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The community of needle endophytes reflects the current physiological state of Norway spruce

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ABSTRACT

This study investigated fungal endophytes in the needles of Norway spruce (*Picea abies*) cuttings in relation to host tree growth. We also determined the prevalence of endophytes in needles incubated for six months. The cuttings originated from clonal origins showing slow- and fast-growth in long-term field trials but the heritable differences in growth rate were not yet detected among the studied cutting. Endophytes were isolated from surface-sterilized needles with culture-free DNA techniques. No significant differences were observed between endophyte communities of slow- and fast-growing clonal origins. However, the endophyte community correlated with the current growth rate of cuttings suggesting that endophytes reflect short- rather than long-term performance of a host. The concentration of condensed tannins was similar in slow- and fast-growing clonal origins but it showed a negative relationship with endophyte species richness, implying that these secondary compounds may play an important role in spruce tolerance against fungal infections. More than a third of endophyte species were detected in both fresh and decomposing needles, indicating that many needle endophytes are facultative saprotrophs. Several potentially pathogenic fungal species were also found within the community of saprotrophic endophytes.

Introduction

Plants can be colonized by endophytic fungi without any indication of their presence or symptoms of disease (Wilson 1995). Depending on the endophyte and host species involved, the interaction can range from antagonistic to mutualistic. Some endophytes are latent pathogens that activate under certain conditions (Carroll 1988; Saikkonen *et al.* 1998), while others can enhance host performance by conferring resistance to herbivores (Clay 1988; Sumarah *et al.* 2008; Albrectsen *et al.* 2010), pathogens (Arnold *et al.* 2003; Ganley *et al.* 2008), and various abiotic stressors (Redman *et al.* 2002; Torres *et al.* 2012), or by affecting plant-associated organisms and multitrophic interactions (Chu-Chou *et al.* 1992; Guo *et al.* 1992; Omacini *et al.* 2001; Mack & Rudgers 2008; Larimer *et al.* 2012). Some interactions can be neutral and certain endophytes (e.g., *Lophodermium piceae*) appear to be latent decomposers which start to act after the death of the host plant tissue (Deckert *et al.* 2001; Korkama-Rajala *et al.* 2008; Promputtha *et al.* 2010). However, most endophyte studies focus on vertically transmitted (i.e., seed) grass-endophytes of economic importance and so far horizontally transmitted (i.e.,

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Table 1 – Shoot height of 18-y-old slow- and fast-growing spruce clones (SGSC: S1–S4; FGSC: F1–F4) and characteristics of 2-y-old juveniles of the same genotypes. Mean values ± standard errors are presented in columns. Significantly different ($\alpha < 0.05$) values between slow- and fast-growing spruce clones are bolded and significantly different clones are marked with different letters.

	Shoot height of 18-y-old genotypes (cm)	Shoot biomass (g, d.w.)	Shoot growth (cm/2011)	Tot. needle biomass (g, d.w.)	Needle length (mm)	Needle surface area (mm^2)	Condensed tannins in needles (g kg^{-1}, d.w.)
SGSC $n = 32$[a]	**380 ± 10.3**	**5.66 ± 0.17**	9.4 ± 0.2	**3.1 ± 0.1**	8.4 ± 0.1	14.7 ± 0.3	77.8 ± 2.4
S1	331 ± 17.5	6.2 ± 0.4 ab	8.1 ± 0.5 b	3.2 ± 0.3 ab	7.8 ± 0.2 bc	12.6 ± 0.5 bc	78.0 ± 3.3 ab
S2	383 ± 21.4	4.4 ± 0.4 bc	9.1 ± 0.5 ab	2.3 ± 0.3 bcd	8.9 ± 0.4 ab	14.6 ± 0.9 ab	73.2 ± 7.9 ab
S3	414 ± 20.0	6.7 ± 0.3 a	10.4 ± 0.8 ab	3.8 ± 0.2 a	8.4 ± 0.3 abc	16.1 ± 0.7 a	86.6 ± 7.6 a
S4	391 ± 21.1	5.4 ± 0.3 ab	9.9 ± 0.6 ab	3.0 ± 0.2 abc	8.4 ± 0.3 abc	15.6 ± 0.7 ab	73.6 ± 8.2 ab
FGSC $n = 32$[a]	**573 ± 11.1**	**3.9 ± 0.2**	9.0 ± 0.3	**2.0 ± 0.1**	7.7 ± 0.2	12.9 ± 0.3	79.0 ± 3.4
F1	551 ± 20.3	2.9 ± 0.7 c	8.6 ± 0.7 ab	1.4 ± 0.4 d	9.4 ± 0.5 a	14.9 ± 0.9 ab	94.6 ± 11.7 a
F2	598 ± 27.3	4.6 ± 0.6 bc	11.4 ± 0.5 a	2.5 ± 0.3 bcd	8.6 ± 0.3 ab	15.1 ± 0.7 ab	53.7 ± 2.7 b
F3	564 ± 22.0	4.1 ± 0.6 bc	8.4 ± 1.0 ab	2.0 ± 0.3 cd	7.0 ± 0.3 c	11.0 ± 0.4 c	81.0 ± 0.9 ab
F4	578 ± 19.2	4.0 ± 0.3 bc	7.4 ± 0.7 b	2.2 ± 0.2 bcd	7.1 ± 0.3 c	10.8 ± 0.5 c	86.7 ± 2.3 a

a Condensed tannins SGSG/FGSC $n = 16$, clonal $n = 4$. Measurements of the 18-y-old genotypes, $n = 26–27$.

