

# Disease Research Report

## *Management of Powdery Mildew and Bunch Rot in British Columbia 2003-2004*

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### **Field Work**

In the 2003 and 2004 growing season work concentrated on the collection of samples (clusters from pre-bloom to harvest) in each vineyard location to determine infection timing of *Botrytis cinerea*. Grape tissue samples were placed in -20°C for later analysis. I-rods were collected weekly or biweekly from Aerobiology spore traps placed in Okanagan Falls and Summerland. I-rods are clear polystyrene rods which rest in a sampling head of the spore trap. When power is on, the motor spins the sampling head and centrifugal force caused the rods to extend downwards at a 90° angle. Airborne particles are trapped on the sticky surface of the rod as it spins. Rods were collected and stored dry until molecular work to determine primary infection periods was completed. Once primary infection timing is determined the parameters used in the present disease forecasting software will be adjusted in the model (Thomas, Gubler, and Leavitt 1994; Weber, Gubler, and Derr 1996) by the company who supplies the software (Spectrum Technologies, Plainfield, Illinois).

Weather stations were placed in 7 vineyards throughout the valley so weather records could continue to be collected and compared. Stations consisted of a Watchdog 450 data logger (Spectrum Technologies, Plainfield, Illinois) with internal sensors for relative humidity and temperature. An external sensor for leaf wetness was added for use with the mildew model. The average temperature remained consistent within the vineyards for 2003 and 2004 (Fig. 1). All locations saw an increase in %Relative Humidity in 2004 (Fig. 2).

Vineyards in 2003 saw an increase in powdery mildew infections exceeding previous years. Even vineyards in the southern Okanagan valley with usual low amounts of powdery mildew were affected. Mildew appeared to get off to an extremely early start in 2003. Colonies were seen in Summerland as early as mid June. Colonies were not observed until the first week in July in 2002. If this occurred in other vineyards early mildew sprays may have been missed allowing disease spores to quickly spread and the disease to increase quickly. The year 2003 was a dry hot year which is the desired conditions for colony growth and spread of powdery mildew. The mean conidial index in June 2003 showed that in all vineyards (except Oliver), that powdery mildew would reproduce every 5 days (Fig. 3). Increased infections in 2003 allowed for the build up of over wintering fruiting bodies (cleistothecia) making sprays for primary infection in 2004 of utmost importance.

### **Laboratory Work**

In the spring of 2003, two sources of powdery mildew were collected: mycelium and spores from greenhouse plants and fruiting bodies from the vineyard at PARC. Samples were placed in extraction tubes and DNA was extracted from four samples for purification of ribosomal DNA (rDNA), amplification of the internal transcribed spacer (ITS) region, and sequencing. ITS gene sequences were compared using the National Centre for Biotechnology Information (NCBI) BLAST search. The BLAST search is used to compare and confirm the identification of our isolates. The sequences of *Uncinular necator* were compared to approximately 40 other sequences of various mildews. From these sequences,

two unique regions were chosen, one 18 base pair forward primer and one 19 base pair reverse primer

Once the primers were received from the company contracted to make them, we tried different experimental protocols to improve their specificity. Different powdery mildews were used alongside grape powdery mildew that included Crabapple, Pansy, Dandelion, Pear, and Rose. More samples may be tried as they are collected (*U. necator* is an obligate parasite and can only grow on its host). A PCR regime was worked out so only powdery mildew from grape was being detected. As a bonus, the protocol used can be completed in 2.5 hours. This may give us the capability of collecting samples in the morning (for example, after a wetness period) and determining if an infection has occurred by noon.

A relatively new PCR process is being used at PARC for amplification and quantification called Quantitative PCR (QPCR or real time PCR). It gives us the capability to determine if the powdery mildew fungus is present and will also estimate the quantity. Numerous experiments were conducted on this machine with varying results. Figure 4 shows an example of an experiment conducted with a dilution series of *U. necator* DNA starting at  $1 \times 10^5$  cells/ml down to 1 cell /ml. The highest amount of DNA present should amplify first (cycle 14) and the lowest should amplify last (cycle 38) or not at all. When unknown samples are run with this standard series the approximate amount of *U. necator* in the sample can be determined.

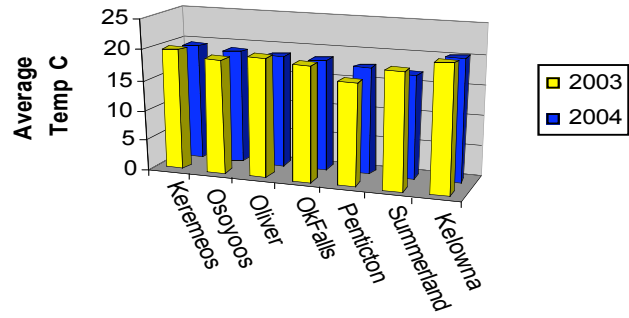
### **Future Work**

We had originally set out to design a probe to be used in a DNA array, an assay where several disease samples can be identified concurrently, for detection of grape pathogens such as *Botrytis cinerea* and *U. necator*. The probe for *B. cinerea* has already been designed and DNA for *U. Necator* has been sequenced and will be used for constructing probes. Two possible probes have already been designed but not tested. This work was put on hold because the PCR assay is working very well for the detection of mildew. It would be more efficient if only a PCR assay is used to determine the presence and approximate amount of mildew (potentially 4 hours) in comparison to the DNA array (2 days).

One disadvantage is the efficiency of the primers; work must be done so that low levels of spores can be detected on I-rods. Using hybridization to design a DNA array may be more useful in low level spore detection although the results take longer.

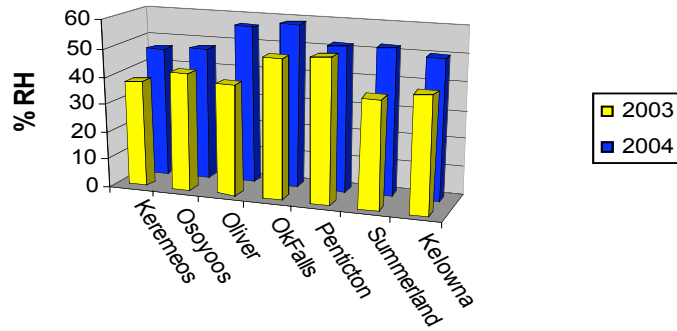
Field and laboratory work will continue in the 2005 growing season.

**Average Temp. 2003-2004**



**Fig. 1**

**% Relative Humidity 2003-2004**



**Fig. 2**

Mean Conidial Index 2003-2004

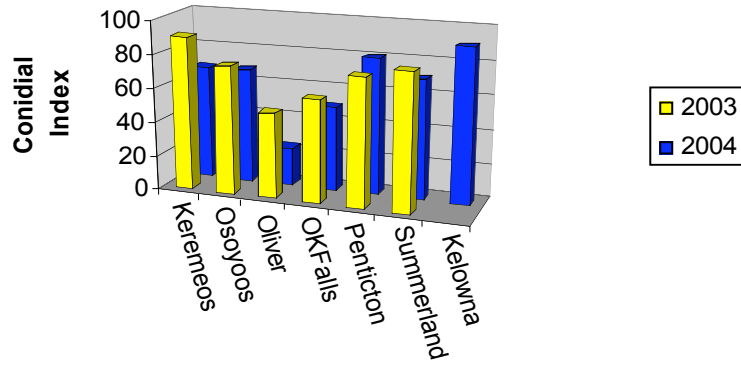


Fig. 3

Fig. 4

