FV 5f Carrot: the biology and control of cavity spot

Conducted on behalf of

Horticulture Research International and ADAS

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Project leader:	Dr T R Pettitt succeeding Dr J G White, HRI Wellesbourne
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Location of project:	HRI, Wellesbourne, HRI Stockbridge House, ADAS Boxworth and commercial grower's sites.
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CONTENTS

PRACTICAL SECTION FOR GROWERS

Background

Subsequent to the identification of several different species of *Pythium* as the primary causal organisms for carrot cavity spot, the most important species in the UK being *P. violae*, reasonably good control of the disease was achieved using the fungicide metalaxyl. However, in recent years control of the disease has declined, and in some fields where metalaxyl was used, has been virtually 'non-existent'. This report describes the third year of work in a project set up by HDC to:

a) determine the reason(s) for the recent apparent decline in the efficacy of metalaxyl.
b) assess new fungicide formulations and new molecules (eg azoxystrobin) for control of cavity spot.
c) find alternative treatments, not based on synthesised fungicides, that might prove more durable (eg calcium-based soil treatments).
d) carry out work to improve our understanding of the biology of the disease with a view to helping with control strategies.

Objectives

- ***Fungicide and associated field trials***

  Field trials were set up at HRI Stockbridge House and at a commercial nursery under ADAS supervision. The objectives of these trial were to:

  i) screen the efficacy of a range of fungicides including SL567, UK876 and Amistar.
  ii) assess the efficacy of calcium compounds, including calcium carbonate, calcium sulphate and calcium nitrate, against cavity spot.

- ***Laboratory screening in pot tests***

  Four groups of pot trials were established to:

  i) assess the 'carry-over' effect of sucessful calcium treatments from the previous season to a second crop.
  ii) determine the impact of rates and timing of applications of calcium carbonate to the soil on disease.
  iii) compare a range of calcium compounds, including calcium carbonate, calcium sulphate, calcium hydroxide and calcium monocarbamide, for their impact on disease.

iv) assess the efficacy of fungicides, UK 876, SL 567 and Amistar for efficacy against cavity spot.

- ***Laboratory screen for fungicide sensitivity***
  Work was continued to determine whether there is resistance to metalaxyl in UK populations of the primary cavity-spot pathogen, *P. violae*. This survey was expanded to include isolates of the (so far) less frequently seen species *P. sulcatum*.

- ***Biology of cavity spot disease of carrots***
  Work was initiated for completion in the final year of the project to:
  i) develop an isolation medium suitable for detection and quantification of *P. violae* in soils.
  ii) produce, extract and carry out germination tests on the overwintering spores (oospores) of *P. violae*.

If successful, the work using these procedures will enable more reliable fungicide efficacy tests to be completed and help us better understand the disease cycle: determining cavity spot risks and improving the timing of fungicide applications.

Summary of Results

The key results of this year's work can be summarised in the following list of conclusions:

Calcium treatments

- In 'carry-over' pot experiments, all the three calcium compounds tested (calcium carbonate, calcium hydroxide and calcium monocarbamide) achieved levels of control of cavity spot that did not significantly vary between the first and the second crop after lime treatment. The pH of each soil remained stable between crops. Generally, the disease control achieved in the calcium carbonate and the calcium hydroxide-treated pots was better than in the calcium monocarbamide pots. The highest pH values were in the Calcium hydroxide-treated pots (pH range = 6.9 – 7.8), followed by calcium carbonate (pH range = 6.3 – 7.3) and the lowest in calcium monocarbamide treatments (pH range = 5.7 – 6.3). These results indicate that control may be achieved in liming treatments *via* a soil pH effect, although calcium availability was not determined and can not therefore be discounted.

- Calcium sulphate had no significant effect on cavity spot disease in either pot experiments or in field trials. In addition, calcium nitrate did not have an effect on disease in field trials. Neither of these compounds increased the pH of test soils in pots, whilst they would both be expected to increase the levels of calcium. This is further evidence in support of a pH as opposed to a calcium effect on *P. violae* cavity spot; a result consistent with Australian findings with the cavity spot caused by *P. coloratum* (El Tarabily *et al.*, 1997).

- Pot trials on the effect of timing and scale of calcium carbonate applications contradicted the previous year's results. Applications immediately prior to drilling (effectively an 'at drilling' application) were the only treatments to significantly reduce cavity spot. Applications 1 and 2 months prior to drilling reduced disease but were not as good as the 'at drilling' application. In addition, there did not appear to be any difference in the effect between the 6 and the 12 tonne/ha applications in either disease control or subsequent soil pH (6 t/ha = pH 6.3 – 6.9 & 12 t/ha = pH 6.6 – 7.1).

- Most of the experimental work in pots, and to some extent field trials, completed so far has been in soils of moderate to slightly acid pH, whereas many UK carrot soils are alkaline. In an attempt to assess the impact of lime on disease in alkaline soils and finally dissociate the possible pH from calcium effects, a series of pot trials have been set up in which lime applications to high and low pH cavity spot 'problem' soils will be compared. In these experiments both the pH and the concentrations of calcium available will be monitored along with the usual disease and yield assessments.

Fungicides

- In all pot tests and field trials, experimental fungicide UK 876 failed to control cavity spot at all rates and timings of application.

- Amistar applications showed little or no effect on cavity spot in the pot experiments. However, fungicide sensitivity tests with representative isolates of *P. violae* did demonstrate significant fungicidal activity, although the ED_{50} was not as low as for metalaxyl. In addition, field trials at both sites used showed some promise for control at the higher rates of application. This merits further investigation.

- The SL 567 metalaxyl formulation gave inconsistent results in pot experiments and disappointing results in both of the field trials. This disappointing performance could

be due to fungicide resistance developing in the pathogen or possibly to metalaxyl degradation in the soil. Assessments of metalaxyl sensitivity in isolates of *P. violae* from both the field sites, and from all over the UK, have so far shown no indications of fungicide resistance in this species. Isolates of alternative cavity spot causing pathogen, *P. sulcatum*, showed a higher degree of metalaxyl tolerance than *P. violae*, and a shift in the field populations in favour of this species could cause a decline in SL 567 efficacy. However, it is also possible that metalaxyl degradation was a factor in the performance of SL 567 and analysis of soil samples from the field sites and from the pot experiments is ongoing.

Pathogen inoculum

- 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 15ºC but not at 20 ºC. The temperature range for germination was 5–30 ºC, with an optimum at 20-25 ºC. 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.

- Hymexazole successfully reduced the *in vitro* growth of *Pythium intermedium, P. sylvaticum* and *Pythium* group HS isolates, whilst having little effect on *P. violae* isolates. This immediately provided an improved medium for isolations from cavities on carrots, but, more importantly, made it possible for the first time to carry out colony forming unit (cfu) assessments on infected soils. Pimaricin at between 2 and 10 ppm with hymexazole at 10 ppm gave the best isolation medium. However, *Mortierella* spp. still caused problems in some samples, and higher rates of hymexazole may need to be tested. *P. violae* inoculum in the cavity spot 'problem' soils so far tested, appears to be very sparse (<100 cfu/g dry weight of soil) and detection required a pre-plating concentration step consisting of wet sieving followed by centrifugation. Work is ongoing on improvements to the pre-plating treatment of samples. More importantly, further work, on a wider range of soil samples is required to determine whether sparse inoculum, even in known 'problem' soils, is a widespread phenomenon as this would influence the development of reliable control strategies.

Action Points for Growers

- Work with calcium additions to soil strongly indicates that increases in soil pH up to approximately pH 7.5 reduced cavity spot disease. The influence of calcium ions may also be important in higher pH soils, but this requires further work. *If considering liming treatments to improve cavity spot control, then carry out a lime/pH response curve to determine the optimum liming rate to achieve pH 7 or above. If the pH is already above 7, then lime treatments may not be effective, although this topic is one of the subjects covered by the final year's experimental work.*

- Assessments of soil populations of *Pythium violae* in cavity spot 'problem' soils have so far shown colonisation to be very sparse. This has strong implications for (a) the determination of disease risks and (b) the control strategies used. We therefore need to assess more field samples from all over the UK. *Whenever possible please send us soil samples together with a sample of affected carrots from any fields that develop severe cavity spot disease. Please send at least 500g of soil plus 3 to 5 affected carrots and information about the location of the field and previous cropping history if available. Please mark samples CAVITY SPOT SAMPLE FOR HDC PROJECT, Tim Pettitt, Plant Pathology & Microbiology, HRI, Wellesbourne, Warwick, CV35 9EF.*

- So far, 31 isolates of *P. violae* have been assessed for their sensitivity to metalaxyl, and none of these has shown resistance to the fungicide. In addition, 5 isolates of *P. sulcatum* have been obtained and tested. *P. sulcatum* isolates tended to show a higher level of tolerance to the fungicide than *P. violae. These results imply that fungicide resistance is not the reason for the recent apparent decline in metalaxyl performance against cavity spot. However, very localised resistance problems may still be occuring, or we may be seeing a shift in the pathogen populations responsible from* P. violae *to the more metalaxyl-resistant* P. sulcatum. *We are therefore very keen to receive samples of carrots plus soil (as outlined in the action point above) from fields showing severe cavity spot, which have received full applications of metalaxyl.*

SCIENCE SECTION

General introduction

Cavity spot has been recognised and studied for 38 years, but for the first 22 of these the cause of the disease was not known. Many theories were advanced, including calcium deficiency, damage by insect larvae, soil ammonification, damage by anaerobic bacteria and others. However, the disease was controlled in studies in Norway using fungicides which specifically targeted Oomycete fungi. The latter group of fungi includes both *Pythium* and *Phytophthora* among the soil-borne plant pathogens, and it was a relatively quick job to isolate *Pythium* from cavities and complete Koch's Postulates by using isolates to cause cavities on healthy carrots. Over the years it has been demonstrated that the slow growing *Pythium violae* is causal in over a dozen countries world-wide and the even slower-growing *P. sulcatum* is causal in a smaller number of countries, particularly on low pH soils. It is now thought that very few other species of *Pythium* are involved primarily in causing cavity spot, although many other species (especially the faster growing species commonly present in soil such as *P. sylvaticum*), may be isolated from old, dark lesions. A more in-depth survey of the history has been presented in the report for HDC-funded project FV5e.

Following on from this work good control of cavity spot was achieved using metalaxyl fungicide treatments, particularly in areas where *P. violae* was the predominant cavity spot pathogen. However, in recent years the efficacy of disease control with metalaxyl appears to have declined in some crops. The current 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 annual report describes the results of the third year of the project and covers:

- results of field trials run by HRI at Stockbridge House and by ADAS at a commercial holding.
- results of pot experiments carried out at HRI Wellesbourne, investigating the effects of calcium compounds on cavity spot.

- an update on ongoing metalaxyl resistance studies, plus tests of sensitivity to the relatively new fungicide azoxystrobin.
- results of promising initial work on pathogen biology in soil in relation to disease; oospore germination and infection, development of isolation media and the measurement of soil inoculum.
- a paper to be presented at the BCPC conference on metalaxyl degradation in carrot fields (Appendix I).

PART 1 – Field trials at HRI, Stockbridge House

Materials and Methods

Site

Horticulture Research International, Stockbridge House, Cawood, Selby, North Yorkshire YO8 3TZ. The trial site being located in field L. This site is known to be infested with *Pythium violae,* the causal agent of carrot cavity spot, following intensive production on this field.

Crop and Cultivar

Carrot cv. Lagor

Trial Design

The experiment was split into two trials with each being a randomised block with 6 replicates. Each plot was comprised of a single split bed (1.8m x 8m long) of 14.4 m^2 . The two halves of the plot were used for sampling purposes with the first half harvested in the autumn and the remaining half strawed down over winter and harvested in February/March 2001.

Treatment Application

Trial 1

The calcium treatments (carbonate, sulphate and nitrate) in Trial 1 were applied 3-4 weeks pre-drilling. The calcium treatments were top-dressed and then immediately incorporated by the bed former. The fungicide treatment in Trial 1 was applied overhead at early post-emergence using an Oxford Precision sprayer with boom attachment.

The amount of nitrate in the calcium nitrate treatment was calculated and extra nitrate was added as a top dressing on the other calcium treatments and the untreated control to

ameliorate any possible treatment differences that would otherwise occur through the addition of nitrate.

Trial 2

The fungicide treatments were applied as soil drenches prior to drilling (within 24 hours of drilling) to allow some incorporation of the fungicides by the drill.

Treatment List

Trial 1

Each treatment was replicated 6 times

Calcium treatments:

1. Untreated

2. Calcium sulphate (incorporation 3 weeks prior to drilling)
   6 tonnes/ha

3. Calcium nitrate (pre-drill and post emergent)
   666kg/ha in a split dose, with half 3 weeks prior to drilling and the second as a top dressing at 2-3 true leaf stage.

4. Calcium carbonate (incorporation 3 weeks prior to drilling)
   10 tonnes/ha

Superimposed on calcium plots:

a) SL567 single post-emergent application at 1-2 true leaf stage at 1.3l product/1000l water/ha.

b) Non treated with SL567.

(Treatments 1, 2 and 4 were amended with Nitrate, as a top dressing, equal to that given in treatment 3).

Trial 2

Each treatment was replicated 6 times

1. Untreated (water) control.

2. **SL567** (metalaxyl) (Syngenta) at 1.3l product/1000l water/ha applied pre-drill as a soil drench.

3. **SL567** (metalaxyl) (Syngenta) as a split dose with 0.65l product/1000l water/ha 1 week prior to drilling and a second dose of 0.65l product/1000l water/ha as a post-emergent application at the 1-2 true leaf stage.

4. **Amistar** (azoxystrobin) (Zeneca) at 6l product/1000l water/ha applied pre-drill as a soil drench.

5. **Amistar** (azoxystrobin) (Zeneca) at 10l product/1000l water/ha applied as a post-emergent application at the 1-2 true leaf stage.

6. **UK876** (experimental product) (Bayer) at a rate of 1.28kg product/1000l water/ha applied pre-drill as a soil drench.

7. **Filex** (propamocarb hydrochloride) 15l product/1000l water/ha applied pre-drill as a soil drench.

The calcium treatments were applied pre-drilling with incorporation at bed forming. Calcium nitrate was applied as a split dose with the first dose applied at the same time as other calcium treatments and the second at 2-3 true leaf stage.

The fungicide treatments were applied overhead using an Oxford Precision sprayer (E-Bar Engineering) with boom attachment modified to operate with compressed air at a pressure of 2.5 bars.

Assessments

All assessments following emergence to the autumn harvest period were performed on the first half of each plot:

1. At the pencil stage and 3-4 weeks later a sample of 25 roots was selected randomly from each of the untreated plots (Trial 2 only) and washed. The number and severity of cavity spot lesions was recorded using the NIAB Provisional Key. This assessment was intended to give an understanding of the expected levels of cavity spot in the trial.

2. In the autumn a random 50-root sample was selected from each plot and was weighted. The roots were then washed and a detailed assessment of cavity spot was performed this included the number of lesions/root and percentage area affected using the NIAB Provisional Key. The second half of the plot was strawed down over winter.

3. In February/march the second half of the split plot, that was strawed down over winter, was harvested using the same method as described above (no. 2) for the autumn harvest assessment.

Crop Diary

Sowing Dates:

Trial 1 – 16 May 00

Trial 2 – 1 June 00

Treatment application Schedule*:*

Trial 1

i) All Calcium treatments applied to soil surface – 8 May 00.

ii) Incorporation of Calcium and bed forming – 11 May 00.

iii) Nitram top dressing to all plots except Calcium nitrate, application calculated to give all plots equal doses of nitrogen (first of two applications) – 30 June 00.

iv) SL 567 post-emergent (1-2 true leaf stage) application – 28 June 00.

v) Calcium Nitrate (3 true leaf stage) – 1 August 00.

vi) Nitram second application – 1 August 00.

Trial 2

i) Pre-drill fungicide application – 15 May 00.

ii) Post-emergent (1-2 true leaf stage) fungicide application – 15 June 00.

Assessment Dates

Sample Harvest Dates for In-Crop Cavity Spot Assessment (Trial 2 only)

25 July 000 & 18 August 00

Major Harvest Dates

- Autumn harvest – 2 November 00 (Trial 1 and 2).

- Post strawing harvest - March 01 (Trial 1 and 2).

Statistical Analysis

A statistical analysis of variance was performed on raw data using a Genstat 5 programme.

Within the tables of results are comments on the significance of data, these comments are based on the comparison between the treaments (Significance Treated). The notation of significance in the tables is based on the following:-

NS = Result not significant
* = Significant result (P at 5 %)
** = Highly significant result (P at 1%)
*** = Very highly significant result (P at 0.1%)

Official Recognition at HRI, Stockbridge House

The study described was undertaken in compliance with the guidelines for Official Recognition of Efficacy Testing Organisations in accordance with EPPO guidelines.
Certificate No. ORETO 020,
Date of Issue: 13 January 1998,
Expiry Date: 31 December 2002.

Results and Discussion

Trial 1

The results for trial 1 (Calcium/SL567 treatments) are displayed in Tables 1-4 and for trial 2 (Fungicide treatments) in Tables 5-8.

The results for trial 1 (Table 1) show a significant reduction in the percentage of roots affected by cavity spot for calcium carbonate (Treatment 4). The other calcium treatments – calcium nitrate and calcium sulphate, were not significantly different from the untreated control. SL567 when applied to calcium sulphate- and carbonate-treated plots, significantly reduced cavity spot incidence, with calcium carbonate plus SL567 (Treatment 4) showing the lowest percentage of roots affected.

The results for the second (post-strawing) harvest show no significant differences between treatments, with all the differences that were observed in the earlier Autumn harvest having evened out between all treatments.

The results in Table 2 show that there were no significant differences between treatments, in the mean number of cavity spot lesions at each of the two harvests.

The results in Table 3 show that there were no significant differences between treatments for the mean severity of cavity spot at the Autumn harvest. However, at the second (post strawing) harvest there were significant differences, with calcium sulphate (Treatment 2) and calcium carbonate (Treatment 4) having higher mean disease severity scores than the untreated control and calcium nitrate (Treatment 3). These differences were observed in both the SL567- treated and untreated plots alike.

There were no significant differences between treatments in the mean total yield at both the Autumn and final (post-strawing) harvests (Table 4).

Trial 2

In trial 2 (fungicide treatments) at the first harvest (Table 5) there was a significantly lower incidence of cavity spot in plots treated with Amistar as a pre-drill application (Treatment 4) and as a post-emergence application (Treatment 5), than the untreated control. These differences, however, were not carried through to the final (post-strawing) harvest, with no significant differences demonstrated between treatments at this final assessment.

There were no significant differences between treatments, in the mean number of lesions and the severity of cavity spot at either of the two harvests (Tables 6 and 7). There were also no significant differences, between treatments, in the mean total yield at both the Autumn and the final (post-strawing) harvests (Table 8).

Table 1. Trial 1 - Mean Percentage of Carrots with Cavity Spot Infection at Two Harvest Intervals [a]

First Autumn Harvest			
Number	**Treatment**	**SL567 application**	**Untreated**
1	Untreated (water) control.	30.1	32.6
2	Calcium sulphate 6 tonnes/ha	22.0	37.3
3	Calcium nitrate 6 tonnes/ha	20.3	22.5
4	Calcium carbonate 10 tonnes/ha	13.9	21.0
Significance Calcium Treatments		*	
LSD 5% Calcium (20df)		10.26	
Significance SL567 Treatments		***	
LSD 5% SL567 (20df)		4.78	
Significance Calcium/SL567 interaction		NS	
LSD 5% Calcium/SL567 (20df)		12.28	
Second (Post-Strawing) Harvest			
1	Untreated (water) control.	61.8	53.9
2	Calcium sulphate 6 tonnes/ha	51.6	48.7
3	Calcium nitrate 6 tonnes/ha	55.5	47.5
4	Calcium carbonate 10 tonnes/ha	56.1	58.9
Significance Calcium Treatments		NS	
LSD 5% Calcium (20df)		7.96	
Significance SL567 Treatments		NS	
LSD 5% SL567 (20df)		6.41	
Significance Calcium/SL567 interaction		NS	
LSD 5% Calcium/SL567 (20df)		12.08	

[a] The results for % roots infected have been angle transformed.

Table 2. Trial 1 - Mean number of Cavity Spot Lesions per Root at Two Harvest Intervals [b]

First Autumn Harvest			
Number	**Treatment**	**SL567 application**	**Untreated**
1	Untreated (water) control.	1.33	1.36
2	Calcium sulphate 6 tonnes/ha	1.37	1.36
3	Calcium nitrate 6 tonnes/ha	1.28	1.28
4	Calcium carbonate 10 tonnes/ha	1.32	1.25
Significance Calcium Treatments		NS	
LSD 5% Calcium (20df)		0.23	
Significance SL567 Treatments		NS	
LSD 5% SL567 (20df)		0.12	
Significance Calcium/SL567 interaction		NS	
LSD 5% Calcium/SL567 (20df)		0.29	
Second (Post-Strawing) Harvest			
1	Untreated (water) control.	1.84	1.62
2	Calcium sulphate 6 tonnes/ha	1.57	1.48
3	Calcium nitrate 6 tonnes/ha	1.70	1.49
4	Calcium carbonate 10 tonnes/ha	1.65	1.69
Significance Calcium Treatments		NS	
LSD 5% Calcium (20df)		0.19	
Significance SL567 Treatments		NS	
LSD 5% SL567 (20df)		0.11	
Significance Calcium/SL567 interaction		NS	
LSD 5% Calcium/SL567 (20df)		0.25	

[b] The results for mean number of lesions per infected root have been square root transformed.

