Project title:	Parsnip: An Improved understanding of root blemishes and their prevention
Project number:	FV 366
Project leader:	Dr G M McPherson, Stockbridge Technology Centre
Report:	Annual report, Year 2 (2011/12)
Previous reports:	Annual report Year 1 (2010/11)
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Date project commenced:	1st January 2010
Date project completed (or expected completion date):	1 April 2013

** Left STC in December 2011*

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The results and conclusions in this report are based on the findings in the 2nd year of a three year investigation. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

This project is helping to identify what pathogens are causing root blemishes in parsnips to allow growers to instigate early and preventative control measures.

Background and expected deliverables

Various root blemishes continue to downgrade the quality of parsnip crops and cause economic damage in some seasons though the incidence in any particular crop can be extremely variable depending on many factors including the cultivar grown, the cropping history of the site, previous incidence of disease and the prevailing weather conditions. In some crops losses can be very significant and for example, in the 2009/10 season crops losses of up to 80% were reported in a few crops. The primary cause(s) of some of these root blemishes were identified in work carried out in Year 1 as being caused by fungal pathogens such as *Fusarium* spp., *Cylindrocarpon* spp., *Itersonilia* sp., and *Phoma* sp. although other blemishes detected were not found to be linked to fungal pathogens.

Black cankers are generally considered to be a result of infection by *Itersonilia pastinaceae* or possibly *Mycocentrospora acerina* (Davis & Raid, 2002). *Phoma complanata* has been associated with brown cankers previously though *Cylindrocarpon destructans,* a relatively common soil-inhabiting fungus, may also be involved in some situations. The orange-brown cankers which have been reported more recently have not been fully investigated and hence a primary cause has not been established. Also, the 'cavity spot'-like symptoms which occur in this crop, unlike in carrots, have not been formally confirmed as due to *Pythium* spp. or other specific pathogens (Gladders,1998).

The identification of the various root blemish symptoms in the field is not entirely reliable, especially using visual inspection alone. Detailed laboratory examination is therefore required to help identify and elucidate primary causal organisms or other factors involved. Hopefully at the end of the project we will have gained sufficient new knowledge to produce a Factsheet to help growers and their advisors quickly identify the primary cause of root blemish in this crop and hence instigate early and preventative control measures.

Summary of the project and main conclusions

A series of pot studies were carried out during the 2nd year of the project with the following outcomes:

- Significant differences in cultivar susceptibility to a range of pathogens was observed.

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- Increasing inoculum from the soil-borne pathogens such as *Cylindrocarpon, Fusarium, Pythium* and *Phoma* could be expected to increase the incidence and severity of blemish development, reduce crop vigour and potentially result in increased fanging in crops. This was demonstrated in pathogenicity tests in the project.

- Some pathogens resulted in decreased germination and increased damping-off of seedlings post-emergence.

- A range of conventional fungicides and biological products performed well in laboratory agar-plate tests and showed activity against the key pathogens responsible for blemishes on parsnips.

- In artificially inoculated pot studies no significant reduction in symptom development was observed following application of the experimental fungicides and biological control products. However, it is important to note though that symptom expression was low and did not provide a strong test. Treatment application methods and frequency may be important factors and these will be examined when testing performance under field conditions,

- Significant increases in average root weight were observed following application of several of the experimental products.

During the first year of this study both ADAS and STC monitored field crops and carried out sampling throughout the autumn period following tap-root formation. Each crop sample was assessed and, where possible, the primary cause of any lesions present was identified. We also collected many samples from pack-houses and growers via the STC Plant Clinic which were also tested and these helped us build a catalogue of blemishes symptoms to which we were able to match to a number of fungal pathogens. Pathogenicity tests were carried out with all of the isolates collected and this provided us with a range of fungal pathogens in culture for use in the work carried out in Year 2.

Work carried out in year 2 of the study took the form of testing the efficacy of a range of chemical and biological control products for control of the primary organisms detected in Year 1 in small-scale *in-vitro* and *in-vivo* studies.

Work carried out by ADAS focused on a series of pot-studies with the following outcomes.

1. Potential differences in the susceptibility of cultivars to each of the pathogens identified as being responsible for the majority of blemishes in Year 1 was investigated. The results indicated that there were significant differences in susceptibility, particularly at higher levels

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of pathogen inoculum. Choice of cultivar in field sites with a history of high blemish incidence may therefore well be an important consideration for growers.

2. The potential efficacy of a range of biopesticides for controlling the known pathogens was investigated. Red flecking symptoms developed on the roots of about 30% of plants in the inoculated and untreated pots and few positive effects following treatment application were observed.

3. ADAS also investigated the efficacy of a range of conventional fungicides applied as a single treatment to the soil surface to control the pathogens and reduce blemish development. Few symptoms developed in this experiment and no significant differences following treatment application were observed in these studies.

STC carried out an *in-vitro* study to test the efficacy of a range of fungicides and biopesticides to inhibit the fungal growth of *Cylindrocarpon, Fusarium* and *Pythium* sp. We identified a number of products that appeared to be very effective against 1, 2 or all of the pathogens.

- Products such as Amistar, Plover, Vivid, Folicur, Beret Gold, and an experimental product all inhibited growth of *Cylindrocarpon* by more than 50% even when used at low concentrations (2ppm of active ingredient). Prestop, HDC F79 and Serenade also showed some activity.

- Vivid, Folicur and the 3 biopesticides also showed good inhibition of *Fusarium* sp. at all concentrations, whilst products such as Amistar, Topsin, Beret Gold and Corbel were effective at the higher concentrations.

- Fewer products tested were effective against *Pythium* sp., Amistar, Vivid and SL567A were the only ones which appeared to be effective.

STC also carried out pot-studies to investigate product efficacy for reduction of blemish development in inoculated pots. However, once again symptom expression was very low and few conclusions could be drawn from the results.

Those products which showed signs of potential activity in the *in-vivo* work, and other products are being taken forward into larger scale field trials at STC (including an existing disease nursery developed for this work and in a commercial crop) and ADAS (in a commercial crop) in year 3 where it is hoped they will result in a reduction in skin blemish problems.

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Financial Benefits

Careful consideration of cultivar for field sites with a history of a high incidence of blemishes may be worthwhile and may help to extend the parsnip-growing life of sites. Although a number of products, conventional and biological, showed good activity against the pathogens in laboratory tests, further field testing is required before firm conclusions can be drawn and recommendations made therefore it is too early to judge the full economic benefit from this project.

Action Points

- Where possible less susceptible parsnip cultivars should be used for sites with a history of high blemish incidence or severity. In this project there were differences in susceptibility between Javelin and Palace.
- Growers should regularly monitor crops for blemish development during the early growth stages and apply already approved products which showed good potential efficacy if required.

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SCIENCE SECTION

Introduction

Approximately 4000ha of parsnips are grown annually in the UK. In 2009/10 the parsnip industry (growers & pack-houses) reported very high wastage figures with average losses in the region of 20% of the crop, but in some cases this has been reported to be much higher in individual crops. At present, growers are unable to take effective targeted action to minimize crop losses due to root blemish and are therefore in the unenviable position of having to invest heavily to maintain production capacity yet risk the possibility that in some years, or in some crops, this effort is wasted due to the late occurrence of blemishes on the washed parsnip roots. If the primary cause(s) of root blemish can be determined it should provide valuable information to guide selection of varieties and to investigate and develop well-targeted cultural, chemical and bio-control measures to help minimize infection and hence reduce wastage in crops in the future.

In Year 2 of the project a range of fungal isolates recovered from parsnip roots in Year 1 were used in laboratory *in vitro* and *in vivo* pot experiments to determine their pathogenicity and to investigate the potential for control with fungicides and biopesticides. Three experiments were carried out at ADAS Boxworth. At STC experiments were carried out to compare the potential of a range of fungicides and biopesticides to control fungal growth of each of the pathogens *in vitro* (agar plate tests). These were followed by pot studies to investigate the efficacy and crop safety of a range of fungicides and biopesticides to control blemish development in young parsnips.

Experiment 1 - pathogen x variety (ADAS)

1. To determine whether pathogens isolated from parsnip roots in 2010 were pathogenic when used as a soil inoculum at two concentrations.
2. To determine whether variety was an influencing factor on blemish development and to which pathogens Javelin, Palace and Pinnacle might be susceptible.

Experiment 2 – Biopesticides (ADAS)

1. To determine whether biopesticide soil treatments are effective against the parsnip blemish pathogens *Fusarium, Cylindrocarpon, Phoma* and *Pythium*.
2. To determine if the applied treatments were safe to use on parsnip crops.

Experiment 3 – Fungicides (ADAS)

1. To determine whether a range of fungicide treatments were effective against the parsnip blemish pathogens *Fusarium, Cylindrocarpon, Phoma* and *Pythium*.
2. To determine if the applied treatments were safe to use on parsnip crops.

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Experiment 4 – Agar plate tests (STC)

1. To determine whether 21 fungicides and 4 biopesticides resulted in inhibition of fungal growth of *Fusarium* and *Pythium*.

Experiment 5 – Fungicide and Biopesticide Pot studies (STC)

1. To determine whether a range of fungicides and biopesticides were effective against blemish-causing pathogens *Fusarium, Cylindrocarpon* and *Pythium*.
2. To determine if the applied treatments were safe to use on parsnip crops.

Materials and methods

1. Pathogen x variety experiment (ADAS)

Three parsnip varieties were inoculated with 7 different fungal pathogens with two rates of inoculation using a vermiculite–maize meal culture system (1% and 10% v/v). Uninoculated controls for each rate of inoculation provided 48 treatments in total (Table 1). The experimental design was fully randomised design with four replicates (192 experimental plots). There was very poor germination of Pinnacle and it has been omitted from analyses as no useful data were collected.

Table 1. Treatments in pathogen x variety experiment at Boxworth 2011.

Treatment number	Factor A – Variety	Factor B – Pathogen isolate x inoculation rate
1.	Javelin	Uninoculated 1%*
2.	Javelin	Uninoculated 10%*
3.	Javelin	*Fusarium* A 1%
4.	Javelin	*Fusarium* A 10%
5.	Javelin	*Fusarium* B 1%
6.	Javelin	*Fusarium* B 10%
7.	Javelin	*Fusarium* C 1%
8.	Javelin	*Fusarium* C 10%
9.	Javelin	*Cylindrocarpon* A 1%
10.	Javelin	*Cylindrocarpon* A 10%
11.	Javelin	*Cylindrocarpon* B 1%
12.	Javelin	*Cylindrocarpon* B 10%
13.	Javelin	*Phoma* 1%
14.	Javelin	*Phoma* 10%
15.	Javelin	*Pythium* 1%
16.	Javelin	*Pythium* 10%
17.	Palace	Uninoculated 1%*
18.	Palace	Uninoculated 10%*
19.	Palace	*Fusarium* A 1%
20.	Palace	*Fusarium* A 10%
21.	Palace	*Fusarium* B 1%

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Treatment number	Factor A – Variety	Factor B – Pathogen isolate x inoculation rate
22.	Palace	*Fusarium* B 10%
23.	Palace	*Fusarium* C 1%
24.	Palace	*Fusarium* C 10%
25.	Palace	*Cylindrocarpon* A 1%
26.	Palace	*Cylindrocarpon* A 10%
27.	Palace	*Cylindrocarpon* B 1%
28.	Palace	*Cylindrocarpon* B 10%
29.	Palace	*Phoma* 1%
30.	Palace	*Phoma* 10%
31.	Palace	*Pythium* 1%
32.	Palace	*Pythium* 10%
33.	Pinnacle	Uninoculated 1%*
34.	Pinnacle	Uninoculated 10%*
35.	Pinnacle	*Fusarium* A 1%
36.	Pinnacle	*Fusarium* A 10%
37.	Pinnacle	*Fusarium* B 1%
38.	Pinnacle	*Fusarium* B 10%
39.	Pinnacle	*Fusarium* C 1%
40.	Pinnacle	*Fusarium* C 10%
41.	Pinnacle	*Cylindrocarpon* A 1%
42.	Pinnacle	*Cylindrocarpon* A 10%
43.	Pinnacle	*Cylindrocarpon* B 1%
44.	Pinnacle	*Cylindrocarpon* B 10%
45.	Pinnacle	*Phoma* 1%
46.	Pinnacle	*Phoma* 10%
47.	Pinnacle	*Pythium* 1%
48.	Pinnacle	*Pythium* 10%

*apply uninoculated vermiculite/maize meal mix to uninoculated plots.

Fungal inoculum was prepared by adding 30 small pieces of actively growing agar cultures (c. 8mm^2) to autoclaved bags of vermiculite + maize-meal (25g/litre of vermiculite). The inoculated bags were incubated in the dark at 20°C for 3 weeks. The bags were turned weekly and checked regularly for signs of contaminants.

The fungal isolates used were as follows:

Fusarium sp. A ex-parsnip blackened crown from Norfolk 2010 - BX11/07 – T25

Fusarium sp. B ex-parsnip dry scar on crown from Norfolk 2010 - BX11/10 – T34

Fusarium sp. C ex-parsnip deep soft rot on root from Norfolk 2010 – T33

Cylindrocarpon destructans A ex-parsnip blackened crown from Suffolk 2010 - BX11/06 – T10

Cylindrocarpon destructans B ex-parsnip ginger blotch from Suffolk 2010 - BX11/06 – T10

Phoma sp. ex-parsnip ginger blotch from Suffolk 2010 – N/A8

Pythium sp. ex-seedling from Boxworth 2010/11

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Each plot consisted of 1 x 5L pot half-filled John Innes No. 1 compost. This was topped by soil inoculated at the required rate with vermiculite-maize meal inoculum (v/v). Each pot was then sown with 25 seeds on 6 September 2011 and positioned in an outdoor plot at ADAS Boxworth. Pots were watered regularly using overhead sprinklers and selectively by hand when appropriate to maintain moist but not wet compost.

