From Forest Nursery Notes, Winter 2011

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Kwasna, H. and Bateman, G. L. Forest Pathology 39:239-248. 2010.

For. Path. 39 (2009) 239–248 doi: 10.1111/j.1439-0329.2008.00583.x

Microbial communities in roots of *Pinus sylvestris* seedlings with damping-off symptoms in two forest nurseries as determined by ITS1/2 rDNA sequencing

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Summary

A methodological molecular procedure, which included extraction and cloning of the ITS1/2 rDNA of root-associated organisms with subsequent transformation and sequencing of representative clones, was effective for detection, discrimination and determination of the frequency of the main damping-off pathogens in roots of *Pinus sylvestris* seedlings growing in different forest-tree nursery soils and exhibiting different rates of disease progress. Roots exhibiting slower damping-off progression were colonized by *Fusarium oxysporum*, *Neonectria radicicola* (Ascomycota) and *Pythium* spp. (Oomycota), which comprised 50% of the microbial community. Roots exhibiting faster damping-off progression were dominated by *Thanatephorus cucumeris* (Basidiomycota), which comprised 80% of the microbial community. The microbial community was more diverse in roots with slower damping-off progression (14 species) than in roots with faster disease progression (seven species).

1 Introduction

The domination of Polish forests by a single species, Scots pine (*Pinus sylvestris* L.), and the programme of afforestation of abandoned farmland (Szujecki 1998; Anonymous 2003) necessitate the production of healthy seedlings of high quality. Damping-off is a serious disease in forest nurseries. In Poland, the disease is caused by fungi that include species of *Fusarium*, *Nectria* and *Neonectria* (Ascomycota), *Thanatephorus* (Basidiomycota) and the oomycetous *Pythium* species (Mańka 1993). In unprotected nurseries, they can cause losses of up to 80% of the planting material (H. Kwaśna, personal communication). Recognition of the damping-off pathogens is of significant practical importance, having consequences for nursery management, particularly in chemical protection (Mańka 1993). The study of the differences in the development of pathogens of various taxonomic groups can not only help determine the particular chemical control agents to be applied but also the different approach options in integrated pest management in forest nurseries.

Identification of damping-off pathogens based on pure-culture isolation is often deceptive because of: (i) dominance of the fast-growing species, (ii) difficulties in induction of growth and sporulation and (iii) overlap of anamorphs in closely related species. Pure-culture isolation and morphotyping often reveal only a small proportion of the total microbial community. The advantages of polymerase chain reaction (PCR)-based molecular methods include the possibility of: (i) discrimination within closely related species and (ii) detection of latent pathogens and slow-growing and non-culturable taxa.

The main objective of the present study was to evaluate a molecular procedure, including transformation of the total root ITS1/2 rDNA and cloning of the representative clones for: (i) detection and discrimination of damping-off pathogens, (ii) determination of the

Received: 26.2.2008; accepted: 14.10.2008; editor: S. Woodward