Scoping study to investigate management of root-rot diseases in parsley

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VIC Department of Primary Industries

Project Number: VG04025

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This report is published by Horticulture Australia Ltd to pass on information concerning horticultural research and development undertaken for the vegetable industry.

The research contained in this report was funded by Horticulture Australia Ltd with the financial support of the vegetable industry.

ISBN 0 7341 1289 0

Published and distributed by:
Horticulture Australia Ltd
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Sydney NSW 2000
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Horticulture Australia VG04025

(April 2006)

Minchinton *et al*

Primary Industries Research Victoria, Knoxfield Centre

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Purpose of project:
This project details the outcomes of a 12-month scoping study of root rot of parsley which investigated primary causes and treatments for root rot in the states of NSW, Queensland and Victoria.

Report completed: April 2006.

Primary Industries Research Victoria

Knoxfield Centre

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Media Summary

Parsley root rot woes controlled

Scientists have identified the cause and control of a root rot disease that severely affects Victorian parsley crops. The disease can cause up to 100% crop losses.

Root rot attacks seedlings and mature plants, generally at the soil line causing a spongy, dull brown rot and a massive loss of roots. It results in the complete collapse of the shoot system.

Field trials conducted on a commercial crop of parsley in Victoria identified two fungicides which completely controlled the disease. These fungicides are from different chemical groups, therefore their use should conform to management of chemical resistance strategies.

Parsley root rot in Victoria was associated with the water mould fungi *Pythium* and *Phytophthora*. The disease is prevalent during the late autumn and winter, especially after heavy rains when soil temperatures are low. Eight-week-old crops are highly susceptible.

Surveys of parsley crops in Queensland, New South Wales and Victoria indicated that a similar disease occurs on parsley in Queensland during periods of warm wet weather. Root rot was of lesser importance on parsley crops in New South Wales.

In laboratory trials conducted in Queensland and New South Wales, a bacterium and a number of fungi, other than water moulds, caused collar rot, root rot and crown rot.

The cause and control of root rots in Queensland parsley crops now needs to be addressed as well as the control of fungi other than water mould, which cause collar and crown rots.

Information resulting from this research is being presented in a poster on parsley diseases and in a notebook that will be distributed nationally to industry 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 and New South Wales Department of Primary Industries. 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 matched 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. In Queensland growers reported root rot was worse during the wet season. Some Queensland growers have established hydroponic production to avoid crop losses and maintain production through the wet season.

This 12 month scoping study:

- Surveyed parsley crops in the major cropping regions of Queensland, NSW and Victoria to identify the main diseases affecting production in Australia,
- Identified the causes of root rot in Queensland, NSW and Victoria by conducting pathogenicity tests (Koch's postulates) on fungi isolated from root lesions,
- Established management strategies to control root rot in Victorian parsley crops and in so doing identified the types of organisms responsible for the disease.

The method of parsley production varies between the states. In Queensland it is either hydroponically grown and hand harvested by removal of older foliage, or in-ground and mechanically harvested, with either trickle or overhead irrigation. Victorian crops are direct seeded, overhead irrigated and hand harvested by cutting all the foliage at ground level. NSW crops are either direct seeded or transplants, overhead irrigated and hand harvested.

Systematic surveys of 31 parsley crops in Queensland, NSW and Victoria showed that root rot and collapse of in-ground parsley plants was the main concern of growers in Queensland and Victoria, but to a lesser extent in NSW. The major foliage disease was Septoria leaf spot, which was more common in field-grown crops than in hydroponically grown crops and more common in Victoria than elsewhere. Leaf blight caused by *Alternaria petroselini* was reported for the first time in Australia, where it caused economic losses to hydroponic parsley crops in Queensland. Root knot nematodes, *Meloidogyne* species, were observed on parsley in NSW and Queensland but not in Victoria.

Pathogenicity tests were conducted on bacteria and fungi consistently isolated from diseased parsley roots in the three states. A Queensland isolate of *Fusarium solani* caused collar rot, whilst *Fusarium* species from NSW regions were only weakly pathogenic producing root browning. The most significant pathogen in NSW crops was *Rhizoctonia solani* which was very pathogenic and caused collar rot. A *Sclerotinia* species isolate from NSW produced a watery petiole and crown rot. *Strentrophomonas maltophilia* was the only pathogenic bacterium producing a crown and root rot of parsley in Queensland.

Isolates of *Pythium* and *Phytophthora* species from Queensland were not pathogenic despite *Pythium* species being consistently associated with root rot and losses in hydroponic parsley. NSW isolates of *Pythium* and *Phytophthora* species were associated with reduced root mass, root browning, collapse of plants and low rates of mortality. In Victoria pathogenicity of *Phytophthora inundata, P. megasperma* and *Pythium sulcatum* was established. All caused stunting, chlorosis, wilt and a dull, soft, brown root rot. *P. sulcatum* caused a rapid rot of lateral roots, whilst *P. megasperma* produced a slower rot of the tap root. Pathogenicity was not established for a *Pythium* sp., *P. oligandrum, P. intermedium, P. ultimum,* an isolate from the *P. diclinum* 'group' and two unidentified *Phytophthora* isolates. *Pythium oligandrum* is known to be a mycoparasite and therefore may have been beneficial.

This project identified that Oomycete fungi from the family Pythiaceae, were the most likely cause of crop losses in Victorian grown parsley. In a field trial, two applications of a metalaxyl fungicide and weekly applications of phosphonic acid after appearance of symptoms, controlled the disease by 87 and 98%, respectively.

Chapter 1

Parsley Diseases

Desmond Auer, Elizabeth Minchinton, Len Tesoriero and Heidi Martin

1.1 The purpose of this scoping study:

- Produce a review for growers on diseases in parsley,
- Survey parsley crops in the major cropping regions of Queensland, NSW and Victoria to identify the main diseases affecting production in Australia,
- Identify the causes of root rot in Queensland, NSW and Victoria by conducting pathogenicity tests (Koch's postulates) on fungi isolated from root lesions,
- Establish management strategies to control root rot in Victorian parsley crops and in so doing identified the types of organisms responsible for the disease.

This chapter reports on parsley production and crop losses in Australia and provides a review of parsley diseases in the following section. The information has been compiled from the scientific literature and the World Wide Web.

1.2 Production of parsley in Australia

The national production of parsley is 1,160 tonnes on 233 ha (ABS 2001) and worth approximately $8.3 million/yr ($35,840/ha/yr). Victoria, New South Wales and Queensland have about equal market share. Parsley is grown as an annual crop either 'in-ground' or hydroponically. In-ground crops are either handpicked 2–3 times per year where the whole shoot is harvested or, alternatively, harvested mechanically. Hydroponically grown parsley is handpicked every 10–14 days by harvesting only the oldest leaves, with production largely confined to southern and southeastern Queensland. Mechanical production is largely located in central Queensland. Most parsley is sold bunched for the fresh market. Mechanically harvested crops are either processed for the fresh or dried market. There is a small export market for organic parsley.

1.3 Crop losses due to disease

Crop failures of up to 100% have been recorded for parsley in both Queensland and Victoria. Diseases causing major commercial losses in Australia are root rots, which occur in Queensland during warm wet weather and in Victoria during cool, wet weather. Leaf spot, caused by *Septoria petroselini*, is the predominant foliage disease of parsley. Leaf blight caused by *Alternaria petroselini* and root-knot nematode damage caused by *Meloidogyne* sp. have caused major economic losses on individual farms. Viral diseases appear to be more of a curiosity, than the cause of crop losses in Australia.

1.4 Parsley diseases

There is little information on parsley diseases, especially in Australia. State herbaria have limited collections of parsley diseases (Appendix 1). A number of diseases occur on parsley in Australia and overseas, with some better documented than others. Whilst it is easy to identify the causal agent of some parsley diseases, others remain elusive. Many diseases that occur on related Apiaceae, such as carrots and celery, also occur on parsley.

Alternaria Leaf Blight / Scorch

Cause:

The main cause of leaf blight is the fungus *Alternaria petroselini,* which has recently been identified for the first time in Australia. Other species of *Alternaria* that cause this disease include *A. selini* and *A. smyrnii* [1].

Symptoms:

Infection begins as a brown to black patch at leaf margins. This patch expands and the entire leaf yellows, then browns and collapses (see Figs 1 & 2 below). In severe cases, complete defoliation occurs. *A. petroselini* can infect at all stages of growth, but very young or very old leaves tend to be more susceptible [1].

Disease development:

The disease is associated with temperatures around 28°C, heavy rains and humid weather. Spores can be dispersed by wind, rain splash or through handling. The disease can be seed-borne. It caused severe crop losses in Queensland during 2005. It is rarely observed in Victorian crops.

Fig 1: *Alternaria petroselini* blight.

Fig 2: Advanced *Alternaria* infection.

Management and control:

Controls for Alternaria leaf blight include:

- Avoid long periods of leaf wetness by overhead irrigating when dew is normally on the leaf.
- Planting in fields where parsley or carrots have not been planted for several years.
- Rotation of crops regularly to discourage re-infection.
- Purchase high quality seed from a reputable source.
- Hot water treat seed at 50°C for 20 min.
- Old plantings should be destroyed and disked in to avoid spread of the fungus to younger plantings [4].

Chemical use:

The only chemical permit available for *Alternaria* on parsley is fungicides containing copper in the form of copper hydroxide or mancozeb to December 2006 [2]. This covers NSW, Tasmania and South Australia only. For the ACT, Queensland, NT and WA, the only registered fungicide for fungal leaf diseases of parsley including Alternaria leaf spot are fungicides containing copper in the form of cupric hydroxide alone [3].

Bacterial Leaf Spot

Cause:

The bacterium *Pseudomonas syringae* pv *apii* causes a leaf spot of parsley.

Comment:

Bacterial spot, caused by a Pseudomonas has been recorded on parsley in Australia. The disease also occurs on celery and fennel. It can be difficult to distinguish from leaf spots caused by the fungus *Septoria*.

Symptoms:

Symptoms initially appear as small angular water soaked lesions on leaves. They turn a rusty brown and develop a greasy appearance, especially at the margins of the lesion. Lesions may coalesce causing extensive leaf death. During dry conditions they have a papery texture and turn a light brown colour.

Disease development:

The bacterium can be seed borne. It can survive on tissue without causing symptoms (epiphytically) until conditions are conducive to a disease outbreak. The bacterium enters plants through wounds and natural openings. It can be transmitted from plant to plant by overhead irrigation, machinery, by insects and by hand. The disease prefers warm temperatures, high humidity and long hours of leaf wetness, at least 7 hrs/day over several days. On celery leaf spots appear 7–10 days after infections.

Photo courtesy of R.M. Davis [20].

Management and control:

- If seed contamination is suspected, soak seed in hot water at 50°C for 25 minutes.
- Irrigate when long periods of leaf wetness can be avoided, such as around sunrise, when dew is normally formed on leaves.
- Avoid fertilisers high in nitrogen as they stimulate lush growth that is very susceptible to bacterial leaf spot.

Chemical use:

No chemicals are registered for control of this disease on parsley.

Bacterial Shoot Blight

Cause:

The cause of this disease in unknown, but appears to be associated with a bacterium, most likely a *Pseudomonas* sp.

Symptoms:

The disease appears as a wet, tan coloured rot of young foliage at the leaf margins which later progresses down the stalk. Symptoms are generally hidden by older, symptomless foliage.

Disease Development:

Little is known of this disease and pathogenicity has not been confirmed. The disease appears to occur in autumn and is associated with dense canopies. It has been an issue on crops grown in Tasmania. In Victorian crops it does not appear to be associated with crop losses. It is rare in NSW and Queensland parsley crops.

Fig 1 and 2: Blight of young leaves.

Management and control:

One grower reported application of copper and mancozeb were beneficial.

Chemical Use:
There are currently no chemicals listed for the control of this disease.

Bacterial Soft Rot or Leaf Drop

Cause:
Soft rot has been associated with the bacteria *Erwinia* species and *Stentrophomonas maltophilia*.

Symptoms:
Above ground symptoms first appear as a wilt of foliage which rapidly progresses to canopy collapse with a distinctive white bleaching of leaves (Fig. 1). Infected plants may be stunted. Infections may be associated with a soft watery basal root rot (Fig. 2) or crown rot. In advance stages a cross section of the crown often reveals complete break down and rot of the cortex (Fig. 3). It is often difficult to remove the root system intact. The disease has been associated with severe crop losses in Queensland.

Disease development:
Little is know of this disease and the cause is not completely certain. It is prevalent on parsley grown in-ground in Queensland during the wet season, especially after heavy rains. Bacteria are ubiquitous in soils. It most likely survives in crop debris in the soil and is probably common in surface water sources. Bacteria enter plants through wounds and natural openings [5].

Fig 1: Above-ground collapse and bleaching of parsley foliage. Fig 2: Basal soft root rot of parsley plant. Fig 3: soft rot of crown associated with bacteria and *Phytophthora.*

(Figs 1 & 2 courtesy of Julia Telford, Fig 3 courtesy of Heidi Martin QDPI&F).

Management and control:
Avoid excessive soil moisture and mechanical damage to roots and maintain a well-drained site.

Chemical Use:
No bactericides are currently registered for leaf drop on parsley.

Botrytis Blight or Grey Mould

Cause:

The fungus *Botrytis cinerea*, is a common pathogen with a wide host range. In Australia it has not yet been reported to cause disease on parsley.

Symptoms:

The disease appears as tan to brown spots on leaves, which can be accompanied by a greyish mould colonising the damaged area [6]. The mould produces masses of grey spores and black irregular shaped sclerotia may form in infected plants. The fungus also causes post-harvest rots.

Disease development:

The fungus is widespread in nature and the disease is highly weather-dependent. Plants are infected when cool wet weather leads to persistent humidity in the canopy. Spores are dispersed by air and need water to germinate. Low temperatures slow disease development.

Fig 1: Grey sporulation of *Botrytis cinerea*. (Photo courtesy of S T Koike [20].

Management and control:

Avoid leaf wetness:

- A short heavy watering in the morning will allow the leaves to dry.
- Increase ventilation by reducing overcrowding, since this will allow rapid leaf drying [6].

This fungus is capable of overwintering, so after harvest, either remove all plants or cleanly plough leftover plant material into the ground.

Chemical use:

The only current permit against Botrytis blight in parsley, covering all states except Victoria, is for fungicides containing copper hydroxide as the sole ingredient, the permit for which is due to run out in February 2006 [3].

Downy Mildew

Cause:

The fungus *Plasmopara petroselini* causes downy mildew on parsley. It has not yet been reported in Australia.

Symptoms:

Initial symptoms consist of white spots on the upper leaf surface. As the disease progresses, the spots enlarge, became angular, and turn yellow. On the under surface of the leaf spots, white-to-greyish white mycelia and spores develop (Fig. 1). Eventually infected leaves and leaf stalks rot [8].

Disease Development:

The fungus requires living tissue to grow. It infects young leaf tissues under cool, wet conditions. Spores are produced overnight on the undersurface of leaf spots and released in the morning as the humidity drops. These airborne spores are dispersed by wind. They are deposited on leaf surfaces and require water for germination and infection. Resting spores (oospores) are produced in leaf tissue and survive in crop debris or in seed.

Fig 1: White-grey spores of downy mildew on the undersurface of a leaf.

Management and control:

- Avoid long periods of leaf wetness.
  If possible irrigate in the early mornings when dew is on leaves, as this will not extend the natural period of leaf wetness. A short heavy watering is preferable to a long light watering as the period of leaf wetness is reduced. If practicable avoid overhead irrigation.
- Increase ventilation.
  Reduce plant densities to decrease the duration of leaf wetness by increasing airflow. Increase drainage to reduce humidity.
- Deter carry-over of crop debris, which may contain fungal spores.
  Plough in crop debris to encourage its decomposition and rotate ground out of parsley crops.

Chemical use:

For control of downy mildew in parsley, there is a temporary permit for copper hydroxide [2] until 31 December 2006 in the states of NSW, Tasmania and South Australia.

Powdery Mildew

Cause:

Powdery mildew is caused by the fungus *Erysiphe heraclei*.

Symptoms:

Powdery mildew causes pale yellow areas on the upper leaf surface associated with whitish sporulation on the lower surface. In the advanced stages, sporulation occurs on the upper surfaces (Figs. 1, 2) and the lesions turn brown. Both petioles and stalks can be colonised (Fig. 3) [9]. The fungus lives on live plant tissue and grows primarily on the outer surface of the plant.

Disease development:

Powdery mildew spores are spread by wind and do not require water to germinate. Conditions of high humidity and moderate temperatures favour infection and disease development. Powdery mildew is more severe under shaded areas since sunlight damages the spores and the mycelium. Older plants tend to be more susceptible to powdery mildew.

Fig 1: Powdery mildew in parsley bunch.

Fig 2: Upper surface of leaf.

Fig 3: Stalk infection.

(Courtesy of ADAS UK Ltd) [11].

Management and control:

- Avoid shady growing conditions, water stress and excess fertilisation.
- Maintaining good plant vigour.
- Heavy rainfall deters powdery mildew.

Chemical use:

Permits for fungicides containing sulphur as the sole component for use against powdery mildew (*Oidium* stage of *E. heraclei*) on parsley and other herbs are current only until February 2006 in all states except Victoria [10].

Comment:

Although common in parsnip, powdery mildew has not yet been reported on parsley in Australia. *E. heraclei* can also infect celery [1].

Root-Knot Nematodes

Cause:

This disease is caused by two nematodes: *Meloidogyne hapla* and *M. incognita*.

Symptoms:

Symptoms include stunted growth, leaf yellowing and wilting during the hottest part of the day. Affected plants are somewhat smaller. Symptoms spread rapidly through a site as the season progresses and succeeding generations of juveniles hatch out. That is why fields affected by root-knot nematodes tend to be patchy (Fig. 1). Plants rarely die prematurely from nematode feeding unless pest pressure is very high. Root-knot nematode feeding stimulates the development of abnormally large cells, resulting in galls 1–20 mm in size (Fig. 2) formation along the roots. These galls prevent adequate water and nutrient uptake resulting in stunted plants. Unlike the nitrogen-fixing nodules of legumes, these galls cannot be rubbed off the root (Fig. 3). Pinhead-sized 'worms' visible to the naked eye may be seen when galls are sliced open [12].

Disease development:

The optimum temperature range for nematode development is 15–30°C. They are generally more severe in sandy and muck soils than in clay soils. Nematodes are less active in cool weather and low soil temperatures. They have not been observed on parsley in Victoria, but can occur in NSW and Queensland.

Fig 1: Field infested with root-knot nematodes.

Fig 2: Parsley affected by root-knot nematodes.

Fig 3: Main taproot of parsley showing galls. (Photograph courtesy of Heidi Martin, QDPI&F).

Management and control:

- Increasing organic matter, such as fowl manure, in the soil encourages organisms that compete with and consume nematodes.
- Remove infested plants to minimise the spread of nematodes to the rest of the crop.
- If possible, rotate crops or leave the area fallow for a year.

Chemical use:

There are currently no permits for the control of root-knot nematodes in parsley.

Fusarium Root Rot

Cause:

Fusarium species.

Comment:

Fusarium species are often implicated along with *Pythium* and *Rhizoctonia* as causing root and crown rot of parsley. However, research reported in Chapter 3 suggests they may only be weak pathogens of parsley.

Symptoms:

In severe infections *Fusarium* fungi produce a crown and root rot (Fig. 1). *Fusarium* fungi are also associated with milder symptoms consisting of yellowing of foliage, especially the older foliage, loss of vigour, wilt, a brown discolouration of roots and a reduction in root mass.

Disease development:

Little is known of *Fusarium* on parsley. *Fusarium* species survive in plant debris, weeds or as spores in the soil. Their ability to cause disease depends on temperature, their density in soil and the susceptibility of host plants. Symptoms develop more rapidly at warmer temperatures. In some vegetable crops symptom development is associated with low nitrogen (N) and phosphorous (P) and high potassium (K), low soil pH, short day lengths and low light intensity. The disease is enhanced by ammonium nitrogen and decreased by nitrate nitrogen.

Fig 1: Crown and root rot of parsley in Queensland. (Courtesy of Heidi Martin QDPI&F).

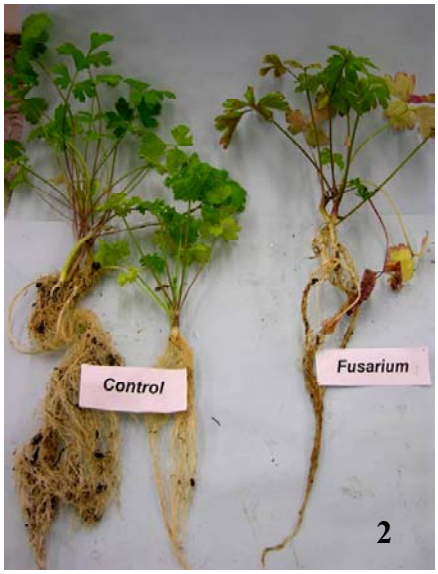

Fig 2: Mild infection of parsley root by *Fusarium* showing browning of roots and some yellowing of foliage. (Courtesy of Len Tesoriero NSW DPI).

Management and control:

- Crop rotation is marginally effective.
- Avoid flooding production areas, as this will spread the fungus.
- Prevent movement of *Fusarium*-infested plants and soils that may cling to machinery, transplants, vehicles and tools.
- In some vegetable crops, raising the soil pH to 6.5–7.0 and using nitrate rather than ammonium forms of nitrogen has been beneficial.

Chemical use:

No fungicides are registered for *Fusarium* diseases of parsley.

Rhizoctonia Crown and Collar Rot

Cause:
The fungus *Rhizoctonia solani*.

Symptoms:
Rot of the root, crown and leaf stalk which leads to plant collapse. It has also been associated with pre- and post-emergence damping off of seedlings. Lesions are reddish-brown and often sunken.

