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Population structure analysis provides insights into the infection biology and invasion strategies of *Kretzschmaria deusta* in trees

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ABSTRACT

Infection biology and invasion strategies of *Kretzschmaria deusta* were investigated through the analysis of patterns of colonization and population diversity in three case studies, comprising different host species. Molecular analysis and isolation assays performed on stem sections at different heights indicated a prevalent heart rot mode of expansion. Random amplified microsatellites – RAMs and somatic incompatibility assays on isolates allowed detection, in one case, of a genet occupying the entire decay column and, in the other cases, of several different genets in each individual tree. Hypotheses on modes of arrival and entrance of *K. deusta* in trees are discussed on the basis of the distribution of genets and population genetics analyses. Significant correlation (Spearman $\rho = 1.0$; $p < 0.001$) between the number of genets and the number of areas delimited by pseudosclerotial plates (PSPs) on the decayed portions of stem sections suggests PSPs are interaction zone lines between different individuals of *K. deusta* occupying adjacent decay columns.

Introduction

Although often regarded merely as a saprotroph, the xylariaceous ascomycete *Kretzschmaria deusta* is locally reported as a primary root rot, butt rot, trunk rot and canker rot of several tree species (Hawksworth 1972; Sinclair *et al.* 1987). In tropical and subtropical regions, *K. deusta* can pose a threat for commercial plantations, e.g. oil palms, red grapefruit, rubber and tea trees (Sinclair *et al.* 1987; Palacios *et al.* 2008), whereas in temperate regions it is reported as one of the main reasons for decreased safety and windthrows of *Acer* spp., *Aesculus* spp., *Fagus* spp., *Tilia* spp. and *Ulmus* spp. trees (Wilkins 1934, 1939; Greig 1989; Gibbs & Greig 1990; Terho & Hallaksela 2008; Michelotti *et al.* in press). Sudden limb failures are also often associated with *K. deusta* (Lonsdale 1999). The detection of *K. deusta* during tree inspection is often problematic, even at an advanced stage of colonization. Indeed, signs of its presence, mainly consisting of inconspicuous conidial and

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perithecial stromata, can easily be missed during visual tree inspections. Even common instrumental analysis, including those based on penetrometers and acoustic diagnostic devices, can often fail to detect wood decay of *K. deusta*, which acts predominantly as a soft rot during tree colonization (Schwarze *et al.* 1995; Brandstetter 2007).

As a consequence, most studies on *K. deusta* have focused either on advanced techniques for the detection of decay (Pearce *et al.* 1994; Schwarze *et al.* 1995; Rabe *et al.* 2004; Deflorio *et al.* 2008a) and its early identification in trees (Nicolotti *et al.* 2009), or on the dynamics of fungal invasion and host response through artificial inoculation assays (Baum *et al.* 2000; Schwarze & Baum 2000; Deflorio *et al.* 2008b, 2009). Based on the results of these studies, the micro-morphological features of the brittle decay caused by this ascomycete and aspects concerning its colonization strategy, i.e. mechanisms of reaction zone penetration and ability to colonize sapwood of certain tree species, have been exhaustively elucidated.

Conversely, although parasitism of *K. deusta* towards several tree species was demonstrated and deeply investigated in earlier case studies performed by Wilkins (1936, 1938, 1939, 1943), no clear evidence defining its infection biology, i.e. the modes of spread and entrance, has been reported so far. Such evidences can be provided by studies on the genetic structure of pathogen populations within and among hosts. The abundance, size and distribution of different genets (genetic individuals) may be considered as an indirect reflection of fungal mode of spread, entrance and establishment among and within the trees (Rayner & Boddy 1986). Briefly, vegetative mycelium through root contacts or soil provides the opportunity for one genet to spread from tree-to-tree, whereas arrival by propagules, i.e. sexual and asexual spores, may give rise to a more heterogeneous population. Similarly, within a tree, population diversity is expected to be higher where infection took place. In fact, during decay progress many genets can be filtered out due to spatial competition for domain and/or selectivity of the environment (Rayner & Boddy 1986). Analysis of population structure can further be useful to improve knowledge of the role of pseudosclerotial plates (PSPs) often characterizing the colonization of *K. deusta* in trees. Although these crust-like aggregations of melanised hyphae are commonly reported as barriers to maintain a dry moisture regime favourable for *K. deusta* growth (Rayner & Boddy 1988), no studies have been performed to test the hypothesis that PSPs may be interaction zone lines between decay columns occupied by different individuals.

