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Community structure of *Phialocephala fortinii s. lat.* in European tree nurseries, and assessment of the potential of the seedlings as dissemination vehicles

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ARTICLE INFO

Article history:
Received 29 May 2007
Received in revised form
26 October 2007
Accepted 10 December 2007
Corresponding Editor: David L. Hawksworth

Keywords:
Cryptic species
Dark septate endophytes
Diversity
Gene flow
Picea abies
Pinus sylvestris
Soil fungus

ABSTRACT

Patterns of colonization of conifer roots by dark septate endopyhtes of the *Phialocephala fortinii s. lat.* species complex in nurseries in Switzerland and Lithuania were studied. The potential for man-mediated genotype flow was estimated for two Swiss nurseries based on customers' addresses and the number of delivered plants. Two hundred and forty-nine strains from three Swiss and five Lithuanian nurseries and an afforestation site were characterized using a combination of inter-simple sequence repeat-anchored PCR (ISSR-PCR), single-copy RFLP analysis, and sequence analysis. *P. fortinii s. lat.* was abundant in nursery seedlings, but the frequency of seedlings colonized varied considerably among and within nurseries. Ten cryptic species (CSP) of *P. fortinii s. lat.* were identified, including four hitherto undiscovered CSP. *P. helvetica* was the dominant species in Swiss nurseries, whereas *P. fortinii s. str.* was the most abundant species in Lithuanian nurseries and the afforestation site. Swiss nurseries deliver plants over distances of more than 200 km indicating the high potential for man-mediated genotype flow in *P. fortinii s. lat.*

Introduction

Recent population genetic studies in Europe suggest that the root endophyte *Phialocephala fortinii s. lat.* is composed of several morphologically indistinguishable cryptic species (CSP) that occur sympatrically in the same forest site and even in the same root fragment (Grünig 2004; Grünig *et al.* 2007; Grünig *et al.* 2006; Sieber & Grünig 2006). Species rank was recently assigned to six CSP (Grünig *et al.* 2008). *P. fortinii s. lat.* is considered to be a mitosporic fungus as no teleomorph has been observed, either in culture or in nature. However, no gametic disequilibrium was found in populations of all CSP tested to date, indicating that recombination occurs or has occurred within CSP (Grünig *et al.* 2006; Grünig *et al.* 2004). *P. fortinii s. lat.* is the dominant dark septate endophyte (DSE) in the roots of members of the *Pinaceae*, but is also

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doi:10.1016/j.mycres.2007.12.003

Table 1 – Preliminary screening of spruce seedling roots for *Phialocephala fortinii s. lat.* in Swiss nurseries

Nursery	Grid reference	Number of trees	Number of root segments	Number of *Phialocephala fortinii s. lat.* strains	Proportion of trees colonized by *P. fortinii s. lat.* [in %]
Walder	47°16′54.81″N, 8°21′9.77″E	25	125	17	32[a]
Court	47°14′21.69″N, 7°20′51.44″E	20	100	9	30
Lobsigen	46°59′5.04″N, 7°18′4.21″E	24	120	1	4

a Trees were collected from four different nursery beds and 16 of the total 17 strains of *Phialocephala fortinii s. lat.* were isolated from the 'Steinenberg' nursery bed.

known to colonize roots of broadleaved trees, shrubs, and herbaceous plants (Addy *et al.* 2000; Ahlich-Schlegel 1997; Ahlich & Sieber 1996; Jumpponen & Trappe 1998; Sieber 2002; Stoyke *et al.* 1992). Despite its wide distribution the ecological significance of *P. fortinii s. lat.* is enigmatic due to the use of genetically different, not clearly defined strains and a multitude of experimental designs used for ecological experimentation (Addy *et al.* 2005; Sieber & Grünig 2006). Moreover, little is known about the mechanisms by which species of this complex are dispersed. Nevertheless, identical clones were found repeatedly in managed forests more than 10 km apart, which was interpreted as a consequence of genotype flow (Grünig *et al.* 2004).

