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Competitiveness of endophytic Phialocephala fortinii s.l. - Acephala applanata
strains in Norway spruce roots
Sophie Stroheker, Vivanne Dubach, Thomas N. Sieber
Sophie Stroheker
ETH Zurich
Institute of Integrative Biology
Forest Pathology and Dendrology
Universitätstrasse 16, }8092\mathrm{ Zurich, Switzerland
Phone.: +41446323253
Fax: +4144 632 1380
sophie.stroheker@alumni.ethz.ch
Vivanne Dubach
Swiss Forest Protection
Eidg. Forschungsanstalt WSL
Zürcherstrasse 111,8903 Birmensdorf, Switzerland
Thomas N. Sieber
ETH Zurich
Institute of Integrative Biology
Forest Pathology and Dendrology
Universitätstrasse 16, }8092\mathrm{ Zurich, Switzerland
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#### Abstract

Dark septate endophytes of the Phialocephala fortinii s.l. - Acephala applanata species complex (PAC) are presumed to be the most abundant root colonizing endophytes of conifers across the Northern hemisphere. To test the competitiveness of different PAC strains, initially PAC-free Norway spruce (Picea abies) saplings were inoculated with five different PAC strains (Phialocephala subalpina (Ps1-Ps3) and P. fortinii s.s. (Pf1-Pf2)) by planting them in pre-colonized substrates. Saplings were left to grow for six weeks and then transplanted crosswise into a second substrate colonized by one of the other four strains for a further two weeks. PAC were isolated from roots and genotyped using microsatellite markers. The power of colonization, i.e. the ability of colonizing roots already colonized by another PAC strain, and the power of retention, i.e. the ability of a resident strain of not being suppressed by an invading PAC strain, were both calculated for each strain in every combination. The experiment was conducted twice, once under a summer climate (run 1) and once under a spring climate (run 2). The two runs differed greatly. In run 2, the competitor in the second substrate almost always suppressed the resident strain, while the situation was more balanced in run 1. In run 1, only strains Ps1 and Ps2 proved to be powerful colonizers. In run 2, Ps1 showed the highest level of retention, and all strains, except Ps3, were powerful colonizers. Strain Ps3 showed the lowest fitness level. Our results show that PAC strains differ (1) in their ability to colonize PAC-free, non-sterile roots, (2) in resistance against being suppressed by another PAC strain and in their ability to invade roots already colonized by another PAC strain. In addition, both the PAC-PAC and the PAC-host interactions depend on the climatic conditions.


## Keywords:

PAC, Root endophyte, Norway spruce, intraspecific interaction, competition, transplantation

## Introduction

Competition has been studied in a vast number of organisms, such as mammals (Kelt et al. 1995), insects (Cerda et al. 2013), and bacteria (Margolis et al. 2010). For many fungal species, competition plays a decisive role in determining their realized niche (Peay et al. 2008). Ectomycorrhizal community structure has been shown to be significantly influenced by inter- and intraspecific competition (Wu et al. 1999; Kennedy 2010). Competitive success has also been found to depend on the time, particularly on the speed of colonization (Kennedy and Bruns 2005). "Priority effects", i.e. the principle of "first come, first served", have been documented in relation to invasive plant species (Dickson et al. 2012), insects (Shorrocks and Bingley 1994), and fungi (Kennedy et al. 2009). For root endophytes, however, little research has been conducted on the topic of competition (Schulz et al. 2006).

