

Hops Feature Edition

Hops joined NCPN in 2010, and the Clean Plant Center Northwest (CPCNW) in Prosser Washington was designated as the headquarters for NCPN-Hops. CPCNW, a program within Washington State University, conducts virus diagnostics, therapeutics, research, and distributes virus-tested planting material to the hop growing industry. CPCNW maintains a collection of virus-tested hop cultivars in a protected environment and tests the foundation plants regularly to ensure they are free of economically harmful viruses and viroids.

The Most Commonly Targeted Pathogens

In keeping with the mission of the National Clean Plant Network, NCPN-Hops focuses on viruses that are most likely to cause negative economic impact to the U.S. hop growing industry.

Four viruses and one viroid are commonly found to infect hops (Humulus lupulus) in the U.S.: Apple mosaic virus (ApMV), Hop mosaic virus (HMV), Hop latent virus (HLV), American hop latent virus (AHLV), Hop stunt viroid (HSVd). Of these, the three Carlavirus species, HMV, HLV and AHLV, are the most abundant, being found in 40-50% of hops grown in the Yakima valley, the largest hop producing area in the country, while HSVd and ApMV are found at lower incidence.

Each of these pathogens causes different disease symptoms on sensitive cultivars, including stunting and reduced cone production, or yellowing, flecking or mosaic/mottle symptoms associated with reduced vigor. One of the most significant effects that these pathogens cause is the reduction or alteration of alpha- and beta-acid content of infected cones, which in turn can negatively impact flavor during the brewing process.



Apple mosaic virus (ApMV) (Photo credit: David Gent)



Hop mosaic virus (HMV) (Photo credit: Ken Eastwell)



Hop stunt viroid (HSVd) (Photo credit: David Gent)

"We've seen first-hand the impact widespread infection by virus and viroids can have on hop production, and therefore cannot overstate the importance of NCPN's role as a critical and trusted link in the hop supply chain."

Newly Released Virus-Tested Hop Varieties

In the last year, NCPN-Hops has tested and released the following varieties to the new 2019 standard, using more sensitive assays to ensure that the plants are free of economically important and harmful hop viruses and viroids:

| Nugget | Glacier |
|-------------|----------|
| Comet | Tahoma |
| Sorachi Ace | Newport |
| Crystal | Columbia |
| Cascade | |

These plants, along with the rest of the CPCNW foundation, are maintained under contained screen house conditions to protect against reinfection by insect vectors, and grown in raised planters isolated from the soil to avoid soil-borne pathogens and insects.



NCPN-Hop Foundation collection maintained in screenhouse

Method Improvements

Over the past two years the CPCNW has been continually updating, optimizing and validating our diagnostic procedures for the improved detection of hop-infecting viruses and viroids. These improved assays have resulted in the detection of latent Carlaviruses (including Hop mosaic virus) that would have otherwise remained undetected by older assays. We have also integrated high-throughput sequencing into the diagnostic protocols to confirm the PCR results, and to search for new or novel viruses. We also updated our workflow to ensure that each variety is fully screened for pathogens at least twice, more for those found to be infected, before it is released to the public. This screening does not stop with release either, because we screen every foundation plant for the most harmful hop-infecting viruses and viroids each year before beginning distribution.

To ensure that we can remove the viruses and viroids detected in Hop germplasm, we have researched where in the plant tissues these pathogens are found. Through in-situ hybridization, we were able to see that, for example, Hop stunt viroid is found in all cells below the L1-L3 meristem, so precise excision is necessary to make a new, clean plant.

Using this methodology, we also found that most 'therapy' treatments, such as heat, cold, or darkness do not really change the pathogen distribution, only the titer. Cumulatively these findings have allowed us to optimize our virus elimination protocols, removing unnecessary steps and ensuring that we know what to do, depending on what viruses or viroids we find. This in turn, allows us to make better, cleaner plants for the U.S. Hop industry.



Meristem shoot tip is excised from the uppermost growing tip of the plant and grown in sterile media to produce virus-free plants.

Citrus bark cracking viroid

With the recent report of Citrus bark cracking viroid (CBCVd) infecting hops in Germany, it's time to review what is known about the disease it causes so that US growers can remain vigilant.

CBCVd has been associated with a range of symptoms, including leaf yellowing and curling (Figure A), premature flowering (Figure B), smaller cone size (Figure C & D), dry rot of roots (Figure E & F), stunting and dieback (Figure G), with plant death occurring 3-5 years after infection. There are no known insect vectors, so spread primarily occurs mechanically on tools via wounding and transfer of infected sap.

The viroid was originally reported in Slovenian hop yards, and has now spread to Germany. Control is achieved by removal of infected bines. Mitigation is complicated by the delayed onset of symptoms, which can take over a year after infection to express. Therefore, if CBCVd infection is detected in a plot, the current recommendation is to remove the entire field to eliminate the chance of asymptomatic plants acting as reservoirs for further spread.

To date, CBCVd has not been observed or reported in US hops. The viroid itself is present in citrus in this country, although it should be noted that there is no obvious means of transmission between citrus and hops so introduction via this route is improbable. It is more likely that CBCVd could be introduced through importation of infected propagative material, so growers are urged not to collect or import material from infected regions, and to have any material they do import tested for this viroid.



Photo credit: Mishra, A.K., Duraisamy, G.S., Matoušek, J. *et al.* Identification and characterization of microRNAs in *Humulus lupulus* using high-throughput sequencing and their response to *Citrus bark cracking viroid* (CBCVd) infection. *BMC Genomics* **17**, 919 (2016). <u>https://doi.org/10.1186/s12864-016-3271-4</u>

Potential virus and viroid transmission through hop seeds



To protect U.S. hop production, the research program run by Dr. Scott Harper (CPCNW Director), has been performing research into whether hop viruses and viroids can be transmitted by seeds. Research found that both Hop stunt viroid and Hop latent viroid were present in both the seed coat and integument, while Hop mosaic virus was found in both tissues plus the embryo. Growth experiments from infected seed lots are underway, but preliminary data suggests that both viroids are transmitted to the progeny. This is significant interest to the hop industry because there are few restrictions around the international movement of hop seeds.





889 plants or "hills" make up one acre of hops, if planted on a standard 3.5 foot by 14 foot spacing.



In the Pacific Northwest, yields average about 2,000 pounds of dried hop cones per acre on mature hop yards, or a little over two pounds per hill (yields vary depending on variety and location).



Hops are typically sold in 200pound bales.



A bale will yield between 135 - 800 barrels of beer (31 gallons each), depending on the recipe.



These facts are provided by USAHops. Visit their website at www.usahops.org

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For more information, visit the <u>NCPN-Hops website</u>



National Clean Plant Network

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