Occurrence of EMARAV and CLRV in tree species native to Finland

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Fig. 1: Betula spp. exhibiting virus-like symptoms: *Betula pubescens ssp. pubescens*, habitus of CLRV infected tree (a), vein banding and leaf roll (b), necrotic lesions (c) of leaves. *Betula pendula*, symptomatic parts of the lower canopy (d), leaf roll and chlorosis (e). *B. pubescens ssp. appressa* vein banding (f). *B. pubescens ssp. czerepanovii*, vein netting and chlorotic leaf patterns (g). *B. nana*, intercostal chlorosis of leaves (h). Finland, July 2006 or 2007.

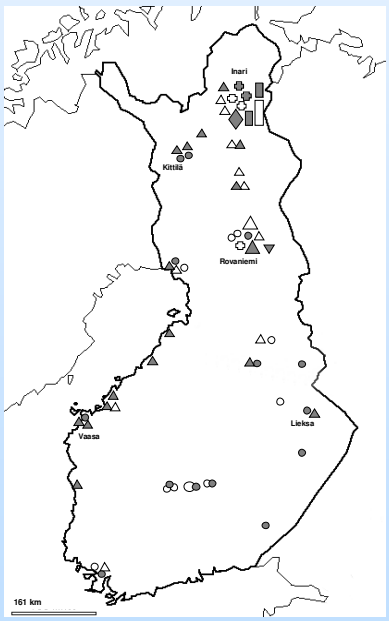

Fig. 3: Locations of sampled trees expressing virus-like symptoms. Species are indicated by the following symbols: *Betula pubescens* subsp. *pubescens* (Δ), *B. pendula* (O), *B. pubescens* subsp. *czerepanovii*, (□) *B. pubescens* var. *appressa* (◊), *B. nana* (+) *B. pendula* var. *carelica* (▽). CLRV infected trees confirmed by IC-RT-PCR are indicated by dark symbols. Small symbols represent one individual tree, middle sized symbols 4-5 trees.

Introduction

Finland is the most densely-wooded country in Europe. More than 76% of the ground is covered by forests which are of great ecological value as well as important industrial resources. Since 2002 an increase of virus-like symptoms in birch such as vein banding, proliferation, chloroses, mottling, and rolling of leaves **(Fig. 1)** was observed. Symptoms could be associated with the Cherry *leaf roll virus*, CLRV (Jalkanen et al. 2007) and a countrywide survey of birch species was initiated. Furthermore, the occurrence of *European mountain ash ringspot-associated virus,* EMARAV, the putative causal agent of chlorotic ringspots and mottling in Finnish mountain ashes **(Fig. 2)** was investigated.

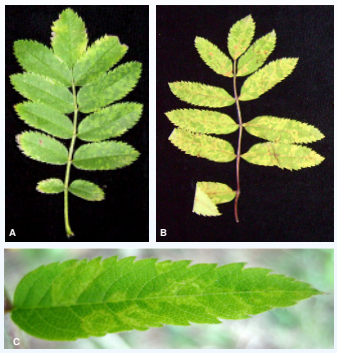

Fig. 2: Symptoms of *Sorbus aucuparia* leaves infected by EMARAV, (A) chlorotic mottling, (B) and (C) chlorotic ringspots

Material and Methods

Leaf material and inflorescences of downy birch (*Betula pubescens*), silver birch (*B. pendula*), curly birch (*B. pendula var. carelica*), dwarf birch (*B. nana*), mountain birch (*B. pubescens subsp. czerepanovii*), Kiilopää birch (*B. pubescens subsp. appressa*), mountain ash (*Sorbus aucuparia*) and red elderberry (*Sambucus racemosa)* were collected in the years 2006-2008 from all over the country. Also water from lakes was included in the sampling. CLRV was detected by an IC-RT-PCR. The detection based on short fragments of the coat protein region on RNA2 (162 bp) or the 3‘ non-coding region of RNA1 and RNA2 (412 bp). Selected samples were sequenced.

For detection of EMARAV in the European mountain ash samples total RNA was extracted according to Mielke et al. 2008 and following by RT-PCR by using a primer pair which amplifies a 204 bp long DNA-fragment from the RNA3, coding for the putative nucleocapsid protein.

Results

It was found that CLRV is able to infect all 6 investigated Betula species (**Fig. 3**). Especially, downy and silver birches were found to be affected by the virus. Furthermore, CLRV could detected in two out of six mountain ashes and in one red elderberry as well as in one surface water sample of a lake. Sequence comparison of a 112 bp long DNA-fragment from six samples with reference sequences **(Fig. 4)** showed a close relation and exhibited highest identities (88.3-89.2%) with an CLRV isolate from Canadian elder which is clustering in the phylogenetic group E (Rebenstorf et al. 2006). Remarkably, CLRV sequences derived from Finnish birches exhibited considerably lower identities (75.0-77.6%) with isolates from German or English birches in group A. This was confirmed by phylogenetic studies performed with the partial 3´non-coding region and indicates towards atypical phylogenetic relations of CLRV originating from Finland.

Furthermore, the six CLRV tested mountain ashes were detected on an EMARAV affection. Four of the six mountain ashes are EMARAV positive **(Fig. 5)**. The infection with CLRV of mountain ash could be confirmed in two sampled trees revealing first-time a mixed infection with the two viruses in a single case.

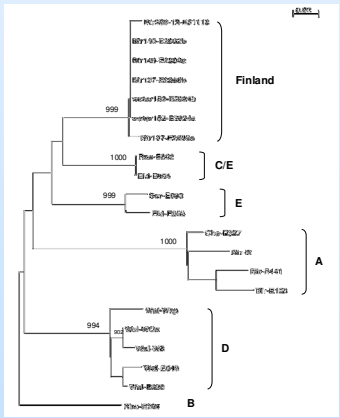

Fig. 4: Neighbour-joining phylogenetic tree of 112 nucleotides of the coat protein-coding region of CLRV. Bootstrap analysis was performed with 1000 replicates and values above 900 are indicated on branches. Phylogenetic groups A-E according to Rebenstorf et al. (2006)

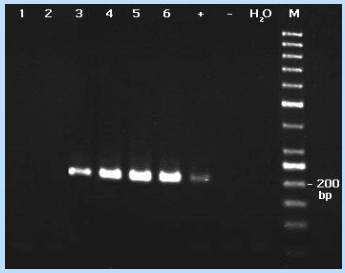

Fig. 5: EMARAV detection by RT-PCR. Line 1-6 = Finnish mountain ashes with symptoms, (+) = positive control EMARAV infected mountain ash, (-) = healthy mountain ash, H_2O = water, M = 50 bp Ladder (Fermentas)

Literature

Jalkanen, R. et al. (2007), *Cherry leaf roll virus* abundant on *Betula pubescens* in Finnland, Silva Fennica 41, 755-762.

Mielke, N. et al. (2008), Detection of *European mountain ash ringspot-associated virus* (EMARAV) in *Sorbus aucuparia* L. by a specific antiserum and reverse transcription-PCR, Forest Pathology 38, 371-380.

Rebenstorf, K. et al. (2006), Host species-dependent population structure of a pollen-borne plant virus, *Cherry leaf roll virus*. Journal of Virology 80, 2453-2462.