Agriculture and Agri-Food Canada | Agriculture et Agroalimentaire Canada

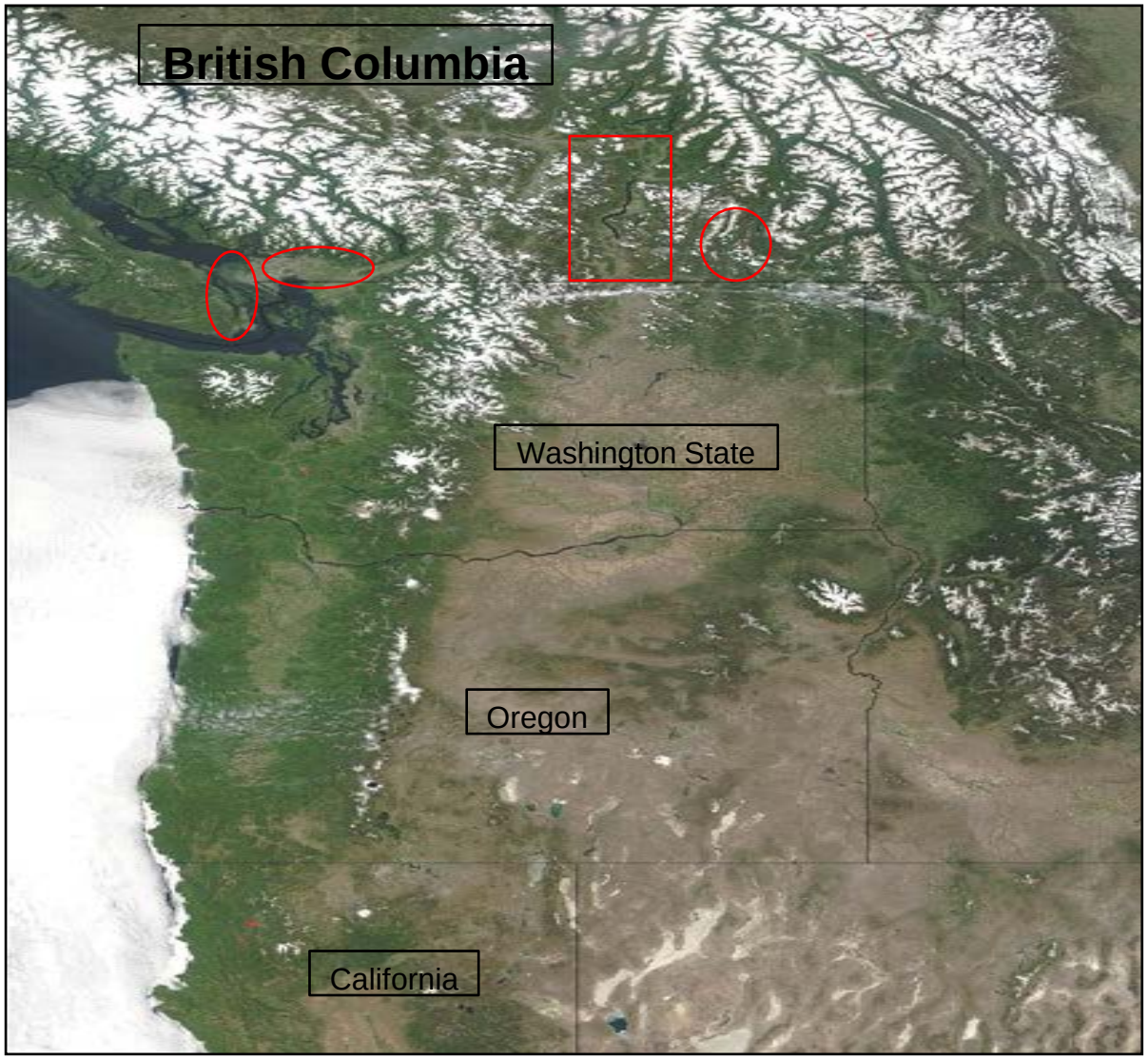
"Out of Thin Air“

Spore Trapping and Other Techniques for Studying Grapevine Trunk Disease

Dan O’Gorman: Agriculture and Agri-Food Canada, Summerland Research and Development Centre (SuRDC)