Disease development
The fungus is ubiquitous in the soil. It survives as either growing or resting mycelium or sclerotia and can colonise dead plant material. Moderate weather conditions and moderate soil moisture promote *Rhizoctonia* infections. Fungal development is inhibited by dry or waterlogged soils.

Management and control:
- Plant good quality seed.
- Maintain optimum growing conditions with respect to temperature, moisture and nutritional requirements.
- Avoid nematode damage as this can provide the fungus with a mode of entry into plants.

Chemical Use:
At present, there are no chemicals registered for use against crown and collar rot caused by *Rhizoctonia* in parsley.

Root Rot caused by *Phytophthora* and *Pythium*

Cause:

In Victoria, the main causes of root rot of parsley are species of *Phytophthora* and/or *Pythium*. Both fungal pathogens have been reported on parsley in Queensland and NSW.

Symptoms:

Shoot symptoms: For *Phytophthora* and *Pythium*, the aboveground symptoms are similar and include pre- and post-emergence damping off of seedlings (Fig. 1) and death and decline of mature plants (Fig. 2). Above ground symptoms are a rapid wilt of foliage (Fig. 3), rot of leaf stalk bases, collapse of the shoot system and plant death. Surviving plants are stunted, show a general yellowing of foliage and are often surrounded by nominally healthy plants.

Fig. 1: Parsley field with post–emergence damping off.

Fig. 2: Plant collapse of mature parsley plants due to *Phytophthora* and/or *Pythium* infection. Note wet conditions.

Fig. 3: Rapid wilt of foliage (left) and collapse of shoot (right).

Fig. 4: Symptoms of *Pythium*. Note, soft watery rot of basal stems, lack of lateral roots and dull, spongy appearance of upper tap root.

Root symptoms: Root rots associated with *Pythium* and *Phytophthora* tend to be a dull brown and spongy in appearance and feel (Fig. 4). *Pythium* tends to attack the tips of lateral roots, and infects at the crown or at the upper root near ground level, leaving little or no lateral root system (Figs. 5 and 6). For *Phytophthora*, infections appear to start at the root tips and travel up the roots, but can occur elsewhere on the root. Infected roots are light to dark brown and lateral roots are still present in many cases (Fig. 7). Anecdotal evidence suggests that *Phytophthora* infections tend to be slower, with *Pythium*-infected plants showing plant collapse and root rot symptoms earlier.

Fig. 5: Parsley seedlings infected with *Pythium*. Note lack of lateral and taproots.

Fig. 6: Close up of parsley plant infected with *Pythium*. Note lack of roots (laterals and taproot) and brown lesion at ground level.

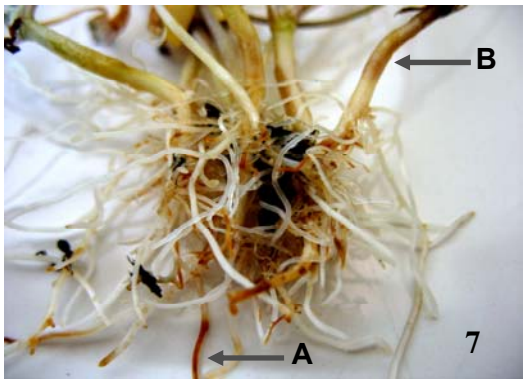

Fig. 7: Parsley seedlings infected with *Phytophthora*. Note rotting of root tips (arrowed A), some rotting of root at ground level (arrowed B), lack of taproot and extensive development of apparently new lateral roots.

Disease development:

In Victoria root rot appears in crops during late autumn and winter when soil temperatures are in the vicinity of 10°C or less and especially after a period of heavy rainfall. Plants of any age appear to be susceptible. In field trials, the disease appeared 8 weeks after emergence. Species of *Phytophthora* and *Pythium* are ubiquitous in soils. They produce two types of spores, oospores and zoospores. The thick walled oospores can survive in soil during adverse conditions and serve to carry the fungus 'over' from one crop to the next and thus one season to the next. The motile zoospores are the principal means of dispersal and infection, enabling the fungus to move in irrigation water or in saturated soils. Both *Pythiums* and *Phytophthoras* can have a broad host range.

Management and control:

Farming practices for management and control of both *Pythium* and *Phytophthora* are identical:

- Raise beds to improve drainage and reduce waterlogging or saturation around roots.
- Time irrigation to avoid wet or dry extremes of soil water.
- Avoid irrigation when heavy winter rain is forecasted.
- Avoid planting on low-lying areas.
- Rapidly incorporate crop debris into soil to encourage breakdown, as *Pythium* and *Phytophthora* have a broad host range and can survive saprophytically.
- Rotating crops, especially with barley, beet or onions was beneficial in Northern Ireland, perhaps due to the addition of lime with the former.

Chemical use:

A temporary permit for the use of phosphonic/phosphorous acid has been granted for parsley and other culinary herbs in all states except Victoria to combat root rot by *Phytophthora* only until September 2006 [7]. There is a one-day withholding period for this fungicide. At this time, no permits have been issued against root rot caused by *Pythium*.

Sclerotinia Rot (Basal Stem Rot)

Cause:

The fungus *Sclerotinia sclerotiorum* causes Sclerotinia rot, also known as white mould.

Symptoms:

The first sign of infection is a white cottony mould at the soil line, which is characteristic for this disease (see Fig. 1). Eventually, the infected tissue turns brown and watery. As the rot progresses, leaves drop off and the parsley plant will decay and collapse [13]. In advanced stages of infection, this fungus produces black irregular shaped sclerotia, which are visible to the naked eye as small black spheres, in infected plant parts. They can also be seen around the parsley plant.

Disease development:

The fungus has a wide host range. Dense canopies and cool wet conditions associated with rain, fog or overhead irrigation as well as temperatures in the range of 15–21°C favour disease development. It survives in the soil as sclerotia or as mycelium on living or dead plants. Sclerotia and mycelium are spread in soil and/or on plant material by implements, animals, in irrigation water and with seed. Sclerotia can over-winter and reinfect the following crop, and can survive in the soil for many years.

Fig 1: Sclerotinia rot. Note white cottony mould.

Management and control:

- Avoid wet conditions in the field as much as possible.
- Weed control is essential in order to eliminate potential hosts for the fungus (note: wide host range).
- Fields must be deeply ploughed (to at least 25 cm) to encourage the decay of plants and sclerotia (the survival form of the fungus) and prevent re-infection in subsequent years.

Chemical use:

There is a current permit valid until December 2006 for the treatment of Sclerotinia rot in parsley as well as other culinary herbs. Fungicides containing procymidone are permitted in all states except Victoria. There is a nine-day withholding period for this particular fungicide [14]. The following restrictions also apply: DO NOT use in protected or covered situations such as glasshouses, greenhouses or plastic tunnels.

Septoria Leaf Spot

Cause:

The fungus *Septoria petroselini* causes a leaf spot on both curly and flat parsley.
It is the most common foliage disease of parsley in Australia. This disease is often called rust by growers, but it is not caused by a true rust fungus.

Symptoms:

This disease appears as small, tan leaf spots (Fig. 1) with black dots across the surface of the spots indicating the presence of black spore cases or pycnidia (Fig. 2). The leaf spots are surrounded with a pronounced dark reddish-brown margin. As the disease progresses the foliar tissue turns yellow and leaves eventually die.

Disease development:

Little is known about the disease cycle of this fungus. The disease can be seed borne and spores may survive and remain infectious on dead or dried leaf material [21]. Wet leaf surfaces are required for spores to emerge from pycnidia where they are splashed dispersed by wind-driven rain, dew and over-head irrigation. Workers and equipment in fields of wet foliage can also transmit the spores to healthy plants in another field. Conditions conducive to disease development include mild temperatures and high humidity. Optimum temperatures for infection are 20–25°C, with a requirement for high humidity after infection (eg. period of leaf wetness with morning dew). Symptoms of the disease manifest 14–21 days after infection [1].

Fig. 1: Leaf Blight on parsley leaves.

Fig. 2: Note black spots inside lesions.

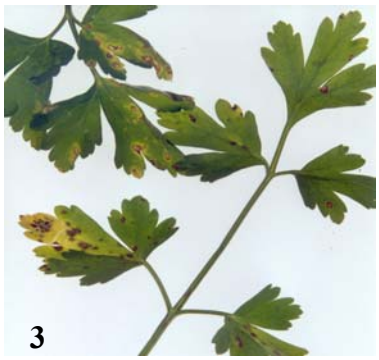

Fig. 3: Flat parsley leaves showing Septoria leaf blight.

Management and control:

- Purchase of quality seed is the best method of disease prevention.
- Flat-leaf parsley varieties are generally more susceptible to the disease than curly-leaf types.
- Use of drip or trickle irrigation rather than overhead sprinklers can reduce the spread of this disease.
- Crop rotation can also assist in preventing the redevelopment of the disease, since pycnidia are known to survive and re-infect the next crop [1]. In the USA, it has also been recommended that a 2-year rotation crop system be implemented to prevent re-infection [16].

Chemical Use:

A temporary permit to use copper-based fungicides containing cupric hydroxide as their only active ingredient has been granted until the end of February 2006 [3].

Apium virus Y

Cause:

Previously thought to be *Celery Mosaic virus* (CeMV). Parsley is also susceptible to CeMV, but its occurrences are rare.

Symptoms:

On young leaves the virus causes vein clearing and a yellow or light green coloured inter-venial mottling. On mature foliage it causes narrow, twisted and mottled leaflets. Plants may be slightly stunted.

Disease development:

Apium virus Y is not seedborne, but can be transmitted mechanically by farming practices and by many species of aphids. Aphids can acquire it from feeding on an infected plant in 5–30 seconds and transmit it to a healthy plant in 5–30 seconds. The virus does not persist in the aphid. Sources of the virus are umbelliferous crop plants such as celery, carrot and dill as well as umbelliferous weeds. Sequential or overlapping crops are considered to be the most important sources of the virus.

Fig 1: *Celery Mosaic virus* on Coriander

Fig 2: *Celery Mosaic virus* on Parsley

Figs 3 & 4: Yellowing of leaves on parsley caused by *Apium virus Y*.
(Photographs 1, 2 & 3 courtesy of Violeta Taicecski, DPI Vic.) [17]

Management and control:

- Remove weed hosts.
- In severe infections implement a host-free period for 1 to 3 months.
- Fungicides will not control viral diseases.
- Systemic insecticides for aphids are not an effective preventative measure for viral disease unless aphids are in plague proportions.

Chemical use:

Viruses cannot be controlled by chemical treatments.

Other viral diseases

To date, the only viruses reported in Australia are CeMV and *Apium virus Y* (parsley virus Y).

A number of other viruses have been recorded on parsley worldwide.

There is carrot motley dwarf (CMD) which consists of carrot red leaf virus (CRLV) and carrot mottle virus (CmoV). Red leaf symptoms consistent with CRLV have been observed in parsley crops in northern Australia, but are rare in Victoria (Figs. 1–3). As yet, CRLV has not been confirmed in Australia.

Other viral diseases recorded on parsley include alfalfa mosaic virus (AMV) (Fig. 4) and chicory yellow mottle virus, which produces a line pattern on leaves. Symptoms of parsley green mottle virus are self-explanatory. A number of other viruses occur on parsley but they are symptomless.

All the above mentioned viruses are aphid-transmitted, so the management and control strategies are similar.

Figs 1, 2 & 3: Suspect carrot red leaf virus. Photograph 1 Courtesy of Robert Baddman, CD Herbs).

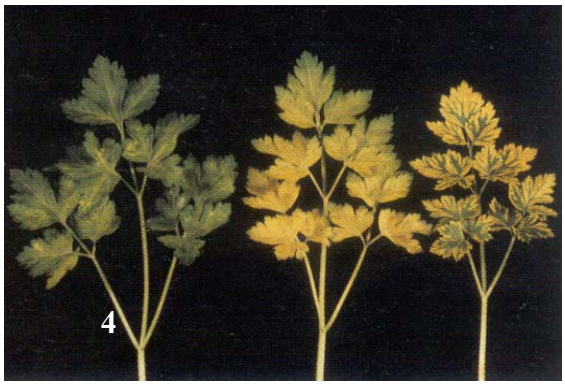

Fig 4: Calico of parsley caused by Alfalfa mosaic virus. Photo courtesy of R N Campbell [20].

Abiotic Disorders

A number of apparently abiotic disorders have been observed on parsley plants in Australia. Their cause is unclear, but they may be associated with nutrient deficiency, salinity or stress.

Root balling:

Root balling of transplants has caused major losses for some hydroponic growers, especially in Queensland. Roots wrap around the plant and fail to spread beyond the cell. In extreme cases plant are stunted and unproductive. It is thought to be associated with stress whilst plants are growing in trays.

Reverse osmosis:

Symptoms consisted of bleached foliage with a completely healthy, white root system. The symptom arose in summer during extremely hot weather in ground where salinity was an issue.

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[20] Courtesy R. M. Davis (plate 11), S. T. Koike (plate 77), and R. N. Campbell (plate 89). Reprinted with permission from the Compendium of Umbelliferous Crop Diseases, 2002, American Phytopathological Society, St. Paul, MN.

Appendix 1: State Herbaria collections of parsley pathogens

Pathogen	Disease
Alternaria petroselini	Alternaria Leaf Spot / Leaf Scorch
Apium virus Y	Leaf chlorosis
Fusarium oxysporum	Root lesions
Fusarium solani	Collar rot
Meloidogyne incognita, *M. hapla*	Root-knot nematodes
Pseudomonas sp.	Bacterial leaf spot
Rhizoctonia solani	Root lesions
Sclerotinia minor, *S. sclerotiorum*	Stem rot
Septoria petroselini	Leaf spot
Stentrophomonas maltophilia	Bacterial crown rot

[A] Formerly called *F. tabacinum* and generally considered a saprophyte.

Chapter 2

Surveys for parsley diseases in Queensland, 2005

Heidi Martin, QDPI&F

Summary

During period of warm wet weather root rot was a major concern to Queensland parsley growers, especially those growing in-ground crops. *Fusarium solani* and the bacterium *Stentrophomonas maltophilia* were demonstrated to cause a collar and crown rot. Species of *Phytophthora* and *Pythium* were consistently isolated from severe crown and root rots. Root knot nematodes were associated with sparse poor parsley stands and *Alternaria petroselini* was reported for the first time in Australia causing leaf blight.

2.1 Introduction

The parsley industry in Queensland is a small, decentralised industry encompassing both large farming enterprises and small family-run businesses. Production practices are extremely variable and include in-ground production with and without plastic mulch with both trickle and overhead irrigation (Fig. 2.1). Hydroponic production is also widely practiced, particularly by the smaller producers (Fig. 2.2).

Figure 2.1: In-ground production of parsley, South-east Queensland.

Figure 2.2: Hydroponic production of parsley in South-east Queensland.

Production in Queensland occurs virtually year-round. Growers in Central Queensland (Biloela) and South-east Queensland districts (Brisbane Metro) plant in the late summer months (March/April) and grow until early November. The cooler climate of the Granite Belt of southern Queensland (Stanthorpe) is ideally suited to summer production.

Relatively few diseases have been officially recorded on parsley in Queensland. There have been numerous recordings of *Septoria* leaf blight of parsley, caused by the fungus *Septoria petroselini* Desm., and this disease is known to be widespread. In addition, the potyvirus *Apium virus Y* has been confirmed as the cause of a leaf chlorosis in parsley. *Stemphyllium vesicarium* and *Colletotrichum gloeosporioides* have also been reported in association with leaf blight and petiole spot symptoms, however, in both cases, the pathogenicity of these fungi have not been confirmed in pathogenicity tests. There is a paucity of information about root rot and vascular wilt diseases. The fungi *Fusarium oxysporum* and *Gibberella intricans* were found to be associated with a vascular wilt and root rot of parsley in plants collected from Wowan (Central Queensland) in 2000. However, again, the pathogenicity of both of these organisms was not confirmed via pathogenicity testing.

In recent years, Queensland growers have reported increasing crop losses, particularly following periods of hot/wet weather at the extremities of the production window. In 2005, we completed a survey of selected Queensland parsley farms in the central, south-east, and southern production districts in an attempt to understand the nature of these losses and characterise any pathogens responsible.

2.2 Materials and methods

2.2.1 Disease surveys

A survey of parsley farms was completed in February 2005. The 'Stratified Random Sampling Method', or a variation of it was employed to survey parsley crops for symptoms of disease. This sampling method divided the bay (area between two sprinkler lines), block of benches or crop into 10 'plots (or strata)' of approximately equal area. Generally a plot consisted of an equal number of rows that were of equal lengths. This method catered for paddocks of varying sizes, shape, and number of lengths of row. In each plot, 10 to 100 adjacent plants were selected to assess for disease, using a system involving the random selection of numbers. The first number indicated which row of plants, and the second number indicated the number of meters from the end of the row at which to begin the monitoring of parsley plants for diseases. The variation on this method was to assess only 5 of the 10 'plots'.

Selected farms were re-visited in the last week of October 2005. Additional samples were received throughout the year from growers seeking diagnosis of diseases via 'Grow Help Australia', the commercial disease diagnostic laboratory of Queensland Department of Primary Industries and Fisheries.

In total, 12 farms were surveyed in February 2005. Two were in the Brisbane Metropolitan district, 7 in the Granite Belt district and 3 in Central Queensland. In October 2005, we re-visited the 2 properties in the Brisbane Metropolitan district. Throughout 2005, additional samples for diagnosis were received from growers from Rockhampton (Central QLD), Tamborine Village (South-east QLD) and Stanthorpe (Southern QLD).

2.2.1.1. Collection of plant and soil samples from survey sites

A selection of plants with representative symptoms was collected from each farm. In most cases, entire plants were collected. Soils and growing media from hydroponic production systems were also sampled. Soil samples were taken from around the root systems of plants showing signs of collapse in the field (Figs. 2.3 and 2.4). Small quantities of soil were collected from multiple sites in each affected field and these sub-samples were combined to give a single composite sample for each affected crop. All samples were placed in clean plastic bags and stored in a cooled Esky for transport to the laboratory. The samples were stored in a cool-room at the laboratory prior to processing. Water samples were also collected in clean plastic bottles from the water supplies of three farms.

Figure 2.3: Collapse of field grown parsley.

Figure 2.4: Close up of collapsed parsley plants.

2.2.2 Pathogen isolation and identification

2.2.2.1 Isolations from plant tissues

Each plant was washed thoroughly in clean water prior to examination. Affected plant tissues were examined microscopically for obvious signs of fungal, bacterial or nematode infection.

Bacterial ooze tests were completed on tissue sections from plants with wet crown, root or petiole rots. Nematode infections were confirmed by dissecting root galls and extracting mature females and egg masses with a dissecting needle.

All plants with suspected virus infections were provided to Dr John Thomas from the QDPI&F Plant Virology Group for diagnosis. Each sample was tested for viral infection via transmission electron microscopy (TEM).

We completed isolations for fungi and bacteria from plants with distinctive rots, lesions and/or tissue discolouration. For fungal isolations, small tissue sections with representative symptoms were dissected from the plants with a sterile scalpel. The tissue sections were washed in clean water and then immersed in a 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 petri dishes of artificial agar media. For root, crown and petiole isolations, tissue sections were plated onto half-strength potato dextrose agar containing streptomycin (PDA + strep), $P_{10}VP$ and $P_{10}VP+T$ ($P_{10}VP$ supplemented with hymexazol). $P_{10}VP$ is a selective medium for the isolation of *Phytophthora* and *Pythium*, and is corn meal agar supplemented with pimaricin (10 µg/mL), vancomycin (200 µg/mL) and pentachloronitrobenzene (160 µg/mL) (Tsao & Ocana, 1969). Foliar isolations were completed on PDA + strep only. All plates were incubated in the dark at 25°C. Plates were monitored for fungal growth and colonies were tentatively identified to genus level on the basis of microscopic morphological characteristics. Fungi that were recovered consistently in culture were subcultured to fresh plates of potato dextrose agar. For long-term storage, all cultures were maintained on PDA slopes.

Bacterial isolations involved excising small tissue sections and macerating them in a few drops of sterile de-ionised water in a sterile petri dish. One loop-ful of the macerate was then 16-streaked onto 2 plates of nutrient agar (NA) with a sterile inoculation loop. Plates were incubated in the dark at 28°C for 48 hours. After 48 hours, bacterial colonies with consistent colony morphology were selected and re-streaked onto fresh plates of NA. Once the bacterial isolates were obtained in pure culture, gram staining was completed on 24-hour-old cultures. For long-term storage, the bacterial cultures were stored on Cryobank® beads (Mast Diagnostics) in a domestic freezer.

Bacterial isolates that caused disease symptoms in pathogenicity tests were further characterised using the BIOLOG system. Briefly, this involved growing each isolate on BUG media and inoculating a 96 well BIOLOG plate with a 10^6 cfu mL^{-1} bacterial suspension. The plate was then incubated for 24 hours at 28°C and the wells were rated visually for colour changes. The well reactions were compared to those in the BIOLOG database to determine the identity of the bacterium.

2.2.2.2 Lupin baiting

Lupin baiting was completed to test for the presence of *Phytophthora* and some *Pythium* spp. in soil samples. For each soil sample, 4 new plastic disposable drinking cups were 1/3 filled with soil. The remaining volume of each cup was filled with de-ionised water. Plastic lids into which 5 small holes had been bored, were then fitted to each cup. Freshly germinated New Zealand Blue lupin seedlings were suspended through the holes so that their radicles were immersed in the water. The cups were placed in a temperature-controlled room at 26°C, under artificial fluorescent lighting operating on a 12/12-hr light/dark cycle, for 7 days. The seedlings were monitored daily for rot development. The radicles of seedlings that developed obvious rot symptoms were examined under a stereomicroscope for signs of infection by *Phytophthora* and/or *Pythium*. In addition, tissue sections from rotted seedlings were plated onto $P_{10}VP$ and $P_{10}VP+T$ media. Resulting colonies were identified

microscopically to genus level and subcultured onto fresh plates of PDA. PDA slope cultures were prepared for long-term storage.

