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# Aspen fungal endophyte communities are enriched after herbivory by leaf eating Chrysomelid beetles

Aspen trees grow abundantly and extensively across the forests of the northern hemisphere. They are early succession species that can quickly establish after fires or storms, and they also support a high biodiversity. While the biodiversity associated with old growth aspen trees has been well described, ecological associations of arthropods and microorganisms of younger aspen stands are less understood. The genetic (DNA) difference between aspen individuals is surprisingly high and typic of outcrossing and expanding populations, which coincides with the short colonization history of aspen in post-glacial time since the glacial ice cap retracted 10000 years ago.

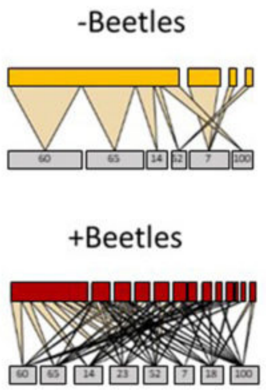


Fig. 1. Bipartite graph of the relationship between the SwAsp genets and the endophyte community that they associated with, respectively in absence (upper panel) and presence (lower panel) of *Chrysomela tremula* leaf beetles. Thicknesses of lines that connect genets with morphotypes are scaled to the abundance with a morphotype occurred in the samples.

This with-in species diversity between aspen individuals is also reflected in the chemical composition of aspen tissues, regarded as is genotypic specific with individual signatures in composition and quantity of single compounds. In a previous study we characterized the content of salicinoid phenolic glycosides of aspen leaves from a Swedish collection of European Aspen (*Populus tremula*) and found an unexpected diversity of these potential bio-active compounds. Associations between the phenolic compounds and arthropod communities are mostly known from studies of North American Aspen (*Populus tremuloides*) and association between phenolic profiles and communities of microorganisms that live inside the plant tissue (also known as endophytes) have been described for a suite of *Populus* species including *P. tremula*. The endophytic fungi do not cause symptoms, but they may be found in any plant organ, and both conservation scientists, plant researchers and plant growers are increasingly interested to determine the function of these plant endophytic communities and learn more about how they may potentially enrich the bio-diversity status of their hosts or support plant health and resistance properties.

We hypothesized that specialist leaf beetles (*Chrysomela tremula*) would directly or indirectly interfere with the endophyte community in the foliar endosphere of *P. tremula*, which we initially expected to associate with host genotype. In particular, we looked for evidence of predictable relationships between fungi of respectively plant and herbivore origin. We grew young clonal aspen plants in a greenhouse environment in northern Sweden and isolated culturable fungi on agar from leaf tissues from plants that had been either "exposed to" or "grown free of" leaf beetles.

We isolated 22 morphotypes of fungi from over 250 leaf segments and documented a strong increase in the number of fungal morphs in the presence of herbivores, as is indicated in the bipartite graphics in Figure 1. Beetle activity added generalist morphs to the mycobiome that overrode the initial host genotype association and herbivory also enriched the fungal community with new fungal community members. Yeast-like genera (*Cryptococcus* and *Rhodotorula*) were isolated only from beetle-damaged tissues and from beetles, whereas fast growing filamentous fungi dominated beetle-free control plants. Moreover, during the culturing from the leaves, in the presence of yeasts, the filamentous *Penicillium* sp. grew less vigorously (Fig. 2). Yeasts that were only isolated from beetle-damaged material were used to conduct experiments that might suggest the kind of relationship that take place between endophytes inside the leaves.  Competition experiments between filamentous fungi of plant origin and beetle-related yeasts further suggested both stimulating and inhibiting modes of action amongst the fungi.

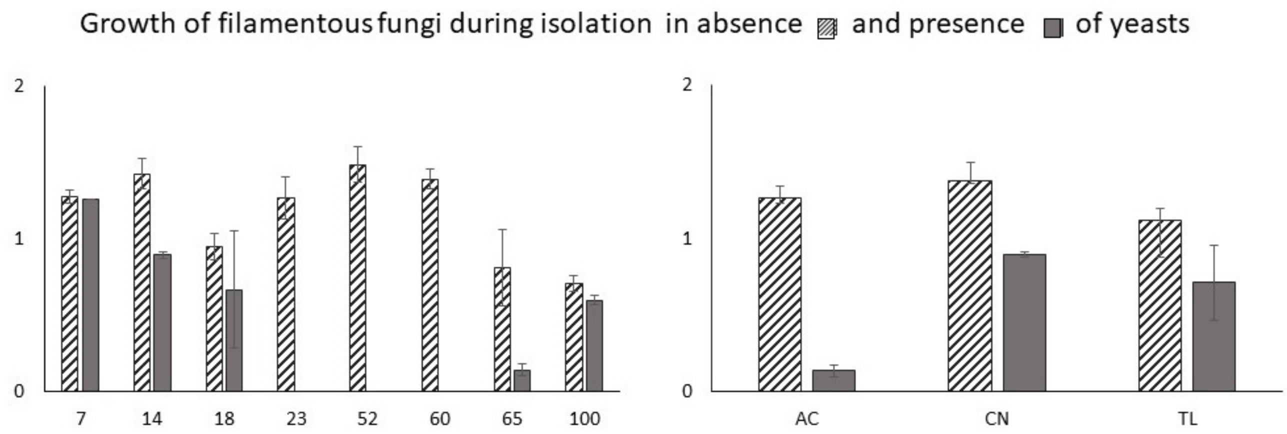


Fig. 2. *Penicillium sp*. grew less vigorously when isolated from aspen leaves together with yeast colonies (average colony diameter, average size ± s.e. in $cm^2$). Host trees had been propagated from the Swedish Aspen (SwAsp) bio resource at Umeå Plant Science Centre and the numbers on the x-axis refer to SwAsp genet number.

As a result, we detected unbalanced relationships between fungal morphs including amensalism, commensalism, parasitism and competition, but we found no evidence of balanced mutualism, and consequently no strong indication that there was a tight positive connection between single filamentous fungal morphs, yeast morphs and leaf beetles. This result suggested a lack of a tight co-evolutionary relationship among the isolated fungal morphs, which might suggest that the beetles served as random vectors of mainly yeasts.

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## Publication

Both plant genotype and herbivory shape aspen endophyte communities.
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