

# **Identification and management of parsley root rot**

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Victorian Department of  
Primary Industries (VICDPI)

Project Number: VG06046

## **VG06046**

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# Identification and management of parsley root rot

**Horticulture Australia VG06046**

**(August 2007)**

**Minchinton *et al.***

**Primary Industries Research Victoria, Knoxfield Centre**



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### **Purpose of project:**

This report details the outcomes of a 12-month project continuing the research from a previous scoping study investigating root rot in parsley (VG04025). This project carried out trials to identify parsley cultivars tolerant to root rot in both Queensland and Victoria and evaluated fungicide alternatives and a biocontrol agent for disease control.

**Report completed: August 2007**

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## Media Summary

### Unravelling parsley root rot

Scientists continue to unravel the problem of root rot in parsley and investigate control options. The disease can cause up to 100% crop losses in Victoria and Queensland. In Victoria, parsley root rot during summer was associated with the fungus *Fusarium*, but in winter, it was caused by the water moulds *Pythium* and *Phytophthora*.

Symptoms of summer parsley root rot, associated with *Fusarium*, were dry, cracked, red-brown lesions on the roots with no above ground symptoms and minimal crop losses. Chemical controls may not be required under such conditions. However, this may not be the case if *Fusarium* has a major impact on crop yields.

Winter parsley root rot, associated with the water moulds *Pythium* and *Phytophthora*, attacks both seedlings and mature plants, causing a spongy dull brown rot resulting in a complete and often rapid collapse of the root system and major crop losses.

The screening of flat-leaf cultivars in Victoria identified that the cultivar Shamrock had up to 70% less root rot than other cultivars. It could be a useful cultivar to grow in areas where both summer and winter root rot is an issue.

A fungal biocontrol preparation of the naturally-occurring mycoparasite water mould, *Pythium oligandrum*, was effective against water moulds in pot trials, but the commercial preparation of the same fungus, Polyversum™, was ineffective in the field under both low and high disease pressure. The mycoparasite may be of use in the hydroponics industry.

Information resulting from this research can be accessed from the HAL final report VG06046 'Identification and management of parsley root rot' and nationally through the Vegetable Industry Development Officer network.

This research was led by scientists at the Department of Primary Industries Victoria Knoxfield Centre, in collaboration with Queensland Department of Primary Industries and Fisheries. The project was facilitated by Horticulture Australia Limited (HAL) in partnership with Federation of Potato and Vegetable Growers Australia Limited (AUSVEG) and was funded by the National Vegetable Levy. The Australian Government provides matching funding for all of Horticultural Australia's Research and Development activities. The researchers gratefully acknowledge the financial support of the Department of Primary Industries through Primary Industries Research Victoria.

## Technical Summary

Growers reported that root rot of parsley caused up to 100% crop losses in Queensland and Victoria for a number of years. In Victoria the problem is worse in late autumn through winter when conditions are cool and wet and is associated with *Pythium* and *Phytophthora*. In Queensland, growers reported root rot was worse during the wet season. Some Queensland growers have established hydroponics production to avoid crop losses and maintain production through the wet season.

This project built on an earlier scoping study to further investigate management of root rot disease on parsley in both Queensland and Victoria by:

- (i) identifying cultivars less susceptible to root rot,
- (ii) seasonal management of the disease with a biocontrol agent and fungicides and
- (iii) pathogenicity trials of Queensland isolates.

Of the six flat-leaf parsley cultivars trialled in Victoria, Shamrock had up to 70% less root rot than other cultivars but root rot symptoms were associated with *Fusarium*, rather than oomycetes. Consequently, the susceptibility of parsley varieties to root rot associated with oomycetes remains undetermined. The susceptibility of 8 curly leaf and 5 flat leaf varieties to root rot in Queensland is also unresolved due to the prevailing drought.

Under summer conditions in Victoria, parsley roots are often associated with dry red-brown wrinkled root lesions, which rarely produce above ground symptoms. In a field trial designed to target oomycetes, chemical treatments had little effect on the incidence of root rot. *Fusarium*, not oomycetes, was responsible for parsley root rot under summer conditions in Victoria and was particularly severe in the summer of 2003/4. Application of chemicals for root rot against oomycetes in Victoria during summer may not be necessary.

The biocontrol agent *Pythium oligandrum* showed efficacy in pot trials as a mycoparasite to protect parsley plants from the pathogen, *P. sulcatum*. However, Polyversum™, a commercial preparation of *P. oligandrum*, did not limit root rot incidence in parsley under low disease pressure in summer associated with *Fusarium*, or high disease pressure in winter associated with oomycetes.

Pathogenicity was demonstrated for *Alternaria petroselini*, two isolates of *Fusarium*, *F. oxysporum*, *Pythium diclinum* and *P. irregulare* on both curly and flat leafed cultivars. The curly leafed cultivar appeared more susceptible than the flat leafed cultivar at higher temperatures. To-date only *A. petroselini* has been associated with an epidemic in the field.

The difficulties experienced in conducting trials during prevailing drought conditions highlights the risks associated with conducting field studies in research projects of short duration.

## Recommendations for further research

- Test Polyversum™ in both hydroponics and glasshouse systems to assess efficacy against parsley root rot.
- Test suitability of other potential biocontrol agents under controlled conditions and in the field.
- Assess cultivars, both flat-leaf and curly-leaf, for resistance against root rot and yield potential in Victoria under low and high disease pressure.
- Establish the pathogenicity of Victorian isolates of *Fusarium* associated with parsley roots during summer, establish if there is a yield loss and if necessary develop management strategies for Queensland and Victorian parsley crops.
- Chemical efficacy trials targeting *Fusarium* under summer conditions in Victoria.

## Chapter 1

# Investigation of biocontrol for management of parsley root rot in Victoria

Desmond Auer, DPI Victoria

## 1.1 Summary

Project VG04025 identified a potential beneficial water mould (oomycete). An extensive literature search showed that *Pythium oligandrum* was a mycoparasitic oomycete capable of parasitising other oomycetes, as well as other potentially pathogenic fungal species capable of causing root rot in parsley. Initial pot trials under controlled conditions were encouraging, protecting parsley root systems from the pathogen *Pythium sulcatum* with a four-fold decrease in root rot incidence. A biofungicide containing *P. oligandrum* oospores (Polyversum™) was sourced from overseas and permission was granted from both AQIS and the APVMA for the import and use of the product in small field trials in both Victoria and Queensland. Polyversum™ failed to suppress root rot in parsley in the field under either low or high disease pressure in Victoria. Due to the biocontrol observed in the glasshouse trials, it may be worth further investigation for disease control under controlled conditions such as hydroponics and nursery production.

## 1.2 Introduction

*Pythium oligandrum* has been the focus of widespread research into biocontrol agents to combat fungal diseases in plants (Brožová 2002). It is a common organism found in soils world-wide and is one of four mycoparasitic oomycetes with spiky oogonia (Jones & Deacon 1995).

Its potential as a biocontrol agent derives from its ability to (i) inhabit the rhizosphere of roots, successfully competing for nutrients against potential pathogens, and (ii) be an active mycoparasite (Al-Rawhali & Hancock 1997). It has been shown to actively parasitise a number of different fungi, including *Fusarium* (Davanlou *et al.* 1999); *Pythium* and *Phytophthora* (Abdelzaher *et al.* 1997; Holmes *et al.* 1998), *Verticillium* (Al-Rawahi 1998) and *Sclerotinia* (Madsen & de Neergaard 1999). There is also evidence of the mycoparasite invading plant tissue and triggering an immune response within the plant itself (Benhamou *et al.* 2001; Hase *et al.* 2006; Mohamed *et al.* 2007; Rey *et al.* 1998).

Due to its mycoparasitic effect on a wide range of fungi, especially those involved in root rot, and its effect on plant defence mechanisms, *P. oligandrum* was investigated as a potential biocontrol agent in this study.

## 1.3 Materials and methods

The efficacy of *P. oligandrum* against parsley root rot was evaluated under controlled environmental conditions in incubator trials in two experiments. In the first incubator trial, the mycoparasite and pathogen were inoculated together and in the second incubator trial, the mycoparasite was inoculated two weeks before the pathogen.

A commercial preparation of *P. oligandrum* (Polyversum™) was tested for its effectiveness against root rot under field conditions in two trials. The first field trial was conducted under low disease pressure during spring-summer and the second field trial was conducted under high disease pressure during autumn-winter.

### 1.3.1 Initial biocontrol trials

#### 1.3.1.1 Preparation of isolates

Both *P. oligandrum* and *P. sulcatum* were sourced from the research team's culture collection (Minchinton *et al.* 2006). *P. sulcatum* was chosen as our test pathogen, because it has been shown to be particularly aggressive on parsley in our previous study. Both isolates were grown on V8 agar at room temperature (20°C) for 5–7 days until microscopic examination of the plate showed development of abundant sporangia and/or oospores.

#### 1.3.1.2 Preparation of parsley plants

Soil was collected from a farm with a history of parsley root rot, sterilised by pasteurisation for six hours and left to cool. Two parsley seedlings (cv. Afro) were transferred to each 10 cm pot containing sterilised soil and plants were then grown at 9°C with a 16/8 hr light/dark cycle. Ten pots (20 plants) were prepared for the first experiment, and three pots (6 plants) were prepared for the second.

#### 1.3.1.2 Treatment and inoculation of parsley plants

In the first experiment, 5 mm agar plugs from the leading (growing) edge of V8 plates of both *P. oligandrum* and *P. sulcatum* were prepared. The root mass of each plant was exposed with a spatula and two agar plugs each of both the pathogen and the mycoparasite were inserted next to the root systems before being reburied with soil. Three types of control plants were also prepared; (i) uninoculated plants; (ii) plants inoculated with *P. oligandrum* alone and (iii) plants inoculated with *P. sulcatum* alone. All plants were then exposed to continuous flooding to simulate the winter conditions suitable for *Pythium* infection.

In the second experiment, *P. oligandrum* plugs were introduced to the soil of parsley plants two weeks prior to inoculation with the pathogen *P. sulcatum* using the method above. Again, three controls consisting of uninoculated plants; plants inoculated with *P. sulcatum* alone and plants inoculated with *P. oligandrum* alone were prepared. All plants were flooded continuously for one week, and then subjected to flooding once per week.

#### 1.3.1.3 Assessment of experiments

In both experiments, after 8 weeks, plants were removed from the pot and roots were washed in tap water to remove as much of the soil as possible.

