



Horticultural
Development
Company

Grower summary

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Detection and quantification of *Verticillium dahliae* and *V. albo-atrum* in soils to determine the risk of verticillium wilt in strawberries

Annual Report 2010

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Headline

A quantitative molecular (QPCR) test has been developed for *Verticillium dahliae*, the cause of strawberry wilt, which is able to detect and quantify inoculum of the pathogen in soil within 24 hours.

Background and expected deliverables

The current method for detecting and quantifying *V. dahliae* in soils is relatively costly and takes 6-8 weeks from sample receipt to reporting. This method relies on wet sieving soil and plating onto culture medium. Colonies that resemble *V. dahliae* growing from the resting structures, microsclerotia, are counted. These counts are used to provide information on risk of wilt and have allowed growers to assess a soil's suitability for strawberry production or the need to fumigate before establishment. However, this method of testing is prone to error, partly because other non-pathogenic verticillium organisms may also be enumerated. The proposed molecular QPCR test, will quantify the amount of target pathogen DNA in a few days, for around half the price of the conventional test. Additionally, the molecular test is capable of detecting *V. albo-atrum*, which the conventional test is unable to do. The project seeks to determine the relationship between soil levels of *V. dahliae* and *V. albo-atrum*, as measured by QPCR, and incidence of verticillium wilt in strawberries grown in the field. The ultimate aim is to provide a rapid, reliable commercial test to growers.

Summary of the project and main conclusions

Molecular test development

Quantitative PCR assays were designed by Fera to detect and quantify *Verticillium dahliae* and *V. albo-atrum* in soil. The assays have so far proved to be specific and have not reacted with non-target micro-organisms tested to date.

*Small-scale pot trial to establish detection of *V. dahliae* and *V. albo-atrum* in soil*

A pot trial was set up to determine the accuracy and sensitivity of the molecular test. Microsclerotia of *V. dahliae* were added to soil to provide a range of levels from 1 to 36 microsclerotia/g. The QPCR test was able to accurately detect the amount of microsclerotia in soil within the range tested. The assay detected down to 1 microsclerotia/g soil but it is likely that the PCR test will detect below this level.

Site selection for field experiments

Thirteen field soils were tested by QPCR (at Fera) and by conventional wet sieving (Harris) method (at ADAS). From these, five were selected to provide a range of inoculum levels. During the 2010 growing season, trials will be set up on these selected fields. Three varieties with differing susceptibilities to verticillium (Elsanta, very susceptible; Symphony, susceptible; and Florence, moderately resistant) will be planted at each site. There was reasonable agreement between the results from the conventional and molecular tests (Figure 1). In seven of the thirteen samples, there was complete agreement. However, in five fields, the Harris method detected microsclerotia at levels sufficient to cause wilt, whereas the QPCR test did not detect any *V. dahliae*. In one field the QPCR test detected *V. dahliae* at levels

equivalent to above 5 microsclerotia/g soil but the Harris method detected less than 0.5 microsclerotia/g soil. In addition, the QPCR test detected *V. albo-atrum* at two sites.

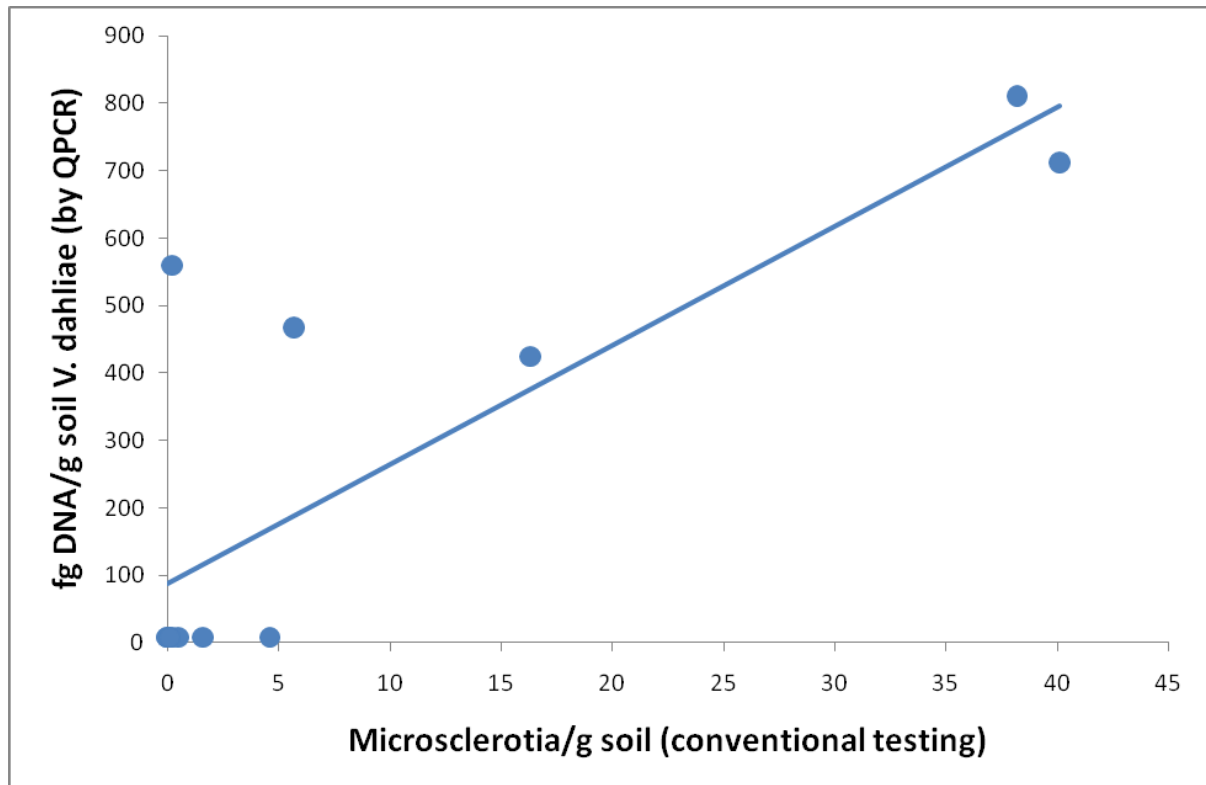


Figure 1. Comparison of results from the QPCR and conventional wet sieving (Harris) tests used to quantify *Verticillium dahliae* levels in field soils.

Disagreement in detection levels using the different methods could be a result of inter-sample variation as well as method error. Work will continue in the 2010 season to test how well the results from each detection method match disease levels. The variability in verticillium levels within fields will also be investigated.

Financial benefits

If the molecular test is sufficiently sensitive and accurate to be used to predict which soils represent a risk to strawberry crops, this would provide a quick and affordable service to growers. However, the methods need to be fully tested and validated before such a service can be offered commercially.

Action points for growers

- This is the first year in a three year project. So far, molecular tests have been designed that detect and quantify the strawberry wilt pathogens, *Verticillium dahliae* and *V. albo-atrum* in soil.
- It is anticipated that the results from this project will be used to refine and develop the molecular tests into a commercial service in the future.

- At present, continue to have soils tested for verticillium levels by laboratories offering the wet sieving (Harris) method.