Project Title	Detection and quantification of *Verticillium dahliae* and *V. albo-atrum* in soils to determine the risk of verticillium wilt in strawberries
Project number:	SF 97
Project leaders:	Dr Jeff Peters, Fera, York Dr Tim O'Neill, ADAS Boxworth, Cambs
Report:	March 2010
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Location of project:	Fera, ADAS Boxworth and commercial sites in Cheshire, Clwyd, Merseyside and Surrey
Project coordinator:	Dr N Boonham, Fera
Date project commenced:	1 April 2009
Date completion due:	31 March 2012
Key words:	Strawberry, wilt, *Verticillium dahliae*, *V. albo-atrum*, soil infestation, detection, quantification

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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

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.

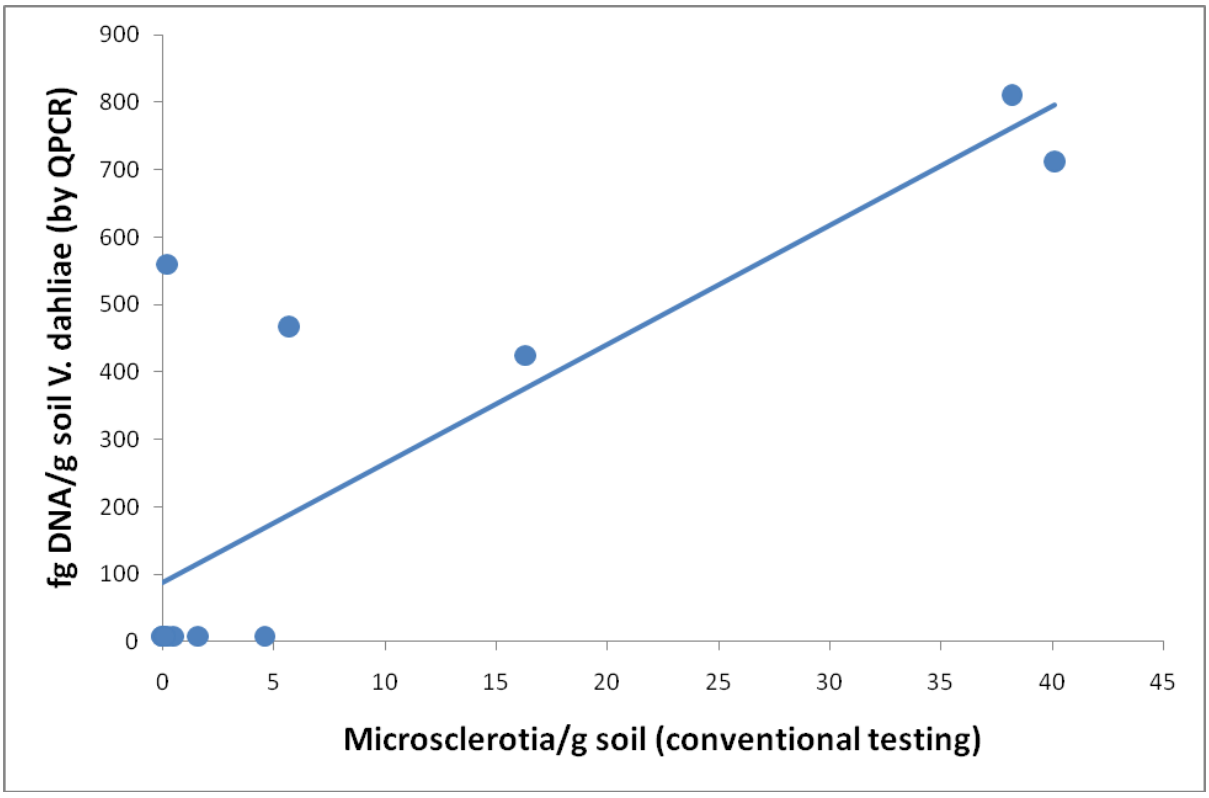

Figure 1. Comparison of results from the QPCR and conventional wet sieving (Harris) tests used to quantify *Verticillium dahliae* levels in field soils.

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.

SCIENCE SECTION

Introduction

Verticillium wilt of strawberries is one of the most serious diseases causing significant yield loss. The major main-season strawberry variety now grown (Elsanta) is highly susceptible to verticillium wilt, and leading new varieties being introduced (eg Sonata, Figaro) appear to be as susceptible. This project seeks to improve disease control and to develop a molecular diagnostic test for detecting and quantifying *Verticillium dahlia* and *V. albo-atrum* in soil. The work builds on successful Potato Council-funded projects (black dot (R249) and diagnostics (R253 and R411)).