2.2.2.3 Soil and water pH and Electrical Conductivity (EC) testing

All soil and water samples collected during the February survey were tested for pH and electrical conductivity using the following Australian Standards™; AS 4419-1998 for all soils and AS 3743-1996 for the hydroponic mixes.

2.2.2.4 PCR identification of Pythium *and* Phytophthora

All *Phytophthora* and *Pythium* isolates obtained during this study were sent to Dr James Cunnington, Department of Primary Industries, Knoxfield, for molecular characterisation.

2.2.3 Pathogenicity tests

Not all microorganisms associated with diseased plants may be responsible for causing the disease symptoms. For this reason, we needed to test if the fungi and bacteria that we isolated from the plants collected during our disease surveys were pathogenic to parsley plants, or just secondary invaders. To do this we inoculated healthy parsley plants in the glasshouse with inoculum prepared from pure fungal and bacterial cultures, and assessed the plants for disease symptoms.

A total of 29 fungal and 9 bacterial isolates were inoculated onto young parsley plants. Fungi isolated from roots and/or crown rots included *Fusarium, Pythium, Phytophthora*, and *Macrophomina*. Three *Colletotrichum* isolates were found to be associated with petiole rots, and *Alternaria, Phoma* and *Exserohilum* were cultured from symptomatic leaf tissues.

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 de-ionised water and scraped with a glass spreader. For each suspension, 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 all *Fusarium, Phoma* and *Colletotrichum* isolates were adjusted to 1 x 10^6 conidia/mL. The *Exserohilum* and the *Alternaria* suspensions were adjusted to 1 x 10^5 conidia/mL.

Pathogenicity testing of each isolate was completed twice, first with curled leaf parsley seedlings cv. Frizz and then with flat leaved Italian parsley. In each case, eight-week-old seedlings were transplanted into individual 100mm diameter plastic pots containing grade 2 vermiculite. One week after transplant, four plants were inoculated with each isolate. Plants treated with sterile deionised water were included as controls.

The *Pythium, Phytophthora* and *Macrophomina* isolates were applied to plants as agar macerates. The *Pythium* and *Phytophthora* isolates were grown on 90mm diameter plates of V8 agar for 14 days. Each culture was finely macerated with a scalpel and half a plate of culture macerate was mixed evenly through the vermiculite mix in each pot. The *Macrophomin*a isolate was cultured on PDA instead of V8 but was applied to plants using this same procedure.

A root dip inoculation method was used to apply the conidial suspensions of *Fusarium*. Seedlings were removed from the pots and their roots were dipped directly into each conidial suspension, then, after inoculation, the seedlings were replanted into the pots of vermiculite.

For fungal isolates cultured from foliar symptoms (*Colletotrichum, Alternaria, Exserohilum, Phoma*), inoculum was misted over seedlings with a hand-held aerosol sprayer. Inoculum was applied until the point of runoff, after which the plants were incubated for 24 hours in moist plastic bags.

Bacterial inoculum was prepared as suspensions of standard turbidity. Pure bacterial cultures were grown on nutrient agar (NA) for 24 hours. Bacterial suspensions were prepared in sterile de-ionised

water and were adjusted to 1.0 x 10^6 cfu/mL with a spectrophotometer. All bacterial isolates were applied as root dips, following the procedure adopted for the *Fusarium* inoculations.

After inoculation the plants were randomly arranged on 3 benches in a glasshouse. Pots were placed in individual plastic saucers. Watering involved periods of mix saturation and water deficit. Initially, the saucers were filled with water to ensure complete mix saturation. Plants were kept saturated for 48 hour periods, after which the saucers were removed and the pots were allowed to dry for 72 hours. This regime was maintained for 14 days, after which the plants were assessed for disease development. Once per week, each pot was fertilised with Aquasol®, a general purpose fertiliser.

2.3 Results

2.3.2 Surveys

Information on parsley production in Queensland is summarised in Table 2.1.

Table 2.1 Production characteristics and disease of parsley in Queensland, February 2005

Survey		Region South-east Queensland	Southern Queensland	Central Queensland
No growers surveyed		2	7	3
Range of area (ha)		5–8	1–14	0.4–2
Type	Curly (%)	100	100	100
	Flat (%)	0	29	0
Cultivars		Inca Triple curled Flamingo	Continential Flamingo Frizz Italian Petro	Flamingo Triple curled Paramount
Planted (%)	Inground	100	57	100
	Hydroponic	50	71	0
Range of pH		5.8–7.1	5.3–8.7	6.8–7.5
Range of EC dS/m		0.09–0.44	0.03–2.8	0.06–2.36
Pythium isolated (% of sites)		50	57	20
Other organisms isolated		*Alternaria* Bacteria Rootknot nematodes *Phoma*	*Alternaria* *Fusarium* *Phytophthora* *Septoria*	Bacteria *Macrophomina*
Estimated loses from root rot (%)		10–80	0–90	5–100

Italian and Continental are flat parsley cultivars and probably the same variety.

2.3.2 Isolations

From the February survey, a total of 33 samples were collected. Isolations from tissue sections were completed for 17 of the samples, 7 samples were provided to plant virology for assessment and 9 samples were tested via lupin baiting. A summary of results for the February survey is given in Table 2.1.

In October 16 additional samples were collected from two properties in South-east Queensland. Isolations from tissue sections were completed for 10 of the samples, 4 of the samples were provided to plant virology for assessment and 2 soil samples were tested via lupin baiting. None of the samples from the October survey were tested for pH or EC. A summary of results are given in Table 2.2.

Table 2.2 Results of second survey on parsley crops in South-east Queensland

Site	Symptoms	Organisms consistently isolated
A	Soil	*Pythium*
	Leaf blight	*Alternaria*
	Stunted and yellowing; collar rot	*Fusarium* spp, *F. solani*
	Taproot: orange-brown coloured stele	Bacteria, *Stentrophomonas maltophilia*
B	Soil	No pathogen detected
	Soft wet rot of roots, crown and leaf bases	No consistent isolation of fungi or bacteria
	Leave: mosaic, mottling	No virus detected
	Grey-brown fungal growth on crown and leaves	Slime mould

A total of 4 *Pythium*, 11 *Fusarium*, 5 *Alternaria*, and 3 *Colletotrichum* isolates were cultured from plants and soil collected during the disease surveys. Single isolates of *Phytophthora*, *Phoma*, *Exserohilum* and *Macrophomina* were also recovered. As described in Table 2.3, DPI Victoria has identified the *pythiums* and the single *Phytopthora* isolate.

Table 2.3 Identification of *Pythium* and *Phytophthora* isolates

Isolate	Isolation method	Identification
1	Lupin bait	Undescribed species in the *Pythium littorale* 'group'
2	Direct root isolation	*Pythium irregulare*
3	Lupin bait & root isolation	*Phytophthora cryptogea*
4	Root isolation	*Pythium diclinum* 'group'
5	Root isolation	*Pythium ultimum*

2.3.2.1 Root Knot nematodes

Severe infection by root-knot nematodes (*Meloidogyne* sp.) was detected in a red clay soil at Rochedale (Brisbane Metro; Figs. 2.5 and 2.6). The species of *Meloidogyne* responsible for this infection was not determined.

Figure 2.5: Severe stunting and wilting of field-grown parsley *plants associated with Meloidogyne* sp.

Figure 2.6: Nodules formed by *Meloidogyn*e sp. on parsley roots.

2.3.2.2 Phytophthora *and* Pythium

Our attempts to fulfil Koch's postulates and prove pathogenicity produced variable results. None of the *Phytophthora* or *Pythium* isolates produced rots on healthy parsley plants when they were applied as pure cultures, even though both were associated consistently with severe root and crown rot symptoms in hydroponic and field-grown plants (Figures 2.7–2.10).

Figure 2.7: Severe crown rot associated with *Phytophthora* and bacterial infection.

Figure 2.8: Bacterial rot of a lower parsley root.

Figure 2.9: Root rot of young hydroponic parsley associated with infection by *Pythium* sp.

Figure 2.10: Healthy hydroponic parsley (right) and plants with *Pythium* root rot (left).

2.3.2.3 Fusarium

Fusarium was recovered consistently from the roots and crowns of many plants showing severe root and crown rot symptoms. Only one *Fusarium* isolate, a *Fusarium solani*, was confirmed as a pathogen in pathogenicity tests. This isolate was cultured from field-grown plants showing dark brown discolouration of the collar tissues (Fig. 2.11). In pathogenicity tests, this fungus produced collar rot symptoms and it was consistently re-isolated from symptomatic tissue.

Figure 2.11: Collar and crown rot of field-grown parsley caused by *Fusarium solani*.

2.3.2.4 Alternaria *leaf blight*

The fungus *Alternaria petroselini* was consistently isolated from a foliar leaf blight of curled-leaf parsley plants collected from growers in the Brisbane metropolitan district (Figs. 2.12 a and b). Unfortunately, this fungus was slow to produce conidia in culture and consequently, we were unable to confirm its pathogenicity to parsley plants because of insufficient inoculum production. The fungus was however identified as *A. petroselini* by Dr James Cunnington (DPI, Victoria) based on morphological features. It is recognised as the causal agent of the disease Alternaria Leaf Blight of Parsley which is a common disease of parsley wherever the crop is grown (Davis & Raid, 2002). Pathogenicity testing is pending.

Figures 2.12 a and b: Foliar symptoms of Alternaria Leaf Blight (caused by *Alternaria petroselini*).

2.3.2.5 Soft bacterial crown rots

Soft bacterial crown rots were common on many of the farms that were surveyed, particularly in field-grown plants. In field, bacterial infections caused a rapid degradation of crown and root tissues (Figs. 2.13 and 2.14). Invasion of the infected tissues by saprophytic bacteria hindered our attempts to isolate primary bacterial pathogens. Only one of the 9 bacterial isolates that we obtained from field plants was confirmed as a crown/root rot pathogen in pathogenicity testing. This isolate was a cream-coloured gram-negative, rod-shaped, oxidase positive bacterium that was identified as *Stentrophomonas maltophilia* by BIOLOG, with a similarity index of 0.87.

Figure 2.13: Bacterial crown rot of field parsley.

Figure 2.14: Internal root discolouration associated with *Stentrophononas maltophilia* infection.

2.3.2.5 Virus-symptoms and nutritional disorders

An assortment of virus-like symptoms including leaf chlorosis, mosaics, mottling, distortion and discolouration were observed in both curled and flat-leaved parsley plants in the 3 Queensland production districts surveyed (Fig. 2.15). No virus particles were detected via TEM in any of the plants that were provided to plant virology for analysis. It seems probable therefore, that many of the virus-like symptoms were caused instead by genetic and/or nutritional disorders.

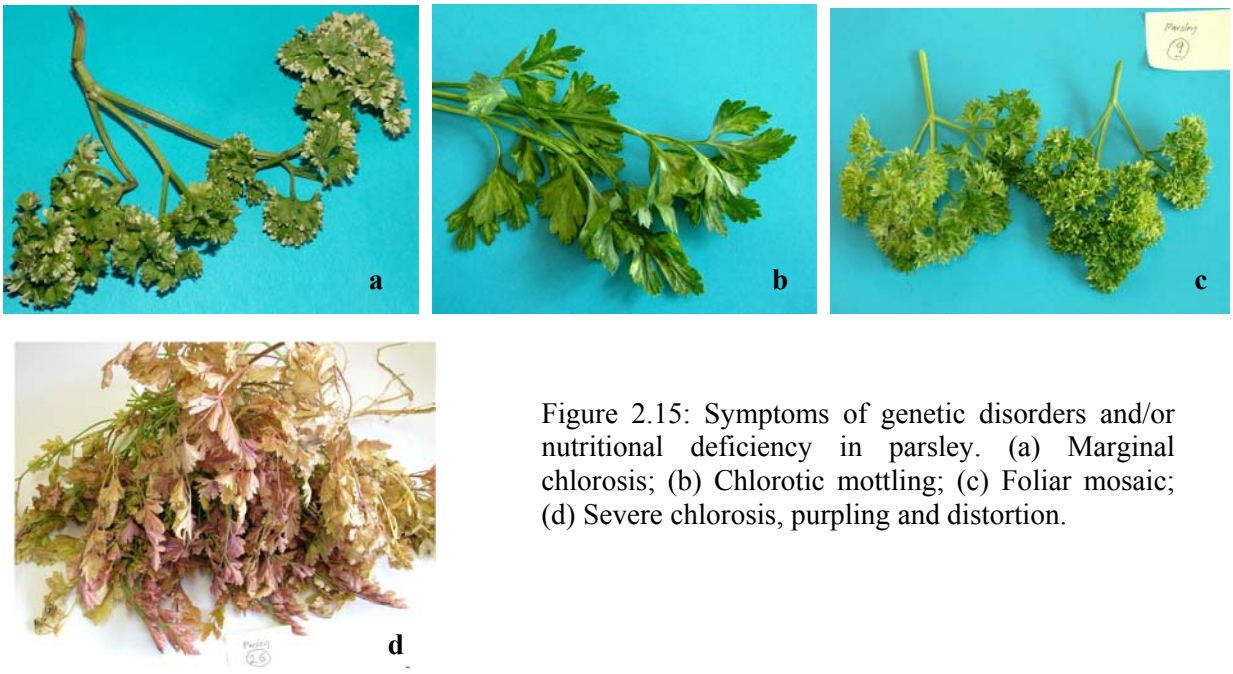

Figure 2.15: Symptoms of genetic disorders and/or nutritional deficiency in parsley. (a) Marginal chlorosis; (b) Chlorotic mottling; (c) Foliar mosaic; (d) Severe chlorosis, purpling and distortion.

2.3.2.6 Root congestions

Root congestion was also commonly encountered in both hydroponically- and field-grown plants. In many cases, plants with severe root congestion were stunted and were also showing foliar symptoms consistent with nutritional deficiencies (Fig. 2.16). It would seem that if root-bound parsley seedlings are transplanted, the root systems are unable to develop sufficiently resulting in stunted plants. Poorly anchored plants may then be more prone to damage and entry by crown rot organisms.

Figure 2.16: Root congestion causes stunted, nutritionally deficient plants.

2.4 Discussion

Plant diseases caused significant losses in parsley crops in Queensland production districts during this survey period. Disease incidence and severity was greatest in field-grown crops following periods of warm, wet weather.

Soil-borne fungi, including *Fusarium oxysporum, Fusarium solani*, *Pythium* spp. and *Phytophthora* spp. were the most commonly isolated organisms associated with disease symptoms. These fungi have also been identified as the causal agents of parsley damping off and root rots in other parts of the world (Hine & Aragaki, 1963; McCracken, 1984a; Hershman et al., 1986; Davis et al., 1994; Nawrocki et al., 2002). Most of the *Pythium* and *Phytophthora* isolates collected in this Queensland survey have been characterised to species level. Outside Australia, *Pythium paroecandum* (McCracken, 1984a; McCracken, 1984b), *Pythium irregulare* and *Pythium ultimum* (Hershman et al., 1986), *Phytophthora nicotianae* (Hine & Aragaki, 1963) and *Phytophthora cryptogea* (Davis et al., 1994) have all been found to be pathogenic to parsley. *Pythium diclinum* has been found to cause pre- and post-damping off in wheat and other crops (Abedelzahar, 2004; El Andrusse, 2005).

The failure of many of the fungal isolates to cause disease in our pathogenicity tests was unfortunate, but not surprising. Other researchers have reported similar difficulties in reproducing disease symptoms in artificially inoculated parsley plants (McCracken, 1984a; McCracken, 1984b). The environment (in particular the temperature and moisture regime) under which pathogenicity tests are conducted can strongly influence symptom expression. As an example, in a previous study with *Phytophthora nicotianae*, the fungus was found to cause a crown rot of parsley at temperatures greater than 30°C, whereas plants incubated at less than 25°C remained symptomless (Hine & Aragaki, 1963). It would be useful to complete additional temperature controlled studies with the isolates collected in our surveys, to elucidate the environmental conditions conducive to infection by each fungal species.

No viral diseases were detected in parsley plants collected in our survey. In Queensland, two viruses are known to affect parsley, *Apium virus Y*, and *Parsley latent virus*, with *Apium virus Y* characterised in Queensland only recently (J.E. Thomas, personal communication). Originally, the virus now known as *Apium virus Y* was identified as *Celery mosaic virus* (CeMV) and hence CeMV was considered a disease of parsley in Queensland until 2002. Transmission studies have now confirmed that parsley is not a host for CeMV (Alberts et al., 1989).

Plants with severe chlorosis and purple discolouration of leaves were identified frequently on a number of parsley farms. These symptoms resembled Carrot Motley Dwarf Disease in the field, however the two viruses responsible for *Carrot Motley Dwarf* (*Carrot mottle virus* and *Carrot redleaf virus*), were not detected in any of the plant material collected. Prior to this survey Carrot Motley Dwarf Disease had not been reported in parsley in Queensland and we did not detect its presence in this survey.

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Chapter 3

Surveys for parsley diseases in New South Wales, 2005

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Summary

Diseases were only responsible for minor losses in commercial parsley crops in NSW. The most significant pathogens were *Rhizoctonia solani* causing crown rot and plant collapse and *Septoria petroselini* causing leaf spot. *Rhizoctonia* isolates were very pathogenic, but isolates of *Pythium*, *Phytophthora* and *Fusarium* were only weakly pathogenic.

3.1 Introduction

Commercial parsley production in New South Wales is restricted to a total area of less than twenty hectares. It is primarily grown in the peri-urban market gardens of the Sydney Basin in rotation with other leafy vegetables and herbs. Production is in-ground, on raised beds with overhead irrigation. Both curly and flat leaf cultivars are grown.

Herbarium records of NSW parsley diseases list several accessions of *Septoria petroselini* associated with Leaf Spots; Mosaic, caused by *Parsley virus Y*; various fungal pathogens (*Rhizoctonia solani*, *Phytophthora* sp., and *Sclerotinia sclerotiorum*) in association with Root and Stem rots; and Root Knot nematodes (*Meloidogyne incognita* and *M. hapla*). Further diagnostic records include associations of *Pythium* and *Fusarium* species with root rots and stunted plants. All of these pathogens have been recorded as pathogens of parsley in overseas studies (Davis & Raid, 2002; McCracken, 1984).

Surveys were conducted between February and August 2005 to update our knowledge of the causes of parsley diseases in NSW.

3.2 Materials and methods

3.2.1 Farm surveys

Eleven properties were surveyed in February 2005; ten were in the Sydney basin and one was near Coffs Harbour on the Mid-North coast. A 'Stratified Random Sampling Method' was employed to survey parsley crops for symptoms of disease and sample collection. Crop areas between two sprinkler lines (crop bays) were divided into 10 plots (or strata) of approximately equal area. Generally a plot consisted of an equal number of rows that were of equal length. This method catered for paddocks of varying sizes, shape, and number of lengths of row. A two-metre length of parsley row, in each plot, was randomly selected to assess for disease. The first number drawn indicated which row to survey. A second number indicated the number of meters from the end of the row at which to begin monitoring for diseases. A variation on this method was to assess only 5 of the 10 plots. Plants showing symptoms of disease were collected from the surveyed plots. Soil from around the root zones was also collected. Where no obvious diseases were recorded, a more general survey was conducted and affected plants were collected for laboratory examination.

3.2.2 Laboratory diagnostics

Plant samples were clinically examined for disease symptoms by Department of Primary Industries Vic. (Crop Health Services) or in-house at NSW Department of Primary Industries. Affected tissue was viewed by light microscopy and plated to a range of general and selective media for fungal and bacterial pathogens. Media included: acidified Potato Dextrose Agar (PDA); Potato Carrot Agar (PCA) with pimaricin (10 ppm) and rifampicin (5 ppm); PCA with pimaricin (10 ppm), rifampicin (5

ppm) and hymexazol (50 ppm); Water Agar (WA) with rifampicin (5 ppm); Kings Medium B; and Sucrose Peptone Agar. Plates were incubated at 25°C for 24–72 hours, examined by light microscopy and sub-cultures made for further identification and pathogenicity testing. Samples with mosaic or red leaf symptoms were examined by Electron Microscopy after sap preparations were negatively stained.

Soil samples were mixed and sub-samples taken for pH and EC testing as well as bioassays. These tests involved potting soil samples into 10-cm pots and seeding with parsley (cvs Italian Plain Leaf or Curled). Pots were placed in a greenhouse (at 20–30°C) and watered as required. Seedlings with disease symptoms were harvested and pathogens isolated as described above.

3.2.3 Pathogenicity testing

Fungal isolates were grown on PDA in an incubator at 25°C and covered with gamma-irradiated oak leaves cut into one-centimetre pieces. Once the leaves were colonised with fungal growth (5–8 days), they were picked off and placed at the base of parsley seedlings (cvs Italian Plain Leaf and Curled) growing in 10-cm pots. Each pot contained five seedlings and there were four replicates for each fungal isolate and cultivar. Pots were placed in a greenhouse at 20–30°C and watered as required (Fig. 3.1). The fungal isolates used were: *Rhizoctonia solani* (five isolates); *Pythium* spp. (nine isolates); *Phytophthora* sp. (one isolate); *Fusarium* sp. (six isolates); and *Sclerotinia sclerotiorum* (one isolate). Seedlings with disease symptoms were harvested and pathogens isolated as described above. After eight weeks of growth, all plants were washed clean of soil with tap water and examined. Rotted or brown roots were plated for fungal pathogens.

Figure 3.1: Pathogenicity tests of fungal isolates were conducted in small pots.

3.3 Results

3.3.1 Farm surveys

Table 3.1 summarises the survey results.

