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Endophytic *Cenangium ferruginosum* (Ascomycota) as a Reservoir for an Epidemic of *Cenangium* Dieback in Austrian Pine

By

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S u m m a r y

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Endophytic *Cenangium ferruginosum* were isolated from symptomless needles and buds of *Pinus nigra* of two age classes (15 and 60-yr-old) from natural stands and plantations in Slovenia. The frequency of colonised needles varied between 0 and 100 % depending on the tree individual, season, needle part (tip, middle part and base of the needles) and age of the needles. However, no statistically significant differences in colonisation could be detected among tree individuals, needle parts and seasons. Thus, age and origin (natural stand or plantation) of trees had no influence on the frequency of colonisation. The variability of colonisation frequencies was mainly due to needle age. One-yr-old needles were significantly ($p < 0.05$) less often colonised by *C. ferruginosum* than older needles. On average, 9 % of the 1-yr-old, 17 % of the 2-yr-old, 21 % of the 3-yr-old, and 22 % of the 4-yr-old needles were colonised. *C. ferruginosum* could not be isolated from buds. These results suggest that the needles represent reservoirs for the fungus from which it can spread to the twigs when environmental conditions change to the advantage of the fungus and/or the disadvantage of the host.

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Introduction

In 1986, an epidemic of *Cenangium ferruginosum* (Fr.) destroyed many Austrian pine trees in the Karst region of Slovenia. Approximately 10,000 m³ of wood had to be harvested (JURC 1986-1987). The epidemic ended quite quickly and declines in Austrian pine caused by *C. ferruginosum* have been only rarely observed since then. However, the question of the origin of such epidemics has remained unanswered.

The existence of a distinct fungal community within healthy plant tissues has been demonstrated for many plant species (PETRINI & al. 1992). Some of these so-called fungal endophytes are known to have pathogenic properties (SIEBER & al. 1991). However, these potentially pathogenic endophytes remain quiescent for most or all of the life cycle of their hosts or host tissues and become pathogenic only under certain circumstances.

Based on the findings of HELANDER & al. 1994 and RACK & SCHEIDEMANN 1987 who detected *C. ferruginosum* as an endophyte in healthy-looking needles of Scots pine (*Pinus sylvestris* L.), we tested the hypothesis that *C. ferruginosum* also occurs endophytically in the needles of *P. nigra*, i. e. that the needles may form a reservoir for *C. ferruginosum* from which an epidemic may start during or after the host has been exposed to unfavourable conditions.

Material and Methods

Collection sites

Eight locations in Slovenia with nine Austrian pine trees were chosen. The locations differ in terms of their ecological parameters and are situated within an area about 150km from the alpine region to the submediterranean phytogeographical region of Slovenia. The characteristics of the sites are: 1po - 800m a.s.l., thinned forest, shallow soil, 2po - 994m a.s.l., windy, south slope, 3po - 125m a.s.l., near main road, edge tree, 4po - 560m a.s.l., solitary tree, shallow soil, 5py, 5po - 790m a.s.l., shallow soil, 6py - 350m a.s.l., dry, hot, 7ny - 450m a.s.l., bottom of deep canyon, wet, 8no - 1075m a.s.l., windy, low winter temperatures. The abbreviations are: p - planted tree, n - natural stand of Austrian pine, o - approximately 60 year old tree, y - approximately 15 year old tree. Needles were collected from one tree at each of the seven sites and from two trees at site 5 in March, June, and October 1993, in March and October 1994, and in January 1995.

Sampling procedure and fungal isolation

At each sampling date a branch facing south was cut from each tree from the lowest whorl of the crown. Needles were separated into annual age classes, transported in polyethylene bags in cold boxes at 4°C and processed within 24 hrs. Eight healthy-looking needles were randomly selected per age class and tree and washed under running tap water for one hour. A 0.3 cm long segment was then excised from the base, the middle and the tip of each needle. The segments were surface-sterilised by the following sequence of immersions: 1 min in 50 % ethanol, 5 min in sodium hypochlorite (2.6 % of active chlorine), and 1 min in 50 % ethanol. Segments were blotted dry and plated onto 2 % (w/v) malt extract agar (MEA) (Malt Extract, Biolife S.r.l., 20 g l⁻¹, Agar Bios Special LL, Biolife S.r.l., 20 g l⁻¹) in 90 mm d Petri dishes. The dishes were incubated at 23°C and examined weekly for six weeks. Mycelial outgrowths from the segments were subcultured and identified. A total of 5592 needle segments were processed (1864 needles).