Seedling emergence was monitored for the first month. A full assessment of the roots for the incidence and severity of blemishes and other symptoms was made on 25 November 2011. Symptoms were assigned to categories for small brown spots and larger blemishes and fanging.

Re-isolations were made from a small selection of roots at the final assessment to confirm that the inoculated pathogen was present.

2. Biopesticide x pathogen experiment (ADAS)

The biopesticide experiment comprised 7 biopesticide treatments tested against 5 pathogen treatments including an untreated/uninoculated control (Table 2). In this experiment the *Fusarium* isolates and the *Cylindrocarpon destructans* isolates were mixed together in equal quantities to give a single bulked treatment for each of the two genera. The 35 treatments were randomised in four complete blocks giving a total of 140 plots.

The isolates used were the same as those in the pathogen x variety experiment.

Fusarium sp. A ex parsnip blackened crown from Norfolk 2010 - BX11/07 – T25

Fusarium sp. B ex parsnip dry scar on crown from Norfolk 2010 - BX11/10 – T34

Fusarium sp. C ex parsnip deep soft rot on root from Norfolk 2010 – T33

Cylindrocarpon destructans A ex parsnip blackened crown from Suffolk 2010 - BX11/06 – T10

Cylindrocarpon destructans B ex parsnip ginger blotch from Suffolk 2010 - BX11/06 – T10

Phoma sp. ex parsnip ginger blotch from Suffolk 2010 – N/A8

Pythium sp. ex seedling from Boxworth 2010/11

Fungal inoculum was prepared by adding 30 small pieces of actively growing agar cultures (c. 8mm^2) to autoclaved bags of vermiculite + maize-meal (2 g per litre of vermiculite). The inoculated bags were incubated in the dark at 20°C for 3 weeks. The bags were turned weekly and checked regularly for signs of contaminants.

Each plot was a 5L pot which was half-filled John Innes No. 1 compost and then 1% of vermiculite–maize meal (v/v) was mixed into the compost. The biopesticides were mixed into compost at the rates advised by the manufacturers and then added to fill the top half of the pot. Each pot was then sown with 25 parsnip seeds on 6 August 2011 and kept in a polythene tunnel at ADAS Boxworth. Pots were watered regularly using overhead sprinklers and selectively by hand when appropriate to maintain moist but not wet compost.

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Seedling emergence and developed was monitored regularly. A full assessment of the roots for the incidence and severity of blemishes and other symptoms was completed on 13 January 2012. Symptoms were assigned to categories for small brown spots and larger blemishes and fanging.

Table 2. Treatments in pathogen x biopesticide experiment 2011.

Trt No.	Factor 1 – Bio-pesticide (code)	Active Ingredient	Rate	Factor 2 - Pathogen
1.	HDC F79	-	750g/m^3	Uninoculated*
2.	HDC F79	-	750g/m^3	*Fusarium* sp.
3.	HDC F79	-	750g/m^3	*Cylindrocarpon destructans*
4.	HDC F79	-	750g/m^3	*Pythium* sp.
5.	HDC F79	-	750g/m^3	*Phoma* sp.
6.	Prestop	*Gliocladium catenulatum* (J1446)	500g/1000L	Uninoculated*
7.	Prestop	*G. catenulatum* (J1446)	500g/1000L	*Fusarium* sp.
8.	Prestop	*G. catenulatum* (J1446)	500g/1000L	*C. destructans*
9.	Prestop	*G. catenulatum* (J1446)	500g/1000L	*Pythium* sp.
10.	Prestop	*G. catenulatum* (J1446)	500g/1000L	*Phoma* sp.
11.	HDC F82	-	25g/m^3	Uninoculated*
12.	HDC F82	-	25g/m^3	*Fusarium* sp.
13.	HDC F82	-	25g/m^3	*C. destructans*
14.	HDC F82	-	25g/m^3	*Pythium* sp.
15.	HDC F82	-	25g/m^3	*Phoma* sp.
16.	HDC F83	-	250g/m^3	Uninoculated*
17.	HDC F83	-	250g/m^3	*Fusarium* sp.
18.	HDC F83	-	250g/m^3	*C. destructans*
19.	HDC F83	-	250g/m^3	*Pythium* sp.
20.	HDC F83	-	250g/m^3	*Phoma* sp.
21.	HDC F78	-	10g/m^3	Uninoculated*
22.	HDC F78	-	10g/m^3	*Fusarium* sp.
23.	HDC F78	-	10g/m^3	*C. destructans*
24.	HDC F78	-	10g/m^3	*Pythium* sp.
25.	HDC F78	-	10g/m^3	*Phoma* sp.
26.	Serenade	*Bacillus subtilis*	200ml/1000L	Uninoculated*
27.	Serenade	*Bacillus subtilis*	200ml/1000L	*Fusarium* sp.
28.	Serenade	*Bacillus subtilis*	200ml/1000L	*C. destructans*
29.	Serenade	*Bacillus subtilis*	200ml/1000L	*Pythium* sp.
30.	Serenade	*Bacillus subtilis*	200ml/1000L	*Phoma* sp.

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3. Fungicide x pathogen experiment (ADAS)

Eight fungicide treatments were tested against 5 pathogen treatments including an untreated/uninoculated control (40 treatments in total) in a randomised block design with four-fold replication.

Fungal inoculum was prepared by adding 30 small pieces of actively growing agar cultures (c. 8 mm2) to autoclaved bags of vermiculite + maize-meal (25 g per litre of vermiculite). The inoculated bags were incubated in the dark at 20°C for 3 weeks. The bags were turned weekly and checked regularly for signs of contaminants.

Each plot was a 5L pot which was half-filled John Innes No. 1 compost and then 1% of vermiculite –maize meal (v/v) was mixed into the compost. The pots were then filled with uninoculated compost. The fungicides were applied to the compost surface at the rates advised by the manufacturers. Treatments as listed in Table 3 were applied using 300 L water/ha using an Oxford precision sprayer with single nozzle at 2 bar pressure with a 02F110 flat fan nozzle.

After spraying, the treatments were incorporated into the surface of the compost by hand. Each pot was then sown with 25 seeds on 6 August 2011 and kept in a polythene tunnel at ADAS Boxworth. Pots were watered regularly using overhead sprinklers and selectively by hand when appropriate to maintain moist but not wet compost.

Seedling emergence and development was monitored regularly. A full assessment of the roots for the incidence and severity of blemishes and other symptoms was completed on 18-19 January 2012. Symptoms were assigned to categories for small brown spots and larger blemishes and fanging.

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Table 3. Treatments in fungicide x pathogen experiment 2011.

Trt No.	Factor 1 – Fungicide (code)	Active Ingredient	Rate/ha	Factor 2 – Pathogen
1.	Untreated	-	-	Uninoculated*
2.	Untreated	-	-	*Fusarium* sp.
3.	Untreated	-	-	*C. destructans*
4.	Untreated	-	-	*Pythium* sp.
5.	Untreated	-	-	*Phoma* sp.
6.	SL567A	metalaxyl-M	1.3L	Uninoculated*
7.	SL567A	metalaxyl-M	1.3L	*Fusarium* sp.
8.	SL567A	metalaxyl-M	1.3L	*C. destructans*
9.	SL567A	metalaxyl-M	1.3L	*Pythium* sp.
10.	SL567A	metalaxyl-M	1.3L	*Phoma* sp.
11.	Topsin M 70WP	thiophanate - methyl	2.24kg	Uninoculated*
12.	Topsin M 70WP	thiophanate - methyl	2.24kg	*Fusarium* sp.
13.	Topsin M 70WP	thiophanate - methyl	2.24kg	*C. destructans*
14.	Topsin M 70WP	thiophanate - methyl	2.24kg	*Pythium* sp.
15.	Topsin M 70WP	thiophanate - methyl	2.24kg	*Phoma* sp.
16.	Signum	pyraclostrobin + boscalid	1kg	Uninoculated*
17.	Signum	pyraclostrobin + boscalid	1kg	*Fusarium* sp.
18.	Signum	pyraclostrobin + boscalid	1kg	*C. destructans*
19.	Signum	pyraclostrobin + boscalid	1kg	*Pythium* sp.
20.	Signum	pyraclostrobin + boscalid	1kg	*Phoma* sp.
21.	HDC F67	-	2.5L	Uninoculated*
22.	HDC F67	-	2.5L	*Fusarium* sp.
23.	HDC F67	-	2.5L	*C. destructans*
24.	HDC F67	-	2.5L	*Pythium* sp.
25.	HDC F67	-	2.5L	*Phoma* sp.
26.	Rudis	prothioconazole	0.4L	Uninoculated*
27.	Rudis	prothioconazole	0.4L	*Fusarium* sp.
28.	Rudis	prothioconazole	0.4L	*C. destructans*
29.	Rudis	prothioconazole	0.4L	*Pythium* sp.
30.	Rudis	prothioconazole	0.4L	*Phoma* sp.
31.	HDC F72	-	4.5kg	Uninoculated*
32.	HDC F72	-	4.5kg	*Fusarium* sp.
33.	HDC F72	-	4.5kg	*C. destructans*
34.	HDC F72	-	4.5kg	*Pythium* sp.
35.	HDC F72	-	4.5kg	*Phoma* sp.
36.	Amistar - drench	azoxystrobin	6 L	Uninoculated*
37.	Amistar - drench	azoxystrobin	6 L	*Fusarium* sp.
38.	Amistar - drench	azoxystrobin	6 L	*C. destructans*
39.	Amistar - drench	azoxystrobin	6 L	*Pythium* sp.
40.	Amistar - drench	azoxystrobin	6 L	*Phoma* sp.

4. Agar Plate Tests - STC

A range of 21 fungicides and 3 biopesticides from a variety of fungicide groups were tested *in vitro* to measure their potential efficacy to inhibit the mycelial growth of *Cylindrocarpon*, *Fusarium* and

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Pythium sp. The pathogens used were from taken from the STC culture collection of isolates retained from parsnip root sampling in Year 1 of this study. For each fungus a range of fungicides/biopesticides which were expected to have a potential for some activity were tested.

Each fungicide was used to amend potato dextrose agar (PDA) at 2, 20 and 100ppm of active ingredient. Products were dissolved in sterile water to produce a stock solution of 1000ppm and then aliquots prepared and added to autoclaved PDA at the appropriate rate. Bio-pesticide products were used at the label rate only. Each fungus was also grown on un-amended PDA to provide a 0ppm control. Details of the products/active ingredients used are shown in Table 4. Following incorporation of the products, the agar plates were allowed to dry in a laminar flow cabinet before 5mm plugs of each of the fungi to be tested were placed in the centre of the plates. Test plates were incubated at 23°C +/- 2°C for 72 hours before being measured. The diameter of the fungal colony was recorded on triplicate plates for each product and at each concentration. If colony growth was uneven, two measurements were recorded and a mean measurement was used.

The recorded values for each fungus/product/concentration were compared to the mean colony diameter of the un-amended plates (0ppm) and the percentage mycelial inhibition was calculated.

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Table 4. Details of products/active ingredients used for the *in vitro* agar plate tests

Treatment No.	Product Name or code No.	Active Ingredient	Pathogen tested against		
			Pythium	***Fusarium***	***Cylindrocarpon***
Conventional fungicides					
1	Amistar	azoxystrobin	✓	✓	✓
2	Scorpio/Swift SC	trifloxystrobin	X	✓	✓
3	Vivid	pyraclostrobin	✓	✓	✓
4	Filan	boscalid	✓	✓	✓
5	Unix	cyprodinil	X	✓	✓
6	Plover	difenoconazole	X	X	✓
7	HDC F81	-	X	✓	✓
8	HDC F65	-	X	✓	✓
9	HDC F70	-	✓	✓	✓
10	Folicur	tebuconazole	X	✓	✓
11	Cercobin	thiophanate-methyl	X	✓	✓
12	Beret Gold	fludioxonil	X	✓	✓
13	Rovral	iprodione	X	✓	✓
14	Corbel	fenpropimorph	X	✓	X
15	HDC F68	-	✓	X	X
16	HDC F76	-	✓	X	X
17	Karamate Dry Flo	mancozeb	✓	X	X
18	HDC F77	-	✓	X	X
19	HDC F75	-	✓	X	X
20	SL567A	metalaxyl-M	✓	X	X
21	HDC F71	-	✓	X	X
Bio-pesticides					
22	Prestop	*Gliocladium catenulatum*	X	✓	✓
23	Serenade	*Bacillus subtilis*	X	✓	✓
24	HDC F79	-	X	✓	✓

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5. Fungicide and biopesticide in vivo bioassay (STC)

Three separate pot studies were carried out to investigate the potential efficacy of a range of different products against each of the main fungal causal agents involved in blemish production in parsnips. The products chosen were tested against *Pythium (*ADAS culture cc234*), Fusarium* (cc225) and *Cylindrocarpon* (cc200) in inoculated pot studies, whilst the tests against *Itersonilia* were carried out in an inoculated field plot.