Table 3. Trial 1 - Mean Cavity Spot Disease Severity Score at Two Harvest Intervals (0-5 Disease Severity Category) [c]

First Autumn Harvest			
Number	**Treatment**	**SL567 application**	**Untreated**
1	Untreated (water) control	0.15	0.54
2	Calcium sulphate 6 tonnes/ha	0.21	0.17
3	Calcium nitrate 6 tonnes/ha	0.28	0.29
4	Calcium carbonate 10 tonnes/ha	0.58	0.11
Significance Calcium Treatments		NS	
LSD 5% Calcium (20df)		0.23	
Significance SL567 Treatments		NS	
LSD 5% SL567 (20df)		0.21	
Significance Calcium/SL567 interaction		NS	
LSD 5% Calcium/SL567 (20df)		0.38	
Second (Post-Strawing) Harvest			
1	Untreated (water) control.	0.55	0.48
2	Calcium sulphate 6 tonnes/ha	0.76	0.66
3	Calcium nitrate 6 tonnes/ha	0.55	0.42
4	Calcium carbonate 10 tonnes/ha	0.87	0.75
Significance Calcium Treatments		***	
LSD 5% Calcium (20df)		0.13	
Significance SL567 Treatments		NS	
LSD 5% SL567 (20df)		0.13	
Significance Calcium/SL567 interaction		NS	
LSD 5% Calcium/SL567 (20df)		0.23	

[c] The results for mean severity per infected root have been log transformed.

Table 4. Trial 1 - Yield – Tonnes per Hectare at Two Harvest Intervals

First Autumn Harvest			
Number	**Treatment**	**SL567 application**	**Untreated**
1	Untreated (water) control.	104.3	103.0
2	Calcium sulphate 6 tonnes/ha	90.9	83.1
3	Calcium nitrate 6 tonnes/ha	116.0	116.9
4	Calcium carbonate 10 tonnes/ha	157.6	112.7
Significance Calcium Treatments		*(6%)	
LSD 5% Calcium (20df)		16.3	
Significance SL567 Treatments		NS	
LSD 5% SL567 (20df)		8.6	
Significance Calcium/SL567 interaction		NS	
LSD 5% Calcium/SL567 (20df)		20.4	
Second (Post-Strawing) Harvest			
1	Untreated (water) control.	122.6	117.2
2	Calcium sulphate 6 tonnes/ha	146.5	129.3
3	Calcium nitrate 6 tonnes/ha	136.9	123.9
4	Calcium carbonate 10 tonnes/ha	140.5	132.5
Significance Calcium Treatments		NS	
LSD 5% Calcium (20df)		17.32	
Significance SL567 Treatments		NS	
LSD 5% SL567 (20df)		14.71	
Significance Calcium/SL567 interaction		NS	
LSD 5% Calcium/SL567 (20df)		27.08	

Table 5. Trial 2 - Mean Percentage of Carrots with Cavity Spot Infection. [a]

Number	Treatment	% carrots with cavities
1	Untreated (water) control.	41.0
2	**SL567** (metalaxyl) (Syngenta) at 1.3l product/1000l water/ha applied pre-drill as a soil drench.	38.0
3	**SL567** (metalaxyl) (Syngenta) as a split dose with 0.65l product/1000l water/ha 1 week prior to drilling and a second dose of 0.65l product/1000l water/ha as a post-emergent application at the 1-2 true leaf stage.	35.9
4	**Amistar** (azoxystrobin) (Zeneca) at 6l product/1000l water/ha applied pre-drill as a soil drench.	27.5
5	**Amistar** (azoxystrobin) (Zeneca) at 10l product/1000l water/ha applied as a post-emergent application at the 1-2 true leaf stage.	24.1
6	**UK876** (experimental product) (Bayer) at a rate of 1.28kg product/1000l water/ha applied pre-drill as a soil drench.	39.1
7	**Filex** (propamocarb hydrochloride) 15l product/1000l water/ha applied pre-drill as a soil drench.	33.8
Significance Treated		*
LSD 5% (30df)		11.68

[a] The results for % roots infected have been angle transformed.

Table 6. Trial 2 - Mean number of Cavity Spot Lesions per Root.[b]

Number	Treatment	No. cavities/root
1	Untreated (water) control.	1.54
2	**SL567** (metalaxyl) (Syngenta) at 1.3l product/1000l water/ha applied pre-drill as a soil drench.	1.62
3	**SL567** (metalaxyl) (Syngenta) as a split dose with 0.65l product/1000l water/ha 1 week prior to drilling and a second dose of 0.65l product/1000l water/ha as a post-emergent application at the 1-2 true leaf stage.	1.45
4	**Amistar** (azoxystrobin) (Zeneca) at 6l product/1000l water/ha applied pre-drill as a soil drench.	1.47
5	**Amistar** (azoxystrobin) (Zeneca) at 10l product/1000l water/ha applied as a post-emergent application at the 1-2 true leaf stage.	1.41
6	**UK876** (experimental product) (Bayer) at a rate of 1.28kg product/1000l water/ha applied pre-drill as a soil drench.	1.65
7	**Filex** (propamocarb hydrochloride) 15l product/1000l water/ha applied pre-drill as a soil drench.	1.66
Significance Treated		NS
LSD 5% (60df)		0.25

[a] The results for mean number of lesions per infected root have been square root transformed.

Table 7. Trial 2 - Mean Cavity Spot Disease Severity Score (0-5 Disease Severity Category). [c]

Number	Treatment	Cavity spot severity score
1	Untreated (water) control.	0.54
2	**SL567** (metalaxyl) (Syngenta) at 1.3l product/1000l water/ha applied pre-drill as a soil drench.	0.47
3	**SL567** (metalaxyl) (Syngenta) as a split dose with 0.65l product/1000l water/ha 1 week prior to drilling and a second dose of 0.65l product/1000l water/ha as a post-emergent application at the 1-2 true leaf stage.	0.54
4	**Amistar** (azoxystrobin) (Zeneca) at 6l product/1000l water/ha applied pre-drill as a soil drench.	0.50
5	**Amistar** (azoxystrobin) (Zeneca) at 10l product/1000l water/ha applied as a post-emergent application at the 1-2 true leaf stage.	0.46
6	**UK876** (experimental product) (Bayer) at a rate of 1.28kg product/1000l water/ha applied pre-drill as a soil drench.	0.58
7	**Filex** (propamocarb hydrochloride) 15l product/1000l water/ha applied pre-drill as a soil drench.	0.66
Significance Treated		NS
LSD 5% (60df)		0.24

[a] The results for mean disease severity score have been log transformed.

Table 8. Trial 2 - Yield – Tonnes per Hectare.

Number	Treatment	Yield (tonnes/ha
1	Untreated (water) control.	200.4
2	**SL567** (metalaxyl) (Syngenta) at 1.3l product/1000l water/ha applied pre-drill as a soil drench.	201.4
3	**SL567** (metalaxyl) (Syngenta) as a split dose with 0.65l product/1000l water/ha 1 week prior to drilling and a second dose of 0.65l product/1000l water/ha as a post-emergent application at the 1-2 true leaf stage.	216.1
4	**Amistar** (azoxystrobin) (Zeneca) at 6l product/1000l water/ha applied pre-drill as a soil drench.	208.8
5	**Amistar** (azoxystrobin) (Zeneca) at 10l product/1000l water/ha applied as a post-emergent application at the 1-2 true leaf stage.	218.4
6	**UK876** (experimental product) (Bayer) at a rate of 1.28kg product/1000l water/ha applied pre-drill as a soil drench.	201.2
7	**Filex** (propamocarb hydrochloride) 15l product/1000l water/ha applied pre-drill as a soil drench.	201.6
Significance Treated		NS
LSD 5% (60df)		55.04

[a] The results for % roots infected have been angle transformed.

Conclusions

- *Pythium* infection established effectively accross the trial site, causing cavity spot infection in all of the untreated control plots.

- A significant reduction in the incidence (percentage of roots affected) of cavity spot was shown with the use of calcium carbonate in pre-strawing assessments in trial 1 (Calcium/SL567 treatments).

- SL567 (single post-emergent application) used together with calcium carbonate and sulphate significantly reduced the incidence of cavity spot in pre-strawing assessments.

- In trial 1 (Calcium/SL567 treatments), there were no significant differences in total yield at both harvests.

- In trial 2 (Fungicide treatments), Amistar, as both a pre-drill and post-emergence application, reduced the incidence of cavity spot at the pre-strawing assessment.

- In trial 2 (Fungicide treatments), there were no significant differences between fungicide treatments in the mean number of lesions per root and the severity of cavity spot at both harvests.

- In trial 2 (Fungicide treatments) there were no significant differences in total yield, either between fungicides or between fungicide treatments and untreated controls.

PART 2 – Field trial run by ADAS at a commercial site

Materials and Methods

Trial design

The experiment was split into two trials in a similar manner to the Stockbridge House field experiment (see Part 1). Each trial consisted of a randomised block with 6 replicates; trial 1 consisted of 4 calcium treatments, arranged in a randomised block with plots split and fungicide applied to one half, giving a total of 8 treatments (see Appendix II, Table i for trial plan), and trial 2 consisted of 7 treatments (Appendix II, Table ii). The plots were 5 m long. Cultivar Nairobi was sown on 2 June at 1.63 million seeds / ha to give a population of 70 – 75 plants per metre row on a four row bed.

Treatments

The experiment was located in a commercial crop of carrots and received standard agrochemical treatments apart from fungicides (see crop diaries Table 9). Soil analysis for the site showed a pH of 8.2, phosphorus 58 mg / l (index 4), potassium 210 mg / l (index 2), magnesium 76 mg / l (index 2) and organic matter 2.06 % m / m. Treatments were applied pre-drilling, or at the appearance of the first true leaf (post emergence). For treatment lists for trials 1 and 2 refer to Part 1 (pages x – y).

Assessments

The trials were checked regularly through the Autumn on 5/10/00, 1/11/00 and 30/11/00, but only minimal disease was observed, a full harvest and assessment was therefore not carried out. The final full harvest, yield determination and disease assessment was carried out on 26/01/01.

For assessments a 2 m row length was lifted from the centre 2 rows of each plot. The carrots were weighed and counted and a 100 root sample was randomly selected, washed and assessed for disease, pest damage and other parameters. Root weights and numbers were assessed first and roots were cold stored until assessments were completed.

Cavity spot lesions were counted and the percentage root area affected was recorded for each root. These absolute percentage area values were converted to the NIAB disease index for subsequent analysis. ‘Old’ cavity spot lesions (which were open lesions with corky strands) and ‘new’ lesions (which showed intact periderm and a water-soaked surface) were distinguished and recorded separately.

Results and Discussion

The incidence and severity of cavity spot were low in both trials 1 and 2 (Figures 1 and 2) and overall there were very few young cavity spot lesions (Tables 10 and 11). Other diseases, including violet root rot and scab, were detected at low levels (Appendix II). These were not affected by the trial treatments. Pest damage was also low and there were

Table 9. Crop diaries for the ADAS Cavity-spot field trial, 2000-2001 (Courtesy Knights Farms)

Farm cultivation records

Date	Operation	Rate (l or kg^{-ha})
01.05.00	Terra disk, sub-soil and plough	n/a
31.05.00	Base fertiliser	n/a
01.06.00	Ridge, stone separate and bedform	n/a
02.06.00	Farm crop drilled c.v Nairobi	n/a
01.07.00	Tractor hoe	n/a
04.07.00	Top dressing	250
01.08.00	Injector hoeing	n/a
02.08.00	Irrigation	n/a
05.08.00	Top dressing	201.0
01.09.00	Irrigation	n/a
10.09.00	Irrigation	n/a
18.11.00	Strawed-down	n/a

Farm spray records.

Date	Chemical	Rate(l or kg^{-ha})
04.05.00	PDQ	4.0
08.06.00	Linuron	1.7
	Stomp 400	5.0
14.07.00	Bittersalz	5.0
	Manganese sulphate	5.0
24.07.00	Copper	0.5
	Manganese sulplate	5.0
25.07.00	Bittersalz	5.0
	Solubor	4.0
27.07.00	Dosaflo	1.9
	Linuron	1.0
	Hallmark Zeon	0.15
07.08.00	Bittersalz	5.0
	Manganese sulphate	5.0
15.08.00	Compass	4.0
	Hallmark Zeon	0.15
26.08.00	Bittersalz	5.0
	Manganese sulphate	5.0
02.09.00	Hallmark Zeon	0.15

no effects from either factor on root numbers harvested or on the yield (Appendix II). There were no significant differences in disease between treatments except in the percentage of roots with NIAB disease category 2 (Tables 12 & 13 and Figures 3 & 4). In trial 1 (calcium treatments), the highest percentage of category 2 severity was seen in the $CaSO_4$ without SL567 and the $Ca(NO_3)_2$ with SL567 treatments (Figure 3). In trial 2 (fungicide treatments), the UK 876 pre-drilling treatment showed the most category 2 disease (Figure 4). Although the differences were not significant, all fungicide treatments except Filex reduced the percentage root area affected (Figure 2). The lowest sevrity of cavity spot in trial 2 followed the Amistar pre-drilling treatment and there were also indications that SL 567 could be effective as a split dose treatment applied pre- and post-emergence (Figure 2). However, the levels of class 2 disease were very low at 5.67 % maximum in trial 1 and 2.67 % in trial 2. The differences in class 2 disease were in some measure reflected by differences in total disease incidence in each trial especially trial 2 (Figures 1 & 2). As in year 2 of this project, the incidence and the severity of cavity spot did not appear related. In trial 1 the highest mean root areas affected by cavity spot were in the $CaCO_3$ treatments (both with and without SL567) whereas in trial 2, the most severe symptoms were seen in the untreated plots.

In terms of yields, the lowest yield in both t / ha and numbers of roots / ha were in the CaCO3 without SL567 treatment in trial 1 (Figure 5). Interestingly, the highest yield was seen in the CaCO3 with SL567 treatment (Figure 5), although in the absence of calcium treatments SL567 did not appear to have any effect on yields. The reduced yield might be related to factors other than cavity spot resulting in early root death or poor emergence, although no obvious causes could be ascertained. Many roots in trial 1 exhibited a ribbing symptom and the percentage of ribbed roots was also the highest in the $CaCO_3$ without SL567 treatment (Figure 6). The cause of this symptom was unknown. Generally there were fewer mishapen roots where fungicide was used (Figure 6 and Table 14). In trial 2, the yields were highest in the untreated, SL567 pre-drilling, Amistar pre-drilling and Filex pre-drilling treatments (Figure 7). The SL567 split dose, Amistar post emergence and the UK876 pre-drilling treatments all appeared to reduce yields both in t / ha and number of roots / ha.

Conclusions

- Despite being in a ‘high cavity spot risk field’, the levels of disease seen in this trial were very low, this means that conclusions from the results of this work need to be considered with caution.

- Under conditions of low disease expression, the treatments did not appear to give disease control, and there were indications that some treatments might had an adverse effect.

- Further work with Amistar is recommended as this appeared to have good activity against cavity spot in this trial.

- SL567 showed limited activity and an assessment will be carried out on the soil from this field site to determine the level of metalaxyl degradation.

- The 3 calcium treatments did not give consistent effects and there is the possibility that calcium nitrate might actually encourage *Pythium* infection.
- There was no clear evidence of calcium treatments controlling cavity spot; this might be a site interaction and other field site may prove more responsive. Many UK carrot soils are alkaline sands and these contrast with the acidic, impoverished sands in Western Australia where the use of calcium incorporations was beneficial.

Table 10. Incidence and severity of new and old lesions of cavity spot on calcium trial at first harvest, Herringswell, 26th January 2001. (Shows effects of calcium and fungicide separately)

Treatment Number	Treatment Description	Mean % roots affected (new lesions)	Mean number of new lesions / 50 roots	Mean % root area affected (new lesions)	Mean % roots affected (old lesions)	Mean numbers of old lesions / 50 roots	Mean % root area affected (old lesions)
1	Calcium sulphate pre-drilling	0.83	0.60	0.30	4.00	2.70	1.80
2	Calcium nitrate pre-drilling and post-emergent	0.50	1.50	0.60	11.00	26.50	11.10
3	Calcium carbonate pre-drilling	3.17	11.20	4.80	8.80	25.30	16.20
4	Untreated	0.33	0.20	0.20	3.50	2.60	2.10
	SED (15df)	2.089	7.930	3.250	5.030	20.150	9.660
	p value	ns	ns	ns	Ns	ns	ns
1	- SL567	1.92	5.80	2.40	7.10	17.00	8.60
2	+ SL567	0.50	0.90	0.50	6.60	11.50	7.00
	SED (20df)	1.449	5.550	2.310	3.690	14.540	7.390
	p-value	ns	ns	ns	Ns	ns	ns

Table 11. Incidence and severity of new and old lesions of cavity spot on fungicide trial at first harvest, Herringswell, 26th January 2001.

Treatment Number	Treatment Description	Mean % roots affected (new lesions)	Mean number of new lesions / 50 roots	Mean % root area affected (new lesions)	Mean % roots affected (old lesions)	Mean numbers of old lesions / 50 roots	Mean % root area affected (old lesions)
1	Untreated	0.00	0.00	0.00	2.00	9.50	2.72
2	SL567 pre-drilling	0.67	0.33	0.35	1.33	1.00	0.68
3	SL567 pre-drilling and post-emergent	0.00	0.00	0.00	1.00	0.50	0.42
4	Amistar pre-drilling	0.67	0.33	0.18	1.00	0.50	0.07
5	Amistar post-emergent	0.33	0.17	0.05	1.67	1.00	0.38
6	UK876 pre-drilling	1.67	1.83	0.40	3.33	2.80	1.83
7	Filex pre-drilling	1.00	2.17	0.47	2.00	6.50	4.32
	Mean	0.620	0.690	0.207	1.760	3.100	1.490
	SED (30df)	0.627	0.974	0.295	1.221	4.460	2.581
	p-value	ns	ns	ns	ns	ns	ns

Table 12. Incidence and severity of cavity spot in trial 1 (calcium trial) at first harvest, Herringswell, 26th January 2001. (Shows interaction between calcium and fungicide treatments).

Treatment number	Treatment Description	Total % roots with cavity spot	% roots with disease severity category 1 (NIAB)	% roots with disease severity category 2 (NIAB)	% roots with disease severity category 3 (NIAB)	% roots with disease severity category 4 (NIAB)	% roots with disease severity category 5 (NIAB)	Mean number of lesions / 50 roots	Mean % area affected by cavity spot
1	Calcium sulphate pre-drill without SL567	7.3	1.33	5.67	0.33	0.00	0.00	0.11	1.20
1	Calcium sulphate pre-drill with SL567	2.0	0.00	2.00	0.00	0.00	0.00	0.02	0.58
2	Calcium nitrate pre-drill and post-emergent without SL567	7.3	2.00	1.33	3.00	1.00	0.00	0.32	0.99
2	Calcium nitrate pre-drill and post-emergent with SL567	14.7	4.00	4.33	3.67	2.33	0.33	0.80	1.47
3	Calcium carbonate pre-drill without SL567	12.7	3.00	2.00	0.67	5.33	1.67	1.36	2.18
3	Calcium carbonate pre-dril with SL567	5.7	2.00	1.67	0.67	0.67	0.67	0.10	2.40
4	Untreated without SL567	2.7	0.67	1.67	0.33	0.00	0.00	0.03	0.81
4	Untreated with SL567	5.3	2.00	1.33	1.67	0.33	0.00	0.08	1.51
	Mean	**7.20**	**1.870**	**2.500**	**1.290**	**1.210**	**0.330**	**0.350**	**1.390**
	SED	**7.23**	**1.858**	**1.789**	**1.909**	**3.011**	**0.920**	**0.772**	**1.139**
	p-value	**ns**	**ns**	**0.035**	**ns**	**ns**	**ns**	**ns**	**ns**

Table 13. Incidence and severity of cavity spot in trial 2 (fungicide trial) at first harvest, Herringswell, 26th January 2001.

