Needle disinfection and bioptic wood sampling achieved with a disposable for drill resistance measurement devices

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Abstract

Needle drill resistance measurement devices (NDRMD) are often used during tree hazard assessment campaigns to detect and measure the extent of wood decay and other defects of wood in trees, despite of the possibility of transmission of potentially pathogenic microbial inoculum from tree to tree through unclean needles. Here, we describe a disposable connectable to NDRMD through an adapter and we report on its efficacy not only at disinfecting the needle, thus reducing the likelihood of disease transmission, but also at collecting wood samples for bioptic purposes, whose subsequent analysis may be pivotal for, or allow to refine, the prognosis. The complete efficacy of the disposable at disinfecting the needle was determined through three different experiments conducted under controlled conditions *in vitro* and *in vivo* using both wood decay fungi and the canker stain pathogen of plane trees, and under field conditions. The disposable combined with NDRMD proved to be as effective as state-of-the-art drilling methods at collecting wood samples for subsequent PCR-based molecular diagnosis of wood decay fungi (Fisher's exact test for count data; $P = 4.846 \times 10^{-7}$) as determined through comparative sampling and diagnostic assays on 42 trees. The disposable allows not only for a routinely, complete and fully standardized disinfection of the needle, but also provides the opportunity to automatically and efficiently collect bioptic wood samples for subsequent phytopathological analyses.

Key words

Diagnosis; disinfection; needle drill resistance measurement devices; resistograph; tree hazard assessment; wood decay fungi.

Abbreviations[1]

1. Introduction

The timely detection of potentially hazardous trees may be of pivotal importance to prevent tree and limb failures in urban environment and hence to reduce the risk of damage to properties and/or people. Such failures are often associated with the structural deterioration of wood caused by decay fungi belonging to basidiomycetes or, less frequently, to ascomycetes (Lonsdale, 1999; Schwarze, 2008). Prognostic decisions during tree hazard assessment campaigns are generally the result of accurate visual inspection of trees often combined with the application of instruments aimed at detecting and measuring wood decay and other defects of wood. Several instruments have been developed for this purpose, including electrical conductivity meters, instruments based on single pulse sonic and ultrasonic techniques, and computerized tomography (Rust and van Wassenaer, 2017). However, Needle Drill Resistance Measurement Devices (NDRMD) (e.g. Resistograph®, IML-PowerDrill®) are utilized most as they are relatively inexpensive and easy to use compared to most of the others. Incidentally, based on a comparative evaluation of several instruments including electrical conductivity meters, instruments based on single pulse sonic and ultrasonic techniques, and computerized tomography, NDRMD were deemed the most accurate in indicating the location and, in some instances, the quantity of decay (Johnstone et al., 2010). NDRMD use a flat spade type drill bit, hereafter referred to as needle, with a 3 mm tip diameter (1.5 mm shaft diameter) to drill and measure the resistance encountered as the drill passes through the wood (Bethge et al., 1996; Rinn et al., 1996; Rust and van Wassenaer, 2017). Therefore, NDRMD are moderately invasive (Johnstone et al., 2010), but whether and in which extent drills may have detrimental effects on trees is still under

[1]NDRMD - Needle Drill Resistance Measurement Devices; PCR - Polymerase Chain Reaction; MUT - *Mycotheca Universitatis Taurinensis*; MEA - Malt Extract Agar; UCG - University Campus of Grugliasco; DBH - Diameter at Breast Height.

