FINAL REPORT

To:
Horticultural Development Council
Bradbourne House
Stable Block
East Malling
Kent, ME19 6DZ

FV 5g

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

D J Barbara and M D Martin[1]
WarwickHRI, University of Warwick, Wellesbourne, Warwick, CV35 9EF
[1]Plantsystems Ltd, 97 Hollycroft Road, Emneth, Wisbech, Cambs, PE14 8BB

June 2007

Commercial - In Confidence

© 2007 Horticultural Development Council

Grower Summary

FV 5g

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

Final report 2007

© 2007 Horticultural Development Council

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
Project leaders:	D. J. Barbara, Warwick HRI M. D. Martin, Plantsystems Ltd.
Report:	Final Report, June 2007
Previous report	Year 1 Annual Report; September 2006
Key staff:	Dez Barbara (Warwick HRI) David Martin (Plantsystems) Colin Noble (Plantsystems)
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 Four commercial farms. Soil sample testing by Carrotech AS (Frederik A. Dahlsvei 20, N-1432 Ås, Norway)
Project coordinator:	-
Date project commenced:	1st September 2005
Date completion due:	30th June 2007
Key words:	Carrot, cavity spot, *Pythium* spp., soil testing, PCR, disease prediction

Signed on behalf of: **Warwick HRI**

Signature:…………………………………… ***Date***:…………………………….

Name Professor Simon Bright
Director and Head of Department

Whilst reports issued under the auspices of the HDC are prepared from the best available information, neither the authors nor the HDC can accept any responsibility for inaccuracy or liability for loss, damage or injury from the application of any concept or procedure discussed.

© 2007 Horticultural Development Council

The contents of this publication are strictly private to HDC members. No part of this publication may be copied or reproduced in any form or by any means without prior written permission of the Horticultural Development Council.

The results and conclusions in this report are based on a series of experiments conducted over a two-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.

© 2007 Horticultural Development Council

CONTENTS

	Page
Grower Summary	1
Headline	1
Background and expected deliverables	1
Summary of the project and main conclusions	2
Financial benefits	4
Action points for growers	5
Science section	6
Introduction	6
Materials and Methods	7
Results and Discussion	9
Conclusions	24
Technology transfer	25
References	26
Appendix 1	27
Appendix 2	28

© 2007 Horticultural Development Council

FV 5g

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

Grower Summary

Headline

- None of the five tests for *Pythium* species as used here provided useful predictive information about the levels of cavity spot recorded in the four fields assessed. On this evidence these Norwegian tests cannot be recommended to growers in the UK.
- Neither crater rot (*Fibularhizoctonia carotae)* or liquorice rot (*Mycocentrospora acerina)* disease occurred in the four fields. The two fungi were detected but it is assumed that the levels found were too low to cause disease by harvest. It is possible that higher levels of the fungi, and consequently disease, may occur in other years and more evidence is needed before strong recommendations can be made for the tests for these two fungi. However, on this evidence these tests cannot be recommended to UK growers.

Background and expected deliverables

Many carrot growers consider cavity spot to be the most important disease problem in this crop and even low levels can cause economic loss. It has been thought that in the UK cavity spot is mainly due to *Pythium violae* or, more rarely, *Pythium 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 *Pythium violae* and *Pythium sulcatum*. It was not clear at the start of this project whether all five of these are important, or even occur, in the UK.

Scientists at Bioforsk (Norwegian Institute for Agricultural and Environmental Research) have developed PCR assays and associated soil extraction procedure that allows detection in soils of the five *Pythium* species they believe important for the disease in that country and this project aimed to assess whether these tests are useful in predicting cavity spot levels in the UK.

The scientific objectives of the project were 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 could be correlated with cavity spot in this country.

© 2007 Horticultural Development Council

The tests are already offered as a commercial service and the prime practical objective of the project was to assess whether soil testing using the Norwegian assays could be a valuable and cost-effective cavity spot disease management tool for UK growers. If disease predictions made in the autumn prior to the crop being grown are accurate then they could be used to determine which fields are most suitable for renting or drilling. Accurate predictions made during growing season of disease levels later in the season would allow more informed choices about crop management, particularly marketing times.

Summary of the project and main conclusions

Four fields in England in which carrots were to be grown on a commercial basis in 2006 were selected. These were sampled six times (October 2005, April, August and November 2006, January and May 2007) over one complete crop cycle. In each field, six sites were located by GPS and field soil taken on all occasions from each of these sites. In December/January, 2006/7, 100 roots were collected from each of the 24 sites and assessed for cavity spot, crater and liquorice rots. Soil from the surface of roots (SOCS) was also collected in January 2007. Weather stations were used to collect environmental data from each field.

All samples were sent to Carrotech AS of Norway for testing in the laboratories of Bioforsk and tested for the presence of five *Pythium* spp. (*Pythium intermedium, Pythium sulcatum, Pythium sylvaticum, Pythium violae* and *Pythium* "vipa"), *Mycocentrospora acerina* (cause of liquorice rot) and *Fibularhizoctonia carotae* (crater rot). The normal commercial service offered suggests that samples from individual fields are mixed together so that the soil extraction/PCR analysis gives a single result intended to represent the entire field for disease management purposes. In this project the six samples from each individual field were tested separately in order to give greater resolution to the results. Soil extraction and the PCR process were carried out according to the Bioforsk protocol for commercial testing.

The potential usefulness of these tests to UK growers was assessed by looking at the correlation of the PCR detection of the various fungi and disease levels, both at individual sites and averaged across fields.

Cavity spot was found on carrots in all four fields although the disease was not seen at all of the 24 individual sites. The variation in the levels of occurrence of the disease was great enough to allow valid comparisons to be made with the PCR detection results.

No liquorice rot or crater rot were found on the carrots samples. The relevant fungi were detected at varying levels in at least some fields so it must be assumed that these levels of fungal inoculum were insufficient to give disease under the prevailing conditions. These two

diseases are primarily storage diseases and so this result was perhaps not surprising as the growers followed the normal UK practice of keeping the crops in the ground rather than putting them into cold-store. On the evidence here these two tests cannot be recommended to UK growers as general management tools useful with these diseases. However, in circumstances where the inoculum levels are higher or conditions different they might prove of value. This is particularly true for crops which are to be cold-stored and it must be stressed that this project did not address the specific application of these tests to such crops.

Both *Pythium sylvaticum* and *P. sulcatum* were detected only sporadically and at low levels during the project. No correlation between the occurrence of these two species and cavity spot was seen at the all sites level.

Pythium "vipa" is a new species (although not yet formally described) discovered by scientists at Bioforsk and thought to be associated with cavity spot in Norway. It had not been previously reported in the UK. This fungus was found at high levels in the samples collected from all four fields in August 2006, but not in earlier samples, and at rapidly declining levels in later samples. The levels seen were not correlated with cavity spot at the all site level. It is not known whether this species is associated with some other disease in the UK but no obvious symptoms of any new disease were seen. The overall importance of the apparent detection of this pathogen in the UK is not known.

Pythium intermedium was widespread, being detected in all four fields and most individual samples, often at high levels. There was a general, if somewhat variable tendency for levels to increase to a maximum in August 2006. There was no correlation between levels of this species detected and disease either at the field level or across individual sites. There appeared to be some increase in levels between the October 2005 and April 2006 (when the carrots were drilled) suggesting that there was no link between this species and carrot as a specific host. Similarly this species seemed to increase in the soil after the removal of the crop, again suggesting it does not have a specific host/pathogen relationship with carrots.

Pythium violae is "traditionally" thought to be the primary pathogen responsible for cavity spot in the UK and it was therefore disappointing that there was little detection of this species in the majority of samples. However, the only positive correlation seen between the detection of any *Pythium* species and cavity spot disease was for the "soil off carrot surfaces" collected in January where the very high level of disease in one field was associated with the highest level of detection of this species. Whilst this gives some support to *Pythium violae* being the primary pathogen causing cavity spot in the UK, it is of no predictive/management value to UK growers.

© 2007 Horticultural Development Council

In recent discussion with scientists at Bioforsk, they suggested that testing “soil off carrot surfaces” in the early part of the season might be useful for predicting final disease levels but this was not tested here.

On the basis of the evidence gathered in this project, none of the five PCR tests for the detection of *Pythium* species, at least in the form tested here and offered commercially, appears to have any value as management tools for UK carrot growers and cannot be recommended to them.

Financial benefits

The Norwegian tests for *Pythium* species as offered commercially provided no predictive information that would be useful to growers in the management of cavity spot and no financial benefit to growers would arise from using them.

The case for testing for the crater rot and liquorice rot pathogens is unproven. However, as these diseases are generally not very important in the UK, they are also unlikely to provide any financial benefit to UK growers. (These two diseases are primarily storage diseases and these tests might provide valuable information for growers intending to cold-store carrots but this was not tested in this project.)

Action points for growers

- The PCR tests are commercially available to growers through Carrotech AS of Norway (at a current cost of approximately £110 per field plus collecting and shipping of the samples).
- For the UK fields used in this project, the tests for the five *Pythium* species, as assessed here, did not give information predictive of the incidence of cavity spot and they cannot be recommended to UK growers when used in their current form.
- Testing soil adhering to carrot surfaces (SOCS) early in the season was not assessed here but might potentially provide useful predictions of later disease levels; this should be further explored before final conclusions about the value of the tests are drawn.