The pathogens were bulked up initially on PDA before being added to a mix of vermiculite, maize-meal and sterile distilled water and incubated at 30°C in the dark. Cultures were turned daily and checked regularly for contaminants. Each plot consisted of a 5L pot filled using John Innes No. 1 compost and inoculated at 1% v/v with each of the pathogens. The only exception to this was in the *Cylindrocarpon* study where the pathogen was incorporated into only the top 2.5L of compost at the product manufacturer's request. There were 4 replicate pots/treatment/pathogen. Pots were each sown with 25 seeds of parsnip cv. Javelin and were laid out in a fully randomised block design on a glasshouse bench.

Treatments

Treatments were applied by pouring 50ml of the prepared solutions evenly over the tops of the pots at the time of sowing. Repeat applications were made after 3-4 weeks and again 3-4 weeks later. All products were applied at 2000L water/ha and at 1% pot volume (50ml diluted product/5L pot).

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Figure 1. Pot study bioassay at STC

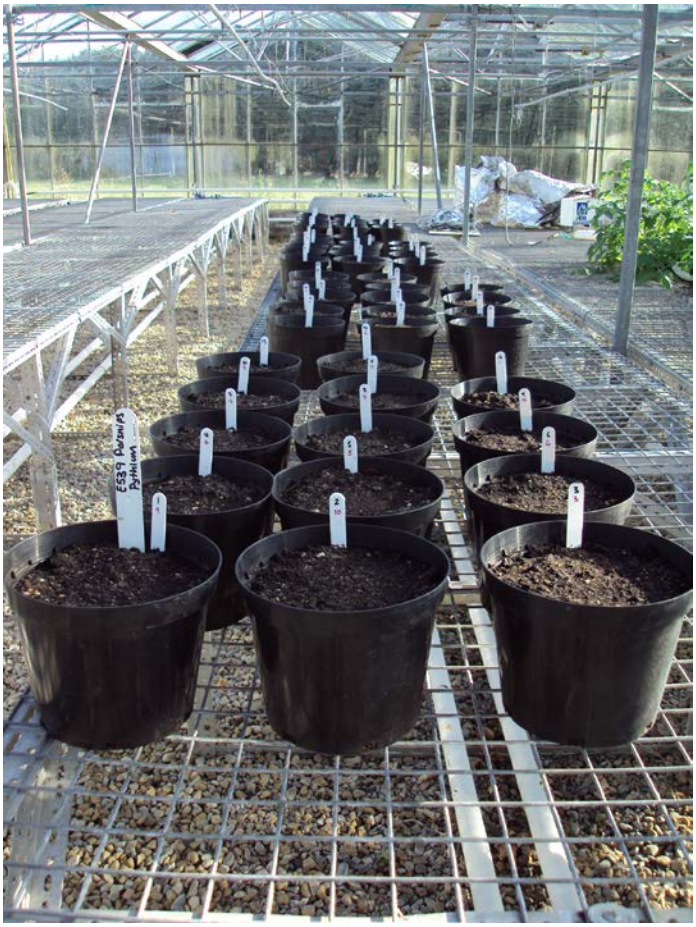

Table 1. Details of products applied for the *Pythium* bioassay.

Treatment Number	Fungicide Active Ingredient	Product	Rate	Amount per litre water
1	untreated uninoculated		-	
2	untreated inoculated		-	
3	metalaxyl-M	SL567A	1.3l/ha	0.65 ml
4	azoxystrobin	Amistar	1.0 l/ha	0.5 ml
5	-	HDC F76	0.5 l/ha	0.25 ml
6	-	HDC F74	0.8 l/ha	0.4 ml
7	-	HDC F68	0.2 l/ha	0.1 ml
8	-	HDC F75	0.6 l/ha	0.3 ml
9	-	HDC F71	4.0 l/ha	2.0 ml
10	-	HDC F80	1.6 kg/ha	0.8 g
11	-	HDC F69	0.375 l/ha	0.1875 ml
12	pyraclostrobin + boscalid	Signum	1.0 kg/ha	0.5 g
13	-	HDC F73	2.4 kg/ha	1.2 g
14	-	HDC F77	0.35 l/ha	0.175 ml

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Table 2. Details of products applied for the *Cylindrocarpon* bioassay.

Treatment number	Fungicide Active Ingredient	Product	Rate	Amount per litre water
1	Untreated uninoculated		-	
2	Untreated inoculated		-	
3	azoxystrobin + difenoconazole	Amistar Top	1.0 l/ha	0.5 ml
4	pyraclostrobin + boscalid	Signum	1.0 kg/ha	0.5 g
5	PRODUCT DID NOT ARRIVE IN TIME FOR STUDY - OMITTED			
6	tebuconazole + trifloxystrobin	Nativo 75WG	0.3 kg/ha	0.15 g
7	cyprodinil + fludioxonil	Switch	0.8 kg/ha	0.4 g
8	PRODUCT DID NOT ARRIVE IN TIME FOR STUDY - OMITTED			
9	iprodione + thiophanate-methyl	Compass	2.0 l/ha	1.0 ml
10	-	HDC F81	0.4 l/ha	0.2 ml
11	-	HDC F65	1.0 l/ha	0.5 ml
12	*Bacillus subtilis*	Serenade	10 l/ha	5 ml
13	*Gliocladium catenulatum*	Prestop	100g/20 l	5 g
14	-	HDC F79	1.5 g/m^2	7.5 g

Table 3. Details of products applied for the *Fusarium* bioassay.

Treatment Number	Fungicide Active Ingredient	Product	Rate	Amount per litre water
1	Untreated uninoculated			
2	Untreated inoculated			
3	azoxystrobin + difenoconazole	Amistar Top	1.0 l/ha	0.5 ml
4	pyraclostrobin + boscalid	Signum	1.0 kg/ha	0.5 g
5	PRODUCT DID NOT ARRIVE IN TIME FOR STUDY - OMITTED			
6	tebuconazole + trifloxystrobin	Nativo	0.3 kg/ha	0.15 g
7	cyprodinil + fludioxonil	Switch	0.8 kg/ha	0.4 g
8	fenpropimorph	Corbel	1.0 l/ha	0.5 ml
9	PRODUCT DID NOT ARRIVE IN TIME FOR STUDY - OMITTED			
10	-	HDC F65	1.0 l/ha	0.5 ml
11	-	HDC F81	0.4 l/ha	0.2 ml
12	PRODUCT DID NOT ARRIVE IN TIME FOR STUDY - OMITTED			
13	*Bacillus subtilis*	Serenade	10 l/ha	5 ml
14	*Gliocladium catenulatum*	Prestop	100g in 20l	5 g

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Germination and damping-off was recorded as the seedlings emerged. Approximately 4 months post-sowing roots were large enough for any blemishes that were present to be recorded. The pots were harvested and full disease assessments were carried out. Roots were then dried to provide dry weight data so that a comparison of potential pathogenicity effects could be recorded.

Crop Diary

27.9.11 *Fusarium* and *Cylindrocarpon* inoculated into vermiculite for bulking-up.

6.10.11 *Pythium* inoculated into vermiculite for bulking-up.

19.10.11 Pot bioassay for *Pythium* set up. First treatment applied.

27.10.11 *Fusarium* & *Cylindrocarpon* bioassays set up and first treatments applied

9.11.11 Seedling emergence recorded on *Pythium* bioassay.

14.11.11 2nd treatment application to *Pythium* bioassay.

17.11.11 Seedling emergence recorded on *Fusarium* & *Cylindrocarpon* tests.

22.11.11 Seedling emergence recorded on *Fusarium*, *Cylindrocarpon* and *Pythium* tests.

24.11.11 2nd treatment application to *Fusarium* & *Cylindrocarpon* bioassays.

30.11.11 Seedling emergence recorded on *Fusarium* and *Pythium* bioassays.

2.12.11 Seedling emergence recorded on *Cylindrocarpon* bioassay.

12.12.11 3rd treatment application to *Pythium* bioassay.

29.12.11 3rd treatment application to *Fusarium* & *Cylindrocarpon* bioassays.

22.3.12 *Pythium* bioassay harvested and roots assessed for blemishes.

27.3.12 Root dry weight recorded in *Pythium* bioassay.

28.3.12 *Fusarium* bioassay harvested and roots assessed for blemishes.

30.3.12 *Cylindrocarpon* bioassay harvested and roots assessed for blemishes.

11.4.12 Root dry weight recorded *in Cylindrocarpon* and *Fusarium* bioassays.

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Results

1. Variety x Pathogen experiment

Table 4. Treatments in pathogen x variety experiment at Boxworth 2011.

Trt No.	*Pathogen inoculum*	*% germination 21 September*			*% damping off 5 October*		
		Javelin	Palace	Mean	Javelin	Palace	Mean
1.	*Cylindrocarpon* shoulder 1%	41.33	32.80	36.00	2.67	10.40	7.50
2.	*Cylindrocarpon* shoulder 10%	57.60	23.00	42.22	8.00	0.00	4.44
3.	*Cylindrocarpon* crown 1%	35.00	13.33	25.71	0.00	8.00	3.43
4.	*Cylindrocarpon* crown 10%	60.00	27.00	43.50	1.00	4.00	2.50
5.	*Fusarium* T25 1%	51.20	29.33	43.00	3.20	5.33	4.00
6.	*Fusarium* T25 10%	35.00	35.00	35.00	13.00	0.00	6.50
7.	*Fusarium* T33 1%	42.00	33.60	37.33	4.00	4.00	4.00
8.	*Fusarium* T33 10%	52.00	25.33	40.57	0.00	4.00	1.71
9.	*Fusarium* T34 1%	45.00	17.00	31.00	4.00	1.00	2.50
10.	*Fusarium* T34 10%	53.00	26.00	39.50	2.00	4.00	3.00
11.	*Phoma* 1%	61.60	40.00	52.00	0.00	2.00	0.89
12.	*Phoma* 10%	58.67	34.67	46.67	2.67	0.00	1.33
13.	*Pythium* 1%	50.00	18.40	27.43	2.00	1.60	1.71
14.	*Pythium* 10%	59.43	10.00	48.44	2.29	6.00	3.11
15.	Uninoculated 1%	56.00	22.00	39.00	5.00	4.00	4.50
16.	Uninoculated 10%	32.00	29.71	30.22	0.00	1.14	0.89
	Mean	50.69	26.88	38.78	3.25	3.31	3.28

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Table 5. Effect of pathogen and inoculum level on germination on 21 September 2011.

	Mean % germination 21 Sept				
	Javelin		Palace		
	Inoculum level		Inoculum level		Mean
Pathogen	1	10	1	10	
Cylindrocarpon shoulder	48	12	26	13	24.8
Cylindrocarpon crown	52	47	24	19	35.5
Fusarium T25	71	76	38	26	52.8
Fusarium T33	56	23	30	19	32.0
Fusarium T34	53	40	19	4	29.0
Phoma	59	58	36	32	46.3
Pythium	49	44	39	8	35.0
Uninoculated	70	53	50	47	55.0
Mean	**57.3**	**44.1**	**32.8**	**21.0**	**38.8**
Mean by Variety	**50.7**		**28.88**		

Factor	Fpr	Sed	LSD
Variety	<0.001	2.37	4.71
Pathogen	<0.001	4.75	9.43
Inoculum level Interaction	<0.001	2.37	4.71
Variety x Pathogen	0.016	6.71	13.33

Germination was assessed on 21, 26 and 30 September and on 5 October: There were significant differences in the percentage germination in relation to variety, pathogen and inoculum level plus an interaction for variety x pathogen (Tables 4 and 5). On 21 September there was more germination in cv. Javelin than in cv. Palace. *Fusarium* T34 adversely affected germination of cv. Palace at both inoculum levels. Germination was low on cv. Palace where high inoculum rates of *Pythium* were used and on cv. Javelin with the higher rate of *Cylindrocarpon* (black shoulder) and *Fusarium* T33 (Table 5).

Table 6. Statistical summary of germination data (pathogen data included in Figure 2).

Factor	Fpr	Sed	LSD
Variety	<0.001	2.783	5.526
Pathogen	<0.001	5.566	11.053
Inoculum level	<0.001	2.783	5.526
Variety x Pathogen	0.043	7.871	15.631
Pathogen x inoculum level	<0.001	7.871	15.631
Time interactions			
Time x pathogen	<0.001	6.193	13.973
Time x inoculum level	<0.001	3.096	6.986
Time x pathogen x inoculum level	0.001	8.758	19.760

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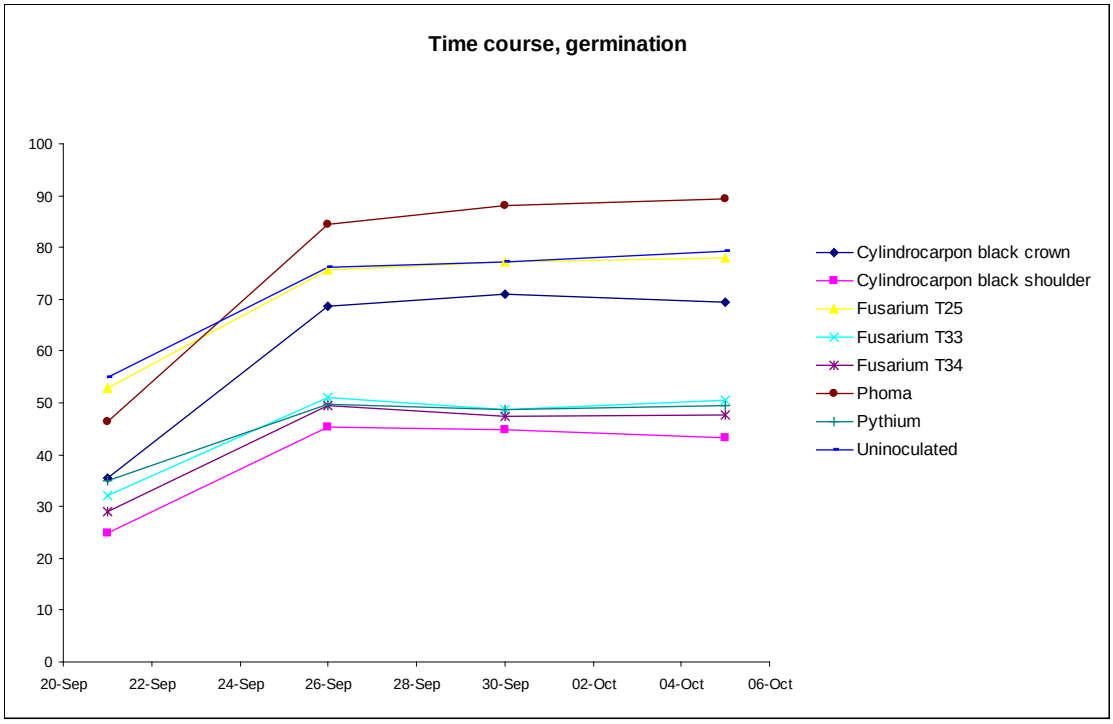

Figure 2. Germination (%) over time in relation to pathogen.

