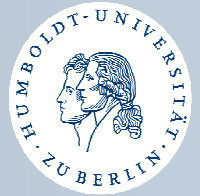


# Cherry leaf roll virus: a threat to Finnish *Betula* spp.

E. Grubits<sup>1</sup>, S. von Bargaen<sup>1</sup>, J. Langer<sup>1</sup>, R. Jalkanen<sup>2</sup>, C. Büttner<sup>1</sup>

<sup>1</sup> Humboldt-Universität zu Berlin, Institute of Horticultural Sciences, Section Phytomedicine, Lentzeallee 55/57, 14195 Berlin, Germany, phytomedizin@agrar.hu-berlin.de

<sup>2</sup> Metla, Finnish Forest Research Institute, Rovaniemi, Finland



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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. CLRV was found in *B. nana* (dwarf birch), *B. pubescens* ssp. *czerepanovii* (mountain birch) as well as *B. pubescens* var. *appressa* (Kiilopää birch) comprising key components of the arctic ecosystem (Fig. 1). A single *B. pendula* var. *carelica* (curly birch) an ornamental tree variety used as expensive veneer wood was also found to be CLRV infected.

## 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 6 different species or subspecies respectively over the country (Fig. 2).

CLRV specific fragments from 3 downy birches from Rovaniemi, 2 silver birch trees (Lieksa, Vaasa) and one mountain birch (Inari) were sequenced. Genetic relationships were investigated by PCR-RFLP as well as sequence comparison with CLRV isolates characterised previously by Rebenstorff 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 (Table 1). 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. 3). Furthermore, from two trees more than one sequence variant of CLRV was detected indicating a higher sequence variability of the virus not only in the Finnish birch population, but also in individual trees.

## *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* var. *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.

## Molecular characteristics of CLRV isolates obtained from Finnish birch trees

Table 1: Phylogenetic grouping of CLRV isolates determined by PCR-RFLP and sequence analysis of the amplified 3' NCR fragment.

no.	Tree species	CLRV isolate, clone	Fragment length <sup>1</sup> (bp)	PCR-RFLP (bp)			RFLP type <sup>2</sup>	Phylogenetic group <sup>3</sup>
				AatI	BspI43I	RsaI		
1	<i>B. pubescens</i> subsp. <i>pubescens</i>	E2485, EG1	412	147/265	ud	83/329	B	B
2	<i>B. pubescens</i> subsp. <i>pubescens</i>	E2485, EG3	412	146/266	ud	ud	D2/C/E	E
3	<i>B. pubescens</i> subsp. <i>pubescens</i>	E2485, EG12	412	147/265	ud	ud	D2/C/E	B
4	<i>B. pubescens</i> subsp. <i>pubescens</i>	E2501, EG9	412	146/266	ud	ud	D2/C/E	E
5	<i>B. pubescens</i> subsp. <i>pubescens</i>	E2501, EG10	412	146/266	ud	ud	D2/C/E	E
6	<i>B. pendula</i>	E2532, EG22	404	ud	ud	ud	A1/D	D
7	<i>B. pendula</i>	E2538, EG28	412	146/266	ud	ud	D2/C/E	E
8	<i>B. pubescens</i> subsp. <i>czerepanovii</i>	E3261, EG31	404	ud	ud	ud	A1/D	D
9	<i>B. pubescens</i> subsp. <i>czerepanovii</i>	E3261, EG32	412	147/265	ud	83/329	B	B

(a) IC-RT-PCR product including primer sequences, (b) according to Buchhop et al. (2006), (c) according to Rebenstorff et al. (2006), (1) ud = undigested

## Phylogenetic relationships of CLRV isolates from Finnish *Betula* species

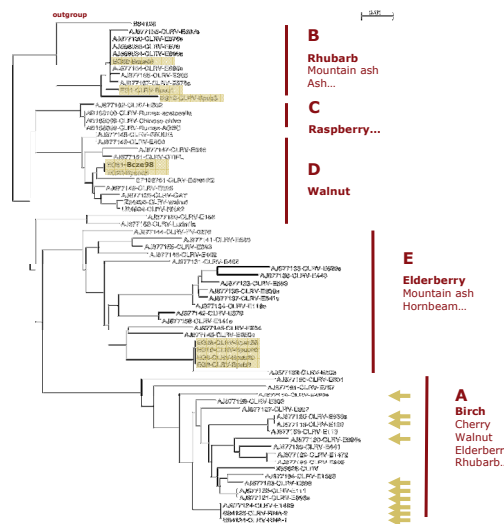


Fig. 3: Phylogenetic tree (ClustalW2 phylip) of the partial CLRV 3' non coding region (approx. 387 bp) exhibit clustering of Finnish CLRV isolates (yellow shaded) in various phylogenetic groups different from previously characterized CLRV isolates originating from birches in other European countries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

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

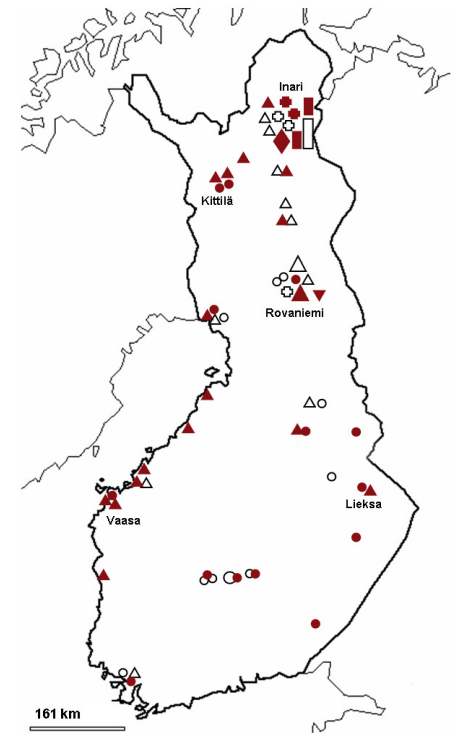


Fig. 2: Locations of sampled trees expressing virus-like symptoms. Species are indicated by following symbols: *Betula pubescens* ssp. *pubescens* ( $\Delta$ ), *B. pendula* (O), *B. pendula* var. *carelica* ( $\nabla$ ), *B. pubescens* ssp. *czerepanovii*, ( $\square$ ) *B. pubescens* var. *appressa* ( $\diamond$ ), *B. nana* (+). 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 is widely distributed in Finland and able to infect all 6 investigated *Betula* species.

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

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