

Alteration of allergen potential by Cherry leaf roll virus (CLRV) in infected birch pollen



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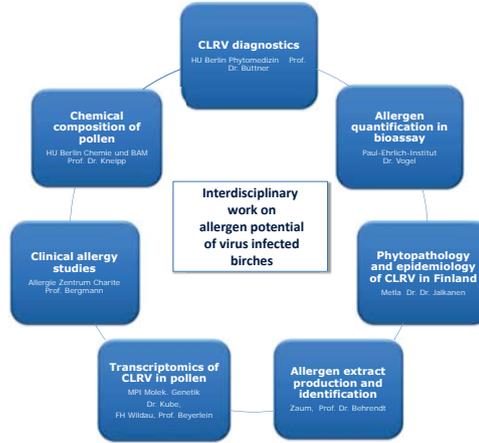
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Working hypothesis

Medical importance of the plant virus CLRV was never investigated and is an innovative topic in allergology as well as in plant pathology.

The plant virus Cherry leaf roll virus (CLRV) infects many woody and herbaceous species and is widespread in temperate regions. Over several years a birch decline is observed in Finland. We detected CLRV in these declining birch trees. As known there is an enormous impact of allergen pollen on human health. We follow the hypothesis that there is a remarkable difference in the impact of pollen from virus infected trees on human being. An interdisciplinary collaboration between forest pathology and human medicine shall answer what alterations of allergic reaction in patients are caused by CLRV infected birch pollen.

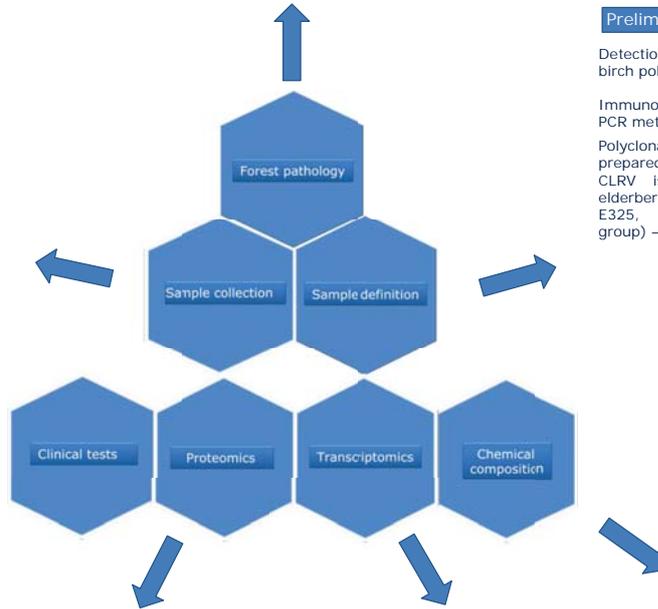
In all likelihood a negative impact on human health has to be seen in an allergen reaction to virus containing or modified plant material such as pollen. The modification of plant proteins or protein expression by CLRV viruses may change and determine the allergen potential of the infected pollen as was shown in grass pollen for Cocksfoot streak potyvirus (CSV) in individuals (Pallett et. al 2009).



Up to 80 % of all allergenic pollen is originated from birch. Around 40-50 % of all hay fever sufferers are sensitized against birch pollen. Birch pollen is of low weight and with a size of 15-20 nm it can be air transmitted several hundred kilometers. Patients may be affected by allergy far away from pollen origin. This demonstrates the global dimension of the pollen distribution. The cooperation aims in the application of strategies for the investigation of the allergen potential of CLRV infected birch pollen.



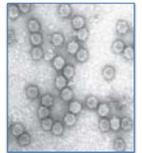
Birch pollen is the most recognized aeroallergen in northern European countries. Exposure to as few as 10 grains/m³ can result in seasonal rhinitis and asthma in birch sensitized individuals. Clinical Skin pric Test/Provocation Test shows response of mast cells to major birch pollen allergen: Bet v 1.



Preliminary work:

Detection of CLRV in birch pollen:

Immuno-Capture-RT-PCR method using Polyclonal antibodies prepared against two CLRV isolates (E603, elderberry group and E325, cherry-birch group) – specific ELISA



Preliminary results: Out of 13 pollen samples

4 positive → IC-RT-PCR with antibody cherry-birch group and 9 negative pollen samples



From 0.2 mg pollen are 3-24 microgram totalRNA extracted.

Major birch pollen allergen

High solubility of Bet v 1 in aqueous systems - easy cross of epithelial barriers

- Subset of *Bet v 1* genes - constitutively expressed in pollen
- Induced in birch cell suspension cultures in the presence of bacterial or fungal pathogens
- Bet v 1 protein activated - only during the final phase of pollen maturation

Literature:

Büttner, C., von Bargaen, S., Bandte, M., Myrta, A. (2011): Cherry leaf roll virus. In: Virus and Virus-Like Diseases of Pome and Stone Fruits (Eds. Hadidi, A., Barba, M., Candresse, T., Jelkmann, W.). 2011, St. Paul, USA.

JALKANEN, R., BÜTTNER, C., VON BARGEN, S. (2007). Cherry leaf roll virus, CLRV, abundant on *Betula pubescens* in Finland. *Silva Fennica* 41, 755-762.

Pallett D. W., Soh E., Edwards M.-L., Bodey K., Lau L. C.K., Cooper J. I., Howarth P. H., Walls A. F. and Wang H. 2009 Proof of concept pilot study: prevalence of grass virus infection and the potential for effects on the allergenic potency of pollen. *Environmental Health* 8(Suppl 1):S10

Schenk M., Cordewener J. HG, America A. HP, van't Westende W. PC, Smulders M. JM, Gilissen L. JWJ 2009 Characterization of PR-10 genes from eight *Betula* species and detection of Bet v 1 isoforms in birch pollen. *BMC Plant Biol.*; 9: 24.

In this part the quality and content of allergen causing proteins in pollen from virus infected trees shall be analyzed and evaluated. It is assumed that the infection of pollen by CLRV virus may influence the allergic reaction in patients, because the main allergen in birch pollen, the Bev1 protein, belongs to the pathogen induced protein family (PR10). The group of PR-10 proteins is induced in plants during infection by pathogens such as viruses, bacteria or fungi, respectively.

Transcriptome profiling or genome-wide expression profiling, will catalogue the complete set of RNA transcripts produced by the pollen and virus. The expression profiling may quantify the changing expression levels of transcripts under different conditions as we hypothesise in the pollen from CLRV infected and non-infected birch trees. Additional information of natural sequence variation in transcripts is provided.

Analysis of birch pollen by normal and surface enhanced Raman scattering (SERS) and or infrared spectroscopy, analytical Raman spectroscopy may enable determination and visualization of differences in chemical structure between Pollen from infected and non-infected birch trees; changes in chemical composition of the pollen grains, comparison of spectra of the different pollen grain bodies revealing alterations in the metabolism; and comparison to quantitative mass spectroscopy (MS).