© 2007 Horticultural Development Council

- The case for testing for the agents of crater and liquorice rots remains unproven as neither disease was found at these four sites. It may be that in fields with higher levels of these pathogens or in other seasons these tests might prove useful. However, these diseases are generally of lesser importance in the UK and the tests are unlikely to give any financial benefit if used as general management tools.
- As crater and liquorice rots are mainly diseases of stored carrots, growers who take up the use of cold stores for carrots may need to reconsider whether these two tests could be of use to them. The usefulness of the tests for managing carrots to be cold-stored was not assessed in this project.

Science Section

Introduction

Carrots are a major crop in the UK (marketed value £125M-£167M 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 disease 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 *Pythium 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 this two 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 *Pythium violae* and *Pythium 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 could potentially 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. With many other soil-borne diseases rapid procedures for the assessment of soil inoculum levels have proved to be effective management tools for disease avoidance and other control strategies. No such tool is available for carrot cavity spot disease. Direct isolation of the fungi is slow and difficult, limited to small numbers of samples and is very poorly quantitative. An immunodiagnostic test for *Pythium violae* has been developed but it lacks sensitivity and specificity, limiting its use to the winter period. For this disease it was felt that quantitative measurements would be particularly important as 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 was superseded by advances in Norway, where scientists at Bioforsk (Norwegian Institute for Agricultural and Environmental Research) developed PCR assays, and associated soil extraction procedures for the five *Pythium* species they believe important for this disease in that country (Hermansen *et al*., 2007; Klemsdal *et al*., 2007).

The project reported on here involved collecting soil and crop samples from the UK and submitting them for testing for the five *Pythium* species in Norway using their PCR-based procedure. The objectives were two-fold. The scientific objectives were (i) to assess whether the five species all occur in the UK, (ii) to determine which of them are associated with cavity spot in the UK and (iii) to study the dynamics of the fungal population through the season in a more extensive way than is being currently done in an intensive way at Warwick HRI in a Defra-funded project.

However, the prime objective of the project was to assess whether soil testing using the Norwegian assays could be a valuable disease management tool for cavity spot for UK growers, allowing them to predict disease levels likely to arise from inoculum levels measured at times when important decisions are to be made.

Materials and Methods

Four field sites were identified in 2005 by Plantsystems. These sites were all to be cropped, on a commercial basis, with carrots 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. Precipitation and soil temperatures were recorded at all four fields using commercial weather stations.

Soil samples were collected from six points in each field and sent to Carrotech AS (Frederik A. Dahlsvei 20, N-1432 Ås, Norway) for testing in the laboratories of Bioforsk. Samples were collected according to the procedure determined by Carrotech except that the samples from individual sites within fields were not amalgamated but were tested individually. The samples were normally field soil collected from carrot beds but not actually on the roots of carrot plants. At the winter 2006/7 sampling, soil off carrot surfaces (SOCS) was also collected by brushing off soil adhering to harvested roots according to the Carrotech protocol. This SOCS material represents the first few millimeters of soil moving away from the root but the precise “depth” of the sample cannot be closely controlled.

Precise details of the locations of the sites are confidential but relevant information on the crops is given in Table 1. Collection dates are given in Table 2.

Table 1. Varieties, drilling dates and other parameters for the four field sites.

County locations of fields	Variety	Drilled	Soil type	Last carrot crop	Approx Field size
Lincolnshire	Nepal	6-5-2006	Sandy loam	1996	15.7 ha
Norfolk	Nairobi	4-6-2006	Loamy sand	2002	32 ha
Nottinghamshire	Nairobi*	3-7-2006*	Sandy loam	Not Known	10-12 ha
Yorkshire	Nairobi	25-5-2006	Loamy sand	Not Known	8-9 ha

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

Table 2. Dates for collections from the four fields. Roots for disease assessments and SOCS were collected on the fifth occasion.

Collection					
1st	2nd	3rd	4th	5th	6th
25-10-2005	10-04-2006	11-08-2006	1-11-2006	9-1-2007 except Yorks 4-12-2006	5-5-2006

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

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

0 – Pathogen not detected

1 – Low levels

2 – Moderate levels

3 – High levels

Disease assessments were done by visual inspection of 100 roots per site by PlantSystems staff. Harvesting of roots for disease assessment and collection of SOCS for three fields was done in January 2007 but at the Yorkshire field the grower harvested earlier and samples were taken in early December.

Correlations between disease levels and PCR detection was analyzed by linear regression using pathogen levels as the explanatory variable. Although data for individual fields was examined informally for correlation of disease and fungus levels, in the majority of formal analyses field information was ignored and all 24 sites used. Linear regressions were only fitted to time and pathogen combinations where the pathogen was detected in at least two separate sites. For each disease measure standard errors, t-probability and power values were generated. The primary t-probabilities considered were based on a one sided test that the slope is positive was used on the assumption that the fungus being assessed is the causal pathogen. On the basis that one or more species (particularly of the *Pythium* spp.) might be acting to suppress the true pathogen and thus be inversely related to disease levels, two-sided t-probabilities were also generated.

Tables of the main statistical analyses are given in appendix 2

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, Norfolk, Nottinghamshire and Yorkshire. All sites were to be managed by commercial growers using normal practices without metalaxyl treatment. Three were drilled with carrots in spring 2006. Due to poor spring weather, one site (Nottinghamshire) was drilled with parsnips by the grower and in order to get an estimate of the cavity spot disease incidence in carrots for that field, six small plots of carrot were established close to the soil sampling points. As much as was available of the previous crop history for the fields was recorded (Table 3).

Table 3. Previous crops where known for the four fields used.

Year	Lincolnshire	Norfolk	Nottinghamshire	Yorkshire
1995	Sugar Beet	-	-	Spring Barley

© 2007 Horticultural Development Council

1996	Carrots	-	-	Spring Barley
1997	Linseed	Winter Barley	-	Potatoes
1998	Sugar Beet	Sugar Beet	-	Winter Wheat
1999	Potatoes	Winter Barley	-	Linseed
2000	Winter Wheat	Potatoes	-	Winter Wheat
2001	Peas	Winter Wheat	-	Sugar Beet
2002	Onions	Carrots	-	Spring Barley
2003	Winter Wheat	Linseed	-	Spring Barley
2004	Sugar Beet	Potatoes	-	Sugar Beet
2005	Winter Wheat	Winter Wheat	Winter Wheat	Winter Wheat

Sample Collection.

Soil samples were collected from the sites on six occasions and SOCS on one occasion (Table 2). 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. Similarly the fourth date could be used to look for potential decline in crops kept for late harvest. The fifth and sixth dates were intended to help understand the full cycle of each of the fungi over a full growing season.

All samples were sent to Carrotech for DNA extraction and PCR testing for the five species of *Pythium* and *M. acerina* and *F. carotae*.

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 (for early examples see Table 4). The reproducibility of the soil sampling by collecting and testing multiple samples from one site on a single occasion was not directly assessed.

Table 4. Typical 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).

Field - Site	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
Field 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
Field 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
Field 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
Field Average	*0.1*	*0.0*	*0.0*	*0.3*	*0.0*

The relationships of the four-point scales to the absolute levels of the various pathogens are not known but 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 amounts of fungal material present.

To allow easy comparison between fields, values for each field/sampling time were averaged (Appendix 1). This is presumed to approximately represent what a grower would receive as a single value for fields. Comparisons with disease levels were made at both the field average and individual site levels.

© 2007 Horticultural Development Council

PCR Testing - Mycocentrospora acerina *(liquorice rot) and* Fibularhizoctonia carotae *(crater rot) up to harvest*

Although not formally part of this project, samples sent to Carrotech are routinely tested for *Mycocentrospora acerina* and *Fibularhizoctonia carotae.* See appendix 1 for results for these two fungi at the field level.

Fibularhizoctonia carotae was detected at a low level in a single field in October 2005 but not in April or August 2006. This fungus was detected in three fields in November and January 2007. Within each field the fungus was detected at only some of the sites. The fungus was also detected in SOCS for the same three fields. The significance of such low level, rather sporadic detection is not clear, particularly as no disease was detected (see below).

Mycocentrospora acerina was detected in soil from two fields (Norfolk and Nottinghamshire) at low to moderate at most sampling times. It was also detected at varying levels in the SOCS collected in January, occurring in SOCS from some sites within these fields at the maximum detectable level. The significance of these levels of pathogen under UK conditions is unknown (no corresponding disease was recorded (see below)) but may warrant further investigation, particularly in relation to crops to be cold-stored as liquorice rot is primarily a storage disease.

PCR Testing - Pythium *species up to harvest*

The primary focus of this project is cavity spot and the occurrence of five *Pythium* species in the UK. See appendix 1 for results for these five species at the field level.