Photographs were taken of all plant root systems and plants were visually assessed for root rot with a simple present/absent rating. To isolate both the pathogen and the mycoparasite, root samples were prepared following the protocol of Davis & McKay (2003). Briefly, small pieces of root were cut and washed in sterile deionised water for 30 s before placement on water agar (WA) plates. Plates were incubated at room temperature for 2 weeks and examined under the microscope for oomycetes (sporangia, oospores, typical hyphae). Isolated oomycetes were submitted to James Cunnington (Biosystematics, DPI Victoria) for formal identification.

### 1.3.2 Parsley Field Trial No. 1 (spring 2006–summer 2007). Biological management of root rot in Victoria: Low disease pressure

#### 1.3.2.1 Chemical application and trial setup

Polyversum™ was sourced from Biopreparaty (Prague, Czech Republic) after approval for importation and use was granted by AQIS and the AVPMA. Polyversum™ consists of *P. oligandrum*

oospores dispersed in anhydrous silicon dioxide at a concentration of no less than  $10^6$  oospores  $g^{-1}$ . The formulation is readily dispersed in water prior to use.

Polyversum™ was applied to the trial using a Silvan Selectra 12 v knapsack (Silvan Pumps and Sprayers, Aus., Pty. Ltd.) as a soil drench using Teejet 8003VP (blue) nozzles in a 3-nozzle boom configuration immediately after dispersing the preparation in water.

Polyversum™ was tested in Victoria on a property with a history of root rot problems against our 'best-bet' chemical regime of one application of metalaxyl (Ridomil Gold 25G®) at seeding and weekly applications of Agri-Fos 600® from week 8 (Table 1.1). Polyversum™ was applied at 400 L/ha. at a concentration of 200 g/400L, while Ridomil Gold 25G® and Agri-Fos 600® were applied at the rates stated in Table 3.1.

The application regime for field trials 1 & 2 is listed in Tables 1.1 & 1.2 respectively. The treatments for both trials were: untreated control (Control); Ridomil Gold 25G® at seeding with weekly sprays of Agri-Fos 600® from 8 weeks onwards (RG + WP); Polyversum was applied two weeks (trial 1) or one week (trial 2) before seeding, at seeding, and then at fortnightly intervals until assessment (PV); and Polyversum applied as before, with Agri-Fos 600® applied weekly after 8 weeks (PV + WP).

The first trial was planted on 27 November 2006 and was finally assessed on 6 March 2007 (week 14). The commercial flat-leaf parsley cultivar Grande was used in this trial. The trial was direct sown at 3 rows per bed on raised beds at a property on North Road in Devon Meadows, Victoria in one bay consisting of 3 lands. This bay was chosen because of its history of parsley root rot. Each land was 1.62 m wide and 30 m long. Each replicate was 4 m long, with a total area of 6.48 m<sup>2</sup> per replicate. Each replicate was arranged in a randomised block formation, with 6 replicates per treatment.

**Table 1.1 Spray regime for spring/summer biocontrol trial in 2006/7**

Treatment	Week -2 13/11/06	0 27/11/06	Week 2 11/12/06	Week 4 22/12/06	Week 6 5/1/07	Week 8 19/1/07	Week 9 25/1/07	Week 10 2/2/07	Week 11 9/2/07	Week 12 16/2/07	Week 13 23/2/07
Control	—	—	—	—	—	—	—	—	—	—	—
RG + WP	—	RG	—	—	—	P	P	P	P	P	P
PV	PV	PV	PV	PV	PV	PV	—	PV	—	PV	—
PV + WP	PV	PV	PV	PV	PV	PV	P	P	P	P	P

PV: Polyversum™; RG: Ridomil Gold 25G®; P: Agri-Fos 600®; WP: Weekly application of Agri-Fos; — no application.

### 1.3.2.2 Trial assessment

Destructive sampling of plants was performed in each replicate plot and each plant was assessed for root rot (presence/absence) at week 14. A 40-cm strip across all three rows in the middle of each replicate was dug up and plants were freed of soil and visually assessed for root rot. The total number of live/surviving plants was counted per defined length (40 cm) as an indicator of pre- and post-damping off.

Parsley plants affected by root rot were cultured for the presence of oomycetes. Following the procedure of Davidson & McKay (2003), root sections were washed in sterile distilled water for 30 s, transferred to water agar (WA) and incubated at room temperature for at least 7 days. Plates were examined microscopically for the presence of oomycetes (sporangia, oospores, typical hyphae) and scored.

### 1.3.3 Parsley field Trial No. 2 (autumn/winter 2007): Biological management of root rot in Victoria under high disease pressure

This trial was planted on the same plot as the previous trial on 19 March 2007 using cv. Grande. Due to time constraints, Polyversum™ was applied one week before seeding, not two weeks as was the case in Trial No. 1. The same layout was used for both trials. Trial No. 2 was finally assessed on 27 June 2007 (week 14, Table 1.2). The trial was assessed for both total number of plants as well as the presence/absence of root rot as per Trial No. 1. Individual plants were cultured for oomycetes as per Trial No. 1.

Table 1.2 Spray regime for autumn/winter biocontrol trial

Treatment	Week -1 12/3	0 19/3	Week 2 2/4	Week 4 16/4	Week 6 30/4	Week 8 14/5	Week 9 21/5	Week 10 28/5	Week 11 4/6	Week 12 12/6	Week 13 18/6
Control	—	—	—	—	—	—	—	—	—	—	—
RG + WP	—	RG	—	—	—	P	P	P	P	P	P
PV	PV	PV	PV	PV	PV	PV	—	PV		PV	
PV + WP	PV	PV	PV	PV	PV	PV	P	P	P	P	P

PV: Polyversum™; RG: Ridomil Gold 25G®; P: Agri-Fos 600®; WP: Weekly application of Agri-Fos; — no application.

### 1.3.4 Data analysis

All data analyses for all trials were carried out using Genstat® for Windows™ 9<sup>th</sup> edition. All incidence of root rot for all trials was scored as a simple ‘yes/no’. The incidence variable analysed was then the presence or absence of root rot expressed as a percentage. The method of analysis for the percentage data was Analysis of Variance. Generalised Linear Models using the binomial distribution were used to analyse the data. LSDs (Least Significant Differences of Means) were calculated at 5%. Similarly, the total number of plants per defined length of row was also analysed by the same method.

## 1.4 Results

### 1.4.1 Initial biocontrol trials

The results for both pot-trial experiments are presented in Table 1.3. There were too few replicates for statistical analysis. In the first pot trial, the results are ambiguous, since a large proportion of the uninoculated control plants showed signs of root rot (M1, Fig. 1.1; Table 1.3) and appeared to be protected when inoculated with *P. oligandrum* (M4, Fig. 1.2).



Fig. 1.1 Root systems of uninoculated control plants (M1); plants inoculated with *P. oligandrum* (M3); plants inoculated with *P. sulcatum* (M2); plants inoculated with both (M4).



Fig. 1.2 Root systems of uninoculated control plants (M1); plants inoculated with *P. oligandrum* (M3); plants inoculated with *P. sulcatum* (M2); plants inoculated with both (M4).

**Table 1.3 Efficacy of *P. oligandrum* against root rot in first biocontrol pot trial**

Treatment	No. of Plants		
	Healthy	Root rot	Root rot (%)
Uninoculated control	8	12	60
<i>Pythium sulcatum</i>	5	15	75
<i>Pythium oligandrum</i>	10	10	50
<i>Pythium sulcatum</i> & <i>Pythium oligandrum</i>	15	5	25

**Table 1.4 Efficacy of *P. oligandrum* against root rot in second biocontrol pot trial**

Treatment	No. of Plants		
	Healthy	Root rot	Root rot (%)
Uninoculated control	6	0	0
<i>Pythium sulcatum</i>	1	5	83.3
<i>Pythium oligandrum</i>	6	0	0
<i>Pythium sulcatum</i> & <i>Pythium oligandrum</i>	5	1	16.7

**Figure 1.3 (L to R) Root systems of uninoculated control plants; plants inoculated with *P. oligandrum*; plants inoculated with *P. sulcatum*; plants inoculated with both.**

In the second trial, no uninoculated control plants exhibited root rot symptoms (Fig. 1.3; Table 1.4). When applied alone, *P. sulcatum* severely affected root systems as there were no lateral roots present. *P. oligandrum* did protect the root system from *P. sulcatum* in the co-inoculated treatment with both lateral roots and the tap root intact and disease incidence was reduced by 80%.

Microbiological isolation managed to re-isolate *P. sulcatum* from roots in all cases where *P. sulcatum* was inoculated, even from roots also inoculated with *P. oligandrum*. However, *P. oligandrum* could not be isolated from either the single-inoculated or the co-inoculated plants.

Due to the high level of biocontrol observed with *P. oligandrum* in the pot trials (up to 80% disease control), it was decided to test *P. oligandrum* as a biocontrol agent in field trials.

#### 1.4.2 Field Trial No. 1 spring–summer 2006

Trial No. 1, grown over the summer months, failed to show any reduction in root rot incidence in either our ‘best bet’ chemical regime, or with the biocontrol agent, either alone or with Agri-Fos 600<sup>®</sup>, compared to the untreated control (Fig. 1.4). There was no significant difference in any treatments when compared to the untreated control (Table 1.5) in either the level of root rot or the number of plants in a defined length.

No oomycetes were isolated from roots exhibiting root rot symptoms. The symptoms of rot were similar for all replicates, showing heavy collar rot sometimes extending down the length of the tap root, with a distinctive ‘dry’ appearance (Fig. 1.5). Root rot symptoms were similar to those caused by *Fusarium* in a previous study (Minchinton *et al.* 2006). Oomycetes such as *Pythium* or *Phytophthora* could not be isolated from roots, but *Fusarium* was isolated from all symptomatic roots.