When time of assessment is taken into account over repeated sampling periods the individual factors of variety, pathogen and inoculum level were again significant at the <0.001 level, but the interaction effects changed so that variety x pathogen were no longer significant. *Cylindrocarpon* black shoulder, *Fusarium* T33 and T34 and *Pythium* isolates showed the lowest percentage germination (Figure 2). Germination increased sharply between 21 and 26 September and there was little new germination thereafter.

Table 7. Effect of pathogen and inoculum level on the % damping off on 5 October.

Pathogen	**Mean % damping off 5 October**				**Mean**
	Javelin		**Palace**		
	Inoculum level		**Inoculum level**		
	1	**10**	**1**	**10**	
Cylindrocarpon shoulder	2	13	4	10	7.3
Cylindrocarpon crown	1	2	0	3	1.5
Fusarium T25	0	0	1	0	0.3
Fusarium T33	5	2	5	3	3.8
Fusarium T34	6	5	1	6	4.5
Phoma	0	0	2	1	0.8
Pythium	2	10	2	7	5.3
Uninoculated	1	3	2	6	3.0
Mean	**2.1**	**4.4**	**2.1**	**4.5**	**3.3**
Mean by Variety	**3.3**		**3.3**		

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Factor	Fpr	Sed	LED
Variety	0.963	1.334	2.649
Pathogen	0.141	2.668	5.297
Inoculum level	0.086	1.334	2.649
Variety x Pathogen	0.996	3.773	7.492

Small numbers of seedlings wilted or rotted off soon after emergence. This was assessed as damping-off. Although there was no significant effects of pathogen, variety or inoculum level on the percentage of damping-off on 5 October (Table 7), there was a significant difference in the % plants suffering damping-off between sampling dates (P <.001) (Figure 3).

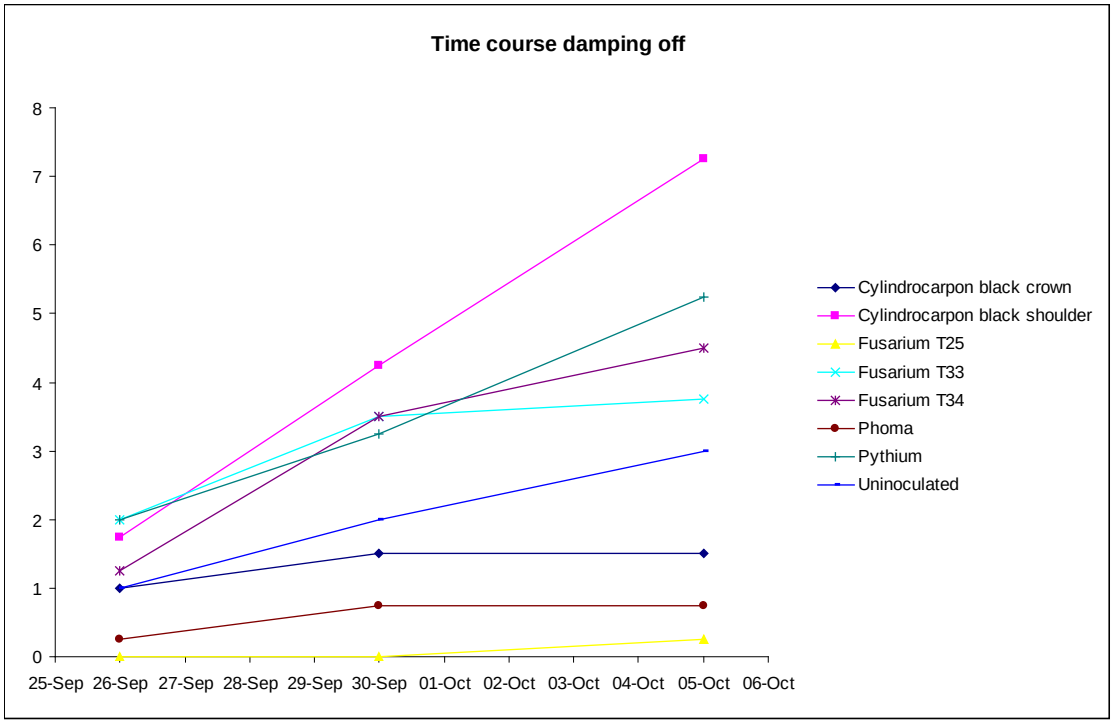

Figure 3. Changes in the Incidence of damping off over time.

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Table 8. Effect of pathogen and inoculum level on seedling vigour on 30 September.

Seedling vigour score	**Variety**				
	Javelin		**Palace**		
	Inoculum level		**Inoculum level**		
Pathogen	**1**	**10**	**1**	**10**	**Mean**
Cylindrocarpon shoulder	4.50	3.75	3.50	3.75	2.75
Cylindrocarpon crown	3.75	1.50	3.75	2.00	3.63
Fusarium T25	3.75	3.50	4.00	2.75	3.88
Fusarium T33	3.75	3.25	3.00	3.00	3.50
Fusarium T34	4.25	4.25	4.00	3.75	3.25
Phoma	3.75	3.50	4.00	2.50	4.06
Pythium	4.75	4.00	4.00	3.75	3.44
Uninoculated	4.00	4.00	3.75	2.75	4.13
Mean	**4.06**	**3.47**	**3.75**	**3.03**	**3.58**
Mean by variety	**3.77**		**3.39**		

Factor	Fpr	Sed	LSD
Variety	0.028	0.1682	0.3341
Pathogen	0.002	0.3364	0.6681
Inoculum level	<0.001	0.1682	0.3341
Variety x Pathogen	0.915	0.4758	0.9449

There were significant effects of variety, pathogen and inoculum level in seedling vigour on 30 September (Table 8). Javelin had greater vigour than Palace and the higher inoculum rate gave lower vigour than the lower rate. *Cylindrocarpon* black crown gave low vigour on both varieties at the higher inoculum rate but there was no variety x inoculum rate interaction.

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Table 9. Incidence of fanging in relation to pathogen inoculum.

	Mean % fang incidence						
	Javelin			Palace			
	Inoculum level			Inoculum level			Grand
pathogen name	1	10	Total	1	10	Total	Total
Cylindrocarpon shoulder	2.14	1.00	1.76	1.00	55.17	19.06	10.41
Cylindrocarpon crown	10.66	10.62	10.64	3.86	18.25	10.03	10.35
Fusarium T25	1.00	4.54	2.77	1.00	4.65	2.82	2.80
Fusarium T33	1.00	21.00	11.00	10.89	17.67	13.79	12.30
Fusarium T34	3.38	14.31	8.85	6.00	9.33	7.67	8.26
Phoma	6.16	7.68	6.92	1.00	3.50	2.25	4.58
Pythium	6.56	18.48	12.52	7.56	81.00	39.04	24.89
Uninoculated	1.00	6.30	3.65	1.00	14.88	7.94	5.80
Grand Total	**4.0**	**11.1**	**7.4**	**4.0**	**21.9**	**12.2**	**9.8**

Factor	Fpr	Sed	LSD
Variety	0.401	0.1772	0.3522
Pathogen	0.019	0.3543	0.7044
Inoculum level	0.002	0.1772	0.3522
Variety x pathogen x inoculum level	0.064	0.7087	1.4088

Back transformed data

	% incidence of fanging						
	Javelin			Palace			Mean
	Inoculum level			Inoculum level			across
Pathogen	1	10	Mean	1	10	Mean	variety
Cylindrocarpon shoulder	0.54	0.06	0.27	0.00	9.91	2.39	1.08
Cylindrocarpon crown	5.72	1.79	3.35	1.61	13.93	5.42	4.32
Fusarium T25	0.00	1.79	0.68	0.00	1.93	0.71	0.70
Fusarium T33	0.00	11.03	2.59	5.66	3.93	4.73	3.52
Fusarium T34	1.44	12.12	4.79	1.23	1.63	1.44	2.77
Phoma	2.46	4.37	3.31	0.00	0.85	0.37	1.44
Pythium	1.30	16.36	5.60	2.66	43.03	13.80	8.98
Uninoculated	0.00	2.26	0.82	0.00	8.62	2.17	1.41
Mean across pathogen	**0.95**	**4.12**	**2.17**	**0.87**	**6.31**	**2.73**	

Factor	Fpr	Sed	LSD
Variety	0.471	0.227	0.452
Pathogen	0.002	0.455	0.905
Inoculum level	<.001	0.227	0.452
Variety x pathogen x inoculum level	0.026	0.910	1.810

The data for fanging was analysed using a logit transformation on 1+ % fanging. The statistical output relates to the transformed data so sed and lsd values cannot be applied to the results in Table 9 above. There were significant differences in fanging between pathogens and inoculum level and a weaker variety x pathogen x inoculum interaction.

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Fanging was most evident with the *Pythium* isolate and was severe on Palace where the 10% inoculation rate was used. Both *Cylindrocarpon* isolates and *Fusarium* T33 also had higher levels of fanging than the controls. There were no varietal differences though Palace showed almost one and a half the incidence of fanging of Javelin at the 10% inoculation rate (Table 9).

Table 10. Severity of fanging in relation to pathogen inoculum.

Raw data

	Average of fang severity						
	Javelin			Palace			
pathogen name	Inoculum level			Inoculum level			Grand Total
	1	10	Total	1	10	Total	
Cylindrocarpon shoulder	16.25	0.00	10.83	0.00	35.00	11.67	11.25
Cylindrocarpon crown	12.50	16.25	14.38	7.50	20.00	12.86	13.67
Fusarium T25	0.00	15.00	7.50	0.00	22.50	11.25	9.38
Fusarium T33	0.00	21.25	10.63	17.50	16.67	17.14	13.67
Fusarium T34	12.50	55.00	33.75	8.75	2.50	5.63	19.69
Phoma	7.50	13.75	10.63	0.00	18.75	9.38	10.00
Pythium	5.00	35.00	20.00	5.00	61.67	29.29	24.33
Uninoculated	0.00	13.75	6.88	0.00	31.25	15.63	11.25
Mean	**6.7**	**22.7**	**14.4**	**4.8**	**24.6**	**13.9**	**14.2**

Factor	Fpr	Sed	LSD
Variety	0.677	3.28	6.52
Pathogen	0.166	6.56	13.04
Inoculum level	<0.001	3.28	6.52
Variety x pathogen x inoculum level	0.019	13.12	26.08

Back transformed data

Severity of fanging	variety level						
	Javelin			Palace			Mean
	Inoculum level			Inoculum level			across
Pathogen	1	10	Mean	1	10	Mean	variety
Cylindrocarpon shoulder	2.63	0.18	1.06	0.00	33.75	5.85	2.81
Cylindrocarpon crown	7.71	2.63	4.68	3.11	16.80	7.79	6.04
Fusarium T25	0.00	4.68	1.41	0.00	7.32	1.93	1.66
Fusarium T33	0.00	11.79	2.70	9.16	3.74	5.98	4.07
Fusarium T34	4.63	54.97	20.59	1.69	0.85	1.23	6.38
Phoma	3.11	8.71	5.36	0.00	3.07	1.02	2.63
Pythium	1.23	33.98	8.98	2.42	61.48	18.62	13.06
Uninoculated	0.00	3.83	1.21	0.00	18.15	3.65	2.23
Mean across pathogen	1.53	8.53	3.97	1.12	11.46	4.27	

Factor	Fpr	Sed	LSD
Variety	0.849	0.301	0.599
Pathogen	0.077	0.602	1.197
Inoculum level	<0.001	0.301	0.599
Variety x pathogen	0.044	0.852	1.693
Variety x pathogen x inoculum level	0.010	0.426	2.395

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The severity of fanging was analysed using a logit transformation and LSD value are not directly applicable to the data in Table 10. The inoculum level was the most important factor with a four-fold increase in severity at the higher rate of inoculum. There was a variety x pathogen interaction, with more severe fanging on Javelin than Palace with *Fusarium* T34. Pathogen differences approached significance (P = 0.077).

Table 11. Incidence of red spotting symptoms in relation to pathogen inoculum.

