Project title:	**Carrots: the biology and control of cavity spot**
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Project Leader:	**Dr Tim Pettitt succeeding Geoff White, HRI Wellesbourne, Warwick CV35 9EF**
Key Workers:	**LH Hiltunen, SR Kenny, JG White and TR Pettitt, HRI Wellesbourne; CM Brewster, C Rockliff, D Varley, F Pomares, C Lambourne and GM McPherson, HRI Stockbridge House; J Fitzpatrick, LJ Rolfe and P.Gladders, ADAS Boxworth.**
Study location:	**HRI Wellesbourne, Warwick CV35 9EF, HRI Stockbridge House, ADAS Boxworth and commercial grower's sites.**
Project Co-ordinators:	**Dr P Wright, Watton Produce Co Ltd, Hargham Road, Shropham, Attleborough, Norfolk, NR17 1DT and Mr J Kenyon, Coe House Farms Ltd, Marsh Farm, Marsh Road, Hesketh Bank, Preston, Lancashire PR4 6XT.**
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CONTENTS

PRACTICAL SECTION FOR GROWERS

Headline

- Metalaxyl performance against cavity spot was inconsistent but no new fungicides have been shown to be as effective.
- The variable performance of metalaxyl is not due to resistance in UK *Pythium violae* populations, and whilst degradation of the fungicide occurs rapidly in some soils, not all the variability can be explained this way. A soil test for degradation is now available.
- Calcium treatments do control cavity spot but different compounds vary in efficacy. Growers can now test provisional criteria for responsive soils.

Background and expected deliverables

Cavity spot of carrot, caused in UK crops by *Pythium violae* and to a lesser extent by *P. sulcatum*, is currently the most economically important disease problem in UK carrot crops. Although good control of the disease was achieved in the early 1980s with metalaxyl and related fungicides, in the late 1990s there were increasing problems in the management of cavity spot using this fungicide. This project was set up to determine the reason(s) for this apparent decline in efficacy and to investigate alternative methods to improve disease control.

The expected deliverables of the project were:

- To identify new fungicides that might be successfully deployed against cavity spot to supplement metalaxyl.
- To identify whether disease control is achievable using calcium compounds.
- To determine whether degradation of metalaxyl occurs and develop, and make available a soil test for it.
- Investigate the biology of the timing of infection of carrots to aid crop management and the development of future control strategies.

Summary of the project and main conclusions

To achieve the expected deliverables above, the project followed a number of lines of enquiry. The most important of these can be summarised as follows:

- Comparing the efficacy of metalaxyl with new formulations and fungicides in the lab, in pot experiments and in field trials
- Assessing the relative efficacy of split applications of the fungicide
- Measurement of possible resistance to the fungicide in cavity spot *Pythium* populations
- Determinations of the rate of metalaxyl degradation in field soils
- Determining the efficacy of various calcium soil treatments against the disease

- Assessing early disease detection and the timing of infection in carrot seedlings
- Measurement of pathogen development in the soil in relation to environment and disease development

The general conclusions of this study are considered in three main areas as follows:

Fungicides

- Metalaxyl is still the best fungicide available for the control of cavity spot.
- No new fungicides have been shown to be better than metalaxyl in either pot or field experiments, although some new chemicals do show promise in laboratory tests.
- There is no resistance to metalaxyl in UK populations of *Pythium violae* and only limited tolerance was seen is the small sample of *P. sulcatum* isolates assessed, so fungicide resistance is not the cause of any decline in metalaxyl efficacy.
- Split doses of metalaxyl did not give improved control of cavity spot over single-dose early applications immediately post seedling emergence.
- The new metalaxyl-M formulation SL 567A was tested and gave comparable control to metalaxyl in pot experiments, but gave disappointing results in some field trials.
- Often field trial results and sometimes pot experiment results can be difficult to explain because the true effects of treatments on the pathogen in the soil cannot be measured fast enough. It is essential that future research concentrates on developing a rapid, reliable and inexpensive way of measuring the pathogen in the soil, probably using molecular techniques. Apart from being essential to the science, such a technique may also provide the basis of an improved disease risk soil test/disease prediction system.
- Metalaxyl degrades in soil. The rate of degradation varies greatly and in some fields it is very rapid (half life < 7 days). Evidence from this project indicates:
    1) degradation is microbial in origin,
    2) all rapid degrading fields assessed so far have had histories of intensive metalaxyl use (so pay careful attention to potato fields!),
    3) neighbouring fields with similar soil but NOT exposed to metalaxyl use (where available), did not degrade metalaxyl as rapidly,
    4) the phenomenon is not limited to one region of the UK, but, since only a limited number of sites have so far been assessed, no prediction on the frequency of rapid degradation can yet be made.
- As part of this project a test for metalaxyl degradation was developed and is available (see 'Action points for growers' below).

Calcium compounds

- Soil amendments with some calcium compounds do significantly reduce the severity and, to a lesser extent, the incidence of cavity spot disease.
- As with any control treatment, the degree of disease control using calcium varies from soil to soil.
- The disease control effects of calcium amendments to soil did consistently carry over to a second crop in pot experiments.
- In some pot experiments lime (calcium carbonate) and hydrated lime (calcium hydroxide) treatments gave better control of cavity spot than SL567A treatments, and

later applications of the fungicide to lime-treated pots did not improve on disease control already achieved.

- Different calcium compounds give different levels of disease control and liming treatments (both lime and hydrated lime) give the best control, whilst gypsum (calcium sulphate) gives no control and calcium nitrate and calcium monocarbamide give an intermediate level of disease control.
- The effects of calcium compounds on disease appear due to both their impact on soil pH and the direct effect of increasing the concentration of available calcium (the change in concentration seems to be what is important and not the actual total). In acid or neutral soils, lime treatments will increase both the pH and the concentration of available calcium whereas in alkaline soils the latter becomes the main effect. Calcium nitrate and calcium monocarbamide only affected the available calcium concentration whilst gypsum had no impact on disease because it increased neither pH nor available calcium concentrations.
- Over the range 3 – 10 t/ha, increasing the liming rate did not appear to improve the efficacy of disease control in the Wellesbourne Cottage Field soil, indicating that benefits of lime application can be achieved with comparatively low rates. The lime/pH response of this soil indicated that no further increases in pH were obtained from applications greater than 6 t/ha.

Pathogen Biology

- *Pythium violae* is still the predominant cavity spot pathogen in UK carrot crops, being present in 90% of all cavities sampled during this project.
- Assessments of infection of seedlings in naturally infected soil demonstrated the presence of *P. violae* infections in roots 6 weeks from drilling by molecular techniques and after 8 weeks by conventional plating methods – demonstrating that infection can occur very early in the development of a crop and that early fungicide applications are essential for effective disease control.
- Oospores have been successfully produced and harvested, and germination successfully achieved in the laboratory. As expected, germination was low (5-14%). This is a normal aspect of oospore survival behaviour and not a reflection on harvested oospore viability. Large numbers of oospores were produced at 10 and 15^{o}C but not at 20 ^{o}C. The temperature range for germination was 5–30 ^{o}C, with an optimum at 20-25 ^{o}C. The pH range for germination was 4.5-9, with optimum germination at pH 8. This latter result indicates that the possible pH effects on disease in soil may be indirect in their action by, for example, encouraging more competitive bacterial growth.
- Infection of carrots was successfully achieved in pot experiments using oospores, with infections clearly detectable in seedling roots 4 weeks after sowing. Further research is required to develop a fully optimised infection system from this work for use as a disease challenge test, which could be used to carry out severe and reproducible tests of fungicides and other proposed new disease control measures, as well as for testing disease resistance in new varieties.
- Addition of hymexazole to selective agar media improved the rate of isolation of *P. violae*, especially from older cavity spot lesions.
- A conventional plating procedure has been developed that allows limited quantification of *P. violae* in soil. However whilst this has produced some very useful scientific information, it is a lengthy, laborious and costly operation (approximately £800 of staff time alone per soil sample).

- The soil plating procedure shows that *P. violae* populations are sparse compared to other soil-borne *Pythium* species, ranging from 0 – 30 spores/g dry weight of soil. The level of colonisation detected in two 'problem' soils was seen to increase to 80 – 200 spores/g dry weight of soil during Autumn 2001. This increase may be linked to the severe cavity spot epidemic seen in that season. The link between quantifiable soilborne *P. violae* populations and disease urgently requires further study to provide accurate and reliable methods of determining the efficacy of potential new disease control methods and possibly even develop a means of disease prediction to aid management.

Financial benefits

It is difficult to quantify the financial benefits of improving the control of such a widespread and important disease as cavity spot, for in years when the disease is bad reduced availability of crop can influence both the crop value and the grading of crops suitable for acceptance. However, some idea of the financial impact can be obtained from simple calculations. For example, in a severe cavity spot season as many as 30% or more of crops can be rejected by pack-houses. This amounts to an estimated loss of £20.5 million based on the 2000-2001 value of the crop. If improved selection of fields combined with informed crop timing and careful use of calcium treatments can reduce rejections by 10% this could cut losses by at least £2.05 million without taking into consideration the savings that would also be made in the reduced wastage at the pack-houses from accepted crops. In addition there should also be reduced losses in crops considered marketable – an increase in 5% marketable roots would be worth over £3 million/annum.

