Project Title	Assessing the effectiveness of a Norwegian developed PCR assay for the prediction of carrot cavity spot levels by measuring soil levels of five *Pythium* species.
Project number:	FV 5g
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Report:	Year 1 Annual, September 2006
Previous report	None
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Location of project:	Warwick HRI, University of Warwick, Wellesbourne, Warwick CV35 9EF, UK Plantsystems Ltd, 97 Hollycroft Road, Emneth, Wisbech, Cambs, PE14 8BB, United Kingdom Commercial farms. Soil sample testing by Carrotech AS (Frederik A. Dahlsvei 20, N-1432 Ås, Norway)
Project coordinator:	John Kenyon, Huntapac
Date project commenced:	1st September 2005
Date completion due:	30th June 2007
Key words:	Carrot, cavity spot, *Pythium* spp., soil testing, PCR, disease prediction

The results and conclusions in this report are based on a series of experiments conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

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

- The usefulness of a PCR-based soil test for the detection of five *Pythium* species as management tools for carrot cavity spot disease in the UK is being investigated using samples from four commercial farms
- To date only *P. intermedium* has been detected.
- The value of the assays and results cannot be judged until carrots from the test sites are assessed for disease in the second half of the project

Background and expected deliverables

Many carrot growers consider cavity spot to be the most important disease problem and even low levels can cause economic loss. It has been thought that in the UK, cavity spot was mainly due to *Pythium violae* or, more rarely, *P. sulcatum*. Recent work in Norway has implicated five distinct Pythium 'species' (one group of isolates has yet to be formally described as a species) including *P. violae* and *P. sulcatum*. It is not clear whether all five of these are important, or even occur, in the UK.

Scientists at Planteforsk (Norwegian Crop Research Institute) have developed PCR assays for the five *Pythium* species they believe important for this disease in that country and this project aims to test whether these tests are useful in predicting cavity spot levels in the UK.

The scientific objectives are to assess whether all the five species occur at four sites in the UK, how detectable levels of them vary over a single season and which of them can be correlated with cavity spot in this country.

The tests are already offered as a commercial service and the more practical major objective of the project is to assess whether soil testing using the Norwegian assays can be a valuable cavity spot disease management tool for UK growers. If disease predictions made in autumn are accurate then they could be used to determine which fields are most suitable for renting or drilling. Growing season predictions will allow more informed choices about crop management, particularly marketing times.

Summary of the project and main conclusions

Four sites have been selected, sampled three times so far (October 2005, April and August 2006) and the first two sets of samples have been tested for the presence of the five *Pythium* species, *Mycocentrospora acerina* (cause of liquorice rot) and *Fibularhizoctonia carotae* (crater rot).

Of the five *Pythium* species, only *P. intermedium* has been detected and this species was present at all sites at both samplings. Levels of *P. intermedium* varied by site and sampling position at the sites.

M. acerina and *F. carotae* were also detected but more sporadically.

The significance of these results will not be known until the disease levels are assessed at the end of the season.

Financial benefits

The level of benefits from this work will be highly dependent on the time of year at which disease predictions can be made and the accuracy of those predictions. As neither of these factors is known at this stage, no useful analysis of the benefits to UK growers can yet be made.

Action points for growers

- No action by growers can be recommended at this stage.
- The PCR tests are commercially available to growers through Carrotech AS of Norway but as there is as yet little information on the relevance of the results to UK conditions they should be used with caution.

Science Section

Introduction

Carrots are a major crop in the UK (marketed value £125M-£160M in each of the last four crop years (Defra: Basic Horticultural Statistics) and the majority of growers consider cavity spot to be their most important crop protection problem. It has been thought that in the UK, cavity spot was due to either of two slow growing *Pythium* species; *Pythium violae* seems to be the most widespread of these but *P. sulcatum* also occurs on carrots. This latter species may be associated with particularly severe symptoms as has been reported overseas (T. Pettitt, personal communication). Some doubt has been cast on a single species cause for the disease by recent work in Norway; this work has implicated five distinct *Pythium* 'species' (one group of isolates has yet to be formally described as a species), including *P. violae* and *P. sulcatum*. It is not clear whether all five of these are important, or even occur, in the UK.

