Department of Primary Industries
Biosciences Research Division
Knoxfield Centre

Invitation to a Project Meeting Thursday 18th March 2010

Pythium Project (VG08026) Update for the Vegetable IPM program

Latest field and lab trial results

Where:

Amstel Golf Club
1000 Cranbourne Frankston Rd Cranbourne
Melway 133 D5

3.00 pm – 5.30 pm

Afternoon tea provided

Speakers:
Joanna Petkowski, Dolf deBoer, Liz Minchinton of DPIVic

For more details contact:
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Research supported by your Vegetable Industry Levy, Horticulture Australia, and the Victorian Department of Primary Industries

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Acknowledgments
HAL (Horticulture Australia Ltd.)
Commonwealth Govt. & State Govt. of Vic.
DW Design

Chemical Use
The chemical use reported in the publication is off label information; consequently the DPI does not recommend it for use. All off label use is at the growers own risk.

Nutritional Characteristics of Parsnip and Parsley Growing Soils

Introduction

In Victoria, parsnip crops are grown on various soil types of different geological origin. There is little documented information on the nutrient status of these soils (critical nutrient concentration values), unlike soils in orchards, vineyards and potatoes for instance. Generally, fertilizer requirements for most vegetable crops are calculated on the basis of the nutrient concentrations in plant material.

Soil samples were collected from one parsley and seven parsnip growing sites. On each site, samples were taken from two depths, 0-15cm and 15-25cm.

Soil textures varied between sites from sandy, sandy loam to medium clay soils. All sites had a low to medium organic matter content, consistent with intensive vegetable production.

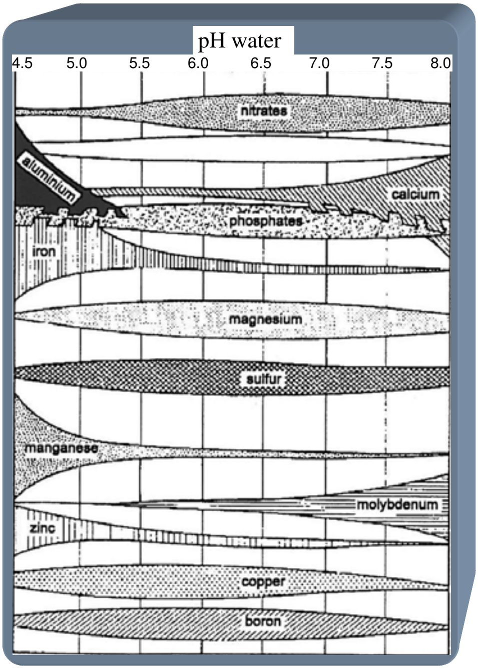


Fig 1 The diagram indicates the availability of nutrients at different pH (water) levels. The diagram was developed for well drained mineral soils in the USA and should be used only as a guide for Australian soils.

General comments

Soil pH ranged from pH 6.4 at the depth of 15-25cm in the sandy loam soil; to pH 8.3 at a depth of 0-15cm in the recently limed sandy soil. The majority of parsnip sites had pHs near to neutral or slightly alkaline. Neutral pH (pH 7.0) is desirable for the optimum availability of the most macro and some micronutrients (Fig 1). All sites were low to moderately saline.

No apparent nutrient deficiencies were detected in the different soils with the exception of low iron in the calcareous and sandy soils. Possible iron deficiencies were observed on young parsnip plants on calcareous and black earth (clay) soils (Fig 2). Low potassium levels were also evident in sandy soil sites, but they were sampled after the crop was harvested.

Fig 2 Possible iron deficiency on parsnip leaves. No virus was detected in affected leaves.

Nutrients and plant diseases

Good soil nutrition is critical to achieving optimum yields. However, deficiencies or excess of soil nutrients or imbalances in the ratios of some of the key nutrients can be linked to the occurrence of some diseases.

There are numerous reports on the effects of nutrient supply on plant diseases, e.g. positive but also negative effects of potassium (K), phosphorous (P) and nitrogen (N) on the incidence of soilborne and airborne diseases of vegetables (tomatoes, brassicas and beans).

A balanced supply of K decreases susceptibility of tissues to maceration and penetration by pathogens.