Table 3.1. Summary of NSW parsley disease survey

Item surveyed		Response
Number of growers surveyed		11
Range of area (ha)		0.5–3
Type of parsley	Curly (%)	64
	Flat (%)	36
Cultivars	Curly	Flamingo, Figaro
	Flat	Italian Plain Leaf
Planting method	Direct seeded (%)	83
	Transplants (%)	16
Irrigation		Overhead
Range of pH		5.8–6.7
Range of EC dS/m		0.2–0.8
Estimated losses from root damage (%)[A]		0–50
Crops with incidence of	*Septoria* (%)	36
	Root knot nematodes (%)	18
	Rhizoctonia (%)	55
	Pythium (%)	55
	Bacterial Soft Rots (%)	55
	Fusarium (%)	73
	Phytophthora (%)	9
	Sclerotinia (%)	9
Incidence in crops	dead plants in crop (%)[B]	<1
	Septoria (%)	<5
	stunting and wilting (%)	<5
	coloured leaves (%)[C]	<1

[A], growers' estimates
[B], all incidence data is based on 11 growers
[C], yellow or white streaking or red foliage thought to be associated with virus but not always confirmed

3.3.2 Laboratory diagnostics

Table 3.1 lists the major fungi found associated with diseased parsley plants collected in the NSW survey. *Rhizoctonia solani* was associated with crown and collar rot symptoms. *Pythium* and *Fusarium* were commonly isolated from brown root systems. Morphological characterisations of *Pythium* isolates using the key of Plaats-Niterink (1981) yielded *P. ultimum* var. ultimum, *P. acanthophoron*, *P. oligandrum*, *P. paroecandrum* and several undetermined species. Nematodes associated with the Root Knot disease symptoms were identified as *Meloidogyne incognita. Septoria petroselini* was identified on leaf spot diseases. *Sclerotinia sclerotiorum* was identified on a watery stem on one farm. *Erwinia carotovora* was consistently isolated from plants affected with soft rots. Soft rots were also found as a secondary rot with fungal diseases and a breakdown of internal crown tissue (Fig. 3.2). This symptom resembles a jelly rot, which is caused by ammonium toxicity in lettuce. Soft rots were also found in conjunction with Root Knot and in plants that were waterlogged (Fig. 3.3).

Figure 3.2: Bacterial soft rot and vascular browning in parsley roots.

Figure 3.3: Waterlogged plants collapsing with Bacterial soft rot.

3.3.3 Pathogenicity testing

All five isolates of *Rhizoctonia solani* caused a collar rot and leaf collapse in both parsley cultivars. One isolate of the *P. diclinum* 'group' (05/466) and a *Pythium* sp. isolate (05/182) were associated with root browning and collapse of curly parsleys in a single replicate of each treatment. Similar low rates of plant mortality were observed with three *Fusarium* isolates on both parsley cultivars. Washed root systems revealed root browning and reduction in root mass when *Fusarium* treatments were compared to uninoculated controls (Fig. 3.4). The single isolate of *Sclerotinia sclerotiorum* reproduced a watery petiole and crown rot in parsley seedlings. The single isolate of *Phytophthora* sp. caused browning and reduced root mass compared with control treatments.

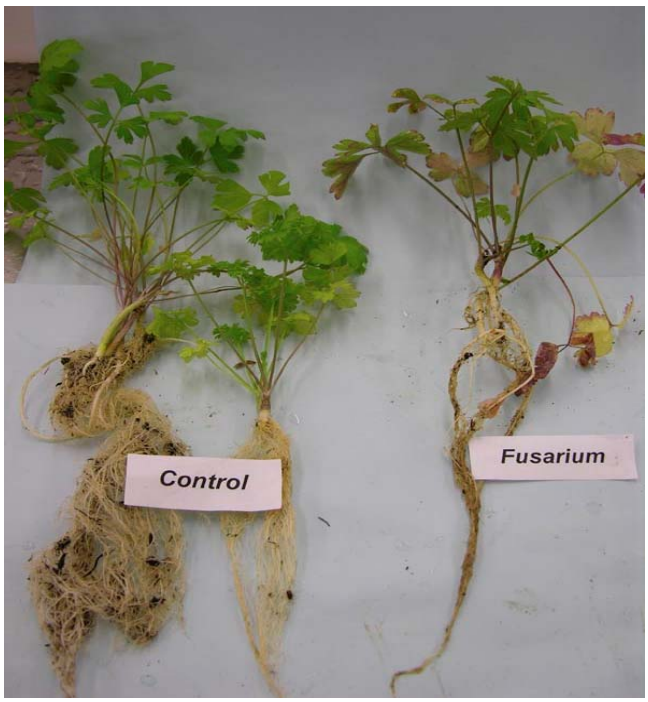

Figure 3.4: Washed out root system of a *Fusarium* treatment with brown and reduced root mass compared with uninoculated controls.

3.4 Discussion

Diseases were responsible for only minor losses in commercial parsley crops in NSW during the survey period. *Rhizoctonia solani* and *Septoria petroselini* were the most significant plant pathogens found. *R. solani* was shown to cause a crown and petiole rot that resulted in plant collapse. *S. petroselini* is known as the cause of a Leaf Spot disease in parsley and is a more significant problem during periods of warm and wet weather (Davis & Raid, 2002). Weather conditions during the survey period were extremely dry. Australian Government Bureau of Meteorology records reveal there were only four rain days in the Sydney Basin in February 2005, which was the second lowest on record.

Pythium, Phytophthora and *Fusarium* isolates were weakly pathogenic in these assays, causing low or no mortalities. This assay system may have underestimated their importance as it differed from field conditions in several respects. It ran for a relatively short period compared to the duration of commercial field crops; the pots were free draining and received regular watering, thus avoiding periods of waterlogging or moisture stress; and there were no extremes in temperature in the greenhouse environment. Environmental stresses are known to exacerbate disease incidence and severity for some these fungi (Davis & Raid, 2002). Further studies are required to characterise the species of these fungi and to determine the significance of *Fusarium* as a potential cause of wilt in field plants.

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Chapter 4

Surveys of parsley diseases in Victoria, 2005

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Summary

Surveys indicated that root rot had caused up to 100% crop losses to commercial parsley production in Victoria. As field trials implicated the Pythiaceae fungi, *Pythium* and *Phytophthora*, either alone or in combination, a series of experiments was performed to confirm isolates' pathogenicity on parsley. Three isolates from our collection were confirmed as pathogenic on parsley: *Pythium sulcatum*, *Phytophthora megasperma* and *Phytophthora inundata*. *Phytophthora inundata* is reported in Australia for the first time.

4.1 Introduction

VPRI Herbarium and Crop Health Services (CHS) databases listed several pathogens that have been associated with parsley root rot in Victoria. These included *Fusarium oxysporum, Microdochium tabacinum, Phoma* spp., *Phytophthora* spp., and *Rhizoctonia solani. Septoria petroselini* and *Pseudomonas* spp. were associated with leaf spot and leaf blight, respectively. A potyvirus that reacted positively to CeMV antisera was detected in parsley grown in Victoria (Brendan Rodoni, PIRVic, personal communication). Based on recent research from Queensland, this virus is most likely to be *Apium virus Y* (J.E. Thomas, personal communication).

A previous project, HAL VG01045 (*Disease management strategies for the production of bunching vegetables)*, identified a number of diseases on parsley: Septoria leaf spot, damping-off, root rot and viruses. The main cause of crop losses was considered to be root rot and post emergence dieback associated with species of *Pythium, Phytophthora, Rhizoctonia* and *Fusarium*. The current project surveyed parsley crops in Victoria to identify diseases on parsley, determine their extent and then identify the cause of the major problem (root rots). The field trials of the present project (see Chapter 5, trial 2, Cochrane; autumn 2005) indicated that Pythiaceae fungi were responsible for root rots in parsley as metalaxyl and phosphonic acid controlled the symptoms. The family Pythiaceae contains obligate and non-obligate parasites and includes the important pathogenic genera *Pythium* and *Phytophthora*. International research (eg. McCracken, 1984; Davis et al., 1994; Davis & Raid, 2002) suggests that Pythiaceae fungi are the main causative organisms of these diseases in parsley.

Consequently, we concentrated on identifying and proving pathogenicity for species of *Phytophthora* and *Pythium* isolated from plants, soils or dam water collected during the survey and field trials, by setting up experiments to prove Koch's postulates.

Koch's postulates can be summarised thus:

- The causative organism must be present in every case of the disease.
- The causative organism must be isolated from the host with the disease and grown in pure culture.
- The specific disease must be reproduced when a pure culture of the causative organism is inoculated into a healthy susceptible host.
- The causative organism must be recoverable from the experimentally infected host.

4.2 Materials and methods

4.2.1 Survey methods

Eight properties around the Clyde/Cranbourne area were surveyed in August 2005. Stratified Random Sampling Methods were employed to survey parsley crops for symptoms of disease (see Chapter 3 for full explanation). Data from the Victorian surveys was collated and will be discussed in section 4.3.1. Plants showing symptoms of disease were collected from the surveyed plots or from elsewhere within the crop and appraised for potential pathogens, either by Crop Health Services (CHS) or by the principal project investigator.

4.2.2 Koch's postulates

4.2.2.1 Isolation methods

Roots of parsley plants collected that exhibited root rot symptoms were treated in the following manner. Small root pieces were suspended in a sample cup in 0.5% hypochlorite for 30 seconds before washing in sterile distilled water. Root pieces were dried on tissue paper before aseptic transferral to water agar (WA) or potato dextrose agar + 0.05% tetracycline (PDAA).

Alternatively, soil samples associated with plants exhibiting root rot were sampled using the pear bait method. Briefly, 5 g of soil is placed in a plastic dish that is then partially filled with tap water. An unripe pear is placed in the dish such that it is partially submerged in the water. Dishes are then placed in direct light and checked for lesions every day. Pears that have not developed lesions after 10 days are discarded. Lesions that develop above the waterline, not below the waterline are of interest. The outside of the lesion is sterilised with 70 % (v/v) ethanol and samples from the lesion are placed on WA or PDAA.

In both cases, plates were incubated at room temperature and fungal colonies exhibiting oomycetes characteristics were subcultured onto V8 plates before Dr. James Cunnington (Biosytematics, DPI Victoria) undertook formal identification by traditional and PCR methods.

4.2.2.2 Choice of isolates

Six *Pythium* and five *Phytophthora* isolates were purified before being transferred to long term storage in sterile distilled water (McGinnis et al., 1974). Results from the field trials (Chapter 5) and isolations indicated that these oomycetes were mainly responsible for root rot in parsley.

4.2.2.2 Preparation of isolates

Fungi isolated in trial work were used to infect healthy parsley plants in order to confirm the isolates' pathogenicity and prove Koch's postulates for some of the above isolates.

In all cases isolates were grown on V8 agar for 5–7 days at room temperature to encourage sporulation. After 5–7 days, agar blocks were used either to inoculate plants or were suspended in sterile pond water for 24–48 hours to induce sporangial formation and zoospores. This method of suspending plugs in pond water tended to form sporangia and zoospores in *Phytophthora*, but produced oospores in *Pythium* isolates, leading to the possibility of a screening tool to determine which oomycetes has been isolated.

4.2.2.3 Preparation of parsley plants

Several methods were employed in this set of experiments, as outlined below:

1. Parsley roots of mature parsley plants (>3 months old) were exposed and scored with a scalpel blade before an agar plug containing the isolate was inserted upper surface-side onto the exposed roots. The plug was then covered with soil.

2. Parsley roots of mature parsley plants (>3 months old) were exposed and left undamaged before an agar plug containing the isolate was inserted upper-surface side onto the exposed roots. The plug was then covered up by soil.

3. Parsley roots of mature parsley plants (>3 months old) were exposed and scored with a scalpel blade and an agar plug that had been suspended in sterile pond water for 24–48 hours at 12°C was placed upper-surface side onto the exposed roots. The pond water was added near the roots after the plug had been covered up by soil. The pond water encouraged sporangial development in *Phytophthora*, but oospore production in *Pythium*.

4. In a variation of the above methodologies, fresh parsley seedlings (<6 weeks old) were used instead of mature parsley plants. In this case, the root mass was gently exposed before the agar plug was placed next to the roots, then the plug was covered with fresh soil.

5. In a second variation of the above, methods 1–3 were repeated with young seedlings (<6 weeks old), and in addition, 'disease-free' parsley roots were integrated into the soil near the root mass according to the techniques of McCracken (1984). The reasoning behind this was that McCracken (1984) found that this methodology produced better and more consistent results with equivalent symptoms to those observed in the field.

In all cases, the pot containing the plant was placed in a plastic dish and the dish was flooded. Flooding conditions were maintained at all times and the plants were grown with a 16/8 hr. light cycle at 9°C until symptoms appeared. As most instances of damping off occurred under trial conditions during the autumn/winter months when free water is prevalent (Chapter 5); these conditions were reproduced in the experiment by maintaining flooding and low temperature.

Plants were checked at regular intervals for symptoms of root rot such as chlorosis, stunting and wilting. When typical symptoms were expressed, the plant was freed from the soil and root samples were placed on water agar (WA) after hypochlorite treatment for 30 sec. in an attempt to re-isolate the causative organism, either *Pythium* or *Phytophthora*.

Plates containing root samples that had fungal colonies exhibiting oomycetes characteristics (eg. formation of sporangia, oospores, typical mycelia) were subcultured onto V8 plates before Dr. James Cunnington (Biosytematics, DPI Victoria) undertook formal identification by traditional and PCR methods.

4.2.3 Antagonism studies

A literature search on the web revealed that one of our isolates, *Pythium oligandrum* was a potential mycoparasite and thus may actually offer plant protection from pathogen infection (Benhamou et al., 1997). It was unknown at this stage of the investigation as to whether the strain of *F. oxysporum* we had in long-term storage was non-pathogenic and thus also capable of mycoparasitic activity (Benhamou et al., 2002). The following experiment was performed in order to screen these isolates for mycoparasitic activity. All strains were grown for 4–7 days on either Potato Dextrose Agar (PDA, Oxoid) or Corn Meal Agar (CMA, Oxoid) at room temperature in continuous light. Discs from two cultures were excised (5 mm) with a sterile cork borer and placed on fresh PDA or CMA plates 5 mm from the edge of the plates. Both *F. oxysporum* and *P. oilgandrum* were tested against each other as well as the other *Pythium* and *Phytophthora* that we had in long term storage. In all, 23 combinations of strains were tested. These plates were incubated at room temperature in continuous light and checked every day for any reactions. Growth of the strains was checked against plates containing single isolates for comparison.

4.3 Results

4.3.1 Survey data

All parsley in Victoria was grown in-ground on raised beds with overhead irrigation. A summary of the surveys is given below in Table 4.1. This summary is by no means meant to be exhaustive. Rather, it is a snapshot of conditions in the field at selected properties at a particular time of the year. The survey was conducted in August 2005 in the winter months, where temperatures are lower and the likelihood of free water is high. At this time, the survey data from 8 growers showed that disease associated with root rot causing stunting, wilting and plant death was a major cause of concern in Victoria.

Several fungi were isolated from root rot in the parsley samples that were submitted to CHS (Table 4.2 below). Fungi such as *Rhizoctonia, Fusarium, Sclerotinia, Mycocentrospora, Cylindrocarpon Microdochium, Pythium* and *Phytophthora* were isolated. *Pythium* species isolated included *Pythium oligandrum, Pythium ultimum* and *Pythium sulcatum*, identified by Dr. James Cunnington. Several *Phytophthora* were also isolated and identified, including *Phytophthora megasperma* and *Phytophthora inundata*. The latter is reported in Victoria for the first time.

Foliar fungi isolated were *Phoma, Septoria* and *Alternaria*. Three out of the eight growers surveyed also had *Septoria*, which occurs on both curly and flat-leafed varieties. Although widespread, the incidence of this pathogen was very low.

Leaf discolouration, possibly associated with viral infections, was also noted in the surveys. Subsequent work by CHS identified the viruses involved as potyviruses using molecular techniques (Brendan Rodoni, PIRVic, personal communication).

No nematodes associated with root rot were found in Victoria during the survey period.

Table 4.1 Survey Data from Victorian Parley Growers in August 2005

Item surveyed		Result
Number of growers surveyed		8
Range of area (ha)		0.8–20.2
Type of parsley	Curly (%)	50
	Flat (%)	50
Cultivar Type	Curly	Shamrock, Limeric, Inca
	Flat	Flamingo, Dutch Verda, Italian Flat Leaf, Grande
Irrigation (Overhead, Trickle)		Overhead
Hydroponics		0
In Ground		8
Range of pH		5.8–7.45
Range of EC dS/m		0.06–0.41
Estimated losses from root damage (%)[A]		0–100
Crops with incidence of	*Septoria* (%)	37.5
	Root Knot nematodes (%)	0
	coloured leaves (%)[B]	37.5
	dead plants/stunting/wilting (%)[C]	100
Incidence in crops	*Septoria* (%)	0.5
	coloured leaves (%)	3.1
	dead plants/stunting/wilting (%)[D]	25.5

[A] growers' estimates
[B] yellow or white streaking or red foliage thought to be associated with virus but not confirmed
[C] all sites, all growers
[D] combined bare ground/root rot data all growers

4.3.2 Koch's postulates experiment

Strains of *Phytophthora* and *Pythium* stored in long term storage were isolated mostly from diseased parsley roots. The exceptions were isolate 3 (from soils associated with parsley showing the symptoms of damping off and root rot) and isolate 11 (dam water from a property that was having problems with root rot). Both 3 and 11 were isolated by the pear bait method. All strains were purified and, in some cases, identified to the species level (see Table 4.2). The last column indicates whether the original pathogen was re-isolated from the diseased parsley thus proving Koch's postulates.

Table 4.2: List of *Phytophthora* and *Pythium* isolates in long term storage

Isolate Number	Fungus	Isolation Method	Original symptoms of root rot	Koch's Postulate Experiment
2	*Pythium oligandrum* [A]	root	+	–
3	*Phytophthora* sp.	Pear bait (soil)	–	–
7	*Pythium* sp.	root	+	–
8	*Phytophthora* sp.	root	+	–
9	*Pythium intermedium*	root	+	–
11	*Phytophthora* sp. [B]	Pear bait (water)	–	–
14	*Pythium ultimum*	root	+	–
15	*Phytophthora megasperma*	root	+	+
16	*Pythium sulcatum* [C]	root	+	+
18	*Phytophthora inundata* [D]	root	+	+
19	*Pythium diclinum* 'group'	root	+	–

[A] Known mycoparasite (eg. Benhamou et al., 1997; Jones & Deacon, 1995)
[B] Isolate from dam water
[C] Pratt & Mitchell (1973)
[D] Brasier et al., (2003)

Throughout this series of experiments, results have been highly variable. The methods described in section 4.2.2.3 did not yield consistent results, with the added complication of waterlogging in some cases masking the disease. Plants exhibiting symptoms of chlorosis and wilting consistent with the disease that the original isolates caused were, on the whole, less severely affected.

At present, Koch's postulates has been successful with three isolates, with all three being re-isolated from artificially infected plants. *Phytophthora inundata* (Fig. 4.1) caused identical symptoms as shown in the field and was re-isolated successfully from the host plant that showed symptoms (front, centre, right). The parsley inoculated with *Pythium sulcatum*, *Phytophthora megasperma* or both also showed signs of stunting and wilting (Fig. 4.2), with *Pythium sulcatum* (Fig. 4.3B) causing more severe symptoms on the parsley roots than *Phytophthora megasperma* (Fig. 4.3A).

Figure 4.1: Parsley plants inoculated with *Phytophthora inundata*.

Back: Left, cut and not inoculated (control), centre inoculated plants, uncut; Right, cut and not inoculated (control).

Front: Left, uncut and not inoculated (control); centre inoculated plants and uncut; Right, uncut and not inoculated (control).

Figure 4.2: Parsley plants inoculated with *Phytophthora megasperma* and / or *Pythium sulcatum*.

Top row: Parsley inoculated with both pathogens.

Second back row: Parsley inoculated with *Pythium sulcatum*.

Second front row: Parsley inoculated with *Phytophthora megasperma*.

Figure 4.3: Parsley plants infected with:

(A) *Phytophthora megasperma*,
(B) *Pythium sulcatum* and
(C) both *Phytophthora megasperma* and *Pythium sulcatum*.

Roots of parsley plants infected with *Phytophthora megasperma* showed brown or red tips, with lateral roots still apparently intact (Fig. 4.3A). In contrast, plants infected with *Pythium sulcatum* had no lateral roots and a severe truncation of the root system. There appeared to be a distinct brown or red band around the soil line in several plants (Fig. 4.3B). Plants infected with both showed more severe symptoms, showing a stronger soil-line band as well as no lateral roots and truncation of the root system (Fig. 4.3C).

4.3.3 Antagonism experiment

Of the 23 combinations attempted, four plates exhibited a visible reaction to each other on PDA plates (Figs. 4.4–4.7). The most visible reaction was *F. oxysporum* against *Pythium ultimum*, with a distinct halo surrounding *Pythium ultimum* (Fig. 4.4). *F. oxysporum* had a less visible 'halo' effect on *Pythium oligandrum* (Fig. 4.5), *Pythium intermedium* (Fig. 4.6) and an unidentified *Pythium* (Fig. 4.7).

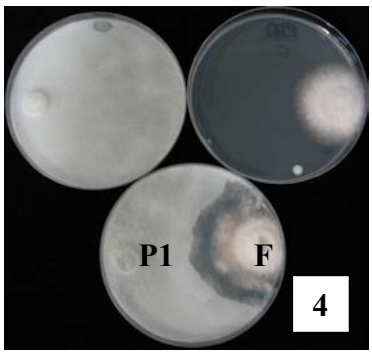

Figure 4.4: *F. oxysporum* with *Pythium ultimum*. Bottom, both on PDA plate (P1, *Pythium*; F, *Fusarium*); left, *Pythium ultimum* alone; right, *F. oxysporum* alone.