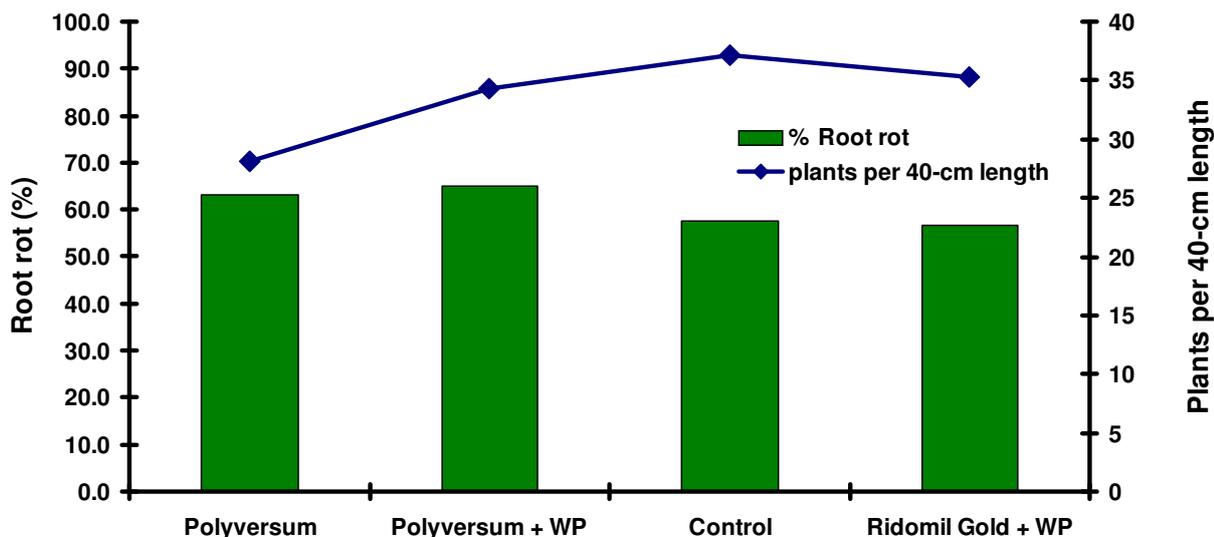


Figure 1.4 Effect of mycoparasite and chemicals to control root rot in flat-leaf parsley in spring/summer. WP: Weekly Agri-Fos 600® spray commencing at 8 weeks

Table 1.5 Statistical analyses of biocontrol field trials

	spring-summer trial (1)		autumn-winter trial (2)	
	Root rot (%)	Total No. plants	Root rot (%)	Total No. plants
Untreated control	63.0	37.1	26.8	14.61
Polyversum	63.3	28.2	39.1	15.06
Polyversum + WP	65.0	34.3	36.7	12.33
Ridomil Gold + WP	56.6	35.3	16.3	16.78
lsd (5%)	7.0	13.6	22.1	5.12

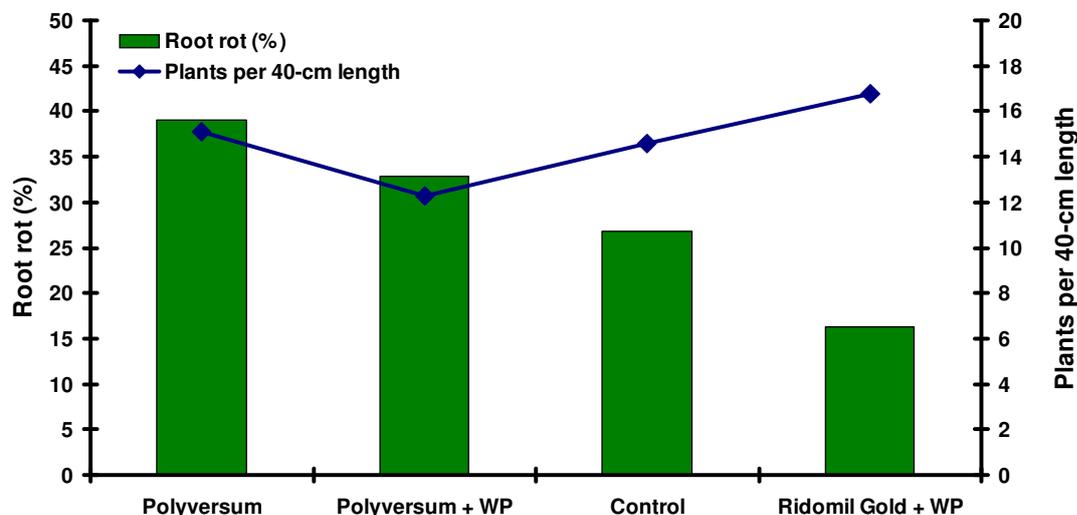


Fig. 1.5 Untreated plants (control) exhibiting root rot symptoms associated with *Fusarium*

### 1.4.3 Field Trial No. 2 autumn-winter 2007

Polyversum™ also failed to reduce root rot in the field in the autumn-winter period (Field trial No. 2, Fig 1.6). However, the incidence of root rot was substantially lower than the previous field trial. There was no significant difference in any treatments when compared to the untreated control in either the level of root rot or the total number of plants in a measured length (Table 1.5). Unlike the spring-summer trial, root rot symptoms were similar to those known to be caused by oomycetes (Minchinton

*et al.* 2006), and oomycetes were isolated from roots of infected plants in all treatments except Ridomil Gold 25G + WP.



## 1.5 Discussion

The interest in the mycoparasite *P. oligandrum* as a potential biocontrol agent is ongoing and active research into its mycoparasitic activity and ability to protect plants or seedlings from pathogenic organisms is continuing (Benhamou *et al.* 1999; Davanlou *et al.* 1999; Mohamed *et al.* 2007; Whipps *et al.* 1993).

The initial pot trials showed that an Australian isolate of *P. oligandrum* was capable of protecting parsley plants from *P. sulcatum*, one of the more aggressive pathogens for this particular crop (Auer *et al.* 2007; Minchinton *et al.* 2006). Root rot incidence was decreased by 80% with co-inoculation of the mycoparasite with the pathogen. *P. oligandrum* was not re-isolated from the roots of any plants challenged with the mycoparasite, but this may be due to several factors, including whether *P. oligandrum* is present in the rhizosphere or in plant tissue (Al-Rawahi & Hancock 1997; Hase *et al.* 2006).

*Pythium oligandrum* appears to have two modes of action for biocontrol activity. Firstly, it has been shown to occupy the rhizosphere in several crops, competing with other organisms in the rhizosphere for nutrients (Al-Rawahi & Hancock 1997). Secondly, it can also form close associations with root cells and actively parasitise pathogenic microorganisms (Benhamou *et al.* 1997).

The initial pot trials were encouraging enough to pursue *P. oligandrum* as a possible biocontrol agent. Polyversum™, consisting of *P. oligandrum* oospores, was sourced from Biopreparaty in the Czech Republic. It is registered for use as a biopesticide in the EU, and has recently been registered in the USA as a biofungicide for use in a variety of crops (Milovsky 2007).

Polyversum™ proved to be ineffective in the field under Victorian conditions in both summer and winter. This may be due to adverse conditions in the field such as lack of adequate water (in the case of the spring-summer trial), or adverse temperature conditions not allowing oospores to germinate. It was impossible to distinguish between the native *P. oligandrum* population and the *P. oligandrum* population 'boost' provided by Polyversum™. In hindsight, a numerical survey before and after application of the biocontrol agent may have been useful.

However, due to the success of the initial pot trial, this mycoparasitic preparation could be effective in other systems such as hydroponics and glasshouse production to protect seedlings from pre-emergence damping off (Whipps *et al.* 1993). The addition of *P. oligandrum* to hydroponics systems has led to an increase in tomato yields (Le Foch *et al.* 2003). That particular study highlighted the

advantages of hydroponics systems being (i) constant temperature and (ii) the abundance of water, both of which optimised *P. oligandrum* colonisation of the root system. In Australia, the addition of *P. oligandrum* to recycling water in hydroponics systems has been proposed as part of an IPM strategy for disease management in the hydroponics industry (NPSI Factsheet 2005/3).

## 1.6 References

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## Chapter 2

### Summer chemical efficacy trial in Victoria

Desmond Auer, DPI Victoria

#### Summary

Extending on our previous years' work (Minchinton *et al.* 2006), chemical options to combat root rot in parsley caused by the water mould fungi *Pythium* and *Phytophthora* in summer were explored. A chemical trial under summer conditions, where the disease pressure from oomycetes is traditionally low, showed that chemical treatments were not effective in preventing root rot. Subsequent analysis showed that, in this case, the disease was not caused by oomycetes, but *Fusarium*, demonstrating that management strategies involving chemicals must be tailored to individual conditions to be relevant and effective. Furthermore, such strategies targeting oomycetes under Victorian summer conditions may not be necessary, since there was little impact on overall crop yields.

#### 2.1 Introduction

The organisms responsible for root rot in parsley were identified and management options were explored in a previous study (Minchinton *et al.* 2006). In summary, oomycetes such as *Pythium* and *Phytophthora*, or a combination of the two were proven to cause root rot in parsley in Victoria under winter conditions. Symptoms included destruction of either the tap root or the lateral roots, or both; stunting, wilting and plant collapse. Chemical management strategies involving judicious use of metalaxyl-m in combination with phosphonic acid provided 87–98% control of the disease in that study.

However, other root rot symptoms had been observed previously in the summer of 2003/2004. Large red lesions appeared on the tap roots of parsley plants with the fine lateral roots mostly intact, with stunting and chlorosis of older leaves evident above ground. The lesions were mostly superficial, only penetrating the surface of the root.

Other chapters in this report detail trials involving a biocontrol agent (Chapter 1) and the use of disease-resistant cultivars to combat root rot in parsley (Chapter 3). This chemical trial was initiated in the spring/summer of 2006/2007, under conditions of low disease pressure, investigating efficacy of the chemical regime which had previously been proven to be effective under winter conditions (Minchinton *et al.* 2006).

#### 2.2 Materials and methods

##### 2.2.1 Chemicals and application

The chemicals used in this trial are listed in Table 2.1. Agri-Fos 600<sup>®</sup> was applied using Teejet SPX No. 12 nozzle (brown), at 30 psi using a Silvan Selectra 12 v knapsack (Silvan Pumps and Sprayers, Aus., Pty. Ltd.) and a 3-nozzle boom at a volume of 500 L/ha. Ridomil Gold 25G<sup>®</sup> was applied as solid granules by hand. Previcur<sup>®</sup> was applied as a soil drench with a watering can. In the seed treatment, parsley seed (cv. Grande) was coated with Apron XL<sup>®</sup> (metalaxyl-m) by the seed treatment company Seed Solutions (PO Box 8239 Carrum Downs Vic 3201).

**Table 2.1 Chemicals used in spring/summer efficacy trial**

Trade name	Active ingredient	Supplier	Application rate	Fungicide Activity Group
Agri-Fos 600 <sup>®</sup>	phosphonic/ phosphoric acid	Agrichem	170 mL/100L	Y
Apron XL <sup>®</sup>	metalaxyl-m (350 g/L)	Syngenta	175 mL/100 kg seed (seed coating)	D
Previcur <sup>®</sup>	600 g/L propamocarb	BayerCropScience	15 mL/10 L at 2L/m <sup>2</sup>	Y
Ridomil Gold 25G <sup>®</sup>	metalaxyl-m	Syngenta	120 g/100 m of row	D

## 2.2.2 Chemical management of root rot under low disease pressure (spring/summer 2006/7)

### 2.2.2.1 Trial layout and spray schedule

A commercial flat-leaf variety (cv. Grande) was used in this trial to test chemical management strategies under spring/summer conditions, which have traditionally been low disease pressure conditions (Minchinton *et al.* 2006). The trial was direct sown in 3 rows on raised beds on a property on North Road in Devon Meadows, Victoria on 22 September 2006 in one bay consisting of 6 lands. This particular bay was chosen because of its history of parsley root rot problems. Each land was 1.62 m wide and 30 m long. Each replicate was 4 m long, with a total area of 6.48 m<sup>2</sup> per replicate. Each replicate was staked out in a randomised block formation, with 6 replicates per treatment, including the control.