Canada

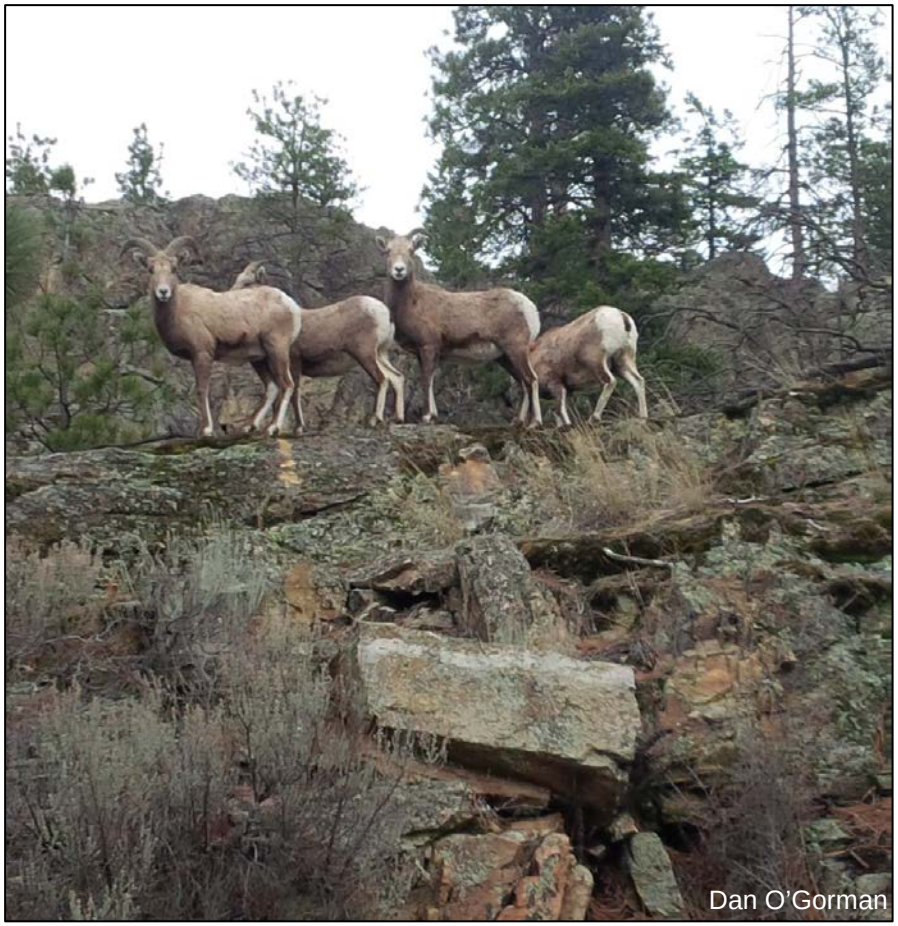
Wine Grapes in British Columbia



Pacific Agriculture Research Centre – Summerland BC

Okanagan Valley, West Kelowna BC

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*

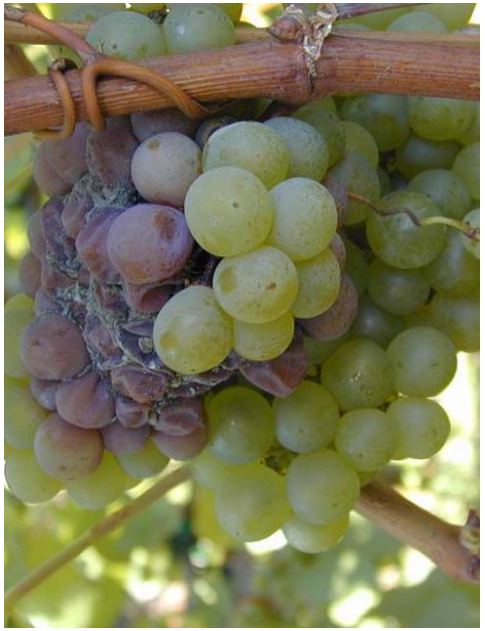
Powdery mildew affects fruit causing splitting and rotting

Sour Rot

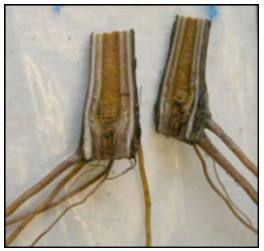
Caused by a yeast and bacterial complex

Botrytis Bunch Rot

Caused by the fungus *Botrytis cinerea*



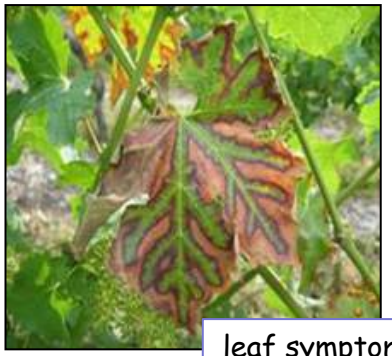
Grapevine Trunk Diseases



Black Foot

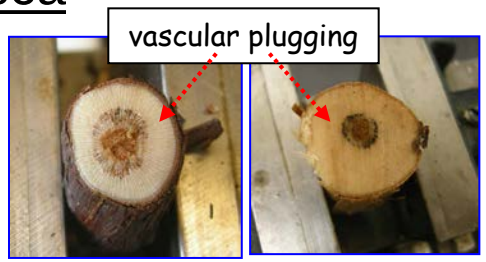
Bot Canker: extending down from old pruning wounds

