Epidemiological investigations on Cherry leaf roll virus

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Introduction

Molecular and serological analyses of 56 CLRV isolates from different origins substantiated genetic heterogeneity in different genome regions that is strongly correlated with the host plant species. The mechanical transmissability of genetically diverse CLRV isolates and therefore the adaptability to different woody host was investigated

Methods and Results

Three phylogenetically distinct CLRV-isolates were inoculated on seedlings of five woody host plant species by stem slashing (Fig.1), 50 woody seedlings for every variant. As control ten seedlings of every species were not treated. Four times within 2 years after inoculation the trees were screened for CLRVinfection by IC-RT-PCR.

Finally, 21 out of 750 inoculated and 50 control trees have been detected being CLRV infected.

5 out of the 21 CLRV infected trees in the experimental plot '(Fig.2) were not inoculated control trees.

CLRV infected trees were found in every species.

CLRV was also detected in aphids which were collected from infected elderberry leaves.

Molecular analyses (Fig.3 & Fig.4) could not identify the inoculated CLRV isolates as the causal agents in the CLRV infected trees.

Conclusions

Since the back transmissions of the CLRV isolates from walnut and elderberry to their original hosts have not been detected we assume that the inoculation method was generally ineffective.

The detected CLRV variants in the infected trees suggest a natural infection due to the fact that CLRV sequences isolated from infected controls strongly resemble those from inoculated trees.

CLRV has been established in the experimental plot presumably by natural, but still unknown modes of transmission.

CLRV contaminated aphids collected from CLRV infected elderberry trees can be considered as a putative vector. Transmissibility of CLRV isolates to different woody host plant species

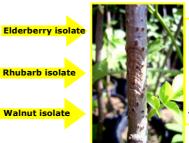


Fig.1: Stem slashing:

mecanical inoculation

of a virussuspension

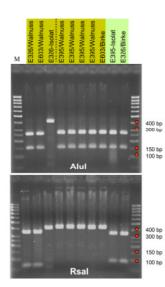
(5µg virus/ml) with a

razor blade

Sambucus nigra Betula pendula Prunus avium Juglans regia Sorbus aucuparia



Fig.2: Experimental plot within an outdoor area surrounded with decidous, fruit and ornamental trees.



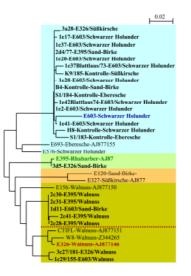


Fig.3: RFLP-analysis by digestion of the approx. 410 bp IC-RT-PCR products with the restriction endonucleases AluI and RsaI displaying the sequence diversities between the inoculated CLRV isolates (E326-walnut isolate, E395-rhuburb isolate) and CLRV variants isolated from CLRV infected walnut and birch trees 2 years after inoculation.

Fig.4: Sequencing of the IC-RT-PCR products revealed 3-11 % sequence diversities between the inoculated CLRV isolates (E326-walnut, E603-elderberry, E395-rhuburb) and the detected CLRV variants.

Within the deduced phylogenetic groups the sequence variants from inoculated and control trees show high identities (blue group) in contrast to the inoculated CLRV isolates.

Walnut infecting CLRV variants apparantly exhibit specific genetic adaptions as exclusively all CLRV sequences from walnut trees clustered in one group (brown).