Average of % red spot incidence	variety level						
	Javelin			Palace			Grand
pathogen name	1	10	Total	1	10	Total	Total
Cylindrocarpon shoulder	17.61	1.00	12.07	39.46	63.50	47.47	29.77
Cylindrocarpon crown	19.94	31.25	25.59	26.54	50.67	36.88	30.86
Fusarium T25	9.96	8.59	9.27	12.89	9.97	11.43	10.35
Fusarium T33	13.27	1.00	7.13	4.41	6.88	5.47	6.36
Fusarium T34	12.19	19.59	15.89	3.50	53.08	28.29	22.09
Phoma	21.85	51.50	36.67	16.04	51.92	33.98	35.33
Pythium	5.66	23.71	14.68	2.67	1.00	1.95	8.74
Uninoculated	7.03	8.39	7.71	3.72	8.94	6.33	7.02
Mean	**13.4**	**19.3**	**16.3**	**13.7**	**29.6**	**20.9**	**18.5**

Factor	Fpr	Sed	LSD
Variety	0.694	0.542	1.077
Pathogen	<0.001	1.084	2.154
Inoculum level	0.295	0.542	1.077
Pathogen x inoculum level	<0.001	1.533	3.047
Variety x inoculum level	0.639	0.766	1.523

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Table 12. Severity of red spot symptoms in relation to pathogen inoculum.

Average of red spot/fleck Severity	variety and inoculum level						
	Javelin			Palace			Grand
pathogen name	1	10	Total	1	10	Total	Total
Cylindrocarpon shoulder	3.25	0.00	2.17	2.50	1.50	2.17	2.17
Cylindrocarpon crown	2.50	2.75	2.63	6.00	9.00	7.29	4.80
Fusarium T25	2.00	1.50	1.75	1.38	0.88	1.13	1.44
Fusarium T33	1.75	0.00	0.88	0.25	0.67	0.43	0.67
Fusarium T34	1.63	3.25	2.44	0.25	10.63	5.44	3.94
Phoma	3.25	8.00	5.63	1.75	6.50	4.13	4.88
Pythium	0.50	3.25	1.88	0.50	0.00	0.29	1.13
Uninoculated	0.88	0.50	0.69	0.50	3.75	2.13	1.41
Mean	**2.0**	**2.6**	**2.3**	**1.6**	**4.4**	**2.9**	**2.6**

Factor	Fpr	Sed	LSD
Variety	0.366	0.788	1.567
Pathogen	0.016	1.577	3.134
Inoculum level	0.082	0.788	1.567
Pathogen x inoculum level	0.114	2.230	4.432
Variety x inoculum level	0.189	1.115	2.216

Back transformed data

% red spot severity	variety and inoculum level						
	Javelin			Palace			
	Inoculum level			Inoculum level			Mean of
Pathogen	1	10	Mean	1	10	Mean	variety
Cylindrocarpon shoulder	2.53	-0.14	0.75	1.48	1.10	1.28	1.00
Cylindrocarpon crown	2.14	2.27	2.20	2.74	6.39	4.27	3.12
Fusarium T25	1.79	0.98	1.35	1.28	0.73	0.99	1.16
Fusarium T33	1.22	0.00	0.49	0.19	0.32	0.25	0.37
Fusarium T34	0.82	3.12	1.74	0.19	3.09	1.22	1.47
Phoma	2.74	5.58	3.97	1.39	5.73	3.03	3.48
Pythium	0.42	2.10	1.10	0.32	0.22	0.27	0.63
Uninoculated	0.73	0.42	0.57	0.42	1.91	1.03	0.78
Mean across pathogen	1.42	1.26	1.34	0.85	1.79	1.27	

Factor	Fpr	Sed	LSD
Variety	0.798	0.1192	0.2370
Pathogen	<0.001	0.2385	0.4740
Inoculum level	0.140	0.1192	0.2370
Pathogen x inoculum level	0.002	0.3372	0.6704
Variety x inoculum level	0.044	0.1686	0.3352

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The logit transformation showed there were significant differences between the pathogen species and individual isolates on the severity of red spotting. The *Phoma* isolate and *Cylindrocarpon* black crown gave the most severe symptoms. Inoculum level effects were significant in the interactions with variety and pathogen but not as a single factor. The 10% inoculum level gave more severe symptoms overall than low inoculum on cv. Palace. Fusarium T34 and *Phoma* both gave more severe spotting at the higher inoculum level on both varieties.

Table 13. Incidence of red lesions (early canker) in relation to variety x pathogen x inoculum.

	Average of % red lesion incidence						
		Javelin			Palace		Grand
Pathogen	1	10	Total	1	10	Total	Total
Cylindrocarpon shoulder	4.41	1.00	3.27	1.00	51.00	17.67	10.47
Cylindrocarpon crown	17.19	10.25	13.72	2.19	1.00	1.68	8.10
Fusarium T25	7.78	8.14	7.96	3.63	5.17	4.40	6.18
Fusarium T33	2.47	13.50	7.99	4.13	1.00	2.79	5.56
Fusarium T34	6.95	2.79	4.87	1.00	9.33	5.17	5.02
Phoma	6.41	2.14	4.27	1.00	1.00	1.00	2.64
Pythium	2.39	7.34	4.86	2.56	1.00	1.89	3.48
Uninoculated	1.00	1.00	1.00	2.47	3.50	2.99	1.99
Mean	**6.1**	**6.1**	**6.1**	**2.2**	**6.9**	**4.4**	**5.3**

	Fpr	Sed	LSD
Variety	0.017	0.213	0.283
Pathogen	0.339	0.425	0.565
Inoculum level	0.282	0.213	0.283
No significant interaction effects			

There was a higher incidence of red lesions on the cv. Palace than on cv. Javelin. This reflected a very high incidence of symptoms (51% of roots affected) with *Cylindrocarpon* black shoulder at the 10% inoculum level on cv. Palace. Palace appeared to have fewer red lesion symptoms than Javelin at the 1% inoculum level, however differences were not significant.

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Table 14. Severity of red lesions in relation to variety, pathogen and inoculum level.

	Mean red lesion/early canker severity						
	Javelin			Palace			Grand
Pathogen	1	10	Total	1	10	Total	Total
Cylindrocarpon shoulder	2.50	0.00	1.67	0.00	0.50	0.17	0.92
Cylindrocarpon crown	7.50	6.25	6.88	0.25	0.00	0.14	3.73
Fusarium T25	5.50	1.75	3.63	1.00	1.25	1.13	2.38
Fusarium T33	0.25	0.25	0.25	3.75	0.00	2.14	1.13
Fusarium T34	4.00	1.25	2.63	0.00	2.50	1.25	1.94
Phoma	5.50	0.25	2.88	0.00	0.00	0.00	1.53
Pythium	2.50	17.50	10.00	0.50	0.00	0.29	5.47
Uninoculated	0.00	0.00	0.00	1.25	2.50	1.88	0.94
Mean	**3.5**	**3.6**	**3.5**	**0.8**	**1.0**	**0.9**	**2.3**

	Fpr	Sed	LSD
Variety	0.021	1.119	2.225
Pathogen	0.397	2.238	4.449
Inoculum level	0.904	1.119	2.225
No significant interaction effects			

Larger red lesions (termed early cankers) were recorded at low levels and were more severe on cv. Javelin than on cv. Palace. The uninoculated compost gave some symptoms on cv. Palace but none on cv. Javelin. There were no significant differences between pathogens or rates of inoculum.

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Table 15. Incidence of canker in relation to variety, pathogen and inoculum level.

	Mean % canker						
	Javelin			Palace			Grand
Pathogen	1	10	Total	1	10	Total	Total
Cylindrocarpon black shoulder	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cylindrocarpon black crown	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Fusarium T25	4.46	2.19	3.33	3.63	1.00	2.32	2.82
Fusarium T33	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Fusarium T34	1.00	1.00	1.00	7.25	9.33	8.29	4.65
Phoma	2.14	1.00	1.57	1.00	1.00	1.00	1.28
Pythium	2.39	2.79	2.59	2.56	1.00	1.89	2.26
Uninoculated	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Grand mean	**1.7**	**1.4**	**1.6**	**2.3**	**2.2**	**2.3**	**1.9**

Factor	Fpr	Sed	LSD
Variety	0.576	0.0602	0.1197
Pathogen	0.015	0.1203	0.2393
Inoculum level	0.098	0.0602	0.1197
No significant interaction effects			

There was a higher incidence of canker lesions with *Fusarium* T34, *Fusarium* T25 *Pythium* and *Phoma* than was observed in the *Cylindrocarpon* inoculated pots, although overall incidence was low. *Fusarium* T34 gave the highest incidence of lesions on cv. Palace (7-9% roots affected). No difference between were observed between the inoculum rate treatments.

Table 16. Canker severity in relation to variety, pathogen and inoculum level.

	Mean canker severity						
	Javelin			Palace			Grand
pathogen name	1	10	Total	1	10	Total	Total
Cylindrocarpon black shoulder	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cylindrocarpon black crown	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fusarium T25	12.00	3.75	7.88	6.25	0.00	3.13	5.50
Fusarium T33	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fusarium T34	0.00	0.00	0.00	22.50	5.00	13.75	6.88
Phoma	1.25	0.00	0.63	0.00	0.00	0.00	0.33
Pythium	0.50	0.50	0.50	2.50	0.00	1.43	0.93
Uninoculated	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	**1.7**	**0.6**	**1.2**	**4.0**	**0.8**	**2.5**	**1.8**

Factor	Fpr	Sed	LSD
Variety	0.514	1.699	3.378
Pathogen	0.190	3.398	6.756
Inoculum level	0.167	1.699	3.378
No significant interaction effects			

There were no significant treatment effects though pathogen differences approached significance (P=0.060) with logit transformed data.

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2. Biopesticides

Table 17. Effect of biopesticides and different pathogens on root blemish symptoms

Factor	Treatment	% roots with fang	Fang % severity	% roots with red fleck	Red fleck % severity	% roots with early canker	Early canker % severity
Biopesticide	Untreated	1.9	0.71	36.3	0.27	10.0	11.2
	HDC F79	1.5	0.27	32.8	0.24	11.9	18.1
	Prestop	8.4	3.46	23.1	0.23	11.8	26.8
	HDC F82	2.8	0.96	32.2	0.27	9.9	13.8
	HDC F83	1.7	0.83	23.3	0.18	11.0	16.1
	HDC F78	1.8	0.53	32.7	0.24	13.1	16.7
	Serenade	2.1	0.52	32.2	0.22	12.7	14.3
Pathogen	Uninoculated	3.2	1.46	33.9	0.24	9.8	15.9
	Fusarium	2.0	0.99	30.5	0.21	14.4	17.7
	Cylindrocarpon	2.9	1.14	30.5	0.25	12.1	21.2
	Pythium	1.6	1.03	28.2	0.29	11.6	14.8
	Phoma	2.6	0.57	31.7	0.19	9.6	13.9
Biopesticide	LSD	***	1.173***	-	0.136	-	0.129
Pathogen	LSD	-	0.991	-	0.115	-	0.109

There were significant increases in fanging in the Prestop treatment, but no effect on other root symptoms.

There were low levels of blemishes in the uninoculated control treatments and few treatments showed significant effects. The methodology differed from the pathogenicity experiments as the inoculum was placed in the lower part of the pots and the biopesticide was in the upper part of the pots to provide protection against spread of pathogens to young parsnip roots.

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Table 18. Interactions of biopesticides and different pathogens on root blemish symptoms.

Factor	Treatment	% roots with fanging	Fang % severity	% roots with red fleck	Red fleck % severity	% roots with early canker	Early canker % severity
Untreated	Uninoculated	2.0	0.32	30.6	0.31	8.4	6.0
	Fusarium	1.0	0.54	41.7	0.23	14.5	15.8
	Cylindrocarpon	3.1	1.49	29.2	0.28	10.7	10.4
	Pythium	0.0	0.00	25.7	0.25	5.4	11.2
	Phoma	3.0	1.19	53.1	0.29	10.4	12.7
HDC F79	Uninoculated	1.1	0.12	48.7	0.24	9.9	23.8
	Fusarium	1.1	0.26	28.0	0.24	13.4	14.7
	Cylindrocarpon	2.3	0.55	36.5	0.30	16.5	23.6
	Pythium	0.0	0.00	33.6	0.26	10.7	17.4
	Phoma	3.0	0.43	16.0	0.17	9.0	11.2
Prestop	Uninoculated	1.7	6.67	22.1	0.12	6.0	31.0
	Fusarium	5.7	2.81	30.9	0.28	8.9	21.0
	Cylindrocarpon	7.6	3.55	15.6	0.21	10.7	37.4
	Pythium	7.8	3.88	32.9	0.45	12.6	7.6
	Phoma	3.3	0.42	15.9	0.08	20.9	36.9
HDC F82	Uninoculated	2.1	0.76	25.6	0.38	8.8	8.3
	Fusarium	1.5	0.79	32.8	0.18	11.3	21.8
	Cylindrocarpon	4.1	1.19	29.8	0.09	9.3	16.3
	Pythium	4.6	1.86	32.8	0.44	7.0	11.8
	Phoma	1.0	0.21	40.3	0.23	12.6	10.6
HDC F83	Uninoculated	0.0	0.00	26.3	0.11	9.5	11.7
	Fusarium	4.5	2.17	21.9	0.19	20.2	25.1
	Cylindrocarpon	2.1	0.31	30.4	0.27	11.5	24.5
	Pythium	2.3	1.51	17.5	0.25	12.0	17.2
	Phoma	0.0	0.14	19.2	0.08	2.2	2.3
HDC F78	Uninoculated	4.2	1.58	45.3	0.21	12.0	13.0
	Fusarium	0.0	0.00	25.8	0.13	14.9	12.8
	Cylindrocarpon	2.2	0.12	30.8	0.40	15.3	22.5
	Pythium	0.0	0.00	22.8	0.12	16.3	26.9
	Phoma	0.0	0.93	36.1	0.35	7.4	8.2
Serenade	Uninoculated	2.2	0.75	30.8	0.31	12.4	17.7
	Fusarium	1.2	0.38	31.1	0.22	15.3	12.5
	Cylindrocarpon	1.1	0.79	33.7	0.18	9.7	14.1
	Pythium	0.0	0.00	33.8	0.26	17.6	11.8
	Phoma	5.6	0.67	31.8	0.11	9.3	15.1
LSD Biopesticide(97 df)		***	1.173***	-	0.136	-	0.129
LSD Pathogen (97df)		-	0.991	-	0.115	-	0.109
LSD Biopesticide x Pathogen (97df)		-	2.622	-	0.304	-	0.289

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3. Fungicides

Results

There was a significant reduction in germination at the earliest assessment following application of Amistar; however germination was high in all treatments by 5 October. Damping-off was evident at low levels with no treatment differences (Table 19).