The causal pathogen, *V. dahliae*, can exist as microsclerotia that can persist in soil for many years. *Verticillium dahliae* and *V. albo-atrum* (Vaa) have a wide host range (c. 300 plant species), including common GB agricultural crops such as potatoes, linseed and brassicas. If those crops become infected with *Verticillium spp.*, the soil can become contaminated with pathogen propagules for up to 25 years.

A pre-planting wilt risk assessment service, the Harris soil test, has been available to GB growers since the early 1990s. This test is based on the detection and enumeration of *V. dahliae* microsclerotia in soil. The assay costs around £165 + VAT and takes 6-8 weeks to complete. This means that considerable forethought is required prior to planting and costs are prohibitive to permit regular pre-planting screening. In addition, as the traditional method is reliant upon the detection of microsclerotia, it overlooks and underestimates the level of inoculum due to pathogenic *Verticillium spp.*, particularly Vaa, surviving in soil in the form of saprophytic mycelium. Currently no test exists for the detection and enumeration of Vaa in soil because the Harris test utilises a sieving assay which detects only inoculum in the form of microsclerotia. The significance of Vaa in soft fruit crops is, therefore, poorly understood due to the lack of reliable diagnostic assays. It is reported to infect strawberry, raspberry and blackberry

This project seeks to develop a rapid PCR-based alternative to the Harris test for detection and quantification of both *V. dahliae* and Vaa in soil. Results using the molecular assay would take days to complete compared with 6 to 8 weeks using the conventional method. Therefore, this project aims to develop a PCR-based test for detecting and quantifying Vaa in soil, to enable soil samples to be tested for both *V. dahliae* and Vaa concurrently. This test could be offered by Fera, and other laboratories, to growers for under £75. Fera has made

advances in sample processing and DNA extraction from large volumes of soil (c. 1 kg). This permits the development of a quantitative PCR assay using current soil sampling methods used by growers.

With the loss of methyl bromide for soil disinfection and increasing concern over the future use of alternative soil sterilants (eg chloropicrin), sound knowledge on levels of *V. dahliae* in soil are increasingly important to aid economically-sound planting decisions.

The main aims of SF 97 are:

- To develop real-time PCR assays for *Verticillium dahlia* and *V. albo-atrum*
- To measure soil inoculum levels in prospective field trial sites
- To establish the reliability of the real-time PCR assay as a means of identifying soils with levels of *Verticillium dahlia* and *V. albo-atrum* that are likely to cause wilt in strawberry.

*Objectives in Year 1***:**

a) To refine and validate real time PCR assays for the rapid detection and quantification of both *V. dahliae* and *V. albo-atrum*.
b) To determine the correlation between soil inoculum levels of target pathogens and levels of DNA as measured by quantitative PCR.
c) To identify field trial sites with a range of inoculum levels of *V. dahlia* for trials work in Year 2.

Materials and methods

Developing and validating a molecular assay for the detection and quantification of *V. dahlia* and *V. albo-atrum*.

QPCR assay development

Using the Broad Institute database of DNA sequence data, an initial search was done of genome regions that were suitable for detecting *V. dahliae* and *V. albo-atrum*. This search identified a conserved region (C1) as a potential candidate target. Assays were therefore designed to use the C1 region of *V. dahliae* and *V. albo-atrum* isolates (Table 1). Nine *V. dahlia* and two *V albo-atrum* isolates were obtained from Fera's culture collection. A further twelve *Verticillium spp.* isolates were obtained from Warwick HRI (courtesy Dr D Barbara). The C1 region from all twenty three isolates of *Verticillium spp.* were sequenced subsequently. Sequences were aligned using Clustal V within Megalign version 4.05 (DNAStar). Individual alignments were completed for each *V. dahlia* and *V. albo-atrum* isolate and a basic distance based cluster tree constructed using Megalign. Exemplar sequences representing all clusters from this provisional alignment were selected and

included in a second alignment. These alignments were used for the design of the primers and probes as follows:

Primers and probes were designed using Primer Express software v2 (Applied Biosystems). All primer and probe sequences were checked for specificity against all published sequences on Gen-Bank using the basic local alignment algorithm implemented as BLASTN. All assays were then validated by testing against a panel of DNA extracts from all available *Verticillium spp.* isolates and a selection of unrelated soil organisms. The two assays were designated VdC1 and VaaC1 for detecting *V. dahliae* and *V. albo-atrum*, respectively

DNA was extracted from fungal cultures using the Wizard® Magnetic DNA Purification System for Food (Promega, FF3750) in conjunction with a Kingfisher ML magnetic particle processor (Thermo Electron Corporation). Mycelium was scraped from the surface of an actively growing culture into a 2 mL screw cap tube containing 0.5 mL of 1 mm zirconia beads (Thistle Scientific) and 1 mL Lysis Buffer A containing 10% antifoam B emulsion (Sigma). Samples were shaken at full speed for 30 s on a mini-beadbeater (BioSpec Products, Inc.) before following the standard Promega and Kingfisher ML extraction protocols. Extracts (typically 1–5 ng uL^{-1}) were stored at -20°C until required. All real-time PCRassays were set up as duplicate 25 uL reactions using Brilliant QPCR Master Mix (Stratagene, Cat. No. 929549) following manufacturer's protocols. All sets of primers were used at 300 nM and probes at 100 nM. TaqMan® PCR reactions were carried out within the ABI Prism7900HTSequence Detector System (PE Biosystems) beginning with 50°C for 2 min, 95°C for 10 min, and 40 cycles of 95°Cfor 15 s and 60°Cfor 1 min.

Soil extraction

Soils were air dried and DNA was extracted from 250 g of soil using a method adapted from Budge 2007.

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

Sclerotia of isolate 12080 (75-150 um diameter; supplied by Warwick HRI) that were suspended in water, were thoroughly mixed into duplicate 50g aliquots of a sandy loam soil (Marsham, Norfolk) to produce concentrations of 1, 5, 10, 10, 20 and 36 microsclerotia/g soil. Single bulk DNA extractions were performed on each duplicate 50 g sample and real-time PCR assays were performed in triplicate as above.

Effect of soil levels of *Verticillium dahliae* determined by a molecular test on incidence of verticillium wilt in field crops

Site selection

During the summer months in 2009, 13 prospective field sites were tested for soil levels of *V. dahliae* by the conventional plate test. Soil samples were collected as recommended for the conventional test (see HDC Factsheet 16/06). A minimum of 50 soil cores to 15 cm depth were collected in a grid pattern using a 2 cm diameter soil auger. The resulting bulk of soil (c. 500 g) was air dried at ADAS High Mowthorpe then divided into two; half was retained at ADAS for determination of *V. dahliae* by the conventional plate test, and half was transferred to Fera for determination of *Verticillium* species by the molecular test. Details of the sites are given in Table 1.

Based primarily on the results of conventional plate tests for *V. dahliae*, five sites were selected with a range of soil levels of *V. dahliae* which are likely to result in differing levels of verticillium wilt when each is planted with a very susceptible, a susceptible and a moderately resistant variety. The basis for selecting the particular sites is shown in Table 2 (Anon., 2005).

Crop details

The varieties used were Elsanta (very susceptible), Symphony (susceptible) and Florence (moderately resistant). Although the variety Hapil is considered to be possibly more susceptible than Elsanta, this variety was not used because Elsanta is very widely grown and growers could more easily relate soil test results to this variety. The new variety

Fenella, a mid-season variety with the same 50% harvest date as Elsanta, is considered to be very resistant to verticillium wilt (D Simpson, pers. comm.). This variety was not used in the replicated experiments in case it proved so resistant that no or little verticillium wilt occurred at the field sites; in this situation, it would not be possible to relate soil levels of *Verticillium spp.* to incidence of verticillium wilt. However, plants of cv. Fenella will be planted at one site, in soil with a relatively high level of *V. dahliae,* to determine its field performance on an infested site.

Plants of Elsanta, Symphony, Florence and Fenella, sufficient for all field sites, were obtained from a UK propagator. The plant specification was A grade with a minimum crown diameter of 12 mm and graded to be as uniform as possible within each variety. Plants were dug in winter 2009 and cold-stored by the propagator until required for planting in spring 2010. A sample of 100 plants of Elsanta, Symphony and Florence were destructively assessed for verticillium wilt (see below) in order to check for possible infection introduced with plants. Crops were grown by the host farmers according to their usual practice.

