

9-14 Sequence diversity of *Cherry leaf roll virus* makes a difference in infected birches in Finland

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INTRODUCTION

Virus-related symptoms were increasingly found on birch leaves throughout northern Fennoscandia. Disease symptoms occurring on downy birch (*Betula pubescens*), silver birch (*B. pendula*), dwarf birch (*B. nana*), Kiilopää birch (*B. pubescens* var. *appressa*), mountain birch (*B. pubescens* ssp. *czerepanovii*), and curly birch (*B. pendula* var. *carelica*) in Finland could be associated with an infection of *Cherry leaf roll virus* (CLRV). The virus is a typical member of the *Secoviridae* family, genus *Nepovirus* and infects primarily deciduous trees and shrubs. The complete genomic sequence of the CLRV has been determined (von Bargaen *et al.* 2012). The bipartite, isometric particles contain positive stranded RNA. Disease symptoms in birch trees are spreading since their first record in 2002 and a countrywide incidence of CLRV could be shown in affected-birch trees tested since 2006 (Jalkanen *et al.* 2007; von Bargaen *et al.* 2009). Symptoms are observed in roadside and urban areas, but are also found in forests. However, the mode of virus dispersal in Fennoscandia is unclear.

MATERIAL AND METHODS

Diseased birch trees all over Finland were inspected for symptoms associated with a CLRV infection such as chlorotic vein banding, leaf roll and necrotic lesions. Total RNA was extracted from symptomatic leaf material according to Boom *et al.* (1990). CLRV was detected by RT-PCR using random hexamers for cDNA synthesis and virus specific primer pairs targeting regions encoding the RNA-dependent-RNA polymerase (RdRp) on RNA1, the coat protein (CP)-coding region on RNA2, and the 3' untranslated region (3' UTR), the

latter being highly conserved among isolates and is present on both genomic segments. Amplified PCR products were sequenced from both directions by Sanger sequencing. Obtained sequences were analyzed and compared to CLRV isolates characterized by Rebenstorf *et al.* (2006) applying ClustalX 2.0 using the incorporated neighbour-joining method for phylogenetic tree construction.

RESULTS AND DISCUSSION

Symptoms observed in CLRV infected birches in northern Europe are more prominent and differ from virus-affected birches from other European countries. It was not possible to obtain a CLRV isolate from a Finnish site by rub inoculation so far. Phylogenetic analyses of a conserved RdRp-sequence fragment showed that grouping of CLRV variants obtained from Finnish birches correspond with host-dependent classification determined by Rebenstorf *et al.* (2006). Partial sequences obtained from the RNA2 encoding the CP as well as the ultimate 3' untranslated regions showed divergence on the nucleotide level in comparison with CLRV isolates from different hosts. This indicates towards the presence of a distinct population of CLRV in Finnish birches.

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