63 debate (Rust and van Wassenaer, 2017). Drill bits may breach the defensive zones and hence increase
64 the likelihood of existing decay to spread further into the tree, as documented for the aggressive
65 canker rot agent *Inonotus hispidus* (Bull.) P. Karst. (Kersten and Schwarze, 2005; Schwarze, 2008),
66 though such a process was deemed relatively unlikely (Lonsdale, 1999). In addition, a major concern
67 is that drill wounds could become infection courts for wood decay fungi, possibly from infective
68 particles carried out on needles that have not been disinfected following previous use (Schwarze,
69 2008; Johnstone et al., 2010). Although only very few studies have been conducted to explore the
70 likelihood of transmission of wood decay from tree-to-tree through unclean needles, attempts to prove
71 cross-infection failed, despite these studies were based on a limited number of fungal species [i.e.
72 *Fomes fomentarius* (L.) Fr. and *I. hispidus*] (Kersten and Schwarze, 2005; Schwarze, 2008).
73 However, wood discoloration and transmission of other microbes in this way, mainly anamorphic
74 fungi, have been documented (Helliwell, 2007; Schwarze, 2008). It should be noted that discolored
75 wood is more likely to be infected by fungi (Rust and van Wassenaer, 2017) and colonization by
76 anamorphic fungi may trigger and is often required for the subsequent infection of wood by decay
77 fungi (Rayner and Boddy, 1988). The fear of transferring microbial inoculum from one tree to another
78 through unclean needles does not refer exclusively to wood decay fungi, but encompasses a wide
79 range of tree pathogens. Concerns are evoked especially about destructive and very infectious
80 pathogens, like the canker stain pathogen of plane trees *Ceratocystis platani* (J.M. Walter) Engelbr.
81 & T.C. Harr., whose infections and spread have been documented to occur easily through pruning
82 tools and other ornamental practices (CABI, 2015; Raupp and Gonthier, 2017).
83 Prognostic decisions within tree hazard assessments also can be based or may be refined by
84 information on which wood decay fungi are colonizing the tree; this has been suggested by a large
85 body of literature (Lonsdale, 1999; Guglielmo et al., 2007; Schwarze, 2008; Gonthier et al., 2015;
86 Mattheck et al., 2015). Indeed, as different fungal species may differ in their ability to colonize a tree,
87 a correct diagnosis can be useful to predict, to some extent, the severity of fungal infection (Lonsdale,
88 1999). The identification of wood decay fungi is generally based on the features of fruiting bodies

89 emerging from trees (Bernicchia, 2005; Gonthier and Nicolotti, 2007). However, fruiting bodies of
90 wood decay fungi are usually present on only a small percentage of infected trees (< 10%), making
91 diagnosis, based on visual inspection of fruiting bodies, unreliable (Giordano et al., 2015). In the last
92 decade, a number of molecular tools based on Polymerase Chain Reaction (PCR) has been developed
93 for the early detection and identification of the most important and widespread wood decay fungi of
94 both conifer and broadleaf trees directly from wood samples (Guglielmo et al., 2007, 2008; Nicolotti
95 et al., 2009, 2010; Gonthier et al., 2015). State-of-the-art for sampling, which is a crucial phase, is
96 based on the collection of wood chips resulting from drillings performed with a 4-mm-diameter, 43-
97 cm-long bit (Guglielmo et al., 2010). While such a drill is more invasive than that of NDRMD, drilling
98 with both instruments may be required if bioptic wood samples have to be collected for diagnosis
99 from wood portions where decay has been previously detected through NDRMD.
100 Here, we describe a disposable connectable to NDRMD through an adapter and we report on its
101 efficacy at both disinfecting the needle and collecting wood samples for bioptic purposes. Both the
102 disposable and the adapter are covered, as a kit, by a pending patent application of the University of
103 Turin (n. 102017000087211 of 28/7/2017).
104

105 **2. Materials and methods**

106 *2.1. The disposable and its working principles*

107 The disposable is made of a plastic microtube with attached cap and with a blind bottom conical in
108 shape (28 mm length, 7 mm maximum internal diameter) (Fig. 1A). A Whatman® qualitative filter
109 paper (415, particle retention 12-15 µm) dampened with 0.2 mL of denatured alcohol (90/10 v/v) is
110 placed in the bottom of the microtube. Above the filter paper, a plastic cone (10 mm length, 7 mm
111 maximum external diameter) is embedded inside the plastic microtube.
112 Once opened and placed into the adapter with the bottom facing the NDRMD, the disposable is
113 pierced by the needle during drill resistance measurement (Fig. 1B-1). The needle, passing through
114 the filter paper dampened with alcohol, becomes disinfected before going through the wood (Fig. 1B-