Action points for growers

- Early application of metalaxyl or metalaxyl-M is still essential for good control of cavity spot but it is important to know how long this protection can be expected to last. If planning to use a field where the failure of metalaxyl has been suspected, or a field not previously used for carrots but that has been used for crops like potatoes, where metalaxyl might have been used intensively, then it would be useful to test the soil for its metalaxyl degradation rate. A test is now available, contact Andy Jukes, HRI Wellesbourne, Warwick, CV35 9EF (email andrew.jukes@hri.ac.uk). If a field is identified as a 'fast degrader' by this test, then a soil test for presence of pathogen is needed. If the pathogen test gives a 'high risk' result, then it is probably best not to use the field, but if a 'moderate to low risk' result is given, it will be best to use resistant varieties and crop early.

- Lime treatments can improve disease control, mainly by reducing the severity of disease. If considering liming a soil for cavity spot control, first determine the soil pH. If this is lower than pH 7, then determine its lime response by following the procedure described in this report (page 9) and from the results of this decide a rate that will achieve a pH of 7-8. If the soil pH is greater than pH 7, consider liming at between 3 and 6 t/ha, whilst taking potential problems in other crops in the rotation (e.g scab in potatoes) into consideration.

- Also refer to HDC factsheet 03/03 'Cavity spot on carrots'.

SCIENCE SECTION

Introduction

In recent years the efficacy of cavity spot disease control in UK carrot crops using metalaxyl appears to have declined in some crops. This project was instigated to determine the reason(s) for this apparent decline and to improve disease control. The project has followed a number of lines of enquiry including, work on metalaxyl resistance in the pathogen populations, degradation of metalaxyl in field soils, timing of infection and general pathogen biology ('looking for weak spots in the life cycle'), plus investigations of alternative control measures, such as the use of calcium compounds, split applications of metalaxyl, and applying new fungicide chemicals. This report describes the results of the fourth and final year of the project and covers:

- results of a field trial of efficacy of commercially available and novel fungicides, run by ADAS at a commercial holding.
- results of the last pot experiments carried out at HRI Wellesbourne, investigating the effects of calcium carbonate on cavity spot in different soil types.
- results of the completed metalaxyl resistance studies, plus tests of sensitivity to the novel fungicide formulations tested in the field trial.
- results of promising initial work on pathogen biology in soil in relation to disease; oospore germination, infection, and the measurement of soil inoculum.
- conclusions from the entire four year project.

PART 1 – Fungicide efficacy field trial run by ADAS at a commercial site

Objective

To investigate the performance of commercially available and novel experimental fungicides in replicated field studies for the control of *Pythium violae*, cause of cavity spot in carrot.

Materials and Methods

The trial consisted of a randomised block design, comprising ten treatments (Table 1) six replicates and located in a field with a history of severe cavity spot. Each plot consisted of a single bed 1.8 m wide by 10 m long with a 1 m guard area. Row spacing was 0.33 m within the bed.

The treatments were applied immediately before drilling or at the first true leaf stage.

Treatments were applied using an Oxford precision sprayer with a 2 m boom fitted with Lurmark F110 02 nozzles operated at a pressure of two bars on 23rd May 2001 and 10th July 2001. Conditions were dry and sunny with little wind at the first application. The soil surface was wet and rain fell after application of treatment 7(heavy shower) and prior to application of treatments 8,9 and 10 on 10 July.

Fleece was used to protect the crop from frost damage on 30 November 2001.

Table 1: Treatment list

Treatment number	Treatment Description
1	Untreated (water) control applied at 1000 litres water/ha
2	SL567A (metalaxyl) split dose applied at 0.65 l/ha in 1000 litres water at both timings, pre-drilling and at first true leaf
3	SL567A applied at 1.3 l/ha in 1000 litres water, first true leaf
4	Amistar (azoxystrobin) applied at 6 l/ha in 1000 litres water, pre-drilling
5	Amistar applied at 6 l/ha in 1000 litres water, first true leaf
6	Amistar split dose applied at 6 l/ha in 1000 litres water at each timing, pre-drilling and first true leaf
7	HDC1 8 l/ha applied in 1000 litres water, first true leaf
8	HDC2 4 kg/ha applied in 1000 litres water, first true leaf
9	HDC3 1.6 l/ha plus 1.2 l/ha adjuvant applied in 1000 litres water, first true leaf
10	Horti 303 4 kg.ha applied in 1000 litres water, first true leaf stage

Assessments

Monthly sampling was carried out from the pencil stage onwards. This sampling involved randomly selecting a total of 100 roots from untreated plots. These roots were washed and assessed for the number of cavity spot lesions per root and the overall severity of infection as a percentage of the root area affected. The initial experimental design proposed harvesting the trial when incidence of cavity spot in any single sample had reached 20 per cent of the roots sampled. However, since the 20 per cent threshold level had not been reached by the start of March 2002 a decision was taken to harvest the trial as the carrots were then in danger of suffering from severe rots due to secondary infection after frost damage. The harvest was carried out on 19th March 2002. Carrots from the two centre rows of each plot were lifted, however due to uneven emergence the length of rows harvested varied per plot to enable 50 roots to be lifted. The roots were washed and had the foliage removed they were then weighed and assessed for disease, pest damage and other parameters. Marketable grades were defined using the NIAB key (Appendix 1 & X).

Statistical analysis

Data were subjected to analysis of variance and transformed to overcome skew distribution where appropriate. When transformation was not successful, untransformed means are presented.

Results

There were no significant differences between treatments in the number of roots affected by cavity spot, the number of lesions present or the area of root affected. Treatment 5 (post emergence Amistar) had the most new lesions whilst treatment 9 (post emergence HDC2) had the most old lesions and the highest number of lesions overall (Tables 2 and 3). The untreated plots had relatively low numbers of both new and old lesions (Table 2).

Table 2: Incidence and severity of new and old lesions of cavity spot at harvest.

	New lesions			Old lesions		
Treatment	Mean % roots affected	Mean no. of lesions per 50 roots	Mean % root area affected	Mean % roots affected	Mean no. of lesions per 50 roots	Mean % root area affected
1 Untreated	0.7	0.3	0.1	1.0	0.8	0.1
2 SL567A (x2)	1.3	6.0	3.5	3.0	1.8	2.3
3 SL567A 1st leaf	0	0	0	2.3	1.5	1.1
4 Amistar pre-em.	0.3	0.3	0.3	0.3	0.2	0.2
5 Amistar post-em.	4.7	5.8	9.6	1.7	1.0	2.3
6 Amistar (x2)	0.7	0.3	0.03	3.3	2.2	2.1
7 HDC1	0	0	0	3.0	1.5	2.2
8 HDC2	1.7	1.8	0.8	2.7	2.2	1.3
9 HDC3	4.3	5.5	3.5	4.3	2.7	2.9
10 H303	2.0	1.8	0.7	3.3	4.2	2.4
SED(45 df)	*	*	*	1.74	*	*
Significance	*	*	*	NS	*	*

* = skewed data unsuitable for analysis
NS = not significant

There were no treatment differences in the severity of cavity spot. Although most of the affected roots fell into categories one and two there were still a few in categories three to five which would be considered as unmarketable. Treatment five had the most unmarketable roots. The affected roots from the untreated plots were all in disease category one and, therefore, all marketable (Table 3, Appendix I gives definitions of disease categories).

There were no significant differences between treatments with regard to yield or root defects (Appendix II) or with regard to the incidence of damage caused by other diseases and pests (Appendix III). Treatment two had the highest yield and lowest number of small

roots. It also had a high incidence of carrot fly damage and roots which had dried brown areas with vertical cracks in these areas, the cause of which was unknown.

Table 3: Incidence and severity of cavity spot at harvest

Treatment	Total % roots with cavity spot	% roots in disease category 1	% roots in disease category 2	% roots in disease category 3	% roots in disease category 4	% roots in disease category 5	Mean % area affected by cavity spot (total per 50 roots)
1 Untreated	1.7	1.7	0	0	0	0	0.2
2 SL567A (x2)	4.0	1.3	1.7	0	0.7	0.3	5.8
3 SL567A 1st leaf	2.3	0	2.3	0	0	0	1.1
4 Amistar pre-em.	0.7	0	0.7	0	0	0	0.4
5 Amistar post-em.	5.7	0.3	2.3	1.3	1.3	0.3	11.9
6 Amistar (x2)	3.7	0.7	1.7	1.3	0	0	2.1
7 HDC1	3.0	0	2.7	0	0.3	0	2.2
8 HDC2	4.0	1.3	1.7	0.7	0.3	0	2.1
9 HDC3	8.0	2.3	2.7	1.7	1.3	0	6.3
10 H303	5.0	1.0	3.0	0.7	0.3	0	3.1
SED (45 df)	*	*	1.50	*	*	*	*
Significance	*	*	NS	*	*	*	*

Discussion

Cavity spot did not develop to a significant extent at this site despite a history of the problem. There were reports of severe cavity spot from September onwards in 2001 and some crops were unmarketable. There was particularly poor establishment of the carrots in the experimental plots in dry conditions. This may have affected the establishment of *Pythium* spp. in the crop and hence final cavity spot development. The influence of soil moisture on *Pythium* spp. early in the life of the crop merits further investigation in future studies of cavity spot.

The low incidence of cavity spot has prevented a full evaluation of new fungicides and further work is required to establish their activity under field conditions. At the very low levels of disease seen, the results indicate that none of the fungicide treatments used had an effect of lowering the incidence and severity of cavity spot compared to the untreated controls. Conversely no adverse effects were recorded.

