

# Grower Summary

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## SF 097a

Using molecular quantification of *Verticillium dahliae* in soil to identify risk of strawberry verticillium wilt

Annual 2014

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**Project Number:** SF 097a

**Project Title:** Using molecular quantification of *Verticillium dahliae* in soil to identify risk of strawberry verticillium wilt

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**Industry Representative:** Mr Richard Stanley, Stanton St John, Oxfordshire

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### **Further information**

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## **GROWER SUMMARY**

### **Headline**

- A new molecular assay for *V. dahliae* designed in this project shows excellent specificity and moderate sensitivity.

### **Background and expected deliverables**

Soil-borne *Verticillium dahliae* is a serious threat to profitable production of soil grown strawberry, especially where suitable land with irrigation is in short supply and crops are grown on a tight rotation. The major main-season variety Elsanta is highly susceptible to verticillium wilt and leading new varieties being introduced appear to be susceptible (e.g. Sonata, Figaro). Strawberry verticillium wilt is difficult to control using fungicides applied to the growing crop and there are few effective products. Chemical fumigation options are limited. Alternative, non-chemical methods of soil disinfestation are not yet available. Host resistance to verticillium wilt is the most effective and sustainable approach, especially when used in combination with other disease management practices. At present genetic resistance plays a minor role in control of strawberry verticillium wilt as varieties are usually selected by growers for characteristics other than verticillium wilt resistance. Previous studies have demonstrated a positive relationship between soil infestation density of *V. dahliae* and occurrence of verticillium wilt in strawberry (Harris & Yang, 1996). A soil test that quantifies soil inoculum levels of *V. dahliae* prior to planting can have a significant role in the management of strawberry verticillium wilt. A soil sieving and agar plate culture test (the Harris test) has been used for this purpose for over 30 years. A quantitative real-time PCR (qPCR) test developed in HDC Project SF 97 offers several advantages over the traditional test, namely: speed (a few days), a high level of specificity and no dependence on expensive and increasingly rare taxonomy expertise. A rapid test is advantageous both for variety/field selection by growers when decision time is short (e.g. with rented land) and to minimise any change in soil inoculum that might occur between soil sampling and planting (e.g. change of microsclerotia distribution in the soil profile with cultivations; decay of crop debris in the soil to release microsclerotia; decline in microsclerotia number with microbial degradation).

The expected deliverables from this project are:

- A molecular test to quantify *V. dahliae* infestation density in soil with a sensitivity equivalent to 0.1 microsclerotia per gram of soil following DNA extraction from a 50 g soil sample.

- A data set relating *V. dahliae* infestation density in soil determined by qPCR to occurrence of verticillium wilt in field crops of strawberry.

The specific objectives in Year 1 were:

1. To improve the sensitivity and reliability of a qPCR test for *V. dahliae* through improved assay design;
2. To initiate validation of the test assay by qPCR testing of soil samples from 50 fields and determining levels of verticillium wilt in strawberry crops planted in these fields in 2013.

## **Summary of the project and main conclusions**

### ***Objective 1 – Improve sensitivity and reliability of qPCR test for V. dahliae***

#### *Sensitivity of test*

Four new real-time PCR assays with putative sensitivity to *Verticillium dahliae* were designed (Table 1). None of these assays, nor the EF assay developed in SF 97, nor a published Californian assay (Bilodeau *et al.*, 2012), had complete specificity for *V. dahliae* under standard Fera PCR conditions. However, by manipulating the reaction conditions assays designed to the rDNA IGS region showed excellent specificity, yet sensitivity was relatively poor (Table 2). This result is encouraging as it shows specificity within the rDNA IGS region can be obtained; future work will aim to boost the sensitivity of this assay.