2). At the end of drill resistance measurement, the needle is retracted inside the NDRMD, allowing for the accumulation of wood particles from the inspected wood inside the plastic cone (Fig. 1B-3). The collected wood particles can serve as bioptic samples for phytopathological analyses (Fig. 1B-4).

[Fig. 1]

2.2. Testing the efficacy of the disposable at disinfecting the needle

The efficacy of the disposable at disinfecting the needle was tested through three different experiments conducted under controlled conditions both *in vitro* (experiment 1) and *in vivo* (experiment 2), and under field conditions (experiment 3) by coupling the disposable with a IML-Resi PD500 (IML, Inc.; needle tip 3.0 mm diameter and shaft 1.5 mm diameter). For all experiments, the disposables were prepared the day before use. The Index Fungorum (2017) and the USDA PLANTS Database (2017) were used as sources of biological nomenclature of fungi and plants, respectively.

In experiment 1, the needle previously disinfected with sterile cotton dampened with denatured alcohol (90:10 v:v), was passed through the aerial mycelium of 10-day-old cultures of the following fungal pathogens: *Ceratocystis platani*, *Ganoderma adspersum* (Schulzer) Donk, *Laetiporus sulphureus* (Bull.) Murrill and *Perenniporia fraxinea* (Bull.) Ryvarden. Fungal isolates were deposited in the *Mycotheca Universitatis Taurinensis* (MUT) with accession numbers MUT5881, MUT5875, MUT5876 and MUT5880, respectively. Caps of 9-cm-diameter Petri dishes filled with MEA (Malt Extract Agar: 31.3 g malt extract agar, 1 L distilled water) were pierced in their centers by using the tip of a scalpel heated in a Bunsen burner flame in order to obtain a hole of approximately 3.0 mm diameter. Subsequently, the needle was inserted through the hole either using (test drilling) or not (control drilling) the disposable, and was rotated so as to transfer any viable inoculum present on the needle itself on the growth medium. For each of the four isolates, 10 test drillings and 10 control drillings were performed, for a total of 80 Petri dishes. Petri dishes were incubated for 20 days

in the dark at 20°C and regularly inspected for the growth of fungal colonies. These fungal colonies were transferred onto MEA and compared to the above fungal pathogens based on macroscopic and microscopic features of the mycelium (Fig. 2A).

In experiment 2, drillings with a needle contaminated by *C. platani* inoculum, as described above, were performed in cuttings of *Platanus* × *hispanica* Mill. ex Münchh. Terminal branches of about 50 cm length and 5-9 mm maximum diameter of a *P.* × *hispanica* on the University Campus of Grugliasco (UCG, North Western Italy) were excised under water, placed in water buckets and brought to the laboratory. They were subsequently placed in 50 mL tubes filled with 30 mL tap water and sealed with Parafilm® as previously described (Garbelotto et al., 2010). For each cutting, one drilling was performed 20 cm above the excised section and 1-2 mm beneath the cambium layer. Drilling points were then wrapped in Parafilm®. Tubes were kept at 21°C on a 12 h light/dark cycle, and they were periodically refilled with tap water. Test and control drillings were performed on 10 cuttings for each of the two categories, for a total of 20 cuttings. Fifteen days after drillings, the thin bark of cuttings was removed and the extent of the necrosis of the cambium layer was measured (Fig. 2B). Attempts of re-isolation of *C. platani* were carried out by transferring small pieces of necrotic tissue into 6 cm diameter Petri dishes filled with MEA.

