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Short communication

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 (NDRMDs) 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 NDRMDs 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.

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 (NDRMDs) 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, NDRMDs were deemed the most accurate in indicating the location and, in some instances, the quantity of decay (Johnstone et al., 2010). NDRMDs 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, NDRMDs are moderately invasive (Johnstone et al., 2010), but whether and in which extent drills may have detrimental effects on trees is still under debate (Rust and van Wassenaer, 2017). Drill bits may breach the defensive zones and hence increase the likelihood of existing decay to spread further into the tree, as documented for the aggressive canker rot agent *Inonotus hispidus*

Abbreviations: NDRMD, Needle drill resistance measurement device; PCR, Polymerase Chain Reaction; MUT, *Mycotheca Universitatis Taurinensis*; MEA, Malt Extract Agar; UCG, University Campus of Grugliasco; DBH, Diameter at Breast Height

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(Bull.) P. Karst. (Kersten and Schwarze, 2005; Schwarze, 2008), though such a process was deemed relatively unlikely (Lonsdale, 1999). In addition, a major concern is that drill wounds could become infection courts for wood decay fungi, possibly from infective particles carried out on needles that have not been disinfected following previous use (Schwarze, 2008; Johnstone et al., 2010). Although only very few studies have been conducted to explore the likelihood of transmission of wood decay from tree to tree through unclean needles, attempts to prove cross-infection failed, despite these studies were based on a limited number of fungal species [*i.e. Fomes fomentarius* (L.) Fr. and *I. hispidus*] (Kersten and Schwarze, 2005; Schwarze, 2008). However, wood discoloration and transmission of other microbes in this way, mainly anamorphic fungi, have been documented (Helliwell, 2007; Schwarze, 2008). It should be noted that discolored wood is more likely to be infected by fungi (Rust and van Wassenaer, 2017) and colonization by anamorphic fungi may trigger and is often required for the subsequent infection of wood by decay fungi (Rayner and Boddy, 1988). The fear of transferring microbial inoculum from one tree to another through unclean needles does not refer exclusively to wood decay fungi, but encompasses a wide range of tree pathogens. Concerns are evoked especially about destructive and very infectious pathogens, like the canker stain pathogen of plane trees *Ceratocystis platani* (J.M. Walter) Engelbr. & T.C. Harr., whose infections and spread have been documented to occur easily through pruning tools and other ornamental practices (CABI, 2015; Raupp and Gonthier, 2017).

Prognostic decisions within tree hazard assessments also can be based or may be refined by information on which wood decay fungi are colonizing the tree; this has been suggested by a large body of literature (Lonsdale, 1999; Guglielmo et al., 2007; Schwarze, 2008; Gonthier et al., 2015; Mattheck et al., 2015). Indeed, as different fungal species may differ in their ability to colonize a tree, a correct diagnosis can be useful to predict, to some extent, the severity of fungal infection (Lonsdale, 1999). The identification of wood decay fungi is generally based on the features of fruiting bodies emerging from trees (Bernicchia, 2005; Gonthier and Nicolotti, 2007). However, fruiting bodies of wood decay fungi are usually present on only a small percentage of infected trees (< 10%), making diagnosis, based on visual inspection of fruiting bodies, unreliable (Giordano et al., 2015). In the last decade, a number of molecular tools based on Polymerase Chain Reaction (PCR) have been developed for the early detection and identification of the most important and widespread wood decay fungi of both conifer and broadleaf trees directly from wood samples (Guglielmo et al., 2007, 2008; Nicolotti et al., 2009, 2010; Gonthier et al., 2015). State-of-the-art for sampling, which is a crucial phase, is based on the collection of wood chips resulting from drillings performed with a 4-mm-diameter, 43-cm-long bit (Guglielmo et al., 2010). While such a drill is more invasive than that of NDRMD, drilling with both instruments may be required if bioptic wood samples have to be collected for diagnosis from wood portions where decay has been previously detected through NDRMD.

Here, we describe a disposable connectable to NDRMDs through an adapter and we report on its efficacy at both disinfecting the needle and collecting wood samples for bioptic purposes. Both the disposable and the adapter are covered, as a kit, by a pending patent application of the University of Turin (n. 102017000087211 of 28/7/2017).

2. Materials and methods

2.1. The disposable and its working principles

The disposable is made of a plastic microtube with attached cap and with a blind bottom conical in shape (28 mm length, 7 mm maximum internal diameter) (Fig. 1A). A Whatman® qualitative filter paper (415, particle retention 12–15 µm) dampened with 0.2 mL of denatured alcohol (90:10 v/v) is placed in the bottom of the microtube. Above the filter paper, a plastic cone (10 mm length, 7 mm maximum external diameter) is embedded inside the plastic microtube.

Once opened and placed into the adapter with the bottom facing the NDRMD, the disposable is pierced by the needle during drill resistance measurement (Fig. 1B-1). The needle, passing through 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).

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

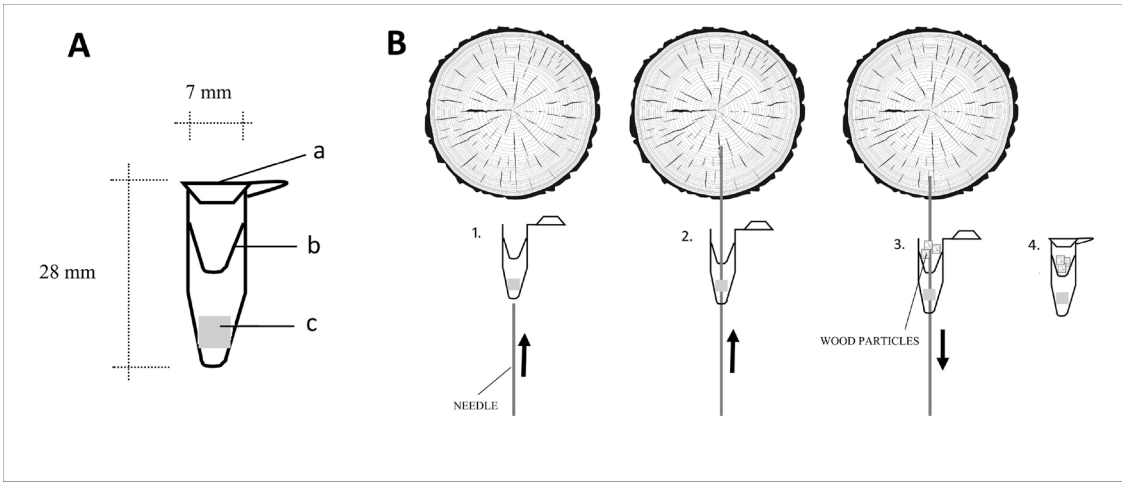

Fig. 1. Graphical representation of the disposable (A) and its working principles (B). In (A), a − cap, b − plastic cone, and c − filter paper dampened with denatured alcohol. In (B), arrows indicate the drive direction of the needle. For details, refer to the text.