The schedule for spraying of chemicals is detailed in Table 2.2, and derived from our ‘best bet’ scenario detailed in the previous report (Minchinton *et al.* 2006). The treatments were; seed coated with metalaxyl-m and weekly phosphonic acid sprays starting 8 weeks after sowing (S + WP); Ridomil Gold 25G<sup>®</sup> at seeding and weekly phosphonic acid sprays starting 8 weeks after sowing (RG + WP); Ridomil Gold 25G<sup>®</sup> at seeding and fortnightly phosphonic acid sprays starting 8 weeks after sowing (RG + 2WP); weekly phosphonic acid sprays throughout the life of the crop until assessment (WP); and application of Previcur<sup>®</sup> at seeding and 4 weeks later (PREV).

**Table 2.2 Application regime for summer chemical efficacy trial**

Treatment	Week 0 22/9	Week 1 29/9	Week 2 6/10	Week 3 13/10	Week 4 20/10	Week 5 27/10	Week 6 3/11	Week 7 10/11	Week 8 17/11	Week 9 24/11	Week 10 1/12	Week 11 8/12	Week 12 15/12
S + WP	S	—	—	—	—	—	—	—	P	P	P	P	P
PREV	PR	—	—	—	PR	—	—	—	—	—	—	—	—
WP	P	P	P	P	P	P	P	P	P	P	P	P	P
RG + WP	RG	—	—	—	—	—	—	—	P	P	P	P	P
RG + 2WP	RG	—	—	—	—	—	—	—	P	—	P	—	P

S: Seed coating of Apron XL; RG: Ridomil Gold 25G<sup>®</sup>; P: phosphoric/phosphonic acid; WP: Agri-Fos 600<sup>®</sup> applied weekly; 2WP: Agri-Fos 600<sup>®</sup> applied fortnightly; PREV: Previcur, — no application

### 2.2.2.2 Trial assessment

The field trial was assessed on 30 October 2006 (5 weeks) and 18 December 2006 (13 weeks). At 5 weeks, each replicate was assessed for plants affected by damping off (wilting, chlorosis, plant collapse).

In the final assessment on 18 December 2006, destructive sampling of plants was performed in each replicate plot and each plant was assessed for root rot. Briefly, a 40-cm strip in the middle of each replicate was scored for root rot and damping off. Two methods of assessment were performed. In the first, a 40-cm length in the middle of each replicate was measured, and then the gaps evident between plants were measured, giving an indication of missing plants/damping off. The total number of plants in that section was also counted. The same 40-cm strip in all three rows was dug up and plants were freed of soil and visually assessed for the presence of root rot (Fig. 2.1).



**Figure 2.1 Chemical efficacy trial assessments (week 13)**

Parsley plants affected by root rot were cultured for the presence of oomycetes. Following the procedure of Davidson & McKay (2003), root sections were washed in sterile distilled water for 30 s, transferred to water agar (WA) and incubated at room temperature for at least 7 days. Plates were then examined microscopically for the presence of oomycetes (sporangia, oospores, typical hyphae).

## 2.3 Results

### 2.3.1 First assessment (week 5) of spring/summer chemical efficacy trial

In the first assessment at 5 weeks, there were no signs of damping off in the first land at any of the replicates, so the average below is taken from 5 replicates (Table 2.3).

**Table 2.3 Parsley seedlings affected by damping off after 5 weeks**

<b>Treatment</b>	<b>Average No. affected (per rep)</b>
<b>S + WP</b>	11.2
<b>PREV</b>	4
<b>Control</b>	2.4
<b>WP</b>	2.2
<b>RG +WP</b>	2
<b>RG + 2WP</b>	1

S: Seed coating of Apron XL; PREV: Previcur; Control: untreated; RG: Ridomil Gold 25G<sup>®</sup>; WP: Agri-Fos 600<sup>®</sup> applied weekly; 2WP: Agri-Fos 600<sup>®</sup> applied fortnightly.

Surprisingly, the seeds coated with metalaxyl-m had higher rates of plant mortality/damping off than the control. Metalaxyl-m was shown to be the most effective chemical for control of oomycetes for about 8 weeks (Minchinton *et al.* 2006). It could be that the seed coating has only a limited protection, as evidenced by the fact that the treatments where Ridomil Gold 25G<sup>®</sup> was applied by hand, there was little evidence of damping off. Previcur was also had higher rates of damping off than the control.

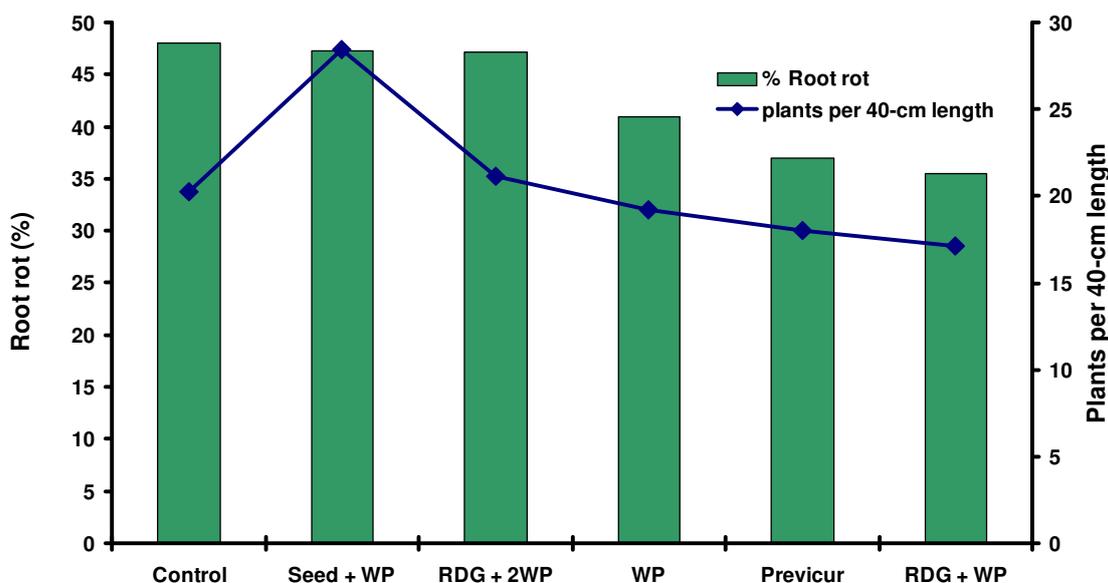
### 2.3.2 Second assessment (week 5) of spring/summer chemical efficacy trial

None of the treatments, including our 'best-bet' treatment (Ridomil Gold 25G<sup>®</sup> followed by weekly Agri-Fos 600<sup>®</sup> after 8 weeks) were effective in reducing the incidence of root rot. There was no significant difference in any treatment compared to the untreated control (Table 2.4) in terms of root rot levels. Root rot levels at week 13 were high for all treatments (Fig. 2.2), which was unexpected, since the chemical treatments targeted oomycetes, previously shown to cause root rot in parsley (Minchinton *et al.* 2006).

**Table 2.4 Statistical analysis of spring-summer chemical trial**

Treatment	Root rot (%)	Total No. of plants
Untreated Control	48.0	20.28 <sup>a</sup>
Seed + WP	47.3	28.44 <sup>b</sup>
RDG + 2WP	47.1	21.11 <sup>a</sup>
WP	40.9	19.22 <sup>a</sup>
Previcur	37.0	18.00 <sup>a</sup>
RDG + WP	35.5	17.11 <sup>a</sup>
lsd (5%)	21.76	5.261

Note: Numbers followed by the same letter do not differ significantly at the 5% level.



**Figure 2.2 Chemical efficacy trials on parsley root rot in spring/summer**

However, the symptoms of root rot in this case were quite dissimilar to conventional root rot symptoms caused by oomycetes (Fig. 2.3). Reddish lesions were evident on affected roots, but there were little or no above-ground symptoms. The roots themselves had a dry, wrinkled appearance, unlike the spongy brown rot caused by oomycetes found in the previous study (Minchinton *et al.* 2006). The lesions were also superficial, not penetrating beyond the surface of the root. In addition, the parsley plants appeared healthy with no evidence of chlorosis, wilting or plant collapse.

Subsequent microbiological analysis of root systems isolated only *Fusarium*, not oomycetes, which this chemical regime was targeting. Similarly, no oomycetes were detected in diseased roots of the untreated control plots. On the other hand, the seed treatment, where seeds were coated with

metalaxyl-m protected the parsley seedlings to some extent (Fig. 2.2), since there was a significant difference in the number of plants per length compared to the untreated control (Table 2.4).



*Healthy roots on the left and roots exhibiting signs of root rot on the right (red lesions, less feeder roots).*

**Figure 2.3 Parsley roots from summer trial**

## 2.4 Discussion

A seed coating of metalaxyl-m also showed some protection from pre-emergence damping off, since the total number of plants in a plot was significantly greater than the untreated control. But this protection is possibly limited, since it also had the highest number of plants affected by damping off post-emergence (Table 2.3). Previcur was also ineffective in the field, but it has been registered for use only under glasshouse conditions against oomycetes (AVPMA PER4970). Resistance to the active ingredient propamocarb has also been observed (Moorman & Kim 2004).

In the final analysis (week 13), the chemical treatments applied under spring/summer conditions proved to be ineffective for the control of root rot, but the root rot symptoms observed in this trial showed little similarity to previously observed characteristics of oomycetes (Minchinton *et al.* 2006). There was also little evidence of the above-ground symptoms usually associated with root rot caused by oomycetes. Microbial isolation showed no presence of oomycetes. Instead, *Fusarium* was the only fungal organism isolated from the diseased roots. Consequently, the chemical regime tailored to manage oomycetes proved ineffective against *Fusarium*.

In Queensland, *Fusarium* has been proven to cause root rot in parsley (Chapter 4). However, when fungicides targeting *Fusarium* were tested in Victoria (iprodione/fludioxonil) they proved to be phytotoxic (Minchinton *et al.* 2006). It is possible that *Fusarium* may not be the primary cause of these root rot symptoms in Victoria, with similar symptoms seen here attributed to the effects of salinity.