In experiment 3, a *Populus nigra* L. (60 cm DBH - Diameter at Breast Height) located in the UCG, in which *P. fraxinea* was previously detected by using molecular diagnostic assays combined with a standardized sampling approach (Guglielmo et al., 2007, 2010), was drilled either using (test drilling) or not (control drilling) the disposable. After each drilling, the needle was rotated so as to transfer any viable inoculum present on the needle itself onto a MEA plate. Ten test drillings and 10 control drillings were performed. Petri dishes were incubated for 20 days in the dark at 20°C and regularly inspected for the growth of fungal colonies (Fig. 2C). Fungal colonies were counted and identified based on the macroscopic and microscopic features of the mycelium.

For each of the three experiments, the frequency of samples leading to fungal growth was compared between test and control drillings by using the Fisher's exact test ($\alpha = 0.05$) (Crawley, 2013). Statistical analyses were performed in R 3.2.3 (R Core Team 2017).

[Fig. 2]

2.3. Testing the efficacy of the disposable at collecting bioptic wood samples

The efficacy of the disposable at collecting bioptic wood samples useful for diagnostic purposes was tested by comparing the outcomes of PCR-based detection and identification of wood decay fungi from wood particles collected by the disposable connected to a IML-Resi PD500 (IML, Inc.) to wood chips collected in Petri dishes with the drilling method described by Guglielmo et al. (2010). The experiment was carried out on a total of 42 *Tilia* ×*europaea* L. with an average DBH of 57 cm located at the UCG. Trees did not show any visible crown symptom. Each tree was drilled at the collar with the two devices approximately at the same point. After use, the disposable was closed immediately, stored in a plastic bag and transferred to the laboratory. Wood particles were removed from the disposable by using sterilized tweezers and placed in 2-mL screw-top tubes. Wood samples were lyophilized over-night, frozen in liquid nitrogen, and homogenized in a FastPrep FP120 Cell Disrupter (Qbiogene, Irvine, CA, USA). DNA extraction was performed by using the E.Z.N.A.™ Stool DNA Isolation Kit (Omega Bio-Tek, Doraville, CA, USA). The DNA was used as template in five multiplex PCR-based assays as described by Guglielmo et al. (2007, 2008).

The frequencies of true positive, false positive, true negative and false negative diagnosis with the disposable using the state-of-the-art method as a reference were cross tabulated and the Fisher's exact test for count data ($\alpha = 0.05$) was used to assess the association between the two methods, and hence the efficacy of the disposable.

3. Results

191 *3.1. Efficacy of the disposable at disinfecting the needle*

192 In experiment 1, while 100% of control drillings resulted in the growth of fungal colonies in Petri
193 dishes that were clearly assignable based on macroscopic and microscopic features of the mycelium
194 to the species used in this study, none of the test drillings performed with the disposable resulted in
195 fungal growth ($P = 1.083 \times 10^{-5}$).

196 In experiment 2, while control drillings always resulted in necrosis of the cambium with length
197 ranging from 0.4 cm to 3.1 cm depending on the cutting (mean 1.9 cm ± 1.4 cm), in the test drillings
198 the necrosis was limited to a narrow (1-2 mm) portion of tissue surrounding the drilling hole. Any
199 attempt of re-isolation of *C. platani* from these narrow necrotic areas failed, whereas the fungus was
200 always re-isolated from the necrosis resulting from control drillings ($P = 1.083 \times 10^{-5}$).

201 In experiment 3, control drillings resulted in the growth of fungal colonies on MEA, which were
202 identified as *Penicillium* spp. (60% of Petri dishes), *Cladosporium* spp. (20%) and *Fusarium* spp.
203 (10%). No fungal growth was observed in the remaining 10% of MEA plates. Conversely, test
204 drillings carried out with the disposable never resulted in fungal growth ($P = 1.191 \times 10^{-4}$).