Experiment design and statistical analyses

At each of the five sites, a randomised block design with fourfold replication was used. Each plot contained 100 plants arranged in 2-row beds at 35 cm intervals (farm-picked sites) or 45 cm intervals (PYO sites); plot size was usually an 18 m length of 0.9 m wide bed. Blocking was done based on *V. dahliae* levels in individual plots determined using the molecular test (i.e. the three plots with the lowest values at each site formed block 1, the next three values formed block 2 and so on). Details of plot values are given in Appendix 1. Results will be examined both within a site and across sites by regression analysis.

Disease assessments

A sample of 100 plants were removed from the cold-stored batches in February 2010 and tested for *V. dahliae* and other pathogens. Fifty plants were examined by ADAS for staining of crown tissue; small pieces of stained tissue were tested for *V. dahliae* or other fungal pathogens by plating onto potato dextrose agar (PDA) after surface disinfection with sodium hypochlorite (1% for 3 mins). The other 50 plants of each variety were tested for *Verticillium spp.* by a molecular test at Fera.

After planting in spring 2010, each crop will be examined for symptoms of verticillium wilt in autumn 2010 and again in 2011.

Table 1: Details of prospective field sites where soil samples were taken and tested for *Verticillium dahliae* by the conventional plate test and a molecular test

Site code	County	Previous crop	Soil type	Date sampled
A1	Lancs	Wheat	Medium loam	14-7-09
A2	Lancs	Wheat	Medium loam	14-7-09
A3	Clwyd	Wheat	Medium loam	14-7-09
A4	Clwyd	Wheat	Medium loam	14-7-09
A5	Merseyside	Barley	Medium loam	14-7-09
A6	Merseyside	Cabbage	Medium loam	14-7-09
A7	Cheshire	Mustard	Sandy loam	14-7-09
A8	Cheshire	Mustard	Sandy loam	14-7-09
A9	Surrey	Broad beans	Medium loam	14-7-09
A10	Surrey	Beetroot	Medium loam	14-7-09
A11	Surrey	Various	Sandy loam	28-7-09
A12	Surrey	Arable	Sandy loam	28-7-09
A13	Surrey	Vegetables	Sandy loam	30-7-09

Table 2: Reported susceptibility of strawberry cvs Elsanta, Symphony and Florence to verticillium wilt at different soil levels of *V. dahliae* as determined by the conventional plate test

Range	Elsanta (Very susceptible)	Symphony (Susceptible)	Florence (Moderately resistant)
None detected	Very low	Very low	Very low
0.1 – 0.2	Low	Low	Very low
0.3 – 0.5	Medium	Low	Low
0.6 – 1.0	High	Medium	Low
1.1 – 2.0	High	High	Low
2.1 – 5.0	Very high	High	Medium
5.1 – 10.0	Very high	Very high	High
> 10.0	Very high	Very high	Very high

Results

QPCR assay development

The results showing the specificity of the *V. dahliae* and *V. albo-atrum* assays are provided in Table 3. The *V. dahliae* assay, ***VdC1*** detected all eleven isolates of *V. dahliae* recovered from strawberry. Also, ***VdC1*** detected *V. dahliae* isolates from chrysanthemum, hops and potato. The assay did not detect a *V. dahliae* isolate from tomato, or any of the non-target fungi tested (including *V. albo-atrum* and *V. longisporum*). The common saprophytic fungus commonly found on strawberry (and often confused with *Verticillium spp.)* was not detected using ***VdC1***. The *V. albo-atrum* assay, **VaaC1** only detected the target pathogen.

Table 3. List of isolates used in developing and validating the assay and specificity of *V. dahliae* (VdC1) and *V. albo-atrum* (VaaC1) assays.