N deficiency or oversupply, affects formation of cellulose in plants and the release of root exudates, which play a very important role in pathogen attraction. N also influences pathogen virulence and growth.

An adequate supply of K and P is very important in development of thicker plant cuticles and cell walls, which are mechanical barriers to pathogen entry and proliferation.

Field Trials

Aim:

Two field trials were set up on sites with different soil textures (sandy loam and medium clay) to determine the role of *Pythium* in the development of parsnip canker. Additionally they aimed to identify different disease management options (Fig 1).

Method:

- The trial on sandy loam was designed as a randomised complete block of seven treatments with eight reps. Treatments included four metalaxyl applications, strobilurin, a biological control agent (*Streptomyces lydicus*) and an untreated contol.
- The trial on clay soil was designed as a randomised complete block of six treatments with six reps. Treatments included four metalaxyl applications, a strobilurin and an untreated control.

Trials were harvested in late October (Fig 2). Incidence and severity of parsnip canker were assessed from all treatments at harvest. Proportion of unmarketable yield was calculated as a percentage of plants with disease symptoms and physiological disorders (skin cracks, forking) (Fig 3 & 4). Disease severity was calculated for each sampled parsnip as a sum of scores for symptom(s) present, using a scale of 0 to 4 (Table 1 & 2). At each site, parsnip roots were sampled every month for six months, from the untreated control plots to determine succession of fungi in the crop (Fig 5 & 6). Different fungi were identified by Crop Health Services (DPI, Victoria).

Table 1 Rating of 0 to 4 for assessment of disease severity on parsnip roots.

0	Healthy parsnip root
1	Superficial brown lesions on the upper tap root or lesion on the lower tap root or forking of tap root
2	Elongated lesion on tap root – ‘growth cracks’
3	Canker – deep lesion on the tap root
4	Crown rot

Fig 1 Trial site on clay soils in April 2009.

Fig 2 Field day at the sandy loam trial site at harvest in October 2009.

Results:

Sandy loam site

All metalaxyl treatments, irrespective of the time of application and number of treatments, reduced the incidence of canker and improved marketable yields. Yields from metalaxyl treated plots were up to 30 % higher than from the untreated control (Fig 4). The biological control agent and strobilurin treatment did not reduce the incidence of canker or improve yields. However, these treatments did reduce the severity of canker (Table 2).

Clay site

None of the treatments controlled canker in the trial at the clay site. More than 90% of parsnips grown at this site were unmarketable (Fig 3 and Table 2).

Conclusions:

- Control of canker with metalaxyl in the sandy soil supports the view that *Pythium* species are important in the development of canker.
- The fact that metalaxyl did not control the disease on clay soil may be due to enhanced degradation of this chemical in the soil. Metalaxyl degradation has been reported to occur in slightly alkaline soils under anaerobic conditions.
- Clay soils may require other options for disease management such as aeration and improved structure using appropriate rotation crops e.g. plants with deep root systems.

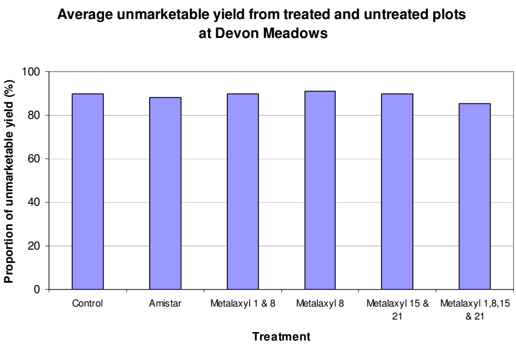


Fig 3 Average unmarketable yields from plots treated with four schedules of metalaxyl applications, strobilurin and untreated control plots on the clay soil site.

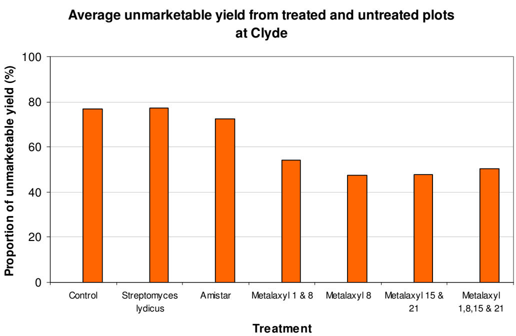


Fig 4 Average unmarketable yields from plots treated with four schedules of metalaxyl applications, strobilurin, *S. lydicus* and untreated control plots on the sandy loam soil site.

