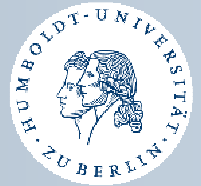


Verifiability and molecular analysis of *Cherry leaf roll virus* infecting Finnish *Betula* species



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Introduction

Cherry leaf roll virus, CLRV, was detected in Finland in several *Betula pubescens* ssp. *pubescens* (downy birch) trees exhibiting symptoms of a viral disease (Jalkanen et al. 2007); the virus could also be confirmed in *B. pendula* (silver birch), both are dominating deciduous tree species in the country. Furthermore, CLRV was found in *B. nana* (dwarf birch), *B. pubescens* ssp. *czerepanovii* (mountain birch) as well as *B. pubescens* ssp. *appressa* (Kiilopää birch) comprising important key components of the arctic ecosystem (Fig. 1).

Methods and Results

Fragments of the 3' non coding region (3'NCR) were amplified by application of CLRV specific IC-RT-PCR.

Testing 76 symptomatic birch trees confirmed CLRV infected birches including 5 different species or subspecies respectively over the country (Fig. 2).

During the year round testing 15 CLRV infected *B. pubescens* trees were sampled seven times during 2006 and 2007. Virus detection was possible during the vegetation period (Fig. 3) between May and September, while CLRV was not detectable in dormant tissues (buds or catkins) in winter.

CLRV specific fragments from 3 downy birches from Rovaniemi, 2 silver birch trees and one mountain birch were sequenced. Genetic relationships were investigated by PCR-RFLP as well as sequence comparison with CLRV isolates characterized previously by Rebenstorf et al. (2006), who established 5 different phylogenetic groups (A-E) depending on the host plant. Nine individual CLRV clones obtained from 6 different *Betula* trees revealed two different fragment sizes, 404 bp and 412 bp, which were in accordance with grouping of Finnish CLRV isolates by PCR-RFLP. Unlike clustering of CLRV strains from birches growing in the UK and Germany exclusively within group A, Finnish CLRV isolates exhibited highest sequence identities to isolates clustered in phylogenetic group B, D or E (Fig. 4).

Betula spp. exhibiting virus-like symptoms



Fig. 1: *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.

Verifiability of CLRV throughout the year

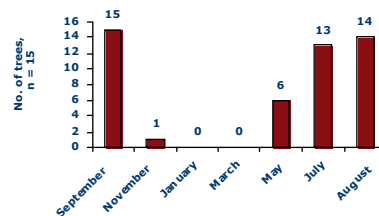


Fig. 3: Detection of CLRV in 15 infected *B. pubescens* trees during the year 2006 and 2007.

Phylogenetic relationships of CLRV isolates from Finnish *Betula* species



Fig. 4: Phylogenetic tree (ClustalW2 phylip) of the partial CLRV 3' non coding region (approx. 387 bp) exhibit clustering of Finnish CLRV isolates (brown shaded) in various phylogenetic groups different from previously characterized CLRV isolates originating from birches in other European countries (arrows).

Distribution of CLRV infected *Betula* spp. trees in Finland

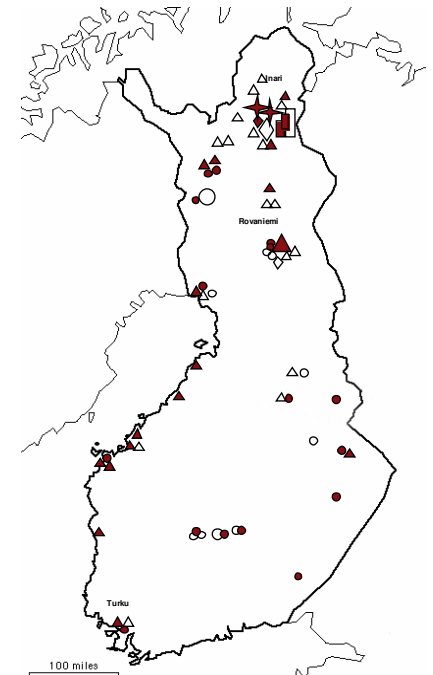


Fig. 2: Locations of sampled trees expressing virus-like symptoms. Species are indicated by following symbols: *Betula pubescens* ssp. *pubescens* (Δ), *B. pendula* (O), *B. pubescens* ssp. *czerepanovii*, (\square) *B. pubescens* ssp. *appressa* (\diamond), *B. nana* (\oplus). CLRV infected trees confirmed by IC-RT-PCR are indicated by red colored symbols. Small symbols represent one individual tree, middle sized symbols 4-5 trees, large symbols 10 or more trees.

Conclusions

CLRV was not detectable by IC-RT-PCR outside the vegetation period in downy birches which may be correlated to a higher freezing tolerance during that stage. Most reliable results were obtained from July until September.

CLRV is widely distributed in Finland and able to infect all 5 investigated *Betula* species.

CLRV populations in Finnish birches differ from other locations and exhibit higher sequence variability.

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