205

206 *3.2. Efficacy of the disposable at collecting bioptic wood samples*

207 Six target fungal species detectable through multiplex PCR were identified by using the state-of-the-
208 art drilling method in samples collected from a total of 23 out of 42 trees: *Armillaria* spp., *Hericium*
209 spp., *Inonotus* spp., *Phellinus* spp., *Stereum* spp. and *Trametes* spp. (Table 1). The same species were
210 detected by using the NDRMD combined with the disposable in 17 out of 42 trees.

211 Overall, the relative frequencies of positive and negative diagnosis with the NDRMD combined with
212 the disposable using the state-of-the-art drilling method as a reference were as follows: true positives
213 40.5% (17 out of 42), false positives 0% (0 out of 42), true negatives 45.2% (19 out of 42) and false
214 negatives 14.3% (6 out of 42). Based on Fisher's exact test for count data, the association between
215 the two sampling methods was significant ($P = 4.846 \times 10^{-7}$). A perfect match in terms of diagnostic
216 outcomes between the two sampling methods was obtained with *Hericium* spp., *Inonotus* spp.,

Phellinus spp., and *Stereum* spp., while *Armillaria* spp. and *Trametes* spp. were detected by NDRMD combined with the disposable in 54.5% and 50% of samples, respectively, found to be positives to the pathogen by using the state-of-the-art drilling method (Table 1).

[Table 1]

4. Discussion

In this work we conceived, successfully developed and tested a disposable serving the dual purpose of disinfecting the needle of NDRMD, thus preventing the transmission of potentially pathogenic inoculum from tree to tree, and of collecting bioptic wood samples for subsequent phytopathological analyses.

The results of experiments on needle disinfection conducted both *in vitro* and *in vivo*, i.e. experiments 1 and 2, clearly show that while there is a significant risk of transferring pathogenic inoculum through non-disinfected needles, at least with the high inoculum loads used in the experiments, such risk was minimized when the disposable was employed. It should be noted that the same results were obtained using both brown and white rot fungi as well as very infectious canker fungal pathogens. We recognize that the risk of disease transmission through non-disinfected needles may be lower in the field as a result of lower inoculum concentrations in wood tissues compared to Petri dishes and/or of heating due to needle rotation during the penetration in wood, as previously suggested (Schwarze, 2008). However, the mechanisms of disease transmission through NDRMD were not within the aims of this study. Rather, we aimed to test the potential efficacy of a disposable at disinfecting needles, hence using high inoculum loads, representing worse-case scenarios, was deemed a highly conservative approach.

In the experiment on needle disinfection conducted in the field, while we failed to demonstrate that a needle may carry, and thus potentially transmit, viable inoculum of the wood decay fungus present in the tree, i.e. *P. fraxinea*, we did detect on needles after drilling viable inoculum of different

243 anamorphic fungal taxa. Such finding is in agreement with observations by Schwarze (2008) who
244 reported isolation of anamorphic fungi from unclean NDRMD needles. However, *P. fraxinea* was
245 detected previously in the tree by using molecular diagnostic assays (Guglielmo et al., 2007), and we
246 cannot exclude that its inoculum, although detectable through DNA-based tools, was no longer viable.
247 In the experiment, the tree was drilled using the disposable before the needle was rotated into the agar
248 medium. Therefore, results of the experiment not only point to a full efficacy of the disposable at
249 disinfecting the needle, but they also support the hypothesis that it is very unlikely that viable fungal
250 inoculum may accumulate into the NDRMD once the needle is pulled back into the device if the
251 disposable is used.
252 While the accumulation of wood particles into the NDRMD also may be minimized by using special
253 felt wheels already available on the market cleaning the needle during retraction into the device, the
254 disinfection intended as a process in which nearly all microorganisms, whether pathogenic or not, on
255 a substrate are killed through the use of chemicals or others means may be rarely achieved unless felt
256 wheels are soaked in disinfectant after every drilling. Such operation is characterized by a low level
257 of standardization and is time consuming, making it rarely used in practical arboriculture. The
258 disposable described here allows overcoming the above flaws, being fully standardized and easy to
259 use.
260 The disposable is not only effective at disinfecting the needle of NDRMD, but if combined with
261 NDRMD through the special adapter, it proved to be as effective as state-of-the-art drillings at
262 collecting wood samples for subsequent diagnostic analyses, as demonstrated by the statistical
263 analysis of the overall data. Indeed, the amount of wood particles, in the order of 10-30 mg, generally
264 collected by the disposable during needle retraction, may be enough for diagnostic purposes, at least
265 if PCR-based methods are used. We did not test whether fungal isolation is possible from wood
266 particles collected in the disposable. While isolation might no longer be feasible because of the
267 presence of alcohol, it should be noted that diagnosis requiring isolation from wood samples is always
268 difficult and time consuming, making PCR-based methods preferable (Nicolotti et al., 2010).

