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ABSTRACT

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New multiplex loop mediated isothermal amplification (LAMP) based assays for the early sequence-specific detection of *Dothistroma pini*, *Dothistroma septosporum* and *Lecanosticta acicola* on pine needles

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Dothistroma pini, *D. septosporum* and *Lecanosticta acicola* needle blights are among the most serious needle fungal diseases affecting pine species and are causing concern in many countries. Because management strategies might vary depending on the pathogen, a fast and accurate diagnosis is crucial. Current DNA-based assays available for their detection require well-equipped laboratories and are time consuming. The possibility to screen for such pathogens directly in the field could significantly expedite a rapid response to the threat. The objective of this work was to develop and validate three real-time monitoring loop mediated isothermal amplification (LAMP) assays that target the three pathogens using a portable device. LAMP primers and fluorescent assimilating probes were designed to recognize the beta tubulin (β-tub2) and elongation factor (EF-1α) regions for the two *Dothistroma* species, and *L. acicola*, respectively. Fifty-five different isolates from various geographical origins and belonging to the species phylogenetically closer to the three target ones were tested and results confirmed that the assays are highly specific. The assays showed high sensitivity as well, being able to detect as low as 3.2 pg μl^{-1} of pure fungal DNA, and to detect the pathogens in naturally infected needles in less than 30 min. The simplicity, sensitivity, specificity, and minimum required equipment make these LAMP assays ideal for in-field routine plant tissue testing and could have great implications for the management of *D. septosporum*, *D. pini* and *L. acicola* both in nurseries and forest systems.

Phytophthora species in rivers and streams of Eastern Sicily

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Phytophthora species were isolated from rivers and streams in eastern Sicily by *in situ* baiting using floating mesh bag-styrofoam rafts and identified by sequence analysis of internal transcribed spacer (ITS) ribosomal DNA (rDNA). The aim of this study was to investigate the ecological diversity, expressed as Shannon Diversity Index (SDI), of *Phytophthora* populations from river systems running nine Protected Natural Areas typical of Sicilian riparian plant communities. The major waterways examined included the Alcantara, Anapo and Fiumefreddo rivers on the eastern coast of Sicily, and streams with torrential water regimes such as Sciambro, on mount Etna. The most prevalent species identified in rivers and streams were *P.* ×*cambivora, P. citrophthora, P. frigida, P. gonapodyides, P. hydropathica, P.* sp. *kelmania, P. lacustris, P. multivora, P. plurivora, P. polonica, P. pseudocryptogea* and *P. thermophila.* High SDI values and a significant proportion of exotic *Phytophthora* species were found in 50% of the examined river systems, particularly in systems highly disturbed by human activities (e.g. the Fiumefreddo river, a small watercourse in a small natural reserve recently established in a highly anthropized area). By contrast, the naturalized systems showed the lowest diversity (e.g. the Sciambro stream, a seasonal water course at high altitude in the Mount Etna regional park, crossing a single-tree-species forest of black pine).

^{1}H-NMR - based metabolomic approach on *Xylella fastidiosa* subsp. *pauca*-infected olive trees: disease biomarkers and a field treatment response characterization

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Xylella fastidiosa subsp. *pauca* is a xylem-limited bacterial phytopathogen associated on many hectares with the "olive quick decline syndrome" in the Apulia region (southern Italy). In a previous work, in an attempt to find out possible compounds showing the capacity to reduce the