification of important wood rotting fungal taxa within *Ganoderma, Inonotus* s.l. and *Phellinus* s.l. *FEMS Microbiol. Lett.* **282**, 228–237.

Guglielmo, F., Gonthier, P., Garbelotto, M. and Nicolotti, G. 2010 Optimization of sampling procedures for DNA-based diagnosis of wood decay fungi in standing trees. *Lett. Appl. Microbiol.* **51**, 90–97.

Guglielmo, F., Michelotti, S., Nicolotti, G. and Gonthier, P. 2012 Population structure analysis provides insights into the infection biology and invasion strategies of *Kretzschmaria deusta* in trees. *Fungal Ecol.* **5**, 714–725.

382 Guillaumin, J.-J. and Legrand, P. 2013 Armillaria root rots. In: *Infectious Forest Diseases.* P.
383 Gonthier and G. Nicolotti (eds). CAB International, pp. 159–177.
384 Jellison, J. and Jasalavich, C. 2000 A review of selected methods for the detection of
385 degradative fungi. *Int. Biodeter. Biodegr.* **46**, 241–244.
386 Johannesson, H. and Stenlid, J. 1999 Molecular identification of wood-inhabiting fungi in an
387 unmanaged *Picea abies* forest in Sweden. *Forest Ecol. Manag.* **115**, 203–211.
388 Hendry, S.J., Boddy, L. and Lonsdale, D. 2002 Abiotic variables effect differential expression
389 of latent infections in beech (*Fagus sylvatica*). *New Phytol.* **155**, 449–460.
390 Hickman, G.W., Perry, E.J. and Davis, R.M. 2011 Wood decay fungi in landscape trees.
391 University of California, Pest Notes, Publication 74109.
392 Hintikka, V. 1993 Occurrence of edible fungi and other macromycetes on tree stumps over a
393 sixteen-year period. *Acta Bot. Fenn.* **149**, 11–17.
394 Hjortstam, K., Larsson, K.H. and Ryvarden, L. 1987 *The* Corticiaceae *of North Europe.*
395 *Introduction and Keys, Vol. 1*. Fungiflora, 59 pp.
396 Korhonen, K. and Stenlid, J. 1998 Biology of *Heterobasidion annosum*. In Heterobasidion
397 annosum*: Biology, Ecology, Impact, and Control*. S. Woodward, J. Stenlid, R. Karjalainen
398 and A. Hüttermann (eds). CAB International, pp. 43–69.
399 Lonsdale, D. 1999 *Principles of Tree Hazard Assessment and Management*. The Stationery
400 Office, 388 pp.
401 Lonsdale, D. 2000 *Hazards from Trees. A general Guide.* Forestry Commission, 28 pp.
402 Lonsdale, D., Pautasso, M. and Holdenrieder, O. 2008 Wood-decaying fungi in the forest:
403 conservation needs and management options. *Eu. J. Forest Res*. **127**, 1–22.
404 Mattheck, C. and Breloer, H. 1992 Tree monitoring with VTA – visual tree assessment.
405 *Baumkontrollen Mit VTA Visual Tree Assessment* **41**, 777–784.