Dark septate endophytes (DSE), characterized by melanized and septate hyphae, are dominant root endophytes in many trees and shrubs (Stoyke and Currah 1991), and are known to colonize roots of more than 600 plant species (Jumpponen and Trappe 1998). Phialocephala fortinii C.J.K Wang \& H.E. Wilcox is an anamorphic ascomycete of the Helotiales and is one of the most prominent DSE colonizing conifers and members of the Ericaceae (Wang and Wilcox 1985; Stoyke et al. 1992; Addy et al. 2000; Grünig et al. 2002). P. fortinii itself is genotypically highly diverse and composed of at least 21 cryptic species (CSPs), which are reproductively isolated (Grünig et al. 2003; Grünig et al. 2004; Grünig et al. 2008). Together with the closely related DSE Acephala applanata, the cryptic species of $P$. fortinii are referred to as Phialocephala fortinii s.l. - Acephala applanata species complex (PAC)(Grünig and Sieber 2005). To this day, the mechanisms of dispersal of the fungus remain a mystery and no sexual state has been discovered, even though Grünig et al. (2004) and Grünig et al. (2006) state that reproduction has occurred or could still occur based on the index of association $\left(I_{A}\right)$. Furthermore, PAC species possess functioning mating type loci (Zaffarano et al. 2010, 2011). PAC communities are diverse, with up to eight CSPs being present at the same study site (Queloz et al. 2008).

Observed shifts in community structure of PAC present in a confined forest plot suggest that different PAC strains compete against each other in the colonization of Norway spruce roots (Stroheker et al. 2016). Reininger et al. (2012) investigated the effect of different PAC strains on the plant biomass of two aseptically grown PAC-host-tree species.

Different PAC strains were inoculated both singularly and paired to measure competition between the strains. Their results indicated that competition was strain dependent. Based on these observations, Hugentobler et al. (2014) investigated the competitiveness of the same PAC strains in a plant-free system and found competition to be similarly strain dependent. However, highly competitive strains in living host-tree roots appeared to be weak competitors in the plant-free system, whereas minimally competitive strains in planta proved more competitive in the plant-free system.

Here, we go one step further by investigating the colonization success rate of different PAC strains of PAC-free, though not sterile, Norway spruce roots and, in a second step, their resistance to pressure of infection by another PAC strain.

## Material and Methods

## Production of inoculum

Polypropylene tubes ( 250 ml, Semadeni, Ostermundigen, Switzerland) were filled with 200 ml of peat:vermiculite:water 1:1:0.5 ( $\mathrm{v}: \mathrm{v}: \mathrm{v}$ ) and autoclaved. Liquid cultures of five PAC strains (Table S1) were produced according to (Tellenbach et al. 2011) and incubated at 20 ${ }^{\circ} \mathrm{C}$ in the dark on a rotary shaker at 80 rpm for 18 days. Liquid cultures were then homogenized with a blender (Polytron ${ }^{\text {TM }}$ PT 10-35 GT Homogenizer, KINEMATICA, Kriens, Switzerland) for 10 s to serve as inoculum (Hugentobler et al. 2014). Tubes were inoculated with 5 ml of one of the strains and incubated at $20^{\circ} \mathrm{C}$ for two weeks prior to the planting of PAC-free three-year-old Norway spruce saplings (Picea abies (L.) Karst.) of Swiss origin grown in a nursery (Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Birmensdorf; previously tested negative for PAC presence through isolation (procedure described below)).

## Experimental setup

The whole experiment was conducted twice. Run 1 was set up in July and run 2 in April of the following year. Accordingly, the climate chamber ran with a summer program for run 1 and a spring program for run 2 . The summer program was defined as follows: 14 h day with $120-140 \mu \mathrm{E} \mathrm{m}^{-2} \mathrm{~s}^{-1}, 18^{\circ} \mathrm{C}$ and $75 \%$ relative humidity [RH] and an 8 h night, with $12^{\circ} \mathrm{C}$ and $85 \%$ RH. 11 h days were run in the spring, with $120-140 \mu \mathrm{E} \mathrm{m} \mathrm{s}^{-1}, 14{ }^{\circ} \mathrm{C}$ and $75 \% \mathrm{RH}$, followed by

11 h nights with $8^{\circ} \mathrm{C}$ and $85 \% \mathrm{RH}$. Day and night were each separated by a 1 h ramp, during which temperature and light either decreased or increased.

The roots of each Norway spruce sapling were washed under running tap water until all soil particles were removed, swirled in $99 \%$ Ethanol ( $\mathrm{v}: \mathrm{v}$ ) for 10 s and transferred into a bath with autoclaved $\mathrm{dH}_{2} \mathrm{O}$ before planting in the substrates described above containing one of the five PAC strains ("resident" strains). The planted Norway spruce saplings were then transferred to a climate chamber.

