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

16

17 **Giordano Luana, Sillo Fabiano, Guglielmo Fabio and Gonthier Paolo**

18

19 *Department of Agriculture, Forest and Food Sciences, University of Torino, Largo Paolo*

20 *Braccini 2, I-10095 Grugliasco, Italy*

21

22

23

24

25 *Corresponding author: P. Gonthier*

26 Telephone number: +39 0116708697

27 Fax number: +39 0112368697

28 e-mail: paolo.gonthier@unito.it

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39 **Summary**

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

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61 **Introduction**

62 In forest ecosystems wood decay fungi play an important role for biodiversity, CO₂ dynamics,
63 nutrient cycling and forest regeneration and as indicators of forest health (Rayner and Boddy,
64 1988; Blanchette, 1991; Stenlid *et al.*, 2008). As a result, in Scandinavia for instance,
65 foresters use management techniques to maximize fungal biodiversity and maintain “red lists”
66 of threatened and endangered fungi (Glaeser and Lindner, 2011). Furthermore, standing and
67 fallen decaying trees are species-rich environments, and deadwood has been recognized as
68 providing resources for a variety of living organisms (Lonsdale *et al.*, 2008). However, wood
69 decay fungi significantly reduce the quality of timber in production forests (Oliva *et al.*, 2010)
70 and they may reduce the protection function of trees against avalanches, rock falls and debris
71 flow in protection forests (Giordano *et al.*, 2012). The loss of wood mechanical properties
72 caused by wood decay fungi can predispose trees to the risk of windthrows or limb failures
73 (Lonsdale, 2000; Hickman *et al.*, 2011). In urban landscapes, tree or limb failures triggered by
74 wood decay can lead hazardous situations, resulting in significant damages of property and/or
75 tragic injuries.

76 The assessment of the presence of wood decay fungi may be required in pristine, production
77 and protection forests, as well as in urban settings for the timely detection of potentially
78 hazardous situations. Since different fungi may differ in their ecology, aggressiveness and
79 ability to colonize wood tissues, an accurate diagnosis of the fungal species involved is
80 usually preferable as it may allow a prediction on how the wood decay will progress and of
81 the probability of failure or decline (Lonsdale, 1999; Guglielmo *et al.*, 2007; Glaeser and
82 Lindner, 2011). During tree hazard assessment, conventional diagnostic methods to detect
83 wood decay fungi are mainly based on visually inspecting trees for the occurrence of external
84 indicators of decay including fruiting bodies (Rayner and Boddy, 1988; Renvall *et al.*, 1991;
85 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).

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

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

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

129

130 **Materials and methods**

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

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

152

153 [Table 1]

154

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

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

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

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

182

$$183 \quad U\% = \frac{\text{infected trees MP} - \text{infected trees VI}}{\text{infected trees MP}} \cdot 100$$

184

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

191

192 **Results and Discussion**

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

198 However, when trees were analysed through multiplex PCR-based methods, the rate of trees
199 infected by at least one target wood decay fungal species raised to 15.7%, 17.9%, 58.0%,
200 45.0% and 45.1% in the city of Genoa, city of Turin and in the Champoluc, Claviere and
201 Aymavilles forests, respectively.

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

206

207 [Figure 1]

208

209 Our results suggest that diagnosis based on visual inspection of fruiting bodies or other signs
210 of wood decay fungi underestimated **on average** more than 90% of infected trees (from 80.4%
211 to 100% underestimation depending on site), with a similar situation in both environmental
212 contexts (91.8% in urban sites vs. 92.4% in forest sites). The number of sampled sites is
213 limited, however, the similar rate of underestimation observed in urban and forest sites ($P >$
214 0.05) and in general in all sites is noteworthy, and may be used to roughly estimate the
215 number of trees infected by wood decay fungi in a site based on the frequency of fruiting
216 bodies or other signs.