Results suggest that the efficacy of the tested method at collecting bioptic wood samples for diagnostic purposes may be lower if the tree is infected by *Armillaria* spp. compared to other fungi, with the exception perhaps of *Trametes* spp., whose low detection frequency implies caution to avoid any further speculation. However, the absence of visible crown symptoms on *Armillaria* spp.-infected trees may support the hypothesis that the decay caused by these pathogens, generally colonizing the external sapwood, was incipient.

Incidentally, all target wood decay fungi detected during the comparative diagnostic assays on *T.* ×*europaea* trees were white rot agents. Therefore, further comparative analyses including brown rot agents as targets and involving both other broadleaf and conifer tree species are needed to confirm the universal efficacy of the disposable combined with NDRMD at collecting bioptic wood samples. Both the adapter and the disposable could be further optimized at the industrial scale. The former may be produced starting from brass or other materials and customized to fit different NDRMD, and the production of the latter can be achieved by assembling standardly filter paper, alcohol, and plastic components. Whether the production at the industrial scale will lead to an improved or a reduced efficacy of the disposable compared to that used in this work will deserve investigation.

In conclusion, the disposable described here may represent a significant improvement for NDRMD, whose harmlessness for tree health has been questioned often. In fact, it allows not only for a complete and fully standardized disinfection of the needle, but also provides the opportunity to automatically and efficiently collect bioptic wood samples for subsequent phytopathological analyses, that may complement the tree hazard assessment and may be pivotal for the prognosis. Whether or not this second application of the disposable is needed may depend on the outcome of the instrumental analysis. Nevertheless, it can be achieved easily by simply closing the disposable and sending it to a plant diagnostic laboratory. Benefits of using the disposable are mainly devoted, but not limited, to the field of arboriculture as they encompass all applications of NDRMD, including inspection of poles, construction timber and indoor wooden structures.

295 **References**

296 Bernicchia, A., 2005. Polyporaceae s.l. Ed. Candusso, Alassio, Italy.

297 Bethge, K.C., Mattheck, C., Hunger, E., 1996. Equipment for detection and evaluation of incipient
298 decay in trees. Arboricultural Journal 20, 13–37.

299 CABI, 2015. *Ceratocystis platani* (canker stain of plane). Retrieved October 5th, 2017 from
300 http://www.cabi.org/isc/datasheet/12144.

301 Crawley, M.J., 2013. The R Book, 2nd ed. JohnWiley & Sons, Chichester, UK.

302 Garbelotto, M., Linzer, R., Nicolotti, G., Gonthier, P., 2010. Comparing the influences of ecological
303 and evolutionary factors on the successful invasion of a fungal forest pathogen. Biological
304 Invasions 12, 943–957.

305 Giordano, L., Sillo, F., Guglielmo, F., Gonthier, P., 2015. Comparing visual inspection of trees and
306 molecular analysis of internal wood tissues for the diagnosis of wood decay fungi. Forestry 88,
307 465–470.

