New Project Summary Report for SF 097a: Identifying strawberry verticillium wilt risk

Project Number	46097100	
Title	Utilising molecular quantification of Verticillium dahliae in soil to identify strawberry verticillium wilt risk	
Short Title	SF 097a: Identifying strawberry verticillium wilt risk	
Lead Contrator	ADAS UK Ltd	
Other Contractors	Food and Environment Research Agency (Fera)	
Start & End Dates	01 February 2013	31 January 2015
Industry Representative	Richard Stanley	Rectory Farm
Project Budget	£71,617	
AHDB Contribution	£71,617	

The Problem

V. dahliae is the primary cause of verticillium wilt, a serious disease found in UK grown strawberry crops. Widespread crop infection will result in significant yield loss. The quantification of V. dahliae soil inoculum prior to planting can be used as a tool to manage the disease. Depending on the levels found, fields and varieties can be selected to limit the risk of infection. The Harris test currently used for V. dahliae is relatively slow (8 weeks), cannot differentiate V. dahliae from V. longisporum and relies on the availability of fungal taxonomy expertise.

A quantitative PCR (QPCR) test, highly specific for V. dahliae and with a sensitivity of 1 microsclerotium/g soil, was developed in SF 97. The test can be used on soil samples of up to 50 g, which greatly increases the reliability of results. A strong positive relationship was found between number of microsclerotia added to soil and the amount of V. dahliae DNA detected. Tests on five field soils correctly identified two sites at high risk of disease; there was a moderately good correlation of soil infestation density of V. dahliae measured by QPCR and the level of verticillium wilt symptoms. The aim of the current project is to develop the test into a practical tool for disease management by (1) increasing the test sensitivity, reliability and speed of sample processing

compared with current standard techniques and (2) validating the improved test in field grown crops.

Aims and Objectives

(i) Project aim(s):

The overarching aim is to improve and validate a novel rapid molecular diagnostic test for strawberry verticillium wilt in soil and reduce the potential for disease transmission to newly planted strawberry plants.

(ii) Project objective(s):

1. To improve the sensitivity of the molecular diagnostic test developed in SF 97 and quantify V. dahliae in soils down to 0.1 microsclerotium of V. dahliae/g soil.
2. To validate the test by further monitoring of the relationship between soil inoculum levels of V. dahliae (measured using QPCR) and the development of verticillium wilt in strawberry.

Approach

Objective 1: Improve sensitivity and reliability of QPCR test for V. dahliae (Fera)

i) Improving QPCR assay design

QPCR primers designed by Bilodeau et al (2012) were tested by Fera. The assay developed by Bilodeau et al (2012) gives the same level of sensitivity as the assay developed in SF 97. However, the primers are based on multi copy IGS sequences, and by combining them with the soil extraction method developed in SF97 the 'hybrid' assay was found to be approximately 100x more sensitive than the complete assay developed in SF 97. Unfortunately, the Bilodeau primers gave significant non-specific reactions to Gliocladium sp. (a common saprophyte present in soil and strawberry plants) which would give false positive reactions when testing soil. Modifications to the primers will be done to improve the specificity of the assay without impairing the improved benefits in sensitivity.

ii) Improving soil sub-sampling for the detection of Verticillium dahliae.

Using methods developed during SF 97 and in Objective 1(i) above, soils, approximately ten samples of 1 kg each, will be split into 6 aliquots of 50 g sub-samples. The level of variance and, thus the measurement of reliability, with increasing the number of replicate samples will be determined. In addition, a sub-set of soils specifically collected by ADAS as part of Objective 2 will be collected using a small amount of multiple, discrete, samples (max. 5 each site). These soils will be tested using the improved sub-sampling method to determine the variability in inoculum levels between different samples in the same field. This is important as it will inform the reliability of the sampling and testing method compared with the required sensitivity of testing down to 0.1 ms/g soil at the field level.

Objective 2: Validate test by assessment of verticillium wilt symptoms in commercial strawberry crops (ADAS and Fera)

Around 50 soil samples from fields due to be planted with strawberry will be tested for V. dahliae by the QPCR test developed in SF 97 and as improved in Objective 1. The soils will consist primarily of samples submitted to Fera or ADAS for a Harris soil verticillium test from which sub-samples will be taken and dried ready for QPCR tests. Additionally, some fields will be soil-sampled by ADAS where growers are planting in fields without conducting a soil verticillium test.

Grower information provided by Fera's Plant Clinic, ADAS's Pest Evaluation Services and ADAS Soft Fruit consultants for this project will be used only for the purposes of this project. Identification of individual growers who have provided soil samples and/or allowed their crops to be monitored for Verticillium wilt will not be disclosed in any report, project publicity or by other means.

Crops will be assessed by ADAS staff for verticillium wilt in their first (2013) and second (2014) year of cropping to determine whether or not the disease is present, and the level of the disease using a simple index (e.g. <1%; 1-5%, 6-20%; 21-50% 51-100%). It is considered important to monitor fields for two cropping seasons as levels of verticillium wilt can be much greater in the second year (see SF 97 results). Where there is doubt about the cause of wilting, samples will be examined for infection by V. dahliae.

It is likely that the traditional V. dahliae soil test results (as ms/g soil) will be available for many of the soils sampled as it is planned that the current wilt test will be the source of many of the soil samples for QPCR assay. It will thus be possible to relate verticillium wilt incidence to both agar plate results and the new QPCR test results, allowing a comparison of the accuracy of the two tests in predicting verticillium wilt for strawberry.

Some potential cropping factors (e.g. variety, soil type) associated with infection and development of verticillium wilt in each crop will be collected. The results of soil tests on V. dahliae infestation density determined by QPCR, incidence of verticillium wilt, variety and soil type will be maintained in an excel spreadsheet format.

This validation will provide valuable feedback on how useful the test is proving in practice. Once sufficient data are available, results will be examined to investigate: (i) the relationship of QPCR results and the incidence of verticillium wilt symptoms in different varieties (i.e. to set preliminary QPCR soil threshold levels for key varieties) (ii) reliability of the test (i.e. are there any unexplained occurrences in verticillium wilt in crops where a pre-planting soil test was negative; (iii) effect of soil type, planting period or other categorisation of samples on infection level. Outputs from the new test which is predicated on the quantification of infection level, will help support grower decisions in commercial site selection. The robustness of this approach and hence uptake by growers will increase over time as results accumulate and the test is commercialised.