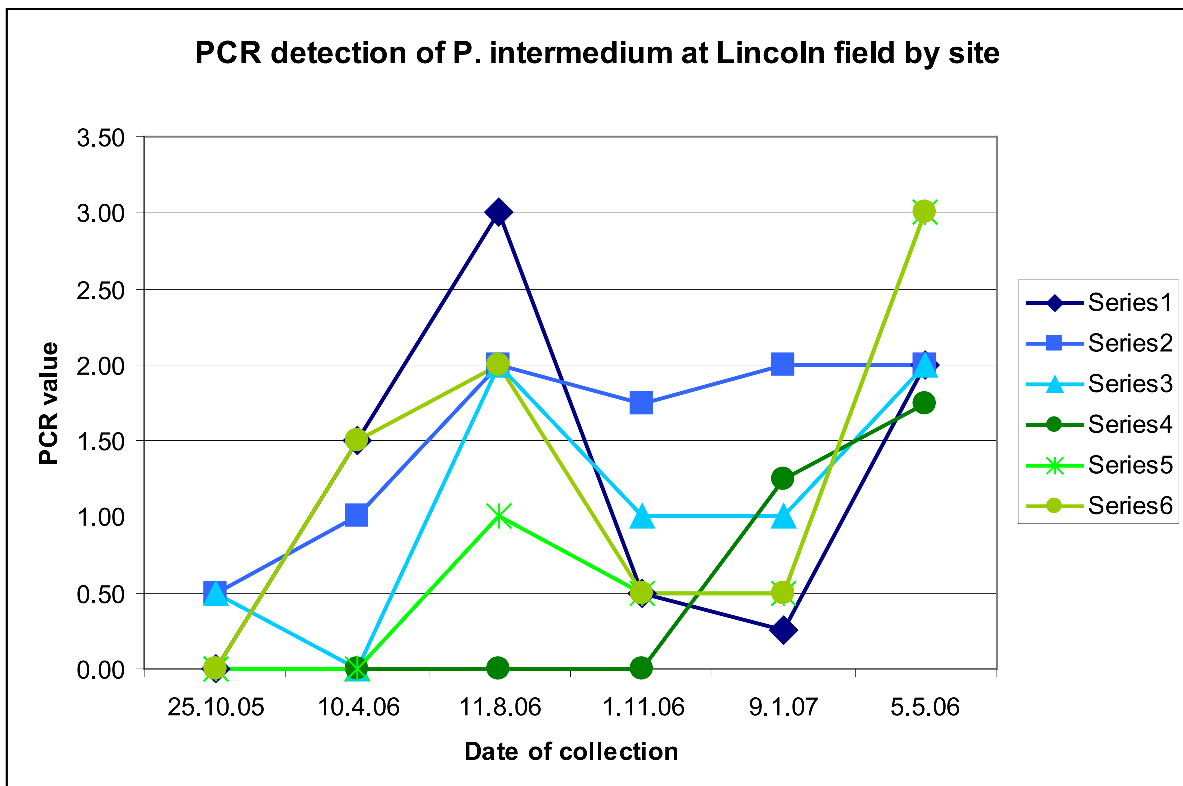
1. *Pythium violae.* Previous work on cavity spot has suggested that this is the primary cause of the disease in the UK but this species was detected only once during the pre-season and growing season soil testing (Lincolnshire field, site 3). In the SOCS at harvest time it was detected at four sites (Nottinghamshire 4 and Lincolnshire 3, 5 and 6). The levels detected in SOCS at the three Lincolnshire sites were quite high and later correlations with disease were strongly influenced buy these three data points.
2. *Pythium intermedium.* This species has been thought a minor cavity spot pathogen in the UK but is now considered to be a significant part of the disease in Norway. This species was detected in all four fields on all occasions and in the majority of sites within fields. There was internal consistency in the results in that sites that were negative on one occasion tended to be those that were negative at other times. But

© 2007 Horticultural Development Council

no site was always negative and this species was detected at all sites in SOCS at harvest.

Levels of *Pythium intermedium* generally increased to a peak in August and fell away slightly but there was considerable variation in this pattern (See Fig 1 for Lincolnshire as an example). At many individual sites and at the fields average level for the three fields with lowest levels in Autumn 2005, there was an apparent increase in levels between the Autumn 2005 sample and Spring 2006, suggesting that growth of this species was not intimately linked with the presence of carrots.

Fig 1. Changes over the year in levels of *P. intermedium* detectable by PCR at six sites in the Lincolnshire field.



----

3. *Pythium* “vipa”. Although never detected before in the UK (it is newly described from Norway) this species was found in all four fields in August 2006 (and at 23 of the 24 sites) but not before that date and in November at only three sites at Norfolk (which had the highest levels in August) and one in January. It was detected at low levels in two SOCS samples in January 2007. This was unexpected and to try to confirm the presence of *Pythium* “vipa” in the UK some of the samples from August were retested in both Norway and UK. The fungus was not detected in these retests, probably because the samples had been stored frozen for several months before

© 2007 Horticultural Development Council

retesting. These results suggest that this species is present in the UK but this needs confirmation.

4. *Pythium sylvaticum.* This species was not detected before August 2006 when it was detected at five sites in two fields (Norfolk and Lincolnshire). It was next detected in two of the same sites in January 2007 (one each field). Surprisingly it was also detected in SOCS in January at three sites from Lincolnshire. Most detection was at low levels with only one result being greater than 1.
5. *Pythium sulcatum*. Was detected on only two occasions up to harvest and on both occasions at one site (Norfolk 5 in November at moderate level and Lincolnshire 2 in January at low level).

PCR testing post harvest.

For growers the potential importance of these tests is as management tools prior to harvest. However, scientifically they can potentially tell us more about the life cycles of the organisms and specifically about the level of return of inoculum to the soil.

All seven species were tested for in early May, 4-5 months after harvest of the carrots from the fields.

1. *Mycocentrospora acerina.* Detected at moderate to high levels at 10 of the 12 sites in the Norfolk and Nottinghamshire fields but not detected at Lincolnshire or Yorkshire. This closely reflects what was detected in SOCS in January but levels were higher than in January soil. Overall the carrot crop appears to return high levels of *M. acerina* inoculum to soils but whether the increase in levels detectable in soil between January and May 2007 reflects growth in the soil or, possibly more likely, redistribution at harvest of the high amounts present close to the roots is not clear.
2. *Fibularhizoctonia carotae.* Detected only in the Norfolk field, at very low levels at one site and very high levels at a second. The one very high site is anomalous; generally *F. carotae* seems to be reducing after the crop is removed but the significance of the one high site is unclear.
3. *Pythium intermedium.* Detected at high levels, frequently at the maximum score of 3, at all sites in all four fields. Levels were slightly lower in the Lincolnshire field than at the other three fields. The levels detected in May were much higher than those recorded for open soil collected in January and somewhat higher than those for January SOCS. This suggests that not only do carrot crops return high levels of *P. intermedium* to soils after harvest but that the levels continue increase after harvest, probably by saprotrophic growth on debris.

© 2007 Horticultural Development Council

4. *Pythium violae.* Detected at generally low levels at four sites spread over three of the four fields. Surprisingly not detected at Lincolnshire where the highest levels were detected in SOCS in January. This suggests that infected carrots do not return large amounts of *P. violae* inoculum to the soil at the end of the growing season.
5. *Pythium* "vipa". Not detected. Only detected sporadically at low level in January.
6. *Pythium sylvaticum.* Detected only at very low levels at one site each at Norfolk and Yorkshire; the same fields and one out two of the same sites where it had been detected in January soil.
7. *Pythium sulcatum*. As expected this species was not detected post-harvest as it had been detected only at very low levels at one site in soil in January.

Disease Occurrences.

The levels of cavity spot are shown in table 5. Two measures of disease were primarily used *viz*. proportion of roots affected and the average number of lesions per affected root. There was clear variation within fields with at worst the proportion of roots affected varying from 0 to 37% (Lincolnshire). At the Lincolnshire field there was an average of 7.6 lesions per affected root whilst the other three sites ranged from 1.6 to 1.9 per affected root.

The worst affected site, both in terms of proportion of roots affected and average lesions per affected root, used the variety Nepal and the high level of disease might reflect varietal susceptibility (all other fields used Nairobi). Similarly the badly affected Lincolnshire site was the first to be drilled (6th May), the second most severely affected (by roots affected) was Yorkshire which was also the second drilled (25th May). With the limited number of fields used and no replication, these effects cannot be differentiated from each other or other field or environmental effects on disease levels. All four sites had similar soils, being either sandy loam or loamy sand (Table 1) but there was no correlation between the two soil types and disease levels.

It is important to note that all lesions fitting the cavity spot type were recorded and used for correlation with fungus detection by PCR. It is possible, probable even, that one ætiologic agent is not responsible for all the lesions, for example *Pythium violae*, *Pythium sulcatum* and *Pythium intermedium* might all cause a proportion of the lesions. However, as neither we or a grower has any simple way of distinguishing lesions caused by different species and commercial growers are concerned with total disease anyway, correlation between PCR detection and lesion numbers used this total figure. Combinations of different PCR measures were used to look for evidence that two or more pathogens were important.

© 2007 Horticultural Development Council

Neither crater rot or liquorice rot was detected in any sample.

Table 5. Disease data for the four fields/24 sites assessed. No crater rot or liquorice rot was recorded at any of the four sites.

	No. Roots	Roots with lesions	Ave. No. Lesions per affected root	
Field - Lincs		**Sample Date**	**09.01.07**	
Site 1	100	0		0.0
2	100	5		2.0
3	100	37		11.0
4	100	6		2.0
5	100	24		5.0
6	100	0		0.0
	Total	*72*	*Average*	*7.6*
Field - Norfolk		**Sample Date**	**09.01.07**	
Site 1	100	4		2.0
2	100	7		1.5
3	100	0		0.0
4	100	0		0.0
5	100	0		0.0
6	100	0		0.0
	Total	*11*	*Average*	*1.6*
Field - Notts		**Sample Date**	**09.01.07**	
Site 1	100	9		1.5
2	100	5		1.0
3	100	0		0.0
4	100	8		3.0
5	100	0		0.0
6	100	0		0.0
	Total	*22*	*Average*	*1.9*
Field - Yorks		**Sample Date**	**04.12.07**	
1	100	10		2.0
2	100	7		1.0
3	100	6		2.0
4	100	11		2.0
5	100	9		2.0
6	100	5		1.0
	Total	*48*	*Average*	*1.7*

Environmental and historical effects on disease levels.