After six weeks, the saplings were transplanted cross-wise into substrates containing the "invading" strain (Table S2). Four replicates of each of the 20 combinations ( 80 tubes in total) were set up for run 1 and five replicates of each of the 20 combinations ( 100 tubes in total) were set up for run 2. When transplanting, each sapling was carefully removed from the substrate containing the resident strain (Substrate 1, Table S2), and the roots were washed under running tap water to remove all peat and vermiculite particles before being transferred to the substrate containing the invading strain (Substrate 2, Table S2). Success of colonization by the resident strain was tested by randomly selecting two woody roots ( diameter $0.5-3 \mathrm{~mm}$ ) from every inoculated sapling prior to transplantation (16 saplings per strain in run 1 and 20 in run 2 ). The roots were pooled strain-wise and surface sterilized according to Ahlich and Sieber (1996). Small root segments (approx. 5 mm in length; 80 segments per strain in run 1 and 50 segments per strain in run 2) were randomly excised from the roots and placed on Terramycin ${ }^{\circledR}$ malt agar (TMA: 15g/I BECOAGAR ${ }^{\text {TM }} ; 20 \mathrm{~g} / \mathrm{l}$ malt, Alpha malt, Klipfel, Rheinfelden, Switzerland; 50mg/l oxytetracycline, Pfizer Ltd., India) for PAC isolation. The saplings were then left to grow in the substrates containing the invading strain for another two weeks before PAC was isolated from the roots again (Ahlich and Sieber, 1996). In run 1, 800 root segments (4 saplings of each combination x 20 combinations $\times 10$ root segments $=800$ root segments) were incubated for PAC reisolation. Thus, the maximum possible number of isolates amounted to 800 in run 1. However, the sampling effort was increased in the second run to provide an even more precise picture of the PAC colonization (5 saplings of each combination $\times 20$ combinations $\times 15$ root segments $=1500$ root segments). The overall rate of colonization success was characterized by visual assessment of the cultures and classification into the three different categories: PAC, nonPAC or root segments without any fungal growth. The colonization frequencies were expressed as percentages in order to make the two runs comparable.

For PAC genotyping, DNA was extracted from mycelium scraped off an area of approximately $1.5 \times 0.5 \mathrm{~cm}$ in the center of each emerging PAC culture (including the root segment) in accordance with the Macherey-Nagel protocol (NucleoSpin ${ }^{\circledR} 96$ Plant II kit; Macherey-Nagel; Düren, Germany). Microsatellite genotyping of eight loci was performed according to Queloz et al. (2010). Analysis of microsatellite data was made using the software GeneMapper ${ }^{\circledR}$ v. 4.0 (Applied Biosystems).

## Assignment probabilities

Allele lengths at the eight microsatellite loci of each DNA extract were compared to the allele lengths of the strains used in the experimental setup (Table S1). Strains could be identified with $100 \%$ certainty in cases where allele length measurements for all eight loci were available.

In a few cases, amplification of loci necessary for strain distinction failed. If amplification failed at a locus where both the resident and the invading strain possess different alleles, it was impossible to determine whether the DNA extract originated from a mycelial mixture of both strains or a pure culture, thus, reducing the chances of correctly identifying the strain(s). For these samples, only loci where the two strains possess different alleles were considered, resulting in the following assignment probability:

[^0]
## Mycelial mixtures

Samples containing alleles of both strains were considered mycelial mixtures. These isolates were treated as fifty-fifty mixtures and were counted for both strains.