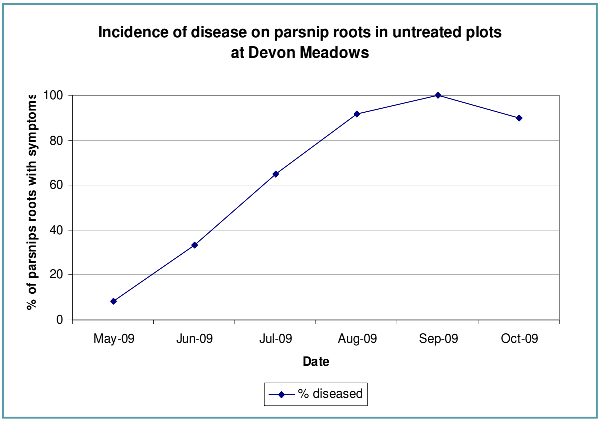


Fig 5 Incidence of disease on parsnip roots in untreated plots at the clay soil site.

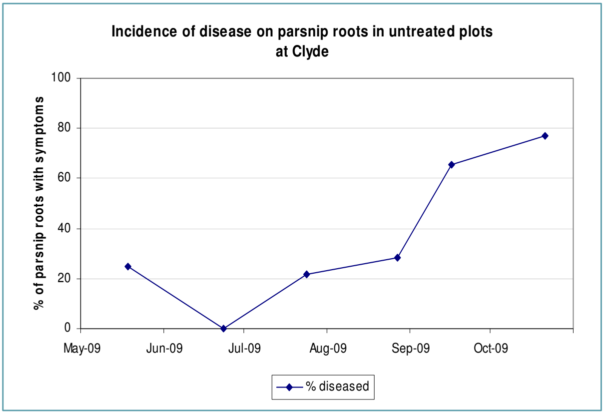


Fig 6 Incidence of disease on parsnip roots in untreated plots at the sandy loam soil site.

Table 2 Average severity scores on parsnip roots from both trial sites on clay and sandy loam soils assessed at harvest.

Site	Clay	Sandy Loam
Treatment	Overall Severity SE=0.1	Overall Severity SE=0.1 *
Control	3.5	2.0
Streptomyces lydicus	na	1.6
Amistar	3.2	1.5
Metalaxyl 1 & 8	3.3	1.1
Metalaxyl 8	2.9	1.0
Metalaxyl 15 & 21	3.6	1.1
Metalaxyl 1, 8,15 & 21	2.9	1.0
F<0.001	F=0.096	
Lsd	n/s	0.3

*) numbers of different colours vary significantly at the 5% level

Monthly surveys of pathogen genera at trial sites at Clyde and Devon Meadows

Aim:
To monitor the succession of pathogens in parsnip crops at the two field trial sites, Clyde and Devon Meadows, Victoria.

Methods:
Isolations of fungi and oomycetes were conducted monthly from symptomatic and non-symptomatic parsnip roots collected from untreated control plots at both trial sites. Sections of symptomatic and non-symptomatic parsnip roots including fine lateral roots were placed on water agar. Oomycete-like hyphae and fungal hyphae were transferred onto V8 agar and potato dextrose agar (PDA), respectively (Fig 1). Identifications of pathogen genera were performed by Crop Health Services (DPI, Victoria).

Results:
At the early crop growing stage (from May to mid July) all cultures were identified as *Pythium* species. and *Pythium*-like species at both trial sites. An increase in populations of *Phoma* species was observed from mid July at both sites. A rapid increase in populations of *Fusarium* species was evident in the August, September and October sampling, especially at the Devon Meadows site (Fig 2).

No Cylindrocarpon species were isolated from the Clyde site but low numbers of *Botrytis cinerea* were found at this site (Fig 3). Populations of *Pythium* species declined when *Phoma* and *Fusarium* species increased. Low numbers of *Rhizoctonia solani* were present at both sites.