Disease control is still very problematic despite cavity spot having been the subject of much, largely industry-funded, research over the last few years. The fungicide metalaxyl has been used for disease control but in some soils enhanced degradation of the fungicide is making it increasingly less effective. Disease avoidance (using only uninfested fields), pathogen elimination (by soil treatments) and early harvesting (before significant damage occurs to roots) are all strategies which can be used to reduce the effects of the disease but their application has been limited by several factors. One of these *viz.* a poor understanding of the dynamics of the relevant pathogens in the soil, is currently being addressed in a Defra funded project at Warwick HRI. However, a major constraint on the more active management of the disease is the lack of an effective quantitative test to determine pathogen levels in soil. Direct isolation of the fungi is slow and difficult, limited to small numbers of samples and is not quantitative. An immunodiagnostic test for *Pythium violae* has been developed but it lacks sensitivity and specificity, limiting its use to the winter period.

It has become clear that a major limitation on UK growers managing cavity spot more effectively is the lack of a rapid test which would allow them to measure levels of the pathogens in soils at various stages of the crop. Quantitative measurements are important because the pathogens are likely to be widespread but growers and advisers need to be able to assess levels at which economically important disease is likely to result.

Work on the development of a rapid, quantitative test (using fluorescent PCR) for *P. violae* in soils was begun in an earlier short-term Defra funded project (HH2302STF) but while

progress was made, this work has probably been superseded by advances in Norway, where scientists at Planteforsk (Norwegian Crop Research Institute) have developed PCR assays for the five *Pythium* species they believe important for this disease in that country.

This project involves collecting soil and crop samples from the UK and submitting them for testing for the five *Pythium* species in Norway using the PCR-based procedure. The objectives are two-fold. The scientific objective is to make an assessment of whether the five species all occur in the UK and which of them are associated with cavity spot here. However, the practical objective is to assess whether soil testing using the Norwegian assays can be a valuable disease management tool for UK growers.

Materials and Methods

Four field sites were identified by Plantsystems. These sites were all to be cropped with carrots in 2006 and programmed for late season harvesting in late 2006 or early 2007. All four sites were considered to be at risk from cavity spot because of previous experience with the disease on the farm or in the specific field.

Soil samples have been collected from six points in each field and sent to Carrotech AS (Frederik A. Dahlsvei 20, N-1432 Ås, Norway) for testing. Samples were collected according to the procedure determined by Carrotech.

Precise details of the locations of the sites are confidential but relevant information on the crops and collection dates is given in Table 1.

DNA was extracted from soils and tested by PCR by Carrotech for five *Pythium* species (*P. violae, P. intermedium, P. sylvaticum, P. sulcatum* and *P*. “vipa”) and two other carrot pathogens (*Mycocentrospora acerina*, cause of liquorice rot, and *Fibularhizoctonia carotae,* crater rot) using proprietary methods.

These tests are semi-quantitative and results are reported on a four point scale (with interpolation) *viz*.:

0 – Pathogen not detected

1 – Low levels

2 – Moderate levels

3 – High levels.

Table 1. Varieties and drilling and collection dates for the four field sites.

Site	Variety	Drilled	Collection dates		
			1st	2nd	3rd
Lincolnshire	Nepal	6-5-2006	25-10-2005	10-04-2006	11-08-2006
Norfolk	Nairobi	4-6-2006	25-10-2005	10-04-2006	11-08-2006
Nottinghamshire	Nairobi*	3-7-2006*	25-10-2005	10-04-2006	11-08-2006
Yorkshire	Nairobi	25-5-2006	25-10-2005	10-04-2006	11-08-2006

*Due to poor spring weather, this site was drilled with parsnips by grower; six plots of carrots established as shown at the sampling points at this site by Plantsystems staff.

Results and Discussion

Field sites and drilling.

Four fields were identified by Plantsystems in autumn 2005 as ones due to be cropped in 2006 with carrots and programmed for late harvest (late 2006/early 2007). Previous experience on the farms or in the specific fields suggested all were at risk of cavity spot disease occurring. These fields were in Lincolnshire, Nottinghamshire, Norfolk, and Yorkshire. All sites were to be managed by commercial growers using normal practices without metalaxyl treatment. Three were drilled in spring 2006. Due to poor spring weather, one site (Nottinghamshire) was drilled with parsnips by the grower; in order to get an estimate of the cavity spot disease incidence for that field in carrots, six small plots of carrot were established close to the soil sampling points.

Sample Collection.

Three sets of soil samples have been collected from the sites in autumn 2005 and in 2006 (Table 1). The first two dates were intended to reflect “field selection” and “pre-drilling” for main crop carrots in a commercial system. The third date might be used by growers to determine whether an established crop should be early harvested or might be safely left in the ground for late harvest. At each site soil was collected from six discrete points located by GPS.