Treatment number	Treatment Description	Total % roots with cavity spot	% roots with disease severity category 1 (NIAB)	% roots with disease severity category 2 (NIAB)	% roots with disease severity category 3 (NIAB)	Mean number of lesions /50 roots	Mean % area affected by cavity spot
1	Untreated	2.00	1.00	0.00	0.67	0.19	2.68
2	SL567 pre-drilling	2.00	0.67	0.67	0.67	0.03	0.74
3	SL567 pre-drilling and post-emergent	1.00	0.00	1.00	0.00	0.01	0.42
4	Amistar pre-drilling	1.67	1.33	0.33	0.00	0.02	0.23
5	Amistar post-emergent	2.00	1.00	1.00	0.00	0.02	0.42
6	UK876 pre-drilling	5.00	2.00	2.67	0.00	0.09	1.05
7	Filex pre-drilling	3.00	1.00	0.67	0.33	0.17	1.88
	Mean	2.380	1.000	0.900	0.240	0.076	1.060
	SED (30df)	1.290	0.925	0.676	0.476	0.096	1.476
	p-value	Ns	Ns	0.015	ns	ns	ns

Figure 1. ADAS Trial 1 (Calcium Field Trial) 2000-01
Incidence and Severity of Cavity Spot

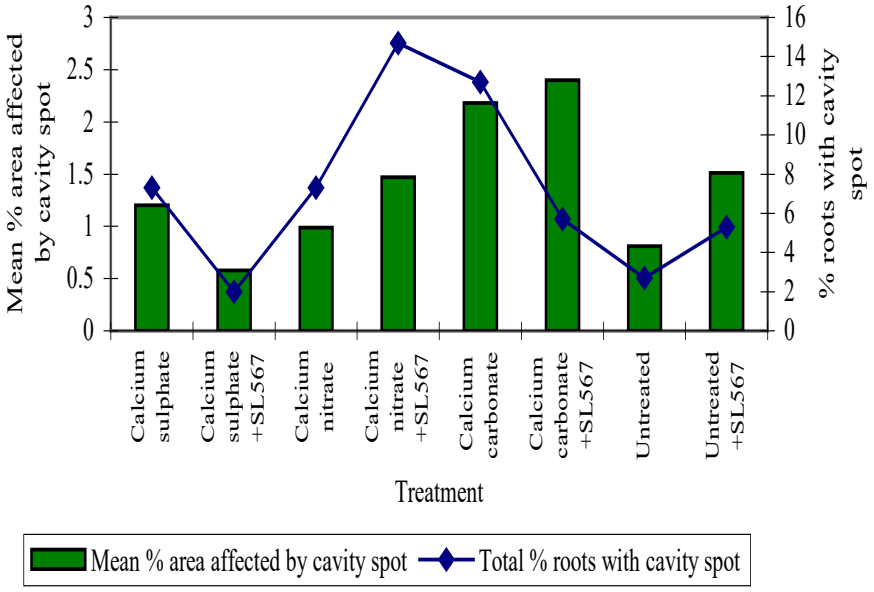


Figure 2. ADAS Trial 2 (Fungicide Field Trial) 2000-01
Incidence and Severity of Cavity Spot

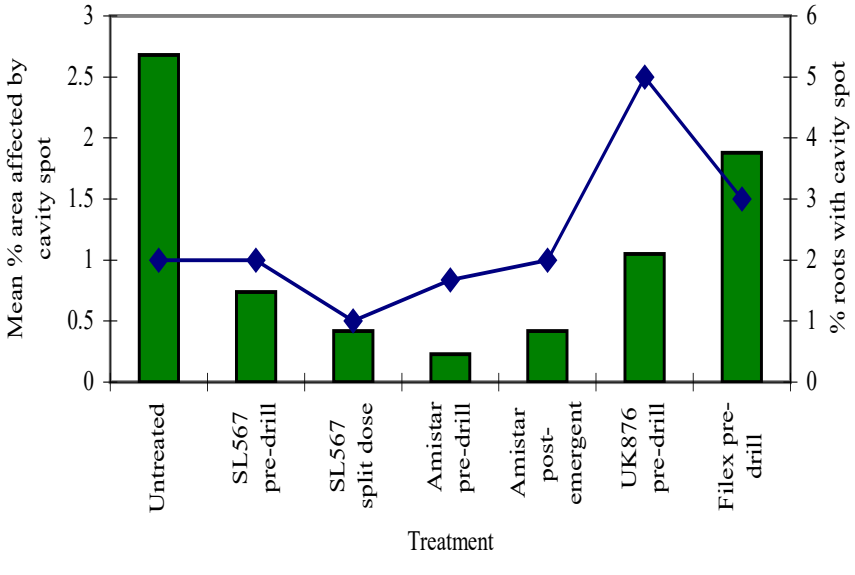


Figure 3. ADAS Trial 1 (Calcium Field Trial) 2000-01
Percentage of Roots with Disease Severity Category 2 (NIAB)
(SED = 0.68; 30 df)

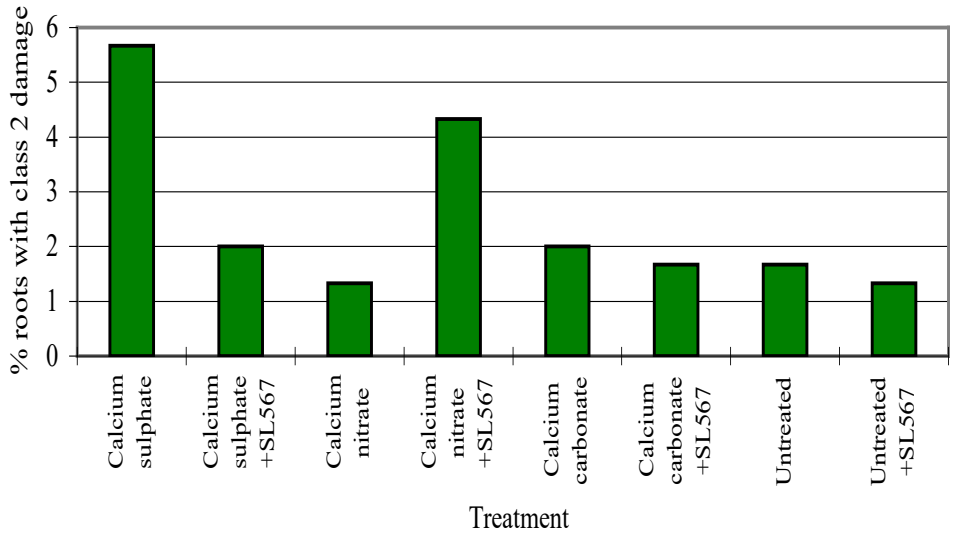


Figure 4. ADAS Trial 2 (Fungicide Field Trial) 2000-01
Percentage of Roots with Disease Severity Category 2 (NIAB)

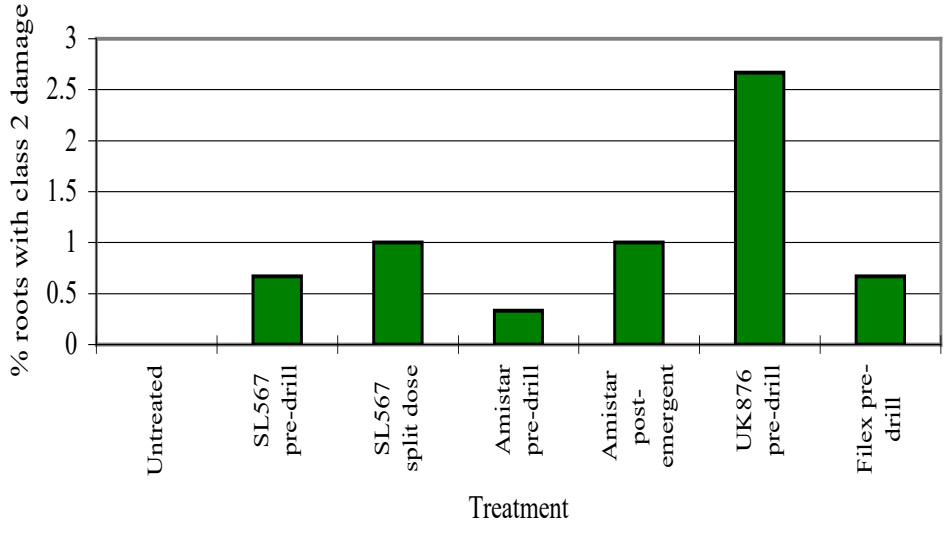


Figure 5. ADAS Trial 1 (Calcium Field Trial) 2000-01
Yield and Root Population at Harvest

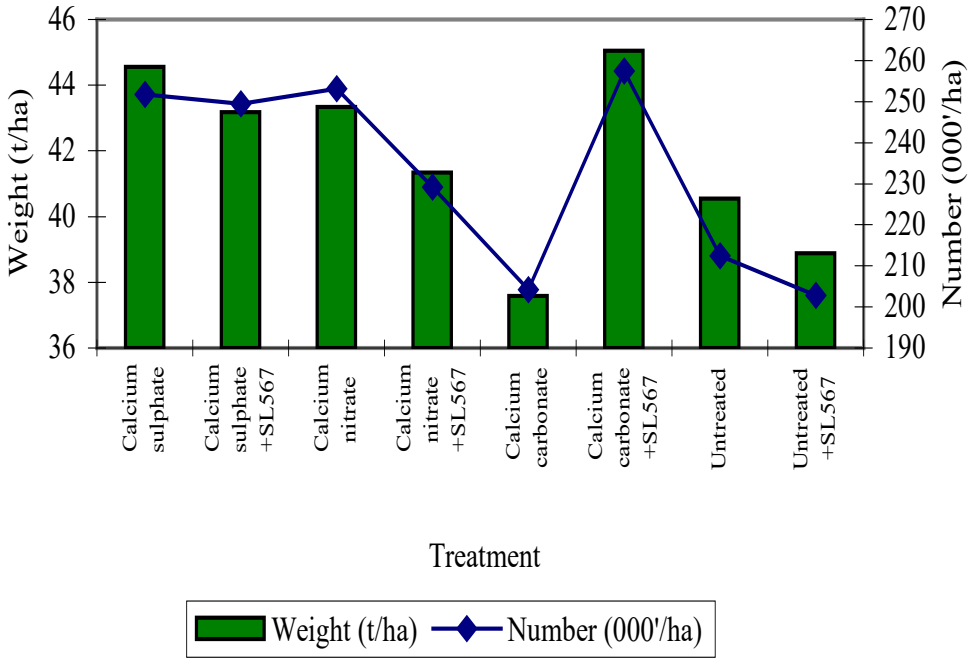


Figure 6. ADAS Trial 1(Calcium Field Trial) 2000-01
Percentage of Ribbed Roots at Harvest

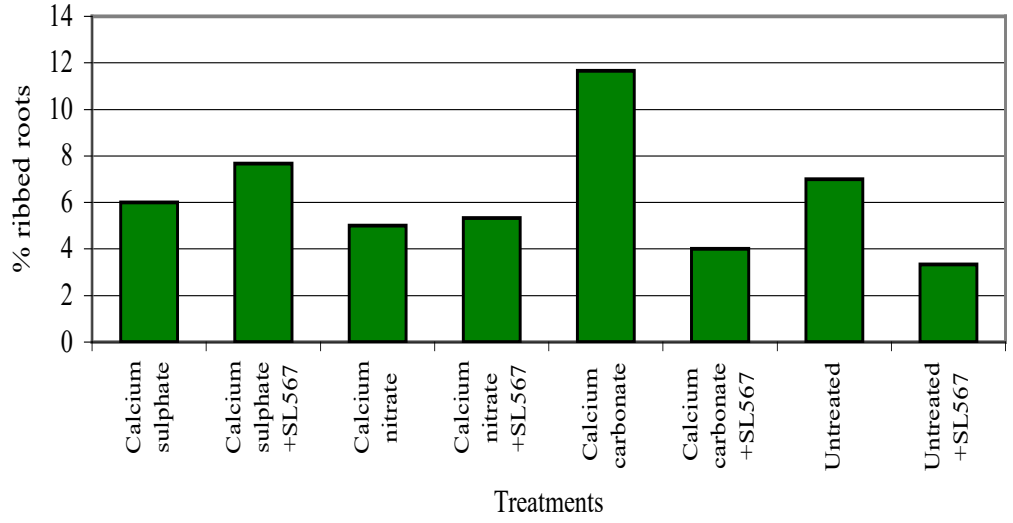


Figure 7. ADAS Trial 2 (Fungicide Field Trial) 2000-01
Yield and Root Population at Harvest

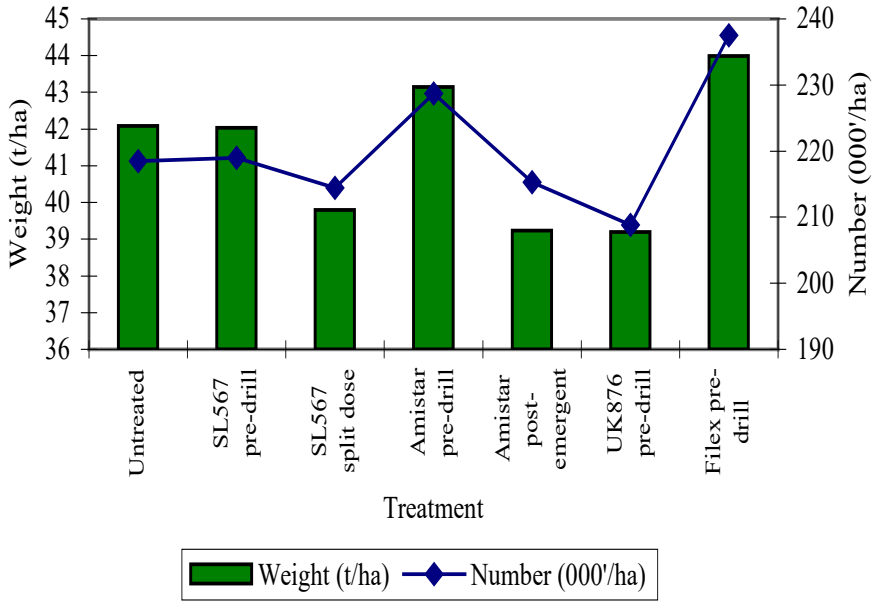


Table 14. Yield, root population and root defects on calcium trial at first harvest, Herringswell, 26th January 2001. (Shows effects of calcium and fungicide separately)

Treatment Number	Treatment Description	% fanged roots	% small roots	% misshapen roots	% ribbed roots	Weight of roots (t/ha)	Number of Roots (000/ha)
1	Calcium sulphate pre-drilling	9.33	3.33	8.80	6.83	43.87	250.70
2	Calcium nitrate pre-drilling and post-emergent	10.83	3.00	6.30	5.17	42.33	241.20
3	Calcium carbonate pre-drilling	7.83	4.00	8.30	7.83	41.32	230.80
4	Untreated	10.33	3.33	7.30	5.17	39.71	207.60
	SED (15df)	1.626	1.361	2.260	2.412	1.560	15.480
	P value	Ns	ns	ns	ns	ns	ns
1	- SL567	8.75	4.08	10.30	7.42	41.50	230.40
2	+ SL567	10.42	2.75	5.10	5.08	42.11	234.70
	SED (20df)	1.369	1.111	2.120	1.282	1.351	7.90
	p-value	Ns	ns	0.022	ns	ns	ns

PART 3 – Fungicide and calcium studies in controlled pot experiments, assessments of fungicide sensitivity, examination of the interaction between *P. violae* inoculum and carrots, and the development of selective media for isolation of *P. violae*

A) Carry-over of 1999 pot experiments

Materials and Methods

Pots from the two experiments carried out in 1999 were retained and re-sown in the following year to study carry-over effects from the previous years calcium treatments. The pots in Experiment A (experiment 2 in 1999) had been treated with different rates of calcium carbonate (Snowcal 10, Omya Croxton and Garry Ltd) and calcium hydroxide (Peakstone lime, RMC Industrial Mineral Ltd). The pots in Experiment B (experiment 3 in 1999) had received high rates of calcium carbonate, calcium hydroxide and calcium monocarbamide (Nitro-Plus 9, Stoller Chemicals Ltd) at two different application times. After harvest in autumn 1999 (Table 15), all the remaining roots and debris were removed and the pots were left on the glasshouse bench until the following spring when the soil was turned and the pots were resown with 40 seeds of carrot cv. Nanco per pot. At completion of emergence, seedling stands were reduced to 20 per pot. No spray applications were made, so all the effects were due to the previous year's treatments. The pots were placed in saucers. Watering was done via the saucers, apart from a light application, when necessary, onto the soil surface to prevent it from drying out. During the growing season the carrots were routinely treated for sciarid and aphids as necessary (Appendix III). The carrots were harvested 140 days after sowing. The washed roots were scored by recording the number of cavities less and greater than 5 mm diameter and assessing a percentage root area affected by cavity spot using NIAB's area assessment chart (Appendix IV). In experiment 2 all roots were weighed. The roots were not weighed in experiment 1, because seedling emergence was uneven, resulting in variable numbers of roots per pot, which then affected the size of the roots. Analysis of variance was carried out on the data using Genstat 5 after angular transformations of the percentage data. In experiment 1 weighted means were used for the analysis to allow for the different numbers of carrots scored for each treatment. Soil pH was measured after the harvest of each crop in a 1:5 mixture of soil and 0.01M calcium chloride.

Table 15. Crop diary for the carry-over experiments.

	Experiment A	Experiment B
Harvest of year 1999 experiment	20.10.1999	10.11.1999
Sowing of the carry-over experiment	10.2.2000	17.2.2000
Harvest of the carry-over experiment	4.7.2000	5.7.2000

Experiment A: Different rates of $CaCO_3$ and $Ca(OH)_2$.
Experiment B: Timing of application of $CaCO_3$, $Ca(OH)_2$ and Ca-monocarbamide. SL567A was not applied for the carry-over crop.

Results and Discussion

In both carry-over experiments the incidence of cavity spot was high, more than 50 % of the carrots having cavities in the untreated controls. In Experiment A, all rates of calcium carbonate reduced the cavity spot incidence measured as percentage of carrots with cavities. However, the reduction was significant only at the rates of 3 and 12 t ha^{-1} (Table 16). The percentage area of carrot affected and number of large cavities per carrot was significantly reduced by all rates of calcium carbonate, and the total number of cavities per carrot was decreased by all rates except one. All rates of calcium hydroxide reduced the cavity spot incidence however the reduction was significant only at the rate of 9 t ha^{-1}. The percentage area of carrot affected was significantly decreased by all rates of calcium hydroxide, whereas the total number of cavities was only reduced at 9 t ha^{-1}. The number of large cavities per carrot was reduced at 3 t ha^{-1}. The soil pHs were similar after the first and second crop (Table 18).

In experiment B, all calcium treatments significantly reduced both the disease incidence and the disease severity measured using different parameters (Table 17). The disease incidence in the untreated controls was 57.5 % and disease severity, measured as total number of cavities per carrot, was 2.01. They were reduced to a mean incidence of 10.6 % and severity of 0.14 across the four calcium carbonate treatments and 9.1 % and 0.17, respectively, across the four calcium hydroxide treatments. For calcium monocarbamide the equivalent data was 19.7 % and 0.45. There were no differences for different times of

applications. Carrot size was reduced by most of the calcium applications. The soil pHs measured after the carry-over crops remained similar to those measured after the first crop (Table 19).

Table 16. Carryover of 1999 experiments. Experiment A: Control of cavity spot on carrots in soil from Wellesbourne treated with different rates of calcium carbonate and calcium hydroxide.

Treatment And rate	Percentage of carrots with cavities	Total number of cavities per carrot	Number of large cavities per carrot	Percentage area of carrot infected
Untreated control	50.0 [a *]	1.08 [ab]	0.86 [a]	4.4 [a]
$CaCO_3$ 3 t/ha	16.3 [bc]	0.31 [cd]	0.20 [bc]	0.8 [b]
$CaCO_3$ 6 t/ha	26.3 [ac]	0.66 [ade]	0.43 [bc]	1.4 [be]
$CaCO_3$ 9 t/ha	19.7 [ac]	0.47 [cef]	0.34 [bc]	0.9 [b]
$CaCO_3$ 12 t/ha	17.4 [bc]	0.45 [ceg]	0.30 [bc]	0.8 [b]
$Ca(OH)_2$ 3 t/ha	27.0 [ac]	0.60 [bdfgh]	0.35 [bc]	0.9 [bf]
$Ca(OH)_2$ 6 t/ha	32.8 [ac]	0.89 [afgi]	0.54 [ac]	1.8 [cefg]
$Ca(OH)_2$ 9 t/ha	17.1 [bcd]	0.55 [cehi]	0.52 [ac]	2.0 [bgh]
$Ca(OH)_2$ 12 t/ha	40.0 [ad]	1.08 [a]	0.87 [a]	2.0 [deh]

* Values in each column followed by different letters are significantly different (P=0.005) based on an analysis of variance and LSD test. For LSDs see Appendix V.

Table 17. Carryover of 1999 experiments. Experiment B: Control of cavity spot on carrots in soil from Wellesbourne treated with calcium compounds. SL567A was not applied for the carry-over crop.

Treatment	Percentage of carrots With cavities *	Total number of cavities per carrot	Number of large cavities per carrot	Percentage area of carrot infected *	Root weight as percentage of Untreated control
Untreated control	57.5 (49.5)	2.01	1.43	3.58 (7.4)	100.0
$CaCO_3$ 12 t/ha 1 month before drilling	12.5 (17.4)	0.20	0.11	0.27 (0.96)	80.3
$CaCO_3$ 12 t/ha Immediately before drilling	23.8 (26.5)	0.30	0.20	0.49 (1.77)	64.8
$CaCO_3$ 12 t/ha 1 month before drilling + SL567A	2.5 (4.6)	0.03	0.01	0.03 (0.13)	74.6
$CaCO_3$ 12 t/ha Immediately before drilling + SL567A	3.7 (5.6)	0.04	0.03	0.08 (0.28)	77.7
$Ca(OH)_2$ 12 t/ha 1 month before drilling	6.3 (8.9)	0.13	0.05	0.16 (0.49)	86.4
$Ca(OH)_2$ 12 t/ha Immediately before drilling	12.5 (14.7)	0.21	0.15	0.46 (1.19)	77.8
$Ca(OH)_2$ 12 t/ha 1 month before drilling	1.3 (2.3)	0.01	0.00	0.00 (0.03)	60.4

Table 17. continued

Treatment	Percentage of carrots With cavities *	Total number of cavities per carrot	Number of large cavities per carrot	Percentage area of carrot infected *	Root weight as percentage of Untreated control
$Ca(OH)_2$ 12 t/ha Immediately before drilling +SL567A	16.3 (20.3)	0.32	0.14	0.35 (1.19)	93.1
Ca monocarbamide 300 l/ha 1 month before drilling	18.8 (21.5)	0.61	0.48	2.29 (3.63)	97.3
Ca monocarbamide 300 l/ha Immediately before drilling	15.0 (18.3)	0.19	0.15	0.41 (1.32)	68.7
Ca monocarbamide 300 l/ha 1 month before drilling + SL567A	16.3 (20.4)	0.32	0.24	0.64 (2.01)	117.2
Ca monocarbamide 300 l/ha Immediately before drilling + SL567A	28.8 (29.8)	0.66	0.41	1.01 (2.72)	73.0
SL567A	27.5 (29.6)	0.64	0.50	1.42 (3.39)	101.1
LSD P ≤ 0.05	13.01	0.27	0.21	1.19	13.88
P ≤ 0.01	17.21	0.36	0.28	1.56	18.24
P ≤ 0.001	22.16	0.49	0.36	2.00	23.30

* arcsin transformation of percentages to which LSD applies in parentheses

Table 18. Carryover of 1999 experiments. Experiment A. pH ($CaCl_2$, 0.01M) of soils treated with different rates of calcium carbonate and calcium hydroxide determined after the first and second crops.