Eutypa dieback

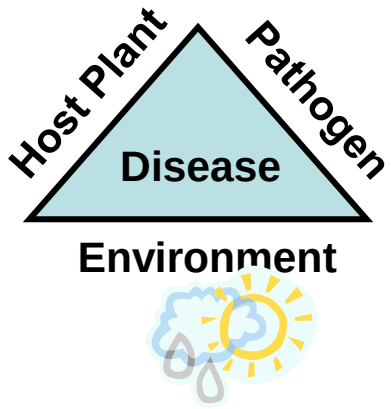


leaf symptom

Esca



Pathogen Biology and Spore Trapping

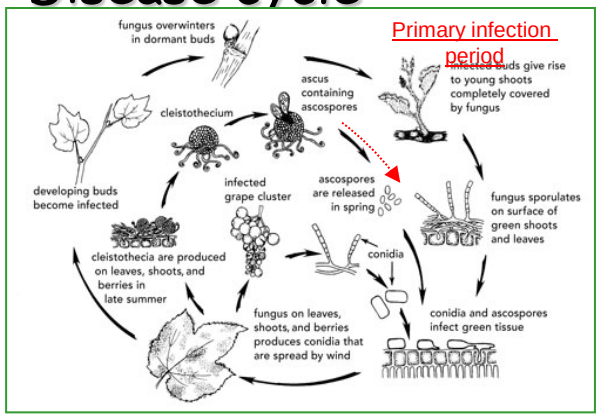


Spore traps

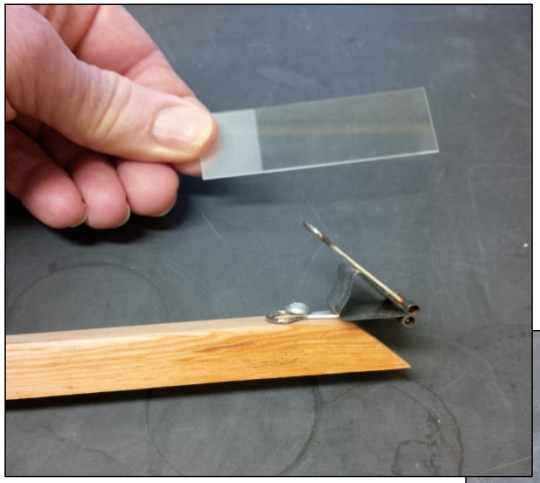
Information used to develop disease management recommendations:

- 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

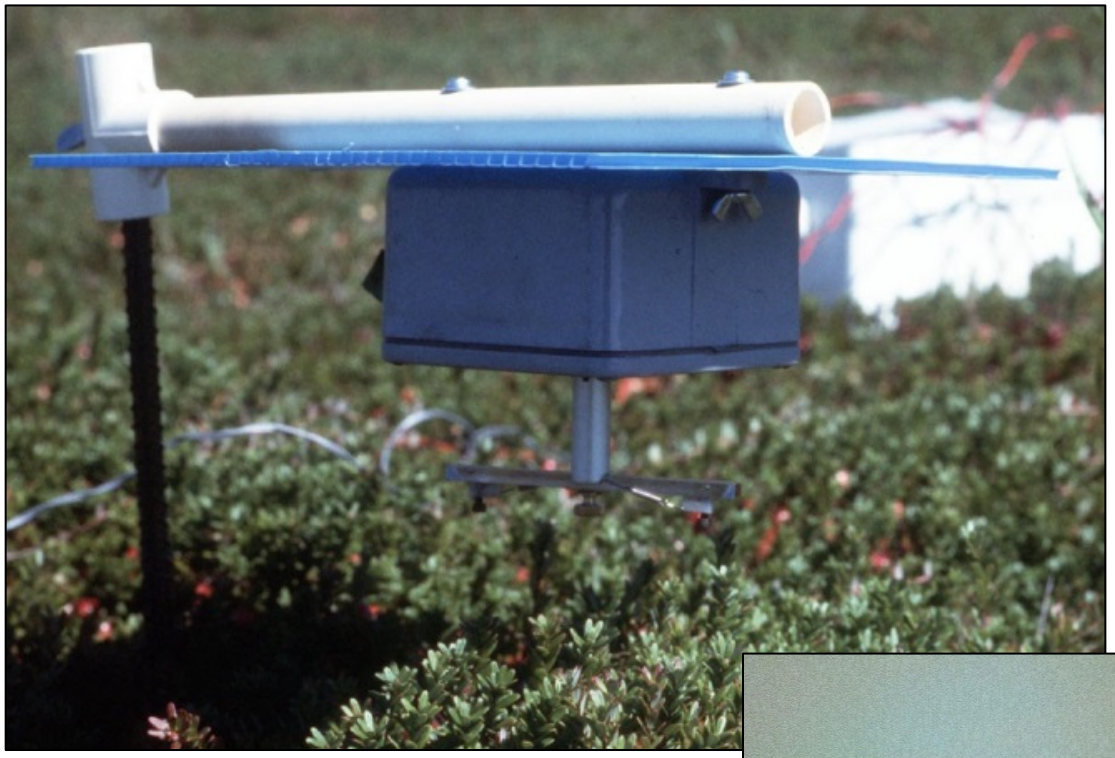
Disease cycle

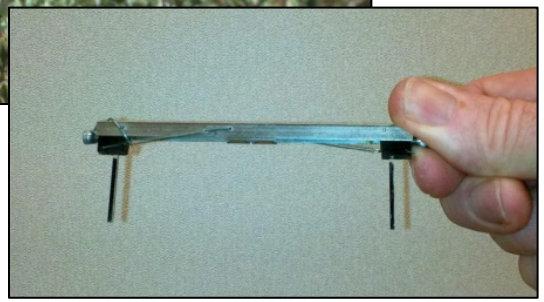


Glass Slide Spore Trap

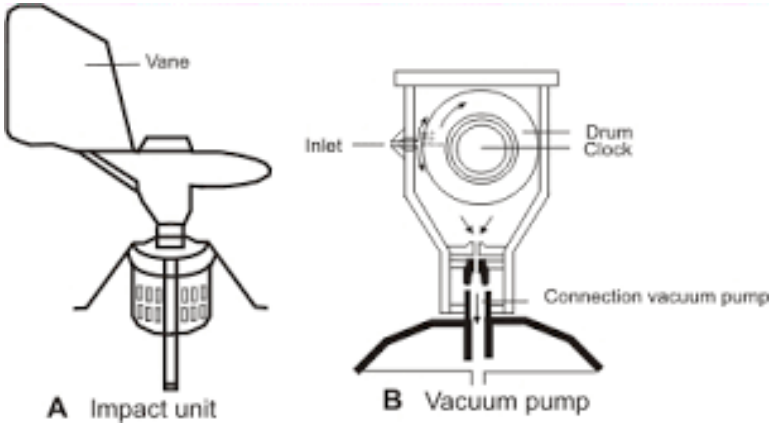


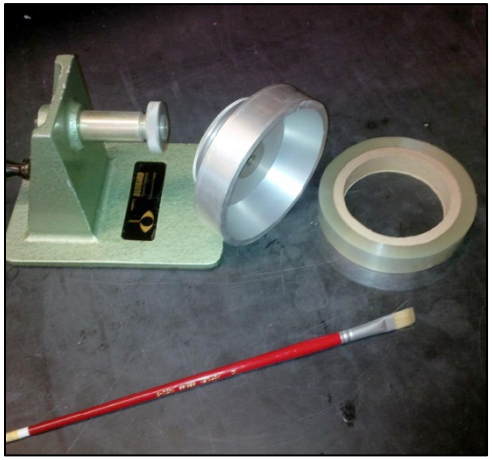
I-Rod Spore Trap





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*

Bee hive

Sugar water

Fire Blight Detection and Disease Prediction

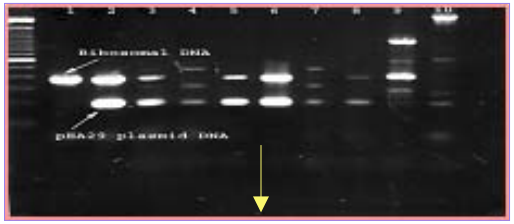
Pathogen detection by qPCR or hybridization correlated well Cougarblight

Field Sample

DNA extraction

DNA array

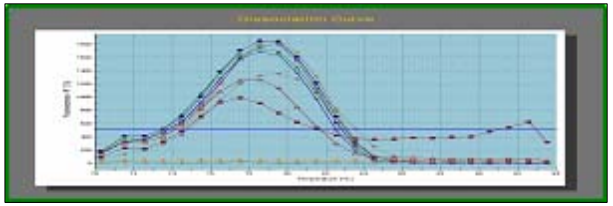
Multiplex PCR: ribosomal & plasmid primers



Hybridization with DNA array membrane

Real time PCR

PCR with pEA29-f1 / AJ76 plasmid primers

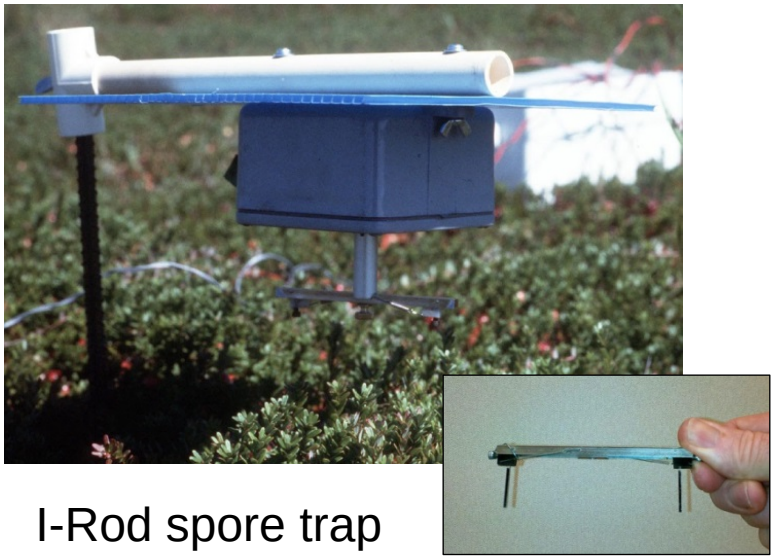


Automated quantitative analysis of results

Detection of Grape Powdery Mildew and Disease Prediction

UC Davis: Grape Mildew Predictive Model

Pathogen: *Erysiphe necator*



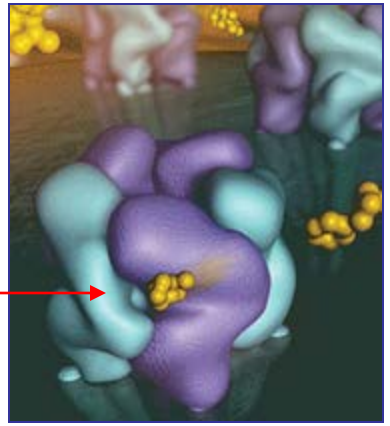
I-Rod spore trap

Model developer and citation. Thomas, C. S., Gubler, W. D., and Leavitt, G. 1994. Field testing of a powdery mildew disease forecast model on grapes in California. Phytopathology 84:1070 (abstr.).