Natural gene and genotype flow has been hypothesized to be restricted in *P. fortinii s. lat.* because suitable propagules or vectors are missing or unknown (Sieber & Grünig 2006). This is supported by several observations: (1) *P. fortinii* rarely sporulates, its conidia are tiny and do not germinate *in vitro*; (2) *P. fortinii* was never detected in arable and grassland soils (Ahlich-Schlegel 1997; Ahlich *et al.* 1998) or in the roots of potted host trees grown outdoors close to forests for several years (O.H., unpubl.); and (3) population structures seem to remain stable for several years (Queloz *et al.* 2005). However, *P. fortinii s. lat.* is known to produce pseudosclerotia in the roots of many host species (Yu *et al.* 2001). These pseudosclerotia are highly resistant against drought and repeated freezing and thawing (Ahlich-Schlegel 1997), indicating that they are suitable survival units, but their role and importance as units of dispersal in undisturbed ecosystems remain unclear. However, pseudosclerotia may be dispersed by the outplanting of colonized nursery plants similar to what has been demonstrated for many pathogens (Jeger *et al.* 2007). In fact, roots of nursery plants were shown to be colonized by DSE (Bloomberg 1966; Danielson & Visser 1990), but the presence of *P. fortinii s. lat.* was rarely demonstrated (Kernaghan *et al.* 2003). Moreover, no data are available about the composition of *P. fortinii s. lat.* communities in nurseries.

The aim of the present study was to investigate some key factors in the hypothesized man-mediated dissemination of *P. fortinii s. lat.* Therefore, the colonization patterns of CSP of *P. fortinii* in several nurseries in Switzerland and Lithuania were studied. In addition, a two-year-old afforestation site, established on former agricultural land with plants produced in a nursery under study, was investigated. The dissemination of nursery plants was quantified for two nurseries based on delivery information to estimate the potential man-mediated genotype flow from nurseries to forests.

Materials and methods

Study sites, sampling design, and isolation of DSE

Three Swiss nurseries were screened for the presence of *Phialocephala fortinii s. lat.* in a preliminary study (Table 1). From each nursery, five root segments each of approximately 20 plants of *Picea abies* were chosen arbitrarily and fungal endophytes were isolated as described by Ahlich & Sieber (1996). For the nursery designated 'Walder', spruce plants were collected from different nursery beds. Whereas 100 % of the plants collected from the nursery bed designated 'Steinenberg' were colonized by *P. fortinii s. lat.* and 16 of the 17 strains where isolated from this site, *P. fortinii s. lat.* occurred only once in one of the other three nursery beds (Table 1). Two nursery beds of the company 'Walder AG' one with high frequencies of *P. fortinii s. lat.* ('Steinenberg') and one in which *P. fortinii s. lat.* was absent ('Äbnet Ost') were chosen for a refined sampling (Fig 1). Roots of 70 randomly selected

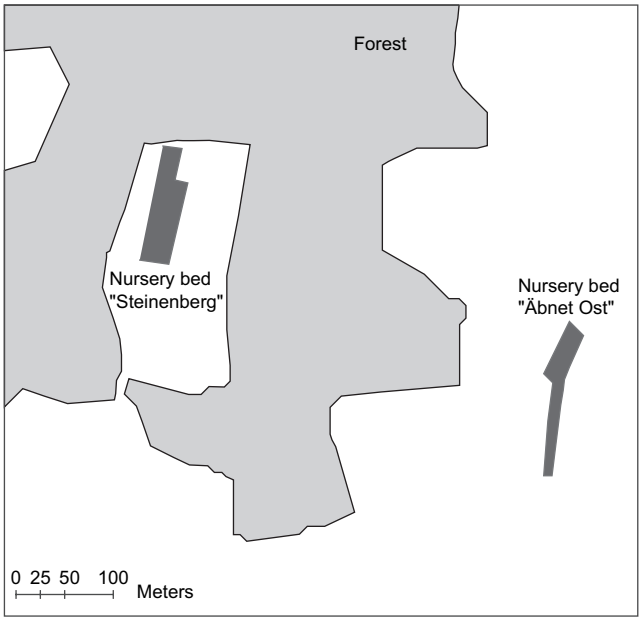

Fig 1 – Map displaying the two nursery beds, 'Steinenberg' and 'Äbnet Ost' of the 'Walder AG' nursery (black areas). The grey area represents forest, whereas the white area represents agricultural land. Forest boundaries were stable for at least 150 y according to historical maps.