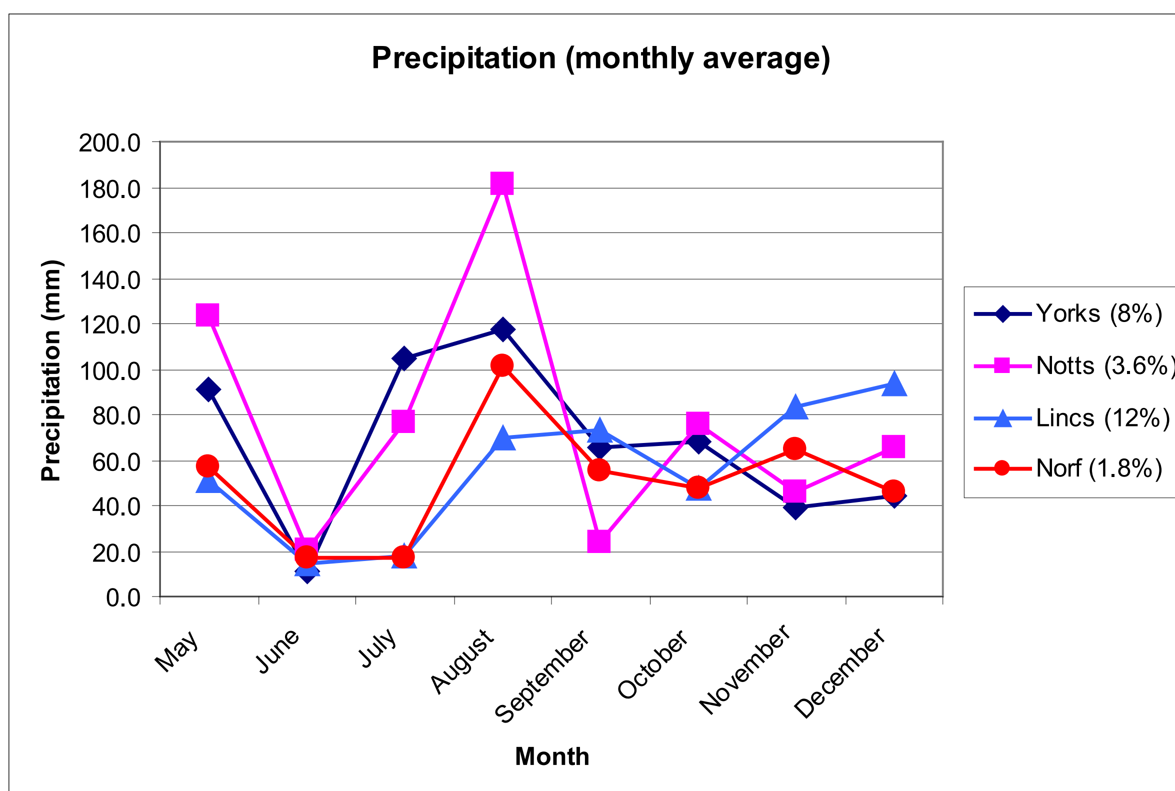
Previous crops are often an important factor in the severity of soil-borne disease and many growers consider this to be the case for cavity spot. Similar ranges of crops (cereals/sugar beet/potatoes/linseed) were grown at all sites except for one crop each of peas and onions in one field and two had been used for carrot crops (Table 3). All had winter wheat in 2005 and so the immediately preceding crop is not a factor in the differing levels of disease seen. However, the two most seriously affected had sugar beet the year previously; whether this crop contributes to high disease levels is being investigated in another project.

© 2007 Horticultural Development Council

The field which had had the most recent carrot crop (Norfolk in 2002) gave the lowest level of cavity spot disease, suggesting a recent history of the crop does not promote the disease. The field with the highest disease level (Lincolnshire) had had a carrot crop 10 years previously but this is unlikely to be significant after such a long interval. (But of course we do not know the initial states of the fields and so we cannot be certain of these conclusions on such limited data.)

Environmental data was gathered only at the field level and so analysis of its relationship to disease levels will only have limited power. There was no clear correlation of average disease for the fields with precipitation over the period of drilling to harvest. There was little difference between the fields with highest and lowest disease (Fig 2). The two fields with intermediate disease levels had higher rainfall than the other two during June to September, the period which (based on concurrent Defra-funded work) is probably crucial for disease development, and were rather similar to each other. Nor was there any relationship to heavy episodic rainfall (data not shown). However, no records of irrigation applied were taken and so there may still be a relation between soil water and disease levels.

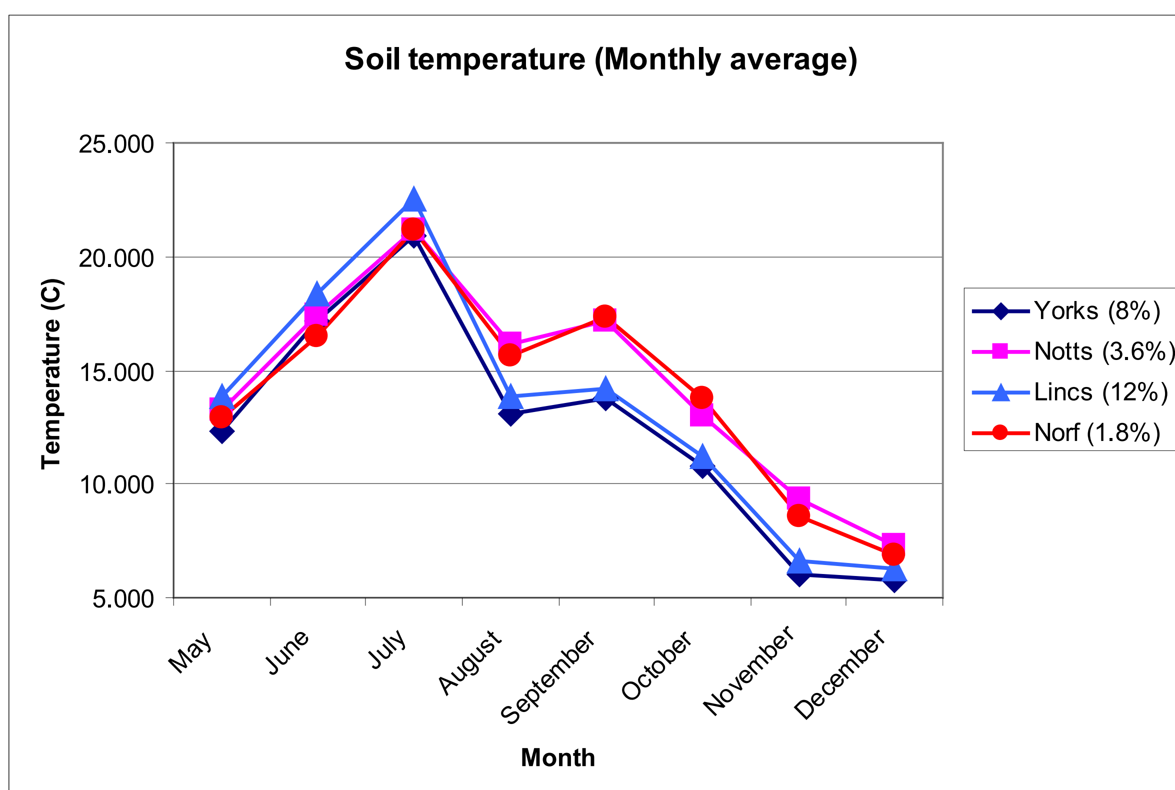
Fig 2. Monthly precipitation at the four fields for the period between drilling and harvest. Figures in parentheses indicate disease levels (as proportion of roots affected) for the four fields at the field average level.



© 2007 Horticultural Development Council

Early in the season soil temperatures at the four sites did not differ greatly but there was a broad correlation between soil temperatures between July and November with disease levels. The two fields with the highest disease levels had lower soil temperatures by about 3-4°C than did the two fields with the lowest disease levels (Fig 3). Previously it has been suggested that soil temperature is not an important factor in the development of cavity spot (see Hiltunen and White, 2002) but the results here are suggestive of a linkage and this will be followed up in other projects to improve our understanding of the disease.

Fig 3. Monthly averages of soil temperatures at the four fields for the period between drilling and harvest. Figures in parentheses indicate disease levels (as proportion of roots affected) for the four fields at the field average level.



Correlation of disease with PCR detection.

1. Crater rot and liquorice rot vs *Fibularhizoctonia carotae* and *Mycocentrospora acerina*

Clearly as neither disease was seen in any sample, no strong conclusions can be drawn for the usefulness of tests for these two pathogens. Levels of *Fibularhizoctonia carotae* detected were generally low but those of *Mycocentrospora acerina* were moderate to high in two of the four fields. It is assumed that these levels were insufficient to cause disease under these conditions. Whether in other conditions or where even higher levels of the fungi are present,

© 2007 Horticultural Development Council

detectable levels of them would correlate with disease is not known. These two diseases are primarily diseases of stored carrots and these tests might be of value to growers intending to cold-store their crop but this was not tested here as no samples from the crops were put into such storage.

2. *Pythium sylvaticum* and *Pythium sulcatum*

Both these species were detected only sporadically and at low levels by PCR in either soil or SOCS. There was no correlation with disease levels except for with *Pythium sulcatum* at the field average level in January at Lincolnshire. However, this was based almost entirely on a single data point (Lincolnshire 2) and there was no correlation at the site level within the Lincolnshire field (for example the highest disease level was associated with no detection by PCR). Similarly there were apparent correlations for both *Pythium sulcatum* and *Pythium sylvaticum* in January SOCS but again these are based on very limited data and low levels of detection.