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

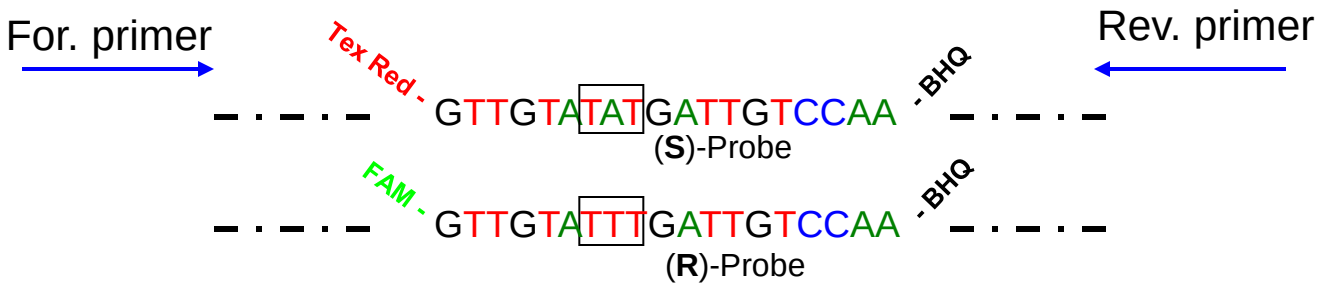


Cytochrome P-450 demethylase

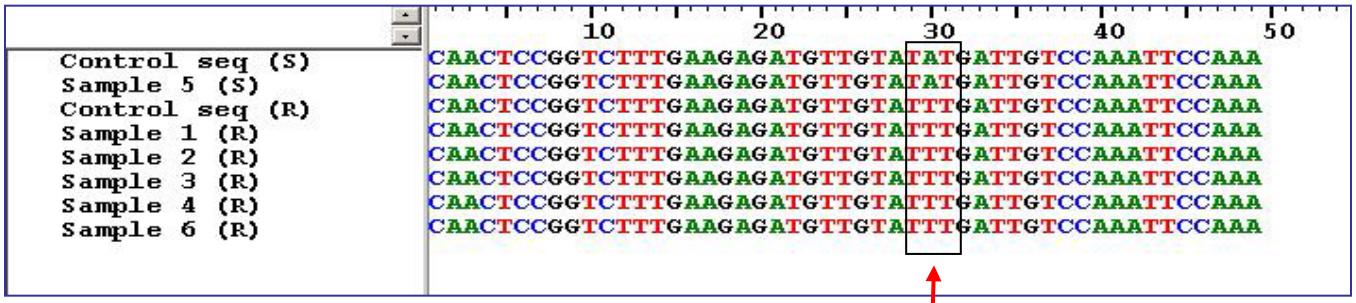
Image from 2n2u.com

Primer and Probe Design for qPCR Detection Assay

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



Cytochrome P-450 sequences



Point mutation

Spore Trapping and qPCR Detection Assay of DMI Resistant Spore

I-Rod Spore trap

2009 spray schedule and spore trap results

Placement Date:	April 9/09	May 5/09	May 9/09	May 15/09	May 22/09	May 28/09	June 5/09	June 19/09	June 26/09	July 8/09	July 20/09	July 24/09	Aug 4/09	Aug 14/09	Aug 21/09	Sept 3/09	Sept 11/09	Sept 18/09	Oct 2/09	Oct 15/09
DMI Resistant:	Yes	No	No	No	No	Yes	Yes	No	No	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	No

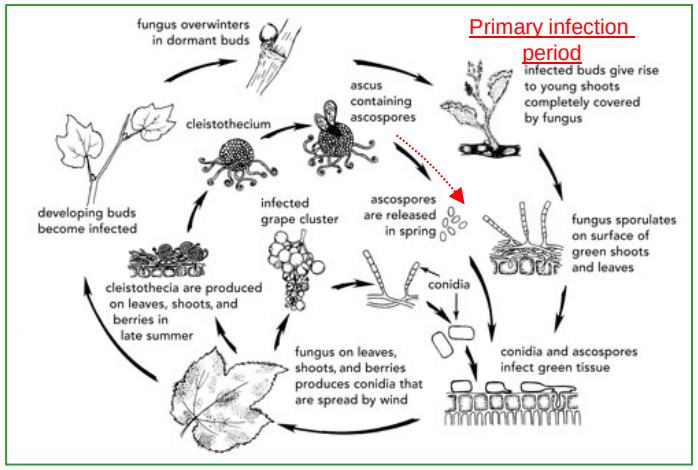
- **Nova** - (DMI fungicide)
- **Sovran** - (non-DMI)
- **Kumulus DF -** (wettable sulfur)
- **Lime sulfur –** (dormant)

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

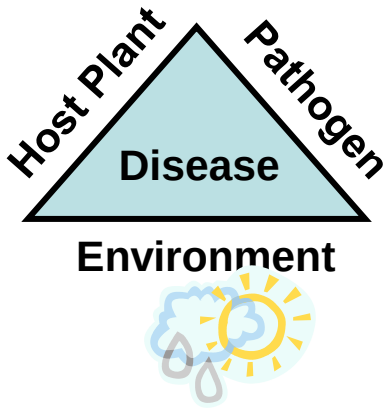
- Disease etiology and pathogen identification
- Biology of grapevine pathogens
- Epidemiology
- Development of molecular diagnostic and detection tools
- Development and implementation of control strategies