## Statistical analyses

The "survival rate", i.e. the frequency of root segments colonized by the resident strain two weeks post transplantation, was defined as the "power of retention " $(y)$ and was calculated for each strain in every combination as follows:

$$
y=\frac{\# \text { of root segments colonized by resident strain }}{\text { total \# of root segments of the strain combination examined }}
$$

Correspondingly, the "invasion rate", i.e. the frequency of root segments colonized by the invading strain two weeks post transplantation, was defined as the "power of colonization" $(x)$ and also calculated for each strain in every combination:

$$
x=\frac{\# \text { of root segments colonized by invading strain }}{\text { total } \# \text { of root segments of the strain combination examined }}
$$

These calculations resulted in four y and four x values for each strain. Scatterplots showing the power of retention $(y)$ versus the power of colonization $(x)$ were rendered in R (Version 3.1.3) using the package ggplot2 (Version 1.0.1). Centroids were calculated for each strain with vertical and horizontal error bars (The R Foundation for Statistical Computing, 2015).
$Z$ - tests were calculated for each strain in relation to its power of retention $\left(Z_{r}\right)$ and its power of colonization $\left(Z_{s}\right)$ :

$$
Z_{r}=\frac{r-n \pi_{r}}{\sqrt{n \pi_{r}\left(1-\pi_{r}\right)}} ; Z_{s}=\frac{s-n \pi_{s}}{\sqrt{n \pi_{s}\left(1-\pi_{s}\right)}}
$$

with $r$ being the number of isolates of the resident strain after transplantation, and $s$ the number of isolates of the invading strain, $n$ the total number of isolates $(=r+s)$, and $\pi_{r}$ the probability of isolating the resident strain and $\pi_{s}$ the probability of isolating the invading strain (Table 2). For $\pi_{r}$, the initial frequency of colonization of each strain was used as an estimate, whereas $\pi_{s}$ was set to 0.5 for all strains. Retention and colonization power was considered significant only when $|Z| \geq 1.96$ (Stahel 2008).

## Results

## Initial colonization

Initial PAC colonization of Norway spruce trees was lower in the first run, with maximum colonization of all root segments reaching $51 \%$ in the case of strain Ps2, followed closely by strain Ps1 with 47\%. The lowest level of colonization was observed for strain Ps3 (32\%) . The overall colonization rate was higher in run 2 , with a maximum colonization of $88 \%$ for strain Ps1, followed by strain Ps2 with 76\%. Again, strain Ps3 showed the lowest colonization frequency ( $42 \%$ ). While the number of root segments showing no fungal growth was relatively high in the first run, very few "sterile" root segments were obtained in the second run (Table 2).

## Colonization post transplantation

In run 1, 71\% of all isolates obtained were classified as PAC, $14 \%$ were non-PAC-fungi, and a further $15 \%$ of the root segments showed no fungal growth. In run $2,66 \%$ of the isolates were classified as PAC, $32 \%$ as non-PAC, and $2 \%$ of the root segments showed no signs of fungal growth. Overall, 860 PAC-isolates were randomly selected for DNA extraction and genotyping (411 in run 1, 449 in run 2), out of which 659 isolates were successfully genotyped. Eighty-nine percent ( 592 isolates) of the genotyped isolates were pure cultures, with 322 isolates in run 1 and 270 in run 2 . Sixty-seven isolates ( $11 \%$ ) were found to be mycelial mixtures of both PAC strains (Figure 1).

The two runs clearly differed: in run 1, $40 \%$ of all PAC isolates were identified as the resident strain and $60 \%$ as the invading strain. In the second run, only $6 \%$ of all PAC isolates were determined as the resident strain and $94 \%$ the invading strain (Figure 2). Considering the power of retention in run 1, only strain Ps3 showed low resistance and proved to be suppressed at a significant rate by the other strains ( $|Z| \geq 1.96$ ). With respect to the power of colonization, strains Ps1 and Ps2 acted as powerful colonizers ( $|Z| \geq 1.96$ ), whereas strain Ps3 proved the weakest colonizer ( $|\mathrm{Z}| \geq 1.96$ ) (Figure 2 , Table 3 ). In run 2, all resident strains showed a significantly low power of retention ( $|Z| \geq 1.96$ ) and were thus easily suppressed by the invading strain. Considering the power of colonization, all strains except Ps3 proved to be powerful colonizers ( $|\mathrm{Z}| \geq 1.96$ ) (Figure 2, Table 4).