3. *Pythium intermedium.*

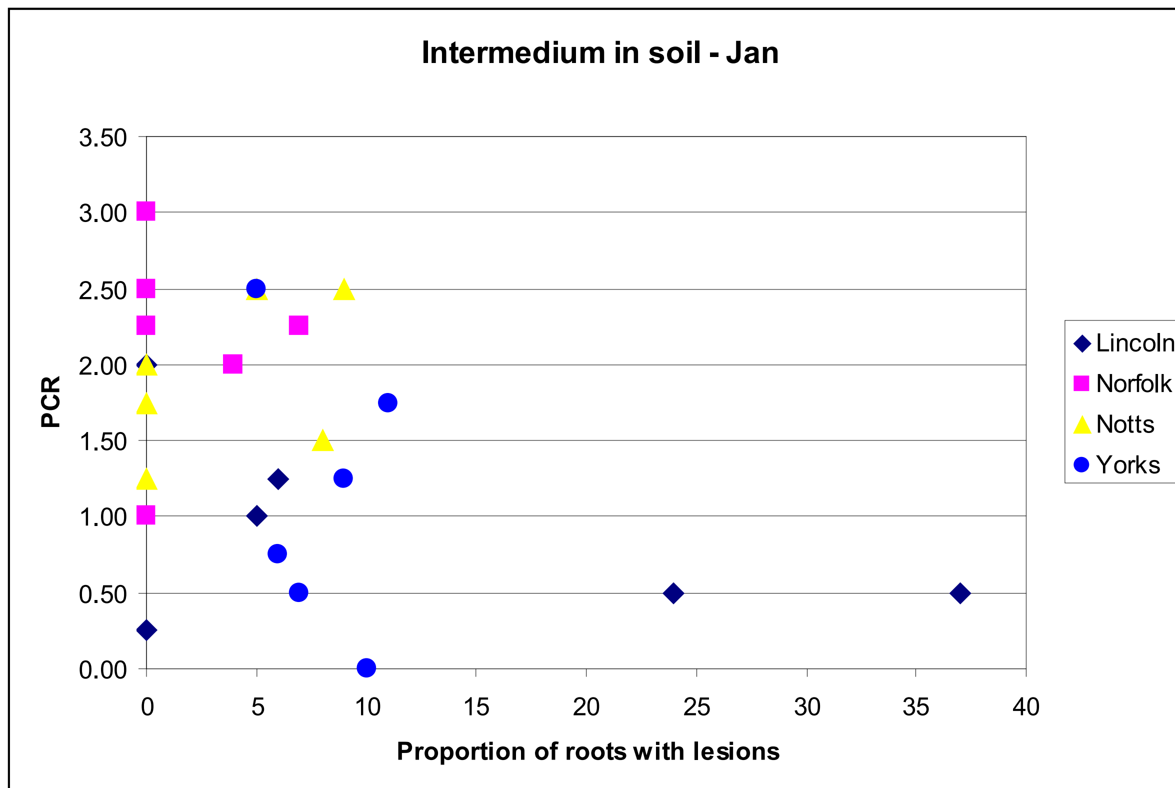
Pythium intermedium was widespread and common but there was no positive correlation with cavity spot levels. As noted the levels seem to increase even before carrots were drilled and in the UK this species seems to be mainly either a rhizoplane inhabitant or saprophyte found more generally in the soil. It may be the specific pathogen in some lesions but its lack of positive correlation with overall lesion numbers suggests that it is at best a minor pathogen in the UK.

There appeared to be a negative correlation of levels of *Pythium intermedium* with average number of lesions per root and with proportion of roots affected for the January collection. This was statistically just significant at the 5% level. This might suggest that this species is somehow antagonistic to the formation of cavity spot (by being antagonistic to *Pythium violae*?). However, this negative correlation is primarily based on just two Lincolnshire data points with high disease levels and low PCR levels (Fig 4) and the low level of statistical significance and limitation to the one collection date suggests this result be treated with great caution.

For all comparisons of PCR detection with disease, the use of cv Nepal at one site may be a confounding factor with varietal susceptibility explaining why this site had higher disease levels than did the other three. For both *Pythium intermedium* and *Pythium* “vipa” (August sampling) there was enough data to look at the correlation over three fields (18 sites) rather than four (24 sites). In both cases this did not reveal a correlation hidden by the inclusion of the Lincolnshire (high disease) data.

© 2007 Horticultural Development Council

Fig 4. Levels of *Pythium intermedium* in soil in January for the 24 sites plotted against proportion of roots with lesions. Note how two data points stand out and affect the correlation.



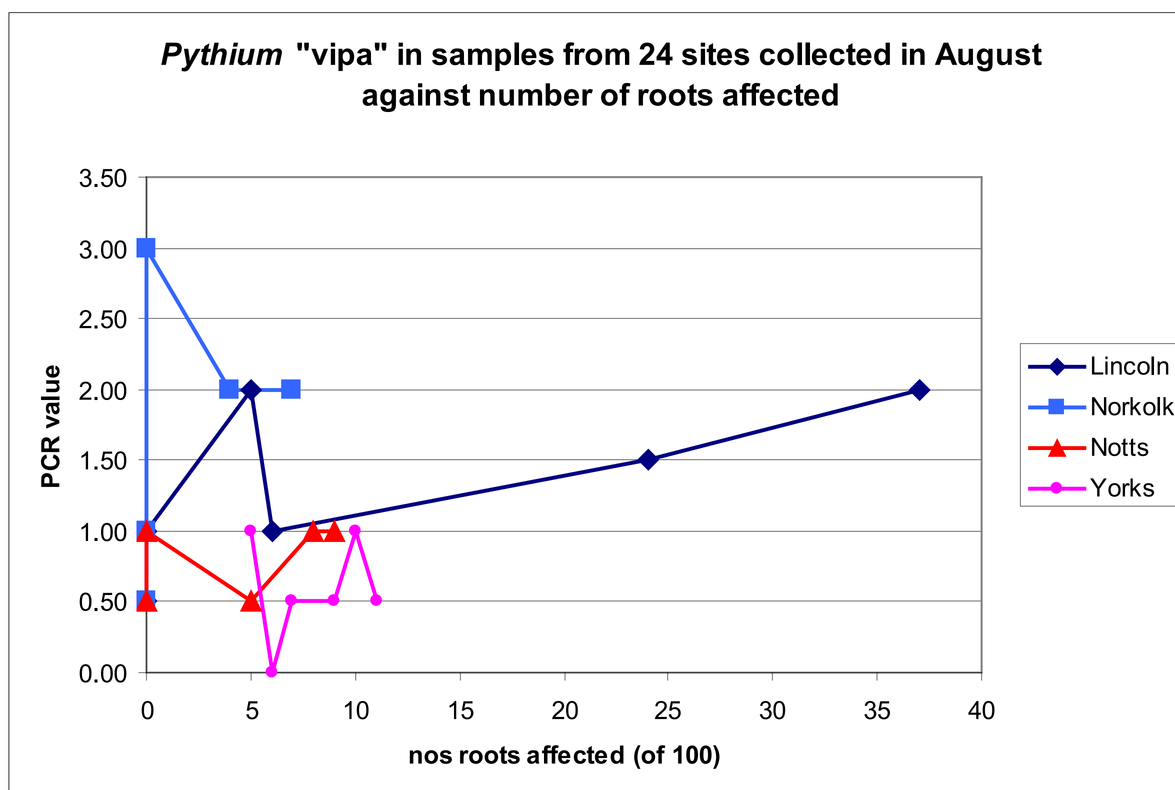
----

4. *Pythium* "vipa"

This species apparently occurred at all four fields but only in high levels on one occasion and showed no correlation with disease levels at the all site level (Fig 5). (As with *Pythium sulcatum* and *Pythium sylvaticum*, there was a correlation at the field average level for the January data but this is based on extremely limited number of detections at very low levels and must be treated with extreme caution).

© 2007 Horticultural Development Council

Fig 5. PCR detection of *Pythium* “vipa” in August 2006 plotted against disease (as number of roots affected) for all 24 sites. (Note there appear to be fewer points because on some occasions two data points fall together.)