Table 19. Effect of fungicides and different pathogens on germination and damping off.

Factor	Treatment	% germination 30 Sept	% germination 5 Oct	% damping-off 5 Oct
Fungicide	Untreated	79	93	0.9
	SL567a	78	93	0.5
	Topsin M 70Wp	73	93	0.7
	Signum	94	93	0.0
	HDC F67	66	90	0.4
	Rudis	65	95	0.0
	HDC F72	65	92	0.5
	Amistar	51	90	0.4
Pathogen	Uninoculated	82	95	0.4
	Fusarium	69	90	0.5
	Cylindrocarpon	67	95	0.4
	Pythium	69	93	0.8
	Phoma	71	89	0.1
Fungicide	LSD	21.7	NS	NS
Pathogen	LSD	NS	NS	NS

There was a low incidence of fanged roots, red spotting and larger blotches classified as 'early cankers' in all treatments including the uninoculated controls (Table 20). There were no significant effects of fungicide or differences between pathogens. There were trends suggesting that *Fusarium* spp. increased early canker symptoms and that levels were higher in the SL567A treatment. The fungicide x pathogen interactions were not significant though there were indications that some fungicides had selective activity. HDC F72 appeared to control early cankers in the *Pythium* treatment (Table 21).

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Table 20. Effect of fungicides and different pathogens on root blemish symptoms.

Factor	Treatment	% roots with fang	Fang % severity	% roots with red fleck	Red fleck % severity	% roots with early canker	Early canker % severity
Fungicide	Untreated	1.9	0.64	3.1	0.18	3.7	0.09
	SL567A	1.3	0.51	3.3	0.22	6.8	0.22
	Topsin M 70WP	2.4	1.04	3.7	0.15	4.2	0.13
	Signum	1.6	0.62	4.2	0.17	2.4	0.13
	HDC F67	1.8	0.43	3.9	0.25	5.4	0.13
	Rudis	1.3	0.36	3.0	0.17	4.9	0.15
	HDC F72	1.3	0.19	3.1	0.17	4.0	0.15
	Amistar	2.0	0.33	4.2	0.28	4.2	0.16
Pathogen	Uninoculated	2.1	0.33	3.3	0.14	4.5	0.13
	Fusarium	1.3	0.37	3.6	0.21	5.2	0.15
	Cylindrocarpon	1.5	0.52	3.6	0.25	4.2	0.12
	Pythium	1.5	0.68	3.7	0.17	4.4	0.15
	Phoma	2.0	0.68	3.6	0.22	3.9	0.17
Biopesticide	LSD	-	0.645	-	0.132	-	0.126
Pathogen	LSD	-	0.510	-	0.104	-	0.100

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Table 21. Interaction of fungicides and different pathogens on root blemish symptoms.

Factor	Treatment	% roots with fang	Fang % severity	% roots with red fleck	Red fleck % severity	% roots with early canker	Early canker % severity
Untreated	Uninoculated	0.0	0.00	3.5	0.06	1.0	0.04
	Fusarium	2.8	0.49	1.7	0.17	6.7	0.13
	Cylindrocarpon	2.2	1.24	4.3	0.29	4.4	0.16
	Pythium	2.3	1.03	3.6	0.19	3.6	0.06
	Phoma	2.1	0.43	2.2	0.19	3.0	0.08
SL567A	Uninoculated	2.3	0.38	2.0	0.13	4.6	0.12
	Fusarium	0.0	0.00	2.4	0.25	1.0	0.19
	Cylindrocarpon	1.1	0.68	3.8	0.27	3.2	0.22
	Pythium	1.1	0.48	4.5	0.25	7.6	0.41
	Phoma	2.2	0.99	3.6	0.19	8.7	0.16
Topsin M	Uninoculated	2.1	0.22	4.9	0.19	2.1	0.12
	Fusarium	3.2	1.44	3.4	0.12	1.8	0.18
	Cylindrocarpon	2.2	0.80	3.0	0.16	1.1	0.01
	Pythium	3.6	1.85	2.7	0.14	7.9	0.19
	Phoma	1.2	0.89	4.6	0.17	3.5	0.15
Signum	Uninoculated	0.0	0.00	3.3	0.12	2.2	0.22
	Fusarium	2.3	0.61	5.3	0.23	1.2	0.06
	Cylindrocarpon	2.2	0.57	3.9	0.14	4.3	0.05
	Pythium	2.3	1.39	4.6	0.25	1.1	0.03
	Phoma	1.0	0.54	4.0	0.09	3.1	0.27
HDC F67	Uninoculated	3.6	0.78	3.4	0.14	9.3	0.13
	Fusarium	0.0	0.00	3.7	0.15	4.4	0.10
	Cylindrocarpon	0.0	0.00	3.6	0.37	4.5	0.13
	Pythium	2.1	0.50	4.1	0.16	6.4	0.18
	Phoma	3.2	0.85	4.5	0.40	2.1	0.12
Rudis	Uninoculated	3.2	0.39	2.0	0.12	5.4	0.11
	Fusarium	0.0	0.00	3.1	0.13	4.1	0.09
	Cylindrocarpon	1.1	0.00	3.8	0.30	4.2	0.10
	Pythium	2.1	0.00	3.1	0.12	5.5	0.21
	Phoma	2.1	0.98	3.1	0.18	5.2	0.25
HDC F72	Uninoculated	1.0	0.12	3.1	0.17	6.2	0.12
	Fusarium	1.1	0.23	4.0	0.11	6.3	0.31
	Cylindrocarpon	1.1	0.11	2.9	0.22	3.4	0.12
	Pythium	0.0	0.00	2.6	0.06	0.0	0.00
	Phoma	2.1	0.50	3.0	0.30	4.2	0.19
Amistar	Uninoculated	4.3	0.74	4.2	0.17	5.3	0.21
	Fusarium	1.2	0.13	5.1	0.54	2.2	0.17
	Cylindrocarpon	2.1	0.33	3.2	0.22	8.3	0.18
	Pythium	1.1	0.21	4.6	0.24	3.3	0.14
	Phoma	1.4	0.23	3.8	0.24	1.6	0.10
LSD Fungicide(117 df)		-	0.645	-	0.132	-	-
LSD Pathogen (117df)		-	0.510	-	0.104	-	-
LSD Fungicide x Pathogen(117df)		-	1.442	-	0.295	-	1.0

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4. Agar Plate Tests (STC)

The mean percentage inhibition in growth observed in the fungicide or bio-pesticide amended agar plates compared to the growth of the same fungus on un-amended agar are shown in the figures below, *Cylindrocarpon* – Figs. 3-6 , *Fusarium* – Figs. 7-9, *Pythium* – Figs. 10-12.

In the tests with *Cylindrocarpon* we observed >50% inhibition in radial growth of the fungus with many of the products tested at 2ppm. Those that fell below this level of inhibition were Cercobin, Unix, HDC F65, Filan, Rovral and HDC F81. At 20ppm all of the products used with the exception of Cercobin and Unix. HDC F81, Folicur and Beret Gold resulted in 100% inhibition of growth at this concentration. At the highest concentration used (100ppm for the fungicides) all products except Unix performed relatively well, with products such as Plover, Vivid, Cercobin, Rovral, Beret Gold, HDC F81 and Folicur inhibiting growth by between 80 - 100%.

The products under test against *Fusarium* were less effective at the lowest concentration of 2ppm. Only 5 products achieved >50% at this concentration; Vivid, Prestop, HDC F79, Serenade and Folicur, however, the fact that the three biopesticides are among these is very encouraging. When used at 20ppm Amistar and HDC F81 joined the group of products achieving >50% inhibition, with Folicur providing 100% inhibition in growth of the fungus. Interestingly, the increase in concentration to 100ppm had little benefit in terms of inhibition. Both HDC F81 and Folicur resulted in 100% inhibition of growth when incorporated into the agar at a concentration of 100ppm.

Overall the range of products tested for efficacy of controlling radial growth of *Pythium* were relatively poor. At 2ppm only the oomycete targeting fungicide SL567A (metalaxyl-M) was effective. Amistar, Vivid (both strobilurins) and SL567A resulted in >50% inhibition at both the 20ppm and 100ppm rates of use, with both Vivid and SL567A resulting in 100% inhibition at 100ppm. Several of the other products used for this *in vitro* screen were chosen for their reported activity against Oomycetes, however it should be remembered that their mode of action may be aimed at other parts of the fungus' lifecyle e.g. sporulation, and therefore they would be unlikely to show a reduction in the production of mycelium of the fungus in this type of test but may still have good activity in *in vivo* tests.

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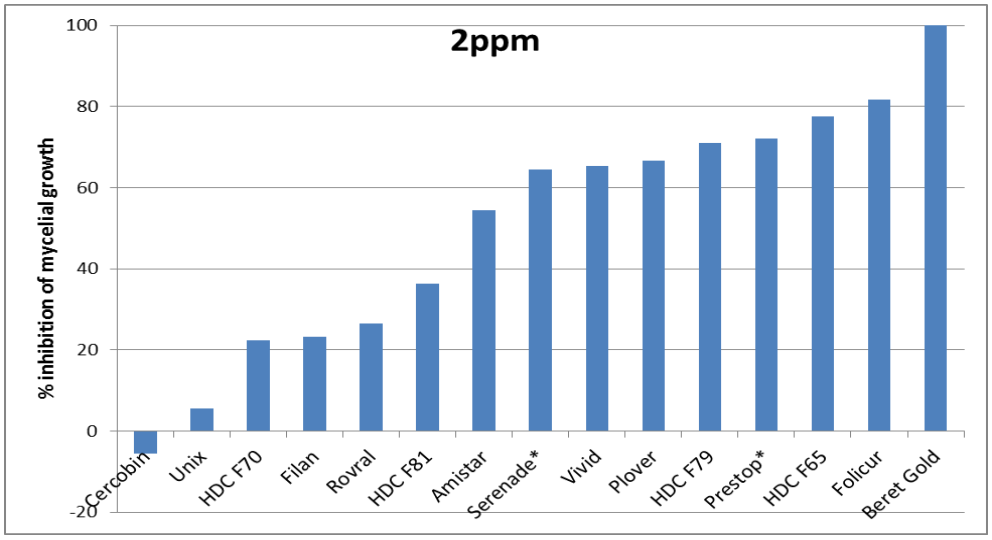
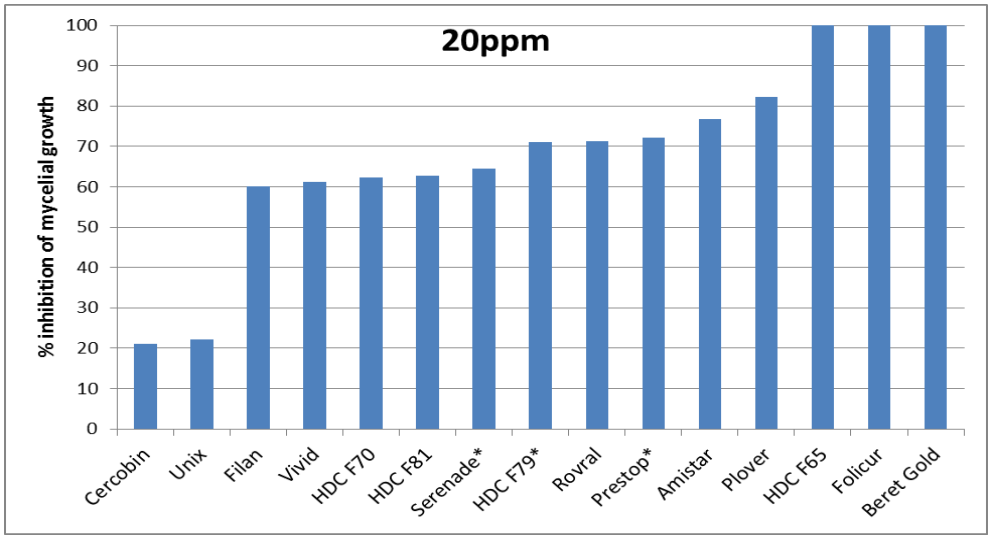
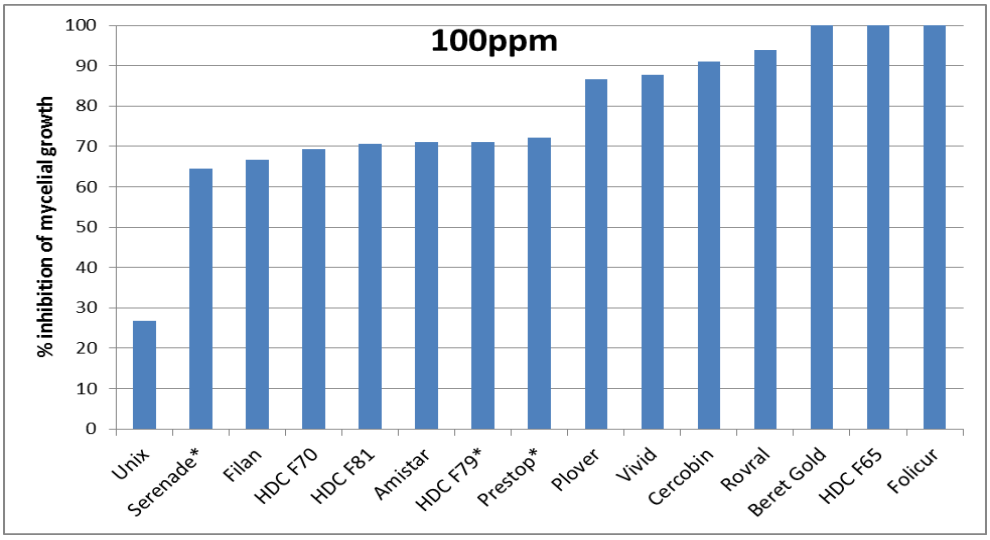
Figs 4-6. The mean % inhibition in radial growth of *Cylindrocarpon* with products used at three concentrations