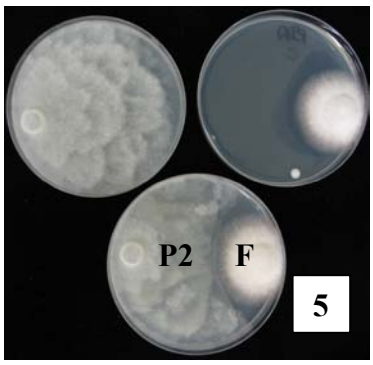

Figure 4.5: *F. oxysporum* with *Pythium oligandrum*. Bottom, both on PDA plate (P2, *Pythium*; F, *Fusarium*); left, *Pythium oligandrum* alone; right, *F. oxysporum* alone.

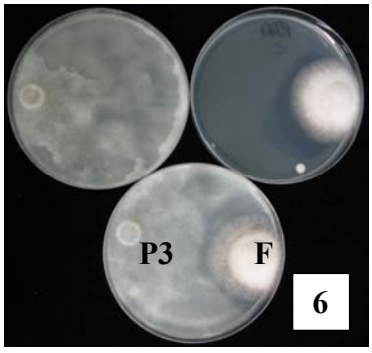

Figure 4.6: *F. oxysporum* with *Pythium intermedium*. Bottom, both on PDA plate (P3, *Pythium*; F, *Fusarium*); left, *Pythium intermedium* alone; right, *F. oxysporum* alone.

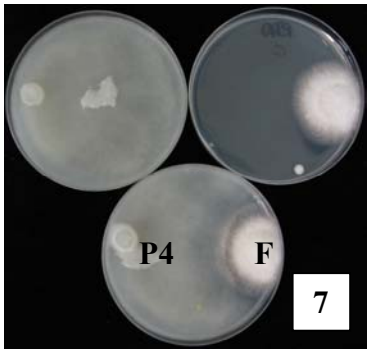

Figure 4.7: *F. oxysporum* with unknown *Pythium*. Bottom, both on PDA plate (P4, *Pythium*; F, *Fusarium*); left, unknown *Pythium* alone; right, *F. oxysporum* alone.

4.4 Discussion

4.4.1 Survey results

In the survey, all growers reported root rot in parsley. From these root rots, several isolates of *Pythium* and *Phytophthora* were isolated. *Phytophthora inundata* is reported for the first time in Australia and the gene sequences of that isolate will be submitted to an appropriate public genebank soon, since the associated paper has been accepted for publication (see Chapter 8 for submission). *Phytophthora megasperma, Phytophthora inundata* and *Pythium sulcatum* are pathogenic in other plants (eg. Brasier et al., 2003; Pratt & Mitchell, 1973; Ryley et al., 1991). *Pythium oligandrum* is a mycoparasite that can be associated with plants or the plant rhizosphere, and is actively being investigated as a potential biocontrol agent (eg. Brožová, 2002), whereas *Pythium ultimum, Pythium diclinum* and *Pythium intermedium* are known pathogens in other plant systems (eg. Benhamou et al., 2002; El-Androusse et al., 2005; Tsror et al., 1997).

Of the fungal genera isolated from root rots in this survey, *Pythium, Phytophthora, Rhizoctonia*, *Fusarium, Sclerotinia* and *Mycocentrospora* contain known pathogenic species associated with root rot. It is likely that both *Cylindrocarpon* and *Microdochium* are secondary or opportunistic pathogens,

since root rots where these were isolated from had a stronger presence of both *Phytophthora* and *Pythium* (CHS, personal communication).

Three fungi isolated from parsley leaves are known foliar pathogens. The fungicides used to control *Septoria* (often referred to as 'rust' by growers) can also control the other two foliar pathogens. *Alternaria petroselini* was formally identified and is the cause of leaf blight in parsley. It is reported for the first time in Australia. However, it is unlikely that this pathogen will be a problem in Victoria due to the unfavourable temperature conditions (see Chapter 2).

Nematodes were not found in Victoria, which may be a consequence of growers applying fowl manure to their crops. Application of fowl manure reduced losses from root-knot nematodes in capsicums by increasing the biological activity of the soil and thus enhancing biological control of the pathogen (Stirling, 2005).

4.4.2 Koch's postulates experiments

Phytophthora inundata, Phytophthora megasperma and Pythium sulcatum have proven to be pathogenic to parsley by Koch's postulates. *Phytophthora inundata* has previously been associated with root and collar roots of trees and shrubs after flooding (Brasier et al., 2003), and is reported for the first time in Australia.

The root symptoms of the infected plants for *Pythium sulcatum* and *Phytophthora megasperma* are typical, in our experience, of *Phytophthora* and *Pythium* infection. *Phytophthora megasperma* infected the root tips, whereas *Pythium sulcatum* infected further up the root and attacked the lateral roots, resulting in no lateral roots and a severely truncated root system (Figs. 4.3A–C). *Pythium sulcatum* had an obviously more deleterious effect on parsley health than *Phytophthora megasperma* (Fig. 4.2), as it affected the root system more severely (Figs. 4.3A & 4.3B). *Pythium sulcatum* is pathogenic in carrots (Pratt & Mitchell, 1973), and has also been isolated from parsley and parsnip (Davidson & McKay, 2001; Plaats-Niterink, 1981). Rotation with a crop such as broccoli reduced severity and incidence of infection in carrot (Davidson & McKay, 2001). *Phytophthora megasperma* causes root rot in lucerne and soybean and is pathogenic on many other hosts (Irwin, 1976; Ryley et al., 1991).

Pythium oligandrum (isolate 2) did not produce symptoms of damping off or root rot in parsley. Although it had been isolated from roots exhibiting root rot, subsequent investigations of the literature showed it to be a virulent mycoparasite, which actively suppresses the infectivity of other fungi and is currently being researched as a biological control agent (eg. Benhamaou et al., 1997; Jones & Deacon, 1995). *P. oligandrum* has been shown to actively infect tissues of plants as well as the surrounding rhizosphere, but not affect the plant itself (Benhamaou et al., 1997; Martin & Handcock, 1987). There are some commercial applications available utilising *P. oligandrum* such as Polyversum, a biofungicide available in the EU for the suppression of damping off in wheat and seed treatment (as a seed coat) containing *P. oligandrum* oospores (Brožová, 2002).

There are several possibilities for the failure of these experiments to prove Koch's postulates. These are outlined below:

- The fungal strains in long-term storage, although isolated from root rots in parsley, may not have been the primary causal organism for the disease.

- Anecdotal evidence suggests that continual subculturing of pathogenic fungi causes the fungus to lose its pathogenicity. Although these fungi have been stored by the recommended method in sterile distilled water (McGinnis, 1974), some isolates have had to be regrown and re-stored from cultures that had been stored in less-than-optimal conditions.

- The pH of the soil mixture present may not have been optimal for the pathogens concerned. A random sample of pH of the potting mixes revealed a pH range of between 5.6 and 6.2. It must be noted that the optimum growth pH for some *Pythium* species is above 6.0, so it could well be that

for those experiments involving *Pythium*, there could have been a problem with germination and subsequent infection because of this.

In retrospect, pre-sterilised soils from the trial sites would have been a better alternative for the above experiments, providing a soil pH close to that of the field trial (pH 6.8–7.4), as well as soil conditions close to the original conditions in which the disease is prevalent. Other studies have shown that damping off caused by *Pythium* is influenced by soil type, with some soils of a low clay content suppressing damping off (Knudsen et al., 2002).

The possibility that the culturing conditions used for *Pythium* yielded a large number of oospores could also have contributed to the poor results. Oospore germination resulting in the formation of zoospores in some *Pythium* species tends to be low unless exacting conditions are met (eg. McQuilken et al., 1990). Conditions may not have been optimal for zoospore production, and thus infection of plant tissue may no have occurred.

4.4.3 Antagonism studies

A routine search focussing on mycoparasites revealed that both *F. oxysporum* and *Pythium oligandrum* have potential to protect certain crops from pathogens (eg. Benhamou et al., 2002; Brožová, 2002; Nelson, 2005). It is clear from our initial results that *F. oxysporum* affects the growth of some of the pythiums in our collection on artificial media (see Figs. 4.4–4.7). The result with *P. ultimum* is also encouraging, since literature suggests that this *Pythium* is especially pathogenic (eg. Georgakopoulos et al., 2002). It has been suggested by other workers that *F. oxysporum* has an inhibitory effect through a combination of anti-fungal compounds and direct mycoparasitism (Benhamou et al., 2002). In that particular study, there was *in planta* evidence of mycoparasitic activity. This may explain a result in Trial No. 2, where a treatment failed to protect parsley at all (see Chapter 5). The chemical regime of iprodione/fludioxonil is touted as being specific against *Fusarium* and *Rhizoctonia*. These fungicides may have also knocked out other beneficial saprophytes as well, allowing the proliferation of *Pythium* and *Phytophthora*, causing the severe symptoms exhibited in the field.

4.5 References

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Chapter 5

Victorian field trials for the identification of the causal fungi and their control options

Elizabeth Minchinton and Desmond Auer

Summary

In Victoria during winter, water mould fungi (oomycetes) caused root rot of parsley. The causal organisms were *Phytophthora*, *Pythium* or a complex of more than one species or genera. Aboveground symptoms appeared in the crop at 8 weeks. Two applications of metalaxyl or weekly applications of phosphonic acid provided 87–98% control of the disease.

5.1 Introduction

Crop losses from parsley root rot and post emergence damping off were identified during project VG01045. Growers reported the problem was most prevalent during late autumn and winter, especially after heavy rain. In one instance a whole bay of parsley was lost to post-emergence damping off. Mature parsley crops were also susceptible to root rot and collapse of shoots. Symptoms were reddish-brown lesions on the neck of plants at the soil line; soft rotting of this root area; necrosis of lateral roots and rot of the taproot. A number of fungi were isolated from diseased roots with the most common being *Fusarium*, *Microdochium*, *Cylindrocarpon*, *Rhizoctonia*, *Pythium*, *Mycocentrospora* and *Phytophthora*. Some growers reported high salinity in the dam water used for irrigation. On one occasion, collapse of parsley during hot summer weather was attributed to reverse osmosis, as the roots were symptomless.

A number of fungi have been reported to cause root rot or damping off in parsley. Root rot of parsley was caused by *Phytophthora cryptogea* in California (Davis et al., 1994) and *P. nicotianae* in Hawaii (Uchida and Kodooka, 2006). However, in Northern Ireland it was associated with *Pythium paroecandrum* (McCracken, 1984a), *P. matophorum* in Germany (Krober and Sauthoff, 1999) and *P. aphanidermatum* on hydroponic parsley in South Africa (Gull et al., 2004).

"Damping off" of parsley in the USA was associated with *Pythium ultimum, P. irregulare* and *Rhizoctonia solani* (Hershman et al., 1986) and *P. debaryanum* (De Zeeuw, 1954), whilst in Belgium and Poland, it was associated with *Alternaria, Fusarium, Phoma, Rhizoctonia, Sclerotinia* and *Pythium* (Nawrocki and Mazur, 2004; Nowicki, 1997). However, Hershman et al., (1986), reported that the *Fusarium* species isolated from parsley were avirulent.

Parsley damping off was successfully controlled with iprodione and metalaxyl when associated with *Alternaria* and *Fusarium* species in Poland (Nowicki, 2002). McCracken (1984b), however, had no success in controlling root rot in Ireland with metalaxyl, furalaxyl, metalaxyl+mancozeb, copper, thiram or Tachigaren™. Reduction in disease was achieved by rotating crops with barley, leeks, beetroot or spring onions (McCracken, 1984a).

Temperature and salinity can influence root rot development in parsley. Hershman et al (1986) showed that pathogenicity of *Rhizoctonia solani* on parsley was influenced by temperature, whereas that of *P. ultimum* and *P. irregulare* was not. Symptoms of root rot caused by excessive fertilizer use and accumulation of high levels of soluble salts were difficult to distinguish from fungal root rots (The Connecticut Agricultural Experiment Station, 2006).

This chapter reports on four field trials designed to:

- Determine which groups of fungi were responsible for damping off and mature plant collapse,
- Establish management options to control the disease with chemicals, a fumigant and a bio-control agent,
- Ascertain if salinity was an issue in parsley production,
- Define a control measure for root rot of coriander, which was causing similar symptoms to root rot of parsley, on request from the steering committee.

5.2 Materials and methods

5.2.1 Chemicals and application

In trial No 1, chemicals were initially applied with a boom fitted with 3 blue hollow cone nozzles SPX No 8 on a Sylvan Slectra 12v knapsack (Silvan Pumps and Sprayers (Aus) Pty. Ltd) at 1000L/ha for the first application and by watering can at 5000L/ha for the second application (Table 5.1).

In trial No 2, granular formulations of chemicals were applied by hand and the bio-control agent was applied by watering can. All other chemical applications were applied with a boom fitted with 3 brown hollow cone nozzles SPX No 12 by knapsack, as previously described. Fungicides were applied at 500L/ha at the seedling stage and 1000L/ha at maturity (Tables 5.1 and 5.2). The seed treatment was courtesy of Seed Solutions (4 Concord Crescent, Carrum Downs, Vic 3201).

In trial No 3, chemicals were applied as described for trial No 2, except hymexazol was applied by watering can at a rate of 1L/1000L and 3L/m^2 of plot (Tables 5.1 and 5.3).

In trial No 4, chemicals were applied as per trial No 2, except that application rate was 500L/ha throughout the trial (Tables 5.1 and 5.4).

Table 5.1 Chemical information and rates

Trade name	Active ingredient	Company	Rate	Activity Group
Agral 600 ™	nonyl phenol ethylene oxide	ICI	0.02%	-
Agri-Fos 600®	phosphonic acid	Agrichem	170 ml/100L(seedling) 3 L/ha (mature)	Y
Apron XL®	metalaxyl	Syngenta	1.75 ml/kg	D
Bavistin®	carbendazim	BASF	40 ml/100L	A
Dynasty®	iprodine, fludioxonil, metalaxyl	Syngenta	200 ml/100kg	B,L,D
Filan®	boscalid	BASF	129 g/100L	G
Perlka®	calcium cyanamide	SKW Trostberg AG	500 kg/ha	-
Plantmate G™	Trichoderma	Agrimm Technologies	15 kg/ha	-
Plantmate WP™	Trichoderma	Agrimm Technologies	4 g/L	-
Ridomil Gold MZ®	mancozeb + metalaxyl	Novatis	2.5 kg/1000L	D,Y
Ridomil Gold 25G®	metalaxyl	Syngenta	120 g/100m of row	D
Rovral®	iprodione	Bayer	100 ml/100L	B
Tachigaren®	hymexazol	Sankyo Agro	1.0 l/1000L	-
Switch®	cyprodinil + fludioxinil	Syngenta	80g /100L	I,L

5.2.2 Field trial No 1 on parsley during summer-early autumn 2004

This trial consisted of two parsley crops; an established young crop (about 8 weeks) and an established old crop near harvest (about 12 weeks). Both crops were cultivar Curly Leaf (South Pacific Seeds) and direct sown at 1435 North Road, Devon Meadows, Victoria. Thiram-treated seed had been planted 3 rows per raised bed at an average of 29 plants/single m of row. In each of the two crops a trial was pegged out on a single bed, in a randomised block design of 5 blocks each containing 3 treatments (plots). Plot sizes were 1.62m wide x 2.5m long. The three treatments consisted of 2 sprays of Ridomil Gold MZ® (metalaxyl + mancozeb), Switch® (cyprodinil + fludioxonil) + 0.02% Agral™ (non-ionic surfactant) and a control (water) applied on 5/2/2004 (week 0) and on 19/2/2004 (week 2).

5.2.3 Field trial No 2 on parsley during autumn-winter 2005

Seeds of cultivar Italian Plain Leaf parsley, (Seminis Vegetable Seeds, Batch 1041566 Lot 303775, thiram-treated), were direct sown at 1435 North Road, Devon Meadows, Victoria at 3 rows per bed on 26/4/2005. The trial was laid out in a randomised block design of 8 blocks each containing 7 treatments (plots). Plots sizes were 1.62m wide x 4m long on raised beds and contained on average 75 seedlings per m length of row on emergence. The treatments were control (water), Ridomil Gold 25G® (metalaxyl), Rovral® (iprodione), Switch® (cyprodinil + fludioxonil), metalaxyl seed treatment plus phosphonic acid (Agri-Fos 600®), Plantmate™ (Trichoderma), nutrient monitored (Rootzone Solutions), Perlka™ (calcium cyanamide) (Table 5.2). All plots, excluding the Rootzone Solutions treatment, were top-dressed with Rustica Plus™ (NPK 12:5:14) at 140kg/ha (Campbells Fertilizer, Australia) on 27/6/2005 (week 9), while the Rootzone Solutions plots were treated with 60g/ha sulphate of potash (NPK 0:0:41) (Incitec Fertilizers) on 5/8/2005 (week 14).

Table 5.2 Schedule of treatment applications for trial No 2 on parsley autumn-winter 2005

	Week (date)																	
	-2	0[P]	2[E]	4	5	6	7	8	9[F]	10	11	12	13	14	15	16	17	18
Treatment	11/4/2005	26/04/2005	12/05/2005	23/05/2005	30/05/2005	6/06/2005	13/06/2005	20/06/2005	27/06/2005	4/07/2005	11/07/2005	18/07/2005	25/07/2005	1/08/2005	8/08/2005	15/08/2005	22/08/2005	29/08/2005
Control (water)	-	-	-	-	W	-	W	-	W	-	W	-	W	-	W	-	W	-
Metalaxyl	-	RG	-	-	-	-	-	-	-	-	RG	-	-	-	-	-	-	-
Iprodione/fludioxonil	-		R	-	S	-	R	-	S	-	R	-	S	-	-	-	-	-
Metalaxyl/ phosphonic acid	-	M	-	-	-	A	-	A	-	A	A	A	A	A	A	A	A	A
Trichoderma	-	Pg	-	-	-	Pd	-	-	-	Pd	-	-	-	Pd	-	-	-	Pd
Nutrient monitored	-	-	+	+	+	+	+	+	+	+	+	+	+	+f	+	+	+	+
Calcium cyanamide	Pe	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[P], planted: [E], emergence; [F], all treatments fertilized except nutrient monitoring; W, water; RG, metalaxyl-m; R, iprodione; S, cyprodinil + fludioxinil; M, metalaxyl (seed); A, phosphonic acid; Pg, trichoderma granular; Pd, trichoderma drench; Pe, calcium cyanamide; +, samples taken for nutrient monitoring; f, fertilizer applied to nutrient monitored treatment; -, not applicable.

5.2.4 Field trial No 3 on parsley during spring-summer 2005

Seeds of parsley Italian Plain Leaf (Seminis) were direct sown at 3 rows per bed on raised beds at a property opposite 200 Moores Road, Clyde, Victoria, on 5/10/2005. The trial was laid out in a randomised block design of 7 blocks, with one block per bed of parsley. Each block contained 5 treatments (plots). Plot sizes were 1.2m wide x 8m long and contained on average 23 seedlings per m length of row on emergence. The treatments were control (unsprayed), seed coated with Dynasty® (azoxystrobin, metalaxyl and fludioxonil), Ridomil Gold 25G® (metalaxyl), AgriFos 600® (phosphonic acid) and Tachigaren™ (hymexazol) (Tables 5.1 and 5.3).

Table 5.3 Schedule of treatment applications for trial No 3 on parsley spring-summer 2005

Treatment	Week (date)											
	0 [P]	1	2	3 [E]	4	5	6	7	8	9	10	11 [S]
	5/10/2005	12/10/2005	19/10/2005	26/10/2005	2/11/2005	9/11/2005	16/11/2005	23/11/2005	30/11/2005	7/11/2005	14/11/2005	21/12/2005
Control (untreated)	-	-	-	-	-	-	-	-	-	-	-	-
Azoxystrobin + metalaxyl + fludioxonil (seed)	D	-	-	-	-	-	-	-	-	-	-	-
Metalaxyl-m	M	-	-	-	-	-	-	-	M	-	-	-
Phosphonic acid	A	-	A	-	A	-	A	-	A	-	A	-
Hymexazol	H	-	H	-	H	-	H	-	H	-	H	-

[P], planted; [E], emergence; [S], assessed; D, Azoxystrobin + metalaxyl + fludioxonil; M, metalaxyl; A, phosphonic acid; H, hymexazol; -, not applicable.

5.2.5 Field trial No 4 on coriander during summer- autumn 2005

Seeds of coriander cultivar Santo (Fiarbanks Selected Seed Company), were direct sown into 3 rows per bed on raised beds at a property opposite 200 Moores Road, Clyde, Victoria, on 5/10/2005. The trial was laid out in a randomised block design of 7 blocks with one block per bed of coriander. Each block contained 5 treatments (plots). Plots sizes were 1.2m wide x 8m long and contained on average 60 seedlings per m length of row on emergence. The treatments were control (unsprayed), Bavistin® (carbendazim), seed coated with Dynasty® (azoxystrobin, metalaxyl and fludioxonil) as previously described, Filan® (boscalid) and Ridomil Gold 25G® (metalaxyl) (Table 5.4).

Table 5.4 Schedule of treatment applications for trial No 4 on coriander autumn-summer 2005

Treatment	Week (date)						
	0 [P]	1	2 [E]	3	4	5	6 [A]
	5/10/2005	12/10/2005	19/10/2005	26/10/2005	2/11/2005	9/11/2005	17/11/2005
Control	-	-	-	-	-	-	-
Carbendazim	-	-	B	-	B	-	-
Azoxystrobin + metalaxyl + fludioxonil (seed)	D	-	-	-	-	-	-
Boscalid	-	-	F	-	-	-	-
Metalaxyl-m	R	-	-	-	-	-	-

[P], planted; [E], emergence; A, assessment; D, azoxystrobin, metalaxyl and fludioxonil; B, carbendazim; F, boscalid; R, metalaxyl; -, not applicable.