217 While multiplex PCR-based methods may be helpful in detecting fungi at an incipient stage of
218 wood colonization (Guglielmo *et al.*, 2007; Nicolotti *et al.*, 2009; Gonthier *et al.*, 2015), **at**
219 **any time of the year and in the absence of external indicators of decay (e.g. fruiting bodies),**
220 the visual inspection of fruiting bodies or other signs does not seem to be a reliable diagnostic
221 method. **According to Vollbrecht and Agestam (1995), it is difficult to classify infected trees**
222 **based on external signs and symptoms of decay.** This observation is **also** supported by
223 previous reports on wood-inhabiting fungal communities (Johannesson and Stenlid, 1999;
224 Rajala *et al.*, 2010; Gonthier *et al.*, 2012; Schmidt *et al.*, 2012; Ovaskainen *et al.*, 2013).
225 Although inventories of wood decay fungi have traditionally been based on inspecting trees
226 for the occurrence of fruiting bodies (Renvall *et al.*, 1991; Hintikka, 1993; Renvall, 1995;
227 Nicolotti *et al.*, 2010), it should be emphasized that fruiting **body** development is highly
228 variable and affected by environmental factors as well as by the fungal species (**Rayner and**
229 **Boddy, 1988**). **Therefore, such inventories should span over several years in order to be**
230 **representative of the fungal diversity (Rayner and Boddy, 1988; Johannesson and Stenlid,**
231 **1999; Vainio and Hantula, 2000).** Furthermore, the absence of fruiting bodies is not
232 **necessarily indicative of the absence of wood decay fungi (Rayner and Boddy, 1988).**

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

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

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

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

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

274 The rates of underestimation (Table 2) of the visual inspection compared to multiplex PCR-
275 based methods were in agreement with the ecological observations concerning the persistence
276 of fruiting bodies of different fungal taxa in the field. In fact, *Armillaria* spp. and *P.*
277 *schweinitzii* form **annual** fruiting bodies that persist only for a short period of the year (usually
278 in autumn) (Lonsdale, 1999), resulting sporadically visible (underestimation rate: 100%); in
279 our study *Armillaria* spp. was observed only in the form of rhizomorphs and/or white
280 mycelial fans. Although *Heterobasidion* spp. form perennial fruiting bodies, they develop
281 almost solely in hidden places: under moss cover at the stem base or on roots of dead standing
282 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**

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

312

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322

323 **Conflict of interest statement**

324 None declared.

325

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334

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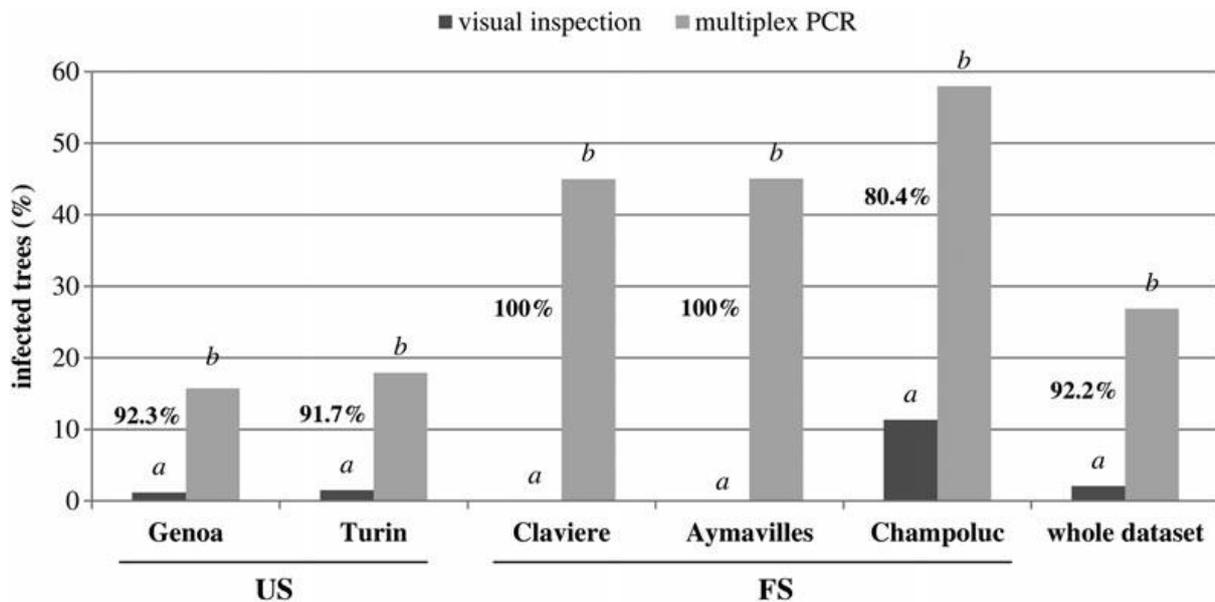
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480 **Figure caption**

481 **Figure 1** Infected standing trees detected in urban sites (Genoa and Turin cities - US) and
482 forest sites (Claviere, Aymavilles and Champoluc - FS) through visual inspection of fruiting
483 bodies or other signs of the presence of wood decay fungi and multiplex PCR-based methods.
484 For each site values of columns with different letters differ significantly (Chi-squared test; $P <$
485 0.05). Underestimation value (percentage) of the visual inspection compared to multiplex
486 PCR-based diagnosis is reported for each site. Values and statistical analysis for the whole
487 dataset are also reported.