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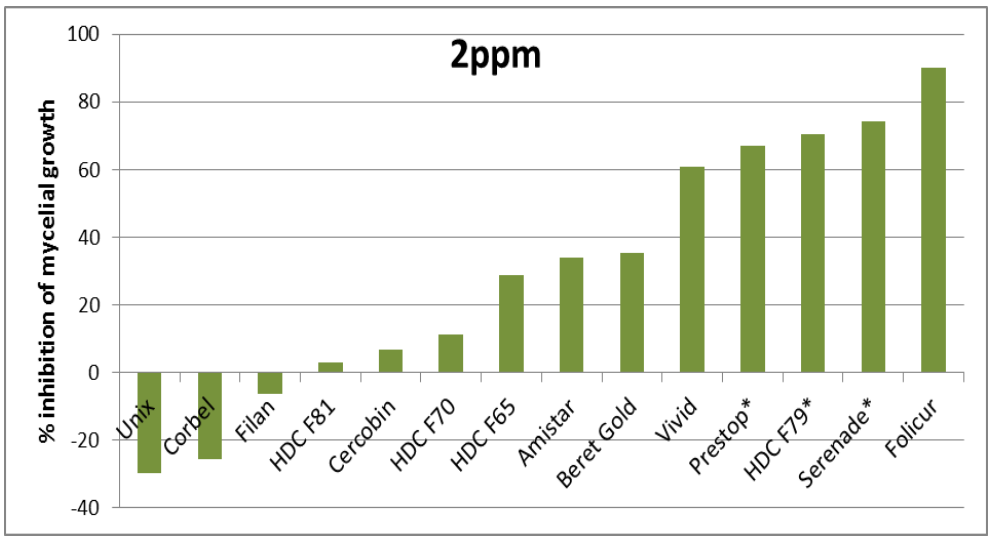
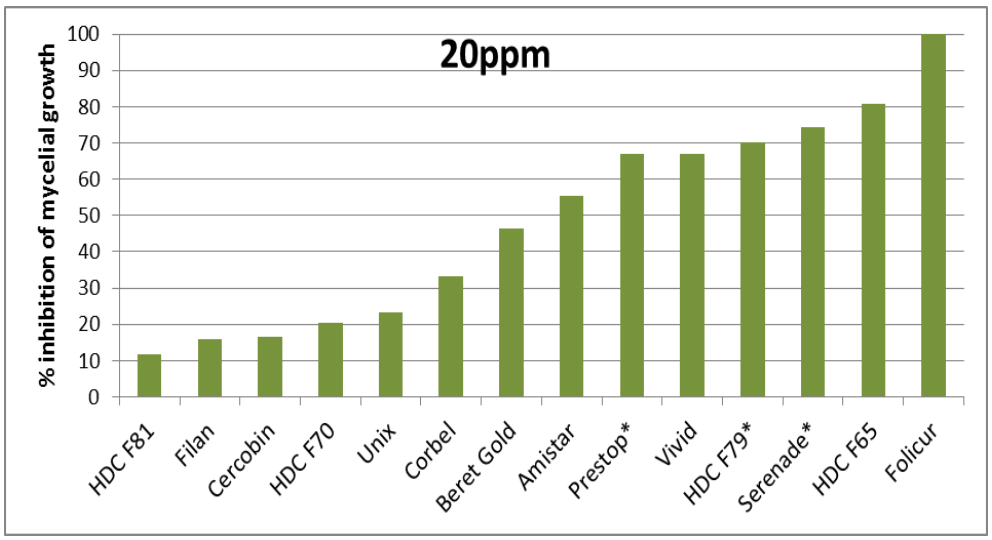
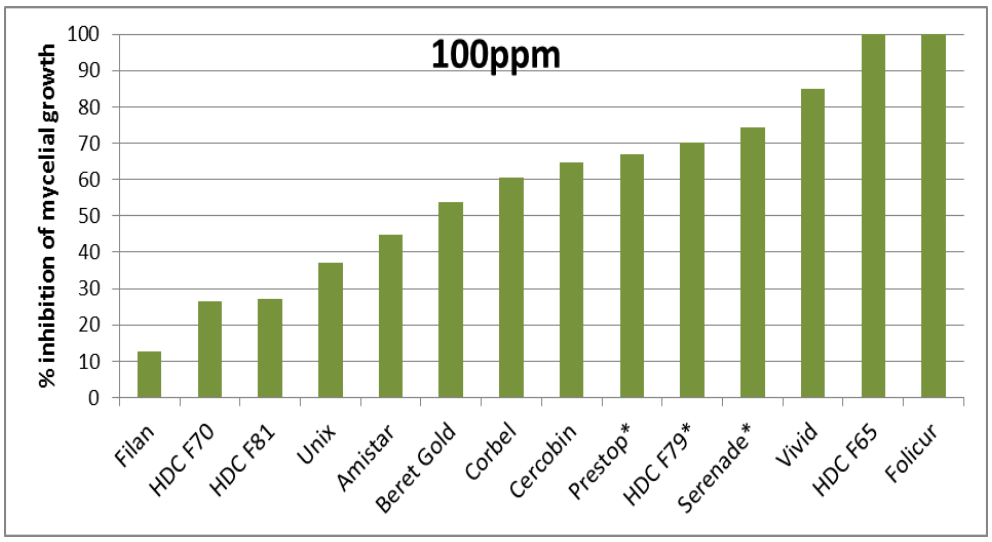
Figs 7-9. The mean % inhibition in radial growth of *Fusarium* with products used at three concentrations







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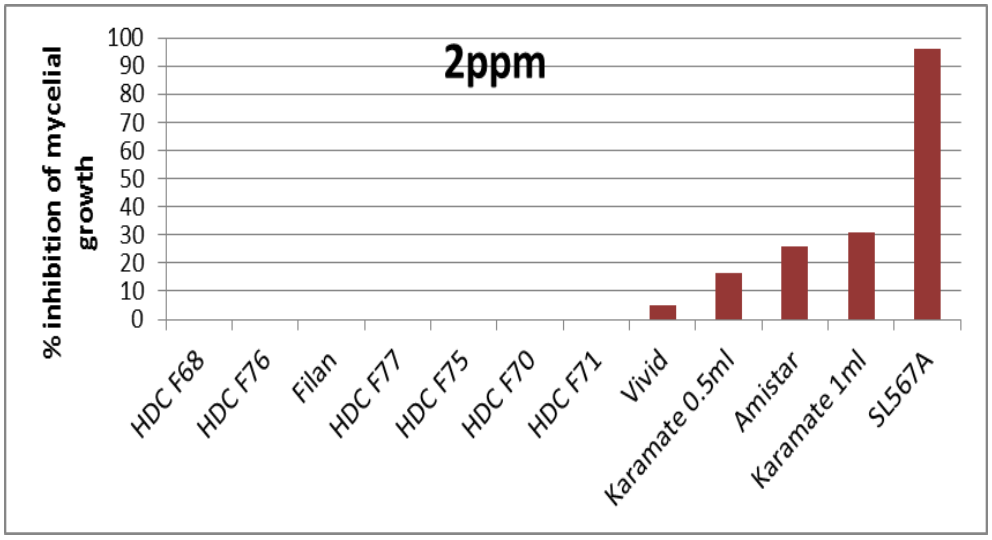
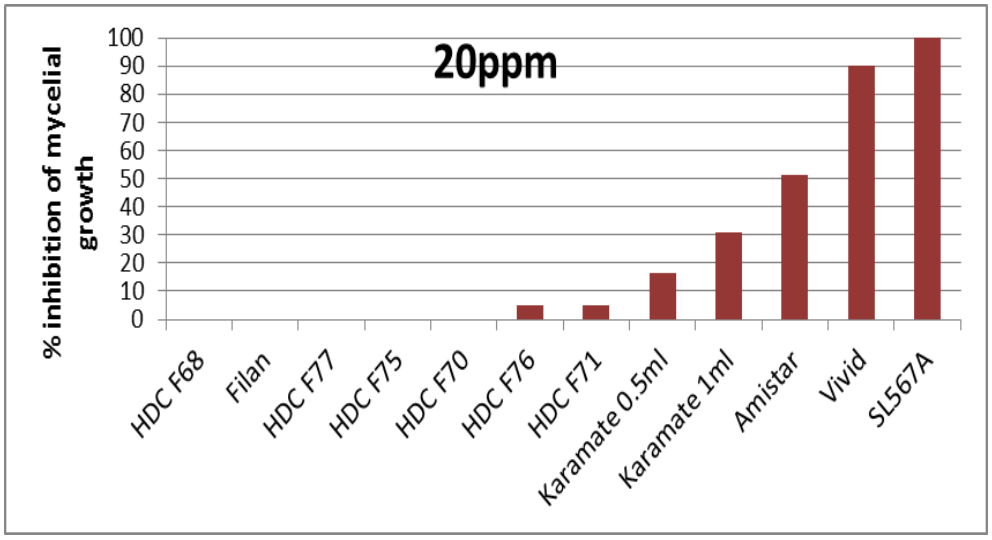
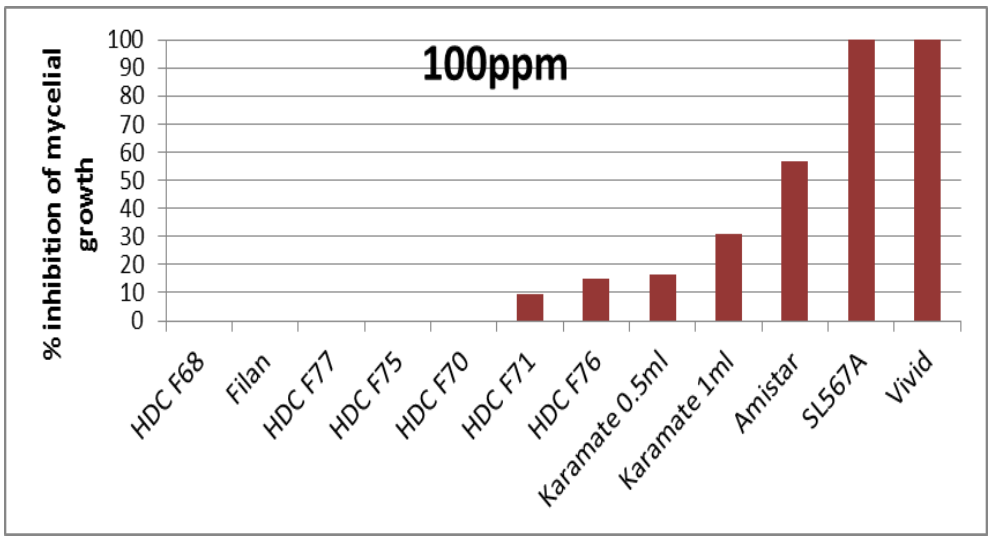
Figs 10-12. The mean % inhibition in radial growth of *Pythium* with products used at three concentrations







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5. In vivo bioassay – STC

As the seedlings emerged regular counts of emergence and any damping-off of seedlings were recorded. A final count of the number of established seedlings/pot was made at the completion of the study.