Treatment And rate	pH after 1st crop	pH after 2nd crop
Untreated control	5.6	5.9
$CaCO_3$ 3 t/ha	6.3	6.7
$CaCO_3$ 6 t/ha	7.3	7.2
$CaCO_3$ 9 t/ha	7.2	7.3
$CaCO_3$ 12 t/ha	7.2	7.2
$Ca(OH)_2$ 3 t/ha	6.9	7.0
$Ca(OH)_2$ 6 t/ha	7.4	7.4
$Ca(OH)_2$ 9 t/ha	7.5	7.6
$Ca(OH)_2$ 12 t/ha	7.7	7.8

Table 19. Carryover of 1999 experiments. Experiment B. pH ($CaCl_2$, 0.01M) of soils treated with calcium compounds or SL567 determined after the first and second crops.

Treatment	pH **After 1st crop**	pH **after 2nd crop**
Untreated control	5.3	5.8
$CaCO_3$ 12 t/ha 1 month before drilling	6.6	6.8
$CaCO_3$ 12 t/ha immediately before drilling	7.0	7.0
$Ca(OH)_2$ 12 t/ha 1 month before drilling	7.5	7.6
$Ca(OH)_2$ 12 t/ha immediately before drilling	7.6	7.6
Ca-monocarbamide 1 month before drilling	6.3	6.0
Ca-monocarbamide immediately before drilling	5.7	5.9
SL567A	5.5	6.0

B) Pot experiments in 2000

Materials and Methods

Three pot experiments were carried out.

Experiment 1:

Experiment 1 investigated the use of calcium carbonate (Snowcal 10, Omya Croxton and Garry Ltd) applied at different times and different rates with or without SL567A (a.i. metalaxyl M, 46 % w/v). The treatments were:

Untreated control
Calcium carbonate, 6 t/ha at drilling
Calcium carbonate, 6 t/ha 1 month before drilling
Calcium carbonate, 6 t/ha 2 months before drilling
Calcium carbonate, 12 t/ha at drilling
Calcium carbonate, 12 t/ha 1 month before drilling
Calcium carbonate, 12 t/ha 2 months before drilling
Calcium carbonate, 6 t/ha at drilling + SL567A, 1.30 l/ha
Calcium carbonate, 6 t/ha 1 month before drilling + SL567A, 1.30 l/ha
Calcium carbonate, 6 t/ha 2 months before drilling + SL567A, 1.30 l/ha
Calcium carbonate, 12 t/ha at drilling + SL567A, 1.30 l/ha
Calcium carbonate, 12 t/ha 1 month before drilling + SL567A, 1.30 l/ha
Calcium carbonate, 12 t/ha 2 months before drilling + SL567A, 1.30 l/ha
SL567A, 1.30 l/ha

Field soil was collected from Cottage Field at HRI Wellesbourne which is known to be a high risk site for *P. violae* induced cavity spot. The soil was bulked and sieved to remove stones before being used in the test. 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 appropriate rates in a concrete mixer and then put into the pots. The treatments were done one or two months before drilling or immediately prior to drilling (Table 20). The pots that were treated before drilling were left in the glasshouse until drilling and the soil kept moist by watering via saucers. Each pot was sown with 40 seeds of carrot cv. Lagor. 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 it from drying out. Where appropriate, SL567A was applied at first true leaf stage with a DeVilbiss atomiser using an electric pump. There were eight replicates of each treatment. During the growing season the carrots were routinely treated for whitefly, aphids and mildew as necessary (Appendix III). The carrots

were harvested 120 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 IV). Analysis of variance was carried out on the data using Genstat 5 after angular transformations of percentages. Soil pH was measured twice, shortly after the calcium incorporation and after harvest in a 1:5 mixture of soil to 0.01M calcium chloride.

Experiment 2:

In experiment 2, different rates of UK876 (active ingredient confidential) were tested along with Amistar (a.i. azoxystrobin, 25 % w/v) and SL567A for their efficacy to control cavity spot. The treatments were:

Untreated control
UK876, 160 g/ha
UK876, 400 g/ha
UK876, 800 g/ha
UK876, 1600 g/ha
Amistar, 4 l/ha
SL567A, 1.30 l/ha

The soil was prepared, the pots set up and the experiment maintained as described above for the Experiment 1, except that there were no treatments that required soil incorporation. All the chemical treatments were carried out at the first true leaf stage with a DeVilbiss atomiser using an electronic pump. There were eight replicates of each treatment. A preliminary harvest was made 130 days after sowing. This showed that cavity spot incidence was low and consequently the full final harvest was postponed to 170 days after sowing. Roots were scored and data analysed as described for Experiment 1.

Experiment 3:

Experiment 3 studied the potential of calcium sulphate ($CaSO_4 \cdot 2H_2O$, SIGMA C3771) for the control of cavity spot. The treatments were:

Untreated control
Calcium sulphate, 3 t/ha
Calcium sulphate, 6 t/ha
Calcium sulphate, 9 t/ha
Calcium sulphate, 12 t/ha

The soil was prepared, the pots set up and the experiment maintained as described above for Experiment 1. There was no chemical standard in this experiment. There were eight replicates of each treatment. The carrots were harvested 130 days after sowing. The roots were not weighed, because seedling emergence was uneven, resulting in variable numbers of roots per pot, which had a confounding impact on root size. Otherwise, roots were scored and data analysed as described for Experiment 1. Soil pH was measured shortly after the calcium incorporation and after harvest in a 1:5 mixture of soil to 0.01M calcium chloride.

Table 20. Crop record of the pot experiments in 2000.

	Experiment 1	Experiment 2	Experiment 3
Sowing	12.7.2000	30.5.2000	17.5.2000
Calcium treatments	11.5.2000 7.6.2000 11.7.2000	NT	15.5.2000
Chemical treatments	2.8.2000	22.6.2000	NT
Harvest	7.-8.11.2000	22.11.2000	20.9.2000

Experiment 1: Calcium carbonate at different timings and rates with or without SL567 compared with SL567A
Experiment 2: Different rates of UK876 compared with Amistar and SL567A
Experiment 3: Effect of calcium sulphate on cavity spot
NT not tested

Results and Discussion

Experiment 1:

The percentage of carrots with cavity spot was 43.7 % in the untreated control (Table 21). All the calcium carbonate treatments applied immediately before drilling significantly reduced the incidence and severity of cavity spot compared to the untreated control. Applications immediately prior to drilling were significantly better than those made 1 or 2 months before drilling. Both rates of calcium carbonate (6 and 12 t ha $^{-1}$) were equally effective and a post-emergence application of SL567A had no effect. However, SL567A alone gave as good control of cavity spot as calcium carbonate applied immediately before drilling. Root weight was increased by some of the calcium carbonate treatments. The pH of soils measured after harvest was increased by 0.7-1.2 units with 6 t ha^{-1} of calcium carbonate and by 0.9-1.4 units with 12 t ha^{-1} of calcium carbonate (Table 24).

Experiment 2:

The incidence of cavity spot was over 40 % in the untreated control pots (Table 22). None of the UK876 or Amistar treatments affected the disease incidence or disease severity. Also SL567A failed to control cavity spot in this experiment. Some of the UK876 and Amistar treatments reduced the root size.

Experiment 3:

The incidence of cavity spot was fairly low (15.0 %) in the untreated control pots (Table 23). None of the rates of calcium sulphate affected the incidence or the total number of cavities per carrot. There were increased numbers of large cavities and a greater surface area of carrot affected with some calcium sulphate treatments. Applications of calcium sulphate did not appear to raise the soil pH (Table 25). These results are consistent with the work carried out in Australia (El-Tarabily *et al*. 1997).

Table 21. Experiment 1. Control of cavity spot on carrots in soil from Wellesbourne treated with calcium carbonate applied at different times and different rates with or without SL567A.

Treatment and rate	Percentage of carrots with cavities*	Total number of cavities per carrot	Number of large cavities per carrot	Percentage area of carrot infected *	Root weight as percentage of untreated control
Untreated control	43.7 (41.2)	0.60	0.13	0.15 (1.34)	100.0
$CaCO_3$ 6 t/ha Immediately before drilling	20.0 (24.5)	0.29	0.03	0.06 (0.59)	99.9
$CaCO_3$ 6 t/ha 1 month before drilling	47.5 (43.7)	1.03	0.19	0.27 (1.85)	138.7
$CaCO_3$ 6 t/ha 2 months before drilling	48.7 (43.9)	0.86	0.08	0.20 (1.68)	157.3
$CaCO_3$ 12 t/ha Immediately before drilling	17.5 (21.1)	0.23	0.01	0.06 (0.52)	111.8
$CaCO_3$ 12 t/ha 1 month before drilling	57.5 (51.8)	1.68	0.21	0.50 (2.52)	170.6
$CaCO_3$ 12 t/ha 2 months before drilling	36.2 (36.6)	0.55	0.05	0.14 (1.20)	165.5
$CaCO_3$ 6 t/ha Immediately before drilling + SL567A[a]	7.5 (11.3)	0.09	0.00	0.01 (0.16)	94.7

to be continued

Table 21. continued.

Treatment and rate	Percentage of carrots with cavities*	Total number of cavities per carrot	Number of large cavities per carrot	Percentage area of carrot infected *	Root weight as percentage of untreated control
$CaCO_3$ 6 t/ha 1 month before drilling + SL567A [a]	36.2 (35.0)	0.53	0.03	0.09 (0.94)	152.0
$CaCO_3$ 6 t/ha 2 months before drilling + SL567A [a]	41.2 (39.7)	0.82	0.19	0.31 (1.73)	156.4
$CaCO_3$ 12 t/ha Immediately before drilling + SL567A [a]	10.0 (14.6)	0.11	0.03	0.05 (0.33)	144.9
$CaCO_3$ 12 t/ha 1 month before drilling + SL567A [a]	35.0 (33.8)	0.69	0.18	0.30 (1.60)	132.2
$CaCO_3$ 12 t/ha 2 months before drilling + SL567A [a]	40.0 (37.6)	0.76	0.10	0.24 (1.44)	173.3
SL567A 1.30 l/ha	7.5 (9.8)	0.09	0.03	0.03 (0.22)	103.8
LSD $P \leq 0.05$	14.63	0.306	0.105	0.562	16.29
$P \leq 0.01$	19.34	0.402	0.138	0.739	21.41
$P \leq 0.001$	24.93	0.513	0.176	0.944	26.66

*arcsin transformation of percentages to which LSD applies in parenthesis

[a] 1.30 l/ha

Table 22. Experiment 2. Control of cavity spot on carrots in soil from Wellesbourne treated with UK876, Amistar or SL567A.

Treatment and rate	Percentage of carrots with cavities*	Total number of cavities per carrot	Number of large cavities per carrot	Percentage area of carrot infected *	Root weight as percentage of untreated control
Untreated control	41.2 (39.3)	0.73	0.06	0.42 (1.95)	100.0
UK876 160 g/ha	36.2 (34.8)	0.62	0.10	0.36 (1.78)	88.2
UK876 400 g/ha	43.7 (39.3)	0.78	0.15	0.27 (1.82)	87.3
UK876 800 g/ha	40.0 (38.4)	0.61	0.13	0.23 (1.66)	98.3
UK876 1600 g/ha	46.2 (42.5)	0.76	0.13	0.29 (1.96)	87.8
Amistar 4 l/ha	37.5 (35.7)	0.50	0.09	0.22 (1.45)	84.4
SL567A 1.30 l/ha	37.5 (35.2)	0.56	0.05	0.19 (1.40)	93.1
LSD $P \leq 0.05$	16.75	0.295	0.098	0.757	11.58

* arcsin transformation of percentages to which LSD applies in parenthesis

Table 23. Experiment 3. Effect of calcium sulphate on cavity spot of carrots grown in naturally infested soil from Wellesbourne.

Treatment	Percentage of Carrots with Cavities *	Total number of cavities per carrot	Number of large cavities per carrot	Percentage area of carrot infected *
Untreated control	15.0 (19.6)	0.21	0.11	0.09 (0.57)
$CaSO_4$ 3 t/ha	11.3 (16.7)	1.45	0.16	0.24 (0.78)
$CaSO_4$ 6 t/ha	20.0 (24.2)	0.44	0.36	0.57 (1.64)
$CaSO_4$ 9 t/ha	26.3 (28.5)	0.63	0.28	0.34 (1.61)
$CaSO_4$ 12 t/ha	15.0 (19.6)	0.19	0.17	0.13 (0.76)
LSD $P \leq 0.05$	14.27	1.66	0.23	0.82

* arcsin transformation of percentages to which LSD applies in parentheses

Table 24. Experiment 1. pH ($CaCl_2$, 0.01M) of soils treated with calciumcarbonate immediately, one month or two months before drilling.

Treatment and rate	pH	
	After incorporation of $CaCO_3$	After harvest
Untreated control	6.0	5.9
$CaCO_3$ 6 t/ha Immediately before drilling [a]	6.3	6.6
$CaCO_3$ 6 t/ha 1 month before drilling [b]	6.5	6.9
$CaCO_3$ 6 t/ha 2 months before drilling [c]	6.9	7.1
$CaCO_3$ 12 t/ha Immediately before drilling [a]	6.6	6.8
$CaCO_3$ 12 t/ha 1 month before drilling [b]	6.7	7.1
$CaCO_3$ 12 t/ha 2 months before drilling [c]	7.1	7.3
SL567A 1.30 l/ha	-	6.0

[a] Calcium carbonate applied immediately before drilling. pH determined one week after incorporation of the compound.

[b] Calcium carbonate applied one month before drilling. pH determined three days after incorporation of the compound.

[c] Calcium carbonate applied two months before drilling. pH determined one month after incorporation of the compound.

Table 25. Experiment 3. pH ($CaCl_2$, 0.01M) of soils treated with calcium sulphate. pH was determined one month after incorporation of $CaSO_4$ and after harvest.

Treatment and rate	pH	
	After incorporation of $CaCO_3$	After harvest
Untreated control	6.1	5.9
$CaSO_4$ 3 t/ha	6.0	5.8
$CaSO_4$ 6t/ha	6.1	5.9
$CaSO_4$ 9t/ha	6.0	6.1
$CaSO_4$ 12t/ha	6.2	6.1

C) Fungicide sensitivity

Materials and Methods

Three isolates of *P. violae* were tested for their sensitivity to azoxystrobin using a laboratory assay. One isolate (PV6A) was from the HRI Wellesbourne culture collection, one isolate (PVC) was recovered from cavities of commercially grown carrots and the other (PVB) from cavities of carrots grown at an ADAS experimental site in 1999. A stock solution of azoxystrobin (1000 μg ml^{-1}) was prepared in sterile distilled water using Amistar (a.i. azoxystrobin, 25 % w/v), 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 azoxystrobin 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 agar 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 fungicide had almost reached the furthest edge of the plate, and the data analysed using Genstat 5 programme which fitted a logistic curve and calculated ED_{50} for azoxystrobin for the different *Pythium* isolates tested.

Results and Discussion

All three isolates of *P. violae* tested showed sensitivity to azoxystrobin in the laboratory assay (Table 26). However, ED_{50} values of azoxystrobin for these isolates were up to fifty times higher than ED_{50} values of metalaxyl for isolates of *P. violae* tested for metalaxyl sensitivity, both in the current year and in Year 2 of this project (in this work ED_{50} values for metalaxyl ranged from 0.023-0.083 μg ml^{-1}).

Table 26. ED_{50} values of (μg ml^{-1}) of azoxystrobin for three isolates of *P. violae*.

Isolate of *P. violae*	ED_{50} (μg ml^{-1})
PV6	3.00 (± 0.444)
PVB	4.63 (± 0.382)
PVC	1.05 (± 0.286)

D) Resistance in *Pythium violae* and *P. sulcatum* to metalaxyl

Introduction

Metalaxyl resistance in populations of P. violae was examined in the first two years of this project and all 20 isolates were found to be sensitive to the fungicide. Further isolates were obtained and tested this year using agar plate tests to produce ED_{50} values. The ED_{50} value indicates the concentration of fungicide required to reduce the growth of the fungus to half that of the untreated control. In addition a number of isolates of *P. sulcatum* from carrot crops were obtained and tested as a comparison.

Materials and Methods

Isolates of *P. violae* and *P. sulcatum* were obtained from cavity spot lesions, found on UK carrots. Many of the carrots were sent to HRI by growers or ADAS, some were lifted directly from fields used in the metalaxyl degradation study, others were grown in the glasshouse in soil from fields used in the metalaxyl degradation study (Table 27).

As in previous years, the isolates were prepared for assay by cleansing through 2 % water agar with rifamycin (30 mg/l) to remove contaminating bacteria. The isolates were then grown on V8 juice agar (3 g agar, 0.4 g calcium carbonate, 20 ml V8 vegetable juice [Campbell Grocery Products Ltd.], 180ml distilled water). 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. Prepared plates were inoculated at the side with one of the test isolates, incubated at 19ºC, 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.

Results

Table 27 shows the origins of the isolates and their ED_{50} values. All of the *P. violae* isolates were sensitive to metalaxyl with ED_{50} values ranging from 0.023 to 0.083 µg/ml. This is consistent with results from years 1 and 2. Of the *P. sulcatum* isolates tested, four had ED_{50} values (0.427-0.658 µg/ml) that were around 10 times higher than those for *P. violae*. One *P. sulcatum* isolate had a ED_{50} value of 3.299 µg/ml.

Discussion

Once again no evidence for the development of resistance to metalaxyl has been found, in the *P. violae* isolates collected this year. These results are consistent with those of White, Stanghellini & Ayoubi (1988). *P. sulcatum* exhibited a higher baseline tolerance to metalaxyl than *P.violae*. Although *P. violae* is currently considered to be the predominant cavity spot pathogen in UK crops, it is important that the balance of these two pathogen populations is regularly monitored, as metalaxyl is likely to be less effective in fields where *P. sulcatum* is present in significant quantities.

Table 27. Origin of *Pythium violae* and *P. sulcatum* isolates, and their ED_{50} ($\mu g\ ml^{-1}$) values for metalaxyl.

***P. violae* isolates**	**Field location**	**Method obtained**	**ED_{50} ($\mu g\ ml^{-1}$)**
21	Lancashire	Carrots from grower	0.048
22	Lincolnshire	Carrots from grower	0.049
23	Nottinghamshire	Carrots from grower	0.060
24	Lincolnshire	Glasshouse grown in soil from degradation study	0.083
25	Unknown	Carrots from ADAS	0.053
26	Nottinghamshire	Field carrots direct from degradation study	0.052
27	Nottinghamshire	Field carrots direct from degradation study	0.023
28	Lancashire	Carrots from grower	0.050
29	Lincolnshire	Glasshouse grown in soil from degradation study	0.038
30	Yorkshire	HDC field trial	0.039
31	Yorkshire	Carrots from grower	0.033
***P. sulcatum* isolates**			
1	Lancashire	Carrots from grower	0.427
2	Unknown	Carrots from ADAS	0.658
3	Staffordshire	Carrots from grower	0.658
4	Unknown	from culture collection	0.586
5	Yorkshire	Field carrots direct from degradation study	3.299

E) Examination of interaction between *P. violae* inoculum and carrots

Materials and Methods

Isolates

Pythium violae isolate 6 (PV6) from the HRI Wellesbourne culture collection and isolate Arnando (PVA) recovered from cavities of commercially grown carrots were used throughout these studies.

Production of oospores

Two different liquid media were compared for oospore production: V8 juice broth (V8B) and V8 juice broth supplemented with oatmeal infusion (V8BO) (Appendix VI). Aliquots of 15 ml of each medium were pipetted into 9 cm diameter Petri dishes. Each dish was inoculated with a 5 mm diameter disc taken from a 3-day-old culture of PV6 or PVA grown at 20°C on corn meal agar (CMA) amended with rifamycin (30 mg l^{-1}). Dishes were incubated at 10°C, 15°C and 20°C in the dark. Oospores were counted after 3 weeks incubation on three replicate plates for each temperature and medium. Oospores were counted in a field of view at ten random points using 100 x magnification, beginning at the colony margin and at 0.7 mm intervals across one diameter of the Petri dish.

To have a continuous supply of oospores, V8B cultures of *P. violae* isolate PVA were set up at 2-4 week intervals.

Bulk production of oospores in conical flasks was also attempted in 50 and 100 ml of V8B in 100 and 250 ml flasks, respectively. However, numbers of oospores produced was very low and their germination rate (1 %) was lower than that of oospores produced in Petri dish cultures (>3 %). Mycelial production was more profuse in flasks and removing it required extra steps in the extraction procedure.

Extraction of oospores from liquid media

Several different methods of extraction were attempted initially. The most efficient procedure was as follows: Petri dish cultures were drained and washed with two changes of sterile distilled water (SDW). Excess moisture was then absorbed with sterile filter paper and the cultures left to dry in a laminar flow hood for 1 hour. The cultures were then macerated in 10 ml of SDW per each Petri dish culture for 1 min at medium speed and 1 min at high speed using Thyristor Regler macerator. The macerated suspension was filtered successively through nylon filter fabric of 500, 255 and 80 µm mesh sizes. Sometimes it was necessary to also use 50 µm mesh to remove

further mycelial fragments. If concentration or cleaning of oospores was required, the filtered suspension was centrifuged at 20,000 *g* for 10 minutes and then resuspended in a small volume of SDW. The number of oospores in the suspension was estimated using haemocytometer counts.

