Objectives and Rationale

To validate efficacy of fungicides and best application methods, it is important to determine fungal growth after treatment. qPCR has made it possible to accurately and sensitively quantify *in planta* fungal levels of selected pathogens. The aim of this study is to determine the efficacy of fungicides based on *Botrytis cinerea* growth after treatment using qPCR.

Methods

B. cinerea qPCR primer/probe set optimisation was carried out by CenGen. Preliminary trials (test fungicide interference, identify most ideal isolation sites and incubation times) were done prior to the fungicide efficacy trial. Leaves harvested from orchards were surface sterilised, inoculated (5 x 105 conidia/ml) and treated with fungicides (1X and 0.1X dose rates of fluopyram and pyrimethanil) after 3h incubation. Leaves were sampled 2h, 48h and 92h after treatment and stored at -18 °C. Samples still need to be sent to CenGen for DNA extraction and qPCR.

Key Results

When tested on grapevine leaves, the presence of fungicides did not interfere with the DNA extraction process. The *B. cinerea* primer/probe set was optimised for simplex qPCR. In addition, a host control (*Pyrus* primer/probe set) was identified, designed and obtained for normalization of *B. cinerea* qPCR data points to expand the work to pear. Optimisation of the *Pyrus* primer/probe set and duplexing with the *B. cinerea* primer/probe set still need to be done. Part of the samples for the remainder of the trials (including efficacy) are in storage while others have been delivered for processing.

Conclusion and Discussion

The *B. cinerea* qPCR primer/probe set showed acceptable performance (R2 and primer efficiency values.) in a serial dilution test analysis using *B. cinerea* DNA only. Samples will be submitted CenGen for qPCR quantification of *B. cinerea* once all primer/probe sets (including duplex reactions with both fungal and plant qPCR reagents) and preliminary trial setups have been optimised.