Disease detection in hop rhizomes and plantlets for clean yard establishment in Wisconsin

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Introduction

- Sustainable hop (*Humulus lupulus*) production in Wisconsin is hindered by pathogens introduced via propagative plant material.
- Growers are interested in screening for several primary pathogens in an effort to improve disease management.
- Multiple testing procedures were used to detect 6 pathogens: *Pseudoperonospora humuli*, the cause of hop downy mildew; *Podosphaera macularis*, the cause of powdery mildew; Apple mosaic virus (ApMV), Arabis mosaic virus (ArMV), Cucumber mosaic virus (CMV), and Carlaviruses (including American hop latent virus, hop latent virus, hop mosaic virus).[1,3]
- Our goals were to 1) determine the feasibility and cost associated with disease assays, and 2) survey diseases in hop propagative material from multiple sources in Wisconsin.

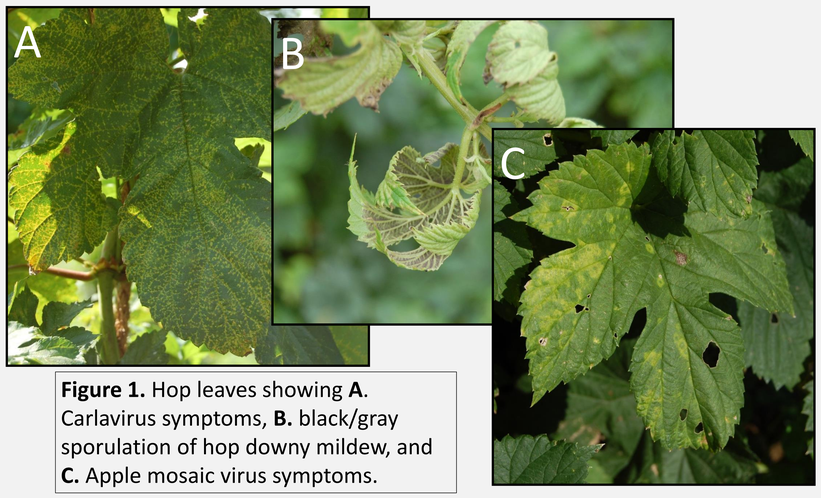

Figure 1. Hop leaves showing **A.** Carlavirus symptoms, **B.** black/gray sporulation of hop downy mildew, and **C.** Apple mosaic virus symptoms.

Total Disease Detections

Source	# Samples Received	Number of samples positive for specific disease (% of total samples received)					
		P. humuli	*P. macularis*	ApMV	ArMV	CMV	Carlavirus
1	8	0 (0%)	0 (0%)	1 (13%)	0 (0%)	0 (0%)	4 (50%)
2	8	3 (38%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (13%)
3	13	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (15%)
Total	**29**	**3 (10.34%)**	**0 (0%)**	**1 (3.45%)**	**0 (0%)**	**0 (0%)**	**7 (10.34%)**

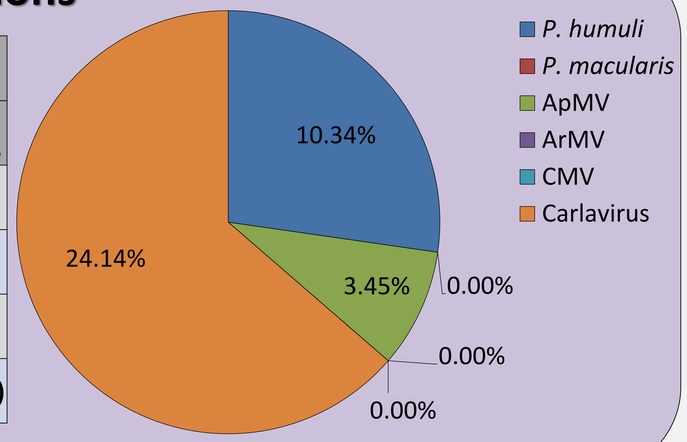

Figure 2. Disease testing results from 10 Dec 2014 to 9 Mar 2015.

Figure 3. Representative hop sample as received from grower.