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491 **Tables**492 **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 8°57'25.20''E	US	<i>Cedrus</i> spp.	45
			<i>Cedrus deodara</i>	14
			<i>Cupressus</i> spp.	35
			<i>Pinus</i> spp.	14
			<i>Pinus halepensis</i>	3
			<i>Pinus pinaster</i>	2
			<i>Pinus pinea</i>	50
			<i>Taxus</i> spp.	2
City of Turin	45°04'16.49''N 7°41'04.73''E	US	<i>Acer</i> spp.	56
			<i>Aesculus hippocastanum</i>	11
			<i>Platanus</i> spp.	401
Claviere (Piedmont)	44°56'22.22''N 6°45'05.60''E	FS	<i>Larix decidua</i>	20
			<i>Pinus uncinata</i>	20
Aymavilles (Aosta Valley)	45°42'04.17''N 7°14'26.99''E	FS	<i>Picea abies</i>	142
Champoluc (Aosta Valley)	45°49'53.65''N 7°43'32.35''E	FS	<i>Larix decidua</i>	14
			<i>Picea abies</i>	74
Total trees analysed		US		633
Total trees analysed		FS		270

493 * UR: Urban sites; FS: Forest sites

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503 **Table 2** Number of infected trees (n.) detected in urban and forest sites through visual inspection of fruiting bodies or other signs and multiplex
504 PCR-based methods. For each fungal taxon the rate of underestimation of the visual inspection compared to multiplex PCR and the P value of
505 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.)
<i>Armillaria</i> spp.	0	40	100	<0.001*	<i>Platanus</i> spp. (30), <i>Acer</i> spp. (4), <i>Aesculus hippocastanum</i> (2), <i>Cedrus</i> spp. (1), <i>Cupressus</i> spp. (1), <i>Pinus pinaster</i> (1), <i>P. pinea</i> (1)
<i>Inonotus/Phellinus</i> spp.	1	19	94.7	<0.001*	<i>Platanus</i> spp. (15), <i>Acer</i> spp. (2), <i>A. hippocastanum</i> (2)
<i>Perenniporia fraxinea</i>	3	13	76.9	<0.001*	<i>Platanus</i> spp. (13)
<i>Fuscoporia torulosa</i>	2	11	81.8	<0.001*	<i>Cedrus</i> spp. (3), <i>P. pinea</i> (3), <i>Pinus</i> spp. (2), <i>Cupressus</i> spp. (1), <i>Pinus halepensis</i> (1), <i>Taxus</i> spp. (1)
<i>Ganoderma</i> spp.	2	9	77.8	0.004*	<i>Platanus</i> spp. (8), <i>Acer</i> spp. (1)
<i>Kretzschmaria deusta</i>	1	7	85.7	0.007*	<i>Acer</i> spp. (5), <i>Platanus</i> spp. (2)
<i>Phaeolus schweinitzii</i>	0	6	100	0.004*	<i>P. pinea</i> (3), <i>Cedrus deodara</i> (2), <i>Cedrus</i> spp. (1)
<i>Porodaedalea</i> spp.	0	3	100	0.102	<i>Cedrus</i> spp. (2), <i>P. pinea</i> (1)
<i>Fomitopsis pinicola</i>	0	2	100	0.317	<i>Pinus</i> 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.)
<i>Heterobasidion</i> spp.	0	86	100	<0.001*	<i>Picea abies</i> (79), <i>Larix decidua</i> (5), <i>Pinus uncinata</i> (2)
<i>Armillaria</i> spp.	10	25	60	<0.001*	<i>Picea abies</i> (13), <i>Pinus uncinata</i> (7), <i>Larix decidua</i> (5)
<i>Armillaria/Heterobasidion</i> (together)	0	22	100	<0.001*	<i>Picea abies</i> (16), <i>Larix decidua</i> (4), <i>Pinus uncinata</i> (2)

506	Total infected trees	10	133	92.5	<0.001*
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* Significant value (P < 0.05)