Sonication and freezing were tested as part of the extraction pocedure to kill the mycelia. However, desiccation followed by maceration of the cultures was found to be equally as effective as sonication (at 33-40 kHz for 2.5 minutes using a Jencons Soniclean). Freezing for 1 hour at –20°C did not kill the mycelia of *P.violae*. Longer periods of freezing were avoided as they may have affected the behaviour of the oospores in subsequent experiments.

Germination of oospores

Germination of oospores produced at 10°C or 15°C was compared on three different media. Oospores of PVA produced in V8B for 9 weeks were extracted as described above. Aliquots (0.3 ml) of oospore suspensions were pipetted onto three replicate plates of V8 juice agar (V8), carrot juice agar (CJA) (Appendix VI) and CMA amended with rifamycin (30 mg l^{-1}) and spread over the surface with a plastic spreader. The plates were incubated at 20°C for 15 hours after which they were examined microscopically. Germination of 250 oospores on each plate was scored, oospores with germ-tubes of a length greater than spore diameter being considered to have germinated.

In a separate experiment which was carried out in the same way as outlined above, germination of oospores on water agar (WA) (Appendix VI) was compared with germination on CMA, both media being amended with rifamycin (30 mg l^{-1}).

Effect of culture temperature and age on production and germination of oospores

Production and germination of oospores of *P. violae* isolate PVA, from liquid media at two different temperatures and after different times, was assessed using cultures of *P. violae* isolate PVA grown in V8B in Petri dishes at 10°C and 15°C. Numbers of oospores were counted in three replicate plates after 4, 6, 8 and 10 weeks growth. At the same time, oospores were extracted as described previously and aliquots (0.3 ml) of oospore suspension were spread on three replicate plates of CMA amended with rifamycin (30 mg l^{-1}). Numbers of germinated oospores were recorded after 16 hours incubation at 20°C in the dark.

Effect of temperature on germination of oospores

Oospores of *P. violae* isolate PVA were produced in V8B in Petri dishes at 10°C or 15°C for 11 weeks and extracted as described previously. To assess the effect of

temperature on oospore germination at 5, 10, 15, 20, 25, 30, 35°C, aliquots (0.3 ml) of oospore suspension were spread over the surface of CMA amended with rifamycin (30 mg l^{-1}). Dishes were placed in sealed plastic bags and three replicate plates were incubated at each temperature for 16 hours after which the oospores were counted as described above. To determine the temperature limits for oospore germination, the plates on which no oospore germination was observed after 16 hours incubation were incubated further for 14 days.

Effect of pH on germination of oospores

Oospores of *P. violae* isolate PVA were produced in V8B in Petri dishes at 15°C for 6 weeks and extracted as described above. The effect of pH on oospore germination was investigated using CMA amended with rifamycin (30 mg l^{-1}). Citrate phosphate buffer (0.05 M citric acid, 0.1 M $Na_2HPO_4 \cdot 7H_20$) and tris(hydroxymethyl)aminoethane (0.1 M Tris, 0.1 M HCl) were used to buffer media over ranges of pH 4-7 and pH 7.5-9, respectively. Solutions with a pH of 4-7 were prepared with respect to citric acid and adjusted to a constant conductivity of 4.6 ms cm^{-1} with distilled water. Solutions with a pH of 7.5-9 were prepared with respect to Tris and adjusted to a constant conductivity of 1.1 ms cm^{-1} with distilled water. These solutions were then used to prepare media over the range pH 4-9. Aliquots (0.3 ml) of oospore suspension were spread over the surface of three replicate plates for each pH. Dishes were placed in sealed plastic bags and incubated at 20°C for 15 hours after which the oospores were counted as described above. To determine the pH limits for oospore germination, the plates on which no oospore germination was observed after 15 hours incubation were further incubated for 14 days.

Activation treatments of immature oospores

Oospores that did not germinate readily after extraction were assumed immature. There was variation between the age of cultures in which oospores became capable of germinating i.e. mature. Oospores from the first batch did not germinate until cultures were 8 weeks old, whereas oospores from the second batch germinated when the cultures were 4 weeks old.

Treatments such as freezing, chilling and potassium permanganate were tested for activating immature oospores. Small aliquots of extracted suspensions of oospores were stored at 5°C or –20°C. A sample from each temperature was recovered at weekly intervals and spread on three replicate plates of CMA amended with rifamycin (30 mg l^{-1}) to check germination. No germination of oospores was observed after freezing at –20°C for up to 18 days, whereas oospores stored at 5°C started to germinate at a low rate (<1 %) after 14 days storage.

Potassium permanganate ($KMnO_4$) has been found to be very effective in inducing germination of oospores of heterothallic species of *Phytophthora* and *Pythium* (Ann & Ko, 1988; Guo & Ko, 1994). The activating effect of $KMnO_4$ treatment is believed to be by the chemical oxidation of wall components (Ruben *et al*. 1980). However, treatment with $KMnO_4$ (0.1 or 0.25 % for 15 minutes) did not induce germination of immature oospores of *P. violae*.

Effect of incubation in non-sterile soil extract on germination of oospores

Soil extracts of three different concentrations were prepared by incubating 10g, 100g and 500g of air dried (for 24 h) Wellesbourne field soil (used in cavity spot experiments in previous years) in 1000 ml of distilled water for 48 hours and subsequently filtering through Whatman No. 1 filter paper. Cultures of PVA were produced in V8B in Petri dishes at 15°C for 11 weeks. The cultures were drained and washed with two changes of SDW. Excess moisture was absorbed with sterile filter paper and the cultures left to dry in a laminar flow hood for 1 hour. The cultures were then covered with the filtered soil extracts or distilled water for the control and incubated at 20°C for 21 days. Lysed and non-lysed cultures were rinsed with SDW and oospores extracted as described above. Aliquots (0.3 ml) of oospore suspensions were spread onto three replicate plates of CMA amended with rifamycin (30 mg l^{-1}). The plates were incubated at 20°C for 16 hours after which they were examined microscopically and germinated oospores counted.

Results and Discussion

Production of oospores

P. violae isolate PV6 produced very few oospores, whereas *P. violae* isolate PVA produced high numbers at 10°C and 15°C within three weeks (Table 28). At 20°C, isolate PV6 produced no oospores and isolate PVA produced few. The addition of oatmeal infusion to the V8 base did not increase oospore numbers produced.

Extraction of oospores from liquid medium

A process was successfully developed for extracting oospores from liquid culture medium. This involved desiccation and maceration of cultures followed by filtration to remove mycelial fragments.

Germination of oospores

The germination of oospores produced at 10°C or 15°C was determined on different

media. There was no significant difference between germination of oospores produced at 10°C or 15°C (Table 29 and 30). The germination rate was highest on CMA and V8. Most of the oospores germinated with one germ tube, but a few had more than one (see Plates 1 and 2). Lateral branching of the germ tube frequently occurred immediately after its emergence. No difference in the way that oospores germinated was observed for different germination media.

Effect of culture temperature and age on production and germination of oospores

The age of the cultures or temperature did not significantly affect the numbers of oospores produced (Table 31). The germination rate varied depending on the age of the culture from which oospores were extracted, however, no clear trend was observed.

Effect of temperature on germination of oospores

After 16 hours of incubation, oospores produced at 10°C germinated over the temperature range 5-30°C with an optimum around 25°C (Figure 8). After 14 days incubation, germination was still inhibited at 35°C.

Oospores produced at 15°C germinated over the temperature range 10-25°C with an optimum around 20°C. After 14 days incubation, germination was still inhibited at 30°C and 35°C, whereas these oospores germinated at 5°C.

Effect of pH on germination of oospores

After 15 hours of incubation at 20°C, germination of oospores occurred over a pH range of 5-8.5, with an around pH 8 (Figure 9). After 14 days incubation, oospore germination was still totally inhibited at pH 4, whereas oospores germinated at a low rate (< 1 %) at pH 4.5 and pH 9.

Effect of incubation in non-sterile soil extract on germination of oospores

Incubation in a non-sterile soil extract prepared from soil conducive to cavity spot reduced the germination of oospores (Table 32). This result was contrary to the findings of previous studies with other *Pythium* species (Ayers & Lumsden, 1975).

Plate 1 **Germinating oospore of *P. violae* with a single branched germ tube**

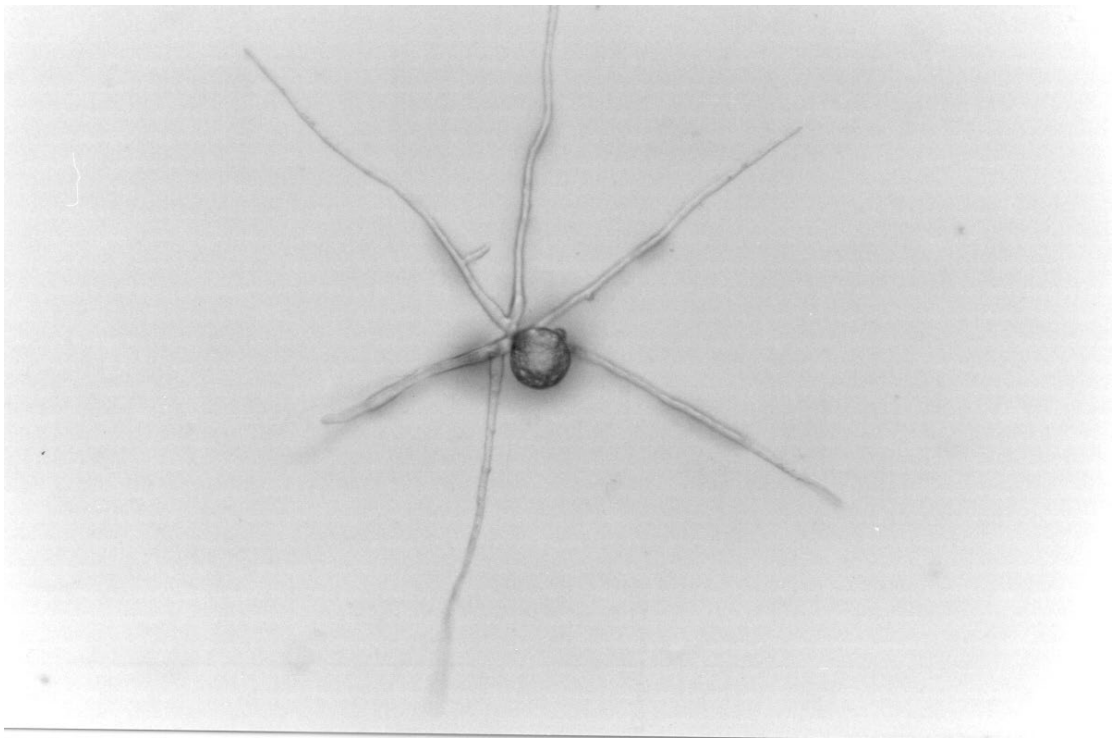


Plate 2 **Germinating oospore of *P. violae* with multiple germ tubes**

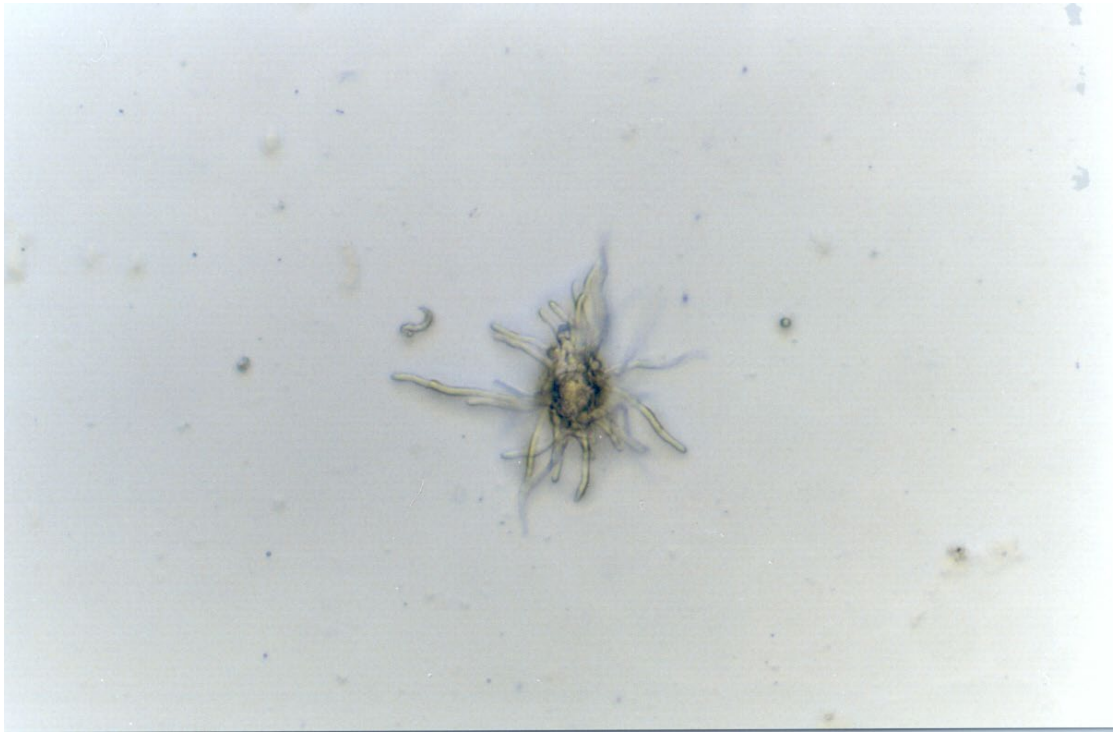


Table 28. Effect of temperature and medium on oospore production of two *P. violae* isolates after 3 weeks incubation.

Medium	Isolate	Mean number of oospores per field of view at 100 x magnification from cultures grown at different temperatures		
		10°C	15°C	20°C
V8B	PV6	0	0.4 (± 0.35)	0
	PVA	16.4 (± 12.69)	34.1(± 8.75)	10.0 (± 9.38)
V8BO	PV6	0.4 (± 0.69)	0.1 (± 0.15)	0
	PVA	35.5 (± 17.06)	25.2 (± 14.27)	1.9 (± 1.25)

V8B V8 juice broth
V8BO V8 juice broth supplemented with oatmeal infusion

Table 29. Germination of oospores of *P. violae* isolate PVA on different media. The oospores were produced in V8B at 10°C or 15°C for 9 weeks and germinated for 15 hours at 20°C in the dark.

Germination medium	Percentage of germinated oospores from cultures grown at different temperatures	
	10°C	15°C
V8 juice agar	10.5 (18.8 ± 2.15)[a]	5.4 (13.4 ± 1.09)[a]
Carrot juice agar	6.8 (15.1 ± 0.88)	5.5 (13.5 ± 2.20)
Corn meal agar	11.3 (19.6 ± 1.36)	8.2 (16.6 ± 0.79)

[a] Figures in parentheses are means with SEM from three replicate plates calculated after angular transformations of percentage data.

Table 30. Germination of oospores of *P. violae* isolate PVA on water agar and cornmeal agar. The oospores were produced in V8B at 10°C or 15°C for 8 weeks and germinated for 15 hours at 20°C in dark.

Germination medium	Percentage of germinated oospores from cultures grown at different temperatures	
	10°C	15°C
Water agar	9.8 (18.3 ± 0.97)[a]	12.9 (21.0 ± 2.12)[a]
Corn meal agar	13.5 (21.5 ± 1.85)	10.8 (19.2 ± 1.35)

[a] Figures in parentheses are means with SEM from three replicate plates calculated after angular transformations of percentage data.

Table 31. Effect of culture temperature and age on oospore production of *P. violae* isolate PVA in V8 juice broth and germination of these oospores.

Culture age	Culture temperature	Mean number of oospores per field of view at 100 x magnification	Percentage of germinated oospores
4 weeks	10°C	33.5 (± 19.82)	12.2 (20.4 ± 1.71)[a]
	15°C	36.2 (± 5.02)	9.1 (17.5 ± 0.78)
6 weeks	10°C	58.6 (± 8.83)	8.6 (17.0 ± 0.74)
	15°C	63.0 (± 15.87)	3.5 (10.6 ± 4.05)
8 weeks	10°C	51.6 (± 7.57)	13.5 (21.5 ± 1.85)
	15°C	60.4 (± 13.61)	10.8 (19.2 ± 1.35)
10 weeks	10°C	58.0 (± 15.40)	13.9 (21.9 ± 0.65)
	15°C	42.1 (± 6.24)	3.0 (10.0 ± 0.81)

[a] Figures in parentheses are means with SEM from three replicate plates calculated after angular transformations of percentage data.

Table 32. Effect of incubation in non-sterile soil extract for 21 days on germination of oospores of *P. violae* isolate PVA.

Treatment	Percentage of germinated oospores
Untreated control	7.1 (15.4 ± 0.62)[a]
Soil extract (10 g l^{-1})	2.0 (7.8 ± 2.31)
Soil extract (100 g l^{-1})	0.3 (2.4 ± 2.09)
Soil extract (500 g l^{-1})	0.5 (3.4 ± 2.96)

[a] Figures in parentheses are means with SEM from three replicate plates calculated after angular transformations of percentage data.

Figure 8. Effect of pH on germination of oospores of *P. violae*.

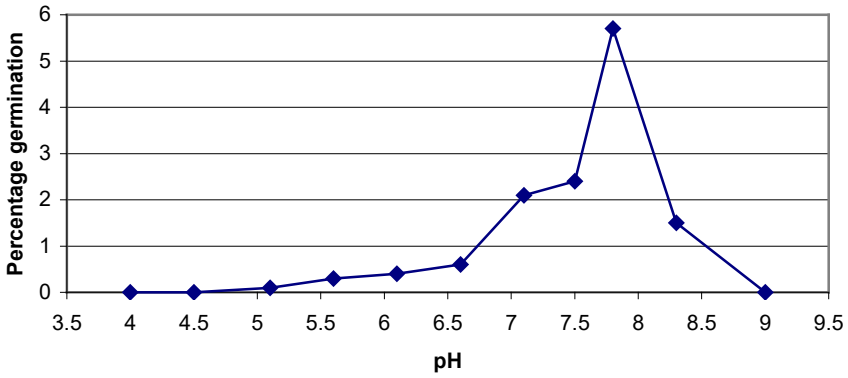
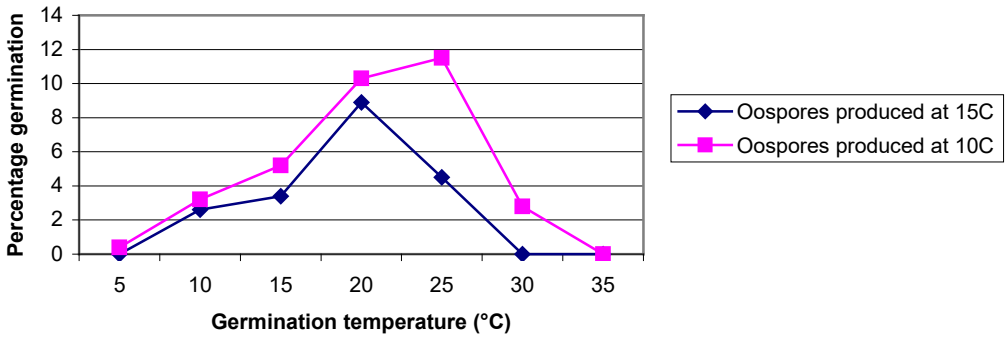


Figure 9. Effect of temperature on germination of oospores of *P. violae* produced at 10°C or 15°C.



F) Development of selective media for isolation of *Pythium violae* from plants and soil

Introduction

Pythium violae can be isolated from carrot cavities using a selective agar containing 30 µg/ml rifamycin (to suppress growth of bacteria) and 100 µg/ml pimaricin. Pimaricin is an antibiotic which at this concentration inhibits growth of almost all common soil fungi with the exception of the oomycetes. However, it is expensive and when used at 100 µg/ml it is known to inhibit spore germination of certain *Pythium* species, making it unsuitable for use in soil isolation media at high concentrations. Many selective media for isolating *Pythium* spp. from soil and diseased tissue have been developed in the past and were reviewed by Tsao (1970). They rely on the use of antibiotics, fungicides and other inhibitory chemicals to suppress the growth of other soil microbes. Modifications suggested and employed since include MPVM (Modified Pimaricin Vancomycin with Minor elements) (Mircetich, 1971) and VP_3 (Ali-Shtayeh et al, 1986). However, most studies have examined *Pythium* populations as a whole without concentrating on one species in particular. *P. violae* is a slow growing fungus and thus difficult to isolate from soil as there is a tendency for faster growing *Pythium* species to overgrow it. Hymexazol is often employed for isolation of *Phytophthora* spp. as it is usually effective against *Pythium* species. However, in previous fungicide screening studies, some tolerance to this fungicide was seen in *P. violae* isolates. Its potential for *P. violae* isolation is examined here along with adjustments to the basal medium to improve the growth rate of this target species on the isolation medium. Media were tested along with plating procedures and sieving techniques for isolations from both plant material and infested soils. This work is ongoing.

The method by which *P. violae* overwinters in soil and infects carrot roots is not clearly understood. It is believed to continue growing when other soil fungi cannot and may well survive harsh conditions as oospores, germinating when temperatures improve. The ratio of oospore to mycelium may vary throughout the season therefore any method for detecting *P. violae* in soil needs to consider both types of propagule.