## Discussion

Our experiment demonstrated the varying ability of closely related members of the Phialocephala fortinii s.I. - Acephala applanata species complex to colonize roots of PACfree, but non-sterile Norway spruce saplings grown in a nursery bed. Although colonization frequencies varied widely between the two runs, the relative colonization frequencies of the five strains were almost identical, with strains Ps1 and Ps2 being the most potent colonizers followed by Pf1 and Pf2. Strain Ps3 was found to be the weakest colonizer (Table 2). Similar strain-dependent colonization intensities (frequency and density) have already been described by Reininger et al., (2012) for strains Ps1, Ps2 and Pf2 colonizing roots of axenically grown Norway spruce seedlings. Other DSE fungi such as Periconia also exhibit strain dependent levels of host colonization (Mandyam et al. 2012). Likewise, genets of Suillus spp. proved to differ in their capability to colonize roots and form ectomycorrhiza (Timonen et al. 1997). Differences in colonization intensity may also be due to interstrain differences in the time span required for root colonization. Such differences have been observed by Kennedy and Bruns (2005) who compared two Rhizopogon species. In their study, R. occidentalis quickly colonized Pinus muricata seedlings, whereas $R$. salebrosus clearly needed more time to thrive.

Strain-dependence has also been observed in relation to the effect of different PAC strains on performance and survival of Norway spruce seedlings. Virulence was strain-dependent with higher variability within, rather than across, PAC species (Tellenbach et al. 2011). In experiments incorporating Norway spruce seedlings, both Tellenbach et al. (2011) and Reininger et al. (2012) observed high virulence of strain Ps1 causing mortality of up to $70 \%$ of the inoculated seedlings. In our experiment, no deterioration of the health status of the saplings at the hands of any of the PAC strains was observed. This could be partially due to the age of the plants, i.e. three-year-old saplings used in this study versus five-to-seven-month-old seedlings in the studies of Tellenbach et al. (2011) and Reininger et al. (2012).

Initial colonization frequencies of the different strains varied strongly between the two runs, with considerably lower colonization rates seen in run 1. This difference in colonization intensity could be the result of seasonality. Run 1 was set up in the summer with a mean temperature of $18{ }^{\circ} \mathrm{C}$ in the climate chamber, whereas run 2 was set up in the spring with a mean temperature of $14{ }^{\circ} \mathrm{C}$. In temperate regions emergence of (axenic) primary roots and secondary growth of older roots are especially pronounced in spring (Hendrick and Pregitzer 1996; Kuehler et al. 1999; Pregitzer et al. 2008). Fresh primary roots are most likely more susceptible to PAC colonization, and the cracks that form in the bark of
older roots due to radial growth could facilitate PAC infection, thus leading to higher colonization rates in run 2 which was set up in April, compared to run 1 which was set up in July. In addition, the frequency of sterile root segments was significantly lower in run 2. As with our observations, Giovannetti (1985) found vesicular-arbuscular mycorrhiza infections to increase from January to June and to decrease in the summer. These seasonal infection variations can, however, also be influenced by soil moisture and nutrient availability in the soil (Rabatin 1979), pH (Sivakumar 2012), and both host and pathogen species considered (Luque et al. 2002; Alvarez et al. 2009). PAC and mycorrhiza both occur within roots, meaning they live in close contact with each other and possibly even compete for the same niche (O'Dell et al. 1993; Menkis et al. 2004).

In nature, PAC colonization frequencies of Norway spruce roots often exceed $80 \%$ (Grünig et al. 2002; Queloz et al. 2011). Therefore, the initial colonization rates of PAC, especially in run 1 of our study, might seem low upon first glance. However, the situation in the forest and in the nursery differs greatly. Whereas forest soils in the northern hemisphere are rich in PAC inoculum, agricultural soils are PAC-free (Ahlich-Schlegel 1997; Brenn et al. 2008). The nursery from which the saplings used in this study originated is situated on former agricultural land and, therefore, the soil is PAC-free. At forest sites, PAC readily colonizes conifer roots, whereas the roots of our saplings acquired a completely different, PAC-free microbial community whilst growing in the nursery bed, as shown through isolations prior to set up of the experiment. This microbial community might behave antagonistically towards PAC and could explain the comparatively low PAC-colonization rates of the sapling roots. Nevertheless, the frequency of colonized root segments reached $88 \%$ for strain Ps1 in the second run. For the first time, our results clearly show the possibility of inoculating non-axenic plants with PAC. Root inoculations have also been successful for other endophytes. Similarly, mycorrhizal fungi can be inoculated in non-axenic plants to enhance the performance of trees grown in nurseries in a process called "controlled mycorrhization" (Garbaye and Churin 1996; Fini et al. 2011).