Pythium species prevailed in the coolest period of the cropping season. They were isolated from both symptomatic and non symptomatic root sections. Fungal genera such as *Phoma*, *Fusarium, Cylindrocarpon* and *Alternaria* were isolated from roots later in the cropping season. They started to occur in July after a period of two weeks of cold weather from mid June to early July. In this coldest period of the entire season *Pythium* species were the most active pathogens and had no competition from other pathogens.

Conclusion:
This study confirms the view that Pythium species are involved in the development of canker on parsnip roots, perhaps by providing a means of entry of other pathogens or saprophytes.

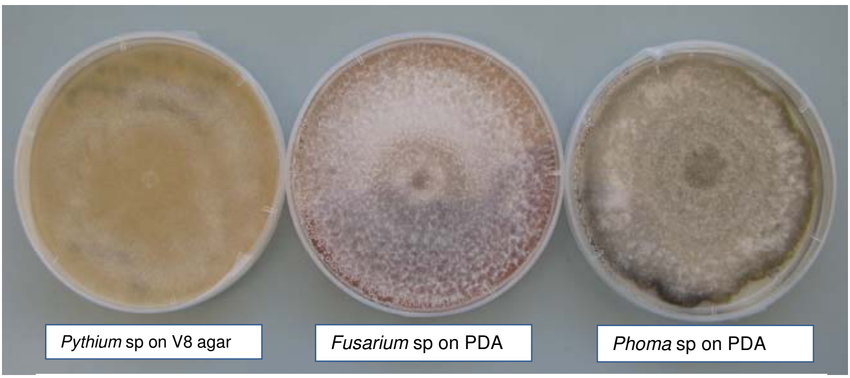


Fig 1 Pathogen cultures

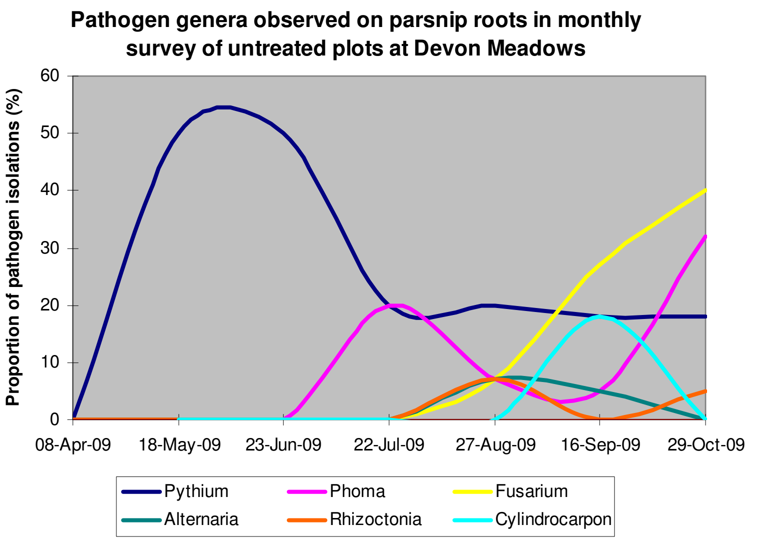


Fig 2 Proportion of pathogen isolations (%) from parsnip roots in monthly surveys of untreated control plots at Devon Meadows.

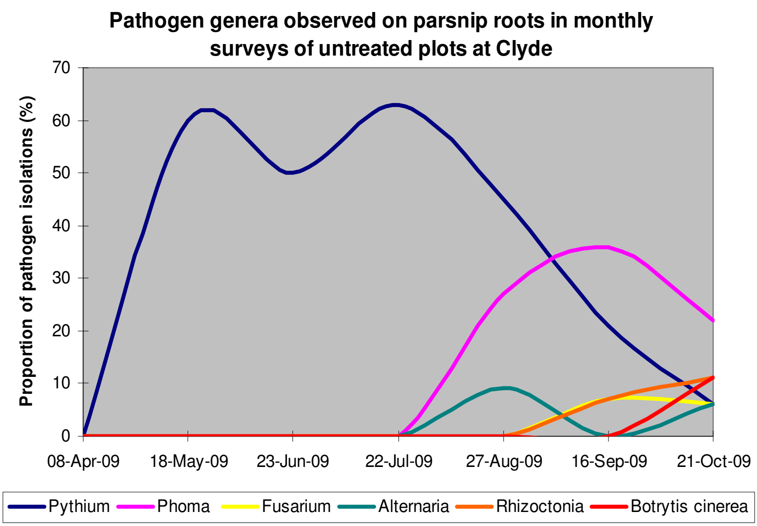


Fig 3 Proportion of pathogen isolations (%) from parsnip roots in monthly surveys of untreated control plots at Clyde.

