Characterization of newly discovered viruses in declining birch – a continuative study in Berlin

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State of the Art

Roadside trees have an immense impact on the health of human population and have to be protected and preserved. Virus-infections of broad leaved trees are widespread in urban areas and in forests, which might play a major role in tree decline. Viral infections are mostly recognized by mosaic-like leaf patterns, chlorotic ringspots, lines and mottling of light and dark green color as well as deformation (Fig 1). To gain a more detailed view on epidemiology of a viral complex in birch, the study was continued in 2017 with 107 birch tree samples in Berlin.

Data from next generation sequencing has shown the complexity of the birch virome. Based on molecular biological diagnostics of *Cherry leaf roll virus*, *Apple mosaic virus*, Carla- and Badnavirus from birch in 2015 and 2016, data on distribution of the viral complex was collected in the urban landscape of southern Berlin. Characterization of newly discovered viruses is one major goal in the next years to determine pathogenicity and evaluation of impact in urban green and forests. Especially interesting for management and maintenance of urban green is the mode of viral transmission.

Fig 1. Examples of symptoms and location: A-1 – Intercostal chlorosis, B-2 – Ringspot/Intercostal chlorosis, C-12 – leaf ringspots, D-1 – Chlorotic line pattern, E-8 – leaf necrosis, F-8 – Intercostal chlorosis, G-9 – Intercostal chlorosis, H-10 – Intercostal chlorosis

Fig 2. Sampling sites in Berlin

List of biotest plant for inoculation	3 weeks after inoculation
Nicotiana benthamiana	No symptoms
Nicotiana tabacum samsun	No symptoms
Chenopodium amaranticolor	No symptoms
Chenopodium quinoa	symptoms

Fig 3. List of biotest plant used for inoculation

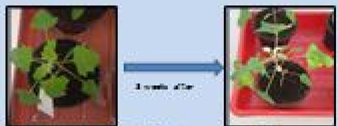

Fig 4. mechanical inoculation of C.quinoa

Methods

Primers were designed for viral detection of infected leaves from different sites in Berlin as shown in map below (Fig 2). Investigation of plant viruses was done by using RT-PCR to test for *Cherry leaf roll virus* (CLRV) and *Apple mosaic virus* (ApMV), which are commonly associated with deciduous trees. The diversity of symptoms and the newly discovered viral species (Carla- and Badnavirus) in *Betula* spp. led to the assumption of a mixed infection with unknown viral origin. Biotest experiment was carried out to check if newly found Carla- and Badnaviruses were mechanically transmissible. This was done using *Chenopodium quinoa* since other plants showed no symptoms (Fig 3). Symptoms like ringspots and intercostal chlorosis were observed three weeks after inoculation and evaluated by RT-PCR (Fig 4).

Results

Fig 5. Gel electrophoresis for detection of *Badnavirus* using RT-PCR with specific primer Badna5G from Birch samples

Fig 6. Detection of BadnavirusSG in C.quinoa using RT-PCR amplified at 242bp

Specific primers	Badnavirus K amplified at 300bp	Badnavirus 5G amplified at 242bp	Carlavirus amplified at 197bp	CLRV amplified at 627bp	ApMV amplified at 204bp
Number of tree samples n=107	39	66	15	20	Not yet completed

Fig 6. Detection of viruses in birch samples by RT-PCR with virus specific primers

Outlook

Transmission experiments for Badnaviruses using biotest plants (*Chenopodium quinoa*) and mechanical inoculation by infected birch leaves proved to be effective. First positive results were obtained for *Chenopodium quinoa* despite the narrow host range of Badnaviruses (Fig 6). This gave the impression that *Badnaviruses* found in birch are mechanically transmissible. Consequences for hygienic practices during tree management and maintenance will be examined once confirmation of *Badnavirus* pathogenicity is known.

Future studies will reveal whether Carlaviruses are mechanically transmissible using biotest experiments in fulfillment of Koch's Postulates. Once this is confirmed, birch leaf symptoms can be correlated with newly found plant viruses. Characterization and localization Apple mosaic virus, newly found Carla- and Badnaviruses will also be carried out using tissue printing technologies. Viruses will be localized within the symptomatic plants based on microscopic technologies to determine if they contribute to birch leaf roll diseases.

7. References

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8. Acknowledgement

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