Materials and Methods

Development of selective media

a) Selecting a basal medium and optimum temperatures

The growth rates of two isolates of *P. violae* were examined on three commonly used agars when incubated at different temperatures. In addition ‘carrot juice agar’ was

devised as a comparison to the standard agars in order to determine whether a carrot-based medium base might favour growth of *P. violae.*

Corn meal Agar (CMA, Difco) and Potato Dextrose Agar (PDA, Oxoid) were prepared according to label recommendations. V8 juice agar contained 15 g agar, 2 g calcium carbonate, 100 ml V8 vegetable juice [Campbell Grocery Products Ltd.], 900 ml distilled water. Carrot juice was prepared by peeling and pulping carrots in a blender, and clarifying by pressure cooking at 15 psi for 15 minutes. Carrot juice agar contained 500 ml carrot juice, 500 ml distilled water, 20 g agar. Isolates were grown on V8 juice agar initially and test plates were inoculated to one side using an agar plug taken from an actively growing colony with a size 2 cork borer. Plates were incubated at 2, 5, 10, 15, 20, 25 and 30ºC. Mycelial extension was measured from day 1 onwards and growth rate calculated. There were 4 replicate plates at each temperature for each isolate.

b) Determining potential of Hymexazol as a selective agent for P. violae

Five species of *Pythium* which are commonly isolated from carrot cavities or periderm were tested for their sensitivity to hymexazol. The species were *P. violae* (Pv), *P. sulcatum* (Ps)*, P. intermedium* (Pin)*, P. sylvaticum* (Psy) and *Pythium Group HS* (HS*)*. Two isolates of each species were tested. The hymexazol test medium was prepared, using CMA made up as per label recommendations, and adding hymexazol (once agar had cooled to 45°C) to give a final concentration in agar of 10ppm. Isolates were initially grown on V8 juice agar, and test plates were inoculated to one side using an agar plug taken from an actively growing colony with a size 2 cork borer. Plates were incubated at 2, 5, 10 and 15°C. Mycelial extension was measured from day 2 onwards and the growth rates were calculated. Three replicate plates were used at each temperature for each isolate.

c) Assessment of mixtures of antibiotics and fungicides on colony formation and growth rate of P. violae

Further modification of the plant isolation medium was required to apply it to soil isolation in order to suppress the wider spectrum of fungi in the soil. Experiments were carried out with solid-substrate inoculum of *P. violae* to determine the effect of various antibiotic / fungicide mixtures on colony formation. In addition the same media were used with soil suspensions to identify mixtures which successfully suppress other soil fungi. The antibiotic mixtures are outlined in table 33.

Table 33. Recipes of antibiotic mixtures added to CMA and tested on growth of *P. violae* for suitability as soil isolation media.

Experiment Number	Code for agar type	Mixture tested (in CMA)
A	R	Rifamycin (30 ppm)
	RH10	Rifamycin (30 ppm) + hymexazol (10 ppm)
	RP100	Rifamycin (30 ppm) + pimaricin (100 ppm)
	RH10P100	Rifamycin (30 ppm) + hymexazol (10 ppm) + pimaricin (100 ppm)
	RH10B10	Rifamycin (30 ppm) + hymexazol (10 ppm) + benomyl (10 ppm)
B	RH10	Rifamycin (30 ppm) + hymexazol (10 ppm)
	RH10N	Rifamycin (30 ppm) + hymexazol (10 ppm) + nystatin (100 units / ml)
	RH10C	Rifamycin (30 ppm) + hymexazol (10 ppm) + PCNB (10ppm)
	RH10P10	Rifamycin (30 ppm) + hymexazol (10 ppm) + pimaricin (10 ppm)
	RH10P2	Rifamycin (30 ppm) + hymexazol (10 ppm) + pimaricin (2 ppm)
	RH10P2C	Rifamycin (30 ppm) + hymexazol (10 ppm) + pimaricin (2 ppm) + PCNB (10 ppm)
	RH10NC	Rifamycin (30 ppm) + hymexazol (10 ppm) + Nystatin (100 units / ml) + PCNB (100 ppm)
	RH10B(5)	Rifamycin (30 ppm) + hymexazol (10 ppm) + Benomyl (5 ppm)

For experiment A, the difference between spreader plating (spreading inoculum suspension on the surface of the agar) and Warcup plating (incorporating inoculum into agar before it sets) was examined. For experiment B only Warcup plating was carried out.

P. violae inoculum was produced by inoculating sand / oatmeal flasks (10:1 w/w) with agar plugs taken from an actively growing colony and incubating at 20°C for 1

or more weeks. Inoculum suspensions were made by mixing 2 g of sand / oatmeal inoculum in 100 ml sterile distilled water (SDW) and shaking for 10 minutes on a wrist action shaker. Soil suspension (experiment B only) was made up by mixing 5 g soil in 50 ml SDW in the same way. Aliquots of 0.5 ml were used for spreader or Warcup plates. All fungicide / antibiotic mixtures were added once the agar had cooled to below 45°C.

Isolations from plant material

Hymexazol at 10 ppm in CMA was tested to determine potential for isolating *P. violae* from carrot cavities. The medium was made up as before, and rifamycin was added (30 µg/ml) to suppress the growth of bacteria. For comparison the basic *Pythium* isolation medium used previously (CMA with rifamycin [30 µg/ml] and pimaricin [100 µg/ml]) was also used. Cavities from a sample of carrots were cut in half, a one half placed (cut edge down) onto each agar type. Plates were incubated at 20°C for 2 weeks.

Isolations from soil

a) Warcup plating

Soil samples from fields which have previously produced carrots with cavity spot were collected, or sent by growers. These samples were processed as described above and plated onto CMA with rifamycin (30ppm), pimaricin (5 or 10ppm) and hymexazol (10ppm).

b) soil sieving

To increase the volume of soil which could be processed, a technique of sieving soil for oospores (Van de Gaag & Frinking, 1997) was adapted to collect particles in the soil of a similar size to *P.violae* oospores. Various volumes of soil and methods were tried: 25 - 100g of soil from a known infested field was mixed in 100 - 1000 ml of tap water. The suspension was sonicated for 5 minutes, and filtered through mesh sizes of 200 µm, 80 µm (or 50 µm) and 20 µm respectively, washing each through with tap water. The residue on the 20 µm sieve was resuspended in tap water. The resulting suspension was poured on top of a sucrose solution (specific gravity 1.35) and centrifuged for 3 minutes at 1800g. Layers were checked for the presence of oospores.

Results

Development of selective media

a) Selecting a basal medium and optimum temperatures

Figures 10 and 11 show growth rates of two *P. violae* isolates on each of the four agars over a range of temperatures. Both isolates grew quickest on carrot juice agar, and CMA was the best of the commonly used agars. Both isolates had a temperature optimum between 15 and 20°C, with growth ceasing at 30°C but not at 2°C.

Figure 10. Growth rate (mm/hour) of *Pythium violae* isolate C on various agars at different temperatures

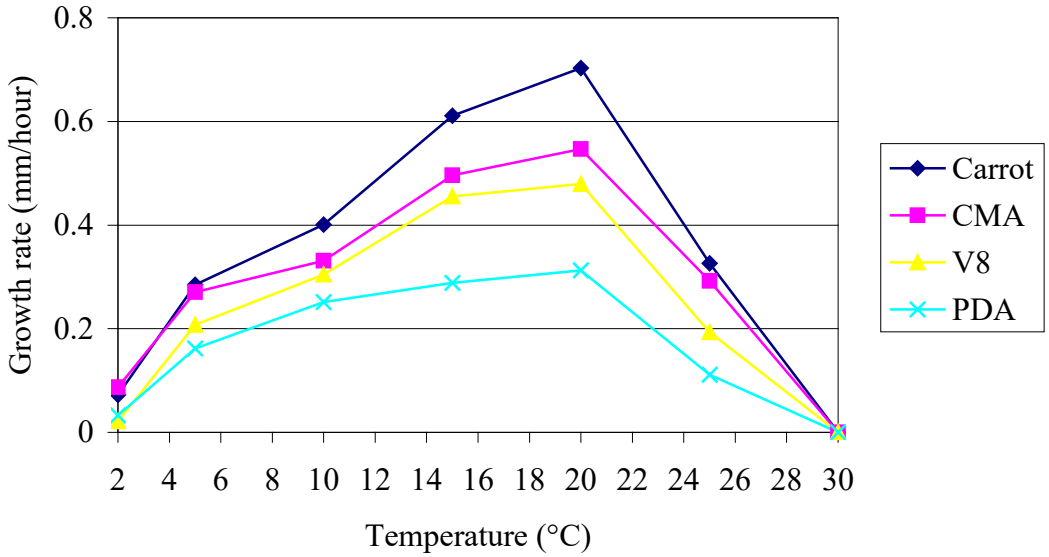


Figure 11. Growth rate (mm/hour) of *Pythium violae* isolate D on various agars at different temperatures

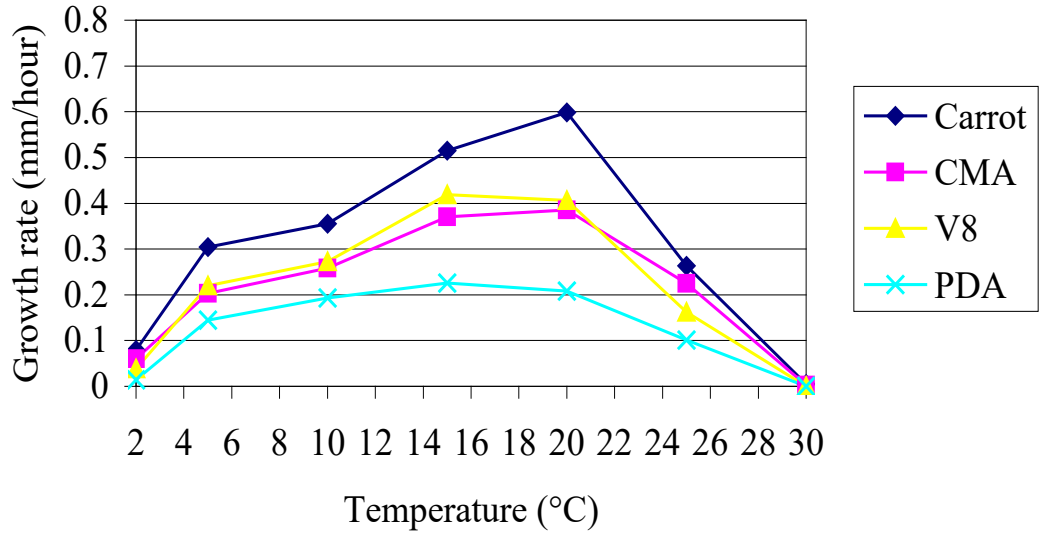
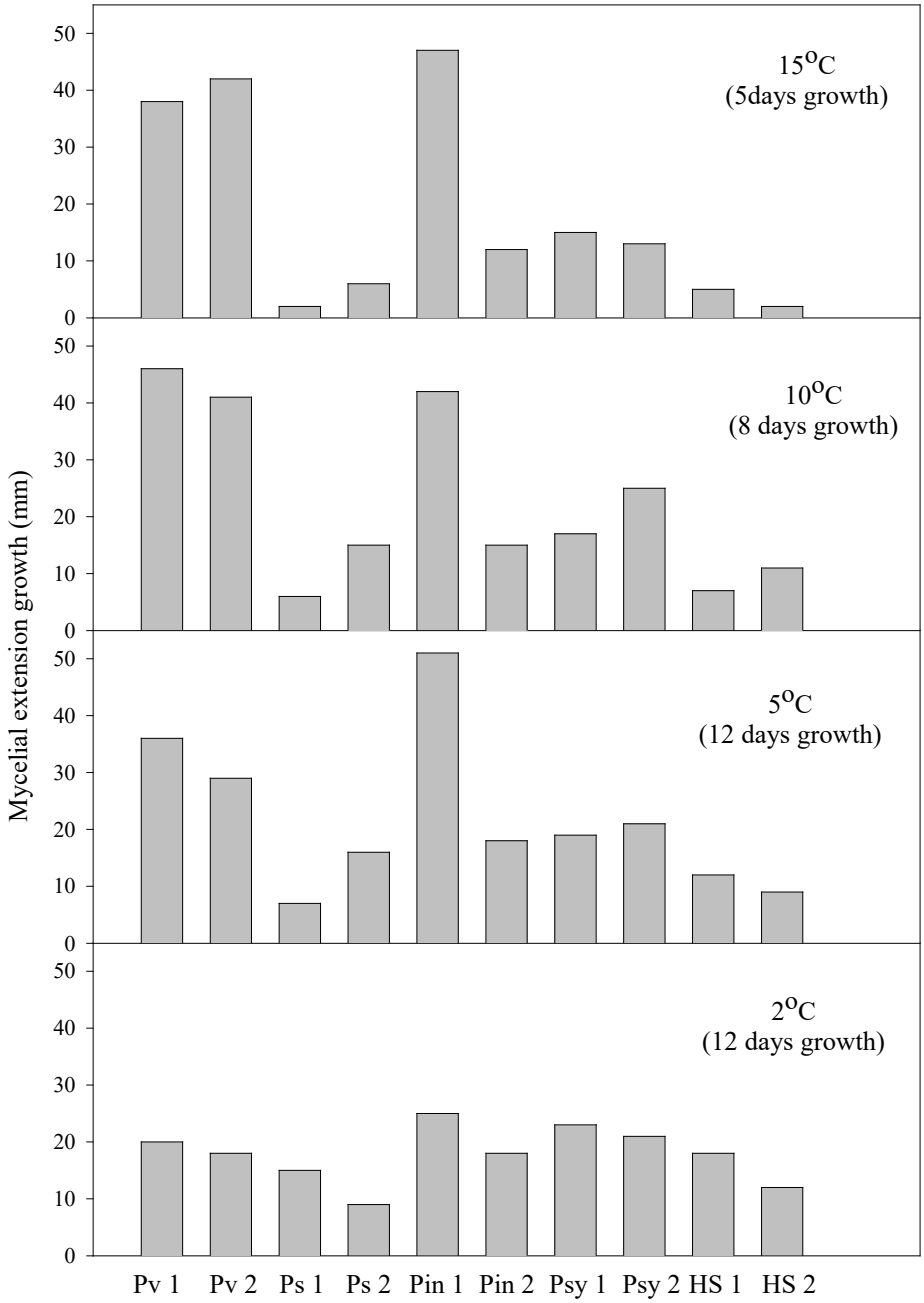


Figure 12. Effect of Hymexazol (10 mg ml^{-1}) on the growth of 10 *Pythium* isolates when incubated at different temperatures



b) Determining potential of Hymexazol as a selective agent for P. violae

Figure 12 compares the growth of the different isolates at each of the four temperatures. At the lowest temperature (2°C) growth of all isolates was very slow, and measurements were made over a 12 day period. At the highest

temperature it took just 5 days for the fastest species to reach the edge of the plate. At temperatures of 10 and 15°C both of the Pv isolates and one of the Pin isolates were considerably faster than the other 7 isolates tested, in the presence of hymexazol. Importantly the Pin isolate grew at a similar rate to Pv, significantly reducing the possibility of overgrowing the *P. violae* at 10 and 15°C (Figure 12).

c) Assessment of mixtures of antibiotics and fungicides on colony formation and growth rate of P. violae

Tables 34 and 35 show colony forming units (cfu) resulting on the antibiotic / fungicide mixtures in experiments A and B. The use of hymexazol at 10 ppm in the agar did not appear to affect the numbers of *P. violae* colonies. However the use of pimaricin at 100 ppm or benomyl at 10 ppm considerably reduced the numbers of colonies formed (table 34). When benomyl was reduced to 5 ppm it still had an adverse effect on the number of *P. violae* colonies retrieved. A combination of PCNB and Nystatin also gave poor colony counts, but when either was added alone to the hymexazol agar recoveries were good. However they offered poor control of other soil fungi. The best control of soil fungi was produced by the addition of low concentrations of pimaricin, and at these concentrations it did not adversely affect recovery of *P. violae*. All of the contaminants which escaped control in these agars (RH10P10 and RH10P2) were *Mortierella* spp. A further experiment tested the addition of ampicillin at 250 ppm to the agar but failed to improve the control of *Mortierella* spp.

Experiment A showed that recovery of colonies arising from mycelium was best achieved using the Warcup method; plating suspensions of material with the molten agar as opposed to spreader plates.

Table 34. Experiment A: Differences in various anti-fungal mixtures on *P. violae* colony forming units using different plating techniques (means of 2 replicate plates)

Agar code[a]	Warcup plate	Spread plate
R	78.0	42
RH10	80	53.5
RP100	5.5	2
RH10P100	0	1.5
RH10B10	0	7

[a] see table 33 for recipes of antibiotic mixtures

Table 35. Experiment B: Differences in various anti-fungal mixtures on *P. violae* colony forming units (means of 3 replicate plates)

Agar code[a]	CFU of *P. violae* artificial inoculum	Approximation of colony numbers of soil fungi (potentially contaminants)
RH10	123.3	200+
RH10N	95.3	50
RH10C	138	200+
RH10P10	140	<10
RH10P2	79.3	20
RH10P2C	69.3	20
RH10NC	7	<10
RH10B(5)	0.7	25

[a] see table 35 for recipes of antibiotic mixtures

Isolations from plant material

The majority of isolations from carrot cavities in the sample assessed on the CMA with rifamycin and pimaricin (CMA++) appeared to be *P. sulcatum*, which was suppressed on the hymexazol agar. *P. violae* was isolated on the CMA++ agar, but isolations on the hymexazol agar were 34% more successful than on CMA. However this sample also produced a hymexazol tolerant isolate of *P. intermedium* on both agars. Although this is a very promising result, further samples are still urgently required from as diverse a selection of cavity spot-affected field sites as possible. When fresh cavities become available from commercial field sites from September onwards, further isolations will allow thorough testing of these media, and possibly further improvements.

Isolations from soil

a) Warcup plating

Dilutions of soil up to 10g of soil (wet weight) in 100 ml SDW produced no colonies of *P. violae* (or *P. sulcatum*) from soils obtained from 3 severe cavity spot fields (including cottage field, HRI Wellesbourne). Small numbers of colonies of other *Pythium* species were detected in all soil samples, these included: *P. intermedium, P. sylvaticum* and *Pythium* HS group. However, none of these colonies grew at rates that would be expected to overrun *P. violae* if present. Additions of *P. violae* propagules to each test soil did give reasonable recoveries of colony forming units (cfu),

indicating that neither soils nor the selective medium were substantially inhibitory to recoveries. This indicated that the concentration of *P.violae* propagules in the soils was too low to be detected by dilution plating and that some form of concentration step was required prior to plating for quantification. In some of the soil plates *Mortierella* spp. still appeared able to grow rapidly, indicating that it may be necessary to increase the concentration of hymexazol in the plating medium to avoid these common soil inhabitants overrunning plates.

b) soil sieving

The work with soil sieving so far has focussed on either oospores or propagules in the same size range as oospores (eg hyphal swellings, mycelial fragments or infected debris particles). Suspensions of particles either plated directly following the sucrose density gradient/centrifugation stage or they were examined under the microscope and individual propagules selected and plated out. In the two soils assessed so far, the highest concentration of propagules was collected in the 20-80 µm sieve mesh size category. The occurrence of *P. violae* was very sparse in both the direct suspension and selected propagule plates, giving cfu estimates of between 0 and 20 cfu g^{-1} dry weight of soil (eg the 'danger level' of *P. sylvaticum* in chrysanthemum greenhouse soils is approximately 3000 cfu g^{-1} dry weight of soil!, Pettitt, 2001). In the plating of selected propagules, the proportions of *P intermedium, P. sylvaticum* and *Pythium* HS were all higher than that of *P. violae*. In the Wellesbourne cottage field soil, 32% of plated propagules were *Pythium* HS, 32% ,*P. sylvaticum*, 23% *P. intermedium* and only 5% *P. violae.* And in the second soil sample from Lancashire, 71% of propagules plated were *P. intermedium* with 7% *P.violae.* The majority of plated propagules were oospores, whereas 48% of colonies in the suspension plates were derived from debris particles, and in the majority of the remaining colonies the original propagule type could not be determined. Examining colonies microscopically to determine their origins was found to be too time-consuming and unreliable. And, with further studies, we propose to use a combination of pimaricin-rich media to exclude oospores and prolonged sonication to exclude mycelium from cfu counts, thereby determining the proportions of each in soil inoculum by elimination.

Discussion

Although *P. violae* is often regarded as a low temperature pathogen it would appear that the isolates tested here have temperature optima for mycelial growth of between 15 and 20°C. As expected, both isolates tested grew best on carrot juice agar. However for the purposes of developing a low-cost detection procedure CMA was chosen as a basal medium for its production speed and ease of use. Hymexazol has

been effective at suppressing growth of the commonly found fast growing *Pythium* species such as *P. sylvaticum* and *Pythium* Group HS, as well as *P. sulcatum*. Use of hymexazol and an incubation temperature of 15°C allowed *P. violae* isolates to compete with the fastest-growing *Pythium* species. This has not eliminated the competative species from plating procedures but does allow quantification of *P. violae* in dilution plates which was previously not possible. Higher concentrations of hymexazol (25 ppm) inhibited the fast growing *Mortierella* spp., often problematic in soil plates for *Pythium* spp, but the effects of these concentrations on *P. violae* growth (and countability of colonies on soil plates) in such plates still needs further investigation.

With high concentrations of pimaricin, oospores can be inhibited and with sonication mycelium can also be eliminated, using these procedures should allow us to determine the proportions of these inoculum types in soils.