**Table 1.** Details and source of real-time PCR assays evaluated in the study

Assay name	Loci	Multiple/Single copy	Source
<u>Established assays</u>			
EF (SF 97)	Elongation factor	Single	HDC project SF 97
Bilodeau	IGS	Multiple	Bilodeau et al., 2012
<u>New assays</u>			
IGS v1	IGS	Multiple	This study
IGS v2	IGS	Multiple	This study
ITS	ITS	Multiple	This study
MtDNA	MtDNA intergenic spacer region	Multiple	This study

**Table 2.** Specificity testing of *Verticillium dahliae* assays showing Ct values when tested against isolates of *V. dahliae* (Vd), *V. tricorpus* (Vt), *V. nigrescens* (Vn), *V. albo-atrum* (Vaa), *V. longisporum* (VI) and *Gliocladium roseum* (Gr)

Assay	Annealing Temperature used	Vd1	Vd2	Vt	Vn	Vaa	VI	Gr
EF	60°C	18.5	21.3	36.5	35.5	33.9	40	37.8
Bilodeau	62°C	16.6	16.2	31.8	nt	30.3	40	33.3
IGS v1	62°C	22.5	18.1	33.9	nt	34.4	35	36.7
IGS v2	64°C	22.8	24.2	40	40	40	40	40
ITS	62°C	31.0	40	40	40	40	40	40
MtDNA	60°C	15.8	17.3	33.7	34.6	31.5	Nt	34.5

Ct = 40 denotes a negative result; a low Ct value denotes good sensitivity. Nt = not tested.

### *Reliability of test*

The effect of testing multiple 50 g sub-samples from a 2 kg field soil sample on variation in test results was examined for seven naturally infested soils. For most samples variance decreased considerably with four sub-samples but showed little further decrease thereafter (Table 3).

**Table 3.** Evaluation of the variation in qPCR results for *Verticillium dahliae* quantification in soil when replicated DNA extractions are compared

Soil sample	Ct value mean of				
	Two replicates*	Three replicates	Four replicates	Five replicates	Six replicates
2a	33.0 (0.3)	32.4 (1.0)	32.7 (1.0)	32.2 (1.4)	32.3 (1.3)
3a	30.5 (0.9)	30.7 (0.8)	30.7 (0.6)	30.9 (0.7)	31.1 (0.8)
14	34.5 (3.2)	35.7 (3.1)	35.6 (2.5)	35.6 (2.2)	35.6 (2.0)
24	31.3 (0.1)	31.3 (0.1)	30.5 (1.5)	30.4 (1.3)	30.5 (1.2)
2b	34.5 (3.2)	35.7 (3.1)	35.6 (2.5)	35.6 (2.2)	35.6 (2.0)
3b	31.3 (0.1)	31.3 (0.1)	30.5 (1.5)	30.4 (1.3)	30.5 (1.2)
43	31.5 (3.4)	32.5 (3.0)	33.1 (2.7)	33.2 (2.3)	33.1 (2.1)

\*Standard deviation given in parenthesis.

## **Objective 2 – Validate test by assessment of verticillium wilt symptoms in commercial strawberry crops**

### *Soil sampling and occurrence of Verticillium wilt symptoms*

In spring and summer 2013 soil samples (2 kg) were taken by ADAS staff using the standard sampling method for *V. dahliae* from 45 fields due to be planted with strawberry; an additional four fields were each sampled by taking and individually testing 50 soil cores to gain information on distribution of the pathogen. The samples were supplied to Fera for determination of *V. dahliae* by qPCR. The samples comprised sites in England (42), Scotland (4) and Wales (3) and covered major soft-fruit production counties including the South east (19), East Anglia (6) and West midlands (9).

When crops with soil sampled by the standard method were examined in autumn 2013 after the end of fruiting, symptoms of verticillium wilt were observed at 34 sites out of 41 sites, with an incidence >5% at 15 sites; four of the sites could not be assessed due to grubbing of the crop. Laboratory tests confirmed *V. dahliae* in symptomatic plant samples taken from five out of nine sites showing a low incidence of verticillium wilt (<5%). A verticillium wilt incidence above 10% was recorded in crops of cvs Camarillo, Eilan, Sonata and Symphony.