In light of the vastly different symptoms occurring in summer and winter conditions, we propose that there are two types of parsley root rot in Victoria: summer parsley root rot (caused by *Fusarium*) and winter parsley root rot (caused by water moulds such as *Pythium* and *Phytophthora*) (Figs. 2.4, 2.5).



Figure 2.4 (left) Summer parsley root rot. Note the leaf chlorosis and the red lesions and the unaffected lateral roots.

Figure 2.5 (above) Winter parsley root rot. Note the lack of lateral roots and soft spongy rot of the tap root. Foliage wilts and rapidly collapses.

The lack of above-ground symptoms and the absence of oomycetes from affected roots demonstrate the need for tailoring management strategies for individual seasons. Chemical treatment of parsley in the spring/summer period appears to be unnecessary against oomycetes. While *Fusarium* could be isolated from plants with root rot symptoms, there was no above-ground symptoms, thus causing minimal economic losses in Victoria.

## 2.5 Conclusion

In Victoria, oomycetes fungi cause root rot of parsley in winter (Minchinton *et al.* 2006). In summer, there was no evidence of pathogenic oomycetes causing root rot and the chemical regime was ineffective for reducing the incidence of root rot. Although root rot was evident, the lack of above-ground symptoms and the fact that there was little difference in yields in the trial suggest that chemical spraying during conditions of low disease pressure from oomycetes is unnecessary and financially wasteful.

*Fusarium* has been shown to be a major root rot pathogen in other plant systems (Benhamou 1992), but it appears to be only a weak pathogen in parsley in Victoria in summer. Depending on the severity or presence of above-ground symptoms, chemical treatment for parsley root rot in Victoria may be reduced or withheld in spring/summer, but if used, should target *Fusarium*, rather than oomycetes.

## 2.6 References

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## Chapter 3

### Cultivar resistance to root rot in Victoria

#### Victorian parsley field trials part 3

Desmond Auer, DPI Victoria

#### Summary

As part of an overall IPM (Integrated Pest Management) strategy for parsley root rot, six commercial flat-leaf parsley cultivars were assessed for root rot resistance in Victoria. The flat-leaf cultivar *Shamrock* was most resistant to root rot under Victorian conditions in winter and had the highest potential yield under conditions in the field that promoted root rot in parsley.

#### 3.1 Introduction

Selection of resistant cultivars has been investigated to combat fungal disease in a variety of crops including broccoli (*Albugo candida*; Minchinton *et al.* 2007) alfalfa (*Verticillium*; Papadopolous *et al.* 1989), chickpeas (*Phytophthora*; Dale & Irwin 1991) and carrots (*Pythium*; Davidson & McKay 2001; Hiltunon & White 2002) to name a few.

This Victorian trial targeted flat-leaf cultivars, since growers report they are more susceptible to root rot than curly-leaf cultivars. All flat-leaf cultivars used in this trial were commercially available to growers and has been grown in Victoria, subject to market trends (Minchinton *et al.* 2006). In a separate trial (see Chapter 4), resistance to root rot in both curly- and flat-leaf cultivars was investigated by Heidi Martin in Queensland.

#### 3.2 Materials and methods

##### 3.2.1 Cultivar sources and trial layout

Six flat-leafed varieties of parsley were trialled for their resistance to root rot under Victorian conditions. Table 3.1 details the source of the cultivars.

Cultivars were planted in a randomised block design in two bays consisting of 6 lands that were 32 m x 1.62 m. Each replicate was 7 m long, resulting in 24 plots per bay at 11.34 m<sup>2</sup>. The trial was direct sown at 3 rows per bed on raised beds at a property on Moores Road, Clyde, Victoria on 14 March 2007. As noted in Table 2.1, both Italian Giant and Rialto were chemically pre-treated by the seed companies prior to delivery, as per those companies' policy.

**Table 3.1 Cultivar source and germination rate**

Cultivars	Source	Address	Germination rate
Grande	SPS Seeds	2/34-38 Ventura Place, Dandenong South Victoria 3175	85% (01/06/05)
Italian Giant <sup>^</sup>	Henderson Seed Group Ltd.	165 Templestowe Road Templestowe Lower 3107	91% (01/09/06)
Plain Leaf	Lefroy Valley	PO Box 97, 18 Dandenong-Hastings Road Tyabb Victoria 3913	85% (01/04/06)
Rialto*	Bejo Seeds	460 Hall Road, Skye, Victoria 3977	N/A
Shamrock	Fairbanks Selected Seed Company	542 Footscray Road Footscray VIC 3011	86% (01/07/06)
Toscana	Terranova Seeds Pty. Ltd.	Unit 13/19 Chifley Street, Smithfield, NSW 2164	85% (01/08/06)

Note: Grande, Plain Leaf & Italian Giant possibly the same variety; \*Thiram & iprodione pre-treated; <sup>^</sup>Thiram pretreated

### 3.2.2 Trial assessment

The trial was assessed at 8 weeks (14 May 2007) for damping off and finally assessed for root rot on June 29 2007 (week 14) using the same methods as before (section 1.2.2.2). Plants showing signs of root rot were also cultured for the presence of oomycetes as before (section 1.2.2.2).

### 3.3 Results

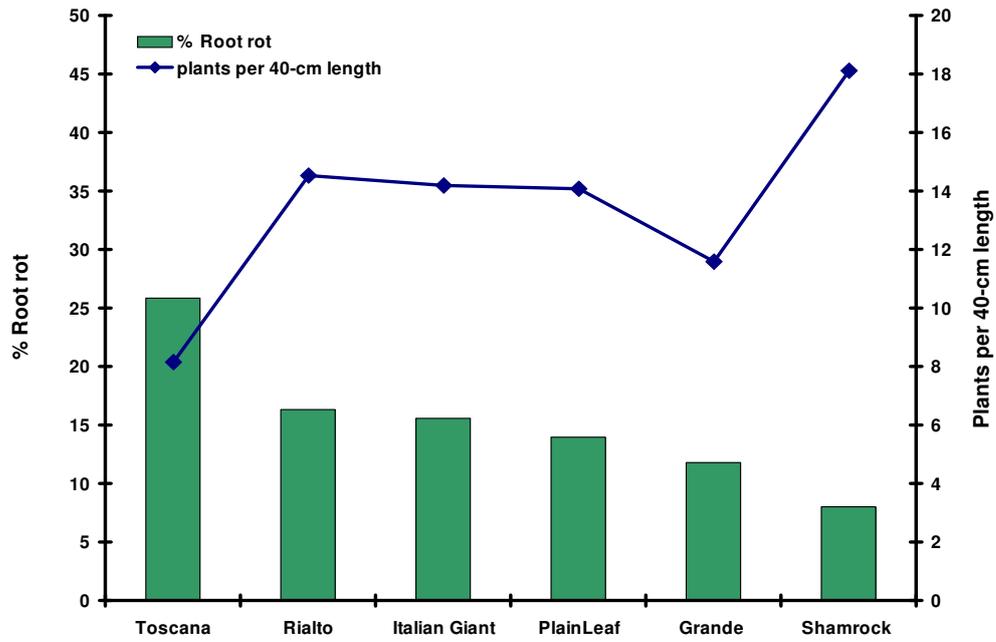
This trial was performed under conditions of high disease pressure from oomycetes, but root rot symptoms were similar to that caused by *Fusarium* (Chapter 2). However, both oomycetes and *Fusarium* were isolated in equal numbers from roots exhibiting symptoms from all samples taken. Toscana was the most susceptible cultivar; both in terms of the number of plants per length (a measure of pre-emergence damping off; Fig 3.1) and root rot incidence (Table 3.2). In contrast, Shamrock was the most resistant cultivar in this trial with the least damping off and lowest incidence of root rot. There was a higher incidence of root rot in Toscana compared to the cultivars Plain Leaf, Grande and Shamrock.

**Table 3.2 Statistical analyses of cultivar trial**

<b>Cultivar</b>	<b>Root rot (%)</b>	<b>log<sub>10</sub> (root rot)</b>	<b>Total plants</b>	<b>Log<sub>10</sub> (Total plants)</b>
<b>Toscana</b>	25.82a	1.554	8.15a	2.098
<b>Rialto</b>	16.28a	1.420	14.53b	2.676
<b>Italian Giant</b>	15.55a	1.407	14.19b	2.653
<b>Plain Leaf</b>	13.96b	1.379	14.08b	2.644
<b>Grande</b>	11.79b	1.338	11.57b	2.449
<b>Shamrock</b>	8.04b	1.256	18.12bc	2.897
<b>Isd (5%)</b>		0.1667		0.2896

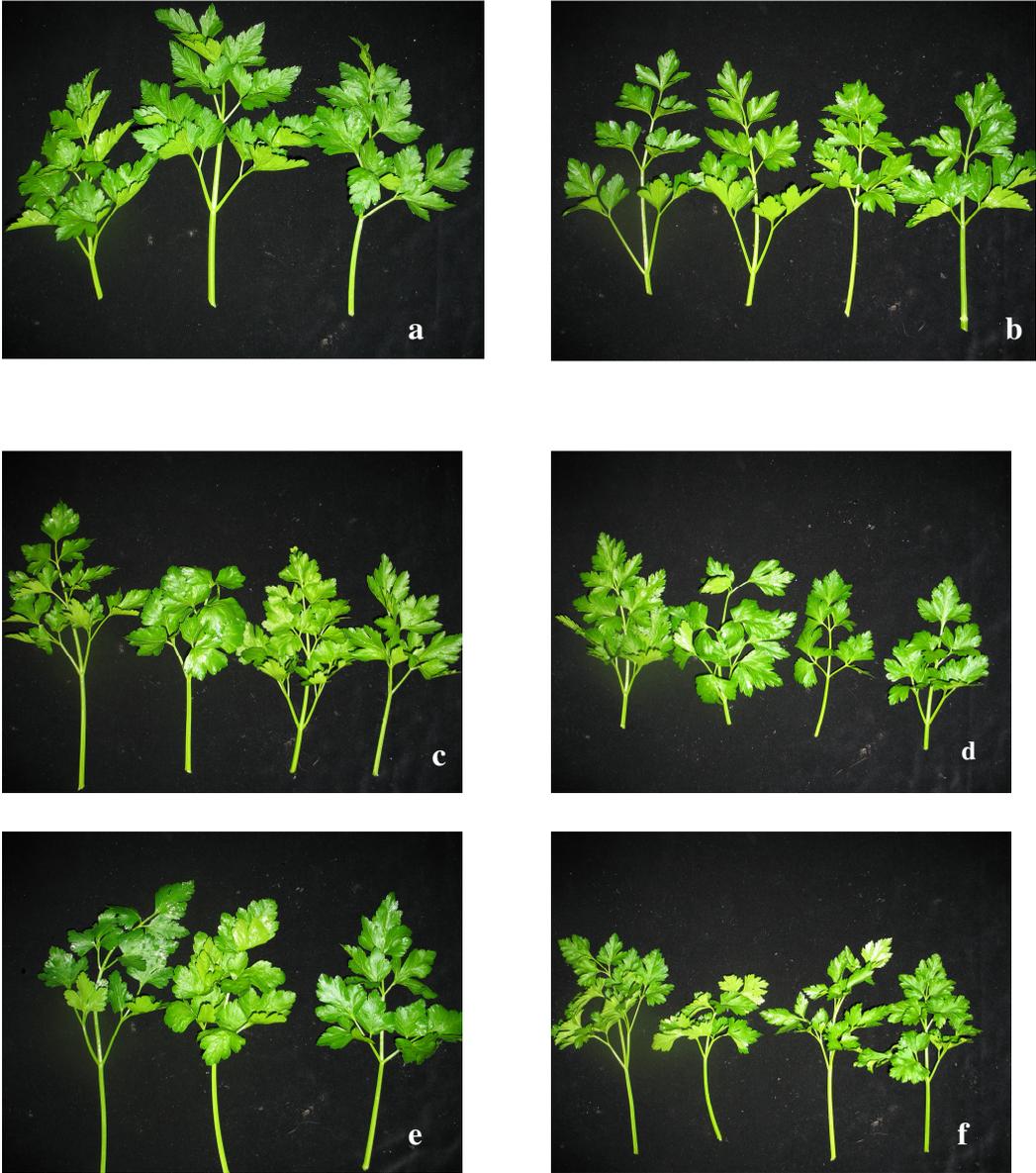
Note: Data transformed logarithmically for statistical analysis. Data presented as log<sub>10</sub> and back-transformed. Numbers followed by the same letter do not differ significantly at the 5% level.

