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The 'birch-leafroll disease' emerging in forests and urban parks in Fennoscandia - Viral agents associated with the disease

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Question: The 'birch-leafroll disease' has emerged the last decade to a serious problem in Fennoscandia. The extensive appearance of virus-infected birches was first reported in 2006 in Finland (Jalkanen *et al.*, 2007). Diseased trees exhibited foliar disorders including vein banding, leaf roll and chlorotic ringspots, the affected trees declining in vigor with time. *Cherry leaf roll virus* has been detected in symptomatic birches, it could however, not be directly correlated with the disease. A natural CLRV population from birches is studied to improve understanding of the emergence of this viral disease. Additionally, the assumption that other viral pathogens are involved in the disease is investigated.

Methods: A natural population of CLRV collected from infected *Betula* trees in Rovaniemi is analyzed and the population features are described. Detection trials targeting other viruses are performed. Samples from affected trees are analyzed with the next-generation sequencing (NGS) technology, allowing the whole microbial population present in each sample to be defined. Detection assays to confirm the presence of the viruses identified by NGS in the RNA samples isolated from the infected tissues are performed.

Results: The birch CLRV population from Rovaniemi is characterised by high genetic variability; considerable haplotype diversity, long genetic distance among haplotypes and high within haplotype diversity are determined. Mixed infections of CLRV strains in single *Betula* trees and recombination events in the CP region are demonstrated. The NGS analysis provided indication of more viruses being present in the birch samples.

Conclusions: CLRV is strongly suggested to be associated with the "birch-leafroll disease" in Fennoscandia. We consider the increased genetic diversity and the coexistence of a complex of highly variable strains in the same host as a significant change in the pathogen population which could constitute a possible factor for the disease emergence. The beneficial effect for the CLRV infection when trees are co-infected with another plant pathogenic virus will be discussed.

Jalkanen, R., Büttner, C. and von Bargaen, S. 2007. *Cherry leaf roll virus* CLRV, abundant on *Betula pubescens* in Finland. *Silva Fennica* 41: 755-762.

O TREE 5

Emaraviruses infecting forest and urban deciduous tree species

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European mountain ash ringspot-associated virus (EMARaV) is the type member of the genus *Emaravirus* comprising plant viruses with a segmented ss(-)RNA genome mainly infecting woody hosts. EMARaV contains at least four monocistronic genome segments within the enveloped spherical particle, encoding the replicase (RNA1), a glycoprotein precursor (RNA2), the viral nucleocapsid protein (RNA3), and a putative movement protein (RNA4), while in other members of the genus up to eight genomic RNA molecules were found. Emaraviruses have a narrow host range usually restricted to few related species. Symptoms induced by EMARaV include chlorotic ringspot, mottle of leaves and decline which may lead to the death of *Sorbus aucuparia* (European mountain ash, syn. rowan). Other broad-leaved tree species are known for decades exhibiting similar virus-like symptoms, but no virus has been associated with the disease, yet.

Distribution and significance of emaraviruses as causal viral agents infecting important broad-leaved tree species of European forests and urban areas are addressed in this study.

Sorbus spp. were assessed for EMARaV infection by visual inspection and RT-PCR. Other deciduous tree species showing virus-like symptoms were investigated for plant virus infection utilizing next-generation RNA sequencing technologies.

Chlorotic ringspots, mottle and dieback occur frequently throughout the *S. aucuparia* population in several European countries including natural stands, managed forest and urban areas. EMARaV is closely associated with the observed disease as demonstrated by RT-PCR. The virus was also detectable in whitebeam species (*S. aria* and *S. intermedia*) with respective symptoms. Further, two previously unknown RNA viruses were identified in two different tree species showing chlorotic ringspots, line pattern and mottle of leaves. Sequence analyses revealed closest relationships to emaraviruses.

EMARaV is the main viral agent which affects rowans throughout Europe and is capable to infect other *Sorbus* species. Related plant viruses are able to infect other broad-leaved tree species in Europe. Thus, members of the genus *Emaravirus* have to be

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considered as serious threats to relevant forest and urban woody species and need to be included into health management strategies of deciduous trees.

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Earlier bud burst in Norway spruce causes shifts in the population dynamics of two eruptive sawfly species

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Lowland stands of pure Norway spruce (*Picea abies*) in Austria are highly prone to mass outbreaks of the small spruce sawfly, *Pristiphora abietina*. Until the mid-1990ies, large forest areas were heavily infested, but outbreaks disappeared thereafter. From 2011 onwards, significant feeding damages were reported from spruce plantations that had suffered severe and repeated defoliation by *P. abietina* in the past. The actual damage is caused by the mountain spruce sawfly, *Pachynematus montanus*, a species considered to be a minor pest with sporadic, local outbreaks in forests above 800 m a.s.l. Within an EU-funded project we investigated the population dynamics of the two sawfly species. We recorded the timing of adult sawfly emergence from the soil (photo-electors) and spruce budburst (stages) on five currently-infested lowland sites and two uninfested sites at higher elevations for 2-3 consecutive years. The data were coupled with air and soil temperature profiles and the results were compared with studies from the 1990ies. Despite year-to-year variations, the beginning of budburst in spruce advanced considerably more than adult wasp emergence. At the lowland sites, *P. abietina* catches were rare while *P. montanus* was the predominant species; both sawfly species were rare at the upland sites.

The phenological window for oviposition is very narrow for *P. abietina*, females accept exclusively the newly-expanding buds. The temporal window is wider for *P. montanus* wasps which lay their eggs on needles of expanding shoots up to 5 cm in length. Accordingly, tight synchrony between budburst and wasp emergence is a major determinant of variability in the abundance of *P. abietina*. We speculate that significant advances in the timing of spruce budburst due to climate warming in recent years outcompeted *P. abietina*, presumably due to the inability of the emerging wasps to find suitable, newly-burst buds for oviposition, but still allow *P. montanus* to colonize the buds.

The shift in population dynamics of the sawfly species has major impacts on forestry; feeding of *P. abietina* larvae is restricted to current-year needles, thus, even high needle loss does not kill the tree. By contrast, *P. montanus* larvae feed both current-year needles and old foliage, and thus predisposing heavily infested trees to attack by bark beetles.