The PAC strains examined in this study exhibited different levels of competitive strength, not only in relation to their ability to "capture" a PAC-free Norway spruce root and "defend" the occupied root tissue against invading mycelia from the surrounding substrate, but also in terms of their ability to colonize a pre-populated root. In both runs, strains Ps1 and Ps2 exhibited the highest power of colonization, meaning that they were particularly adept at invading roots, which had already been colonized by another (resident) PAC strain (Figure 2). Interestingly, even though Ps1 showed one of the highest colonization rates as a
resident in formerly PAC-free roots, it was easily suppressed by invading PAC strains in the run 1 , thus having a low power of retention (though not significantly so). In run 1, both $P$. subalpina strains Ps1 and Ps2 acted as powerful colonizers, whereas the two P. fortinii s.s. strains Pf1 and Pf2 took an intermediate position. Interestingly, all resident strains in run 2 were significantly suppressed by the invading strains, and all strains except $P$. subalpina strain Ps3 acted as powerful colonizers. However, tracing the behavior of the different strains back to the species is not possible, since P. subalpina strain Ps3 was on the losing end, not only in terms of resistance, but also in terms of colonization in both runs. Correspondingly, the frequency of strain Ps3 as a "resident" in formerly PAC-free roots was the lowest. Reininger et al. (2012) investigated competition between the same PAC strains inoculated both singularly and dually into the substrates and their effects on biomass of five-month-old Norway spruce seedlings in axenic conditions. Again, competition was seen to be strain dependent. The strongest interaction occurred between strains Ps2 and Pf2, with Pf2 significantly reducing biomass of strain Ps2. Strain Ps2 reduced strain Ps1 significantly at 18 ${ }^{\circ} \mathrm{C}$ but not at $23^{\circ} \mathrm{C}$. This contrasts sharply with the strong inhibition of Ps2 and simultaneous growth stimulation of Ps1 in a plant-free interaction test of the two strains on malt extract agar (Hugentobler et al. 2014), indicating that PAC strain competitiveness depends on whether the interaction occurs in the saprotrophic or biotrophic phase. Moreover, the colonization power of PAC strains may depend on the temperature since the colonization frequency was much higher in run 2 of our experiment, which was set up in April, i.e. performed at a lower temperature $\left(14{ }^{\circ} \mathrm{C}\right.$ vs. $18^{\circ} \mathrm{C}$ ).

Suppression or replacement was never observed directly, and we do not know how often replacements really occurred. On a microscopic level, roots are huge microcosms composed of different tissue types, each of them composed of thousands of cells which may be living or dead, i.e. offering a vast number of habitats and niches. Individual thalli may colonize only a few cells. Thus, there is room for a multitude of PAC genotypes within one single root (Sieber \& Grünig 2006; Sieber \& Grünig 2013). We therefore used the expression "colonization" to circumscribe the reduction of the frequency of colonization of the resident strain, observed after the addition of the invading strain. The observed reduction in frequency of the resident strain could be the result of a stochastic effect, but might also be caused by a competitive advantage of the invading strain during re-isolation from the root segments on malt agar. Also niche partitioning with the resident strains colonizing interior parts of the roots, and invading strains colonizing the outer parts of the roots is a possible outcome, preventing detection of the resident strain. Wardle et al. (1993) investigated
interspecific competitiveness between Trichoderma harzianum and Mucor hiemalis in agricultural soil, as well as between $T$. polysporum and M. hiemalis in forest soil. T. harzianum was out-competed by M. hiemalis in agricultural soil, but never totally replaced. These findings indicate specific microhabitats/niches colonized by T. harzianum so it could not be totally excluded, with both species showing stable co-occurrence. In forest soils, $T$. polysproum had a competitive advantage over M. hiemalis.