Testing the pathogenicity of different fungi to parsnip

Aim:

To determine if infection by *Pythium sulcatum*, which is pathogenic to carrot and parsley roots, predisposes parsnips to canker.

Methods:

Parsnip roots, taken from a September 2009 harvest, were surface sterilised with 70% ethanol and placed 2 per box on moist paper towel. A plug of agar of each test fungus was placed on the upper and lower root. One parsnip root and agar plug was damaged with a needle, whilst the other was left undamaged. Boxes were incubated at 10 °C in the dark and roots assessed for disease symptoms at week 6, 10, 11 and 15 weeks after inoculation.

Fig 1 *Cylindrocarpon* inoculated onto parsnip (U undamaged bottom; D damaged top)

Fig 3 *P. sulcatum* + *Clylindrocarpon* inoculated onto parsnip (U undamaged bottom; D damaged top)

Results:

A fungus is considered to be pathogenic if it causes a lesion on an undamaged root and only a week pathogen if it causes damage only on a damaged root. Damage only became evident 10 weeks after inoculation.

- *Cylindrocarpon* was a weak saprophyte and even weaker pathogen as few lesions were produced on parsnip roots (Figs 1 & 2).
- *Clylindrocarpon* +*P. sulcatum* produced similar results to the above suggesting no interaction (Figs 3 & 4).
- *P. sulcatum* was a moderate saprophyte but weak pathogen. Symptoms were a localised reddish discolouration of the roots (Figs 5, 7& 6).
- *F. oxysporum* was strongly pathogenic and saprophytic (Figs 7 & 8). Lesions contained white hyphae.
- *P. sulcatum* + *F. Oxysporum* (Figs 9 & 10).
- When Figs 6, 8 & 10 are compared it suggests:
    a) The combination is highly saprophytic, requiring damage for lesion development.
    b) The combination produces a higher incidence of lesions earlier.

Fig 2 *Cylindrocarpon* inoculated onto parsnip

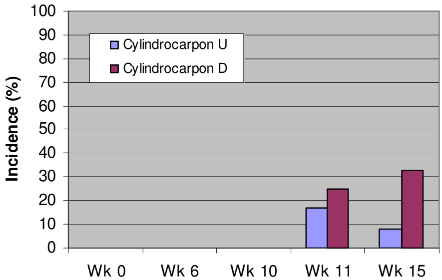
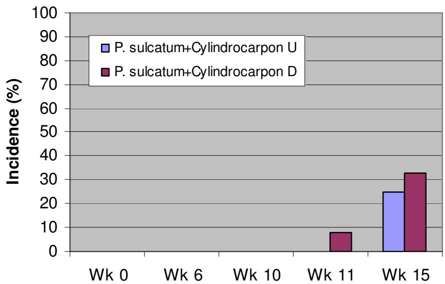


Fig 4 *P. sulcatum* + *Clylindrocarpon* inoculated onto parsnip



Conclusion

- *F. oxysporum* proved to be the most pathogenic of the fungi tested on parsnip roots, but produced relatively small lesions. Similar symptoms have been observed in the field.
- *Pythium sulcatum* was not particularly pathogenic on the mature parsnip roots. This experiment will be repeated on parsnip seedlings to determine whether infection by *Pythium* depends on the age of the plant.