----

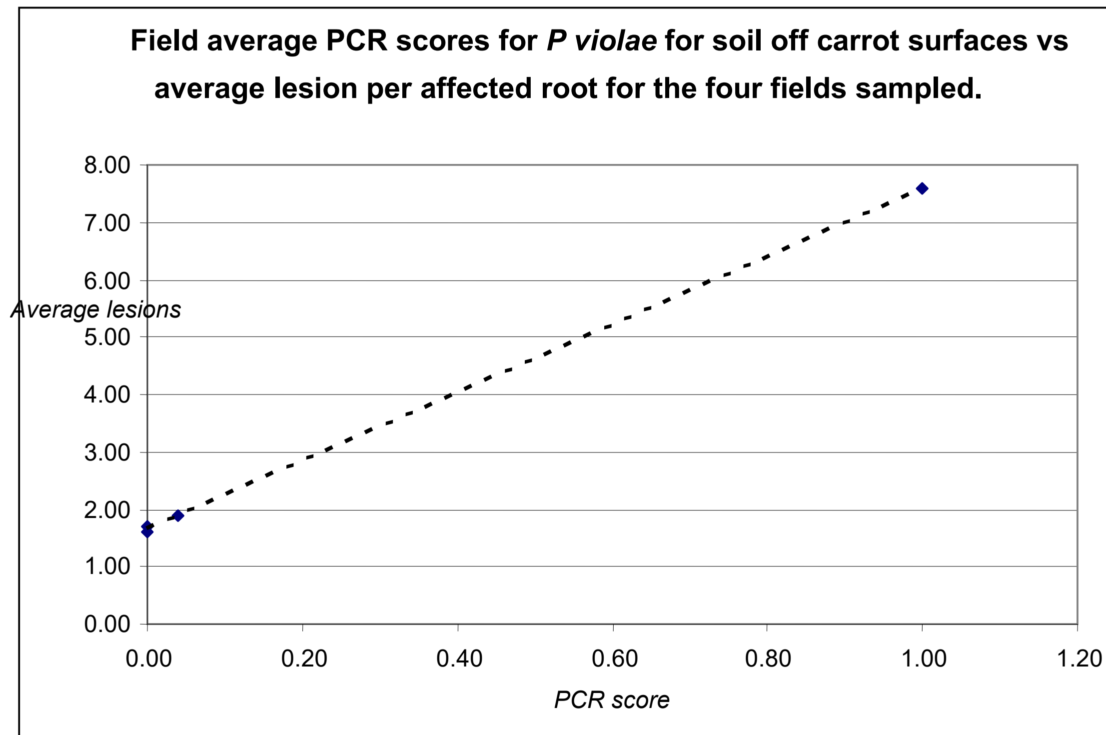
5. *Pythium violae*

The virtual lack of detection of this species in soil samples obviously meant that no correlation of this measure with disease levels was seen. However, the only highly significant ($t < 0.001$), correlation with disease seen in this project at the 24 site level was of this species in SOCS with both measures of disease. As all detections except one were at the Lincolnshire field then this correlation is possibly only relevant to the variety Nepal. A correlation was also seen at the whole field level but this is largely based on the high disease levels in the single field at Lincoln (Fig 6). However, backed by the correlation at the 24 site level, this correlation is probably more meaningful than the other correlations at the whole field level. Clearly any correlations found at this stage of the season might be of scientific interest but is of no value as a predictive management tool.

Overall the correlation even within this one field supports the suggestion that *Pythium violae* is the primary pathogen, but this should be regarded as an indication only because whilst in the other three fields there was variation in disease (average lesions/affected root) there was limited low detection (one site only). Whether *Pythium violae* is the primary pathogen at these sites is not clear from this data.

© 2007 Horticultural Development Council

Fig 6. Correlation of detection of *Pythium violae* in January SOCS with average lesions for the four fields



----

6. *All* Pythium *species.*

The possibility of combinations of levels of two species being better predictors than levels of any single species was considered. However, the combinations giving statistically significant correlations were with *Pythium violae* in SOCS which was itself statistically significant (see appendices) and combining fungal assessments did not improve the predictive power of the tests and will not be considered further here. Similarly the possible combination of levels of *Pythium intermedium* at all assessments times with the levels of *Pythium "vipa"* seen in August was specifically considered but did not give significant positive correlation with cavity spot levels but did give a negative correlation for January *Pythium intermedium* detection. As above this was not an improvement on the *Pythium intermedium* results alone.

© 2007 Horticultural Development Council

Conclusions

Practical objectives

- The lack of either crater or liquorice rot diseases meant that we cannot draw firm conclusions as to these two tests. However, as they are primarily storage disease it seems unlikely that they will be of value as a general management tool for UK growers. These two tests may be value for crops intended for cold-storage but this was not specifically addressed in this project.
- No correlation of predictive management value to UK growers between levels of any of the five *Pythium* species and cavity spot disease was found. On the basis of this evidence, none of these five tests can be recommended to UK carrot growers in the current form. (In discussions with Norwegian scientists it was suggested that in-season testing of SOCS may provide a prediction of future disease. However, data emerging from a parallel Defra-funded project suggests that in the UK the time window for such testing will be rather limited and harvesting decisions might be better based on a disease assessment in late August. Further assessments of the modified protocols would be needed to determine if such tests would be truly useful in the UK.)

Scientific objectives

- Environmental monitoring did not show any link with precipitation (although this was compromised by not having a record of the levels of irrigation) but did suggest a possible link between lower temperatures soil between July and December and higher levels of disease. This will be further investigated elsewhere.
- The sole highly significant correlation seen for any *Pythium* species was between *Pythium violae* in soil off carrot surfaces collected in mid-winter 2006/7 and disease levels; this supports the idea that this species is the primary cavity spot pathogen in the UK. This conclusion is however largely based on the two sites within the Lincolnshire field which had high disease levels and it cannot be regarded as strongly supported here.
- A possible confounding factor is that cv Nepal was used in the field with the highest disease levels (cv Nairobi was used at the others) and this may be the reason for disease in the Lincolnshire field being so high. There is no “varietal susceptibility” consideration built into the commercial testing offered and so this factor was not considered here, particularly as reliable data on the field resistance of varieties is not available anyway. Correlations for the other three fields alone were considered but no useful predictive results were noted.

- Although *Pythium intermedium* is obviously common in the UK, being present in all four fields, often at high levels, the lack of any correlation between levels of detection and disease suggest that in the UK it is not a major primary pathogen for cavity spot.
- The lack of correlation with disease and apparent increase in levels at many sites prior to the planting of carrots suggest that in the UK *P. intermedium* is primarily either a rhizoplane inhabitant or more general soil saprophyte. This is reinforced by the apparent increase in levels in the soils after the crop was removed.
- The sporadic detection and generally low levels of *Pythium sylvaticum* or *Pythium sulcatum* in fields where cavity spot occurred suggests that neither of them are important in cavity spot disease in the UK (although this may not be the case is some other fields as *Pythium sulcatum* has been previously associated with the disease – see review by Hiltunen and White, 2002).
- The patterns of detection of *Pythium intermedium* have given us a greater understanding of the life cycle in soil of this species but for the other species the sporadic (and for *Pythium violae* disappointingly low) level of detection has meant we have learned little new about their life cycles. It is particularly noteworthy that *P. intermedium* appeared to increase in the soil after the removal of the crop.
- All five *Pythium* species now seem to be present in the UK; four were already known to be present but this is the first record for *Pythium* "vipa". As high levels of this species were only found on one occasion and no confirmatory isolations have been made, the detection of *Pythium "*vipa*"* needs to be confirmed. The significance of its presence and whether it is associated with disease need to be established.

Technology transfer

Presentations to events attended by growers and crop consultants

- International Onion and Carrot Conference & Exhibition; East of England Showgrounds, Peterborough: November 23/24, 2005. Dr Arne Hermansen (Bioforsk /Norwegian Institute for Agricultural and Environmental Research) described the development of the PCR tests and Norwegian experience with them. Dr Hermansen is part of the team at Bioforsk which developed the PCR tests used by Carrotech AS.
- BCGA/HDC Carrot Technical Seminar, PGRO, Thornhaugh, Cambs. 25/1/2007. Carrot cavity spot (CP46 & FV 5g). (D.J. Barbara)
- Syngenta Carrot Agronomy Meeting, Norton Disney, Lincoln 29/3/2007. Cavity spot – the latest research findings, future directions and possible applications. (D.J. Barbara).

© 2007 Horticultural Development Council

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.

Hermansen, A., Herrero M.-L., Gauslaa, E., Razzaghian J., Nærstad, R. and Klemsdal S.S. (2007). *Pythium* species associated with cavity spot on carrots in Norway. Annals of Applied Biology, 150:115-121

Klemsdal S.S., Herrero M.-L., Wanner, L.A., Lund, G. and Hermansen, A. (2007). PCR based identification of *Pythium* spp. causing cavity spot in carrots and sensitive detection in soils. Plant Pathology (in press).

Appendices

Appendix 1.

PCR results for the four fields as averages over the six sites per field for the seven PCR assays carried out on the samples by Carrotech AS.

	Soil 25/10/05	Soil 10/4/06	Soil 11/8/06	Soil 1/11/06	Soil 9/1/07 (4/12//06 Yorks)	Soil 5/5/12007	SOCS 1/1/07 (4/12/06 Yorks)
F carotae							
Lincoln	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Norfolk	0.08	0.00	0.00	0.04	0.08	0.54	0.17
Notts	0.00	0.00	0.00	0.12	0.08	0.00	0.25
Yorks	0.00	0.00	0.00	0.08	0.17	0.00	0.29
M acerina							
Lincoln	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Norfolk	0.42	0.17	0.25	0.00	0.42	1.50	1.54
Notts	0.00	1.08	1.66	0.17	0.62	2.08	1.62
Yorks	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P sulcatum							
Lincoln	0.00	0.00	0.00	0.00	0.08	0.00	0.00
Norfolk	0.00	0.00	0.00	0.25	0.00	0.00	0.00
Notts	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Yorks	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P intermedium							
Lincoln	0.17	0.67	1.67	0.71	0.92	2.29	2.37
Norfolk	1.67	1.62	1.50	0.71	2.20	2.92	2.50
Notts	0.58	1.04	2.50	1.96	1.92	2.67	2.83
Yorks	0.12	0.29	1.83	0.17	1.12	3.00	1.46
P violae							
Lincoln	0.00	0.00	0.00	0.12	0.00	0.00	1.00
Norfolk	0.00	0.00	0.00	0.00	0.00	0.12	0.00
Notts	0.00	0.00	0.00	0.00	0.00	0.08	0.04
Yorks	0.00	0.00	0.00	0.00	0.00	0.21	0.00
P sylvaticum							
Lincoln	0.00	0.00	0.00	0.00	0.00	0.00	0.29
Norfolk	0.00	0.00	0.25	0.00	0.04	0.08	0.00
Notts	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Yorks	0.00	0.00	0.42	0.00	0.04	0.00	0.00
***P* "vipa"**							
Lincoln	0.00	0.00	1.33	0.00	0.00	0.00	0.04
Norfolk	0.00	0.00	1.92	0.42	0.04	0.00	0.00
Notts	0.00	0.00	0.75	0.00	0.08	0.00	0.00
Yorks	0.00	0.00	0.58	0.00	0.04	0.00	0.04

If more detailed results are required they can obtained by contacting D J Barbara at Warwick HRI.

