From Forest Nursery Notes, Summer 2013

81. © Distribution and genetic diversity of the root-rot pathogen *Neonectria macrodidyma* in a forest nursery. Menkis, A. and Burokiene, D. Forest Pathology 42:79-83. 2012.

Forest Pathology WILEY-BLACKWELL

For. Path. 42 (2012) 79–83 doi: 10.1111/j.1439-0329.2011.00712.x

SHORT COMMUNICATION

Distribution and genetic diversity of the root-rot pathogen *Neonectria macrodidyma* in a forest nursery

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Summary

The aim of this study was to assess genetic diversity and spatial distribution of 123 strains of the root-rot pathogen *Neonectria macrodidyma* isolated from fine living roots of bare-root nursery cultivated *Pinus sylvestris*. We found that ITS rDNA sequences were 100% identical for all strains. Arbitrary primed PCR fragment analysis showed the presence of two distinct *N. macrodidyma* types (Type 1 and Type 2), which included respectively 11 and 14 genotypes composed of 1–29 strains. Results of this study indicate that *N. macrodidyma* is commonly associated with fine living roots of pine seedlings, is largely disseminated by asexual means of local genotypes and has even distribution in the forest nursery soils. In living roots, *N. macrodidyma* is likely present as dormant propagules but under favourable conditions, it may develop rapidly and have a significant negative effect on plant health and productivity.

1 Introduction

Neonectria macrodidyma Halleen, Schroers & Crous was recently described as a new species (Halleen et al. 2004), commonly associated with black foot disease of grapevines in a wide geographical area (Alaniz et al. 2007; Auger et al. 2007). Disease symptoms included drying and dying shoots, abnormal development and necrosis of roots, black discoloration of the wood and overall reduction in root biomass. In our previous studies in northern Europe, this species was also commonly isolated from healthy looking and diseased roots of conifer seedlings planted in forest nurseries, clear-cuts and farmland (Menkis et al. 2005, 2006). This suggests that similarly as many other *Neonectria* spp., *N. macrodidyma* is a plant pathogen well adapted to a wide range of hosts and habitats, and can be of great economic importance.

Previously, *Neonectria* spp. have been reported among the major causal agents of the root dieback in forest nurseries (Beyer-Ericson et al. 1991; Hamelin et al. 1996; Lilja et al. 1992; Menkis et al. 2006). Majority of those fungi are considered to be opportunistic, necrotic pathogens that produce toxins to invade and kill the plant tissues (Beyer-Ericson et al. 1991; Unestam et al. 1989). They may often act as saprotrophs while being attached to the surface of living roots. But stress and reduction in seedling vitality may induce rapidly pathogenic behaviour in those fungi (Unestam et al. 1989). Although there is a substantial amount of information on incidence of root pathogens in forest nurseries (Galaaen and Venn 1979; Kope et al. 1996; Lilja et al. 1992; Menkis et al. 2006), information on their genetic diversity and spatial distribution is scarce. Such information would also be of practical importance allowing further optimization of disease-management practices in forest nurseries. Moreover that virulence of the plant pathogen studied in this study was shown to vary significantly depending on the fungal genotype (Alaniz et al. 2007). The aim of this study was to assess genetic diversity and spatial distribution of the root-rot pathogen *Neonectria macrodidyma* – the most commonly isolated fungal species from roots of healthy looking *Pinus sylvestris* L. seedlings grown in a confined nursery plot (Menkis and Vasaitis 2011).

2 Materials and methods

A total of 123 strains of *N. macrodidyma* analysed in this study were isolated during our preceding work from fine living roots of 100 two-year-old bare-root cultivated seedlings of *P. sylvestris* sampled using a 1.5 m × 1.5 m grid design in a confined nursery plot 225 m^2 in size (Menkis and Vasaitis 2011). Sampled root systems were washed with tap water, and 20 individual root tips from each plant were randomly collected from different parts of the root system using forceps. In total, 2000 individual root tips were used for isolation of fungal cultures.

In this study, we compared several different methods to assess genetic diversity of *N. macrodidyma* strains. First, to confirm the identity of the species, all strains were subjected to sequencing of the internal transcribed spacer of the fungal ribosomal DNA (ITS rDNA) using primers ITS1F and ITS4 (White et al. 1990). Extraction of DNA, amplification and sequencing followed established methods (Rosling et al. 2003). To reveal intraspecific diversity among all ITS rDNA sequences, those were aligned and analysed in BioEdit (Hall 1999). Then, to identify possible clones of *N. macrodidyma* and their distribution at the study site, we carried out somatic incompatibility tests by pairing the fungal isolates in all combinations on vegetable juice agar plates (Vasiliauskas and Stenlid 2001). In ascomycetes, somatic incompatibility can delimitate incompatible mycelia and prevent the somatic exchange of genetic material between members of the same species, and it must frequently occur in nature, because in many species studied compatibility groups were found (Nauta and Hoekstra 1994). Furthermore, genetic diversity of the isolates was studied by using PCR-based restriction fragment length polymorphism (PCR-RFLP) of intergenic spacer (IGS) of

Received: 9 October 2010; accepted: 7 January 2011; editor: R. N. Sturrock