Isolate identifier	**Identification**	**Supplied by**	**Isolated from**	**Reaction with *VdC1* assay**	**Reaction with VaaC1 assay**
12001	*V. dahliae*	Fera	Chrysanthemum	pos	neg
12002	*V. dahliae*	Fera	Tomato	neg	neg
12005	*V. albo-atrum*	Fera	Unknown	neg	pos
12006	*V. longisporum*	Fera	Oil seed rape	neg	neg
DC59	*V. dahliae*	Warwick HRI	Soil	pos	neg
321-3	*V. dahliae*	Warwick HRI	Strawberry	pos	neg
1871	*V. albo-atrum?*	Warwick HRI	Strawberry	neg	pos
1875	*V. dahliae*	Warwick HRI	Strawberry	pos	neg
1877	*V. dahliae*	Warwick HRI	Strawberry	pos	neg
2341	*V. dahliae*	Warwick HRI	Hop	pos	neg
332-1	*V. dahliae*	Warwick HRI	Strawberry	pos	neg
12078	*V. dahliae*	Warwick HRI	Soil	pos	neg
12079	*V. dahliae*	Warwick HRI	Strawberry	pos	neg
12080	*V. dahliae*	Warwick HRI	Soil	pos	neg
12085	*V. dahliae*	Warwick HRI	Strawberry	pos	neg
12086	*V. dahliae*	Warwick HRI	Strawberry	pos	neg
12008	*V. dahliae*	Fera	Potato	pos	neg
12701 Symphony bx09/77a	*V. dahliae*	Fera	Strawberry	pos	neg
12701 Eros bx09/77b	*V. dahliae*	Fera	Strawberry	pos	neg
12701 Bx 09/76	*V. dahliae*	Fera	Strawberry	pos	neg
12702	*V. dahliae*	Fera	Strawberry	pos	neg
13526	*V. dahliae*	Fera	Potato	pos	neg
CC505	*Gliocladium* sp.	Fera	Strawberry	neg	neg
-	*Colletotrichum coccodes*	Fera	Soil	neg	neg
-	*Rhizoctonia solani* AG3	Fera	Soil	neg	neg

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

There was a good linear relationship between the amount of DNA detected by QPCR and the amount of microsclerotia of *V. dahliae* per g soil within the range 1 to 36 microsclerotia/g (Figure 1; *P*=0.009; R^2=0.982). The assay detected down to 363 fg DNA/g soil. This represented 1 microsclerotia/g soil. It is likely that the PCR test will detect below this level as the theoretical limit of detection using the current extraction method and assay is around 250 fg DNA/g soil.

At the time of writing this report, the soil experiments using *V. albo-atrum* had not been completed. However, a dilution series of culture extract produced a good linear relationship (Figure 2). In addition, *V. albo-atrum* was detected in two field soils as part of the site selection work (Table 4). Pot trials will be carried out in 2010 to determine the effect of *V. albo-atrum* and *V. dahliae* inoculum levels on wilt incidence and severity.