Disease cycle

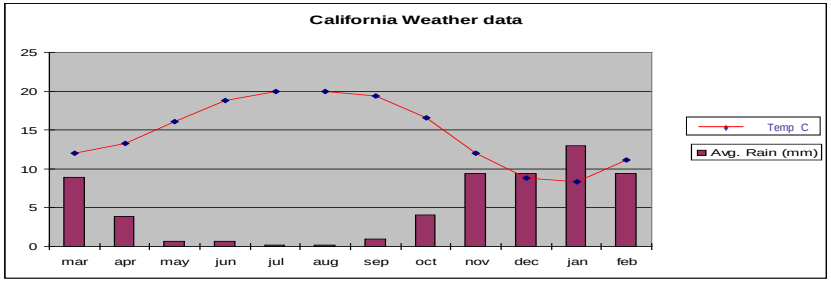


Spore traps

Disease management and the Weather

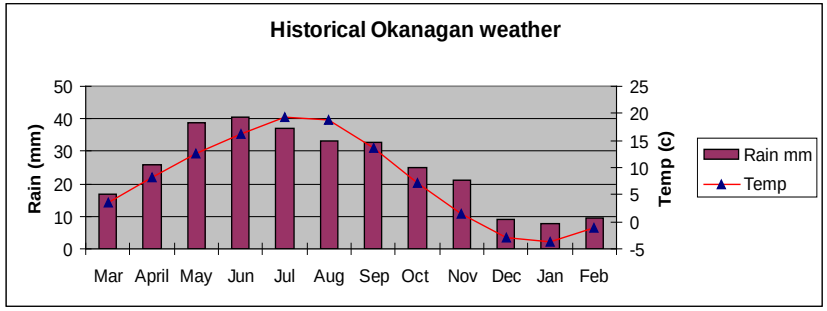


California weather – Napa Valley

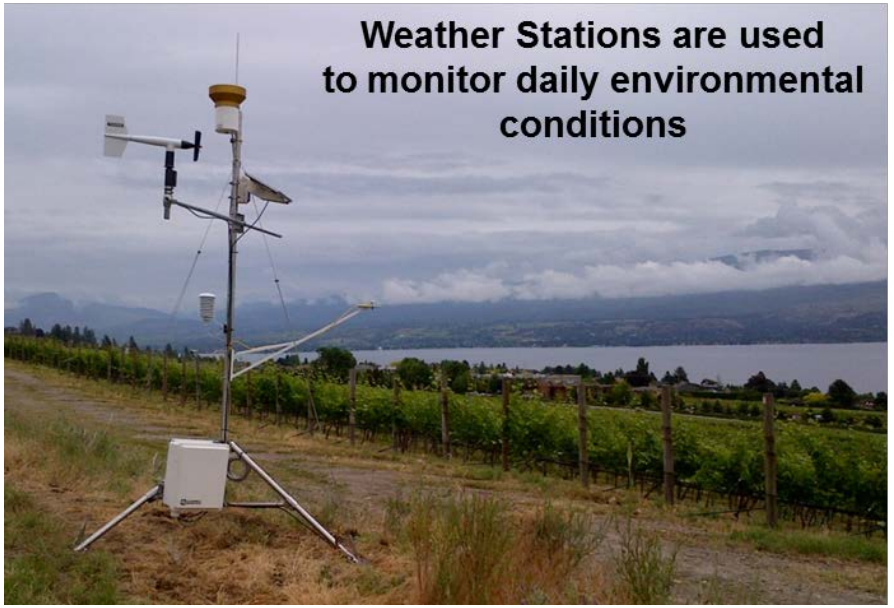


- When are fungal spores release in the environment?
- Which environmental conditions favor spore release and infection?
- Determine high risk infection periods throughout the year
- Target chemical and/or biological control product timing and cultural practice

Okanagan Valley weather



Spore Trap Locations in the Okanagan Valley



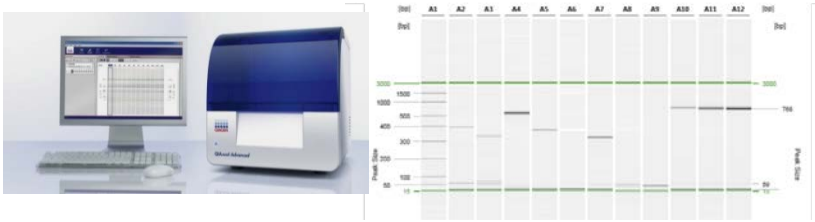
Weekly collection

Total genomic DNA extraction

PCR with Species/genus specific primers

5’-ATCGAATTGCATGCGATT-3’
5’-GGTCAATCGAATTGCG-3’