There was no significant difference in the incidence of root rot in Rialto, Italian Giant, Plain Leaf, or Grande. It is suspected that Italian Giant, Plain Leaf and Grande are either derived from the same stock or are the same cultivar, repackaged. There was a significant difference between Toscana and all other cultivars in terms of number of plants per plot, and there was also a significant difference between Grande and Shamrock in terms of total plants per plot. All cultivars were seeded with exactly the same equipment, so any differences in the number of plants per length would be due to the cultivars' individual properties, including resistance to disease and resistance to pre-emergence damping off.



**Figure 3.1 Cultivar responses to root rot under Victorian autumn/winter conditions**

There were no appreciable differences in either plant and leaf size in the various cultivars, apart from Shamrock, which was a smaller bush and had smaller leaves (Fig. 3.2 a–f).



**Figure 3.2 Leaf stalks of cultivars. a) Grande, b) Italian Giant, c) Plain Leaf, d) Rialto, e) Toscana and f) Shamrock.**

### 3.4 Discussion

The winter conditions of 2007 did not lead to characteristic root rot symptoms associated with oomycetes, with root rot similar to that caused by *Fusarium* in summer evident (see Chapter 2). However, both oomycetes and *Fusarium* were isolated from diseased roots. The drier conditions contributed to an atypical winter and may have been factors contributing to root rot symptoms being more consistent with *Fusarium*, rather than oomycetes.

The Victorian cultivar trial demonstrated that Shamrock was the most resistant cultivar to root rot under winter conditions, although there is no significant difference between Shamrock, Grande or Plain Leaf. There was a significant difference in plants density between Shamrock and both Grande and Rialto, but not the other cultivars. This cultivar trial would have to be performed under true summer conditions, typical winter conditions (i.e. cold and wet), as well as a variety of soil types for a complete analysis of the suitability of this cultivar for root rot resistance and yield performance.

Shamrock performed better than all other cultivars in the assessed parameters of root rot incidence and plant density. The trial property routinely plants Shamrock and claims it has always had the best growth, yield and vigour for their market (C. Arnott, pers. comm.). If other attributes of this cultivar are also acceptable, in terms of yield (i.e. how many harvests per planting season), bolting (i.e.. is more than one harvest possible, etc.) and marketability, Shamrock may be a suitable cultivar to grown under conditions of high disease pressure in Victoria.

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## Chapter 4

### Identification of cause and management of parsley root rot in Queensland

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#### Summary

Chemical control and causes of root rot in parsley were investigated in parsley as part of an ongoing study extending from previous work (Minchinton *et al.* 2006). Due to adverse conditions of drought and the lack of disease, conclusions could not be drawn from field trials involving chemical treatment or cultivar testing. Six of nine Queensland isolates from parsley root rot were confirmed as pathogenic, including *Fusarium oxysporum*, *Pythium irregulare* and *Pythium diclinum*. A foliar isolate of *Alternaria petroselini* was also confirmed as pathogenic at several different temperatures. In general, curly-leafed parsley was more susceptible to confirmed pathogens than flat-leafed parsley. The lack of successful field trials highlights the risks associated with conducting trials in projects of short duration.

#### 4.1 Introduction

In recent years, Queensland parsley growers have reported increasing crop losses due to soil-borne diseases, particularly following periods of hot/wet weather at the extremities of the production window. In a previous project VG04025 ‘Scoping study to investigate management of root rot diseases in parsley’, *Fusarium*, *Pythium* and *Phytophthora* species were the most commonly isolated organisms associated with root rot disease symptoms in Queensland parsley plants.

*Pythium* and *Phytophthora* spp. were also identified as the major causes of root rot in Victoria and NSW and furthermore, the fungicides Ridomil Gold 25G<sup>®</sup> or Agri-Fos 600<sup>®</sup> were shown in Victorian field trials to adequately control root rot in winter parsley production.

This project was conducted to continue and finalise aspects of work that were commenced in project VG04025. Specifically, the Queensland component of the work aimed to:

- Determine if control measures identified for parsley crops affected by root rot during cool wet winters in Victoria will also control root rot in Queensland during the wet season
- Complete analysis of pathogenicity tests (Koch’s Postulates) with prospective pathogens collected from Queensland parsley crops
- Investigate the beneficial mycoparasite organism *Pythium oligandrum* as an alternative to metalaxyl
- Investigate the relative susceptibility of flat and curly-leafed parsley varieties to root rot in a Queensland field production system

#### 4.2 Materials and methods

##### 4.2.1 Cultivar trial

Thirteen parsley cultivars, 8 curl leaf types and 5 flat leaved types, were assessed in a field trial planted by Mr Rodney Dunn on-farm at Rochedale, Brisbane at a site with a previous history of disease. Seed lots were provided directly by seed companies (Table 4.1).

**Table 4.1 Parsley cultivars assessed in Queensland on-farm**

Variety	Type	Seed Company
Triple Curled	curl	Terranova
San Marino	curl	Terranova
Green Market	curl	Terranova
Flamenco	curl	Terranova
Darki	curl	Terranova
Forest Green	curl	Terranova
Inca	curl	SPS
Petra	curl	Bejo
Rialto	flat leaf	Bejo
Grande	flat leaf	SPS
Italian Dark Green	flat leaf	Terranova
Large Leaf Italian	flat leaf	Terranova
Toscana	flat leaf	Terranova

All varieties were assessed both as direct seeded plants and as transplants. The trial was planted as a two-way factorial arrangement on a randomised complete block design, with 3 replications (blocks). Blocks were divided into 26 plots, each of which was 4m long. Seeds and transplants were planted by hand on beds 1m wide. Each bed was planted with 3 rows, spaced 30cm apart. Within each row plants were spaced approximately 40cm apart. All direct-seeded plots were sown on 4 January 2007. Remaining plots were planted with seedlings produced by Withcott Seedlings P/L. Plots planted with seedling transplants of flat leaf parsley varieties were planted on 15 January 2007, while seedlings of curl-leaf varieties were planted into plots on 23 January 2007 (Figure 4.1).

The trial was maintained and managed by Mr Rodney Dunn using his usual growing practices. In addition, the trial was monitored regularly for plant development and the development of root rot diseases.



**Figure 4.1: Parsley variety trial at Rodney Dunn's Farm (January 2007)**

#### 4.2.2 Chemical efficacy trial (Queensland, summer)

Ten treatments were compared in a field trial planted at Stockleigh, QLD on 8<sup>th</sup> January 2007, on a site with a previous history of disease (Tables 4.2 and 4.3). All treatments were applied as soil drenches at planting and as foliar sprays 8 weeks after the trial was seeded. Some treatments (Treatments 1, 8, 9, 10) involved additional sprays to those applied at planting and 8 weeks. Plots treated with Agri-Fos 600<sup>®</sup> (Treatment 1) were sprayed weekly, while those treated with the Ridomil Gold 25G<sup>®</sup> and Agri-Fos 600<sup>®</sup> combination (Treatment 10) involved a Ridomil spray at planting, and weekly Agri-Fos 600<sup>®</sup> treatments from week 8 onwards. Plots treated with Polyversum<sup>™</sup> (Treatments 8 and 9) were drenched every 2 weeks commencing 2 weeks prior to seeding. For the Polyversum<sup>™</sup> and Agri-Fos 600<sup>®</sup> combination (Treatment 9), the applications of Polyversum<sup>™</sup> were discontinued after week 8 and weekly applications of Agri-Fos 600<sup>®</sup> were applied from week 8 onwards.

Treatments were applied to plots at the rate of 1670 L ha<sup>-1</sup> with a motorised backpack sprayer, fitted with a 1m-wide boom and 4 twin-jet nozzles.

The trial was planted as a randomised complete block design with 3 replications. Plots were 5m long and 2 bed widths (2.30m) wide, with 2 m x 2.30 m untreated bed sections at the plot ends. The trial was direct seeded 6 rows per bed by tractor with cv. Flamenco seed spaced 15 cm apart.

A regular irrigation program for the trial was initiated after seeding using overhead sprinkler irrigation. The trial was monitored weekly for seedling emergence and subsequent disease development.