406 Nicolotti, G., Socco, L.V., Martinis, R., Godio, A. and Sambuelli, L. 2003 Application and
407 comparison of three tomographic techniques for detection of decay in trees. *J. Arboric.* **29**,
408 66–78.
409 Nicolotti, G., Gonthier, P., Guglielmo, F. and Garbelotto, M. 2009 A biomolecular method for
410 the detection of wood decay fungi: a focus on tree stability assessment. *Arboric. Urban For.*
411 **35**, 14–19.
412 Nicolotti, G., Gonthier, P. and Guglielmo, F. 2010 Advances in detection and identification of
413 wood rotting fungi in timber and standing trees. In *Molecular Identification of Fungi*. Y.
414 Gherbawy and K. Voigt (eds). Springer, pp. 251–276.
415 Nobles, M.K. 1965 Identification of cultures of wood-inhabiting hymenomycetes. *Can. J.*
416 *Botany* **44**, 1097–1165.
417 Oliva, J., Thor, M. and Stenlid, J. 2010 Reaction zone and periodic increment decrease in
418 *Picea abies* trees infected by *Heterobasidion annosum* s.l. *Forest Ecol. Manag.* **260**, 692–698.
419 Oliva, J., Romeralo, C. and Stenlid, J. 2011 Accuracy of the Rotfinder instrument in detecting
420 decay on Norway spruce (*Picea abies*) trees. *Forest Ecol. Manag.* **262**, 1378–1386.
421 Ouis, J. 2003 Non-destructive techniques for detecting decay in standing trees. *Arboric. J.* **27**,
422 159–177.
423 Ovaskainen, O., Schigel, D., Ali-Kovero, H., Auvinen, P., Paulin, L., Nordén, B. and Nordén,
424 J. 2013 Combining high-throughput sequencing with fruit body surveys reveals contrasting
425 life-history strategies in fungi. *ISME J.* **7**, 1696–1709.
426 Parfitt, D., Hunt, J., Dockrell, D., Rogers, H.J. and Boddy, L. 2010 Do all trees carry the
427 seeds of their own destruction? PCR reveals numerous wood decay fungi latently present in
428 sapwood of a wide range of angiosperm trees. *Fungal Ecol.* **3**, 338–346.
429 R Core Team 2013 *R: a Language and Environment for Statistical Computing*. R Foundation
430 for Statistical Computing, Vienna, Austria.

Rajala, T., Peltoniemi, M., Pennanen, T. and Mäkipää, R. 2010 Relationship between wood-inhabiting fungi determined by molecular analysis (denaturing gradient gel electrophoresis) and quality of decaying logs. *Can. J. For. Res.* **40**, 2384–2397.

Rayner, A.D.M. and Boddy, L. 1988 *Fungal Decomposition of Wood: Its Biology and Ecology*. John Wiley & Sons, 602 pp.

Renvall, P. 1995 Community structure and dynamics of wood-rotting basidiomycetes on decomposing conifer trunks in northern Finland. *Karstenia* **35**, 1–51.

Renvall, P., Renvall, T. and Niemelä, T. 1991 Basidiomycetes at the timberline in Lapland: 2. An annotated checklist of the polypores of northeastern Finland. *Karstenia* **31**,13–28.

Schmidt, O. 2007 Indoor wood-decay basidiomycetes: damage, causal fungi, physiology, identification and characterization, prevention and control. *Mycol. Prog*. **6**, 261–279.

Schmidt, O., Gaiser, O. and Dujesiefken, D. 2012 Molecular identification of decay fungi in the wood of urban trees. *Eur. J. Forest Res.* **131**, 885–891.

Schwarze F.W.M.R and Ferner D. 2003 *Ganoderma* on trees – differentiation of species and studies of invasiveness. *Arboric. J.* **27**, 59–77.

Stalpers, J.A. 1978 Identification of wood-inhabiting aphyllophorales in pure culture. *Stud. Mycol.* **16**, 1–248.

Stenlid, J., Penttilä, R. and Dahlberg, A. 2008 Wood-decay basidiomycetes in boreal forests: distribution and community development. In *Ecology of Saprotrophic Basidiomycetes*. L. Boddy, J.C. Frankland and P. van West (eds). Academic Press, pp. 239–262.

Terho, M., Hantula, J. and Hallaksela, A.M. 2007 Occurrence and decay patterns of common wood-decay fungi in hazardous trees felled in the Helsinki City. *Forest Pathol*. **37**, 420–432.