© 2007 Horticultural Development Council

Appendix 2

Tables of main statistical results for correlations of disease with PCR results.

Linear regressions were only fitted to time/pathogen combinations where the pathogen was detected in at least two separate samples. Even so some of the relations must be treated with caution as they are reliant on very few positive detections.One-sided T-tests were used to look for positive correlations (causal relationships) but many slopes were negative and two-sided T-tests were used to test negative correlations (antagonistic relationships).

A2-1. PCR detection from soil for the five pre-season to harvest sampling times.

Disease Measurement	Pathogen	Time	Slope	Standard Error	T-prob (1-sided)	T-prob (2-sided)	Power
Number of Roots Affected	F carotae	4	-7.760	9.740	0.783	0.434	0.191
		5	-0.30	9.566	0.513	0.975	0.053
	M acerina	1	-2.285	2.539	0.811	0.378	0.220
		2	-2.231	1.960	0.867	0.267	0.294
		3	0.016	4.372	0.499	0.997	0.050
		4	-10.929	11.194	0.830	0.340	0.242
		5	-1.310	2.722	0.683	0.635	0.119
	P intermedium	1	-1.792	2.468	0.762	0.476	0.173
		2	-0.821	2.044	0.654	0.692	0.105
		3	-3.823	2.165	0.955	0.091	0.526
		4	-2.539	2.040	0.887	0.226	0.331
		5	-4.269	1.963	0.980	**0.041**	0.678
	P sylvaticum	2	0.150	4.783	0.488	0.975	0.053
		5	2.727	25.79	0.459	0.917	0.061
	P vipa	2	1.404	2.255	0.270	0.540	0.149
		4	-8.011	6.163	0.897	0.207	0.350
		5	0.375	15.125	0.490	0.980	0.053
Average Number of Lesions on Diseased Roots	F carotae	4	-2.480	2.668	0.819	0.363	0.229
		5	0.3000	2.632	0.455	0.910	0.062
	M acerina	1	-0.674	0.6971	0.828	0.344	0.240
		2	-0.677	0.5362	0.890	0.220	0.337
		3	-0.0157	1.2035	0.505	0.990	0.051
		4	-2.786	3.091	0.812	0.377	0.220
		5	-0.482	0.7462	0.738	0.525	0.154
	P intermedium	1	-0.1917	0.6863	0.609	0.783	0.085
		2	-0.241	0.5623	0.664	0.672	0.110
		3	-0.730	0.6175	0.875	0.250	0.309
		4	-0.476	0.5720	0.793	0.414	0.201
		5	-1.141	0.5436	0.977	**0.047**	0.652
	P sylvaticum	2	-0.375	1.3142	0.611	0.778	0.086
		5	-1.636	7.093	0.590	0.820	0.078
	P vipa	2	0.5640	0.6145	0.185	0.369	0.225
		4	-1.911	1.713	0.862	0.277	0.287
		5	1.125	4.157	0.395	0.789	0.083
Average Number of Lesions on 100 Roots	F carotae	4	-0.5104	0.9666	0.699	0.603	0.129
		5	-0.3460	0.9389	0.642	0.716	0.099
	M acerina	1	-0.1649	0.2521	0.740	0.520	0.156
		2	-0.1542	0.1959	0.781	0.189	0.189
		3	-0.1185	0.4297	0.608	0.785	0.084
		4	-0.4957	1.1207	0.669	0.663	0.112
		5	-0.1384	0.2678	0.695	0.610	0.126
	P intermedium	1	0.0759	0.2453	0.380	0.760	0.089
		2	-0.0367	0.2018	0.572	0.857	0.071
		3	-0.2433	0.2218	0.858	0.285	0.280
		4	-0.1271	0.2060	0.728	0.544	0.148
		5	-0.3477	0.1997	0.952	0.096	0.517
	P sylvaticum	2	-0.2278	0.4684	0.684	0.632	0.120
		5	-0.8800	2.533	0.634	0.732	0.095
	P vipa	2	0.2417	0.2179	0.140	0.279	0.284
		4	-0.3770	0.6245	0.724	0.552	0.145
		5	-0.3575	1.4871	0.594	0.812	0.079

© 2007 Horticultural Development Council

A2-2. PCR detection from soil off carrot surfaces (SOCS) at the harvest sampling.

Disease Measurement	Pathogen	Time	Slope	Standard error	T-prob (1-sided)	T-prob (2-sided)	Power
Number of Roots Affected	F carotae	SOCS	-2.274	5.130	0.669	0.662	0.112
	M acerina	SOCS	-2.928	1.479	0.970	0.060	0.608
	P intermedium	SOCS	-2.187	2.249	0.830	0.341	0.241
	P sylvaticum	SOCS	8.123	8.864	0.185	0.369	0.225
	P violae	SOCS	8.794	1.860	**<0.001**	**<0.001**	0.998
	P vipa	SOCS	7.091	25.75	0.393	0.786	0.084
Average Number of Lesions on Diseased Roots	F carotae	SOCS	-0.772	1.4088	0.706	0.589	0.133
	M acerina	SOCS	-0.815	0.4063	0.972	0.618	0.618
	P intermedium	SOCS	-0.458	0.625	0.765	0.471	0.175
	P sylvaticum	SOCS	2.306	2.437	0.177	0.354	0.233
	P violae	SOCS	2.295	0.5376	**<0.001**	**<0.001**	0.994
	P vipa	SOCS	1.636	7.093	0.410	0.820	0.078
Average Number of Lesions on 100 Roots	F carotae	SOCS	-0.3302	0.5024	0.741	0.518	0.157
	M acerina	SOCS	-0.1708	0.1538	0.861	0.279	0.285
	P intermedium	SOCS	0.0193	0.2261	0.467	0.933	0.059
	P sylvaticum	SOCS	0.2449	0.8876	0.393	0.785	0.084
	P violae	SOCS	0.8590	0.1845	**<0.001**	**<0.001**	0.998
	P vipa	SOCS	-0.5636	2.537	0.587	0.826	0.076

A2-3. PCR detection from soil for the five pre-season to harvest sampling times combined.

Disease Measurement	Pathogen	Time	Slope	Standard error	T-prob (1-sided)	T-prob (2-sided)	Power
Number of Roots Affected	F carotae	1 - 5	-27.80	32.88	0.797	0.407	0.205
	M acerina	1 - 5	-4.114	4.142	0.835	0.331	0.248
	P intermedium	1 - 5	-6.248	3.145	0.970	0.060	0.610
	P sulcatum	1 - 5	-21.96	28.20	0.778	0.444	0.187
	P sylvaticum	1 - 5	0.949	21.10	0.483	0.965	0.055
	P vipa	1 - 5	1.279	9.108	0.445	0.890	0.066
Average Number of Lesions on Diseased Roots	F carotae	1 - 5	-7.317	9.062	0.786	0.428	0.194
	M acerina	1 - 5	-1.268	1.1336	0.862	0.276	0.287
	P intermedium	1 - 5	-1.342	0.895	0.926	0.148	0.424
	P sulcatum	1 – 5	-4.821	7.801	0.729	0.543	0.148
	P sylvaticum	1 - 5	-1.679	5.798	0.613	0.775	0.086
	P vipa	1 - 5	1.116	2.497	0.330	0.659	0.113
Average Number of Lesions on 100 Roots	F carotae	1 - 5	-2.434	3.248	0.770	0.461	0.179
	M acerina	1 - 5	-0.3264	0.4109	0.782	0.436	0.191
	P intermedium	1 - 5	-0.3378	0.3284	0.843	0.315	0.259
	P sulcatum	1 – 5	-1.1321	2.804	0.655	0.690	0.105
	P sylvaticum	1 - 5	-1.0044	2.066	0.684	0.632	0.120
	P vipa	1 - 5	0.5969	0.8881	0.254	0.508	0.160

© 2007 Horticultural Development Council

A2-4. PCR detection from SOCS for combined species using soil or SOCS samples. Only combinations where there was a positive correlation are given and hence in this table only SOCS results appear – all other comparisons were negative). All statistically significant pairings involved *Pythium violae* SOCS and did not improve on this species alone.