Soils tested so far have shown very sparse population by *P. violae*. This may be a reflection on the sensitivity of the plating method and we are currently assessing this using measured additions of oospores and mycelium. However, sparse inoculum may be the natural situation, even in severe cavity spot fields. Alternatively, propagules may be naturally sparse at the times of year so far assessed. Either of these two situations would have a profound effect on the development of reliable diagnostic tests for affected fields and require further work.

GENERAL CONCLUSIONS

Calcium treatments

- In 'carry-over' pot experiments, all the three calcium compounds tested (calcium carbonate, calcium hydroxide and calcium monocarbamide) achieved levels of control of cavity spot that did not significantly vary between the first and the second crop after lime treatment. The pH of each soil remained stable between crops. Generally, the disease control achieved in the calcium carbonate and the calcium hydroxide-treated pots was better than in the calcium monocarbamide pots. The highest pH values were in the Calcium hydroxide-treated pots (pH range = 6.9 – 7.8), followed by calcium carbonate (pH range = 6.3 – 7.3) and the lowest in calcium monocarbamide treatments (pH range = 5.7 – 6.3). These results indicate that control may be achieved in liming treatments *via* a soil pH effect, although calcium availability was not determined and can not therefore be discounted.

- Calcium sulphate had no significant effect on cavity spot disease in either pot experiments or in field trials. In addition, calcium nitrate did not have an effect on disease in field trials. Neither of these compounds increased the pH of test soils in pots, whilst they would both be expected to increase the levels of calcium. This is further evidence in support of a pH as opposed to a calcium effect on *P. violae* cavity spot; a result consistent with Australian findings with the cavity spot caused by *P. coloratum* (El Tarabily *et al.*, 1997).

- Pot trials on the effect of timing and scale of calcium carbonate applications contradicted the previous year's results. Applications immediately prior to drilling (effectively an 'at drilling' application) were the only treatments to significantly reduce cavity spot. Applications 1 and 2 months prior to drilling reduced disease but were not as good as the 'at drilling' application. In addition, there did not appear to be any difference in the effect between the 6 and the 12 tonne/ha applications in either disease control or subsequent soil pH (6 t/ha = pH 6.3 – 6.9 & 12 t/ha = pH 6.6 – 7.1).

- Most of the experimental work in pots, and to some extent field trials, completed so far has been in soils of moderate to slightly acid pH, whereas many UK carrot soils are alkaline. In an attempt to assess the impact of lime on disease in alkaline soils and finally dissociate the possible pH from calcium effects, a series of pot trials have been set up in which lime applications to high and low pH cavity spot 'problem' soils will be compared. In these experiments both the pH and the

concentrations of calcium available will be monitored along with the usual disease and yield assessments.

Fungicides

- In all pot tests and field trials, experimental fungicide UK 876 failed to control cavity spot at all rates and timings of application.

- Amistar applications showed little or no effect on cavity spot in the pot experiments. However, fungicide sensitivity tests with representative isolates of *P. violae* did demonstrate significant fungicidal activity, although the ED_{50} was not as low as for metalaxyl. In addition, field trials at both sites used showed some promise for control at the higher rates of application. This merits further investigation.

- The SL 567 metalaxyl formulation gave inconsistent results in pot experiments and disappointing results in both of the field trials. This disappointing performance could be due to fungicide resistance developing in the pathogen or possibly to metalaxyl degradation in the soil. Assessments of metalaxyl sensitivity in isolates of *P. violae* from both the field sites, and from all over the UK, have so far shown no indications of fungicide resistance in this species. Isolates of alternative cavity spot causing pathogen, *P. sulcatum*, showed a higher degree of metalaxyl tolerance than *P. violae*, and a shift in the field populations in favour of this species could cause a decline in SL 567 efficacy. However, it is also possible that metalaxyl degradation was a factor in the performance of SL 567 and analysis of soil samples from the field sites and from the pot experiments is ongoing.

Pathogen inoculum

- 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 15ºC but not at 20 ºC. The temperature range for germination was 5–30 ºC, with an optimum at 20-25 ºC. 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.

- Hymexazole successfully reduced the *in vitro* growth of *Pythium intermedium, P. sylvaticum* and *Pythium* group HS isolates, whilst having little effect on *P. violae* isolates. This immediately provided an improved medium for isolations from cavities on carrots, but, more importantly, made it possible for the first time to carry out colony forming unit (cfu) assessments on infected soils. Pimaricin at between 2 and 10 ppm with hymexazole at 10 ppm gave the best isolation medium. However, *Mortierella* spp. still caused problems in some samples, and higher rates of hymexazole may need to be tested. *P. violae* inoculum in the cavity spot 'problem' soils so far tested, appears to be very sparse (<100 cfu/g dry weight of soil) and detection required a pre-plating concentration step consisting of wet sieving followed by centrifugation. Work is ongoing on improvements to the pre-plating treatment of samples. More importantly, further work, on a wider range of soil samples is required to determine whether sparse inoculum, even in known 'problem' soils, is a widespread phenomenon as this would influence the development of reliable control strategies.

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APPENDIX I

Evidence for the enhanced degradation of metalaxyl in UK carrot soils

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ABSTRACT

Laboratory studies were carried out to determine the rates of degradation of metalaxyl in soils from nine fields in which metalaxyl had been used extensively as a soil treatment for the control of cavity spot disease in carrots. In all these fields, the fungicide had failed to control the disease in recent years. A further carrot field was sampled to which metalaxyl had never been applied. Soil samples were taken from 10 stations within each field and each sample was processed individually. Sub-samples of the 10 were also bulked to produce a composite sample for each field. The time taken for 50 % of the fungicide to be degraded (DT_{50}) was calculated using GENSTAT 5, fitting Gompertz or linear regressions to the data. Comparisons were made between the regressions fitted for the composite samples and the average of those fitted for the 10 stations. In fields where the fungicide failed, the DT_{50} values varied from 4 to 14 days. The composite samples produced DT_{50} values that were comparable (4 to 15 days). In the field where no metalaxyl had been applied the average DT_{50} for the 10 stations was 46 days compared with 43 days from the composite sample. A second study was carried out with soil samples from two fields adjacent to one another. One field had no previous history of metalaxyl application whilst the other had a history of application and failure in the control of cavity spot. The DT_{50} values for metalaxyl degradation in soils from these fields were 39.3 and 13.2 days, respectively.

INTRODUCTION

Cavity spot disease of carrots in the UK is largely due to the metalaxyl-sensitive fungus *Pythium violae*. It produces sunken lesions on the carrot root, and is particularly damaging in years of high rainfall. Control of the disease has relied on the use of metalaxyl and, more recently, metalaxyl-M, applied between drilling and first true leaf stage. In recent years deterioration in the performance of metalaxyl has been observed in some fields, both by growers on their own field sites and by scientists during cavity spot field experiments (McPherson, pers. comm.). Populations of the pathogen have been continuously monitored for metalaxyl resistance, using the method of White *et al*. (1988), but no resistance to metalaxyl has been found.

Various studies have established that metalaxyl is subject to degradation by soil microorganisms (Bailey & Coffey 1985; Droby & Coffey 1991). Recent studies in Western Australia have shown that reduced persistence of metalaxyl in fields used for carrot production is associated with previous metalaxyl use (Davison & McKay

1999). This study aims to examine the persistence of metalaxyl in fields used for carrot production in the UK, and to compare persistence in two adjacent fields with different metalaxyl treatment histories.

MATERIALS AND METHODS

Site selection and sampling methods

Nine fields were identified by UK carrot growers, as having received metalaxyl applications over several years, and recent crop failure due to cavity spot despite metalaxyl use. One field was also identified at HRI Wellesbourne as having no previous history of metalaxyl use.

For each field, approximately 1 kg of top soil was collected from each of 10 stations within the field, along a 250 – 300 m transect. The trowels used for sampling were washed and disinfected between stations and fields to prevent cross contamination between samples. A composite sample was produced for each field by bulking together an equal quantity of soil from each of the 10 station samples, and mixing well. All samples were stored at 5°C prior to the laboratory incubations.

Control soils were difficult to obtain, and despite collecting soil samples from untreated areas like headlands, comparison of these soils with the field samples often revealed considerable differences in characteristics such as pH, making the soils unsuitable as controls.

To address this, a second study was carried out with soil from a further two fields located adjacent to one another. One field had received a number of metalaxyl applications together with a recent metalaxyl-treated carrot crop with cavity spot; the other field had no metalaxyl pre-treatment history. Soil samples were collected from 5 stations within the treated field. These samples were processed separately and bulked to produce a composite sample. Soil was also collected from 5 stations in the untreated field and processed as one bulked sample which was divided to produce 2 replicate subsamples.

Sample preparation and residue analysis

When handling soil, new or autoclaved equipment was used for each sample, and the bench was sprayed with industrial methylated spirit between samples. All soils were sieved to 3mm, and the maximum water holding capacity (MWHC) of the soil from each field was assessed using soil from the composite samples. In addition the moisture content of individual samples was determined and, where necessary, they were air-dried to reduce the water content to below 40 % of the MWHC.

Analytical (99.6 %, Syngenta) and technical (97.4 %, Syngenta) grade metalaxyl were used throughout the study. A solution of technical metalaxyl (0.5 g/l in water) was pipetted onto the soil sample to give a concentration of 10 mg/kg dry soil and the samples were thoroughly mixed and then transferred to 500 ml pots. For fields 1-4 there were two replicate pots per soil sample. For fields 5-10 this was reduced to one pot per sample. Sterilised distilled water (SDW) was pipetted around the edge of the pots to increase the moisture level to 40 % of the MWHC. The lids were replaced

loosely and pots were incubated at 15°C. Sub-samples of soil (15 g) were taken on d 0 and at regular intervals thereafter. On each sampling occasion any water lost from pots was replaced with SDW.

Metalaxyl was extracted from each 15 g sub-sample by shaking with 20 ml methanol for 50 min on a wrist-action shaker. The soil samples were allowed to settle for at least 10 min. Samples of clear supernatant were removed and analysed by hplc using a LiChrospher-RP18 (5µm) column and acetonitrile: water: orthophosphoric acid (70: 30: 0.25 by volume) eluant at a flow rate of 1 ml min^{-1}; detection was by UV absorbance at 210 nm. The retention time of metalaxyl was 3.5 min. The response on hplc was calibrated against a 5 mg/l analytical grade metalaxyl standard.

Determination of DT_{50} values

Using GENSTAT 5, the time taken for 50 % loss of the metalaxyl (DT_{50}) was determined by fitting either a Gompertz curve or a linear regression as appropriate. This was carried out for each of the composite samples and by grouping data for each of the 10 stations within the field.

RESULTS AND DISCUSSION

Table 1. Comparison of the DT_{50} (d) derived from fitting of the Gompertz equations or linear regressions to data from the composite sample and 10-station samples.

	DT_{50} (d) of fitted curves / linear regressions	
Field identification number	10 stations processed individually	**Composite sample**
1	14.4	14.9
2	8.1	8.8
3	8.2	9.0
4	9.2	8.9
5	9.7	8.3
6	3.7	3.6
7	4.0	3.5
8	6.8	5.5
9	7.5	7.1
10	45.7	42.7

The estimated DT_{50} values are listed in Table 1. Considerable variation in the rate of degradation of metalaxyl was seen between soils from the different fields. The highest DT_{50} value (45.7 d) was recorded in soil from the field (number 10) with no history of metalaxyl treatment, with metalaxyl persisting in all samples for 72 d or more. Despite the variable degradation rate within this field (Figure 3), the DT_{50} of metalaxyl in the composite sample was similar to that based on the regression for the data from the 10 stations.

In soil from nine fields with metalaxyl treatment histories, eight (fields 2-9) had half-

lives of less than 10 d (e.g. Figure 2). This parallels the work of Davison & McKay (1999) in Western Australia where half-lives in soil from 3 fields, each with a metalaxyl treatment history and a failed crop, was 10 d or less. In soil from their field with no previous metalaxyl use and successful control of cavity spot, the half-life was 82 d.

Field 1 exhibited the greatest variability in degradation rates between soil samples from the different stations, with metalaxyl persisting in soil samples from 3 stations for over 60 d but at other stations disappearing within 30 d (Figure 1). In soil from all nine fields where performance of the chemical had been poor and disease levels were at or near crop write-off, the DT_{50} was between 4 and 14 d. By bulking together soil samples from individual stations within a field to produce a composite sample, a good indication of metalaxyl performance was still achieved (DT_{50} values of 4 to 15 d). This would appear to be a good approach for soil sampling to predict the behaviour of the fungicide in a particular field.

In the second study with soil from two adjacent fields with similar properties, the degradation rate of metalaxyl was considerably faster in soil from the treated field compared to the untreated field (Figure 4), with DT_{50} values of the bulked composite samples of 13.2 and 39.3 d respectively. Since these fields were located next to each other, were of the same pH, and other properties, yet differed in their metalaxyl treatment histories, this would suggest enhanced microbial degradation of the fungicide was occuring at this site.

Studies have shown that the timing of metalaxyl application is an important factor in the control of cavity spot disease of carrots, and there appears to be a crucial time early in the life of a crop when protection from the pathogen is essential. Gladders & McPherson (1986) found the best control was achieved with metalaxyl (+ mancozeb) applications made between sowing and four weeks post-crop emergence. Clearly, if the fungicide persists for less time in the soil, crop protection will be reduced.

Metalaxyl, and the recently introduced metalaxyl-M, are the only reliable fungicides for the control of cavity spot and are still effective in the vast majority of carrot production areas. Reports of failure of the fungicide on a small number of fields is a cause for concern and these experiments show that enhanced biodegradation could be factor at some of these sites.

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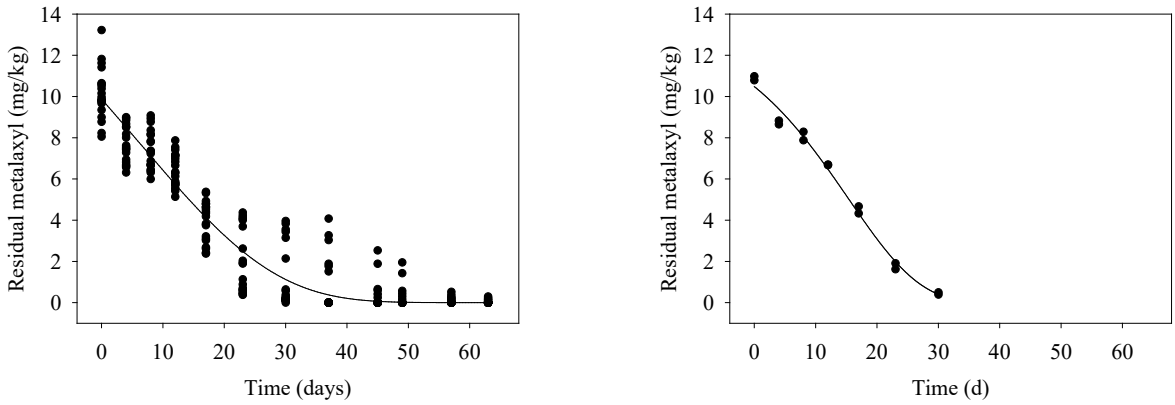

Figure 1. Comparison of metalaxyl degradation between the 10-station (left) and composite (right) soil samples from field 1.

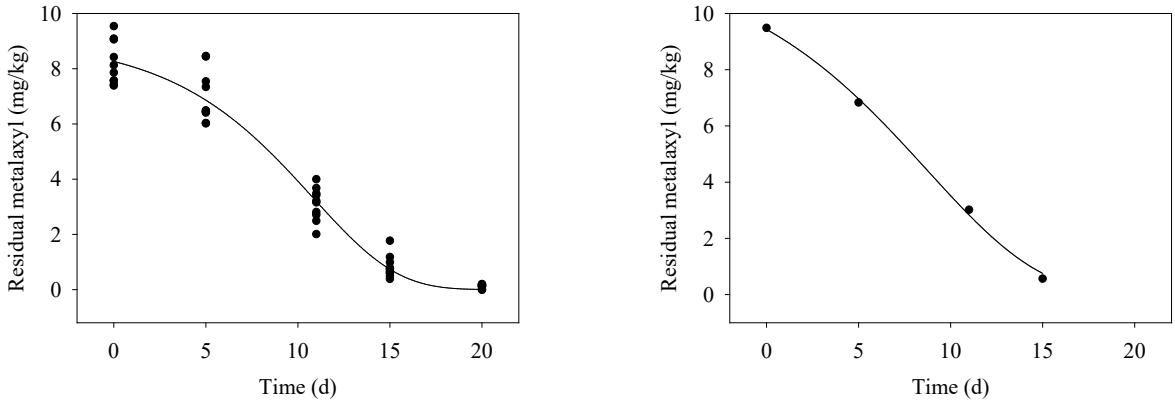

Figure 2. Comparison of metalaxyl degradation between the 10-station (left) and composite (right) soil samples from field 5.

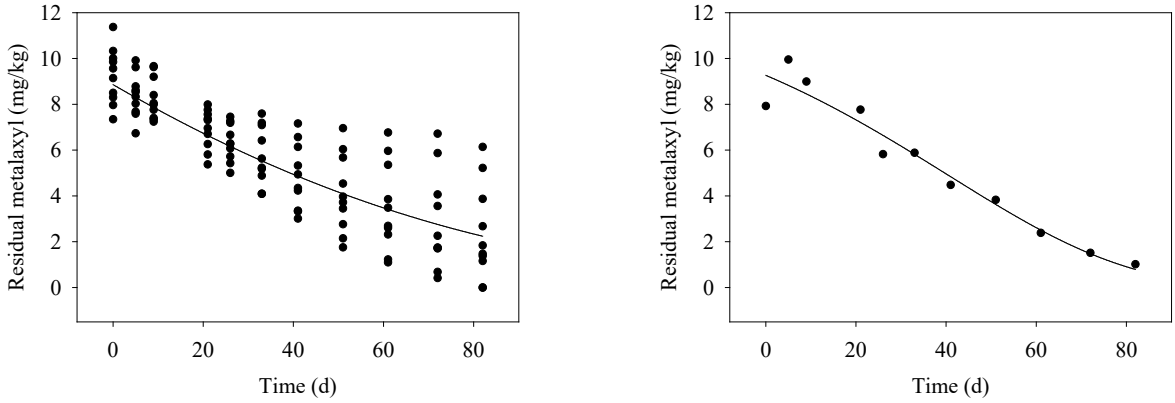

Figure 3. Comparison of metalaxyl degradation between the 10-station (left) and composite (right) soil samples from field 10.

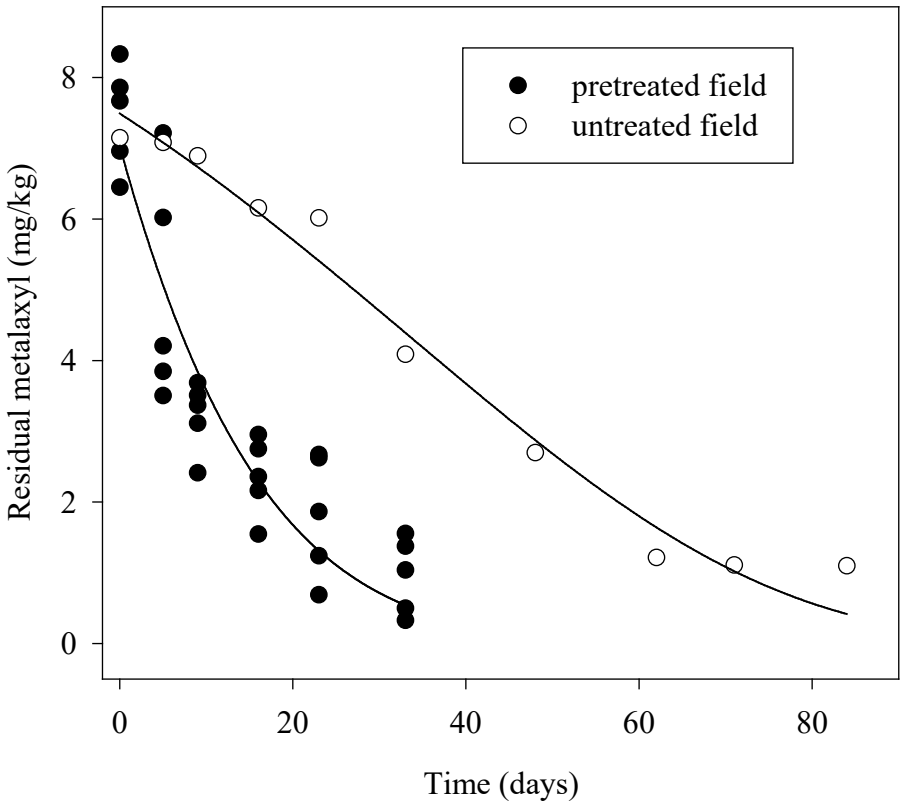

Figure 4. Comparison of metalaxyl degradation between soil samples from fields with and without pretreatment histories.

APPENDIX II

Table i. Trial plan for cavity spot calcium treatments 2000.