308 Gonthier, P., Nicolotti, G., 2007. A field key to identify common wood decay fungal species on
309 standing trees. Arboriculture & Urban Forestry 33, 410–420.

310 Gonthier, P., Guglielmo, F., Sillo, F., Giordano, L., Garbelotto, M., 2015. A molecular diagnostic
311 assay for the detection and identification of wood decay fungi of conifers. Forest Pathology 45,
312 89–101.

313 Guglielmo, F., Bergemann, S.E., Gonthier, P., Nicolotti, G., Garbelotto, M., 2007. A multiplex PCR-
314 based method for the detection and early identification of wood rotting fungi in standing trees.
315 Journal of Applied Microbiology 103, 1490–1507.

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

319 Guglielmo, F., Gonthier, P., Garbelotto, M., Nicolotti, G., 2010. Optimization of sampling procedures
320 for DNA-based diagnosis of wood decay fungi in standing trees. Letters in Applied Microbiology
321 51, 90–97.
322 Helliwell, D.R., 2007. A short note on effects of boring holes in trees. Arboricultural Journal 30, 245–
323 248.
324 Index Fungorum, 2017. Retrieved October 10th, 2017 from http://www.indexfungorum.org.
325 Johnstone, D., Moore, G., Tausz, M., Nicolas, M., 2010. The measurement of wood decay in
326 landscape trees. Arboriculture & Urban Forestry 36, 121–127.
327 Kersten, W., Schwarze, F.W.M.R., 2005. Development of decay in the sapwood of trees wounded by
328 the use of decay-detecting devices. Arboricultural Journal 28, 165–181.
329 Lonsdale, D., 1999. Principles of tree hazard assessment and management. Research for Amenity
330 trees. The Stationery Office, London, UK.
331 Mattheck, C., Bethge, K., Weber, K., 2015. The body language of trees: encyclopedia of visual tree
332 assessment. Karlsruhe Institute of Technology, Karlsruhe, Germany.
333 Nicolotti, G., Gonthier, P., Guglielmo, F. Garbelotto, M., 2009. A biomolecular method for the
334 detection of wood decay fungi: a focus on tree stability assessment. Arboriculture & Urban
335 Forestry 35, 14–19.
336 Nicolotti, G., Gonthier, P. Guglielmo, F., 2010. Advances in detection and identification of wood
337 rotting fungi in timber and standing trees. In: Gherbawy, Y., Voigt, K. (Eds.), Molecular
338 Identification of Fungi. Springer, Berlin, Germany, pp. 251–276.
339 R Core Team, 2017. R: A language and environment for statistical computing. R Foundation for
340 Statistical Computing, Vienna, Austria.
341 Raupp, M., Gonthier, P., 2017. Biotic factors: pests and diseases. In: Ferrini, F., Konijnendijk van
342 den Bosch C., Fini A. (Eds.), Routledge Handbook of Urban Forestry. Routledge, Oxon, UK,
343 pp. 251–272.

Rayner, A.D.M., Boddy, L., 1988. Decomposition of wood: its biology and ecology. John Wiley, Chichester, UK.

Rinn, F., Schweingruber, F.H., Schar, E., 1996. Resistograph and X-ray density charts of wood comparative evaluation of drill resistance profiles and X-ray density charts of different wood species. Holzforschung 50, 303–311.

Rust, S., van Wassenaer, P., 2017. Tools for tree risk assessment. In: Routledge Handbook of Urban Forestry, Edited by Ferrini, F., Konijnendijk van den Bosch C., Fini A. Routledge, Oxon, UK, pp. 489–498.

Schwarze, F.W.M.R., 2008. Diagnosis and prognosis of the development of wood decay in urban trees. ENSPEC Pty Ltd, Rowville, Australia.