Soil inoculum levels

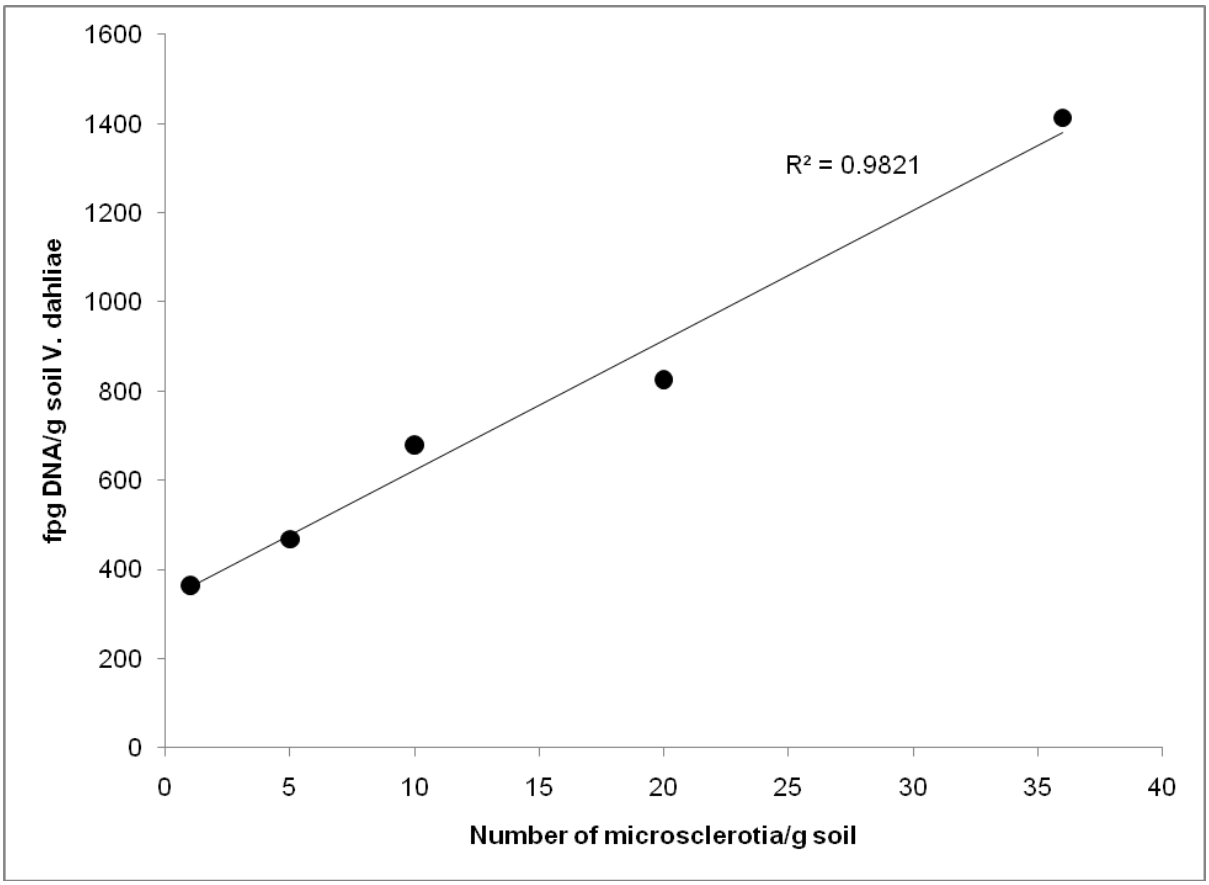

Figure 1. Relationship between the number of microsclerotia of *V. dahliae* (ranging from 1 to 36) per g soil and amount of target DNA detected by quantitative PCR.

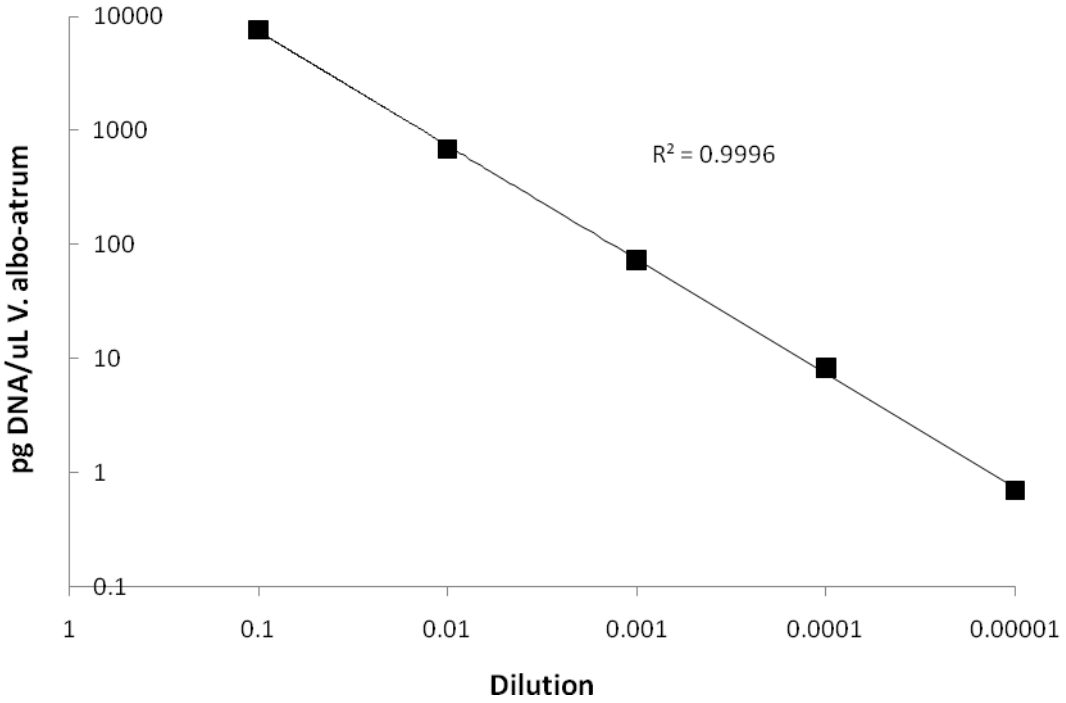

Figure 2. The amount of *V. albo-atrum* DNA/uL detected by quantitative PCR from a ten-fold dilution series using nucleic acid extracts from mycelial cultures.