**Table 4.3: Schedule of treatment applications for parsley fungicide trial**

Treatment	Week (date)											
	-2	0 <sup>P</sup>	2	3	4	5	6	7	8	9	10	11
	2/12/2006	8/1/2007	22/1/2007	29/1/2007	5/2/2007	12/2/2007	19/2/2007	26/2/2007	5/3/2007	12/3/2007	19/3/2007	26/3/2007
1. Phosphorous acid	—	A	A	A	A	A	A	A	A	A	A	A
2. Carbendazim	—	C	—	—	—	—	—	—	C	—	—	—
3. Metalaxyl	—	M	—	—	—	—	—	—	M	—	—	—
4. Iprodione	—	I	—	—	—	—	—	—	I	—	—	—
5. Captan	—	Cp	—	—	—	—	—	—	Cp	—	—	—
6. Tolclophos-methyl	—	T	—	—	—	—	—	—	T	—	—	—
7. Quintozene	—	Q	—	—	—	—	—	—	Q	—	—	—
8. <i>Pythium oligandrum</i>	Po	Po	Po	—	Po	—	Po	—	Po	—	Po	—
9. <i>Pythium oligandrum</i> / phosphorous acid	Po	Po	Po	—	Po	—	Po	—	Po	A	A	A
10. Metalaxyl / phosphorous acid	—	M	—	—	—	—	—	—	A	A	A	A
11. Control (water)	W	W	W	W	W	W	W	W	W	W	W	W

<sup>P</sup> = planted; A = phosphorous acid; C = carbendazim; M = metalaxyl; I = iprodione; Cp = captan; T = tolclophos-methyl; Q = quintozene; Po = *Pythium oligandrum*; W = water control; — no application

**Table 4.2: Chemical information and application rates**

Trade Name	Active Ingredient	Company	Rate	Activity Group
Agri-Fos 600 <sup>®</sup>	phosphorous acid	Agrichem	170 mL/100L	Y
Spin Flo <sup>™</sup>	carbendazim	Nufarm	50 mL/100L	A
Ridomil Gold 25G <sup>®</sup>	metalaxyl	Syngenta	120 g/100m row	D
Rovral <sup>®</sup> Aquaflo	iprodione	Bayer	100 mL/100L	B
Captan <sup>®</sup>	captan	Farmoz	3.75 g in 3L water/m <sup>2</sup>	Y
Rizolex <sup>®</sup> Liquid Fungicide	tolclophos-methyl	Sumitomo	12 mL/100L	X
Quintozene 750	quintozene	Barmac	0.5 g/m <sup>2</sup>	Y
Polyversum	<i>Pythium oligandrum</i>	Biopreparáty	50 g/100L	N/A

### 4.2.3 Pathogenicity Tests

A total of 29 fungal isolates were collected in disease surveys of Queensland parsley crops in 2005 as a component of the previous study (Minchinton *et al.* 2006). Initial pathogenicity testing was completed in the glasshouse to determine which of the fungi had the capacity to cause disease on parsley plants.

As an extension of this work, we selected 9 of these isolates (Table 4.4) and investigated their pathogenicity to both curl and flat-leaved parsley plants in controlled environment cabinets at three different temperatures.

**Table 4.5: Summary of isolates tested for pathogenicity on parsley**

Isolate	Isolation Method	Identification
1	Root isolation	<i>Fusarium</i> sp.
2	Petiole isolation	<i>Colletotrichum gloeosporioides</i>
3	Crown isolation	<i>Fusarium</i> sp.
4	Crown isolation	<i>Fusarium oxysporum</i>
5	Leaf isolation	<i>Alternaria petroselini</i>
6	Root isolation	<i>Pythium irregulare</i>
7	Root isolation	<i>Pythium diclinum</i>
8	Root isolation	<i>Macrophomina phaseolina</i>
9	Collar isolation	<i>Fusarium solani</i>

Testing was completed using the curl parsley variety cv. Petra and the flat-leaved type cv. Rialto. In each case, eight-week-old seedlings were transplanted, 2 per pot, into 10-cm plastic pots containing a peat/sand mixture. Two weeks after transplant, 6 pots of cv. Petra and 6 of cv. Rialto were inoculated with each isolate.

Fungal inoculum was applied to plants either as conidial suspensions, or as agar culture macerates. To prepare conidial suspensions, 14-day-old cultures grown in the dark at 26°C on half-strength potato dextrose agar (PDA) were flooded with sterile deionised water and scraped with a glass spreader. The number of conidia per mL of inoculum was determined using a haemocytometer, and the spore concentration of each was adjusted by adding sterile de-ionised water. Conidial suspensions of *Fusarium*, *Colletotrichum* and *Alternaria* cultures were all adjusted to 10<sup>6</sup> conidia mL<sup>-1</sup>.

The *Pythium* and *Macrophomina* isolates were applied to plants as agar macerates. *Pythium* isolates were grown on 90mm plates of V8 agar for 14 days and *Macrophomina* was similarly grown on half-strength PDA. Each isolate was finely macerated with a sterile scalpel and half a plate of culture macerate was incorporated into the upper portion of the peat/sand mixture in each pot. Pots treated with sterile de-ionised water were included as controls.

Two pots of each plant type x isolate combination were then incubated in 3 CEC cabinets set at 15°C, 25°C or 35°C, for 21 days. Pots were watered daily, 30 mL per pot, and were fertilised with Aquasol<sup>®</sup> soluble fertiliser once per week at the recommended dose. After 21 days, plants were assessed for disease development and attempts were made to re-isolate the causal pathogens from symptomatic plants onto half-strength PDA, amended with streptomycin (200 µg/mL, PDAA).

#### 4.3.2.1 Additional diseased specimens collected

Samples of diseased parsley plants were received from several farms during this project. Specifically, curl and flat-leaved plants with foliar leaf blight were obtained from a parsley producer at Caboolture; and curl and flat-leaved plants showing stunted, unthrifty growth with root and crown rot symptoms, were obtained from in-ground production at Rochedale.

Fungal isolations were completed for the plants with foliar lesions as follows: Small tissue sections with representative symptoms were dissected from the leaves with a sterile scalpel. The tissue sections were rinsed in sterile water and then immersed in 1% (v/v) sodium hypochlorite solution for

approximately 1 minute. They were then aseptically removed from the sodium hypochlorite and blotted dry on sterile blotting paper. Next, the tissue sections were plated onto PDAA.

Fungal isolations were also completed for the root and crown tissues of the stunted, unthrifty plants from Rochedale. For these plants, tissue sections were plated onto PDAA as well as P<sub>10</sub>VP and P<sub>10</sub>VP + T (P<sub>10</sub>VP supplemented with hymexazol). P<sub>10</sub>VP is a selective medium for the isolation of *Pythium* and *Phytophthora*, and is corn-meal agar supplemented with pimarinic acid (10 µg/mL), vancomycin (200 µg/mL) and pentachloronitrobenzene (160 µg/mL) (Tsao & Ocana 1969).

All plates were incubated in the dark at 25°C. Plates were monitored for fungal growth and colonies were identified on the basis of morphological characteristics. Representative cultures were retained on PDA slopes for long-term storage.

A nematode extraction was also completed by QDPI & F nematologist Jenny Cobon from soil collected from around the root systems of the stunted plants from Rochedale. Small quantities of soil were collected from close to the roots of 10 symptomatic plants in the crop. The soil was combined in a plastic bag and a 200 mL sub-sample was removed and placed in a Whitehead tray for 3 days. Nematodes were quantified and identified to genus level based on microscopic characteristics.

## 4.4 Results

### 4.4.1 Cultivar trial

This trial was conducted to assess the relative susceptibility of parsley varieties to root rot diseases. Unfortunately, no root rot developed in the trial, presumably because drought conditions prevailed during the production of the crop. The mean rainfall total for Brisbane (between the years 1840 and 1994) for the months January through July is 749.1 mm. For 2007, the total rainfall received January–July (332.6 mm) was only about half that expected in an average year.

Environmental conditions favourable for infection processes are an essential requirement for the development of disease epidemics. For the majority of pathogens, high soil and/or leaf wetness periods are necessary for epidemics to develop if susceptible hosts and pathogenic organisms are present. This site had a previous history of root rot diseases, and it was planted with a susceptible host (curl and flat-leaved parsley). It can be assumed therefore, that disease failed to develop because of a lack of appropriate environmental conditions. In particular, low rainfall during the course of the trial seems to have been a major limiting factor to disease development.

Because disease failed to develop in this trial, we were not able to assess the varieties for susceptibility to root rot diseases.

### 4.4.2 Chemical efficacy trial

This trial was completed to assess the efficacy of a selection of fungicides for control of root rot diseases of parsley. The trial site was selected because high levels of root rot had developed in parsley plants at the site in previous seasons.

Two weeks after the site was seeded, heavy rain in an afternoon thunderstorm washed many of the young seedlings from the plots. Subsequent to this event, below average rainfall was recorded for the remainder of the crop cycle.

No root rot developed in this trial, presumably due to environmental conditions that were again unfavourable for disease epidemic development. As for the variety trial, low rainfall seems to have been a major limiting factor for disease development. Because disease failed to develop at the site, we were unable to assess the relative efficacy of fungicides for root rot disease control.

### 4.4.3 Pathogenicity tests

**Table 4.5: Disease symptom development and pathogenicity confirmation**

Isolate	Variety	Temperature (°C)			Symptoms
		15	25	35	
1. <i>Fusarium</i> sp.	Rialto	–	–	–	No disease symptoms
	Petra	–	–	–	
2. <i>Colletotrichum gloeosporioides</i>	Rialto	–	–	–	Slight root browning in cv. Petra at 35 °C
	Petra	–	–	X	
3. <i>Fusarium</i> sp.	Rialto	–	X	X	Severe root rot in cv. Petra Slight root rot in cv. Rialto at 25 °C and 35 °C
	Petra	K	X	X	
4. <i>Fusarium oxysporum</i>	Rialto	–	X	X	Severe root & crown rot in cv. Petra & moderate root rot in cv. Rialto at 25 °C and 35 °C
	Petra	–	K	K	
5. <i>Alternaria petroselini</i>	Rialto	K	K	K	Moderate leaf lesion development in cv. Rialto at all temperatures
	Petra	–	–	–	
6. <i>Pythium irregulare</i>	Rialto	K	K	X	Severe root rot at all temperatures in cv. Rialto and cv. Petra
	Petra	K	K	X	
7. <i>Pythium diclinum</i>	Rialto	–	–	X	Slight root rot at 35 °C in cv. Rialto Rot at all temperatures in cv. Petra, severe symptoms at 35 °C
	Petra	K	K	X	
8. <i>Macrophomina phaseolina</i>	Rialto	–	–	–	No disease symptoms
	Petra	–	–	–	
9. <i>Fusarium solani</i>	Rialto	–	–	X	Severe root rot at 35 °C in cv. Petra Mild root browning at 35 °C in cv. Rialto
	Petra	–	–	K	
10. Uninoculated control	Rialto	–	–	–	No disease symptoms
	Petra	–	–	–	

– = no disease symptoms; X = disease symptoms present but pathogenicity not confirmed; K = disease symptoms present & re-isolation of original isolate (Koch's postulates fulfilled)

Six of the nine isolates assessed in the pathogenicity tests produced disease symptoms on parsley plants and were successfully re-isolated from symptomatic plant tissues. Symptoms tended to be more severe on the curly parsley variety Petra than the flat-leaved variety Rialto, for all the pathogenic isolates except *Alternaria petroselini* (Isolate 5) and *Pythium irregulare* (Isolate 6). In the case of *A. petroselini*, foliar lesions developed on cv. Rialto, but failed to develop on cv. Petra, whereas symptoms of disease caused by *P. irregulare* were of equal severity on both varieties. For both of these pathogens, symptoms developed with equivalent severity at all temperatures.