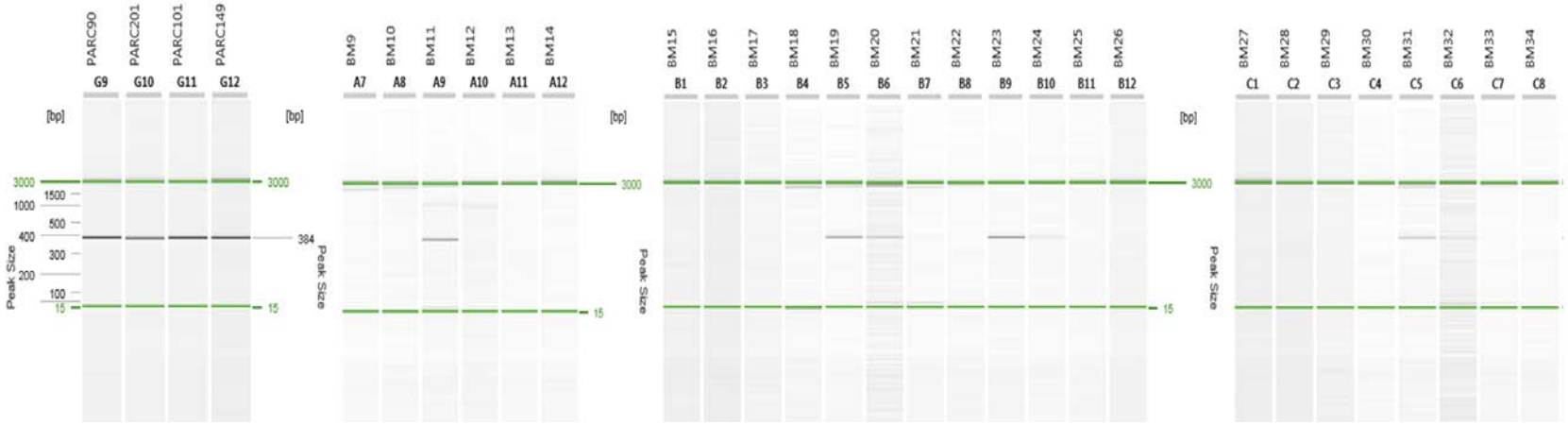
Detection



Quantification

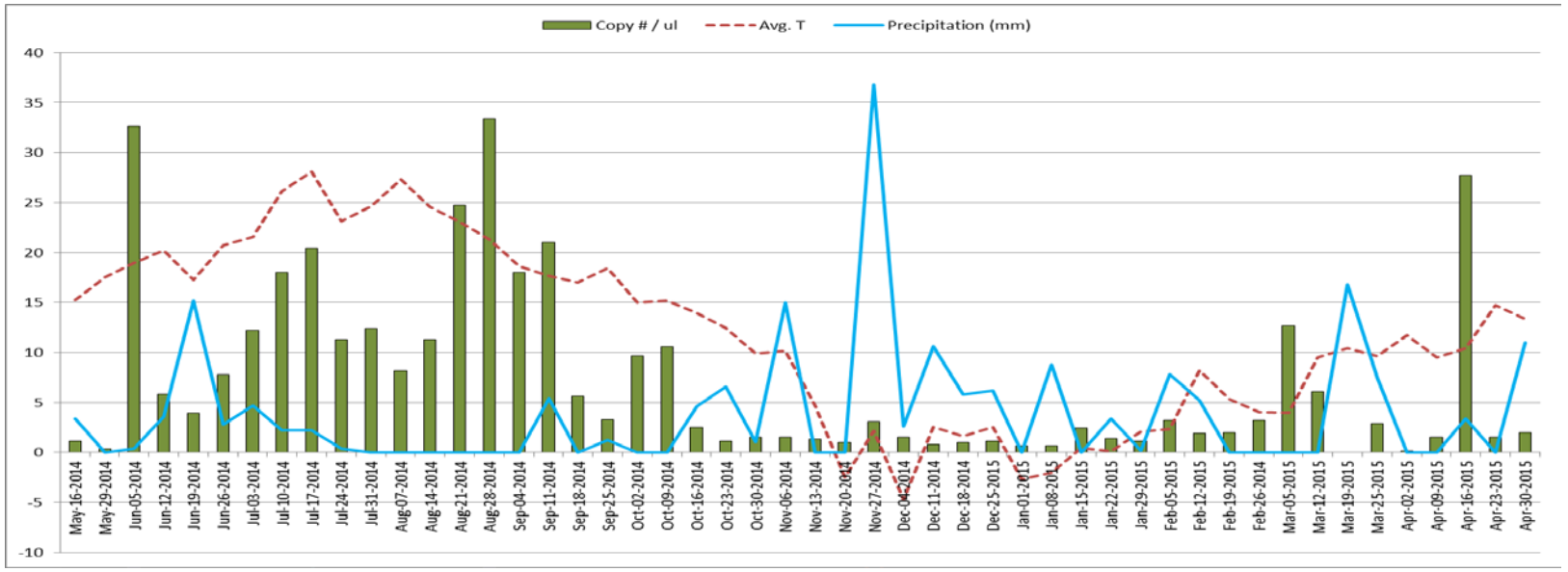
Botryosphaeriaceae spp. spores detection May-2014

Botryosphaeriaceae species-specific primers (Up to 6 different species)

