Epidemiological investigations on *Cherry leaf roll virus*

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The world-wide distributed pathogen *Cherry leaf roll virus* (CLRV), which is occurring primarily on deciduous and fruit trees from al least 17 genera, is the only plant virus with such a wide host range among woody plants so far. Host range and geographical distribution of the virus indicates a fast adaptability to different hosts and therefore a genetic heterogeneity among CLRV-isolates of different origins. This was confirmed by sequence analyses of various coding and non coding genomic regions of CLRV-isolates derived from different host plants and geographical origins and suggested a prevalent host dependent phylogenetic relation (Rebenstorf et al. 2006).

This is substantiated by the fact that CLRV is naturally transmitted through pollen and seeds requiring a high degree of host adaption of CLRV-isolates. However, this is not a strict principle as some CLRV-isolates from one host plant species clustered in different phylogenetic groups. Therefore, other modes of transmission may be involved in virus dispersal in natural habitats. Within the present investigations on CLRV this study focuses on the epidemiological relevance of mechanical transmission of CLRV between host plant species as well as on host range of selected phylogenetically diverse isolates.

CLRV-isolates (elderberry-isolate E603, walnut-isolate E326, rhuburb-isolate E395) were mechanically inoculated on five natural woody host plant species by stem slashing (Fig.1): *Sambucus nigra* (black elderberry), *Juglans regia* (English walnut), *Prunus avium* (sweet cherry), *Betula pendula* (common birch), *Sorbus aucuparia* (mountain ash).

A CLRV-infection screening of all 50 inoculated plants per CLRV-isolate was done by Immunocapture-RT-PCR 3, 9 and 14 months after inoculation. To specify results, IC-RT-PCR products of the last sampling (14 month a.i.) were reamplificated by a semi-nested PCR and RFLP analysis (Fig.2). Putative CLRV-positive IC-RT-PCR products will be sequenced to confirm infection of woody hosts and determine viral isolate.

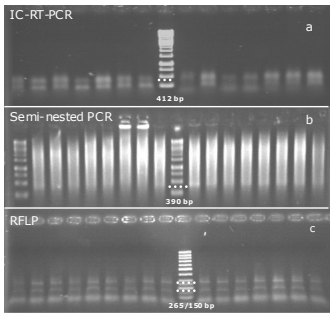

Fig.1: inoculation by stem slashing. 10 cuts with a razor blade immersed into virus suspension

Fig.2: CLRV-infection screening of birch seedlings inoculated with the rhubarb-isolate E395. **a:** IC-RT-PCR with CLRV-specific primers (fragment size: 412 bp) **b:** reamplification of IC-RT-PCR product by semi-nested PCR; expected product: 390 bp. **c:** RFLP-analysis with AluI-endonuclease. IC-RT-PCR products are cleaved by the enzyme into smaller fragments; restriction pattern of 265 and 150 bp specifies CLRV.

One year after inoculation it could be proved the three CLRV-isolates E603, E326 and E395, propagated on experimental test plants over years, are still infectious on natural woody hosts.

The CLRV-isolate E603 from elderberry was able to infect all five tested woody plant species. In contrast, for the isolates E326 (walnut) and E395 (rhubarb), CLRV-infected plants were detected only in two out of five species so far (see Fig.3). As the CLRV-detection system resulted in unreliable signals from several samples, testing of these trees has to be repeated or assayed by alternative methods (for instance DAS-ELISA). However, CLRV-infection of selected samples was confirmed by sequence analysis.

Generally, results suggest that the CLRV-isolate derived from black elderberry exhibit a wider host range than the isolates from walnut or rhubarb. Host adaption may not be stringent and/or transmission barriers between host species are developed differentially.

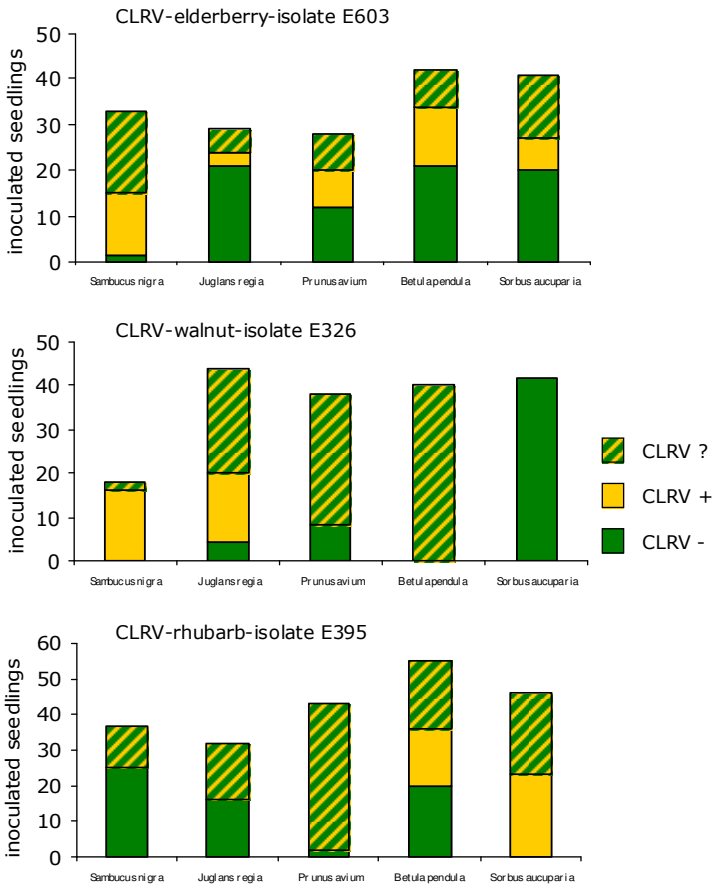

Fig.3: CLRV-detection in five woody plant species inoculated with three different CLRV-isolates. Twenty months after inoculation, tentative calculation of CLRV-infection status within the variants after screening at three sampling dates by IC-RT-PCR, semi-nested PCR and RFLP. Samples were scored positive if CLRV specific products were at least generated twice from individual trees by two different methods.

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K. Rebenstorf, T. Candresse, M. J. Dulucq, C. Büttner, and C. Obermeier (2006): Host Species dependent Population Structure of a Pollen-Borne Plant Virus, Cherry leaf roll virus. Journal of Virology 80, 2453–2462