5.2.6 Assessment

5.2.6.1 Scoring symptoms

Field trial No 1 was assessed at 4 weeks on 4/3/2004 by randomly harvesting 10 plants per plot and scoring the first 10cm of taproot below ground level for percentage of root covered by lesions. Data was grouped into 2 categories:
Proportion of plants with <20% symptoms of root rot
Proportion of plants with >20% symptoms of root rot
and analysed by ANOVA using Genstat 8.1 Lawes Agricultural Trust (Rothamsted Experimental Station).

Field trial No 2 was assessed visually at week 10 on 8/7/2005 for average number of plants with dieback, average length of row with dieback, average number of infection sites of dying plants and average plant vigour. At week 14 on 5/8/2005 and week 20 on 13/9/2005; the trial was assessed for average length of row with dieback; average number of infection sites of dying plants and average plant vigour. Data were analysed as previously described. Yield was estimated at 60 decks/3 rows/40m of parsley bed, where one deck equals 10 bunches of parsley (P. Cochrane, pers. comm.) which equates to 0.5 deck of parsley per 1m length of a single row. Vigour was assessed on a scale of 0-3, where 0 = no plants, 1 = poor growth, 2 = moderate growth, 3 = lush growth.

Field trial No 3 was assessed on 21/12/2005 for percentage of plants with symptoms of dieback (the total number of parsley plants and number of parsley plants with symptoms of wilt or death) as well as vigour, as for trial No 2. Data were analysed as previously described.

Field trial No 4 was assessed on 17/11/2005 by randomly harvesting a 1m length of row in each row of a plot and counting the total number of plants, assessing the number of plants with necrotic symptoms on roots and vigour of plants in the plots, on a scale of 0 – 3, as previously described. These data were analysed using logistic regression with Genstat 8.1 Lawes Agricultural Trust (Rothamsted Experimental Station).

5.2.6.2 Nutrient monitoring, pH and EC

In trial No 1, soil and irrigation water from the mature crop were tested for pH and EC by Crop Health Services (DPI Vic. Knoxfield). In trial No 2, nutrient monitoring, pH and EC testing were undertaken by Rootzone Solutions (3 Rainer St Karrinyup, WA 6018). The collection probes were located at depths of 15 cm and 30 cm. The researchers conducted pH and EC tests in trials Nos 3 and 4.

5.2.6.3 Pathogen identification

In trial No 1, parsley plants from the mature crop showing roots with red-brown lesions were sampled and sent to Crop Health Services for pathogen testing, whilst in trials 2, 3 and 4, pathogen testing was undertaken by the researchers. Diseased root pieces from control plots were surfaced-sterilised in 0.5% sodium hypochlorite for 30 sec then plated onto water agar (WA) and either potato dextrose agar with 0.05 g/l acromycin (PDAA) or V8 agar (Johnson and Booth, 1983). Isolates of potential pathogens were sent to Dr James Cunnington (DPI Vic., Knoxfield) for identification.

5.2.6.4 Meteorological data

Soil temperature data were collected during trial 2 by an Environdata Mark 4 weather station (Environdata Australia Pty. Ltd.), with a TA10 temperature sensor buried to 10cm depth. This soil temperature data was compared with data collected in the same parsley growing area during 2002 by Oscar Villalta (DPI, Victoria). Rainfall data was obtained from the Bureau of Meteorology.

5.2.6.5 Residue analysis

Residue analysis was undertaken by the National Measurement Institute (Australian Federal Government, 51–65 Clarke Street, South Melbourne Vic. 3205), for metalaxyl and phosphorous acid present as mono- and di-potassium phosphonate for trials 2 and 3. The trial No 2 samples were frozen

at –20°C for approximately 10 weeks prior to analysis, whilst the trial No 3 samples were frozen at the same temperature then sent for analysis within a week of the final sample collection.

5.3 RESULTS

5.3.1 Field trial No 1 parsley

There were no above ground symptoms on either the young or old parsley plants. In the young parsley plant trial there was no significant difference between any of the treatments for the proportion of plants with a score <20% or a score >20% of plant roots with symptoms of root rot. In the old parsley plant trial, metalaxyl + mancozeb had a significantly lower proportion of plants with a score <20% of plant roots with symptoms of root rot and consequently a corresponding significant higher proportion of plants with >20% of root rot (Table 5.5). The soil pH and EC were 6.72 and 0.54 mS/cm, respectively. In irrigation water, pH and EC were 7.36 and 3.3 mS/cm, respectively. Crop Health Services frequently isolated Fusarium and Pythium, whilst Microdochium and Cylindrocarpon were less frequently isolated from reddish-brown lesions on mature parsley roots in all treatments.

Table 5.5 Effect of fungicides on symptoms of root rot in 'old' parsley, summer – autumn 2004

Treatment	Proportion of plants with root rot scores	
	<20%	>20%
Control (water)	90.8a	9.2
Cyprodinil + fludioxinil + Agral™	84.8a	15.2
Metalaxyl + mancozeb	66.5b	33.5
lsd (5%)	18.58	–

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

5.3.2 Field trial No 2 parsley

5.3.2.1 Efficacy of treatments to control dieback

There were no symptoms of pre-emergence dieback. Above ground symptoms of dieback first appeared in control plants of the trial at 8 weeks as wilt, necrosis and collapse of plants (Table 5.6). Roots of collapsed plants showed a neck and shoulder rot with loss of laterals. The metalaxyl and phosphonic acid treatments were far more effective than all other treatments in controlling dieback throughout the trial. At harvest these two treatments had controlled parsley dieback by 87–98%, respectively (Table 5.7).

The metalaxyl seed treatment was reported to be effective for 4 weeks and was followed by the phosphonic acid treatment from week 4. As symptoms of dieback did not appear until week 8, it will henceforth be referred to as the phosphonic acid treatment.

At week 8 phosphonic acid and metalaxyl treatments significantly reduced the length of parsley row with dieback, reduced the number of infection sites and did not decrease vigour of plants. At week 12, metalaxyl was significantly more effective in controlling parsley dieback than phosphonic acid for the length of row and number of infection sites showing dieback. At week 18, plants treated with phosphonic acid appeared more vigorous than those treated with metalaxyl, but there were no significant differences between these treatments. Both treatments were superior to all the other treatments at week 12 and week 16.

Trichoderma and calcium cyanamide treatments had no effect on parsley dieback as they did not differ significantly from the control treatment for any of the factors measured throughout the trial (Table 5.6). This is also the case for the nutrient monitoring treatment, which had no fungicides

applied and where top-dressing was delayed by 5 weeks. The use of iprodione/fludioxonil significantly increased the amount of disease compared with the control treatment or showed no significant differences from the control. Leaving parsley untreated (control) resulted in a 75% crop loss (Table 5.7).

5.3.2.2 Residue analysis

Residues of metalaxyl and phosphorous acid present as mono and di-potassium phosphonate were below Maximum Residue Levels (MRL). The lack of a spike in the residue of phosphorous acid present as mono and di-potassium phosphonate at Day 0 just after the spray application is of concern (Table 5.8). It may be attributed to the storage of frozen parsley leaves at –20°C for several months.

5.3.2.3 Meteorological data

In this parsley growing area soil temperatures range from 17–25°C during summer and fall to below 10°C during winter (Fig 5.1). Soil temperature on trial site No. 2 showed temperatures at and below 10°C during the time parsley root rot is prevalent (Fig 5.2). There was 10mm or more of rainfall on the 11th and 14th of June, the 4th, 20th and 31st of August (Fig. 3). The heavy rainfall in June was a month before the disease appeared in the field trial.

5.3.2.4 Nutrient monitoring, pH and EC

Refer to chapter 6 for a description of the nutrient monitoring results on the trial site.

5.3.2.5 Pathogen identification

Pythium and *Phytophthora* species were consistently isolated from pear baits of soil and from upper parts of diseased plant roots. Difficulty was experienced in obtaining pure cultures for identification from both sources especially from the plant roots, although, *Pythium ultimum* and *Phytophthora megasperma* were identified. About 12 months earlier Crop Health Services DPIVic isolated *Pythium oligandrum* and *P. intermedium* from parsley roots, and an unidentified *Phytophthora* sp. was isolated from pear baits of soil, from a diseased parsley crop on the same site during HAL project VG01045 (unpublished).

Table 5.6 Effect of treatments on parsley dieback Trial No 2

Treatment	Week 8 (8/7/2005)					Week 12 (5/8/2005)					Week 16 (28/8/2005)			
	Log mean length of row of diseased plants	Mean length of row of diseased plants (cm)	Log mean No. infection sites	Mean No. infection sites	Mean vigour	Log mean length of row of diseased plants	Mean length of row of diseased plants (cm)	Log mean No. infection sites	Mean No. infection sites	Mean vigour	Log mean length of row of diseased plants	Mean length of row of diseased plants (cm)	Log mean vigour	Mean vigour
Control (water)	2.84b	16.10	1.12ab	2.12	1.62b	5.67b	279.33	2.56a	12.48	1.59b	6.82a	903.59	–0.80b	0.35
Metalaxyl	0.17c	0.19	–0.42c	0.16	2.38a	2.79d	6.20	–0.05c	0.45	2.78a	5.10b	154.58	0.94a	2.45
Metalaxyl/ phosphonic acid	0.88c	1.41	–0.13c	0.38	2.53a	3.55c	24.96	0.88b	1.91	2.56a	3.49b	22.83	1.09a	2.88
Nutrient monitored	2.20b	8.05	0.86b	1.86	1.84b	5.24b	179.61	2.36a	10.07	1.69b	6.76a	852.65	–0.71b	0.39
Calcium cyanimide	2.49b	11.10	0.78b	1.69	1.66b	5.38b	206.15	2.53a	12.00	1.72b	6.91a	995.02	–0.80b	0.35
Trichoderma	3.15ab	22.33	1.16ab	2.69	1.59b	5.94ab	370.31	2.77a	15.45	1.56b	6.95a	1037.3	–1.24bc	0.19
Iprodione/fludioxonil	3.74a	41.15	1.86a	5.92	1.41b	6.30a	536.72	2.55a	12.28	1.05c	7.03a	1125.3	–1.59c	0.10
l s d	0.973		0.6628		0.5045	0.6220		0.6410		0.4535	0.6310		0.5630	

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

Table 5.7 Effect of treatments on the estimated yield of parsley in trial No 2 at 16 weeks

Treatment	Mean length of 12m single row of parsley lost to dieback[A]	Mean percentage of parsley lost to dieback[B]	Yield[C] (decks/ha)	
Control (water)	9.04	75.3	2287.04	a[A]
Metalaxyl	1.55	12.92	8062.96	b
Metalaxyl / phosphonic acid	0.23	1.92	9081.48	b
Nutrient monitored	8.53	71.08	2677.78	a
Calcium cyanimide	9.95	82.92	1581.48	a
Trichoderma	10.37	86.42	1257.41	a
Iprodione/fludioxonil	11.25	93.75	578.70	a

[A], data from Table 5.6; [B], Data are based on mean losses of parsley in a 12m single row of parsley plants; [C], The optimum yield is based on 60 decks of parsley from 3 rows of a bed 40m long by 1.62m wide that produces 0.5 decks of parsley per 1m length of row and therefore 9259.26 decks/ha. The yield for each treatment was calculated as optimum yield minus the percentage yield loss for that treatment.

Table 5.8 Residue analysis of fungicides in parsley tissue for treatments that controlled parsley dieback in trial No 2

Treatment	Active	Day	Date sampled	Level of detection (mg/kg)
Control (water)	Water	na	13/9/2005	<0.05 metalaxyl <1.0 phosphorous acid
Ridomil Gold 25G	Metalaxyl	Day + 64	13/9/2005	<0.05
Agri-Fos 600	Phosphorous acid present as mono and di potassium phosphonate	Day - 1	12/9/2005	<1.0
Agri-Fos 600	Phosphorous acid present as mono and di potassium phosphonate	Day 0	13/9/2005	<1.0
Agri-Fos 600	Phosphorous acid present as mono and di potassium phosphonate	Day + 7	20/9/2005	<1.0

Fig 5.1 Average daily soil temperature (°C) for a parsley growing area in Cranbourne during 2002, courtesy of Oscar Villalta. This graph shows the general trend in soil temperature data in the Cranbourne parsley-growing area for a whole year.

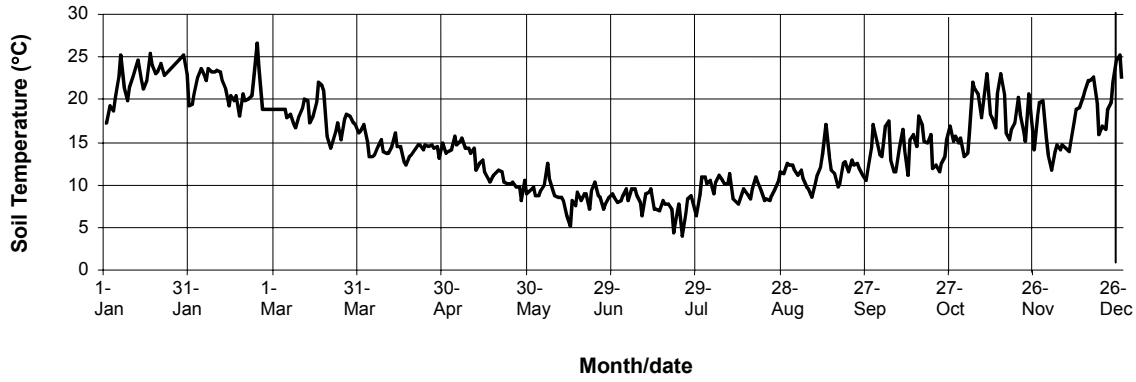


Fig 5.2 Average daily soil temperature (°C) on trial No 2 site, winter 2005

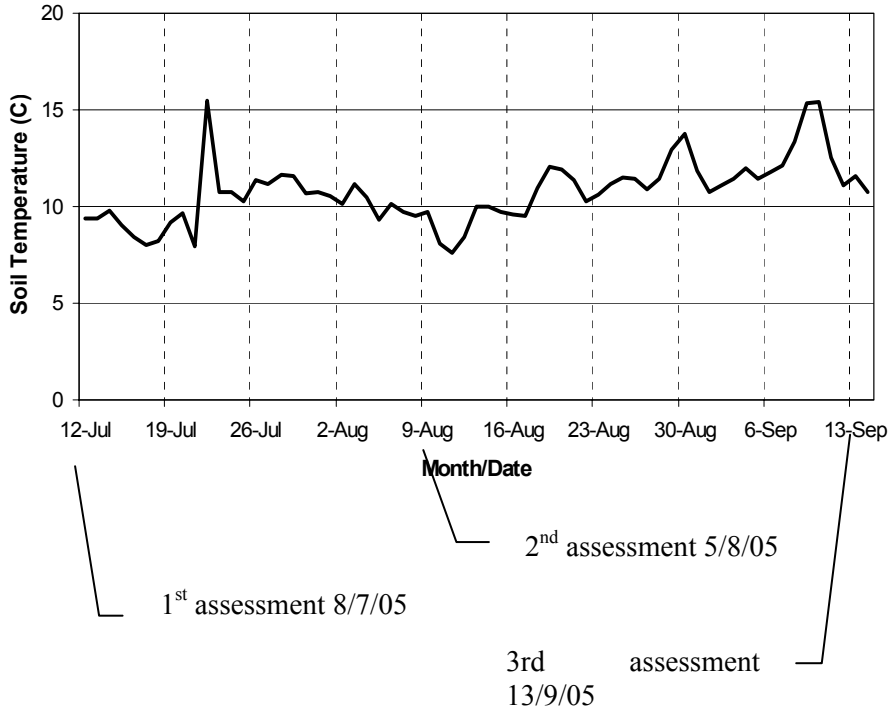
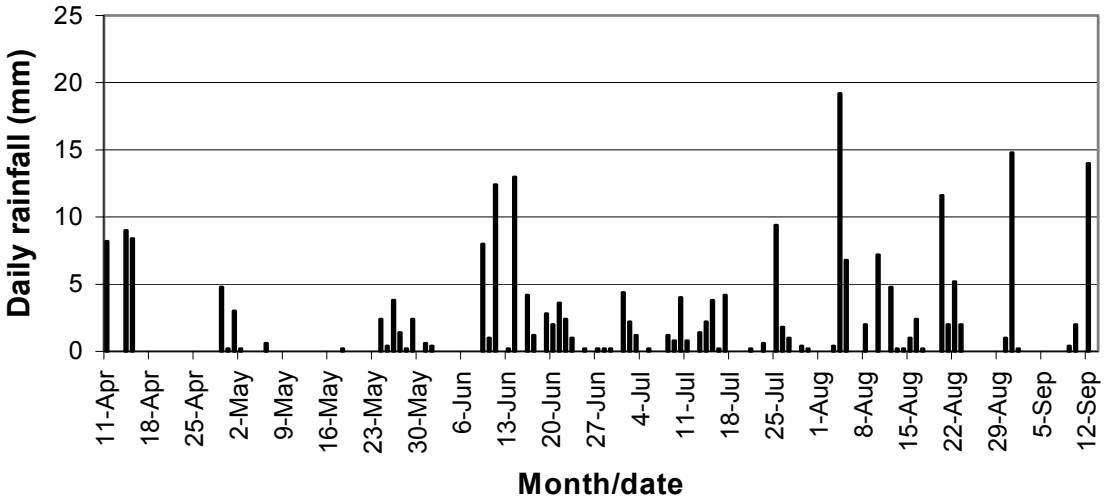


Figure 5.3 Rainfall observations at Moorabbin airport 2005 (20km north-east of Cranbourne)



5.3.3 Field trial No 3 parsley

5.3.3.1 Effect of treatments to control dieback

Aboveground symptoms, expressed as dieback of foliage, first appeared at week 8 on plants in the control and seed coated (azoxystrobin + metalaxyl + fludioxonil) treatments, but disease pressure was low at harvest, week 11 (Table 5.9). Symptoms of rot on roots were similar to trial No. 2. Two applications of metalaxyl produced the best control of dieback. Fortnightly applications of phosphonic acid, fungicide coated seed or hymexazol did not control dieback. Up to and including week 6, hymexazol-treated parsley displayed slower growth, compared with all other parsley treatments and by harvest at week 11 it had the lowest plant vigour. The site had a pH of 7.28 and EC of 0.37 mS/cm.

Table 5.9 Effect of treatments for controlling parsley dieback in Trial 3 at week 11 during spring-summer 2005

Treatment	Mean percentage of plants with dieback	Mean plant vigour (scale 0-3)
Azoxystrobin + metalaxyl + fludioxonil (seed)	5.52a	2.49 b
Control	5.21a	2.72 ab
Phosphonic acid	5.02a	2.62 b
Hymexazol	5.06a	1.96 c
Metalaxyl	0.92b	2.81 a
lsd	1.756	0.1319

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

5.3.3.2 Residue analysis

Residue analysis showed that plants in both the unsprayed control plots and the phosphonic acid treated plots (Agri-Fos 600) had a high phosphonic acid level (phosphorous acid present as mono- and di-potassium phosphonate) on the last day phosphonic acid was sprayed. The day after spraying there was a sharp decrease in phosphonic acid levels below the MRL (Table 5.10). In contrast, the residue levels of metalaxyl persisted until at least day 19 and fell below MRL from day 22.

Table 5.10 Residue analysis of parsley for fungicides which controlled parsley dieback in trial No 3

Treatment	Active	Day	Date sampled	Level of detection (mg/kg)
Control	Water	na	14/12/2005	3.5 phosphorous acid, <0.05 metalaxyl
Control	Water	na	15/12/2005	<0.05 metalaxyl <1.0 phosphorous acid
Control	Water	na	19/12/2005	<0.05 metalaxyl <1.0 phosphorous acid
Control	Water	na	22/12/2005	<0.05 metalaxyl <1.0 phosphorous acid
Control	Water	na	28/12/2005	<0.05 metalaxyl <1.0 phosphorous acid
Ridomil Gold 25G	25g/kg metalaxyl	Day + 15	15/12/2005	0.29 metalaxyl
Ridomil Gold 25G	25g/kg metalaxyl	Day + 19	19/12/2005	0.12 metalaxyl
Ridomil Gold 25G	25g/kg metalaxyl	Day + 22	22/12/2005	<0.05 metalaxyl
Ridomil Gold 25G	25g/kg metalaxyl	Day + 28	28/12/2005	<0.05 metalaxyl
Agri-Fos 600	Phosphorous acid present as mono and di potassium phosphonate	Day 0	14/12/2005	3.7 phosphorous acid
Agri-Fos 600	Phosphorous acid present as mono and di potassium phosphonate	Day + 1	15/12/2005	<1.0 phosphorous acid
Agri-Fos 600	Phosphorous acid present as mono and di potassium phosphonate	Day + 5	19/12/2005	<1.0 phosphorous acid
Agri-Fos 600	Phosphorous acid present as mono and di potassium phosphonate	Day + 8	22/12/2005	<1.0 phosphorous acid
Agri-Fos 600	Phosphorous acid present as mono and di potassium phosphonate	Day + 14	28/12/2005	<1.0 phosphorous acid

5.3.4 Field trial No 4 coriander

There were no above ground symptoms of dieback in the coriander trial at harvest, week 6 (Table 5.11). One application of metalaxyl at planting and the seed treatment (azoxystrobin + metalaxyl + fludioxonil) significantly reduced symptoms of root rot measured as percentage of plants with symptoms of necrotic banding on the first 10cm of coriander roots, compared with control plants. All other treatments did not significantly reduce symptoms of root rot compared with control plants. The site had a pH of 7.23 and EC of 0.21 mS/cm.

