HOP VIROIDS, HLVd AND HSVd BIOLISTIC INFECTION AND QUASIFORMS ANALYSIS

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Molecular analysis of two hop viroids, HSVd and HLVd is performed with respect to their variability and ability to infect various weed species potentially important for viroid spreading and microevolution (see Figs on the side of the poster). While HLVd was referred to have the host range limited to hop, HSVd forms very invasive group with many molecular variants or quasispecies and ability to infect various plant species. Recent analyses (Matoušek J. e.a.:Virology 287:349, 2001) show, however, that HLVd forms quasispecies, too, providing its replication under temperature stress. Moreover, it was found that certain thermomutants of HLVd can undergo microevolution in *Solanaceous* species forming "low level" populations (Matoušek J.: Biol. Plant., 46: 607, 2003). Hence, in principle, under certain conditions both these viroids could have some potential to propagate in various weed species, perhaps forming "low level" populations representing latent danger for various hop genotypes.

Fig.3

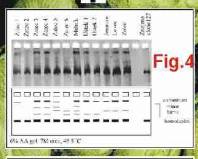
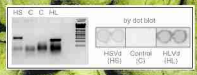
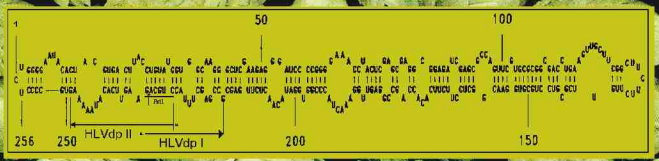


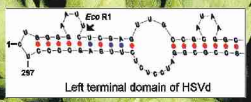
Fig.4



HLVd infectious construct: cDNA with duplication of *Pst* I cleavage site, amplification with primers as shown in Fig.1 below.



Eco R1 restriction site was used to prepare infectious cDNAs of HSVd Fig. 2



Galinsoga ciliata (Raf.) Blake

Epilobium parviflorum Schreb.

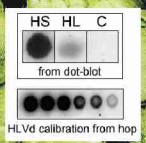
Chenopodium hybridum L.

Artemisia vulgaris L.

Galium aparine L.

Lycium arvense (L.) SCOP.

Symptoms on *G. ciliata* leaves



Matricaria matricaria L.

Arctium palula L.

In the present work biolistic methods for viroid trasfer in infectious cDNA (Fig. 1 and 2) and RNA (12-20 % PEG fraction as described by Matoušek e. a. J. Plant Physiol. 133:401,1988) forms were developed. It was found that a minute amount of viroid RNA is sufficient to promote viroid infection by this approach, which is about 1000x more efficient than classical inoculation using carborundum method. HSVd variants that were collected from grapevines surrounding hopgardens (Matoušek e. a.:Plant Soil Environ. 49:168, 2003) were easily transmissible to hop, in which it promotes severe symptoms and developmental distortions (Fig. 3). Using TGGE and cDNA heteroduplex analysis, we found that at least 70 % of grapevine samples from locations close to hopgardens in Northern Bohemia [Žatec and Úštěk growing areas], but also in Slovenia [Žalec growing area] were infected with HSVd forming populations containing quasispecies (Fig. 4). Particular variant of HSVd corresponding to AC E01844 was experimentally transmissible from these samples to Czech hop Osvald's 72 also by classical mechanical inoculation. First attepmpts to inoculate various weed species led to finding that the strongest hybridisation signals are detectable in some biolistically inoculated members of *Compositae* family, such as *Galinsaga ciliata* (designated by the asterisk). In some plants like in *Urtica* sp. extremely low viroid concentration led to storng pathogenesis effect. Analysis of HLVd and HSVd propagation in approx. 15 weed species collected in hopgarden agrobiotype is in progress.

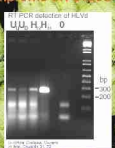
Conclusions

1. Highly efficient method of biolistic inoculation of hop viroids was developed.

2. Biolistic transfer of natural quasiforms of HSVd/HLVd to Osvald's 72 clone resulted in strong viroid infection and HSVd symptoms development on this hop.

3. Analysis of viroid infections after biolistic inoculation into various weed plant species was performed. Strong hybridization signals were detected in samples from *G. ciliata* and in some other plants mainly from *Compositae* family. Complex RT PCR analysis is in progress.

Symptoms on Urtica



Amaranthus retroflexus

Urtica dioica L.

Erysimum cheiranthoides L.

Capsella bursa-pastoris (L.)

Sonchus arvensis L.

Taraxacum officinale L.

Senecio vulgaris L.