Inoculum size can also determine the outcome of interactions between fungal thalli, as shown by Holmer and Stenlid (1993) for wood decomposers. Furthermore, Holmer and Stenlid (1993) observed mycelial growth of the invading strain towards the other strain, and mycelial growth of the invaded strain away from the invader. In our experiment, the inoculum size and hence inoculation pressure of the invading strain was much higher compared to the inoculum size of the resident strain present within the roots at the time of transplantation, thus potentially forcing the resident strain deeper into the root tissues.

To conclude, our results indicate that PAC strains differ in their ability to colonize roots of Norway spruce saplings. The five strains belonging to two PAC species differed in their capability of offering resistance against an invading PAC strain, and also in their power to suppress an established strain within living roots. Moreover, we were able to inoculate unsterile Norway spruce saplings with PAC, which offers an opportunity for natureorientated field experiments with PAC. Such inoculations should be performed in the spring, where roots of Norway spruce seem to be more susceptible to PAC infections. Further research is required to link the colonization of PAC to Norway spruce defense mechanisms, which are activated upon fungal infection (Felicijan et al. 2016). As endophytes have major implications for tree health (for review see Pautasso et al. (2014)), the interactions between roots and PAC-endophytes and the successional processes during root endophyte community formation warrant further attention.

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## Figure labels

Fig. 1 Percentage colonization for the twenty combinations, data for both runs combined, showing the proportion of the resident strain (black), mycelial mixtures (dark grey) and the invading strain (light grey). The strain mentioned first in the "Combination label" represents the resident strain.

Fig. 2 Power of retention versus power of colonization of each strain for run 1 and run 2 . Centroids were calculated for each strain with vertical and horizontal error bars

Table 1: Number of differing loci in each strain combination used for strain identification. Numbers in parentheses indicate the minimum number of successfully amplified differing loci required to guarantee an assignment probability of $\geq 70 \%$.

|  | Ps2 | Ps3 | Pf1 | Pf2 |
| :--- | :--- | :--- | :--- | :--- |
| Ps1 | $4(3)$ | $2(2)$ | $7(5)$ | $7(5)$ |
| Ps2 | - | $2(2)$ | $7(5)$ | $7(5)$ |
| Ps3 |  | - | $7(5)$ | $7(5)$ |
| Pf1 |  | - | $6(5)$ |  |

Table 2: Colonization of Norway spruce saplings by the resident PAC strain prior to transplantation: percentage of root segments colonized by the resident strain (PAC), other fungi (Non-PAC), and root segments with no fungal growth (Sterile), for both run 1 and run 2 . Number of root segments examined per strain: 80 in run 1; 50 in run 2.

| Run 1 |  |  | Run 2 |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :---: |
| Strain | PAC [\%] | Non-PAC [\%] | Sterile [\%] | PAC [\%] | Non-PAC [\%] | Sterile [\%] |  |
| Ps1 | 47 | 14 | 39 | 88 | 12 | 0 |  |
| Ps2 | 51 | 19 | 30 | 76 | 22 | 2 |  |
| Ps3 | 32 | 39 | 29 | 42 | 54 | 4 |  |
| Pf1 | 38 | 26 | 36 | 52 | 48 | 0 |  |
| Pf2 | 43 | 40 | 18 | 50 | 50 | 0 |  |

Table 3: Z- tests calculated for each strain in run 1 with respect to the power of retention and the power of colonization. Critical value $|Z| \geq 1.96$. The probability of isolating the resident strain (power of retention) or the invading strain (power of colonization) is represented by $\pi_{r}$ and $\pi_{s}$, respectively. $r$ represents the number of resident strain isolates for the power of retention, and $s$ the number of invading strain isolates for the power of colonization. $n$ represents the total number of isolates.