Site selection for field experiments

Soil infestation with *V. dahliae* and *V. albo-atrum* at the 13 prospective field sites is shown in Table 4. *V. dahliae* was detected at 10 of the sites by the plate test, at infestation levels ranging from 0.1 to 40.1 cfu/g; and at 5 of the sites by the molecular test, at infestation levels ranging from 424 to 810 fg DNA/g. There was a reasonable relationship between infestation levels as determined by the two methods (Figure 3; P <0.05, R^2 = 0.668). In addition, *V. albo-atrum* was found in two of seven fields tested by the molecular method.

In order to relate soil infestation by *V. dahliae* with observed verticillium wilt incidence in the field it is necessary to obtain a wide range of disease severity values without minimal clustering around zero or 100%. Five sites were therefore selected for field experiments (A1, A7, A8, A11 and A12) to provide a broadly even spread of risk categories (very low – 4; low – 4; medium/high – 3; very high – 4) (Table 5). No *V. albo-atrum* was detected in the initial soil samples from any of these sites. Further details of sites selected for field experiments, including dates of plot samples are given in Appendix 2.

Table 4: Soil infestation with *V. dahliae* and *V. albo-atrum* at 10 prospective field sites as determined by a conventional plate test and a molecular test

Site code	Conventional plate test	Molecular test	
	V. dahliae **(cfu/g)**	***V. dahliae*** **(fg DNA/g**	***V. albo-atrum*** **(fg DNA/g)**
A1	0.2	560	<250
A2	<0.1	<250	739
A3	38.2	810	726
A4	40.1	711	<250
A5	0.2	<250	<250
A6	1.6	<250	<250
A7	0.5	<250	<250
A8	<0.1	<250	<250
A9	<0.1	<250	<250
A10	0.1	<250	<250
A11	4.6	<250	<250
A12	5.7	468	<250
A13	16.3	424	<250

Limits of Detection of *V. dahliae* by the plate and molecular test are <0.1 cfu/g and <250 fg DNA/g respectively.

