Characterisation of an unknown putative virus from *Ulmus laevis* PALL.

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INTRODUCTION

Scientific findings indicate that viruses are widespread in trees of forests and urban green, and may cause losses of economic importance (Büttner *et al.* in press).

Over many years a monitoring was carried out with over 100 years old European White Elms (*Ulmus laevis* PALL.) in the park of Caputh near Berlin. During the last years, they showed leaf symptoms such as chlorotic ringspots, necroses and dieback, suggesting a virus as causal agent (Büttner & Führling 1993). Fungal and bacterial pathogens as causal agent of these symptoms could be excluded by microbiological assays. Also an infection by well studied viruses such as *Cherry leaf roll virus* (CLRV), *Elm mottle virus* (EMV), *Arabis mosaic virus* (ArMV) and *Tobacco ringspot virus* (TRSV) could not be confirmed. However, some facts point to a member of the genus *Carlavirus* (Bandte *et al.* 2004).

The aim of the present study is the isolation and characterization of a putative viral pathogen which is so far unknown. Based upon the assumption that the causal agent belongs to the genus *Carlavirus*, we apply different specific molecular biological and biochemical methods to identify the putative virus.

MATERIAL AND METHODS

Leaf material from 15 elms showing characteristic symptoms was collected in late spring over 3 years. The causal agent was transmitted mechanically from elm to *Chenopodium quinoa* by inoculation of leaves with homogenized bulbs from symptomatic elms. In order to scrutinize the hypothesis of a *Carlavirus* infection, nucleic acids were isolated from collected elm leaves as well as from inoculated *C. quinoa*. For cDNA synthesis i) random hexamers, ii) oligo-dT-primers or iii) *Carlavirus*-specific primers were used (Nie *et al*. 2008). Subsequently, RT-PCR was conducted with material from elm and *C. quinoa* applying *Carlavirus*-specific primers (Nie *et al*. 2008). Material infected with *Potato latent virus* (PotLV) and *Potato virus M* (PVM) provided by the Deutsche Sammlung Mikroorganismen und Zellkulturen (DSMZ) was used as positive control. For a partial virus purification symptomatic elm leaf material was used. To visualize the coat protein of the putative member of the *Carlavirus* aliquots of partial virus purification were

electrophoretically separated by SDS-PAGE. Furthermore, RNA was isolated from the purification and used for RT-PCR as described above.

RESULTS AND OUTLOOK

Meanwhile the European White Elm belongs to the endangered woody species of Germany. Therefore is a need to investigate diseases on this species and viral infections play an important role in the degeneration process.

Using described methods, it was not possible to confirm the pathogen as a member of the genus *Carlavirus* by now. Successfully inoculated *C. quinoa* showed deformation, chloroses and necroses on leaves. By RT-PCR with material from elm and *C. quinoa* several fragments of different sizes were generated. After sequencing, they did not show accordance to published *Carlavirus* sequences.

Current and future studies will have a focus on dsRNA. Hence, dsRNA will be isolated and used for random PCR according to Froussard (1992). Amplicons will be cloned, sequenced and compared with sequence information from the NCBI-database. Furthermore, virus particles will be purified directly from symptomatic elm leaves as well as from inoculated *C. quinoa*. The morphology of the virus particles will be investigated by transmission electron microscopy.

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