### *Association of soil infestation with verticillium wilt symptoms*

Each soil sample was tested for *V. dahliae* by established qPCR assays using sets of primers from a UK test (Fera EF assay) and a Californian test (Bilodeau assay). In the 41 fields sampled by the standard 50-core bulk soil method and assessed for wilt, *V. dahliae* was detected in five and 29 soils by the Fera and Bilodeau tests respectively (Table 4). There was not a good correspondence of incidence or severity of Verticillium wilt symptoms with soil density of *V. dahliae* determined by either the Fera or Bilodeau molecular assays.

Of the 15 sites with obvious verticillium wilt (symptoms present in >5% of plants), *V. dahliae* was detected in soil from two and 13 of these sites by the Fera and Bilodeau tests respectively. The high level of apparently false negative results from the Fera EF soil test may reflect the known lower sensitivity of this assay. There were seven sites where no verticillium wilt symptoms were observed; three of these were reported to have *V. dahliae* present in the soil by the Bilodeau test, none by the Fera EF test. The apparently false positive results from the Bilodeau test may reflect the lower specificity of this assay. In 2014, occurrence of verticillium wilt symptoms will be further examined in the 41 crops assessed in 2013. Additional crops planted in 2014 will also be soil sampled and assessed to increase the data set on soil levels of *V. dahliae* and associated levels of verticillium wilt.

The soils from all 41 sites assessed in 2013 and additional sites sampled in 2014 will be tested for *V. dahliae* infestation density using one of the new qPCR assays developed in this project.

**Table 4.** Occurrence of verticillium wilt symptoms in strawberry crops in 2013 and associated levels of *V. dahliae* in soil pre-planting as determined by qPCR tests

Incidence of Verticillium wilt symptoms (% plants)	No. crops in this category	Number of sites where <i>Verticillium</i> was detected in soil	
		EF primers	Bilodeau primers
0	7	0	3
0.1 – 1	12	0	6
1.1 – 5	7	3	7
5.1 – 10	7	0	6
>10	8	2	7
Total	41	5	29

#### *Distribution of V. dahliae in fields*

Examination of the distribution of *V. dahliae* in four fields by testing 50 soil samples taken on a grid pattern showed that infestation was highly clustered. Kriging was possible for three of the four sites and this analysis will inform the development of a sampling strategy.

#### *Main conclusions*

- In 2013, using a molecular assay developed in California, the presence or absence of verticillium wilt symptoms in strawberry was correctly predicted by pre-plant soil test results for *Verticillium dahliae* at 29 out of 41 sites.

- The level of verticillium wilt symptoms was greater in crops grown in soils where *V. dahliae* was detected (8.8% of plants) than in soils where it was not detected (2.2% of plants).
- Some sites where no *V. dahliae* was detected had symptoms of verticillium wilt and *vice-versa*; such results are not unexpected given the differences between sites in variety and cropping factors, potential errors associated with assessing wilt symptoms and taking representative soil samples, and specificity of the molecular assay.
- A new molecular assay for *V. dahliae* designed in this project shows excellent specificity and moderate sensitivity. Soils from around 50 sites will be tested using this assay in 2014 to determine if it more accurately predicts verticillium wilt than either the Californian assay or the EF assay developed in SF 97.

## Financial benefits

Verticillium wilt, caused primarily by *V. dahliae*, is one of the most serious diseases of field grown strawberry causing significant yield losses, and is a significant driver to soft fruit production being shifted into substrate and table top systems. Quantifying 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 risk.

If a field is not tested for *V. dahliae* prior to planting a susceptible variety, and the fungus is present at levels sufficient to cause infection, potential losses are around £12,000/ha assuming 50% of the crop is affected. If a field is treated with Basamid (dazomet) or Custofume (chloropicrin) as a precaution against verticillium wilt, and the fungus is not present at levels sufficient to cause disease, unnecessary costs of £3-5,000/ha may be incurred. An accurate assessment of *V. dahliae* soil infestation density can thus provide significant savings.

## Action points for growers

- There are no action points at present.