Fig 7 *F. oxysporum* inoculated onto parsnip
(U undamaged bottom; D damaged top)

Fig 8 *F. oxysporum* inoculated onto parsnip

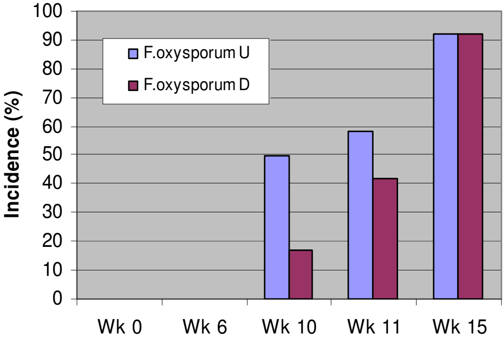


Fig 9 *P. sulcatum* + *F. oxysporum* inoculated onto parsnip (U undamaged bottom; D damaged top)

Fig 10 *P. sulcatum* + *F. oxysporum* inoculated onto parsnip

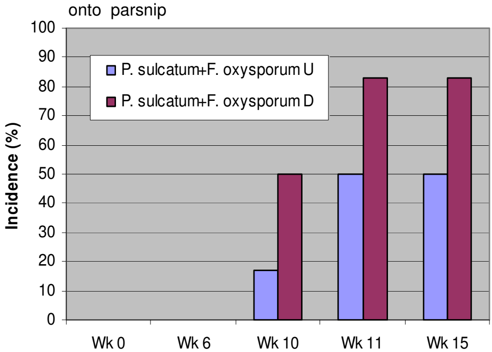
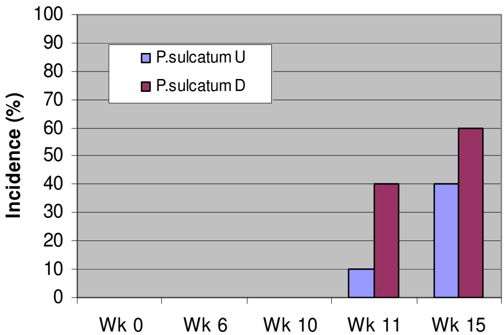


Fig 5 *P. sulcatum* inoculated onto parsnip
(U undamaged bottom; D damaged top)

Fig 6 *P. sulcatum* inoculated onto parsnip



Efficacy of biological control agents for Pythia in hydroponic coriander crops

Len Tesoriero, IINSW NSW Department of Primary Industries EMAI, RMB 8, Camden NSW 2570

Isolation and pathogenicity testing

Several species of *Pythiu,m* were isolated from diseased coriander roots. Eleven of these were screened for pathogenicity to coriander growing in a hydroponic system. *Pythium sulcatum* caused the most damage to coriander roots but some isolates of *P. coloratum* and *P. dissotochum* also caused some root damage.

Glasshouse trials for efficacy of bio-control agents

Aim:

Two biological control organisms and a SAR chemical (systemic activator of resistance), were evaluated for their effect on *P. sulcatum* root rot of coriander in hydroponics.

The Trial:

The trial was a randomized complete block design consisting of 6 replicates. The treatments were single applications of the bio-control agents *Bacillus subtilis* (Fulzyme Plus™), *P. oligandrum* (mycoparasite) or Bion™ (SAR chemical) and included a positive (*P. sulcatum*) and negative (uninoculated) control treatments. Nutrient solution temperatures in the hydroponic system ranged from 15 ºC to 20 ºC.

Results:

Three trials were conducted. In the first two trials, *Pythium* levels were so high that all plants died. In the third trial *Pythium* concentrations were adjusted to more realistic levels.
In this trial (Fig.1)

- Neither Bion nor *P. oligandrum* had any effect on root rot levels in the hydroponic system. Bion was phytotoxic at the concentrations used in this experiment. Plants treated with this chemical were smaller than untreated plants.
- Treatment with *B. subtilis* protected plants from root rot for the first 3 weeks. However, this protection gradually wore off and by harvest time the yield of plants treated with the bacteria were 50% less than in the control plants (no *Pythium*).
- The activity of the *P. oligandrum* isolate may have been low, a fresh culture should be retested.

Conclusion:

- A number of *Pythium* species proved to be very damaging to coriander grown in a hydroponics system.
- *Bacillus subtilis* showed promise in controlling root rot of coriander. However, this treatment may need to be applied regularly throughout the growing season to provide effective protection against root rot.

Fig 1 Hydroponic trial set up in the glasshouse at EMAI Camden, NSW.