	REP 1	REP 2	REP 3
	1 T4 -FUNG	9 T1 -FUNG	17 T2 -FUNG
	2 T4 +FUNG	10 T1 + FUNG	18 T2 + FUNG
	3 T3 +FUNG	11 T2 - FUNG	19 T1 - FUNG
GUARD	4 T3 -FUNG	12 T2 + FUNG	20 T1 + FUNG
	5 T1 -FUNG	13 T4 - FUNG	21 T3 + FUNG
2.5M MIXING AREA→	6 T1 + FUNG	14 T4 + FUNG	22 T3 -FUNG
↑ 5 M ↓	7 T2 + FUNG	15 T3 - FUNG	23 T4 - FUNG
↑ 5 M ↓	8 T2 -FUNG	16 T3 + FUNG	24 T4 + FUNG
	REP 4	**REP 5**	**REP 6**
	25 T2 -FUNG	33 T4 - FUNG	41 T1 + FUNG
	26 T2 + FUNG	34 T4 + FUNG	42 T1 - FUNG
	27 T3 + FUNG	35 T2 + FUNG	43 T3 + FUNG
GUARD	28 T3 - FUNG	36 T2 - FUNG	44 T3 - FUNG
	29 T4 + FUNG	37 T1 + FUNG	45 T2 - FUNG
	30 T4 -FUNG	38 T1 - FUNG	46 T2 + FUNG
	31 T1 + FUNG	39 T3 + FUNG	47 T4 - FUNG
	32 T1 - FUNG	40 T3 - FUNG	48 T4 + FUNG

→ Fungicide Trial

Treatments:

1. Calcium sulphate at 6 tonnes/ha (incorporate 1 month before drilling).

2. Calcium nitrate 666 kg/ha. (333 kg/ha 1 month before drilling and 333 kg/ha as top dressing at 2-3 true leaf stage)

3. Calcium carbonate at 10 tonnes/ha (incorporate 1 month before drilling)

4. Untreated.

CALCIUM APPLIED TO A 15 m LENGTH. EACH PLOT WILL BE 5 m WITH A 2.5 m 'MIXING AREA' BETWEEN MAIN TREATMENTS.

Split plot treatment:

+ FUNG SL 567 at 1.3l product in 1000l water/ha (2-3 true leaf stage)

- FUNG No fungicide to be applied

APPENDIX II

Table ii. Trial plan for fungicide treatments 2000.

	REP 1	REP 2	REP 3
↑ 5 M ↓	1 T3	8 T7	15 T5
	2 T2	9 T6	16 T6
← CALCIUM TRIAL	3 T5	10 T1	17 T1
	4 T7	11 T4	18 T2
	5 T1	12 T5	19 T3
	6 T4	13 T3	20 T4
	7 T6	14 T2	21 T7
	REP4	**REP5**	**REP6**
	22 T3	29 T5	36 T2
	23 T1	30 T3	37 T5
	24 T2	31 T2	38 T3
	25 T5	32 T4	39 T1
	26 T4	33 T7	40 T6
	27 T6	34 T6	41 T7
	28 T7	35 T1	42 T4

GUARD BED

← 1 BED→

T1 = Untreated
T2 = SL567 pre-drilling 1.3l in 1000l water/ha
T3 = SL567 Split dose. I week prior to drilling and post emergent at 1-2 true leaves at 0.65l in 1000l water/ha
T4 = Amistar pre drilling at 6l in 1000l water/ha
T5 = Amistar post emergent at 1-2 true leaf stage at 10l in 1000 l water/ha
T6 = UK876 pre drilling at 1.28 kg in 1000l water/ha
T7 = Filex pre drilling at 15l in 1000l water/ha

APPENDIX II

Table iii. Yield, root population and root defects on fungicide trial at first harvest, Herringswell, 26th January 2001.

Treatment number	Treatment description	% fanged roots	% small roots	% misshapen roots	Weight of roots (t/ha)	Number of Roots (000/ha)
1	Untreated	11.00	2.00	7.7	42.09	218.5
2	SL567 pre-drilling	9.00	2.67	5.7	42.03	219.0
3	SL567 pre-drilling and post-emergent	7.00	3.33	5.7	39.80	214.4
4	Amistar pre-drilling	12.67	3.67	5.7	43.14	228.7
5	Amistar post-emergent	7.67	4.67	7.0	39.23	215.3
6	UK876 pre-drilling	7.33	3.00	2.3	39.20	208.8
7	Filex pre-drilling	9.67	2.00	4.3	43.99	237.5
	Mean	9.190	3.050	5.50	41.350	220.30
	SED (30df)	3.334	1.484	4.16	2.771	20.02
	p-value	Ns	ns	ns	ns	Ns

Table iv. Yield, root population and root defects on calcium trial at first harvest, Herringswell, 26th January 2001. (Shows interaction between calcium and fungicide).

Treatment number	Treatment description	% fanged roots	% small roots	% misshapen roots	% ribbed roots	Weight of roots (t/ha)	Number of roots (000/ha)
1	Calcium sulphate pre-drill without SL567	8.00	4.33	6.70	6.00	44.55	251.8
1	Calcium sulphate pre-drill with SL567	10.67	2.33	11.00	7.67	43.18	249.5
2	Calcium nitrate pre-drill and post-emergent without SL567	8.33	3.33	8.30	5.00	43.33	253.2
2	Calcium nitrate pre-drill and post-emergent with SL567	13.33	2.67	4.30	5.33	41.34	229.2
3	Calcium carbonate pre-drill without SL567	9.67	4.67	14.30	11.67	37.59	204.2
3	Calcium carbonate pre-dril with SL567	6.00	3.33	2.30	4.00	45.05	257.4
4	Untreated without SL567	9.00	4.00	12.00	7.00	40.55	212.5
4	Untreated with SL567	11.67	2.67	2.70	3.33	38.88	202.8
	Mean	**9.580**	**3.420**	**7.700**	**6.250**	**41.810**	**232.60**
	SED	**2.529**	**2.078**	**3.750**	**3.018**	**2.466**	**19.10**
	p- value	**ns**	**ns**	**ns**	**ns**	**ns**	**0.013**

Table v. Incidence and severity of new and old lesions of cavity spot on calcium trial at first harvest, Herringswell, 26th January 2001. (Shows interaction between calcium and fungicide treatments).

Treatment numbers	Treatment description	Mean % roots affected (new lesions)	Mean number of new lesions / 50 roots	Mean % root area affected (new lesions)	Mean % roots affected (old lesions)	Mean numbers of old lesions / 50 roots	Mean % root area affected (old lesions)
1	Calcium sulphate pre-drill without SL567	1.33	1.0	0.5	6.3	4.5	2.7
1	Calcium sulphate pre-drill with SL567	0.33	0.2	0.1	1.7	0.8	0.8
2	Calcium nitrate pre-drill and post-emergent without SL567	0.00	0.0	0.0	7.3	16.0	6.8
2	Calcium nitrate pre-drill and post-emergent with SL567	1.00	3.0	1.1	14.7	37.0	15.5
3	Calcium carbonate pre-drill without SL567	6.00	22.2	9.2	12.3	45.8	24.0
3	Calcium carbonate pre-dril with SL567	0.33	0.2	0.5	5.3	4.7	8.4
4	Untreated without SL567	0.33	0.2	0.1	2.3	1.5	1.2
4	Untreated with SL567	0.33	0.2	0.3	4.7	3.7	3.1
	Mean	**1.210**	**3.40**	**1.50**	**6.80**	**14.30**	**7.80**
	SED	**2.926**	**11.16**	**4.61**	**7.25**	**28.79**	**4.24**
	p-value	**ns**	**ns**	**ns**	**ns**	**Ns**	**ns**

APPENDIX II

Table vi. Summary of pest and minor disease incidence on fungicide trial at first harvest, Herringswell, 26th January 2001.

Treatment number	Treatment description	% roots with carrot fly damage	% roots with scab	% roots with violet root rot
1	Untreated	1.00	5.67	0.00
2	SL567 pre-drilling	1.00	4.00	0.00
3	SL567 pre-drilling and post-emergent	1.33	3.00	0.00
4	Amistar pre-drilling	0.33	5.33	0.00
5	Amistar post-emergent	1.00	2.33	0.33
6	UK876 pre-drilling	0.33	1.33	0.00
7	Filex pre-drilling	0.33	3.00	0.00
	Mean	0.760	3.520	0.048
	SED (30df)	0.912	1.735	0.178
	p-value	ns	ns	ns

Table vii. Summary of pest and minor disease incidence on calcium trial at first harvest, Herringswell, 26th January 2001. (Shows effects of calcium and fungicide separately).

Treatment number	Treatment Description	% roots with carrot fly damage	% roots with scab
1	Calcium sulphate pre-drilling	0.67	2.17
2	Calcium nitrate pre-drilling and post-emergent	1.00	2.17
3	Calcium carbonate pre-drilling	1.50	3.00
4	Untreated	0.67	3.33
	SED (15df)	0.714	1.404
	p value	ns	ns
1	- SL567	1.17	3.17
2	+ SL567	0.75	2.17
	SED (20df)	0.528	0.938
	p-value	ns	ns

Table viii. Summary of pest and minor disease inidence on calcium trial at first harvest, Herringswell, 26th January 2001. (Shows interacion between calcium and fungicide treatments).

Treatment numbers	Treatment Description	% roots with carrot fly damage	% roots with scab
1	Calcium sulphate pre-drill without SL567	0.67	2.00
1	Calcium sulphate pre-drill with SL567	0.67	2.33
2	Calcium nitrate pre-drill and post-emergent without SL567	1.67	3.33
2	Calcium nitrate pre-drill and post-emergent with SL567	0.33	1.00
3	Calcium carbonate pre-drill without SL567	1.33	4.00
3	Calcium carbonate pre-dril with SL567	1.67	2.00
4	Untreated without SL567	1.00	3.33
4	Untreated with SL567	0.33	3.33
	Mean	**0.960**	**2.670**
	SED	**1.034**	**1.932**
	p-value	**ns**	**ns**

APPENDIX II

Table ix. Incidence and severity of cavity spot on fungicide trial at first harvest, Herringswell, 26th January 2001.

Treatment Number	Treatment description	Total % roots with cavity spot	% roots with disease severity category 1 (NIAB)	% roots with disease severity category 2 (NIAB)	Mean number of lesions /50 roots	Mean % area affected by cavity spot
1	Untreated	2.00	1.00	0.00	0.19	2.68
2	SL567 pre-drilling	2.00	0.67	0.67	0.03	0.74
3	SL567 pre-drilling and post-emergent	1.00	0.00	1.00	0.01	0.42
4	Amistar pre-drilling	1.67	1.33	0.33	0.02	0.23
5	Amistar post-emergent	2.00	1.00	1.00	0.02	0.42
6	UK876 pre-drilling	5.00	2.00	2.67	0.09	1.05
7	Filex pre-drilling	3.00	1.00	0.67	0.17	1.88
	Mean	2.380	1.000	0.900	0.076	1.060
	SED (30df)	1.290	0.925	0.676	0.096	1.476
	p-value	Ns	ns	0.015	ns	ns

Table x. Incidence and severity of cavity spot on calcium trial at first harvest, Herringswell, 26th January 2001. (Shows effects of calcium and fungicide treatments separately).

Treatment Number	Treatment description	Total % roots with cavity spot	% roots with disease severity category 1 (NIAB)	% roots with disease severity category 2 (NIAB)	Mean number of lesions /50 roots	Mean % area affected by cavity spot
1	Calcium sulphate pre-drilling	4.80	0.67	3.17	0.07	0.89
2	Calcium nitrate pre-drilling and post-emergent	11.50	3.00	2.83	0.56	1.23
3	Calcium carbonate pre-drilling	12.0	2.50	1.83	0.73	2.29
4	Untreated	3.80	1.33	1.50	0.06	1.16
	SED (15df)	6.660	1.323	1.339	0.545	0.765
	p value	ns	ns	ns	ns	ns
1	- SL567	9.00	1.75	2.33	0.46	1.30
2	+ SL567	7.10	2.00	2.33	0.25	1.49
	SED (20df)	4.830	0.923	0.707	0.387	0.596
	p-value	ns	ns	ns	ns	ns

Table xi. Incidence and severity of cavity spot on calcium trial at first harvest, Herringswell, 26th January 2001. (Shows interaction between calcium and fungicide treatments).

Treatment Number	Treatment Description	Total % roots with cavity spot	% roots with disease severity category 1 (NIAB)	% roots with disease severity category 2 (NIAB)	Mean number of lesions / 50 roots	Mean % area affected by cavity spot
1	Calcium sulphate pre-drill without SL567	7.7	1.33	4.33	0.11	1.20
1	Calcium sulphate pre-drill with SL567	2.0	0.00	2.00	0.02	0.58
2	Calcium nitrate pre-drill and post-emergent without SL567	7.3	2.00	1.33	0.32	0.99
2	Calcium nitrate pre-drill and post-emergent with SL567	15.7	4.00	4.33	0.80	1.47
3	Calcium carbonate pre-drill without SL567	18.3	3.00	2.00	1.36	2.18
3	Calcium carbonate pre-dril with SL567	5.7	2.00	1.67	0.10	2.40
4	Untreated without SL567	2.7	0.67	1.67	0.03	0.81
4	Untreated with SL567	5.0	2.00	1.33	0.08	1.51
	Mean	**8.00**	**1.870**	**2.330**	**0.350**	**1.390**
	SED	**9.54**	**1.858**	**1.671**	**0.772**	**1.139**
	p-value	**ns**	**ns**	**Ns**	**ns**	**ns**

APPENDIX III

Pesticides used in the carry-over pot experiments in 2000:

Sciarid	Intercept (a.i. imidacloprid, 70 % w/w)
Aphids	Dovetail (a.i. lamda-cyhalothrin + pirimicarb, 5:100 g/l) Pirimor (a.i. pirimicarb, 50 % w/w)

Pesticides used in the pot experiments in 2000:

Whitefly	Nicotine (a.i. nicotine 40 % w/w) Talstar (a.i. bifenthrin, 100g/l) Lindane (a.i. gamma-HCH, 800 g/l) Savona (a.i. fatty acids, 49 % w/w)
Aphids	Dovetail (a.i. lamda-cyhalothrin + pirimicarb, 5:100 g/l) Pirimor (a.i. pirimicarb, 50 % w/w)
Mildew	Thiovit (a.i. sulfur, 80 % w/w).

APPENDIX IV

APPENDIX V

Table i. Carryover of 1999 experiments. Experiment A. Control of cavity spot on carrots in soil from Wellesbourne treated with calcium compounds: percentage of carrots with cavities. LSD in Bold indicates significant difference between treatments.

		Control	$CaCO_3$ 3t/ha	$CaCO_3$ 6 t/ha	$CaCO_3$ 9 t/ha	$CaCO_3$ 12 t/ha	$Ca(OH)_2$ 3 t/ha	$Ca(OH)_2$ 6 t/ha	$Ca(OH)_2$ 9 t/ha	$Ca(OH)_2$ 12t/ha
Treatment and rate	Percentage of carrots with cavities *	**LSD at p 0.05**								
Control	50.0 (47.4)									
$CaCO_3$ 3 t/ha	16.3 (16.0)	**24.34**								
$CaCO_3$ 6 t/ha	26.3 (28.5)	22.04	22.18							
$CaCO_3$ 9 t/ha	19.7 (24.2)	**22.04**	22.18	19.63						
$CaCO_3$ 12 t/ha	17.4 (18.5)	**22.49**	22.62	20.13	20.13					
$Ca(OH)_2$ 3 t/ha	27.0 (30.8)	22.92	23.06	20.61	20.61	21.09				
$Ca(OH)_2$ 6 t/ha	32.8 (30.7)	23.09	23.22	20.81	20.81	21.28	21.74			
$Ca(OH)_2$ 9 t/ha	17.1 (20.0)	**22.49**	22.62	20.13	20.13	20.62	21.09	21.28		
$Ca(OH)_2$ 12t/ha	40.0 (39.5)	23.17	**23.31**	20.90	20.90	21.37	21.82	22.01	21.37	

* arcsin transformation of percentages to which LSD applies in parentheses

Table ii. **Carryover of 1999 experiments. Experiment A. Control of cavity spot on carrots in soil from Wellesbourne treated with calcium compounds: total number of cavities per carrot. LSD in Bold indicates significant difference between treatments.**

		Control	$CaCO_3$ 3t/ha	$CaCO_3$ 6 t/ha	$CaCO_3$ 9 t/ha	$CaCO_3$ 12 t/ha	$Ca(OH)_2$ 3 t/ha	$Ca(OH)_2$ 6 t/ha	$Ca(OH)_2$ 9 t/ha	$Ca(OH)_2$ 12t/ha
Treatment and rate	Total number of cavities per carrot	**LSD at p 0.05**								
Control	1.08									
$CaCO_3$ 3 t/ha	0.31	**0.51**								
$CaCO_3$ 6 t/ha	0.66	0.46	0.46							
$CaCO_3$ 9 t/ha	0.47	**0.46**	0.46	0.41						
$CaCO_3$ 12 t/ha	0.45	**0.47**	0.47	0.42	0.42					
$Ca(OH)_2$ 3 t/ha	0.60	0.48	0.48	0.43	0.43	0.44				
$Ca(OH)_2$ 6 t/ha	0.89	0.48	**0.49**	0.44	0.44	0.44	0.45			
$Ca(OH)_2$ 9 t/ha	0.55	**0.47**	0.47	0.42	0.42	0.43	0.44	0.44		
$Ca(OH)_2$ 12t/ha	1.08	0.49	**0.49**	0.44	**0.44**	**0.45**	**0.46**	0.46	**0.45**	

Table iii. Carryover of 1999 experiments. Experiment A. Control of cavity spot on carrots in soil from Wellesbourne treated with calcium compounds: number of large cavities per carrot. LSD in Bold indicates significant difference between treatments.

		Control	$CaCO_3$ 3t/ha	$CaCO_3$ 6 t/ha	$CaCO_3$ 9 t/ha	$CaCO_3$ 12 t/ha	$Ca(OH)_2$ 3 t/ha	$Ca(OH)_2$ 6 t/ha	$Ca(OH)_2$ 9 t/ha	$Ca(OH)_2$ 12t/ha
Treatment and rate	Number of large cavities per carrot	**LSD at p 0.05**								
Control	0.86									
$CaCO_3$ 3 t/ha	0.20	**0.35**								
$CaCO_3$ 6 t/ha	0.43	**0.37**	0.37							
$CaCO_3$ 9 t/ha	0.34	**0.37**	0.37	0.33						
$CaCO_3$ 12 t/ha	0.30	**0.38**	0.38	0.34	0.34					
$Ca(OH)_2$ 3 t/ha	0.35	**0.39**	0.39	0.35	0.35	0.35				
$Ca(OH)_2$ 6 t/ha	0.54	0.39	0.39	0.35	0.35	0.36	0.37			
$Ca(OH)_2$ 9 t/ha	0.52	0.38	0.38	0.34	0.34	0.35	0.35	0.36		
$Ca(OH)_2$ 12t/ha	0.87	0.39	**0.39**	**0.35**	**0.35**	**0.36**	**0.37**	0.37	0.36	

Table iv. **Carryover of 1999 experiments. Experiment A. Control of cavity spot on carrots in soil from Wellesbourne treated with calcium compounds: percentage area of carrot infected. LSD in Bold indicates significant difference between treatments.**

		Control	$CaCO_3$ 3t/ha	$CaCO_3$ 6 t/ha	$CaCO_3$ 9 t/ha	$CaCO_3$ 12 t/ha	$Ca(OH)_2$ 3 t/ha	$Ca(OH)_2$ 6 t/ha	$Ca(OH)_2$ 9 t/ha	$Ca(OH)_2$ 12t/ha
Treatment and rate	Percentage area of carrot infected *	**LSD at p 0.05**								
Control	4.4 (7.2)									
$CaCO_3$ 3 t/ha	0.8 (1.8)	**2.03**								
$CaCO_3$ 6 t/ha	1.4 (3.0)	**1.84**	1.85							
$CaCO_3$ 9 t/ha	0.9 (2.0)	**1.84**	1.85	1.64						
$CaCO_3$ 12 t/ha	0.8 (1.5)	**1.87**	1.81	1.68	1.68					
$Ca(OH)_2$ 3 t/ha	0.9 (2.3)	**1.91**	1.92	1.72	1.72	1.76				
$Ca(OH)_2$ 6 t/ha	1.8 (3.7)	**1.92**	**1.94**	1.73	**1.73**	**1.77**	1.81			
$Ca(OH)_2$ 9 t/ha	2.0 (3.1)	**1.79**	1.89	1.68	1.68	1.72	1.76	1.77		
$Ca(OH)_2$ 12t/ha	2.0 (4.5)	**1.93**	**1.94**	1.74	**1.74**	**1.78**	**1.82**	1.83	1.78	

* arcsin transformation of percentages to which LSD applies in parentheses

APPENDIX VI

V8 juice broth (V8B)

100 ml V8 vegetable juice (Campbell's Soups, King's Lynn, Norfolk, UK)
2 g $CaCO_3$
900 ml distilled water

V8 juice agar (V8)

100 ml V8 vegetable juice (Campbell's Soups, King's Lynn, Norfolk, UK)
2 g $CaCO_3$
15 g agar
900 ml distilled water

Corn meal agar (CMA)

17 g corn meal agar (Difco)
1 l distilled water

Carrot juice agar (CJA)

500 ml carrot juice
500 ml distilled water
18 g agar

> **Carrot juice** was prepared by liquidising x g peeled carrots in x ml distilled water and then straining them through muslin. After autoclaving the homogenate at 121C for 15 min, the clarified solution on the top was used to make CJA.

APPENDIX VI

V8 juice broth supplemented with oatmeal infusion (V8B)

100 ml V8 vegetable juice (Campbell's Soups, King's Lynn, Norfolk, UK)
25 ml oatmeal infusion
2 g $CaCO_3$
875 ml distilled water

> **Oatmeal infusion** was prepared by boiling 60 g of fine oatmeal (Stratford Health Foods, Starford-upon-Avon) inside a muslin bag in 500 ml of distilled water for 20 min. The solution was used to supplement V8 broth.

Water agar (WA)

20 g agar
1000 ml distilled water