Disease Measurement	Pathogen	Time	Slope	Standard error	T-prob (1-sided)	T-prob (2-sided)	Power
Number of Roots Affected	Carotae + Violae	SOCS	15.816	3.942	**<0.001**	**<0.001**	0.987
	Sylvat + Violae	SOCS	15.418	3.444	**<0.001**	**<0.001**	0.996
	Violae + Vipa	SOCS	17.98	3.686	**<0.001**	**<0.001**	0.999
Average Number Of Lesions on Diseased Roots	Carotae + Violae	SOCS	4.037	1.1396	**0.001**	**0.002**	0.963
	Sylvat + Violae	SOCS	4.050	0.9859	**<0.001**	**<0.001**	0.990
	Violae + Vipa	SOCS	4.686	1.0696	**<0.001**	**<0.001**	0.995
Average Number of Lesions on 100 Roots	Carotae + Violae	SOCS	1.4901	0.3999	**<0.001**	**0.001**	0.975
	Intermd + Violae	SOCS	0.6557	0.2841	**0.016**	**0.031**	0.723
	Sylvat + Violae	SOCS	1.4298	0.3560	**<0.001**	**<0.001**	0.988
	Violae + Vipa	SOCS	1.7292	0.3716	**<0.001**	**<0.001**	0.998

A2-4. PCR detection of *Pythium* “vipa” in August with *Pythium intermedium* in soil at all collection times

Disease Measurement	Pathogen	Time	Slope	Standard error	T-prob (1-sided)	T-prob (2-sided)	Power
Number of Roots Affected	*P intermedium + P Vipa (August)*	1	-3.583	4.936	0.762	0.476	0.173
		2	-1.641	4.087	0.654	0.692	0.105
		3	-7.646	4.330	0.955	0.091	0.526
		4	-5.077	4.080	0.887	0.226	0.331
		5	-8.54	3.926	0.980	**0.041**	0.678
Average Number of Lesions on Diseased Roots	*P intermedium + P Vipa (August)*	1	-0.383	1.373	0.609	0.783	0.085
		2	-0.483	1.125	0.664	0.672	0.110
		3	-1.461	1.2350	0.875	0.250	0.309
		4	-0.953	1.1440	0.793	0.414	0.201
		5	-2.283	1.087	0.977	**0.047**	0.652
Average Number of Lesions on 100 Roots	*P intermedium + P Vipa (August)*	1	0.152	0.491	0.380	0.760	0.089
		2	-0.0734	0.4036	0.572	0.857	0.071
		3	-0.4866	0.4436	0.858	0.285	0.280
		4	-0.2542	0.4120	0.728	0.148	0.148
		5	-0.6953	0.3994	0.952	0.096	0.517

A2-5 Correlation of PCR detection with disease levels at the whole field average level for the four fields. * in the Time column indicates the pathogen was found in only one field. Several pathogen-time combinations are presented in the table which were not analysed previously. These are combinations where the pathogen was found in only one location at one field and are identified by a # in the Time column. The low number of sample fields (4) means that correlations given here and in A2-6 must be treated with caution.

Disease Measurement	Pathogen	Time	Slope	Standard Error	T-prob (1-sided)	T-prob (2-sided)	Power
Number of Roots Affected	F carotae	3*#	-72.67	57.75	0.833	0.335	0.221
		4	-45.20	50.63	0.767	0.466	0.156
		5	-24.00	44.19	0.680	0.641	0.105
	M acerina	1	-4.552	5.294	0.760	0.480	0.150
		2	-2.939	3.446	0.759	0.483	0.149
		3*	-14.53	11.550	0.833	0.335	0.221
		4*	-21.67	35.48	0.698	0.604	0.114
		5	-12.24	5.600	0.920	0.160	0.428
	P intermedium	1	-6.162	3.619	0.885	0.231	0.315
		2	-2.133	7.19	0.603	0.795	0.076
		3	-5.179	2.591	0.908	0.184	0.384
		4	-2.465	3.866	0.706	0.589	0.117
		5	-7.35	1.192	0.988	**0.025**	0.976
	P sulcatum	4*#	-24.22	19.25	0.833	0.335	0.221
		5*#	90.00	43.88	0.089	0.177	0.396
	P sylvaticum	2	-3.667	15.564	0.582	0.836	0.070
		5	-70.00	124.40	0.685	0.630	0.107
	P violae	4*#	60.00	29.25	0.089	0.177	0.396
	P vipa	2	-2.076	5.106	0.638	0.724	0.088
		4*	-14.53	11.550	0.833	0.335	0.221
		5	-100.00	62.95	0.874	0.253	0.290
Average Number of Lesions on Diseased Roots	F carotae	3*#	-16.667	17.140	0.784	0.433	0.169
		4	-14.800	12.251	0.825	0.350	0.211
		5	-10.000	10.595	0.778	0.445	0.164
	M acerina	1	-1.161	1.451	0.746	0.508	0.141
		2	-0.750	0.9442	0.745	0.510	0.140
		3*	-3.333	3.428	0.784	0.433	0.169
		4*	-5.667	9.597	0.693	0.615	0.111
		5	-2.993	1.793	0.882	0.237	0.308
	P intermedium	1	-1.319	1.205	0.806	0.388	0.190
		2	-0.711	1.912	0.627	0.746	0.084
		3	-1.202	0.8570	0.852	0.296	0.250
		4	-0.525	1.079	0.663	0.675	0.098
		5	-1.828	0.6222	0.951	0.099	0.604
	P sulcatum	4*#	-5.556	5.713	0.7835	0.433	0.169
		5*#	27.33	7.688	**0.036**	0.071	0.729
	P sylvaticum	2	-1.944	4.017	0.662	0.676	0.097
		5	-24.00	31.78	0.736	0.529	0.134
	P violae	4*#	18.222	5.126	0.036	0.071	0.729
	P vipa	2	-0.205	1.422	0.551	0.899	0.062
		4*	-3.333	3.428	0.784	0.433	0.169
		5	-29.00	15.116	0.903	0.195	0.365
Average Number of Lesions on 100 Roots	F carotae	3*#	-4.133	6.494	0.705	0.590	0.117
		4	-5.816	3.677	0.873	0.254	0.288
		5	-4.650	2.865	0.877	0.246	0.297
	M acerina	1	-0.3448	0.5161	0.714	0.573	0.122
		2	-0.2229	0.3356	0.713	0.575	0.121
		3*	-0.8267	1.2989	0.705	0.590	0.117
		4*	-1.747	3.340	0.674	0.653	0.102
		5	-0.8332	0.7447	0.810	0.380	0.195
	P intermedium	1	-0.2820	0.4823	0.691	0.618	0.110
		2	-0.2841	0.6465	0.649	0.703	0.092
		3	-0.3054	0.3524	0.761	0.478	0.151
		4	-0.1110	0.3827	0.601	0.799	0.076
		5	-0.5226	0.3235	0.876	0.248	0.296
	P sulcatum	4*#	-1.3778	2.165	0.705	0.590	0.117
		5*#	10.013	0.7653	**0.003**	**0.006**	1.000
	P sylvaticum	2	-1.0139	1.2646	0.747	0.507	0.141
		5	-9.780	10.214	0.781	0.439	0.166
	P violae	4*#	6.676	0.5102	**0.003**	**0.006**	1.000
	P vipa	2	0.0803	0.4862	0.442	0.884	0.064
		4*	-0.8267	1.2989	0.705	0.590	0.117

© 2007 Horticultural Development Council

		5	-10.130	4.977	0.911	0.179	0.392

A2-6 Correlation of PCR detection with disease levels at the whole field average level for the four fields for SOCS. * in the Time column indicates the pathogen was found in only one field.

Disease Measurement	Pathogen	Time	Slope	Standard error	T-prob (1-sided)	T-prob (2-sided)	Power
Number of	F carotae	SOCS	-25.07	16.39	0.867	0.266	0.277
Roots	M acerina	SOCS	-4.542	1.443	0.956	0.088	0.649
Affected	P intermedium	SOCS	-3.177	4.993	0.705	0.590	0.117
	P sylvaticum	SOCS*	25.71	12.537	0.089	0.177	0.396
	P violae	SOCS	7.546	3.759	0.092	0.183	0.386
	P vipa	SOCS	174.00	52.80	**0.042**	0.081	0.679
Average	F carotae	SOCS	-7.967	3.236	0.934	0.133	0.494
Number of	M acerina	SOCS	-1.098	0.5428	0.910	0.180	0.389
Lesions on	P intermedium	SOCS	-0.475	1.435	0.614	0.772	0.080
Diseased	P sylvaticum	SOCS*	7.810	2.197	**0.036**	0.071	0.729
Roots	P violae	SOCS	2.298	0.6663	**0.038**	0.075	0.709
	P vipa	SOCS	42.00	20.40	0.088	0.176	0.398
Average	F carotae	SOCS	-3.047	0.5528	0.985	**0.031**	0.950
Number of	M acerina	SOCS	-0.3000	0.2454	0.827	0.346	0.214
Lesions on	P intermedium	SOCS	0.0042	0.5044	0.497	0.994	0.051
100 Roots	P sylvaticum	SOCS*	2.861	0.2186	**0.003**	**0.006**	1.000
	P violae	SOCS	0.8443	0.07218	**0.004**	**0.007**	1.000
	P vipa	SOCS	11.440	9.312	0.172	0.344	0.215

Care is needed when interpreting the data for the soil on the carrot surface (SOCS). *P vipa* was only found at two sites, one at Lincs and one at Yorks, *P sylvaticum* was found only at Lincs and *P violae* was found at Lincs and at one site at Notts. The following table gives the minimum and maximum pathogen levels for the SOCS field averages. For all pathogens there is a very small range of values and it would not be wise to use these regressions for predicting the relationship of pathogen levels and disease over the full pathogen range of 0 to 3.

		Pathogen Levels	
		Min	Max
F carotae	SOCS	0	0.292
M acerina	SOCS	0	1.625
P intermedium	SOCS	1.458	2.833
P sylvaticum	SOCS*	0	0.292
P violae	SOCS	0	1.000
P vipa	SOCS	0	0.042