Eight buds were collected from each tree in January 1995 and processed in the same manner as the needles after the removal of dry bud scales.

Statistical analyses

The Kruskal-Wallis test was used to compare tree individuals, needle parts (base, middle and tip of the needles), and needle age classes (1- to 4-yr-old needles) regarding colonisation by *C. ferruginosum*. The signed-ranks test was employed to evaluate changes in the frequency of colonisation between sampling dates (SOKAL & ROHLF 1981).

Results

The frequency of needles colonised by *C. ferruginosum* varied between 0 and 100 % depending on tree individual, season, needle part and age of the needles. However, no statistically significant differences in colonisation could be detected among trees and needle parts, although Figs. 1 and 2 suggest that some differences may exist. The variability of colonisation frequencies was mainly due to needle age. One-yr-old needles were significantly ($p < 0.05$) less often colonised by *C. ferruginosum* than older needles. Two- to 4-yr-old needles were not statistically different with respect to colonisation by *C. ferruginosum*. Five year old needles, however, seemed to be less frequently colonised than younger (except 1-yr-old needles) or older needles (Fig. 2). This differences could not be evaluated by a statistical test since the number of examined 5-yr-old or older needles was too low. On average, 9 % of the 1-yr-old, 17 % of the 2-yr-old, 21 % of the 3-yr-old, and 22 % of the 4-yr-old needles were colonised by *C. ferruginosum*.

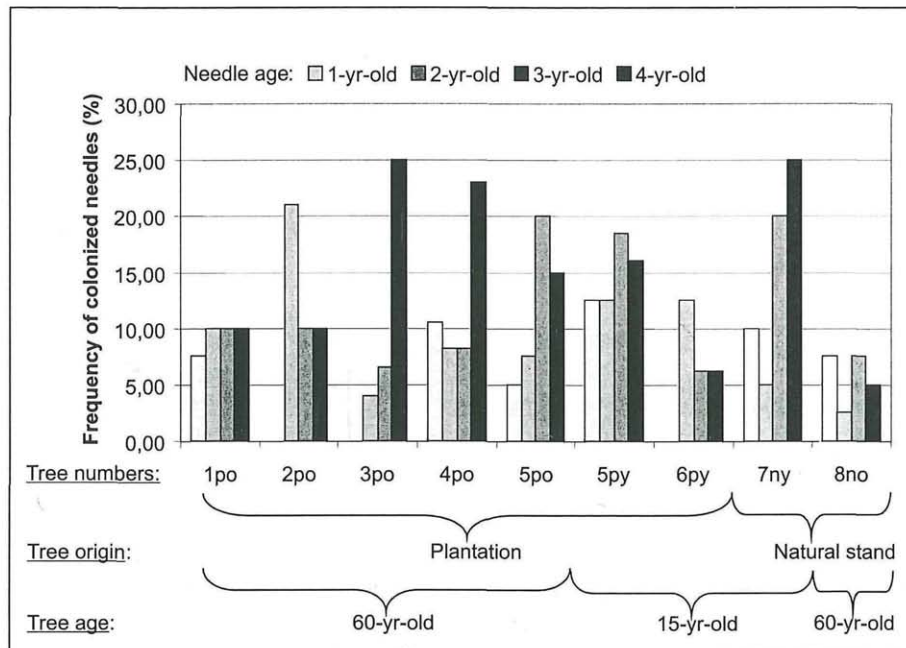


Fig. 1. Frequency (%) of 1- to 4-yr-old needles colonized by *Cenangium ferruginosum*.