PART 2 – Pot experiments studying effect of lime (calcium carbonate) on cavity spot in different representative carrot soils

Materials and methods

Selection of soils and measurement of lime-pH response

Five soils from fields with known histories of severe cavity spot were selected for comparisons of the effects of lime additions on disease. These are listed in Table 4). Prior to setting up the calcium carbonate pot experiment, lime-pH response curves were generated for each of the five soils. Calcium carbonate (Snowcal 10, Omya Croxton and Garry Ltd) was added to each soil at the rates of 0, 0.5, 1, 2.0, 4.7 and 9.3 t ha^{-1}. The pots were placed on trays and the soil kept moist by watering via the trays. There were three replicate pots for each soil and calcium carbonate concentration. The pots were incubated at 20 ± 2°C for 14 days after which the soil pH was measured in a 1:5 mixture of air-dried soil to 0.01M calcium chloride and the lime response curves drawn.

Table 4 Sources of the soils used in the calcium carbonate pot experiment

Soil code name	Source	OS grid reference
A1	Shropham, Norfolk	TL 996940
A6	Marham, Norfolk	TF 701105
B	Brigg, Lincolnshire	TA 078006
SH	Stockbridge House, North Yorkshire *Site for field trials in years 1-3 of this project*	SE 558367
W	HRI Wellesbourne, Warwickshire – 'Cottage field' *Soil used for the majority of pot experiments in this project*	SP 268571

Calcium carbonate pot experiment

The calcium carbonate pot experiment investigated the effect of calcium carbonate on the incidence and severity of cavity spot in the five different soils selected above. Each soil was assessed without a lime amendment and with a lime amendment set at 6 t ha^{-1}, the latter was decided upon based on the results of the soils' lime responses.

Soils were sieved to remove stones before being used. The soil was either used untreated to fill 25 cm diameter pots containing 1.5 kg of gravel, or first treated with calcium carbonate at the rate of 6 t ha^{-1} in a concrete mixer and then put into the pots. The pots were sown a day after the calcium carbonate incorporation with 40 seeds per pot of carrot cv. Nairobi. There were eight replicate pots of each treatment. Following emergence, seedling stands were reduced to 20 per pot. The pots were placed in saucers and watering was done via the saucers apart from a light application onto the surface when necessary to prevent drying out. During the growing season, the carrots were routinely treated for

aphids and powdery mildew as necessary. The carrots were harvested at 190 days after sowing. The washed roots were weighed and then scored for the number of cavities less than and greater than 5 mm diameter and the percentage root area affected by cavity spot using NIAB's area assessment chart (Appendix X). Analysis of variance was carried out on the data using Genstat 5 after angular transformations of percentages.

Soil pH was measured at sowing, two weeks after sowing, two months after sowing and at harvest in a 1:5 mixture of air-dried soil to 0.01M calcium chloride. Soil water extractable, cation exchangeable and total calcium were measured two weeks after the calcium carbonate incorporation. Analyses of electrical conductivity, soil phosphorous, potassium, magnesium and nitrogen contents were carried out on all soils two months after sowing.

Results & Discussion

The five soils assessed showed a range of pH responses to lime additions (Figure 1). The two East Anglian soils (A1 and A6) remained virtually unchanged at pH 7.6-7.8. Soils SH and B showed moderate responses increasing from pH 6.7 to pH 7.3. The biggest pH shift was seen in the more acidic soil W from Wellesbourne (from pH 5.7 to pH 7.3). All the pH response curves appeared to have reached an assymtote by 6 t ha^{-1} added lime and, as stated above, this concentration was selected for the main comparison pot experiemnt.

In the main pot experiment the pH of all soils except A1 remained stable throughout (Appendix VII). In soil A1, there was a steady rise in pH in both the amended and un-amended treatments of 0.5 over the 190 day trial period. In all the lime-amended treatments, the pH rapidly rose, being more or less at its final level one day after incorporation, except in the initially most acidic soil W, which had reached its final pH by the 2 weeks post-drilling pH assessment (see Appendix VII).

Lime treatments at 6 t ha^{-1} reduced cavity spot disease in all five test soils. The severity of disease, as measured by the mean percentage surface area of carrots affected by cavity spot, was significantly reduced in all treated soils (Figure 2A). The effects of lime on disease incidence were less marked, but a general reduction was seen in all lime-treated soils. Disease incidence was assessed by two methods: the percentage of carrots showing cavities, and the total number of cavities per carrot (the latter is more a measure of incidence of infection). With the percentage of carrots showing cavities, significant reductions were seen with lime additions to soils A1, A6 and SH, but only a slight reduction was seen in soil B and no effect in soil W (Figure 2B). Whilst the total number of cavities per carrot was reduced in soils A1, A6, SH and W, there was an increase in cavity numbers in soil B. Nevertheless, as stated above, the cavities in all lime-treated soils were significantly smaller and yields in terms of root weights were generally greater in these soils (Figure 2 D & E).

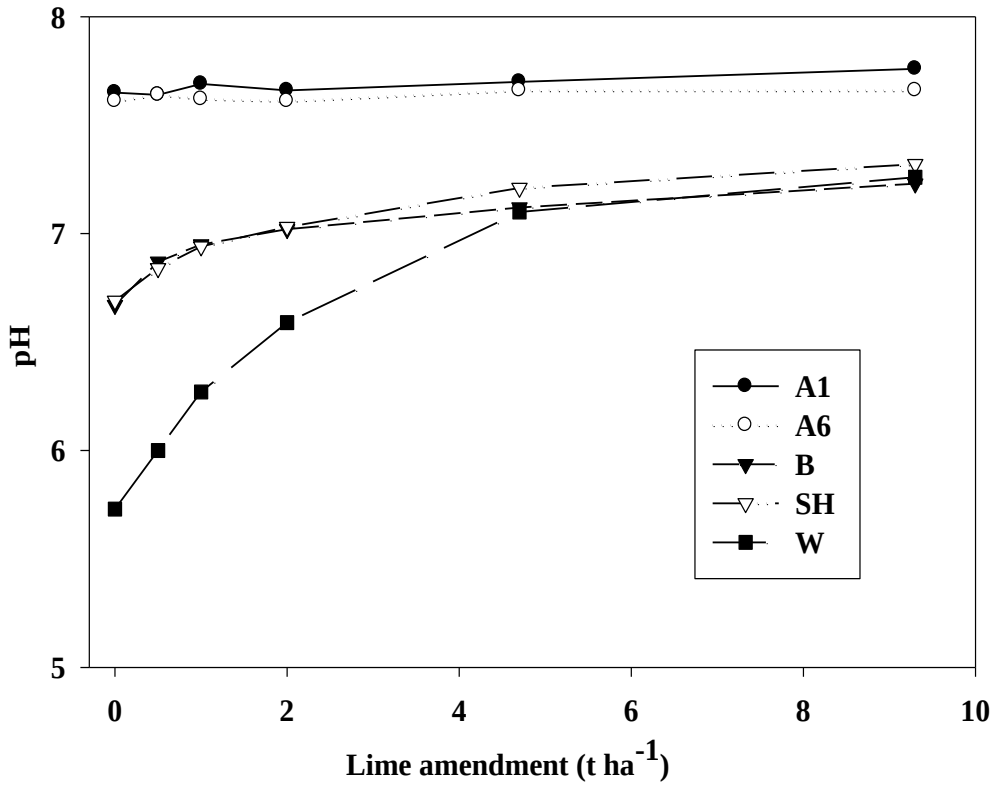
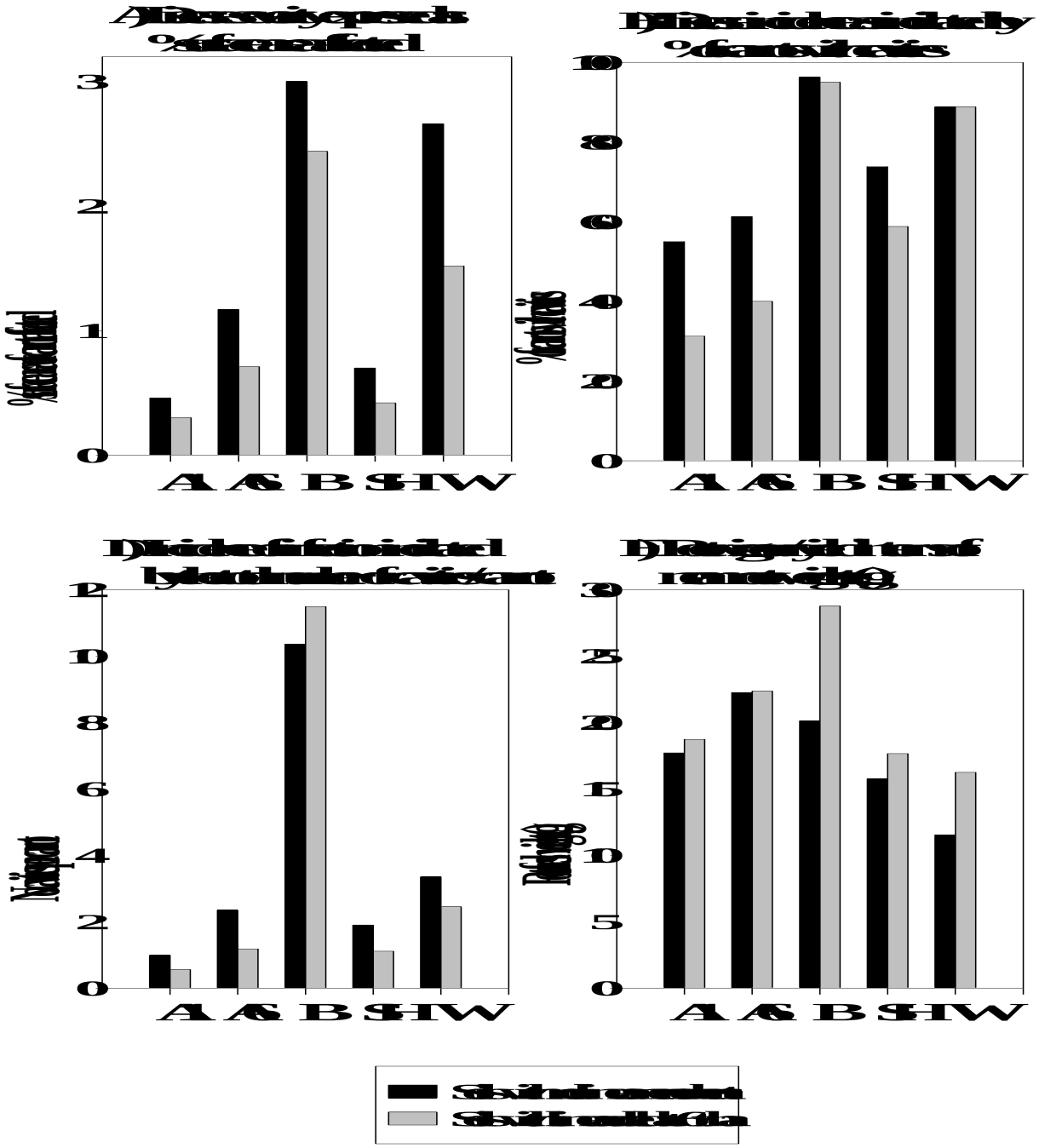


