"Out of Thin Air“
Spore Trapping and Other Techniques for Studying Grapevine Trunk Disease

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Wine Grapes in British Columbia
Okanagan Valley,
Oliver BC
Fraser Valley, Abbotsford BC
Crown Gall
Caused by the Bacterium
*Agrobacterium vitis* (syn.: *Rhizobium vitis*)
Powdery Mildew
Caused by the fungus *Erysiphe necator*

Powdery mildew affects fruit causing splitting and rotting.
Sour Rot
Caused by a yeast and bacterial complex

Botrytis
Bunch Rot
Caused by the fungus *Botrytis cinerea*
Grapevine Trunk Diseases

Black Foot

Bot Canker: extending down from old pruning wounds

Eutypa dieback

Esca

vascular plugging

leaf symptom
Pathogen Biology and Spore Trapping

Information used to develop disease management recommendations:

- When are fungal spores released in the environment?
- Which environmental conditions favor spore release and infection?
- Determine high risk infection periods throughout the year
- Target chemical and/or biological control product timing and cultural practices
Glass Slide Spore Trap
I-Rod Spore Trap
Burkard Drum Spore Trap
Burkard Cyclone Air Sampler: spore trap
Fire Blight Detection and Disease Prediction

COUGARBLIGHT
WSU Fire Blight Flower Infection Risk Assessment Model

Fire Blight Cause: *Erwinia amylovora*
Fire Blight Detection and Disease Prediction
Pathogen detection by qPCR or hybridization correlated well Cougarblight

DNA array

Field Sample

Real time PCR

DNA extraction

Multiplex PCR: ribosomal & plasmid primers

PCR with pEA29-f1 / AJ76 plasmid primers

Automated quantitative analysis of results

Hybridization with DNA array membrane

Hybridization with DNA array membrane
Detection of Grape Powdery Mildew and Disease Prediction

UC Davis: Grape Mildew Predictive Model

Pathogen: *Erysiphe necator*

Powdery Mildew: DMI Fungicide Resistance

- Fungal populations resistant to demethylation-inhibiting fungicides (DMIs) is due in part to a single DNA point mutation.

- The target gene encodes the enzyme Cytochrome P-450 demethylase.

- DNA point mutation (an A to T transition at codon 136) is presumed to produce a conformational change in the protein active site.

![Active site / target binding](Image from 2n2u.com)

![Cytochrome P-450 demethylase](Image from 2n2u.com)
Primer and Probe Design for qPCR Detection Assay

- Outside primers with internal hydrolysis probe for detection of point mutation

Cytochrome P-450 sequences

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<tr>
<th>Sample Type</th>
<th>Sequence</th>
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<tr>
<td>Control seq (S)</td>
<td>CAACTCCGGTCTTTGAAGAGATGGTGTATGATTGTCGAATTCGAAA</td>
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<tr>
<td>Sample 5 (S)</td>
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<tr>
<td>Sample 4 (R)</td>
<td>CAACTCCGGTCTTTGAAGAGATGGTGTATGATTGTCGAATTCGAAA</td>
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<tr>
<td>Sample 6 (R)</td>
<td>CAACTCCGGTCTTTGAAGAGATGGTGTATGATTGTCGAATTCGAAA</td>
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Spore Trapping and qPCR Detection Assay of DMI Resistant Spore

2009 spray schedule and spore trap results

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<tr>
<td>DMI Resistant:</td>
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<td>No</td>
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- **Nova** - (DMI fungicide)
- **Sovran** - (non-DMI)
- **Kumulus DF** - (wettable sulfur)
- **Lime sulfur** – (dormant)

The length of the coloured lines corresponds to the Time period over which the sprays are effective.
Epidemiological and Diagnostic Studies of Grapevine Trunk Diseases

- Disease etiology and pathogen identification
- Biology of grapevine pathogens
- Epidemiology
- Development of molecular diagnostic and detection tools
- Development and implementation of control strategies

Disease cycle

Spore traps
Disease management and the Weather

- When are fungal spores release in the environment?
- Which environmental conditions favor spore release and infection?
- Determine high risk infection periods throughout the year
- Target chemical and/or biological control product timing and cultural practice
Spore Trap Locations in the Okanagan Valley

Vineyards of the Okanagan and Similkameen Valleys

Spore Traps Location

Burkard Spore Trap

Weather Stations are used to monitor daily environmental conditions
Weekly collection

Detection

Total genomic DNA extraction

Quantification

PCR with Species/genus specific primers

5’-ATCGAATTGCATGCGATT-3’
5’-GGTCAATCGAATTGC-3’
Botryosphaeriaceae spp. spores detection May-2014

Okanagan Falls

Precipitation (mm)

Average Daily Temperature (°C)

Day

Botryosphaeriaceae species-specific primers (Up to 6 different species)
2015 Spore Trap Data Analysed using ddPCR

Osoyoos BC
ddPCR Assay Development (*Botryosphaeria*)

- Oil is added to the PCR mix
- Droplet generator creates \(~20,000\) droplets
- Each droplet contains rxn mix and 1 DNA molecule
ddPCR Assay Development: Multiplex (Botryosphaeriaceae & Diatrypeaceae)

This is the ddPCR Multiplexed Gradient with the BOT Beta F1+R1 primers (150nM), and DIA5S 16F+89R (50nM) with Parc91 (1/40) and Parc131 (1/20,000)

If you look at the Multiplex, you can see that the lower temperatures are better.
Epidemiological and Diagnostic Studies of Grapevine Trunk Diseases

ddPCR

- Botryosphaeriaceae assay looks like it working
- Diatrypaceae assay also working well
- *Phaeomoniella* assay it looks like it working
- *Phaeoacremonium* assay it looks like it working

Spore trap sampling

- We have about 1½ years of spore trap samples collected
- We will continue trapping spores for another 2 years
Thank You

Plant Path Lab 2015

Thanks to the BCWGC for funding in collaboration with AAFC GF2 AIP funds