Table 5.11 Effect of chemical treatment on coriander root rot at week 6, trial 4, during spring 2005

Treatment	Percentage of plants with symptoms of root rot (%)	
Control	27.05	a
Carbendazim	21.79	ab
Boscalid	19.34	ab
Azoxystrobin + metalaxyl + fludioxonil (seed)	16.56	b
Metalaxyl	11.11	b

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

5.4 Discussion

5.4.1 Trial No 1 - parsley

Disease pressure was low in trial No 1 during summer 2004 as there were no above ground symptoms of dieback and severe symptoms of root rot, similar to trial No. 2 were not present on harvested roots. Neither fungicide treatment controlled the little root rot that was present in the form of reddish brown lesions. It is probable that the causal organism was not very active at this time of the year, possibly because soil temperatures are high in during spring and summer (Oscar Vilalta, pers. comm.). Growers have reported that root rot was more prevalent during cooler periods of the year such as winter. The higher levels of root rot with the metalaxyl + mancozeb treatment compared with the unsprayed control is of concern, and suggests it is not appropriate for control of root rot symptoms during summer.

The reddish-brown root lesions observed on the parsley roots may be associated with soluble salts injury, which was reported to have similar symptoms to root rot (Dimson and Agnew, 2005; The Connecticut Agricultural Experimental Station, 2006). The very high EC (3.3 mS/cm) of irrigation water, which was applied every day, was well above the acceptable level (1 mS/cm), may have increased the susceptibility of parsley to root damage. Given the history of high salinity on the field site it is possible that soluble salts injury may be contributing to root damage in summer grown parsley crops.

5.4.2 Trial No 2 - parsley

Oomycete fungi were responsible for root rot in parsley during winter on the trial site. Symptoms of dieback first appeared in plots at 8 weeks as wilt, necrosis and collapse of plants. Metalaxyl or phosphonic acid controlled it by 87-98%, respectively. Both chemicals are commonly used to control oomycete root rots but are from different activity groups which should aid pesticide resistance management strategies by enabling growers to rotate fungicides between different activity groups (Cohen and Coffey, 1986; Lyr, 1995). Phosphonic acid was effective as a weekly spray after emergence of the disease. Metalaxyl may only have efficacy for 8 weeks as at 16 weeks (8 weeks after the last application) symptoms of dieback were reappearing in the crop. The use of metalaxyl may not be a long lasting solution for control of parsley root rot. Davidson and McKay (1999; 2001) found repeated used of metalaxyl for control of cavity spots of carrot caused by *P. sulcatum* was

unsuccessful in some areas due to degradation of the fungicide in WA soils. Bailey and Coffee (1985) reported a half-life of 70 days or 10 weeks for metalaxyl. On the trial site, metalaxyl started to fail at 8 weeks, so caution will need to be exercised when using this fungicide. In Ireland metalaxyl did not control *Pythium paroecandrum* causing root rot of parsley.

As there were no symptoms of pre-emergence dieback until week 8, it is possible that chemical controls may not be necessary until a week or two prior to week 8. Pre-emergence dieback, however, has been observed in the Victorian industry. Consequently, it may be prudent to apply this fungicide at planting.

It is unlikely that the seed treatment with azoxystrobin + metalaxyl-m + fludioxonil had any effect on controlling dieback in parsley as it only has efficacy for 4–6 weeks (Meibush, pers. comm). Symptoms of the disease were not observed for another 2–4 weeks, until week 8. Seed coating may be more useful for crops with a shorter growing period or where disease appears earlier in the crop's life.

The complete collapse of plants with the iprodione/fludioxonil treatment indicates *Fusarium* and *Rhizoctonia* are not the cause of dieback in parsley. It also suggests these fungicides could be phytotoxic or detrimental to beneficial organisms. *Fusarium* and *Rhizoctonia* are frequently isolated from root rots of parsley (Hershman et al., 1986; Nawrocki and Mazur, 2004; Nowicki, 1997), but pathogenicity tests by Hershman et al. (1986) showed that *Fusarium oxysporum* and *F. solani* associated with parsley root rot were not pathogenic.

Low plant vigour due to cold growing conditions combined with wet soil increases the susceptibility of the plants to infection. Pre-emergence damping off in sugar beet was worst when the ratio of the growth rate of the host to the pathogen was lowest (Leach, 1947). *Phytophthora* and *Pythium* species recorded on parsley have a wide temperature range (Hershman et al., 1986; Stamps, 1978; Waterhouse and Waterston, 1964a; Waterhouse and Waterston, 1964b), but *P. ultimum* only causes damage in wet soil conditions which reduce plant vigour (Kraft and Roberts, 1969). Oomycetes require water for spore dispersal at water potentials at or close to zero (Gisi et al., 1980; Pieczarka and Abawi, 1978). Growers report that parsley root rot is most prevalent after winter rains, but not after irrigation. It is probable that the duration of soil water potentials at or close to zero is longer after rains than after irrigation. Parsley on this trial site was irrigated most days so soil water potentials would be at or close to zero for only short periods of time. The combination of the duration of soil water potentials near saturation produced with the winter rains, low soil temperatures and slow parsley growth was probably responsible for promoting root rot in parsley.

5.4.3 Trial No. 3 parsley

In this spring to summer grown parsley crop, low levels of root rot, with above ground symptoms appeared at 8 weeks, similarly to the winter parsley trial. Oomycete fungi were determined to be responsible for the root rot symptoms as the metalaxyl treatment controlled symptoms. The phosphonic acid was unsuccessful as a fortnightly spray in this trial.

The hymexazol treatment was detrimental from the beginning of the trial, whereas the other treatments including the control were not. Hymexazol is specific for *Pythium, Fusarium*, *Aphanomyces* and *Corticium* (Tomlin, 2003). It was very successful in controlling *Pythium* on red beet in Queensland (Martin et al., 2001). It suggests that *Pythium* is not entirely responsible for the symptoms; the rate of hymexazol was too high; it was detrimental to beneficial organisms; or the *Pythiums* present were hymexazol-insensitive (Ali-Sharayeh et al., 2003).

Phosphorous acid could only be detected in frozen plant samples taken at Day 0. Unfortunately, due to adverse weather conditions, drift occurred such that the control plots had phosphonic acid residues comparable to the test. However, this would not have affected the results, since assessment was performed later that week. Metalaxyl was not detected in frozen plant samples twenty-two days after application. The rapid decline in phosphorous acid residue and the slower decline of metalaxyl residues in plant tissue supports the use of these chemicals with appropriate withholding periods.

5.4.4 Trial No 4 coriander

Despite the lack of significant symptoms of root rot, there was a visually quantifiable difference in the metalaxyl and Dynasty seed treatments compared to the control. The near-absence of any root rot symptoms achieved with the metalaxyl treatment suggests that the cause of the symptoms were oomycete fungi, rather than *Mycocentrospora*. *Mycocentrospora* is a somewhat similar fungus to *Ascochyta*, which carbendazim and boscalid would probably control.

5.5 Conclusion

In Victoria oomycete fungi caused root rot of parsley in winter. Symptoms may be associated with *Phytophthora*, *Pythium* or a complex of more than one species or genera. Above ground symptoms appeared at 8 weeks.

The disease was adequately controlled by 87–98% with either:

- Two applications of metalaxyl; the first at planting and the second applied 8 weeks later,
- Weekly applications of phosphonic acid, after symptoms appeared.

Care will have to be exercised in using metalaxyl due to the risk of fungi developing resistance and the potential for fungicide degradation (Bailey and Coffey, 1985).

Given the reported incidence of root rot associated with low temperature during late autumn and winter following heavy rainfall, growers should also consider scheduling their irrigation to reduce the potential for over-watering. The duration of water potentials at or near saturation, which promotes infection requires further evaluation to develop a management strategies to reduce its impact.

The reported influence of salinity on parsley root rot and the high levels of salinity on trial sites 1 and 2 needs further investigation to determine if it is increasing susceptibility of parsley plants to root rot.

Subsequent to these trials, growers of Dutch carrots and silver beet applied metalaxyl to control damping off. One grower reported yields of Dutch carrots more than doubled from 8–10 decks to 25 decks with one application of metalaxyl applied by gandy at planting. The effects on silverbeet are still being determined.

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Chapter 6

Nutrient evaluation of parsley trial No 2

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Summary

Analysis of dam water indicated that salinity was high during summer but declined during winter. Salinity levels were high at the commencement of trial No. 2 and remained high in the control (standard industry fertilized plot) but declined in the root-zone (reduced fertilizer plot). These nutrient levels could deliver meaningful fertilizer value to the parsley, but their high levels could lead to crop stress.

6.1 Introduction

Plants extract their nutrient requirements from the soil solution in the root-zone. Their feeder roots absorb dissolved elements and these are conveyed to the leaves via the xylem transport vessels.

What becomes available in the "soil, root-zone solution" depends upon what constituents are supplied via:

- Irrigation water: all irrigation water sources contain varying levels of dissolved solutes, some of nutrition value and some that can cause "trouble".
- Soil applied fertilizer dressings.
- Fertigation applications: fertilizer elements dissolved in irrigation water.
- Soil matrix C.E.C.: soils release nutrients into solution depending upon their inherent matrix fertility, cation exchange capacity (C.E.C.).

Supplying the correct balance of nutrients to the plants at the correct concentration and time requires understanding and careful management of all the above contributing factors.

6.2 Material and methods

6.2.1 Root-zone soil solution monitoring (RSSM)

Point lysimeters are employed to extract the aforementioned solutions from around the roots. Nutrient content of these root-zone solutions can be tracked via laboratory analysis.

Regular RSSM allows dissolved salt and nutrient trends to be monitored, in real-time, over the life of the crop. Normal soil analysis does not allow this, as typically only one sample is taken, usually before the crop is planted. The solutions extracted are literally the dissolved food source that the plants access via their active feeder roots.

Such solution monitoring is standard practice in hydroponics vegetable production. Norms for levels of various dissolved plant nutrients are well documented. Overall, salt (dissolved fertilizers, plus constituents of the irrigation water) concentrations are easily measured using electrical conductivity (EC) meters measuring mS/cm.

6.2.2 Analysis of dam water

Prior to the start of the trial, the dam water was tested to ascertain what levels of dissolved nutrients were in it and available to the crop via irrigation applications during growth.

The dam water was sampled in summer and winter during 2005 on trial No 2 site, to gauge seasonal variation in the dissolved solutes and to determine what the irrigation water source would supply as useful crop nutrients over the irrigated growing period.

6.2.3 Analysis of trial No. 2 field site

In trial No. 2, two point lysimeters were established in one of the control plots (standard industry fertilization treatment) and in one of the Root-zone plots. The point lysimeters were set in the soil profile at two depths shallow (15 cm) and deep (30 cm) in the two plots. The Control plots were top-dressed with Rustica Plus™ (NPK 12:5:14) 140 kg/ha. (Campbells Fertilizer, Australia) on 27/6/2005 (week 9), while the Root-zone Solutions plots were treated with 60 g/ha sulphate of potash (NPK 0:0:41) (Incitec Fertilizers) on 5/8/2005 (week 14). Weekly samples were collected from each of the point lysimeters and sent to Root-zone Solutions (3 Rainer St., Karrinyup, WA 6018) for analysis.

6.3 Results

6.3.1 Analysis of dam water: irrigation water quality perspectives

This section of the report was compiled on 14 July 2005 and reports on aims to put the nutrient load of the irrigation water at trial site No. 2 into context by comparing it with recommended fertilizer rates for parsley growth. Table 6.1 and Figs. 6.1 and 6.2 summarise the analysis of results received from the lab for water samples taken in January 2005 and June 2005. The dam water supplied large amounts of crop nutrients, summer and winter.

Table 6.1 Dam water analysis results from January and June tests on trial site No. 2

DATE	MONTH	GROWER	SITE	pH SH	EC SH	NO_3 SH	N SH	PO_4 SH	P SH	K^+ SH	Ca SH	Mg SH	Na SH
31 January 2005	JAN	DEVON MEADOW	DAM	7.5	2.3	667	150	40	13.1	69	110	30	
28 June 2005	JUN	DEVON MEADOW	DAM	8	1.3	220	50	9	2.9	52	100	48	

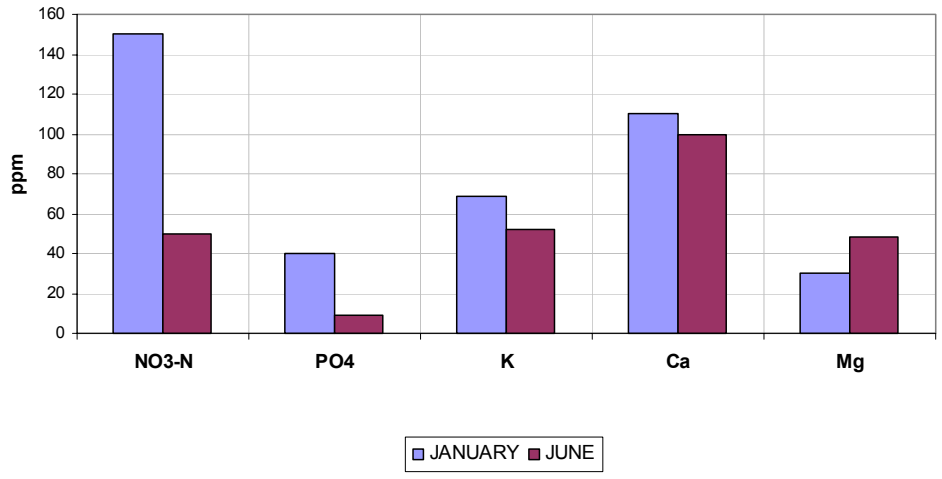


Figure 6.1 Comparison of January and June dam water nutrient analysis

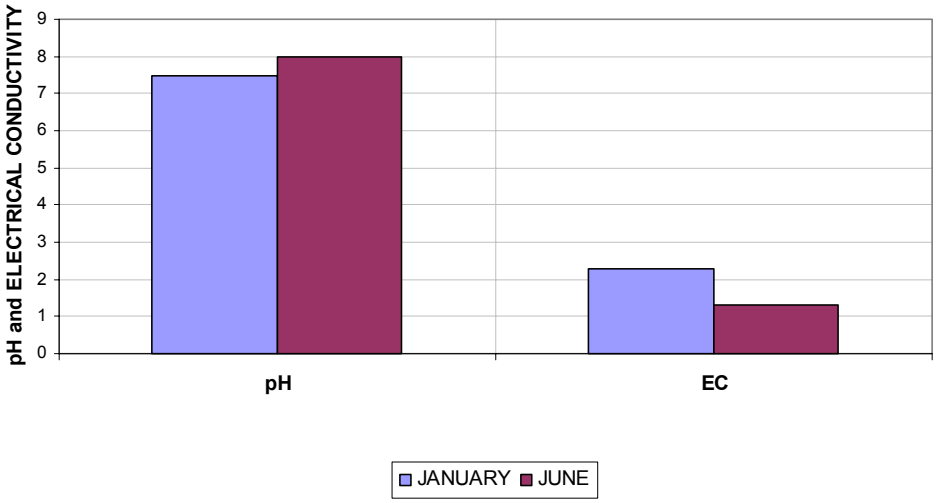


Figure 6.2 Comparison of January and June dam water pH and EC

Except for magnesium, all of the major nutrients have dropped in concentration over the period of time examined. It is likely that magnesium follows this same trend since the first reading was an indirect calculation based on the Hardness result and the corresponding calcium reading. The hardness reading is restricted at the top end and it is almost certain that the reading would be higher if the lab could read it in a higher range. EC has also dropped significantly, reflecting the overall decrease in total salts. It should be a simple procedure to correlate these trends with increasing or decreasing inflow to the dam whether this is directly from rain, evaporation and usage, or indirectly from increased stream flow. The dynamics of this are fairly straightforward with dilution and concentration being a function of water quality inflow (ie. nutrient rich runoff or low EC rain) and volume of inflow.

Table 6.2 and Fig. 6.3 compare the total recommended fertilizer rates over a growth cycle with the equivalent applied nutrition from the irrigation water over the same time period.

Table 6.2 Comparison of recommended fertilization rates with applied nutrition supplied in the irrigation water in January and June

		NO_3-N	P	K	Ca	Mg
JAN/FEB/MAR/APR	**Applied nutrition per day (kg/ha)**	7.50	0.65	3.45	5.50	1.52
	Applied nutrition per 3 month growth cycle (kg/ha)	675.34	58.73	310.50	495.00	136.96
JUN/JUL/AUG/SEP	**Applied nutrition per day (kg/ha)**	2.48	0.15	2.60	5.00	2.40
	Applied nutrition per 3 month growth cycle (kg/ha)	222.75	13.22	234.00	450.00	216.00
RECOMMENDED FERTILISATION RATES FOR PARSLEY (Maynard and Hochmuth, 1997)		126	49	93		

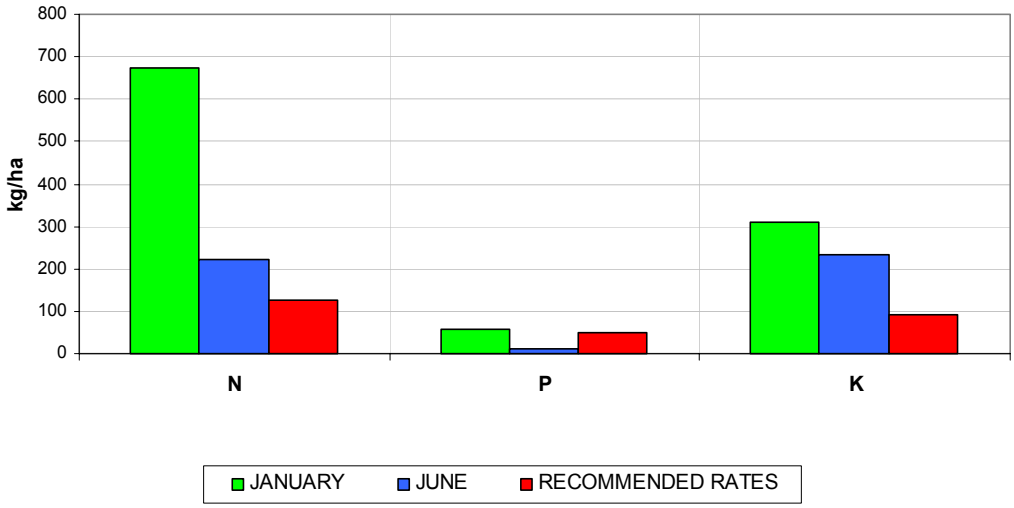


Figure 6.3 Comparison of recommended nutrition rates with accumulated nutrient load applied through the irrigation in January and June.

Assumptions are as follows:

- Irrigation supplied is an average of 5mm per day
- There is 100% wetted area
- The growing cycle is over 90 days

It is immediately obvious from Fig. 6.3 that the accumulated N and K applied through the irrigation in both January and June is more than sufficient to supply the needs of the crop without any further top dressings. Supplied P in January was sufficient, according to recommended rates, but additional fertilizer was needed in June to supplement what was available in the irrigation water.

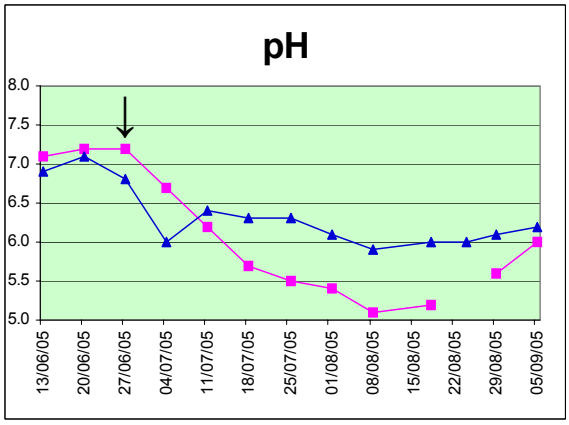
These observations don't take into account the contribution of the soil to the nutrition of the crop and therefore only look at the nutritive inputs from an external point of view. Depending on the profile's physical and chemical properties, the soil may contribute a significant amount of nutrition and its ability to accumulate or to lose fertility is a further factor to consider.

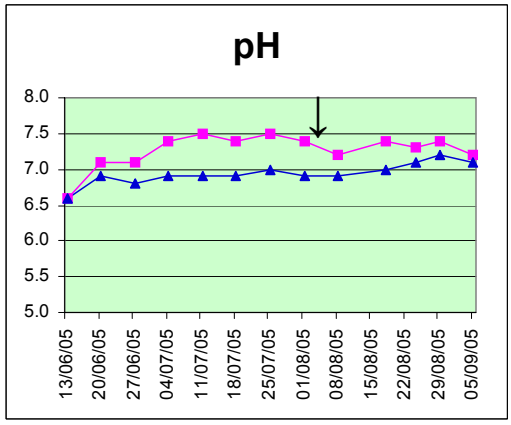
6.3.2 Analysis of field trial No. 2 nutrient solutions

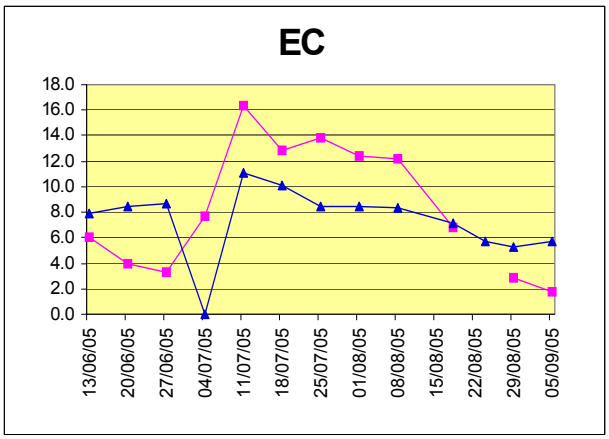
Results from the analysed, weekly solutions were graphed according to the treatment and soil profile depth, ie. Shallow (15-cm) and Deep (30-cm) for the control plot and for the Root-zone plot (Fig. 6.4). Dates of respective fertilizer top dressings are indicated in the top graphs.