Figure 1 Lime response curves for the five soils assessed in the calcium carbonate pot trial (see Table 4).

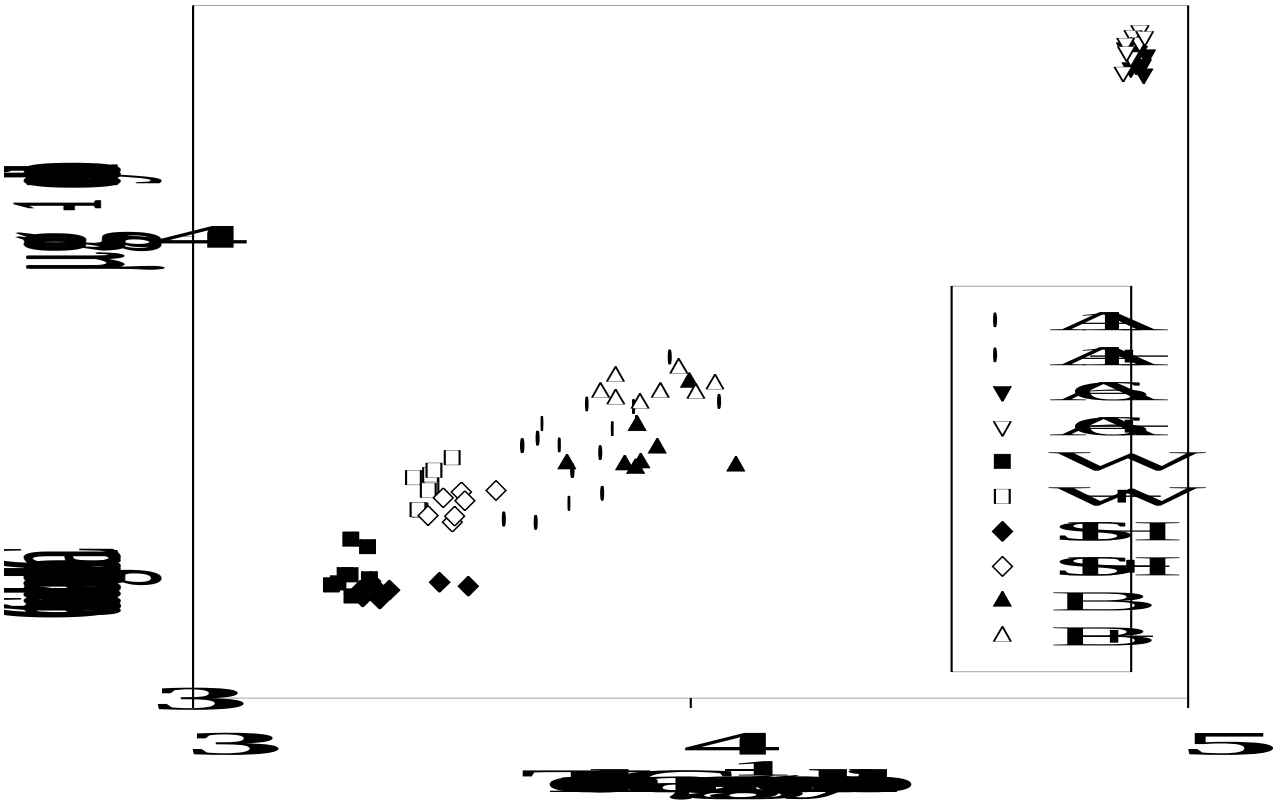
Whilst not contradicting the findings of previous workers on the effects of increased pH from liming in the reduction of disease (Scaife *et al.*, 1983, Perry & Groom, 1984, White, 1988), the results of this study show that the impact of liming is more than a simple pH effect. In previous years experiments in this project (Hiltunen *et al.*, 2000, White *et al.*, 2000), increasing the pH alone has been shown to be important for reducing cavity spot when working with low pH soil. However, the disease is often prevalent in soils with a naturally high pH (Soroker *et al.*, 1984, Jakobsohn *et al.*, 1984) and this was seen in the current study in soils A1 and A6. In soils A1 and A6 the addition of lime still reduced both the incidence and severity of disease whilst having little impact on pH. The addition of calcium carbonate to all five soils resulted in modest increases in water-soluble calcium (0 - 84 μg g^{-1} see Appendix Table) and relatively consistent increases of about 1000 μg g^{-1} of cation exchangeable ('available') calcium (Figure 3). This is consistent with the findings of Scaife *et al.* (1983) who observed sharp reductions in cavity spot incidence when surface soil exchangeable calcium exceeded 8 milliequivalents per 100g soil. How exchangeable calcium acts on the disease is unclear. Australian work primarily in impoverished acid sand soils (quite different to even the lightest UK soils), indicated that calcium did not have a direct role in the reduction of disease and that exchangeable calcium levels were not important (El-Tarabily *et al.*, 1997). Similarly Vivoda *et al.* (1991) concluded that there was no relationship between total and exchangeable calcium in soils and the occurrence of cavity spot. Considering the high levels of both total and exchangeable calcium present in soil

Figure 2 Effect of lime amendment at 6 t ha^{-1} in five representative soils (see Table 4), on cavity spot disease incidence and severity and on the vigour of the carrot roots.



A6 and the levels of disease, the results of the current study would support this conclusion. However, it appears that it is the change in exchangeable calcium that might be of importance in reducing disease. Such a result implies an effect on either the host plant or possibly on the soil microflora. El-Tarabily *et al.* (1996) found that in limed soil, microbial activity was increased, especially populations of aerobic bacteria, fluorescent

Figure 3 Change in cation exchangeable calcium concentration with total calcium concentration in the five test soils with and without lime amendment at 6 t ha^{-1}. All data have been square root transformed.



Pseudomonas spp. and other Gram negative species as well as actinomycetes. This increase in bacterial activity was correlated with a decline in populations of filamentous fungi and of the incidence of cavity spot disease. It is possible that calcium could also act directly on the host tissues' reaction to pathogen (or secondary rotting micro-organisms) attack, which might be important, especially in soils where the severity of cavity spot, not the incidence of lesions is affected by lime treatments. This has not yet been considered in any detail and there are two possible mechanisms by which it might operate. The first is by the action of calcium on the pectins in cell walls that would effectively 'harden' them by reducing the impact of pathogen cell wall degrading enzyme activity (Zamski & Peretz, 1996). The second involves the role of calcium ions as an important part of the elicitation system for plant cell resistance, generating the extracellular alkalinization needed to induce an oxidative burst (Bolwell *et al.,* 2002). Whilst not reducing the number of infections, the presence of increased concentrations of cation exchaneable calcium may increase the speed of plant resistance responses and thereby reduce the severity of cavity spot lesions. This is an area that requires further research involving

quantification of the pathogen in the soil and the use of molecular techniques in monitoring cellular resistance mechanisms.

PART 3 – Fungicide resistance/sensitivity studies

Materials and Methods

Completion of assessment of resistance in U.K. P. violae *populations to metalaxyl*

36 isolates of *P. violae* were obtained in 2001/2 from cavity spot lesions, found on UK carrots, brining the total number of isolates collected in this project to 67 (see Appendix IX). The majority of the carrots were taken from metalaxyl-treated crops, many of these were sent to HRI by growers or ADAS, whilst others were lifted directly from fields where the soil was collected for the calcium carbonate pot experiment.