The third set of samples was collected in August 2006 but testing results are not yet available.

All samples were sent to Carrotech for DNA extraction and PCR testing.

PCR Testing - General.

Results from duplicate PCR assays for each sample showed that this part of the assay procedure is reproducible as paired results rarely differed by more than half a unit on the scale. The reproducibility of the soil extraction was not directly assessed by using multiple extraction of single sample soils. Comparisons within sets of six samples suggests that in most cases the results were consistent across fields but there were exceptions; for example at Nottinghamshire, values for *M. acerina* in spring ranged from undetectable to high and in the same field *P. intermedium* ranged from undetectable to moderate. Further investigations are necessary to understand whether these are genuine variations across the fields or variation in the sampling/extraction process. Some tests of this type are being undertaken in a parallel DEFRA funded project.

Although the relationships of the four-point scale to the absolute levels of the various pathogens are not known (but are almost certainly not linear), values for each field/sampling time were averaged to allow easy comparison between fields. However, it is important to realise that a value of “2” does not indicate twice the absolute amount of pathogen present for a value of “1” but simply that there is measurably more pathogen present. Almost certainly the linear four-point scale reflects some form of logarithmic relationship for actual values.

PCR Testing - M. acerina *(liquorice rot) and* F. carotae *(crater rot).*

Although not formally part of this project, samples sent to Carrotech are routinely tested for *M. acerina* and *F. carotae.*

M. acerina was detected in autumn in two samples from the Norfolk site at low to medium levels and at the same sampling points from the same field at equivalent or slightly lower levels in spring (Table 2). In spring it was also found in five of the six samples from the Nottinghamshire site at low to high levels, despite being not found in the autumn. The significance of these levels of pathogen under UK conditions is unknown but may warrant further investigation, depending on whether it is detected in later samplings in this project and whether any disease attributable to these pathogens is noted in these samples when assessed.

F. carotae was detected in a single autumn sample at a low level but not in any spring samples. The significance of such low level, sporadic detection is not clear.

PCR Testing - Pythium *species*.

The primary focus of this project is cavity spot and the occurrence of five *Pythium* species in the UK. Two species not previously thought to be associated with cavity spot in the UK (*P. sylvaticum*) or not known to occur in the UK (*P.* "vipa") were not detected at either sampling date. (*P. sylvaticum* has only been detected sporadically in the parallel DEFRA project and *P.* "vipa" not at all – albeit in the latter case for a more limited number of samples.) Similarly neither of the two species thought to be mainly (*P. violae*) or occasionally (*P. sulcatum*) associated with cavity spot have yet to be detected in this project, although both have been detected at other sites in the DEFRA project.

P. intermedium has not previously been thought to be associated with cavity spot in the UK but is considered to be part of the disease in Norway. This organism was detected at all four sites in both autumn and spring. Importantly for this project the levels at the four sites were quite varied in both autumn (being low, 0.1 and 0.2, or somewhat higher 0.6 or quite high, 1.7) and spring (0.3 to 1.6) opening the possibility of finding a correlation with disease levels at the end of the project. Interestingly, the three lower levels in autumn seem to have increased by the spring sampling, whilst the highest has stayed constant.
The value of measuring the levels of *P. intermedium* and the meaning of the undetectably low levels (or absence of) the other species cannot be assessed until the completion of the project when disease levels for these crops is known.

Later in the project it is intended not only to test soil from the open field (*i.e.* prior to drilling or, later, not adjacent to the growing roots carrots) but also the levels of the pathogens in soil brushed from the surface of the maturing roots. In tests at Warwick HRI using the same basic test procedures as part of the parallel DEFRA project, *P. violae* has been detected at much higher levels in soil from the surface of roots than in "open field soil" and it will be interesting to see if a similar pattern emerges at the sites used here, despite the apparent absence of *P. violae* in early samples.

Table 2. Results of PCR testing four sets of soil samples from two sampling dates.
Six soil samples were taken from each field. DNA was extracted and tested in duplicate for seven fungi by Carrotech AS using PCR.
P. sylvaticum, P. sulcatum, P. violae and *P.* "vipa" were not detected in any samples. *F. carotae* was not detected in the April samples. Results scored on a 4-point scale (0-3).