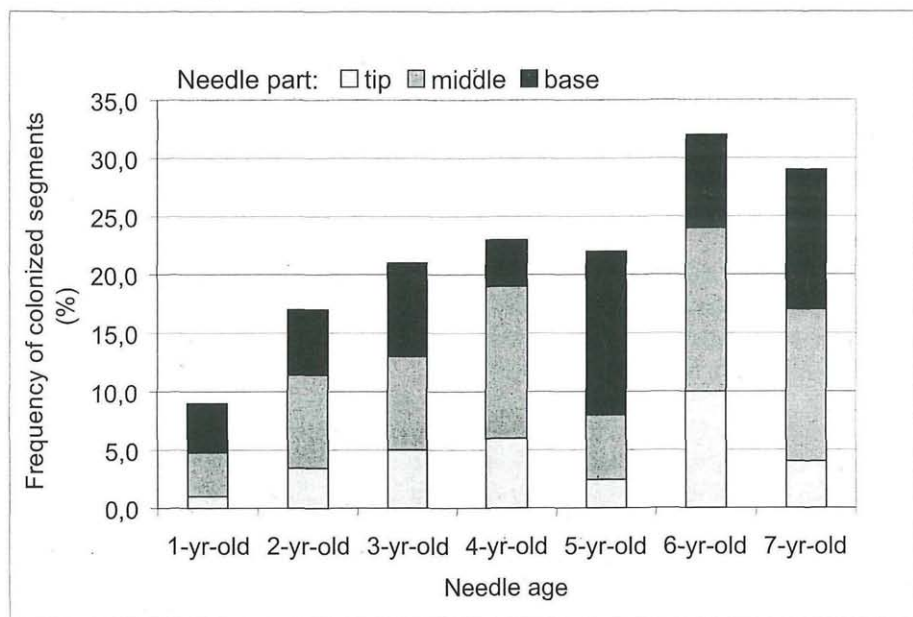


Fig. 2. Frequency (%) of segments colonized by *Cenangium ferruginosum* with respect to needle age and needle part.

The frequency of colonisation by *C. ferruginosum* seemed to depend on the season with maxima in October and minima during the winter months. This dependence could, however, not be proven statistically.

C. ferruginosum could not be isolated from buds.

Discussion

RACK & SCHEIDEMANN 1987 and HELANDER & al. 1994 detected *C. ferruginosum* as an endophyte in needles of *P. sylvestris*. *P. nigra* can now be added as another host of endophytic *C. ferruginosum*. This fungus was the second most frequently isolated endophyte in needles of *P. nigra*, with *Cyclaneusma niveum* (Pers.: Fr.) DiCosmo, Peredo & Minter being the most frequently isolated one (JURC & al. 1995).

No statistically significant difference was found in the colonisation of needles among trees, although needle colonisation was very low for the trees at sites 6 and 8 compared to other trees (Fig. 1). Consequently, age and origin (plantation or natural stand) of the trees did not differentially influence needle colonisation by *C. ferruginosum*. The only statistically significant difference existed between 1-yr-old and older needles. The frequency of colonisation was much lower in 1-yr-old needles. An increase in endophyte colonisation with increasing needle age has been shown for many conifers (JOHNSON & WHITNEY 1992).

HELANDER & al. 1994 detected the highest number of endophytic *C. ferruginosum* in the basal part of Scots pine needles. By contrast, in the present study, the frequency of colonisation in the tip and middle part of the needles was found to be higher - though not to a statistically significant level - than that found in the needle base (Fig. 2). HELANDER & al. 1994 hypothesised that *C. ferruginosum* either systemically colonises the needles from the twigs through the petioles or that spores preferentially infect the needle base because of more favourable conditions for infection (e. g. higher humidity resulting from the presence of bud scales). The results of this study support the view that *C. ferruginosum* infects the needles by means of spores to establish endophytic thalli. As soon as the environmental conditions change to the advantage of *C. ferruginosum* and/or the disadvantage of the host, the fungus may spread from the needles into the twigs and lead to the disease syndrome typical for *C. ferruginosum*. This may happen after long periods of drought and may explain why epidemics can develop suddenly and quite quickly over large areas, as was the case in Slovenia in 1986 (JURC 1986-1987). The role of the insect *Thecodiplosis brachyntera* as an inciting factor for disease development is emphasized by KOWALSKI 1998, but it occurs mostly in *Pinus sylvestris* and was not observed in Austrian pine at the time of this study. The hypothesis of endophytic *C. ferruginosum* as a reservoir for epidemics of cenangium dieback is in accordance with the observation of GREMMEN 1959. He regards the fungus as a pioneer organism on branches still attached to the tree and reports that in many cases it seems to inhabit branches suffering from a primary parasitical attack of the fungus *Gremmeniella abietina*.

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