As in previous years, the isolates were prepared for assay by cleansing through 2 % water agar with rifamycin (30 mg l^{-1}) to remove contaminating bacteria. The isolates were then grown on V8. A dilution series of metalaxyl in corn meal agar was prepared to give concentration of 0, 0.01, 0.1, 1, 5, 10, 50 or 100 µg ml^{-1}. Prepared plates were inoculated at the side with one of the test isolates, incubated at 20 ± 2ºC, and the colony diameter was measured until the untreated controls had grown across the plates. There were four replicates of each concentration for each of the isolates. The Genstat statistical programme was used to fit a logistic curve to the data and to calculate ED_{50} values with standard errors.

Efficacy in vitro *of chemicals tested in the ADAS field trails (see PART 1)*

All the chemicals tested in the ADAS field trial were assessed for their efficacy *in vitro* against three representative recent isolates of *P. violae*. A stock solution of each chemical (1000 µg ml^{-1}) was prepared in sterile distilled water, then diluted 2- and 10-fold and mixed with CMA to achieve the required concentrations (100, 50, 10, 5, 1, 0.1, 0.01, 0 µg a.i. ml^{-1}). There were four replicate plates for each concentration/isolate combination. Each plate was inoculated at the side with a 5 mm diameter mycelial disc cut from V8 juice agar (15 g calcium carbonate, 100 ml V8 vegetable juice [Campbell Grocery Products Ltd.], 900 ml DW) cultures. The plates were incubated at 20 ± 2°C for up to 7 days. Colony growth was measured as mycelial extension (mm). Growth measurements were taken when the first colonies in the dilution series of chemicals had almost reached the furthest edge of the plate, and the data analysed using Genstat 5 programme which fitted a logistic curve and calculated the ED_{50} for each chemicals for the different *Pythium* isolates tested.

Results and Discussion

Completion of assessment of resistance in U.K. P. violae *populations to metalaxyl*

No evidence for the development of resistance to metalaxyl was found in any of the 67 *P. violae* isolates tested during the year 2001-2002. All of the *P. violae* isolates were sensitive to metalaxyl with ED_{50} values ranging from <0.010 to 0.053 µg ml^{-1}. This is

consistent with results from the previous years of this project, and records of the origins of all isolates tested throughout the project, together with their ED_{50} values, are presented in Appendix Table X. These results are also consistent with the previous work of White, Stanghellini & Ayoubi (1988) and indicate that there has been no development of resistance to metalaxyl in U.K. poulations of *P. violae*.

Efficacy in vitro *of chemicals tested in the ADAS field trails*

All three isolates of *P. violae* tested were sensitive in the laboratory assay to HDC1, HDC3 and Horti 303, but not to HDC2 (Table 5). ED_{50} values of the active ingredient of HDC3 were similar to those of metalaxyl for isolates of *P. violae* tested in the current and previous years of this project (in this work ED_{50} values for metalaxyl ranged from <0.013-0.052 μg ml^{-1}). However, in the ADAS field trial none of the chemicals could be shown to affect the incidence or severity of cavity spot. The low incidence of cavity spot prevented a full evaluation of these chemicals in the field conditions.

HDC1 and HDC3, which are both strobilurin type compounds, showed better efficacy in the laboratory assay against *P. violae* than azoxystrobin the active ingredient of Amistar that was tested last year (Pettitt *et al*. 2001). ED_{50} values of the active ingredients of HDC1 and HDC3 were 10 and 20 times lower, respectively, than that of azoxystrobin (ED_{50} ranged from 1.05 to 4.63 μg ml^{-1}).

Horti 303 was more effective against *P. violae* than mancozeb alone. The ED_{50} for mancozeb against *P. violae* was found to be 17.5 μg ml^{-1} by White *et al*. (1992).

Table 5. ED_{50} values of chemicals used in ADAS field trial for three isolates of *P.violae*.

Chemical	Active ingredient and its concentration	Isolate of *P. violae*	ED_{50} (μg ml^{-1})
HDC1	Strobilurin type 25 %	A	0.15
		B	0.44
		C	0.89
HDC2	Not known 50 %	A	> 100
		B	> 100
		C	> 100
HDC3	Strobilurin type 40 %	A	< 0.01
		B	0.03
		C	0.03
Horti 303	Mancozeb 70 % (Horti 303 1.75 %)	A	1.67 (0.04)
		B	2.19 (0.05)
		C	1.81 (0.05)

PART 4 – Studies on pathogen biology

Objective

To study pathogen inoculum to develop future methods for carrying out controlled inoculations for tests of potential disease control methods and to develop procedures for quantifying natural populations of the pathogen in soil and determining how they respond to control strategies.

Materials and Methods

Oospore inoculum – attempts to improve spore germination by enzyme pre-treatment

P. violae isolate Arnando (PVA), recovered from cavities of commercially grown carrots from a farm in Lancashire, was used for this experiment. Oospores were extracted from PVA culture produced in V8 juice broth (V8B) (2 g calcium carbonate, 100 ml V8 vegetable juice [Campbell Grocery Products Ltd.], 900 ml distilled water) grown at 15°C for 8 weeks. The culture was drained, washed with three changes of sterile distilled water (SDW) and left to dry in a laminar flow hood for 1 hour. It was then macerated in SDW for 1 minute at medium speed and 1 min at high speed using an Ultra Turrax® (TP 18/10) macerator (Janke & Kunkel KG). The macerated suspension was filtered successively through nylon filter fabric of 500, 255 and 80 µm mesh sizes to remove mycelial fragments. Solutions of 0.5 % cellulase and 0.5 % ß-glucuronidase were prepared in 0.1 M $NaH_2PO_4 \cdot 2H_2O$ (pH 5.1). Aliquots of oospore suspensions were mixed with the enzyme suspensions and these were then incubated along with the untreated controls at 20, 25 and 30°C. Samples were taken after 24 and 48 hours incubation from each solution and pipetted onto three replicate plates of corn meal agar (CMA) amended with rifamycin (30 mg l^{-1}) (Sigma Chemical Co. Ltd.). The plates were incubated at 20 ± 2°C for 16 hours after which they were examined microscopically. Germination of 250 oospores on each plate was scored, oospores with germ-tubes longer than the spore diameter were considered to have germinated.

Oospore inoculum – infection studies using carrot seedlings

Sixty modules (50 x 45 x 170 mm) in a modular tray were each filled with a rockwool block (12 x 12 x 17 mm), 12 g of sterile pea gravel added and then filled up to the top with sterile silver sand. The modular tray was placed on capillary matting in a tray. The sand was moistened with tap water by watering from above, and the surface was kept wet by spraying. Four carrot seeds cv. Nanco were sown in each module and covered by sand. The tray was covered with wet tissues and polythene until emergence. After emergence, the seedlings were thinned to three per module. The watering was done from below with tap water until emergence and with feed prepared in DW (0.5 g l^{-1} Vitax Vitafeed III, 0.023 g l^{-1} $Ca(NO_3)_2$) after that. The temperature control in the glasshouse was set for heating at 5°C and venting at 20°C, but the temperature varied greatly according to ambient temperature (June-August). Inoculum was produced by extracting the oospores from a PVA culture grown in V8B for 5 weeks at 15 ± 2°C. Oospore suspension (1 ml, containing approx. 3.6 x 10^5 oospores) was pipetted into each module immediately after sowing. The viability of the oospores was determined by checking the germination of the oospores on CMA. The experiment was repeated in a similar manner with the following exceptions: in the second experiment the inoculation was done twice i.e. after sowing and

after emergence; the glasshouse temperature stayed lower because the second experiment was run over winter (December-March).

From three weeks after sowing onwards, 16-20 seedlings were harvested at weekly interval. The roots were washed using tap water and then rinsed with distilled water (DW). The whole root system was plated on CMA amended with rifamycin (30 mg l^{-1}) and pimaricin (100 mg l^{-1}) (Sigma Chemical Co. Ltd.). The plates were incubated at 20 ± 2°C in the dark for up to two weeks and checked microscopically for the presence of *P. violae.*

Comparison of infectivity of three* P. violae *inocula in carrot seedling bioassays

Extracted oospores, sand oatmeal inoculum and naturally infested soil were compared in a seedling test for causing cavity spot infection in carrot roots.

Oospore inoculum was produced from PVA culture grown in V8B at 15°C for 8 weeks. The culture was drained, washed with three changes of SDW and left to dry in a laminar flow hood for 1 hour. It was then macerated in SDW for 1 minute at medium speed and 1 min at high speed using an Ultra Turrax® (TP 18/10) macerator. The macerated suspension was filtered successively through nylon filter fabric of 500, 255 and 80 μm mesh sizes to remove mycelial fragments. Two modular blocks with 20 cells (30 x 30 x 60 mm) in each were filled with sterile sand. Oospore suspension (1 ml) was pipetted into each cell.

Oatmeal inoculum was produced by inoculating a flask of sterile sand oatmeal medium (160 g silver sand, 40 g medium oatmeal [Prewett's], 40 ml distilled water) with three mycelial plugs of PVA culture and then incubated at 20 ± 2°C for two weeks. Inoculum was then mixed with sterile sand (1:5) and moistened with SDW. The mixture was used to fill two modular blocks with 20 cells.

Naturally infested soil collected from a field with a high incidence of cavity spot was used as an inoculum. It was stored at 5°C after collection, sieved to remove stones and break up lumps and then used to fill two modular blocks of 20 cells.