spores) tree endophytes are less understood (Carroll 1988; Saikkonen *et al.* 1998; Sieber 2007; Rodriguez *et al.* 2009).

Endophyte communities are known to vary among individual trees of a given species (Deckert & Peterson 2000; Ganley & Newcombe 2006). Microclimate factors such as humidity and temperature are believed to affect endophyte community structure and abundance, which often peak in dense forests (Helander *et al.* 1994; Müller & Hallaksela 1998). Genetic profile of the host is also known to affect foliage endophytes (Todd 1988; Elamo *et al.* 1999; Ahlholm *et al.* 2002; Saikkonen *et al.* 2003; Saikkonen 2007; Rajala *et al.* 2013). Variation in plant defense compounds (Saunders & Kohn 2009) such as condensed tannins (Bailey *et al.* 2005), and other host characteristics such as needle size (Rajala *et al.* 2013) or nutritional status (Martín-García *et al.* 2011; Larkin *et al.* 2012) also influence endophytes.

Previously we have investigated saprotrophic endophytes inhabiting needle litter from 11-y-old slow- and fast-growing spruce clones (Korkama-Rajala *et al.* 2008). We exposed the needles to endophytic decomposition for 2 y, and found the endophyte community to be more diverse among slow-growing clones, revealing a possible relationship between host tree growth and richness of saprotrophic endophytes. However, factors that could regulate the host growth–endophyte community interaction remained unanswered. Thus our aim was to study the community of endophytic fungi in needles of the slow- and fast-growing Norway spruce (Korkama-Rajala *et al.* 2008) rooted cuttings before the genetically determined differences in their growth rate became evident. As the concentration of condensed tannins has been previously found to vary among different tree genotypes and affect the endophytes (reviewed by Schweizer *et al.* 2008), we hypothesized that the concentration of condensed tannins in Norway spruce clones will influence the needle endophyte community. An additional goal of the study was to estimate which endophytic fungi are the early colonizers of dead decomposing needles. Especially the role of *L. piceae*, the most frequently isolated endophyte from healthy spruce needles (Barklund 1987; Sieber 1988; Müller *et al.* 2001), was of interest since it has been found to be metabolically active in needle litter of fast-growing spruce clones (Korkama-Rajala *et al.* 2008). We utilized culture-free molecular techniques that allow rapid and sensitive screening of tissues that contain very low endophyte densities and chemical compounds that interfere with DNA extraction (e.g., fresh needles).

Materials and methods

Plant material

Cuttings were taken in 2009 from Norway spruce (*Picea abies*) genotypes grown at a clonal trial site established in 1994 (see Korkama *et al.* 2006 for details of the study site). Four of the origins are considered as slow-growing spruce clones and four as fast-growing after 11 (Korkama *et al.* 2006) and 18 y growth (Table 1). Cuttings were rooted in the nursery in *Sphagnum* peat-vermiculite (7:3) substrate by keeping the air temperature in +16 to +19 °C, the bed temperature was set to +22 to +24 °C and humidity of 80 %. In summer 2010, rooted cuttings were transplanted into containers filled with forest humus. In spring 2011, the 2-y-old cuttings were planted randomly in a 6 m × 11 m site in Tuusula, southern Finland (60°21′32.60″, 25°0′12.94″), adjacent to a mature Norway spruce stand representing *Vaccinium myrtillus* fertility site type.

In October 2011, eight cuttings from each clone were taken and needles were sampled (ca. 1 g f.w.) from twigs of age class 1 and 2 (flushed in the spring of 2011 and 2010, respectively) in the laboratory. Needle samples intended for community profiling were washed with detergent (0.2 % Tween) in ultrasonic bath and surface-sterilized as in our earlier study (Rajala *et al.* 2013) *via* rinsing in 70 % ethanol and 5 % sodium hypochlorite.

Measurement of needle size

Needle length and projection area were estimated by scanning (Epson Perfection V700 Photo, Seiko Epson Corporation, Nagano, Japan) nonsterilized needles (ca. 100 per cutting) and using the image analysis software ASSESS (American Phytopathological Society, Minnesota, USA). Surface area was