Site/ Sample	25th October, 2005				10th April, 2006	
	P.intermedium	*M. acerina*	*F. carotae*		*P. intermedium*	*M. acerina*
Lincoln 1	0 / 0	0 / 0	0 / 0		1.5 / 1.5	0 / 0
Lincoln 2	0 / 0	0 / 0	0 / 0		0 / 0	0 / 0
Lincoln 3	0.5 / 0.5	0 / 0	0 / 0		2 / 1	0 / 0
Lincoln 4	0 / 0	0 / 0	0 / 0		0 / 0	0 / 0
Lincoln 5	0 / 0	0 / 0	0 / 0		0 / 0	0 / 0
Lincoln 6	0.5 / 0.5	0 / 0	0 / 0		1/1	0 / 0
Average	*0.2*	*0.0*	*0.0*		*0.7*	*0.0*
Notts 1	0.5 / 0	0 / 0	0 / 0		1.5 / 1.5	1 / 0.5
Notts 2	0.5 / 0	0 / 0	0 / 0		0.5 / 0.5	1.5 / 2
Notts 3	0 / 0.5	0 / 0	0 / 0		0.5 / 1	3 / 3
Notts 4	2 / 2	0 / 0	0 / 0		1 / 1.5	0 / 0
Notts 5	0.5 / 1	0 / 0	0 / 0		1.5 / 1.5	0.5 / 0.5
Notts 6	0 / 0	0 / 0	0 / 0		0.5 / 1	0.5 / 0.5
Average	*0.6*	*0.0*	*0.0*		*1.0*	*1.1*
Norfolk 1	2 / 2	0.5 / 0.5	0 / 0		2.5 / 2	0.5 / 0
Norfolk 2	0.5 / 1.5	2 / 2	0 / 0		2 / 2	0.5 / 1
Norfolk 3	2.0 / 1.5	0 / 0	0 / 0		2.5 / 2	0 / 0
Norfolk 4	2.5 / 2.5	0 / 0	0 / 0		1 / 1	0 / 0
Norfolk 5	1.5 / 0.5	0 / 0	1 / 0		1 / 1	0 / 0
Norfolk 6	2.0 / 1.5	0 / 0	0 / 0		1.5 / 1	0 / 0
Average	*1.7*	*0.4*	*0.1*		*1.6*	*0.2*
Yorks 1	0 / 0	0 / 0	0 / 0		0 / 1	0 / 0
Yorks 2	0 / 0	0 / 0	0 / 0		0 / 0	0 / 0
Yorks 3	0 / 0	0 / 0	0 / 0		0 / 0	0 / 0
Yorks 4	0 / 0	0 / 0	0 / 0		0 / 0	0 / 0
Yorks 5	0.5 / 0.5	0 / 0	0 / 0		0 / 0.5	0 / 0
Yorks 6	0.5 / 0	0 / 0	0 / 0		1 / 1	0 / 0
Average	*0.1*	*0.0*	*0.0*		*0.3*	*0.0*

Conclusions

Few general conclusions can be drawn from the results at this stage as the “message” from this work will only emerge after the levels of disease have been assessed in the early part of 2007 and correlated with the PCR results.

It is clear that the PCR testing procedure regularly detects *P. intermedium* and this species is present at all four field sites at variable levels. The two non-*Pythium* fungi have also been detected, but at only at one or two sites and generally at low levels.

Parallel work in a DEFRA funded project using essentially the same tests at Warwick HRI support the conclusion that the Norwegian tests are effective in UK conditions for three of the remaining *Pythium* species and it must be assumed that *P. violae, P. sulcatum* and *P. sylvaticum* are either absent from the four sites used here or present at levels too low to be detected at the first two sampling dates. To date *P.* “vipa”, an as yet undescribed species, has not been detected in the UK in either project. Norwegian experience suggests this test is also effective in detecting the target species and we conclude that at present there is no evidence this species occurs in the UK. This species may of course be detected during further testing.

Technology transfer

Dr Arne Hermansen (Planteforsk/Norwegian Crop Research Institute) described the development of these tests and Norwegian experience with them at the biennial International Onion and Carrot Conference & Exhibition (East of England Showgrounds, Peterborough: November 23/24, 2005). Dr Hermansen is part of the team at NCRI which developed the tests used by Carrotech AS.

References

A recent general review of cavity spot and its association with specific *Pythium* species, with emphasis on the UK situation, can be found in:

Hiltunen, L.H. and White J.G. (2002). Cavity spot of carrot (*Daucus carota*). Annals of Applied Biology, 141: 201-223.