Two carrot seeds of cv. Nanco were sown in each cell. Each modular block was placed in a tray in a propagator and transferred to an incubator room. The temperature was kept at 18°C and the daylength at 10 h. The trays were watered weekly using SDW. The modular blocks inoculated with the oospore and sand oatmeal inoculum were given feed (0.5 g l^{-1} Vitax Vitafeed III, 0.023 g l^{-1} $Ca(NO_3)_2$) from three weeks after sowing.

The seedlings were harvested four weeks after sowing. The roots and the ungerminated seeds were washed using tap water and then rinsed with DW. The whole root system and the seeds were plated on CMA amended with rifamycin (30 mg l^{-1}) and pimaricin (100 mg l^{-1}). The plates were incubated at 20 ± 2°C in the dark for up to two weeks and checked microscopically for the presence of *P. violae.*

Assessment of the effect of soil moisture on the cavity spot infection of carrot seedlings

A sub-sample of infective soil from site B (see Table 4 above) was used. The soil had been stored at 5°C until needed (approx. 2.5 months). The soil moisture content and maximum water holding capacity were determined. Pots (15 cm diameter) were filled with 300 g of gravel and 1400 g of the above soil and the weight of each pot was recorded. Each pot was then sowed with 30 carrot seeds cv. Nairobi and covered until emergence with brown paper. The surface of the soil was kept moist by spraying it with water. After emergence the pots were watered to 50, 55 and 60 % moisture content. These moisture contents were maintained gravimetrically every 3 to 5 days. There were eight replicates for each treatment.

Ten seedlings were pulled from each pot, 6 and 14 weeks after sowing. The seedlings were washed thoroughly with DW to remove all soil particles. The whole root system was plated on CMA amended with rifamycin (30 mg l^{-1}) and pimaricin (100 mg l^{-1}). The plates were incubated at 20 ± 2°C in the dark for up to two weeks and checked microscopically for the presence of *P. violae.*

Results and Discussion

Oospore inoculum – attempts to improve spore germination by enzyme pre-treatment

Pre-treatment of oospores with enzymes at 25 and 30°C did not affect the germination (Table 6). Incubation for 24 hours at 20°C with cellulase doubled the germination percentage compared to the untreated control. Longer incubation time (48 hours) slightly reduced the germination in all treatments.

Table 6. The effect of cellulase and ß-glucuronidase pre-treatments on the germination of oospores.

Incubation temperature of the pre-treatment	Enzyme pre-treatment	Percentage of germinated oospores	
		After 24 h pre-treatment	After 48 h pre-treatment
30°C	Untreated (buffer)	0.5	NT
	Cellulase	0	NT
	ß-glucuronidase	0.1	NT
25°C	Untreated (buffer)	2.3	0.4
	Cellulase	1.9	0.5
	ß-glucuronidase	1.2	0.3
20°C	Untreated (buffer)	2.1	1.3
	Cellulase	4.7	2.3
	ß-glucuronidase	2.0	1.6

NT = not tested

The level of germination in all treatments was considerably lower than the germination of oospores normally, when the oospores were suspended in water and plated shortly after extraction. The buffer or the time gap between the extraction of oospores and plating them may have reduced the germination rate. It is also possible that the oospores start germinating whilst being incubated with the enzymes, but are then damaged when plated for germination on the solid medium.

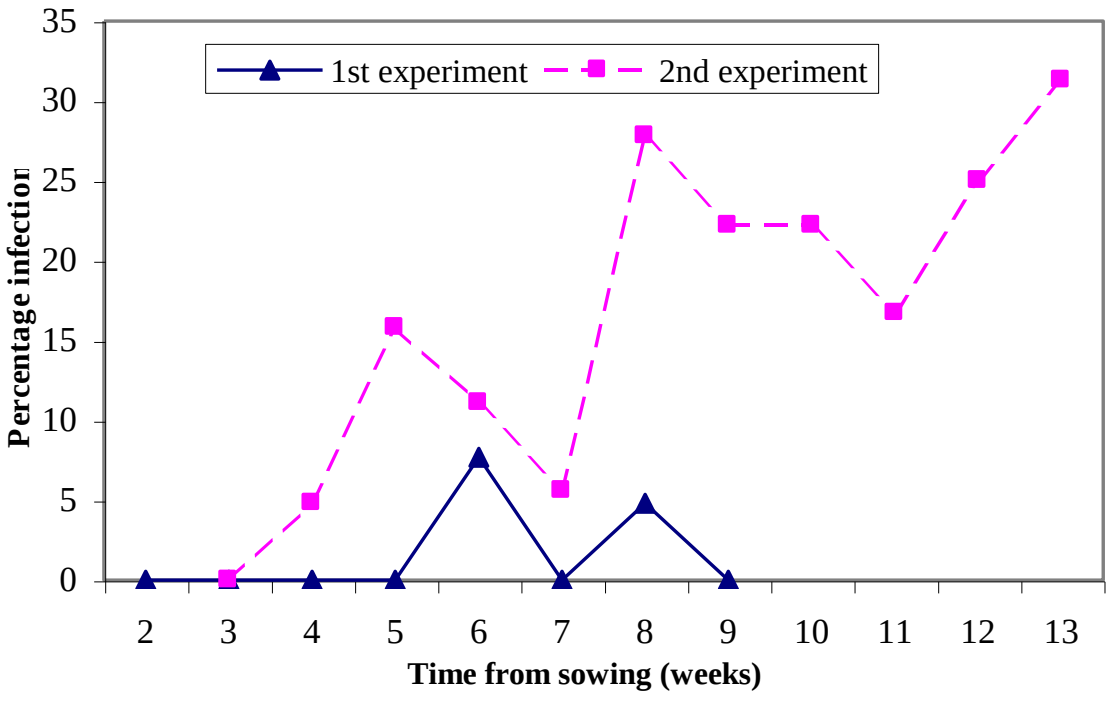
Cellulase has potential as a pre-treatment to enhance the germination of *P. violae* oospores. However, use of different buffers should be investigated and the concentration of cellulase as well as the incubation temperature and time should be optimised. Liquid germination media would be beneficial as a germination medium to avoid possible damage to the germinated oospores during plating on solid medium.

Oospore inoculum – infection studies using carrot seedlings

In the first experiment *P. violae* was only isolated from the roots of the seedlings at 6 and 8 weeks after sowing (Fig. 4). On both occasions, less than 8 % of the seedlings were infected. The first experiment was run in the summer and despite the temperature control, the temperature varied greatly (daily min: 7.5-20.3°C; daily max: 17.0-35.5°C).

In the second experiment, *P. violae* was first isolated four weeks after sowing and at every sampling after that. The percentage infection increased from 5 % at four weeks to 31 % at 13 weeks after sowing. As the second experiment was run over winter, the temperatures stayed lower and did not vary as much as in the first experiment (daily min: 5.5-11.4°C; daily max: 11.8-18.6°C).

Figure 4 Infection of seedlings inoculated using extracted oospores.



Higher levels of infection in the second experiment may have been due to the lower temperature during the second experiment, the double dose of inoculum and/or different timing of the inoculation.

Comparison of infectivity of three* P. violae *inocula in carrot seedling bioassays

Extracted oospores of *P. violae* gave a high level of infection in the roots of carrot seedlings (Table 7). This confirmed the result of the seedling test in the glasshouse, which indicated that oospores extracted from liquid culture could be used to produce infection in carrot seedlings. The method can be used in future experiments to establish the inoculum threshold required for infection as well as to study the effect of environmental factors on infection and symptom expression.

None of the seeds emerged in the treatment inoculated with oatmeal sand indicating that the inoculum rate may have been too high. No *P. violae* was isolated from any of the seeds recovered from this growing medium. No *P. violae* was isolated from the seedlings or seeds grown in naturally infested soil. The level of inoculum in the soil may not have been high enough to establish detectable levels of infection at the early seedling stage when the sampling was done, or possibly were overgrown by faster growing colonies of other *Pythium* spp.. A high percentage of seedlings grown in the soil were infected by other *Pythium* spp..

Table 7. Isolation of *Pythium* spp. from the roots of 4-week-old carrot seedlings inoculated using different inocula of *P. violae*.

Inoculation method	**Percentage emergence**	**Isolation of *Pythium* spp. from carrot roots (%)**	
		P. violae	**Other *Pythium* spp.**
Oospores	80	44	0
Oatmeal sand inoculum	0	0	0
Naturally infested soil	68	0	98

Assessment of the effect of soil moisture on the cavity spot infection of carrot seedlings

P. violae was not isolated from the roots of any carrot seedlings at either sampling time or at any of the soil moisture levels assessed. Although soil B was demonstrated to contain active inoculum by presence of disease in the field, measurements of cfu (see 'Measurement of soil-borne inoculum of *P. violae*' below) and high levels of disease in the calcium pot experiments, conditions were not conducive to infection in this pot experiment. In the absence of knowledge on inoculum levels and how this relates to pathogenicity, it is difficult to explain this result. Storage of the soil for two months did not eliminate viable pathogen propagules as seen in isolations from the soil, but it may have reduced their pathogenicity.