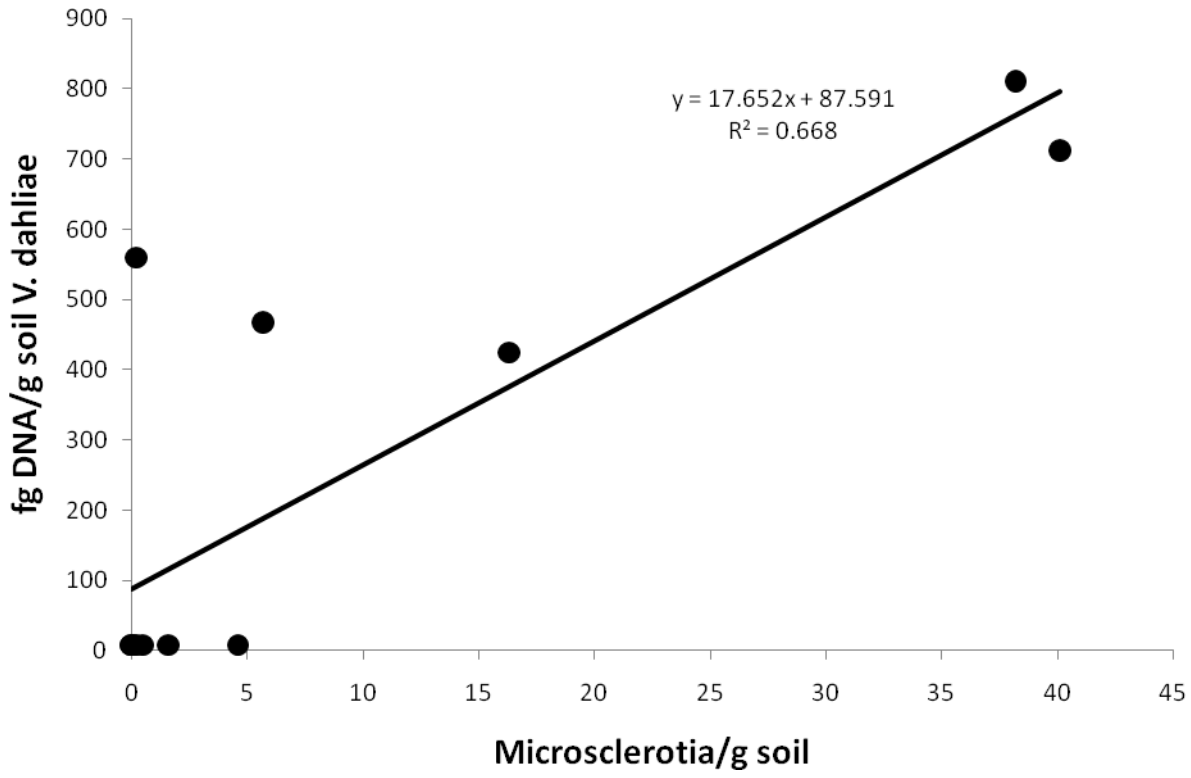

Figure 3. A comparison of *V. dahliae* quantification using the quantitative PCR assay (fg DNA/g soil) and the conventional Harris method (microsclerotia/g soil).

Table 5: Predicted risk of verticillium wilt in cvs Elsanta, Symphony and Florence at five field sites based on soil infestation of *V. dahliae* (as determined by a conventional plate test) and reported varietal susceptibilities

Site code	*V. dahliae* (cfu/g)	Predicted risk of verticillium wilt in:		
		Elsanta	Symphony	Florence
A8	<0.1	Very low	Very low	Very low
A1	0.2	Low	Low	Very low
A7	0.5	Medium	Low	Low
A11	4.6	Very high	High	Medium
A12	5.6	Very high	Very high	Very high

Conclusions

During this reporting period, molecular QPCR assays have been developed for both *V. dahliae* and *V. albo-atrum,* the causes of verticillium wilt of strawberry. The assays have so far been shown to be specific, only testing positive against target pathogen. The *V. dahliae* assay was sensitive, detected down to 363 fg DNA/g soil or 1 microsclerotia/g soil. When the results from field soil testing using the QPCR assay and conventional Harris method were

compared, there was general agreement. However, in four (out of thirteen) soils the Harris method detected *V. dahliae* at levels above the 0.1 microsclerotia/g threshold, whereas the QPCR assay was not able to detect target pathogen. Field trials and further pot experiments will be carried during 2010 to ascertain which of those methods most accurately correlate inoculum levels with disease.

References

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Budge, G (2007). Detection and characterisation of Rhizoctonia solani affecting UK brassica crops. PhD Thesis, University of Reading.

Technology transfer

Poster

Detection and quantification of *Verticillium dahliae* and *V. albo-atrum* in soils to determine risk of verticillium wilt in strawberry. Verticillium Symposium, Corfu, 16-19 November 2009.

News items

Verticillium wilt of strawberry. Fera *Plant Clinic News*, July 2009, p 2.

Presentations

New developments in soil disinfestation. ADAS Soft Fruit Group, Oxford, 8 February 2010.

Appendix 1: Soil infestation with *V. dahliae* in individual plots at five field sites prior to planting. () = dates when samples were received by Fera. DNA extractions were done within 48 hours of receipt.

Plot Number	*V. dahliae* (fg DNA/g) at sites (sample date)				
	A8 (8/12/2009)	**A1 (29/4/2010)**	**A7 (8/12/2009)**	**A11 (7/5/2010)**	**A12 (7/5/2010)**
1	<250	<250	<250	<250	<250
2	<250	<250	<250	<250	<250
3	<250	<250	<250	<250	700
4	<250	<250	<250	**480**	<250
5	<250	<250	<250	<250	<250
6	<250	<250	<250	310†	<250
7	<250	<250	<250	<250	1650
8	<250	<250	<250	<250	<250
9	<250	<250	<250	<250	520
10	<250	<250	<250	<250	<250
11	<250	<250	<250	<250	2620
12	<250	<250	<250	<250	<250
Mean	<250	<250	<250	250	600
Original site mean (fg/g)	<250	560	<250	<250	468
Original site mean (cfu/g)	<0.1	0.2	0.5	4.6	5.6

† *Verticillium spp*. (not *V. dahliae*)

Appendix 2: **Details of sites selected for field experiments**

Site code	County	*V.dahliae** (cfu/g)	*V.dahliae** (fg DNA/g)	*V. albo-atrum** (fg DNA/g)	Date plots sampled	Date planted
A8	Cheshire	<0.1	<250	<250	2/12/09	
A1	Lancs	0.2	560	<250		
A7	Cheshire	0.5	<250	<250	2/12/09	
A11	Surrey	4.6	<250	<250		
A12	Surrey	5.7	468	<250		

* Overall site value, sampled July 2009.