Table 22. Mean seedling establishment in *Pythium* bioassay

Treatment	Mean no. of germinated seedlings (22.11.11)	% of seedlings which damped-off/TRT (pre or post-emergence)
1. Uninoculated control	23.75	6
2. Inoculated control	25.00	6
3. SL567A	23.75	8
4. Amistar	24.75	3
5. HDC F76	24.25	5
6. HDC F74	24.50	2
7. HDC F68	24.25	6
8. HDC F75	24.50	2
9. HDC F71	23.50	9
10. HDC F80	24.25	4
11. HDC F69	24.25	5
12. Signum	24.50	3
13. HDC F73	23.75	6
14. HDC F77	23.25	7
SED	**0.762**	**0.857**
F: Fcrit	**0.848 : 1.96 NS**	**0.797 : 1.961 NS**

Table 23. Mean seedling establishment in *Cylindrocarpon* bioassay

Treatment	Mean no. of germinated seedlings (2.12.11)	% of seedlings which damped-off/TRT (pre or post-emergence)
1. Uninoculated control	24.25	3
2. Inoculated control	24.50	4
3. Amistar Top	23.75	4
4. Signum	24.5	5
5. NOT APPLIED	-	-
6. Nativo	24.75	2
7. Switch	24.24	5
8. NOT APPLIED	-	-
9. Compass	24.50	2
10. HDC F81	24.75	4
11. HDC F65	24.00	4
12. Serenade	23.75	5
13. Prestop	23.75	6
14. HDC F79	23.75	5
SED	**0.656**	**0.759**
F: Fcrit	**0.730 : 2.067 NS**	**0.348 : 2.067 NS**

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Table 24. Mean seedling establishment in *Fusarium* bioassay

Treatment	Mean no. of germinated seedlings (30.11.11)	% of seedlings which damped-off/TRT (pre or post-emergence)
1. Uninoculated control	24.25	3
2. Inoculated control	24.75	3
3. Amistar Top	23.25	6
4. Signum	22.75	18 SIGNIFICANT
5. NOT APPLIED	-	-
6. Nativo	23.75	4
7. Switch	25.00	3
8. Corbel	23.25	27 SIGNIFICANT
9. NOT APPLIED	-	-
10. HDC F65	24.50	6
11. HDC F81	23.75	8
12. NOT APPLIED	-	-
13. Serenade	24.25	4
14. Prestop	24.50	4
SED	**0.72**	**1.279**
F: Fcrit	**1.997 : 2.132 NS**	**4.52 : 2.13 SIG**
LSD		**2.58**

The recorded germination data for each of the pot studies indicates that there was little effect on the germination rate of the parsnip seed following inoculation with the respective pathogens in each study. Little pre or post-emergent damping-off was observed in either the *Pythium* or *Cylindrocarpon* studies. However, in the *Fusarium* inoculated pots, there were significant levels of damping-off observed in the pots treated with Signum (T4) and Corbel (T8) compared to the uninoculated and inoculated control. As the levels of damping-off in the inoculated control remained low this effect is likely to be associated with phytotoxicity from the applied treatment rather than the introduced *Fusarium* inoculum.

A full assessment of root blemishes was carried out on each of the roots during the final assessment in late March 2012. Roots were assessed for symptoms such as ginger blotch, cavity-spot, brown canker, and shoulder lesions (Tables 25-27).

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Table 25. Observed lesion development in *Pythium* pot study (22nd March 2012)

Treatment	Mean No. of roots with cavity spot lesions	Mean No. of roots with sunken lesions of shoulder	Mean No. of roots with brown cankers developing	Mean No. of roots with orange/ginger blemish	Mean dry weight of individual root (g)
1. Uninoculated control	0.50	3.25	0.25	0	0.268
2. Inoculated control	0.75	2.50	0	0	0.266
3. SL567A	0.50	1.50	0	0.25	0.273
4. Amistar	0.50	2.25	0	0.25	0.319
5. HDC F76	1.50	3.75	0	0.25	0.227
6. HDC F74	0.25	2.00	0	0	0.317
7. HDC F68	1.25	2.25	0.25	0.75	0.256
8. HDC F75	1.00	5.50	0.25	1.00	0.309
9. HDC F71	0.75	1.75	0	0.25	0.330
10. HDC F80	1.50	3.50	0	0.25	0.272
11. HDC F69	0.75	2.75	0	0	0.286
12. Signum	0.25	2.25	0	0	0.337
13. HDC F73	0.75	1.25	0	0	0.290
14. HDC F77	0.50	1.75	0	0	0.349
SED	**0.629**	**1.304**	**0.166**	**0.443**	**0.445**
F : Fcrit	**0.848 : 1.961 NS**	**1.467 : 1.961 NS**	**0.846 : 1.961 NS**	**0.965 : 1.961 NS**	**1.231 : 1.961 NS**

A total of 100 roots/treatment were assessed. P = 0.05

Lesion development was low overall and, perhaps surprisingly, some lesions developed in the uninoculated pots. No significant differences in the level of infection were observed following application of the treatments. In general the mean root weight was slightly higher in the treated pots, with the exception of those treated with HDC F76 (T5), however the increase in weight was not significant.

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Table 26. Observed lesion development in *Cylindrocarpon* pot study (30th March 2012)

Treatment	Mean No. of roots with cavity spot lesions	Mean No. of roots with sunken lesions of shoulder	Mean No. of roots with orange/ginger blemish	Mean dry weight of individual root (g)
1. Uninoculated control	1.25	1.75	2.50	0.379
2. Inoculated control	0	1.50	5.00	0.339
3. Amistar Top	0.5	0.75	4.75	0.425
4. Signum	0.5	0.75	5.50	0.440
5. NOT APPLIED	-	-	-	-
6. Nativo	0.25	0.25	2.50	0.388
7. Switch	1.00	1.25	4.75	0.412
8. NOT APPLIED	-	-	-	-
9. Compass	*	*	*	*
10. HDC F81	1.25	1.25	3.75	0.411
11. HDC F65	0.25	1.25	5.00	0.468
12. Serenade	*	*	*	*
13. Prestop	0.25	1.50	6.25	0.392
14. HDC F79	0.25	4.25	4.75	0.538
SED	**0.472**	**1.035**	**9.05**	**0.048**
F : Fcrit	**1.507 : 2.151 NS**	**1.777 : 2.151 NS**	**0.472 : 2.151 NS**	**2.082 : 2.165 NS**

A total of 100 roots/treatment were assessed. P = 0.05

* Due to experimental error in this trial the raw data for treatments 9 & 12 is not available.

The incidence of lesion development was very low in all the plots including the inoculated untreated control (T2). No significant reduction in lesion development was observed following application of treatments in this study. As was observed in the *Pythium* pot study (Table 25) there was some increase in the mean weight of roots following treatment application, however the differences were not statistically significant.

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Table 27. Observed lesion development in *Fusarium* pot study (28th March 2012)

Treatment	Mean No. of roots with cavity spot lesions	Mean No. of roots with sunken lesions of shoulder	Mean No. of roots with orange/ginger blemish	Mean dry weight of individual root (g)
1. Uninoculated control	0.50	1.75	0.75	0.283
2. Inoculated control	0.75	0	0	0.214
3. Amistar Top	1.00	0.50	0.25	0.307 SIG DIFF
4. Signum	0.25	0.75	0.75	0.322 SIG DIFF
5. NOT APPLIED				
6. Nativo	1.00	0.75	1.00	0.275
7. Switch	0.75	1.5 SIG DIFF	0.25	0.316 SIG DIFF
8. Corbel	0	0	0	*
9. NOT APPLIED				
10. HDC F65	0.25	1.5 SIG DIFF	0.50	0.404 SIG DIFF
11. HDC F81	1.25	1.25	0.25	0.331 SIG DIFF
12. NOT APPLIED				
13. Serenade	0.50	1.5 SIG DIFF	0	0.375 SIG DIFF
14. Prestop	0.75	2.5 SIG DIFF	1.00	0.332 SIG DIFF
SED	**0.634**	**0.738**	**0.504**	**0.032**
F: Fcrit	**0.707 : 2.132 NS**	**2.167 : 2.132 SIG**	**0.604 : 2.132 NS**	**5.361 : 2.211 SIG**
LSD	**-**	**1.491**	**-**	**0.065**

A total of 100 roots/treatment were assessed. P = 0.05

* Due to experimental error the data for dry weight of roots in T8 is not available.

In the *Fusarium* pot study the development of lesions in the inoculated control pots was very poor. However, there was some development of sunken shoulder lesions in the remainder of the pots, with the exception of T8 (Corbel), and significantly higher levels of these lesions were observed in T7 (Signum), T10 (HDC F65), T13 (Serenade) and T14 (Prestop). This would suggest that these treatments may not be effective against the pathogen causing this type of lesion. All of the treatments, with the exception of T6 (Nativo) resulted in a significant increase in the mean root weight compared to the inoculated control (P=0.05).

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Conclusions

In the ADAS pot studies a number of combinations of tests were undertaken to determine what affect each of the pathogens under scrutiny had on seedlings of different cultivars. In these tests we used only 2 cultivars, but the results indicate that the choice of variety may well be important as germination rates were significantly reduced by addition of *Cylindrocarpon* and 2 isolates of *Fusarium* during early assessments, although later germination in the inoculated pots did result in the differences losing their significance. No significant differences were observed in the level of damping-off of seedlings post-emergence following inoculation in either cultivar tested. Unsurprisingly, high levels of inoculum (10%) resulted in significantly reduced vigour in cv Javelin following inoculation with the *Cylindrocarpon* crown isolate, *Fusarium* isolates T25 and T33 and with *Pythium* sp. Cultivar Palace was less affected with a significant reduction in vigour only apparent following a 10% inoculation with the *Cylindrocarpon* crown isolate.

The tests also indicate that the incidence of fanging was statistically significant at the higher inoculum rate in cv. Javelin following inoculation with *Fusarium* T33 and T34, *Phoma* and *Pythium*, and in cv. Palace when inoculated at 10% with the both the *Cylindrocarpon* isolates and *Pythium* sp.

The incidence of red spotting of roots was significantly higher in pots inoculated at the 10% rate with both the *Cylindrocarpon* isolates, *Fusarium* T34 and *Phoma* in both cultivars. The severity of the spotting was worse in the pots inoculated with *Phoma* and the *Cylindrocarpon* crown isolate.

The development of early canker symptoms was more frequent on cv. Palace than on cv. Javelin following inoculation with the *Cylindrocarpon* black shoulder isolate at the higher inoculum level. There appear to be differences in pathogenicity between isolates of the same species and more extensive studies of this may be required in future.

None of the biological products or conventional fungicides under trial had a significant positive effect on reducing symptom development in our studies. Demonstrating the activity of these products in soil is challenging and more elaborate methodology should be considered. In the biological control experiments the pathogens were mixed into compost in the lower part of the pot and the biocontrol treatments mixed into the upper half of the pot. Thus the pathogens were not enveloping the young seedlings as they were in the pathogenicity tests. This almost certainly kept disease pressure low during the test and was oriented to give the biocontrol treatments the most favourable conditions in which to work. The fungicides were applied to the compost surface and lightly mixed into the compost to simulated incorporation by the seed drill. The capability of the various products to move further into the soil profile and persist sufficiently long to protect seedlings is unknown. In the absence of specific activity in this experiment, further work should consider high volume applications and repeated treatments.

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Several products showed good activity at controlling mycelial growth of the isolates of *Cylindrocarpon, Fusarium* and *Pythium* during the agar-plate tests (Table 28). Both Amistar and Vivid showed good activity against all 3 pathogens in these tests and therefore might be worthwhile treatments for crop application. However, several other products resulted in reduced fungal growth of individual pathogens and may well be worth considering for evaluation in field-scale trials. All 3 biological products showed good activity against *Cylindrocarpon* and *Fusarium* sp.

Table 28. A summary of efficacy of products tested using the agar plate test.

Product	Products providing >50% inhibition of radial growth								
	Cylindrocarpon sp.			*Fusarium* sp.			*Pythium* sp.		
	2ppm	20ppm	100ppm	2ppm	20ppm	100ppm	2ppm	20ppm	100ppm
Amistar	✓	✓	✓	x	✓	✓	x	✓	✓
Filan	x	✓	✓	x	x	x	x	x	x
Unix	x	x	x	x	x	x	Not tested		
Plover	✓	✓	✓	Not tested			Not tested		
HDC F81	x	✓	✓	x	x	x	Not tested		
HDC F65	✓	✓	✓	x	✓	✓	Not tested		
HDC F70	x	✓	✓	x	x	x	x	x	x
HDC F68	Not tested			Not tested			x	x	x
Vivid	✓	✓	✓	✓	✓	✓	✓	✓	✓
Folicur	✓	✓	✓	✓	✓	✓	Not tested		
Topsin	x	x	✓	x	x	✓	Not tested		
Beret Gold	✓	✓	✓	x	x	✓	Not tested		
Rovral	x	✓	✓	Not tested			Not tested		
HDC F77	Not tested			Not tested			x	x	x
Karamate	Not tested			Not tested				x	x
HDC F76	Not tested			Not tested			x	x	x
HDC F75	Not tested			Not tested			x	x	x
SL567A	Not tested			Not tested			✓	✓	✓
HDC F71	Not tested			Not tested			x	x	x
Corbel	Not tested			x	x	✓			
Prestop	✓			✓			Not tested		
HDC F79	✓			✓			Not tested		
Serenade	✓			✓			Not tested		

The *in-vivo* pot study tests carried out with a range of the same products were rather disappointing as it would appear that inoculum levels may well have been too low. The tests carried out by ADAS now provide evidence that a higher level of inoculum, closer to 10% than the 1% used, was required to produce good symptom expression. Some significant results were observed in the *Fusarium* sp. pot study. Damping-off was reduced following application with Signum and Corbel. There was a significant increase in the dry weight of roots following treatment with Amistar Top, Signum, Switch, HDC F81, HDC F70, Serenade and Prestop, despite the fact that symptom expression was low overall.

The findings from the Year 2 studies will be taken forward into 4 full-size field trials to be carried out during the final year of the project. Three of the sites will be in commercial crops, whilst the 4th

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trial will be carried out in the disease bed which has been maintained and cropped during the first 2 years of this study.

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