Measurement of soil-borne inoculum of P. violae

The range of procedures tested in the work following on from that reported in 2001 (Pettitt *et al.,* 2001) on the topic of quantification of *P. violae* from soil using conventional plating techniques will not be considered in detail here. Soil sieving as described previously (Pettitt *et al.,* 2001) worked well, although the process produced large numbers of non-target colonies (> 90% of all colonies). The use of sucrose density gradient centrifugation proved disappointing as the germination of the majority of *P. violae* propagules was inhibited by the concentrations of sucrose required for good separations. Nevertheless, this procedure may prove a useful concentration step in future molecular approaches to quantification of soil-borne inoculum of this pathogen. Finally, a relatively simple plating procedure was adopted. This consisted of plating 0.5 ml aliquots of a dilution series of the selected soil sample onto selective agar plates (see Pettitt *et al.,* 2001) at a rate of 50 plates per dilution. A dilution of soil equivalent to 0.1g in 100 ml was generally selected for further analysis (selection was based on the number of colonies developing on plates after 2 days). The further analysis consisted of daily sub-culturing from the original plates over 4 days from 24h after plating (at 24 h all plates were assessed). All colonies on the original plates of the selected dilution were sub-cultured onto fresh selective agar for identification. This procedure provided accurate determinations of the numbers of *P. violae* propagules in soil, but it was slow, laborious and expensive in both materials and staff time, costing an estimated £800 per soil sample in staff time alone. However, some useful results were obtained (Table 8) that demonstrate that there may be some value in developing new (less expensive!) techinques for monitoring changes in *P. violae* populations in relation to the environment and disease development. Both the Wellesbourne Cottage Field soil (soil W) and samples from Brigg (soil B) showed marked increases in the number of colony forming units (cfu) present from late Summer to Autumn 2001 (Table 8). This would appear to match the development of the generally severe cavity spot epidemic in the 2001 season as well as the severe disease in the fields sampled. A further two samples from the north of England were assessed when the epidemic had already started and both of these showed comparatively high levels of *P. violae* cfu. All of these sites were not harvested until November.

Table 8 Numbers of *P. violae* cfu recovered per gramme dry weight of soil from four cavity spot problem soils during late in the 2001 season showing changes in the populations that might be linked to the development of the severe epidemic of that season.

Soil	Assessment date		
	Aug 2001	Oct 2001	Nov 2001
Wellesbourne Cottage Field (‘soil W’)	15	0	127
Brigg (‘soil B’)	-	32	185
Lancashire 1	-	-	80
Yorkshire 2	-	-	164

Conclusions

Fungicides

- Metalaxyl is still the best fungicide available for the control of cavity spot.
- No new fungicides have been shown to be better than metalaxyl in either pot or field experiments, although some new chemicals do show promise in *in vitro* tests.
- There is no resistance to metalaxyl in UK populations of *Pythium violae* and only limited tolerance was seen is the small sample of *P. sulcatum* isolates assessed.
- Split doses of metalaxyl did not give improved control of cavity spot over single-dose early applications immediately post seedling emergence
- The new metalaxyl-M formulation SL 567A was tested and gave comparable control to metalaxyl as Fubol 58WP in pot experiments, but gave disappointing results in some field trials.
- Field trials generally gave inconsistent, disappointing and sometimes confusing results. Whilst much more consistent than field trials, pot experiments also gave some unclear results. This was largely because the direct impact of treatments on the gpathogen could not be accurately determined. In the absence of good spatial and temporal detection and quantification of soil-borne inoculum and how this relates to disease, such experiments and field trials will continue to be difficult to interpret. and of comparatively limited value. New treatments require detailed evaluation to optimise dose and timing using a range of different accurately quantified epidemics.
- Metalaxyl degrades in soil. The rate of degradation varies greatly and in some fields it is very rapid (half life < 7 days). Evidence from this project indicates:
    1) degradation is microbial in origin,
    2) all rapid degrading fields assessed so far have had histories of intensive metalaxyl use,
    3) neighbouring fields with similar soil but NOT exposed to metalaxyl use (where available), did not degrade metalaxyl as rapidly,
    4) the phenomenon is not limited to one region of the UK, but, since only a limited number of sites have so far been assessed, no prediction on the frequency of fast degradation can yet be made.
- As part of this project a test for metalaxyl degradation was developed and is available (Contact Andy Jukes, HRI Wellesbourne, Warwick, CV35 9EF, email andrew.jukes@hri.ac.uk)..

Calcium compounds

- Soil amendments with some calcium compounds do significantly reduce the severity and, to a lesser extent, the incidence of cavity spot disease.
- As with any control treatment, the degree of disease control using calcium varies from soil to soil.
- The disease control effects of calcium amendments to soil did consistently carry over to a second crop in pot experiments.
- In some pot experiments lime (calcium carbonate) and hydrated lime (calcium hydroxide) treatments gave better control of cavity spot than SL567A treatments, and later applications of the fungicide to lime-treated pots did not improve on disease control already achieved.

- Different calcium compounds give different levels of disease control and liming treatments (both lime and hydrated lime) give the best control, whilst gypsum (calcium sulphate) gives no control and calcium nitrate and calcium monocarbamide give an intermediate level of disease control.
- The effects of calcium compounds on disease appear due to both their impact on soil pH and the direct effect of increasing the concentration of cation exchangeable calcium ions. In acid-neutral soils, lime treatments will increase both the pH and the concentration of exchangeable calcium whereas in alkaline soils the latter becomes the main effect. Calcium nitrate and calcium monocarbamide only affect the concentration of exchangeable calcium and gypsum has no impact on disease because it increases neither pH nor exchangeable calcium concentrations.
- Over the range 3 – 10 t/ha, increasing the liming rate did not appear to improve the efficacy of disease control in the Wellesbourne Cottage Field soil indicating that benefits of lime application can be achieved with comparatively low rates. The lime/pH response of this soil indicated that no further increases in pH were obtained from applications greater than 6 t/ha.

Pathogen Biology

- *Pythium violae* is still the predominant cavity spot pathogen in UK carrot crops, being present in 90% of all cavities sampled during this project.
- Assessments of infection of seedlings in naturally infected soil demonstrated the presence of P. violae infections in roots 6 weeks from drilling by molecular techniques and after 8 weeks by conventional plating methods – demonstrating that infection can occur very early in the development of a crop and that early fungicide applications are essential for effective disease control.
- Oospores have been successfully produced and harvested, and germination successfully achieved *in vitro*. As expected, germination was low (5-14%). This is a normal aspect of oospore survival behaviour and not a reflection on harvested oospore viability. Large numbers of oospores were produced at 10 and 15^{o}C, but not at 20 ^{o}C. The temperature range for germination was 5–30 ^{o}C, with an optimum at 20-25 ^{o}C. The pH range for germination was 4.5-9, with optimum germination at pH 8. This latter result indicates that the possible pH effects on disease in soil may be indirect in their action by, for example, encouraging more competitive bacterial growth, otherwise high pH mightbe expected to aggravate cavity spot.
- Infection of carrots was successfully achieved in pot experiments using oospores, with infections clearly detectable in seedling roots 4 weeks after sowing. Further work is required to develop a fully optimised infection system from this work for use as a pathogenicity test to provide quantified disease challenges to new potential control methods and possibly for development into a plant resistance assay.
- Addition of hymexazole to selective agar media improved the rate of isolation of *P. violae*, especially from older cavity spot lesions.
- A conventional plating procedure has been developed that allows limited quantification of *P. violae* in soil. However this is a lengthy, laborious and costly operation (approximately £800 of staff time alone per soil sample).
- The soil plating procedure shows that *P. violae* populations are sparse compared to other soilborne *Pythium* species, ranging from 0 – 30 colony forming units (cfu)/.g dry weight of soil. The level of colonisation detected in two ‘problem’ soils was seen to increase to 80 – 200 cfu/g dry weight of soil during Autumn 2001. This increase may

be linked to the severe cavity spot epidemic seen in that season. The link between quantifiable soilborne *P. violae* populations and disease urgently requires further study to provide accurate and reliable methods of determining the efficacy of potential new disease control methods.

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APPENDIX

Appendix I Definitions of disease categories used in the ADAS field trial.

% root surface area infected	Disease category	Marketability Score
0.0	0	1 Marketable (excellent)
0.0 - 0.3	1	1 Marketable (excellent)
0.3 - 1.5	2	2 Marketable (Good)
1.5 - 3.0	3	3 Umarketable
3.0 - 10.0	4	3 Unmarketable
10.0 +	5	3 Unmarketable

Appendix II Yield and root defects at harvest in the ADAS field trial.