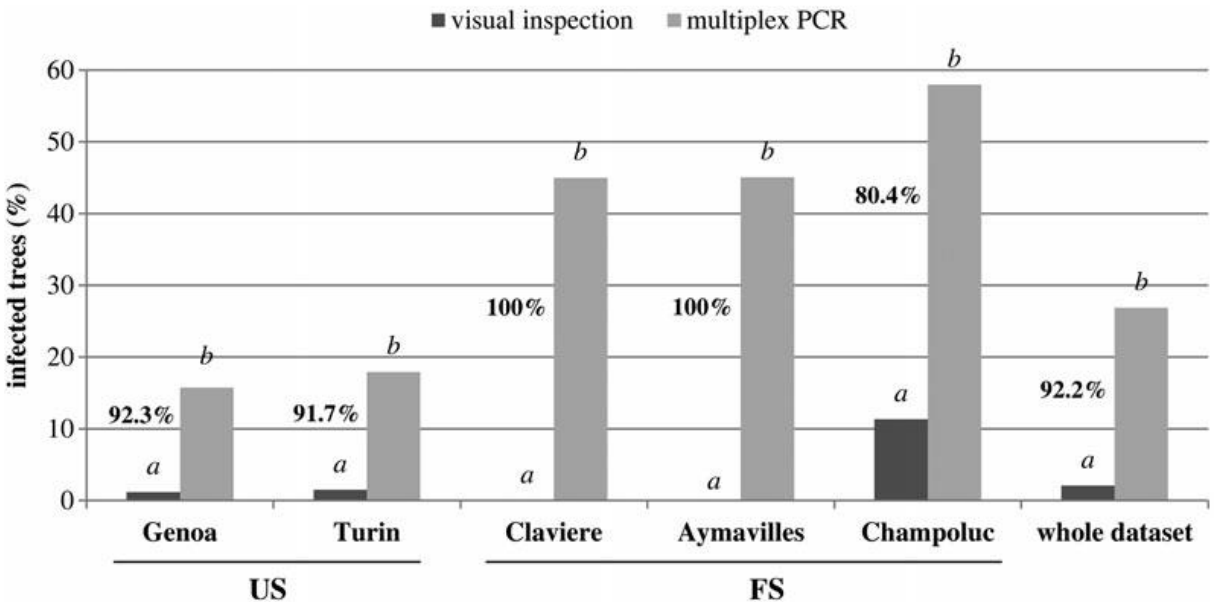
Vainio, E.J. and Hantula, J. 2000 Direct analysis of wood-inhabiting fungi using denaturing gradient gel electrophoresis of amplified ribosomal DNA. *Mycol. Res.* **104**, 927–936.

Vasaitis, R. 2013 Heart rots, sap rots and canker rots. In: *Infectious Forest Diseases.* P. Gonthier and G. Nicolotti (eds). CAB International, pp. 197–229.

Vollbrecht, G. and Agestam, E. 1995 Identifying butt rotted Norway spruce trees from external signs. *For. Landascape Res.* **1**, 241–254.

**Figure caption**

**Figure 1** Infected standing trees detected in urban sites (Genoa and Turin cities - US) and forest sites (Claviere, Aymavilles and Champoluc - FS) through visual inspection of fruiting bodies or other signs of the presence of wood decay fungi and multiplex PCR-based methods. For each site values of columns with different letters differ significantly (Chi-squared test; $P < 0.05$). Underestimation value (percentage) of the visual inspection compared to multiplex PCR-based diagnosis is reported for each site. Values and statistical analysis for the whole dataset are also reported.