The presence of different genets within a population can be detected by testing for somatic incompatibility (SI) between vegetative mycelia (Lane 1981). This method has allowed determination of intraspecific diversity in populations of xylariaceous fungi both on a local scale, i.e. within single ascomata or trees and among adjacent trees in a woodland site (Chapela & Boddy 1988; Griffin *et al.* 1992; Rodrigues *et al.* 1995; Hendry *et al.* 1998; Johannesson *et al.* 2001), and on a larger scale, i.e. throughout wide geographical areas (Pérez Jiménez *et al.* 2002). Molecular methods based on randomly amplified polymorphic DNA (RAPD) markers proved to be even more effective in discriminating genets within fungal populations (Jacobson *et al.* 1993). While the SI assays can reveal the genetic differences associated with the loci involved in the self/non-self recognition, molecular genotyping can access genome-wide polymorphisms (Douhan *et al.* 2011). Although reproducibility of RAPD markers is often questionable, a variant of this technique, based on random amplification of DNA regions flanked by microsatellites (random amplified microsatellites – RAMs or inter simple sequence repeats – ISSR), allows more reproducible fingerprints (Gente *et al.* 2002) and has proved to be a valuable tool for assessing genetic diversity within populations of parasitic, saprotrophic and mutualistic symbiotic fungi (Vainio *et al.* 1998; Gherbi *et al.* 1999; Paavolainen *et al.* 2001).

The present study was aimed at investigating the infection biology and invasion strategies, i.e. modes of arrival, entrance and establishment, of *K. deusta* in three case studies, each involving a different tree species located either in urban environment or in a forest site. Each case study was thus focused on: (i) assessing the patterns of colonization of *K. deusta* in trees through both isolation assays and multiplex PCR (M-PCR)-based molecular analysis performed on wood samples collected from stem sections at different heights and on soil samples surrounding trees; (ii) determining the population structure of *K. deusta* through RAMs analysis and SI assays within each tree and stem section, and among trees in one site. Results of population structure analysis have further been used to test the hypothesis that PSPs are the result of mycelial interaction between different individuals occupying adjacent decay columns.

Material and methods

Tree material and sampling procedures

The three case studies comprised four trees in which *K. deusta* had been previously detected through a drill-based technique combined with M-PCR-based molecular analysis (Guglielmo *et al.* 2010). The first case included an *Acer platanoides* (ID: A1) and an *Acer pseudoplatanus* (A2) tree located in the Turin city area 15 m apart along the same street (45°03′40″N; 7°39′22″E); the second case consisted of a *Fagus sylvatica* tree (F) located in the forest of the nature reserve of Sacro Monte di Varallo, northern Italy (45°49′08″N; 8°15′21″E); the third case concerned a 200-yr-old roadside *Platanus acerifolia* tree (P) on a central street in the city of Cirié, northern Italy (45°13′58″N; 7°36′14″E). All trees were first examined for external signs and symptoms of disease, as well as for the presence of wounds. After felling, three to four transverse trunk sections, about 5 cm thick, were collected from the basal stem of each tree up to the upper edge of the visible decay column, transferred to the laboratory and maintained in a plastic sample bag at 4 °C prior to sampling. Height above ground level and diameter were recorded for each trunk section (Table 1). Cross-sectional extents of wood visually classified as decayed, discoloured or sound-looking, as well as hollow, were calculated using the program ArcGIS 9 (Esri, Redlands, CA, USA) applied on photographs of each trunk section (Table 1). Number of areas delimited by PSPs in each stem section was assessed to calculate their density on the total surface area of decayed wood (density = No of areas delimited by PSPs/m^2 decayed area).