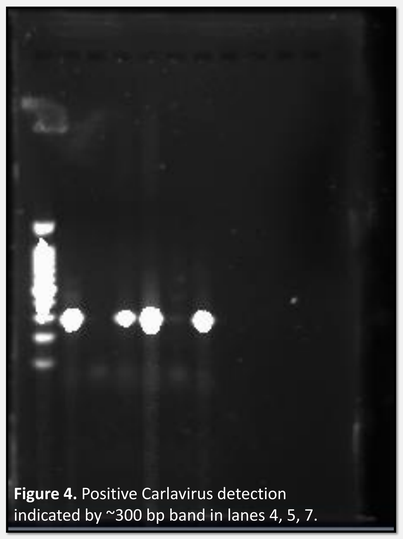

Figure 4. Positive Carlavirus detection indicated by ~300 bp band in lanes 4, 5, 7.

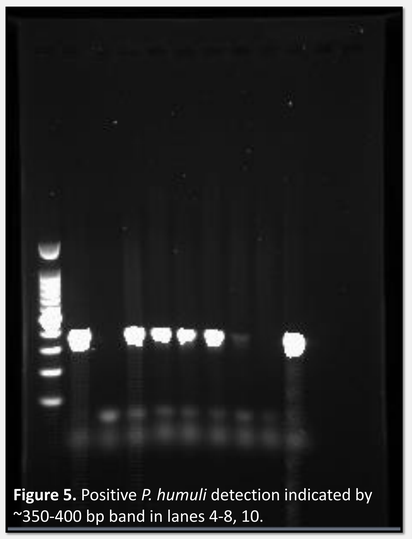

Figure 5. Positive *P. humuli* detection indicated by ~350-400 bp band in lanes 4-8, 10.

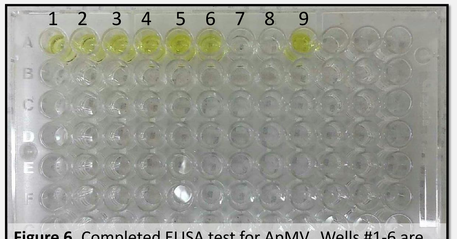

Figure 6. Completed ELISA test for ApMV. Wells #1-6 are positive, well #7 is negative, wells 8 & 9 are negative and positive controls, respectively.

Figure 7. Agdia Immunostrip test for ArMV.

Figure 8. DNA extraction for hop downy mildew PCR-based test.

Materials & Methods

- All propagative material (leaves and stems of plantlets) was asymptomatic upon receipt, and was maintained at 4°C until processed.
- Immunostrip® tests were used for the detection of Arabis mosaic and Cucumber mosaic viruses.
- Apple mosaic virus was tested by ELISA.
- Carlaviruses were detected using RT-PCR with Carlavirus-specific primers.
- *P. humuli* was detected in total genomic DNA from asymptomatic plants with specific primers.[2]
- Plant tissues were incubated on water agar amended with antibiotics and examined microscopically for signs of *P. humuli* & *P. macularis*.

Conclusions

- The pathogens *P. humuli*, ApMV, and Carlavirus were detected in asymptomatic plantlets, reinforcing the need for continued and more extensive disease screening of hop propagative material.
- The disease panel was repeatable and could be completed within a reasonable time period (~8 days).
- No rhizomes were sent by collaborators. In the future, this panel will have to be tested for the ability to accurately detect pathogens in below-ground material.
- Future goals include adding two viroid tests to the panel; hop latent viroid and hop stunt viroid have been reported in the United States but few facilities are capable of testing for these pathogens.[1,3]

References

1) Johnson, D.A., Engelhard, B., Gent, D.H. 2009. Downy Mildew. *Compendium of Hop Diseases and Pests*. APS Press. 18-22.
2) Patzak, J. 2005. PCR detection of *Pseudoperonospora humuli* and *Podosphaera macularis* in *Humulus lupulus*. *Plant Protect. Sci*. 41:141-149.
3) Pethybridge, S.J., Hay, F.S., Barbara, D.J., Eastwell, K.C., Wilson, C.R. 2008. Viruses and Viroids Infecting Hop: Significance, Epidemiology, and Management. *Plant Dis.* 92:324-338.

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