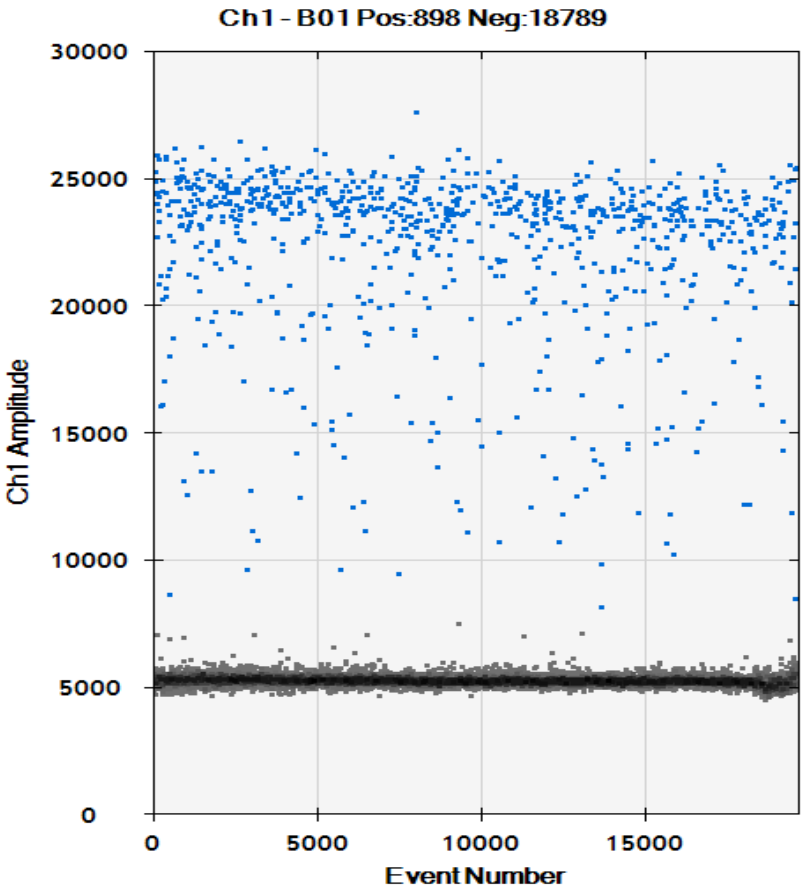


2015 Spore Trap Data Analysed using ddPCR

Osoyoos BC

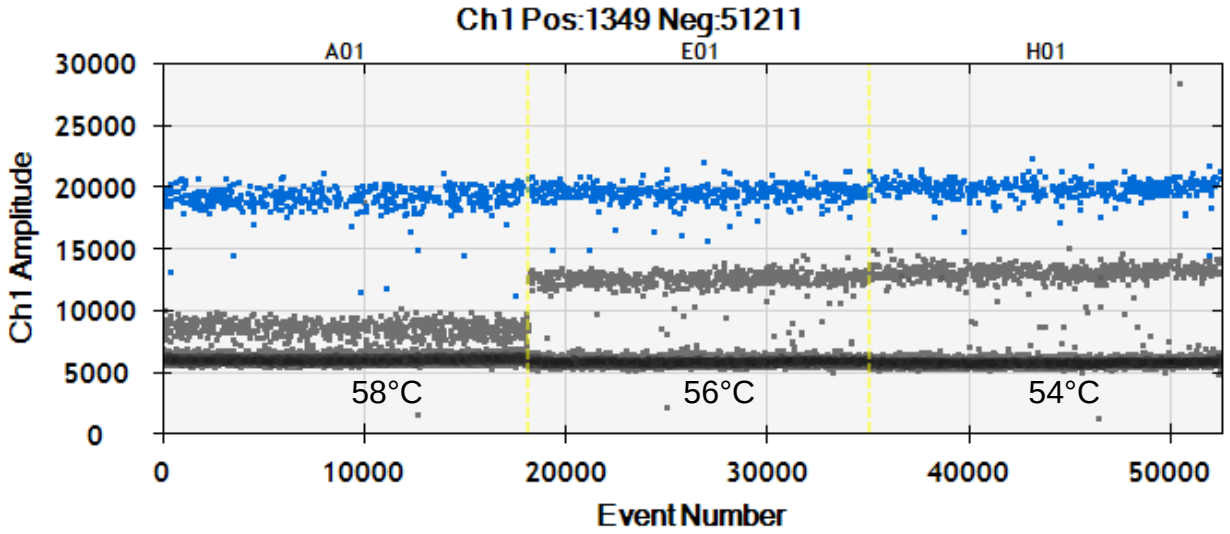


ddPCR Assay Development (*Botryosphaeria*)



- 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 & Diatrypaceae)



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

multiplex

- *Phaeomoniella* assay it looks like it working
- *Phaeoacremonium* assay it looks like it working

multiplex

Spore trap sampling

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

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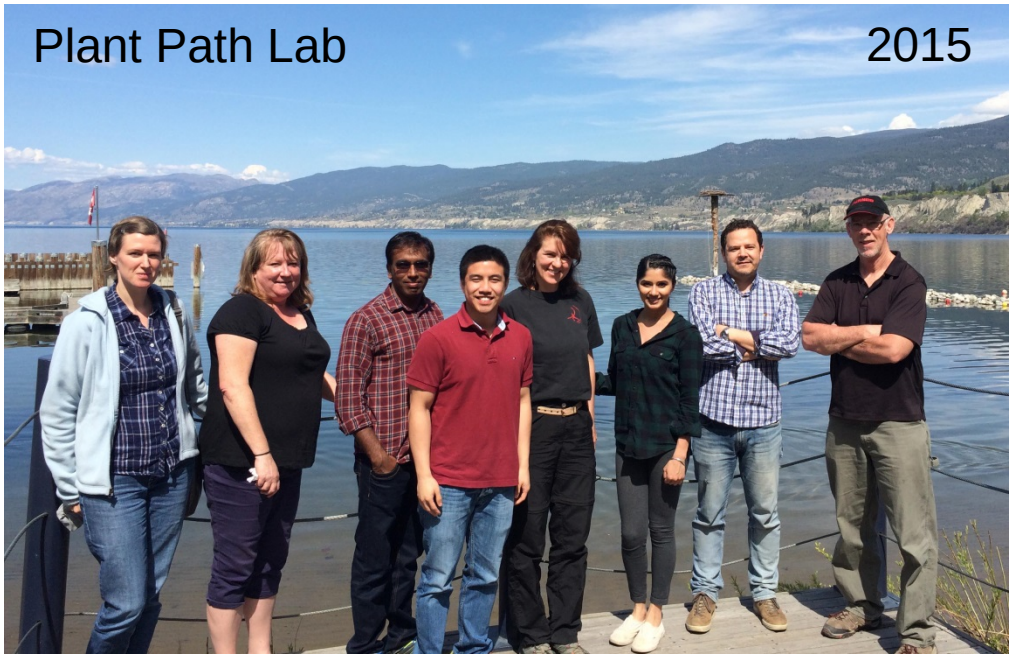
Thank You



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Canada