USDA PLANTS Database, 2017. US Department of Agriculture. Natural Resources Conservation Service PLANTS Database. Retrieved October 10th, 2017 from https://plants.usda.gov.

Tables

Table 1. Comparison of the outcome of the multiplex PCR assays on wood samples collected by two sample methods, a state-of-the-art drilling method and NDRMD using disposable connectable. N = number of positive diagnosis.

Fungal taxa	**State-of-the-art drilling method (N)**	**NDRMD combined with the disposable (N)**	**Relative frequency of positive diagnosis with NDRMD combined with the disposable compared to the state-of-the-art drilling method (%)**
Armillaria spp.	11	6	54.5
Hericium spp.	1	1	100.0
Inonotus spp.	2	2	100.0
Phellinus spp.	2	2	100.0
Stereum spp.	5	5	100.0
Trametes spp.	2	1	50.0
Total N of positive samples	23	17	73.9
Total N of negative samples	19	25	
Total N of analysed samples	42	42	

Figure legends

Fig. 1. Graphical representation of the disposable (A) and its working principles (B). In (A), a – cap, b – plastic cone, and c – filter paper dampened with denatured alcohol. In (B), arrows indicate the drive direction of the needle. For details, refer to the text.

Fig. 2. Three experiments conducted under controlled conditions to test the efficacy of the disposable at disinfecting the needle. (A) *In vitro* (experiment 1), representative example comparing test and control drillings with needle passed through inoculum of *Laetiporus sulphureus*; (B) *in vivo* (experiment 2), test and control drillings of *Platanus* × *hispanica* cuttings with needle passed through inoculum of *Ceratocystis platani*; (C) under field conditions (experiment 3), phases of the experiment and, in the bottom right picture, general view of the disposable and the adapter.

406 **Figures**

407 **Fig. 1.**

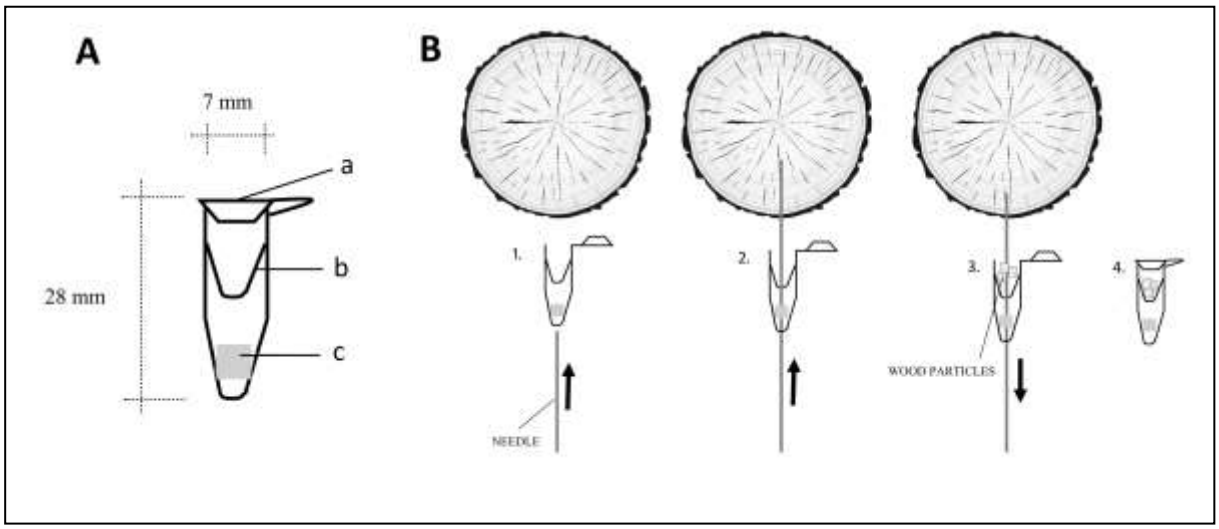

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Fig. 2.