## Tables

**Table 1** Sites and tree species analysed for the detection of wood decay fungi

| Site | Coordinates Lat-Long | US/FS* | Tree species | N. of trees analysed |
|---|---|---|---|---|
| **City of Genoa** | 44°24’10.12’’N | US | *Cedrus* spp. | 45 |
| | 8°57’25.20’’E | | *Cedrus deodara* | 14 |
| | | | *Cupressus* spp. | 35 |
| | | | *Pinus* spp. | 14 |
| | | | *Pinus halepensis* | 3 |
| | | | *Pinus pinaster* | 2 |
| | | | *Pinus pinea* | 50 |
| | | | *Taxus* spp. | 2 |
| **City of Turin** | 45°04’16.49’’N | US | *Acer* spp. | 56 |
| | 7°41’04.73’’E | | *Aesculus hippocastanum* | 11 |
| | | | *Platanus* spp. | 401 |
| **Claviere (Piedmont)** | 44°56’22.22’’N | FS | *Larix decidua* | 20 |
| | 6°45’05.60’’E | | *Pinus uncinata* | 20 |
| **Aymavilles (Aosta Valley)** | 45°42’04.17’’N | FS | *Picea abies* | 142 |
| | 7°14’26.99’’E | | | |
| **Champoluc (Aosta Valley)** | 45°49’53.65’’N | FS | *Larix decidua* | 14 |
| | 7°43’32.35’’E | | *Picea abies* | 74 |
| **Total trees analysed** | | US | | 633 |
| **Total trees analysed** | | FS | | 270 |

* UR: Urban sites; FS: Forest sites

**Table 2** Number of infected trees (n.) detected in urban and forest sites through visual inspection of fruiting bodies or other signs and multiplex PCR-based methods. For each fungal taxon the rate of underestimation of the visual inspection compared to multiplex PCR and the P value of Chi-squared test or Fisher exact test are reported. Association with host species is also indicated

| **Fungal taxa in urban sites** | **Visual inspection (n.)** | **Multiplex PCR (n.)** | **Underestimation (%)** | **P value** | **Trees species (n.)** |
|---|---|---|---|---|---|
| *Armillaria* spp. | 0 | 40 | 100 | <0.001* | *Platanus* spp. (30), *Acer* spp. (4), *Aesculus hippocastanum* (2), *Cedrus* spp. (1), *Cupressus* spp. (1), *Pinus pinaster* (1), *P. pinea* (1) |
| *Inonotus/Phellinus* spp. | 1 | 19 | 94.7 | <0.001* | *Platanus* spp. (15), *Acer* spp. (2), *A. hippocastanum* (2) |
| *Perenniporia fraxinea* | 3 | 13 | 76.9 | <0.001* | *Platanus* spp. (13) |
| *Fuscoporia torulosa* | 2 | 11 | 81.8 | <0.001* | *Cedrus* spp. (3), *P. pinea* (3), *Pinus* spp. (2), *Cupressus* spp. (1), *Pinus halepensis* (1), *Taxus* spp. (1) |
| *Ganoderma* spp. | 2 | 9 | 77.8 | 0.004* | *Platanus* spp. (8), *Acer* spp. (1) |
| *Kretzschmaria deusta* | 1 | 7 | 85.7 | 0.007* | *Acer* spp. (5), *Platanus* spp. (2) |
| *Phaeolus schweinitzii* | 0 | 6 | 100 | 0.004* | *P. pinea* (3), *Cedrus deodara* (2), *Cedrus* spp. (1) |
| *Porodaedalea* spp. | 0 | 3 | 100 | 0.102 | *Cedrus* spp. (2), *P. pinea* (1) |
| *Fomitopsis pinicola* | 0 | 2 | 100 | 0.317 | *Pinus* spp. (2) |
| **Total infected trees** | **9** | **110** | 91.8 | <0.001* | |
| **Fungal taxa in forest sites** | **Visual inspection (n.)** | **Multiplex PCR (n.)** | **Underestimation (%)** | **P value** | **Trees species (n.)** |
| *Heterobasidion* spp. | 0 | 86 | 100 | <0.001* | *Picea abies* (79), *Larix decidua* (5), *Pinus uncinata* (2) |
| *Armillaria* spp. | 10 | 25 | 60 | <0.001* | *Picea abies* (13), *Pinus uncinata* (7), *Larix decidua* (5) |
| *Armillaria/Heterobasidion* (togheter) | 0 | 22 | 100 | <0.001* | *Picea abies* (16), *Larix decidua* (4), *Pinus uncinata* (2) |

| | | | | |
|---|---|---|---|---|
| **Total infected trees** | **10** | **133** | 92.5 | <0.001* |

* Significant value (P < 0.05)