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Investigating Seed and Pollen Transmission of Hop Latent Viroid in Hops

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Hop latent viroid (HLVd - *Cocadviroid latenshumuli*; genus *Cocadviroid*, family *Pospiviroidae*) is a pathogen that infects hop (*Humulus lupulus*) and hemp (*Cannabis sativa*) plants. It can severely impact yield and quality of the cultivation. In hemp the potential role of the pollen in the spread of the viroid **was described** (Atallah et al., 2023; Punja et al. 2025). Regarding hops negligible seed transmission rate was reported (Matoušek et al. 2000). Since the transmission rate of viroids has always been a controversial topic, further investigations were conducted to assess the HLVd presence on pollen and the rate of horizontal and vertical transmission on the mother plants and the F1 plants, respectively.

Pollen

To detect the presence of HLVd on pollen, the granules were separated from anthers (Fig. 1).

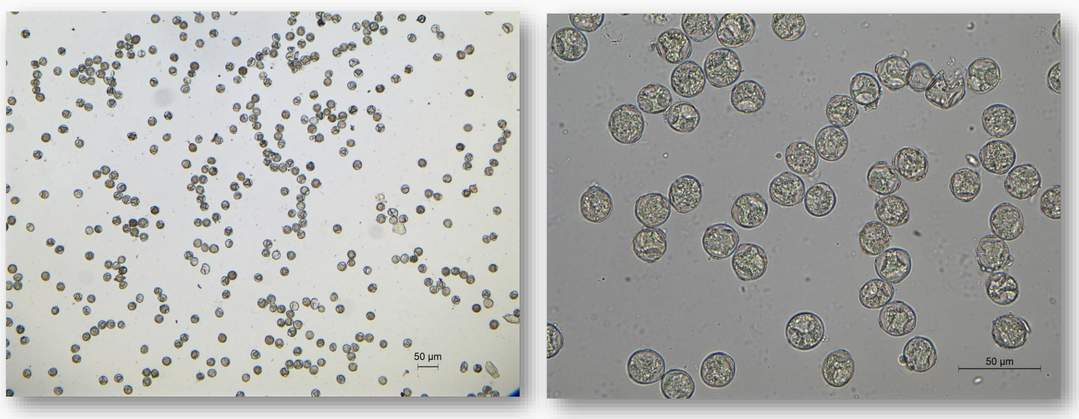

Figure 1. Pollen from cv. Chinook at different magnifications.

200 mg of hop pollen cv. Chinook was collected and washed with 450 µl of RLT buffer. After centrifugation (6000 g for 1 min) the supernatant was collected and the pellet was subjected to other two washing rounds for a total of three (Fig. 2). Then the TRNA was extracted from the three recovered supernatants and pellet using RNeasy Plant mini kit (Qiagen).

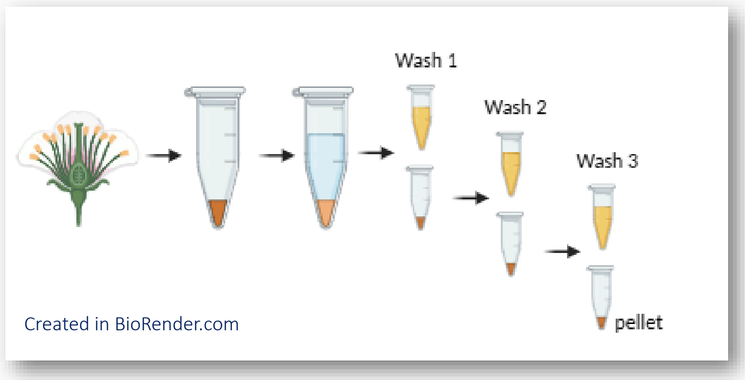

Figure 2. Schematic representation of the pollen preparation before TRNA extraction

Specific RT-PCR (Matousek et al., 2003) was performed on the TRNA extracted from the 3 washes (W1, W2, W3) and from the pellet (P). A positive band of the expected size was obtained both from the washes and from the pellet (Fig. 3), suggesting that the viroid contaminated the surface of the granules.

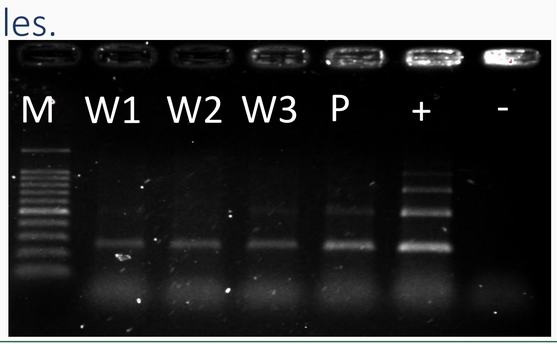

Figure 3. RT-PCR amplification of HLVd on pollen. M = 100 bp marker; + = positive control; - = negative control.

Transmission

Female parental plants (cv. Chinook and Comet) that were pollinated with naturally infected pollen were collected along with all the F1 plant generated (440), as shown in Fig. 4.

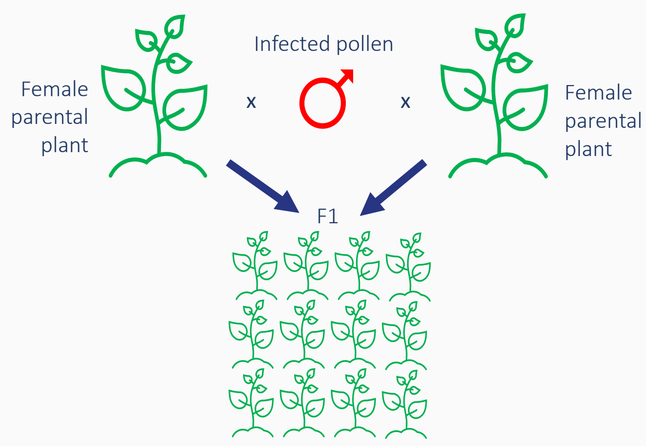

Figure 4. Schematic representation of the plants tested in this study

TRNA was extracted starting from 1 g of hop leaves and using GH+ buffer and RNeasy Plant mini kit (Qiagen). RT-PCR for detection and identification of HLVd was performed employing specific primer pairs (Matousek et al., 2003). The test results are reported in Table 1.

Sample origin	cv.	sex	no. Infected/ no. tested
parental plant	Comet	female	**1/1**
parental plant	Chinook	female	**1/1**
F1	Chinook	female	0/20
F1	Comet	male	0/20
F1	Comet	female	0/270
F1	Comet	unidentifiable	0/130

Table 1. Results of HLVd testing on parental and F1 plants from pollination with HLVd-contaminated pollen

Both the female parental plants (cv. Chinook and Comet) tested positive to the presence of HLVd. Instead, none of the 440 plants collected from the F1 generation resulted infected by HLVd. These results were in line with with previous findings, indicating that HLVd should only be able to contaminate the surface of pollen granules.

In conclusion, the transmission of HLVd seems to involve at least only pollen and not seeds. These findings agreed with those previously reported in hop (Matoušek in 2000) but disagreed with those reported in hemp (Atallah et al., 2023 and Punja et al. 2025). Specific transmission trials involving healthy female plants and HLVd-infected male plants acting as pollen donors, performed under controlled growing conditions, are ongoing. Considering the widespread presence of HLVd in the hop germplasm a clear evaluation of the role played by pollen in the transmission of HLVd, should be very useful to improve the monitoring of the phytosanitary status of the hop plants.

References

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