PERMITS

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Below is a list of all registered products for *Pythium* species in selected Permits as of January 2010.

Pythium control options in selected vegetables

CROP	ACTIVE INGREDIENT	PRODUCT	PERMIT	DISEASES	RATE	APPLICATION TIMING	WHP
CARROTS	Metalaxyl + mancozeb	Ridomil Gold MZ & other	PER10301 (expires 31/3/13)	Pythium spp. and Phytophthora spp.	2.5 kg/ha	Early post planting	7 days
CARROTS	Metalaxyl	Ridomil Gold 25G & other products	registration	Damping-off: Pythium spp. and Phytophthora spp.	40 kg/ha	Granule application to soil surface at	NA
PARSNIPS	Metalaxyl + mancozeb	Ridomil Gold MZ & other	PER10301 (expires 31/3/13)	Pythium spp. and Phytophthora spp.	2.5 kg/ha	Early post planting	7 days
PARSLEY	Metalaxyl	Ridomil Gold 25G & other products	PER11425 (expires 31/10/11)	Pythium root rot and Phytophthora root rot	40 kg/ha	Granule application to soil surface at	NA

Other fungicides with registrations for *Pythium* control in other crops

- Azoxystrobin
- Captan
- Propamocarb
- Thiophanate-methyl
- Thiram

USA registered products in carrots

Carrots / Cavity Spot - Pythium sulcatum and P. violae

- Ridomil Gold (metalaxyl)
- Reason (fenamidone) - not available in Australia
- Metham sodium

Carrots / Root Dieback (Forking and Stubbing): Pythium ultimum and P. irregulare

- Ridomil Gold (metalaxyl)
- Metham sodium
- Agrifos (phosphorous acid) ????

Milestone No. 103: Review & stop-start.

Date: 31/03/2010

Achievement Criteria

No.	Item	Achieved	Comment
1	Pathogenicity (inoculations in vitro with single and combinations of pathogens) completed	✓	▪ Unsatisfactory results, ▪ Revise method
2	Growth chamber studies (for moisture and temperature relationships) in progress	✓	▪ Field data suggest concentrate on temperature ▪ Slow start – limited growth cabnets ▪ Student project
3	Report on monitoring of moisture and temperature relationships in parsley and parsnip	✓/x	▪ Temperature important for parsnip ▪ Equipment failed in parsley
4	Field trial (biologicals and chemicals) completed	✓	▪ Suggests *Pythium* affects all stages ▪ Need a biological active at low temperatures
5	Report on completed hydroponics trial	✓	▪ Final report November 2010 ▪ Len Tesoriero has had a heart attack so it may be delayed
6	Report to industry in one: ▪ field day, ▪ an article for a grower magazine, and ▪ a steering committee meeting	✓	▪ Parsnip field day 21/10/2009 ▪ Vegetables Industry Report 08-09 p38. ▪ 18/03/2010
7	Project review undertaken with stakeholders & any redirections put in place		

Milestone No. 104: Hydroponics trials completed

Date: 30/11/2010

Achievement Criteria

No.	Item	Comment
8	Report hydroponics work to industry	Final report pending - may have to be revise as Len Tesoriero had a heart attack and his 2 IC is relocating to QLD
9	Monitoring of moisture and temperature relationships commenced in parsley and parsnip crops	Relocate weather stations and soil moisture sensors (dealing directly with supplier in QLD) ▪ Locate parsnip sensors in the field trial
10	Field succession of *Pythium* and *Fusarium* etc populations commenced in the laboratory.	▪ Succession monitoring commenced 2009
11	Meet with and consulted steering committee to develop 2010 field trials. Trials for e.g.: ▪ Irrigation, ▪ cover crops, ▪ cultural practices and ▪ initiation of infection in the field (in 2009)	Options: ▪ Metalaxyl early – *Phoma /Fusarium/Itersonilia* ▪ Mulch ▪ High pH ▪ Biologicals – *P. oligandrum*, *B. subtilis* ▪ Irrigation
12	Commence interstate trials with biologicals in WA on carrots	▪ Evaluate or proceed?
13	Commence interstate trials with biologicals in QLD on coriander (Herb Industry)	▪ Warmer soil temperature – send up biologicals for field testing