Treatment	Weight (kg)	% fanged roots	% misshapen roots	% small roots	% ribbed roots
1 Untreated	7.02	8.7	3.7	2.3	1.3
2 SL567A x2	8.43	12.0	2.7	1.0	1.0
3 SL567A 1lf	7.53	17.3	5.3	2.7	1.0
4 Amistar pre-em	6.78	8.0	2.7	5.7	0.3
5 Amistar post-em	7.60	12.7	5.3	1.7	0.7
6 Amistar x2	8.28	13.0	3.0	2.0	4.0
7 HDC1	7.61	11.7	5.0	2.7	0.3
8 HDC2	7.45	13.7	5.0	6.0	1.3
9 HDC3	7.54	12.7	4.7	4.7	0.3
10 H303	6.99	10.0	4.7	3.0	2.7
SED (45 df)	0.737	5.30	2.27	1.89	*
Significance	Ns	Ns	Ns	Ns	*

Appendix III Summary of pest and minor disease incidence at harvest in the ADAS field trial

Treatment	% roots with carrot fly damage	% roots with shoulder rot	% roots with violet root rot	% roots with vertical cracks
1 Untreated	11.0	1.7	0.7	4.7
2 SL567A x2	14.0	1.0	0.7	11.0
3 SL567A 1lf	10.0	1.0	0.3	4.4
4 Amistar pre-em	11.7	1.0	0	5.3
5 Amistar post-em	12.0	0.3	0.7	6.1
6 Amistar x2	15.0	0.7	0	8.3
7 HDC1	4.0	1.7	1.3	3.0
8 HDC2	13.7	0	0	5.0
9 HDC3	9.3	1.0	0	5.4
10 H303	11.0	2.0	0	7.1
SED (45 df)	5.08	*	*	3.07
Significance	Ns	*	*	Ns

Appendix IV Disease progress during the ADAS field trial 2001/2002

Date sampled	Date assessed	% roots with *cavity spot	% roots with Disease Index 1	% fanged roots
07/08/01	08/08/01	0	0	0
07/09/01	11/09/01	0	0	0
12/10/01	12/10/01	1	1	0
15/11/01	15/11/01	0	0	0
11/01/02	15/01/02	0	0	0
13/02/02	13/02/02	1	1	0

* based on sample of 50 roots

Appendix V Site details and treatments for the ADAS field trial, 2001/2002

Site Shropham Norfolk (Field Low Road North East)
Grid Reference: TL991923
Crop: Carrot cv. Lagor (located in a farm crop of parsnip cv. Javelin)
Date of drilling: 23/05/01
Seed rate: 1.63million per hectare
Seed treatment: Rovral and Thiram
Soil type: Sandy loam
Soil analysis (at drilling): pH: 8.0
Phosphorus: 43 mg/l (Index 3)
Potassium: 111 mg/l (Index 1)
Magnesium: 45 mg/l (Index 1
Previous cropping: 2000 Bulb onions
1999 Sugar beet
1998 Winter wheat
1997 Potatoes

Treatments:

Fertilisers: Seedbed (16 March 2001) N 17 kg/ha, P 50 kg/ha, K 200 kg/ha, Mg 100kg/ha
Top dressing (3 June 2001) 40 kg/ha
Trace elements OptE-Man (manganese) 1.50 l/ha on 17 August 2001 (with Hallmark)
OptE-Man (manganese) 2.50 l/ha + Opt-E-Cu (copper) 0.50 l/ha + Headland Zinc 0.50 l/ha on 1 September 2001 (with Hallmark)

Herbicides:
Linuron Flowable 1.35 l/ha + Stomp 400 5.0 l/ha in 1000 l water/ha 3 June 2001
Afalon 0.70 l/ha + Dosaflo 2.00 l/ha in 400 water/ha on 28 July 2001 (tank mix with Hallmark)

Fungicides: Only experimental treatments applied to carrots.

Insecticides:
Temik 10G 10 kg/ha 31 May 2001
Toppel 0.25 l/ha in 300 l water/ha 6 July 2001
Pirimicarb 280 g/ha in 200 l water/ha 17 July 2001
Hallmark with Zeon Technology 0.15 l/ha in 400 l water/ha 28 July 2001
Hallmark with Zeon Technology 0.15 l/ha in 400 l water/ha 17 August 2001
Hallmark with Zeon Technology 0.15 l/ha in 400 l water/ha 1 September 2001

Harvest date: 19 March 2002

Appendix VI Crop Diary for the ADAS field trial

Date	Action
23/05/01	Set-up trial, drilled and applied pre-drill treatments. Watton will apply pre-em herbicides. Soil samples taken. (LR)
22/06/01	Visited site - very little emerging (less than 50%). Some have reached 1st true leaf stage but most still at cotyledon stage. Lots of volunteer potatoes. Whole field showing low emergence not just our trial area. 48.5mm rainfall (10th-17th). (LR)
10/7/01	2nd treatments applied. Soil surface wet. Rain after spraying plot 7. Treatment 8,9,10 after a heavy shower. (PB)
14/7/01	Heavy thunderstorms in the area.
15/7/01	Hand rogue potato volunteers. Obvious erosion of bed edges after heavy rain on 14 July. Seedling population very sparse. (PG)
17/7/01	Site visited. Very heavy shower 16/7/01 - signs of surface erosion, water standing on headland. Plants o.k. but seems to have 1st leaf removed, just stalk remains. 27mm rain recorded on th 18th (evening) in nearby raingauge. Potatoes have been rogued by hand. (PB)
07/08/01	Plots at bottom of the trial ('ie' end by hedge) have much higher emergence than those at the top of the field. Potato volunteers have been sprayed. (LR).
30/11/01	Experimental area covered with fleece to protect from frost. (PB).
11/01/02	Site visit. Fleece had blown off part of experimental area - bottom of slope near the headland. Sample dug of unsprayed area: no cavity spot just a few small vertical cracks on some roots. (PB).
13/02/02	Site visit. Some roots now rotting from top downwards (5%). Sample washed - 1% cavity spot and some with CRF. (PB).
19/03/02	Harvested all plots - took 60 roots from each. Counted carrots in 1m2 of each untreated plot. Took overall soil sample. NO harvest length set due to variation in emergence. (LR).

Appendix VII Measurements of soil pH throughout the lime treatments pot experiment (see PART 2, page 9 above).

Soil	Lime @ 6 t/ha	Soil pH			
		At drilling	2 weeks later	2 months later	At harvest
A1	-	7.57	7.73	7.76	8.07
	+	7.71	7.75	7.92	8.02
A6	-	7.62	7.61	7.61	7.47
	+	7.63	7.62	7.61	7.45
SH	-	6.74	6.68	6.69	6.23
	+	7.22	7.21	7.20	6.77
B	-	6.86	6.77	6.77	6.44
	+	7.19	7.19	7.13	7.00
W	-	5.76	5.80	5.79	5.88
	+	6.83	7.17	7.25	7.29

AppendixVIII Basic mineral analysis of the soils used in the lime treatments pot experiment (see PART 2, page 9 above).

Soil	Lime (6 t/ha)	P (µg/ml)	K (µg/ml)	Mg (µg/ml)	N (µg/ml)	Total Ca (µg/g)	Water soluble Ca (µg/g)	Cation exchange-able Ca (µg/g)
A1	-	29	96	73	129	5978	122	3009
	+	-	-	-	-	7345	119	4148
A6	-	62	568	243	383	79388	288	23985
	+	-	-	-	-	77463	338	25878
SH	-	79	697	567	119	2561	35	1722
	+	-	-	-	-	3408	61	2622
B	-	96	343	207	92	8419	90	3514
	+	-	-	-	-	8529	114	4679
W	-	40	274	408	107	2076	136	1880
	+	-	-	-	-	2975	220	2911

Appendix IX Fungicide resistance tests: origin of *P. violae* isolates and their ED_{50} (μg ml^{-1}) values for metalaxyl.

***P. violae* isolate**	**Field location**	**ED_{50} (μg ml^{-1})**
1	Culture directly from collection	0.081
2	ADAS, Arthur Rickwood	0.072
3	ADAS, Arthur Rickwood	0.062
4	ADAS, Wolverhampton	0.080
5	Norfolk	0.053
6	Lancashire	0.074
7	Norfolk	0.066
8	Norfolk	0.070
9	Suffolk	0.067
10	Suffolk	0.090
11	Merseyside	0.073
12	Yorkshire	0.073
13	Merseyside	0.067
14	Suffolk	0.066
15	Yorkshire	0.076
16	Lancashire	0.062
17	Norfolk	0.061
18	ADAS, origin unknown	0.072
19	Lancashire	0.063
20	Norfolk	0.064
21	Lancashire	0.048
22	Lincolnshire	0.049
23	Nottinghamshire	0.060
24	Lincolnshire	0.083
25	Unknown	0.053
26	Nottinghamshire	0.052
27	Nottinghamshire	0.023
28	Lancashire	0.050
29	Lincolnshire	0.038
30	Yorkshire	0.039
31	Yorkshire	0.033
32	Scotland	0.021
33	Scotland	0.019
34	Yorkshire	0.020
35	Yorkshire	0.028
36	Yorkshire	0.021
37	Yorkshire	0.026
38	Yorkshire	0.013
39	Yorkshire	0.016
40	Lincolnshire	<0.010
41	Lancashire	0.038
42	Lancashire	0.048
43	Lancashire	0.026
44	Lancashire	<0.010

45	Yorkshire	<0.010
46	Yorkshire	0.020
47	Yorkshire	0.018
48	Yorkshire	0.030
49	Yorkshire	0.018
50	Lancashire	0.015
51	Yorkshire	0.026
52	Yorkshire	0.034
53	Cupar, Scotland	0.022
54	Lancashire	0.046
55	Yorkshire	0.034
56	Yorkshire	0.032
57	Lincolnshire	0.014
58	Yorkshire	0.030
59	Yorkshire	<0.010
60	Yorkshire	0.015
61	Yorkshire	0.034
62	Yorkshire	0.030
63	Yorkshire	0.042
64	Yorkshire	0.025
65	Lancashire	0.053
66	Lancashire	0.052
67	East Anglia	0.017
***P. sulcatum* isolates**		
1	Lancashire	0.427
2	Unknown	0.658
3	Staffordshire	0.658
4	Unknown	0.586
5	Yorkshire	3.299

Appendix X NIAB disease severity score chart used in all experiments in parts 2 & 4 above.