Symptoms tended to be more severe at higher temperatures for *Fusarium oxysporum* (Isolate 4), *Pythium diclinum* (Isolate 7), *Fusarium* sp. (Isolate 3) and *Fusarium oxysporum* (Isolate 9).

*Macrophomina phaseolina* (Isolate 8), *Colletotrichum gloeosporioides* (Isolate 2) and *Fusarium* sp. (Isolate 1) were not pathogenic to the parsley plants in this study. In addition, no symptoms of disease developed in the un-inoculated parsley plants which were included in the trial as a control.

#### 4.4.4 Diseased plant (parsley) specimens



**Figure 4.2: Flat-leaved parsley plants showing symptoms of *Alternaria petroselini* leaf blight**

The fungus *Alternaria petroselini* was isolated consistently from plants showing foliar leaf blight symptoms that were collected from Caboolture (Figure 4.2).

Although *A. petroselini* was first identified in Australia in 2005 during project VG04025, the pathogenicity of isolates collected from Queensland was not confirmed during this earlier project. Consequently, we completed a pathogenicity bioassay with the *A. petroselini* isolate collected from the Caboolture plants.

A conidial suspension of *A. petroselini* was prepared using 14-day-old cultures grown in the dark at 26°C on half-strength potato dextrose agar (PDA). The cultures were flooded with de-ionised water and scraped with a glass spreader and the number of conidia per mL of inoculum was determined using a haemocytometer. The spore concentration was adjusted to 10<sup>6</sup> conidia/mL by adding sterile de-ionised water.

Five-week old parsley plants were misted until run-off with the suspension and then incubated in a moist plastic bag at 25°C for 24 hours. After incubation, the plants were placed on a glasshouse bench and monitored for symptom development for 14 days. Foliar lesions typical of leaf blight developed after 10 days in the glasshouse and *A. petroselini* was successfully re-isolated from the lesions onto half-strength potato dextrose agar – confirming Koch's Postulates.



**Figure 4.3: Field-grown parsley plants showing damage due to *Meloidogyne* spp. infestation**

No fungal organisms were consistently isolated from the stunted plants with root rot that were collected from Rochedale. Instead, the nematode extraction from soil collected from the root-zones of these plants yielded more than 3500 root knot nematodes (*Meloidogyne* sp.) per 200 mL of soil (Figure 3). Although disease threshold levels for nematodes on parsley have not been well-characterized (Jenny Cobon, personal communication), it is highly likely that such high numbers of root knot nematodes would result in economic damage to parsley crops. PCR identification is currently being conducted to determine the species of *Meloidogyne* responsible for the damage.

## 4.5 Discussion

The work reported here represents a continuation of studies on diseases of parsley that commenced in 2006 (Minchinton *et al.* 2006).

Two *Pythium* species and three *Fusarium* isolates were successfully shown to be pathogenic to parsley in bioassays completed in controlled environment cabinets. Furthermore, the curly-leaved variety cv. Petra was more susceptible to these pathogens than the flat-leaved type cv. Rialto – particularly when the assays were completed at higher temperatures (> 25°C).

In addition, we successfully confirmed the pathogenicity of Queensland isolates of *Alternaria petroselini* on curly and flat-leaved parsley for the first time and root knot nematodes (*Meloidogyne* spp.) were found to be responsible for large yield losses in field-grown parsley plants. Additional work is now being completed to identify the species of *Meloidogyne* responsible for the yield losses.

The difficulties we experienced with both the cultivar and fungicide trials due to prevailing drought conditions highlight the risks associated with conducting field studies in research projects of short duration. To guard against the possibility of trial failure, field studies of the type attempted here should preferably be completed over several seasons.

With regards to the cultivar trial, the grower Rodney Dunn plans to keep the trial for several seasons to assess the relative performance of the different varieties. It may be that in subsequent years root rot may develop in the trial and differences in cultivar susceptibility to root rot pathogens may become apparent, particularly if average or above average rainfall events occur.

## 4.6 References

- Minchinton, E.M., Auer, D.P.F., Thomson, F., Martin, H., Tesoriero, L., Kirton, L. and Trapnell, L. N. (2006). Final report on HAL project VG04025. 'Scoping study to investigate management of root-rot diseases in parsley'. 87 pp.
- Tsao, P.H. and Ocana, G. (1969). Selective isolation of species of *Phytophthora* from natural soils on an improved antibiotic medium. *Nature* **223**: 636–638.

## Chapter 5

### Technology transfer and recommendations

#### Summary

This chapter reports on the benefits of a project advisory group established to oversee research projects. This group increased communication and cooperation between growers, researchers and allied support businesses and resulted in an accelerated impact of research and development within the parsley industry. Recommendations for future research are presented.

#### 5.1 Introduction

The research reported herein is the result of collaboration between industry advisory groups and project steering committees. These groups consisted of vegetable growers, crop consultants and chemical resellers, with diverse experiences which they brought to the project. The groups provided an opportunity for researchers to describe their approach and current progress thus promoting the impact of research and development projects. They also enabled growers and allied industries to ensure their needs are being met by the research project. The advisory group approach worked very well and is DPI's preferred method of involvement with the Vegetable Industry.

This interaction and collaboration with growers, vegetable industry development officers (IDOs) and the subcontracting of sections of work to industry experts has been of enormous benefit to the project. Growers in The Australian Herb and Spice Industry Association were identified through contacts with parsley growers in Queensland. The IDOs identified parsley growers in other states. The advisory committee encouraged the researchers to promote the results of the research to growers nationally in industry publications.

#### 5.2 Industry advisory group

The Department of Primary Industries Victoria has taken the approach of inviting growers and private allied support business representatives to volunteer their time and join with researchers to plan and discuss parsley disease issues first hand. Not all growers are in the position of being able to volunteer their time due to the demands of growing and marketing vegetables and consequently the researchers are extremely grateful to those who were able to contribute.

The advisory group members who supported project VG06046 were:

Craig Arnott – Market Gardener – Arnotts Vegetable Farms – Clyde, Vic.

Peter Cochrane – Market Gardener – P.J. and J. Cochrane Pty Ltd – Devon Meadows, Vic.

Rocky and Tony Lamattina – Market Gardeners – A. D. Lamattina & Sons – Clyde, Vic.

Karl Riedel – Vegetable Crop Agronomist – E.E. Muir & Sons – Cranbourne, Vic.

Lisa and Ray Crooks – Riverview Herbs – Chamber Flat, QLD

Rodney Dunn – Market Gardener– Rochdale, QLD.

#### 5.3 Dissemination of information to industry

Adults acquire information in different ways such as reading, talking and visual cues. Some forms of information distribution will be more useful or accessible than others. There are many methods to distribute information to growers, such as field days, industry publications, workshop meetings and steering committees. During the course of this project we have endeavoured to utilise a broad range of information delivery methods and take every opportunity to report to industry. The Appendix lists the steering committee meetings, field days, workshops, industry publications and technical publications.

## 5.4 Recommendations

*The major recommendations to growers from this work are:*

- The flat leaf parsley variety Shamrock is less susceptible to root rot in Victoria and may be a suitable cultivar for growing under conditions of expected high disease pressure.
- Root rot in summer in Victoria is associated with *Fusarium* which appears to have a low impact on production, consequently control may not be warranted.
- Control of root rot of parsley associated with oomycetes is only necessary during winter when disease pressure is high.
- The application of metalaxyl-m has some efficacy to control damping off during summer but should be used judiciously to comply with resistance management strategies.
- The use of the biocontrol agent in-ground is not justified as it did not control root rot in these trials.

*Areas of future research which would benefit the industry are:*

- Test Polyversum™ in hydroponics systems to assess its efficacy against parsley root rot caused by water moulds (oomycetes).
- Establish the pathogenicity of Victorian isolates of *Fusarium* associated with parsley roots during summer; establish if there is a yield loss and if necessary develop management strategies for Queensland and Victorian parsley crops.
- The Queensland field trials to identify (i) cultivars less susceptible to root rot and (ii) chemical management options need to be repeated, as they were abandoned due to drought.
- Trials to identify cultivars less susceptible to winter (oomycete) root rot under consistent winter conditions are required in Victoria.

## 5.5 Publications

Anon (2006). The cause of root rot in parsley uncovered. *Vegetables Australia Review* 2006. p 32.

Auer, D., Minchinton, E., Cunnington, J., Thomson, F., Martin, H., Forsberg, L. and Tesoriero, L. (2006). Identification and control of root rot of parsley in Victoria. *Proceedings of the 4<sup>th</sup> Australasian Soilborne Diseases Symposium*, Millennium Hotel, Queenstown, New Zealand 3–6 September 2006, p 24–25.

Cunnington, J.H., Minchinton, E.J., Auer, D.P.F. and Martin, H.L. (2007). First record of *Alternaria petroselini* sensu lato causing leaf blight on parsley in Australia. *Plant Pathology* **56**: 723.

Minchinton, E. and Martin, H. (2007). Parsley rot study set to continue. *Good fruit and Vegetables, Vegetable Platter*, **17**: 19.

Minchinton, E.J. and Auer, D. (2007). Parsley root rot research set to continue. *Vege Link, Victorian Vegetable Growers*, autumn issue **29**: p 6.

### Steering committee meeting:

12<sup>th</sup> September 2006, Cranbourne, Vic.

### Field day:

29<sup>th</sup> June 2007, Moores Rd, Clyde, Vic.

### Conferences:

4<sup>th</sup> Australasian Soilborne Diseases Symposium, Millennium Hotel, Queenstown, New Zealand, 3–6 September 2006. Oral presentation of current project.

Australian Herb and Spice Industry Association Conference, Hahndorf, SA, 4–5 September 2006.

National Vegetable Expo, Werribee, Vic., 3–4 May 2007.

Ausveg Conference, Sydney, NSW, 30–31 May 2007.

Australian Herb and Spice Industry Association Workshop, Melbourne Victoria, 25–26 September 2007. Oral presentation of current project.

### Posters:

Auer, D.P.F., Minchinton, E., Cunnington, J., Martin, H. and Fiona Thomson (2007). Root rot in parsley: effect and cause.