Run 1

## Power of retention

| Strain | $\boldsymbol{\pi}_{\boldsymbol{r}}$ | $\boldsymbol{r}$ | $\mathbf{n}$ | $\boldsymbol{Z}_{\boldsymbol{r}}$ | Significance |
| :--- | :--- | :--- | :--- | :--- | :--- |
| Ps1 | 0.5 | 13.5 | 38 | -1.78 | n.s. $^{\text {a }}$ |
| Ps2 | 0.5 | 47.5 | 87 | 0.85 | n.s. |
| Ps3 | 0.35 | 18.5 | 93 | -3.05 | $*$ |
| Pf1 | 0.4 | 31.5 | 64 | 1.5 | n.s. |
| Pf2 | 0.45 | 35 | 72 | 0.61 | n.s. |


| Power of colonization |  |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :--- |
| Strain | $\boldsymbol{\pi}_{\boldsymbol{s}}$ | $\boldsymbol{s}$ | n | $\boldsymbol{Z}_{\boldsymbol{s}}$ | Significance |
| Ps1 | 0.5 | 41.5 | 52 | 4.29 | $*$ |
| Ps2 | 0.5 | 68.5 | 91 | 4.82 | $*$ |
| Ps3 | 0.5 | 18.5 | 59 | -2.86 | $*$ |
| Pf1 | 0.5 | 54 | 96 | 1.22 | n.s. |
| Pf2 | 0.5 | 26.5 | 56 | -0.40 | n.s. |

[^1]
## ACCEPTED MANUSCRIPT

Table 4: Z- tests calculated for each strain in run 2 with respect to the power of retention and the power of coloization. Critical value $|Z| \geq 1.96$. The probability of isolating the resident strain (power of retention) or the invading strain (power of coloization) is represented by $\pi_{r}$ and $\pi_{s}$, respectively. $r$ represents the number of resident strain isolates for the power of retention, and $s$ the number of invading strain isolates for the power of coloization. $n$ represents the total number of isolates.

## Run 2

| Power of retention |  |  |  |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :--- | :---: | :---: |
| Strain | $\boldsymbol{\pi}_{\boldsymbol{r}}$ | $\boldsymbol{r}$ | $\mathbf{n}$ | $\boldsymbol{Z}_{\boldsymbol{r}}$ | Significance |  |  |
| Ps1 | 0.9 | 11.5 | 40 | -12.91 | $*$ |  |  |
| Ps2 | 0.8 | 11.5 | 51 | -10.25 | $*$ |  |  |
| Ps3 | 0.4 | 1.5 | 108 | -8.19 | $*$ |  |  |
| Pf1 | 0.5 | 4.5 | 35 | -4.39 | $*$ |  |  |
| Pf2 | 0.5 | 4.5 | 71 | -7.35 | $*$ |  |  |

Power of colonization

| Strain | $\boldsymbol{\pi}_{\boldsymbol{s}}$ | $\boldsymbol{s}$ | $\mathbf{n}$ | $\boldsymbol{Z}_{\boldsymbol{s}}$ | Significance |
| :--- | :--- | :--- | :--- | :--- | :--- |
| Ps1 | 0.5 | 65 | 67 | 7.69 | $*$ |
| Ps2 | 0.5 | 89.5 | 93 | 8.91 | $*$ |
| Ps3 | 0.5 | 20 | 35 | 0.84 | n.s. $^{\text {a }}$ |
| Pf1 | 0.5 | 64.5 | 72 | 6.71 | $*$ |
| Pf2 | 0.5 | 32.5 | 38 | 4.37 | $*$ |

${ }^{a}$ n.s. $=$ not significant at $p \leq 0.05$.



Run 2



[^0]:    Assignment probability $=$ $\frac{100}{\text { Number of differing }{ }^{1} \text { loci }} \times$ Number of amplified differing ${ }^{1}{ }^{1}$ loci
    ${ }^{1}$ loci where the two strains possess different alleles (Table 1, Table S1).

    If assignment probability was $\geq 70 \%$, the isolate was included in the analyses and otherwise excluded (Table 1).

[^1]:    ${ }^{\mathrm{a}}$ n.s. $=$ not significant at $\mathrm{p} \leq 0.05$