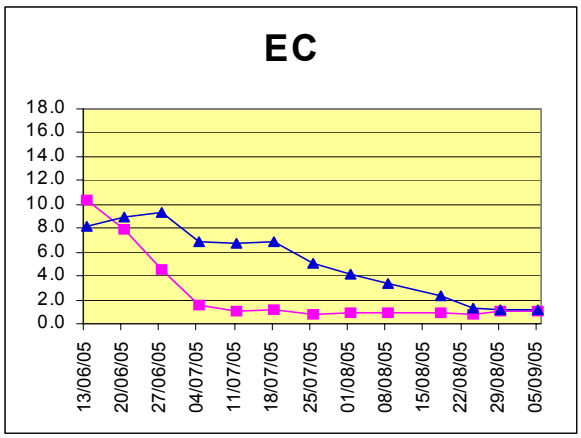
Both treatments had unusually high EC start levels on 13/06/05. Note how the Rootzone Shallow treatment EC levels (Fig. 6.4) were in fact higher than the Control, until the consequences of the NPK topdressing had a dramatic effect.

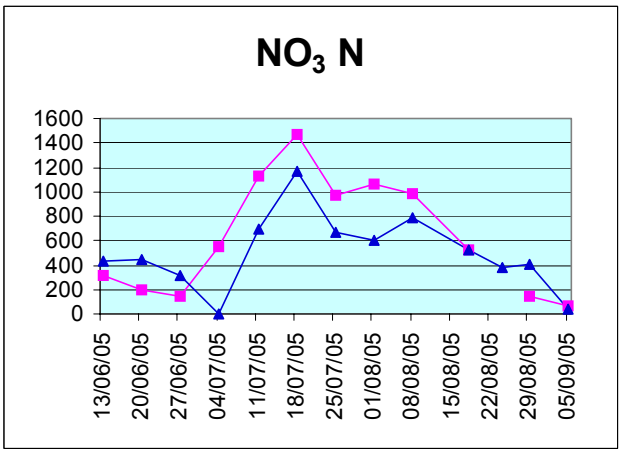
Figure 6.4 Analysis of the shallow (15cm) [–■–] and deep (30cm) [–▲–] profile from the Control solution plots (standard industry treatment) (left side) and the Root-zone solution treated plot (right side). Arrows indicate fertilizer treatment (see 6.2.3). EC is measured in mS/cm, NO_3 in ppm (mg/L).











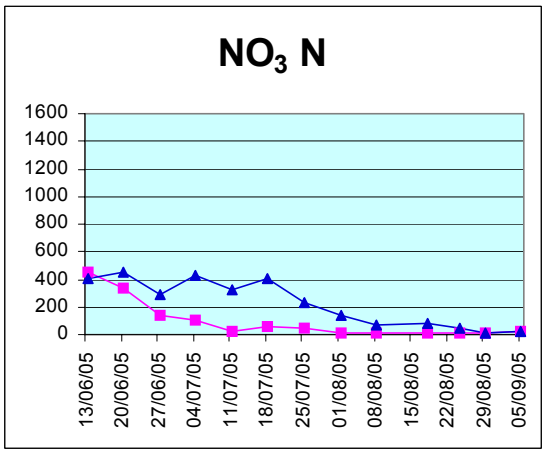
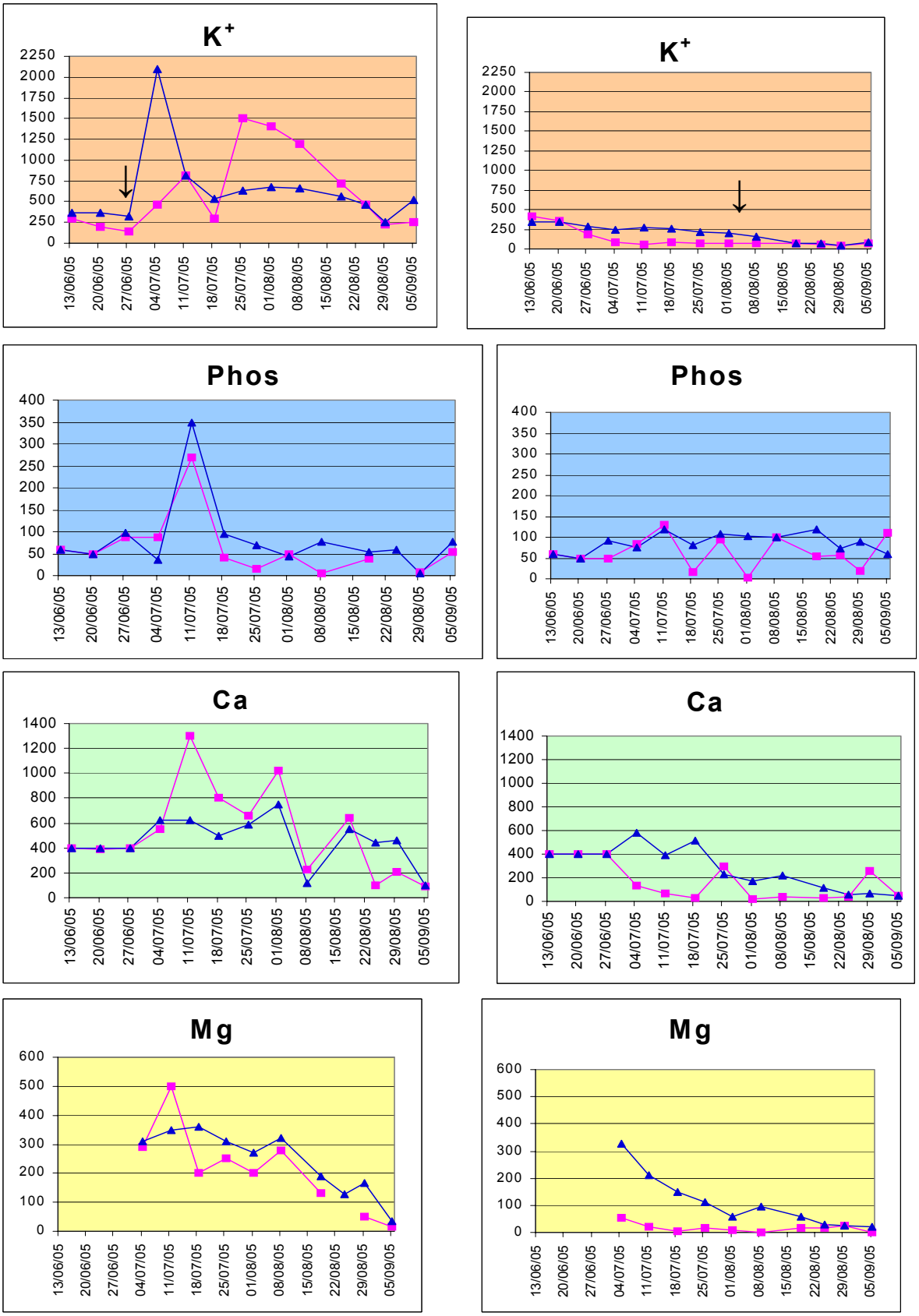


Figure 6.4 [Continued] Analysis of the shallow (15cm) [–■–] and deep (30cm) [–▲–] profile from the Control solution plots (standard industry treatment) (left side) and the Root-zone solution treated plot (right side). Arrows indicate fertilizer treatment (see 6.2.3). All ions measured in ppm (mg/L).



The most notable differences between treatments occurred after the NPK topdressing that the Control treatment received on 27/06/05 (Fig. 6.4). Note the rapid change in the Shallow solution analyses over the next few weeks. EC and nitrate (NO_3) rose in concert to very high levels, whilst potassium (K^+) took longer to peak, as expected because as a cation it moves slower through the negative soil matrix. The fertiliser application of 140 kg/ha was a large single topdressing causing a "peak level" in EC both in the Shallow (16.4) and the Deep (11.1) (Fig. 6.4). This occurred two weeks after application.

After the topdressing of 27/06, levels of NPK were more than double in the Control treatment compared with the Root-zone treatment (Fig. 6.4). Nitrogen (N) and potassium (K) in particular were at extreme levels in the Shallow Control treatment, whilst the nitrogen and potassium in the Root-zone treatment were moderate and then possibly a little low in the last few weeks.

In the Control treatment at the Deep (30-cm) level, EC never dropped below 5 mS/cm, and peaked at 11.1 mS/cm a week or two after the 140 kg/ha top dressing. In the Root-zone treatment the peak EC level was 9.3 mS/cm at the end of June. Only in the last four weeks did the EC fall below 2.5 mS/cm. To illustrate the meaning of the above EC levels, we can compare them to Dutch hydroponics norms for EC levels on a 1.5:1 (water volume to media extraction) (Bunt, 1988) in Tables 6.3 and 6.4 below.

Table 6.3 Dutch salinity readings for extracted media solutions (Bunt, 1988)

EC Readings in solution (mS/cm (1:1.5))	Interpretation of salinity rating
< 0.7	Low
0.7–1.2	Fairly Low
1.3–1.8	Moderate
1.9–2.7	Fairly High
2.8–3.6	High
> 3.6	Very High

Table 6.4 Treatment solution EC readings in mS/cm (Dutch Salinity Interpretation)

Probe level	Treatment	Item	Observation		
			Lowest	Average	Highest
Shallow (15cm)	Control	EC	1.8	7.7	16.4
		Comment	moderate	very high	extreme
		Time	end of trial	–	2 weeks post top dressing
		Date	5/9/2005	–	11/7/2005
	Root-zone	EC	0.8	2.5	10.3
		Comment	fairly low	fairly high	extreme
		Time	–	–	start of trial
		Date	25/7 & 24/8/2005	–	13/6/2005
Deep (30cm)	Control	EC	5.3	8.1	11.1
		Comment	very high	very high	extreme
		Time	-	–	2 weeks post top dressing
		Date	29/8/2005	–	11/7/2005
	Root-zone	EC	1.2	5.0	9.3
		Comment	fairly low	very high	extreme
		Time	end of trial	–	–
		Date	29/8 and 5/9/2005	–	27/6/2005

6.4 Discussion

6.4.1 Analysis of dam water: irrigation water quality perspectives

Irrigation water salt content varied markedly from summer to winter, i.e. 2.3 mS/cm in summer to 1.3 mS/cm in winter. This could be due to rainfall causing a dilution factor. Both winter and summer nutrient levels in the irrigation water would have delivered meaningful fertilizer nutrient value to the parsley.

6.4.2 Nutrient levels in parsley trial No. 2

It appears that in trial No 2, nutrient (salinity) levels were high from the outset both in the soil and irrigation water. Levels remained high throughout the trial, except in the Root-zone Shallow plot solutions.

These high levels could arguably lead to crop stress and root damage and dieback. In a wide range of crops, EC levels above 4.0 mS/cm are known to have deleterious effects, and this is also true for parsley (The Connecticut Agricultural Experiment Station, 2006).

In the hydroponics growing of parsley, a leafy soft crop, EC levels in solutions would generally be maintained between 1.5–2.5 mS/cm. (Harris, 1994).

6.5 Future directions

It is suggested that a pot trial be undertaken at DP1 Knoxfield using low fertility soil mixtures and irrigating the plants using trial 2 dam water, to ascertain how much extra additional nutrients are required to supply the parsley with its total nutrient requirements over a growth cycle. Disease incidence could possibly be rated in this trial.

The above results also indicate that trial site 2 soil effectively constitutes a high fertility (high salinity) soil (see Table 6.4). Thus, a pot trial can be designed where soil is taken from trial site 2 and pots are irrigated via trial site 2 dam water to elucidate the effects of high salts on incidence of fungal infection and root rots.

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Chapter 7

Economic analysis of field trial No 2 on parsley during autumn-winter 2005

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Summary
An economic analysis of treatments to control root rot of parsley indicated 10 applications of phosphonic acid would increase profitability by $26,751/ha and 2 applications of metalaxyl would increase profitability by $24,670/ha. The phosphonic treatment assumes applications can commence at the first sight of symptoms.

7.1 Introduction

This chapter reports an economic analysis of field trial No 2 (Chapter 5) carried out on Italian Plain Leaf parsley at a site on North Road Devon Meadows. The purpose of the trial was to determine the efficacy of various fungicides in controlling pathogens responsible for causing dieback in the crop. Parsley was sown on 26/4/2005 and the last treatments were applied 18 weeks later on 29/8/2005. Details about this trial have been discussed in the previous chapter.

7.2 Method

The method used was to calculate the net economic benefits of the treatments used in trial No 2 as the increases in their contributions to net profit above that of the control. This approach assumed that the efficacy of the treatments were reflected in their yields, and changes in variable costs for the treatments comprised changes in the cost of fungicides and their application for minimizing dieback in parsley, together with changes in harvesting and packaging costs. All other variable costs such as the costs of tillage and bedding, herbicide costs for controlling weeds, costs of fertilizer, costs of labour and any other variable costs for growing parsley, would be the same for the control and the treatments.

7.3 An economic analysis of various treatments to minimize the incidence of dieback in parsley

7.3.1 Assumptions
- A deck of parsley comprises 10 bunches and had a farm gate price of $6.00 per deck.
- The cost of spraying the fungicides was estimated at $28 per ha.
- The granular fungicide Ridomil Gold 25G® in the metalaxyl treatment, and Perlka® in the calcium cyanide treatment were applied through a gandy at a cost of $100 per ha.
- Harvesting and packaging was estimated to cost $2 per deck.

7.3.2 The cost of chemicals for minimizing the incidence of root rot in parsley
Table 7.1 shows the costs per ha. of the chemicals used for the various treatments and Table 7.2 reveals the costs per ha. of the chemicals and their application. A 'Hypothetical' treatment of combining metalaxyl (at planting) and phosphonic acid (commencing at week 8) has been included in Table 7.2. In Table 7.3, differences in the contribution of the treatments to profitability relative to that of the control and their rankings are shown. The rankings for the treatments are displayed in Figure 7.1.

Table 7.1 The cost of chemicals to for treatments to reduce the incidence of dieback in parley

Chemical	Active ingredient	Rate/ha.	Cost/unit	Cost/ha.
			$	$
Agri-Fos 600®	Phosphonic acid	170 ml	4.36/L	0.74
Agri-Fos 600®	Phosphonic acid	3.0 L	4.36/L	13.08
Perlka®	Calcium cyanamide	500 kg	1.40/kg	700
Plantmate G™	Trichoderma	15 kg	16.80/kg	252
Plantmate WP™	Trichoderma	400 g	60/kg	24.00
Ridomil Gold 25G®	Metalaxyl	120 g	22.80/kg	2.74
Rovral®	Iprodione	100 ml	55.24/L	5.52
Switch®	Cyprodinil + fludioxinil	80 g	151.20/kg	12.10

Table 7.2 Cost per ha. of treatments to reduce the incidence of dieback in parsley

Treatment	Chemicals	Number of applications	[A]Cost per application	Total cost of application	Cost of chemical per application	Total cost of chemicals	Total cost of treatment
			$/ha.	$/ha.	$/ha.	$/ha.	$/ha.
Metalaxyl	Ridomil Gold 25G®	1		[B]	2.74	2.74	105
	Ridomil Gold 25G®	1	100	100	2.74	2.74	
Phosphonic acid[C]	Agri-Fos 600®	2	28	56	0.74	1.48	427
	Agri-Fos 600®	9	28	252	13.08	117.72	
Iprodine/fludioxonil	Rovral®	3	28	84	5.50	16.5	221
	Switch®	3	28	84	12	36	
Trichoderma	Plantmate G™	1		[B]	252	252	460
	Plantmate WP™	4	28	112	24	96	
Calcium cyanamide	Perlka®	1	100	100	700	700	800
	Ridomil Gold 25G®	1		[B]	2.74	2.74	471
Hypothetical[D]	Agri-Fos 600®	2	28	56	0.74	1.48	
	Agri-Fos 600®	10	28	280	13.08	130.80	

[A], Includes labour plus machinery variable costs.
[B], No cost because application was through a gandy pulled behind the seeder.
[C], Does not include cost of seed coating as only commercially available to seed companies.
[D], Hypothetical treatments = one application of metalaxyl at planting and 10 applications of phosphonic acid commencing at week 8.

Table 7.3 Difference in the contribution to profitability of the treatments compared to that of the control and their rankings

Treatment	Total cost of chemicals	Labour for harvesting and packaging	Yield	Farm gate income	Contribution to profitibility	Difference from control	Ranking
	$/ha.	$/ha.	Decks/ha.	$/ha.	$/ha.	$/ha.	
Control	–	4,574	2,287	13,722	9,148	–	–
Metalaxyl	105	16,126	8,063	48,378	32,146	22,998	3
Iprodine/fludioxonil	221	1,157	579	3,472	2,094	–7,054	6
Phosphonic acid	427	18,163	9,081	54,489	35,899	26,751	1
Trichoderma	460	2,515	1,257	7,544	4,570	–4,579	5
Calcium cyanamide	800	3,163	1,581	9,489	5,526	–3,622	4
Hypothetical[A]	471	17,144	8,572	51,433	33,818	24,670	2

[A] The hypothetical case assumes a median value for all parameters lying between that of metalaxyl and phosphonic acid. There was no significant difference between the metalaxyl treatment and the phosphonic acid treatment (Chapter 5, see also fig. 7.1 below).

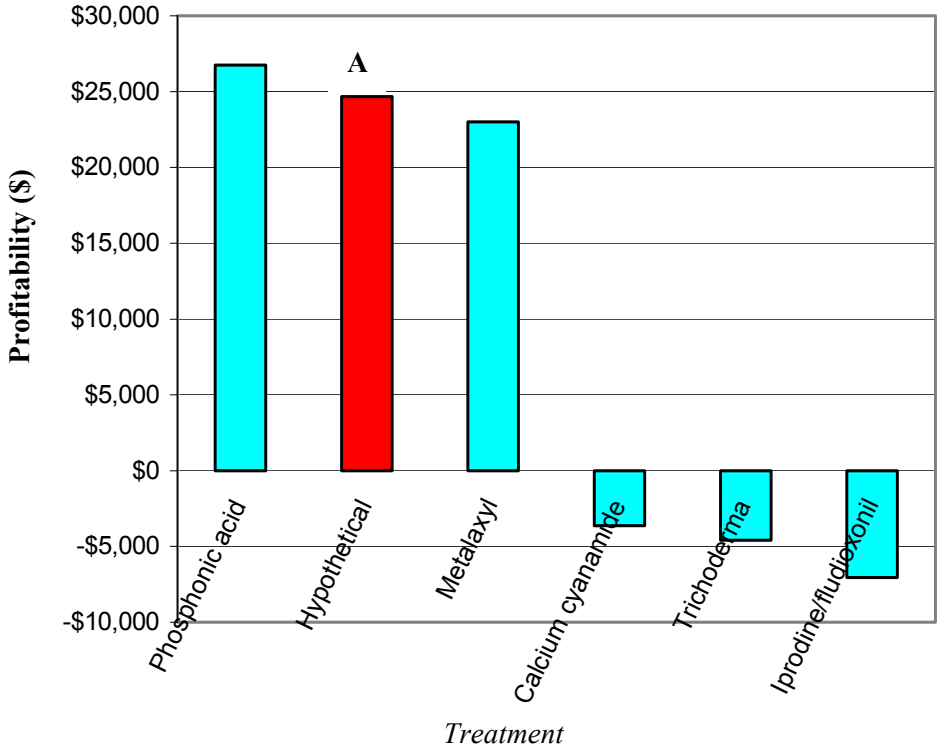

Figure 7.1 Differences in contribution of the treatments to profitability compared to that of the control

[A] The hypothetical case assumes a median value for all parameters lying between that of metalaxyl and phosphonic acid. There was no significant difference between the metalaxyl treatment and the phosphonic acid treatment.

7.4 Discussion and conclusions

The order of increasing profitability of the treatments over that of the control were: the phosphonic acid treatment, followed by the hypothetical (a combination of the phosphonic acid and the metalaxyl treatments) and the metalaxyl treatment, clearly allowing large increases in contribution to profitability per ha for growing parsley to be made as a result of their application with increases being $26,751, $24,670 and $22,998 respectively (Table 7.3 and Figure 7.1). The figures in Table 7.3 are derived from the absolute differences in the various parameters; being the total cost of applied fungicides, the yield of parsley leading to changes in the absolute costs for labour and harvesting, and farm gate income resulting in changes in the contribution to farm profitability by deducting expenses from income.

Another way of looking at the economic benefits of using those three treatments to reduce the impact of pathogenic fungicides on dieback in parsley is shown in Table 7.4.

Table 7.4 Changes in extra costs of applying treatments resulting in extra yields, extra harvesting and packaging costs, extra gross income and extra net contributions to profitability per ha. compared to those achieved by the control

Treatment	Extra cost of treatment	Extra yield	Extra cost of harvesting and packing	Extra gross income	Extra contribution to profitability
	$/ha.	Decks/ha.	$/ha.	$/ha	$/ha
Phosphonic acid	427	6,794	13,589	40,767	26,751
Hypothetical	471	6,285	12,570	37,711	24,670
Metalaxyl	105	5,776	11,552	34,656	22,998

Table 7.4 sets out changes in the major parameters affecting changes in net contribution to profitability. In that respect, the calculations are set out as three partial budgets, a type of analysis frequently used by agricultural economists to determine the best options for alternative investments. Note: Increases in net contribution to profit would be expressed as percentage returns to extra capital employed for the three treatments under consideration, but since they employ the same amount of capital, that step is not necessary. The important issue is that the values for extra return to profitability, and hence their rankings, are the same in Table 7.3 as they are in Table 7.4.

As with nearly all treatments where an ameliorant is applied to prevent disease in a crop, the returns in extra yield and hence the value of extra income, are large compared to the extra expense of applying the ameliorant.

Metalaxyl is more attractive for use by growers for the treatment of root rot in parsley than phosphonic acid, possibly because they only have to apply two applications of Ridomil Gold 25G® instead of 10 applications of Agri-Fos 600®. They may perceive that the extra time could be better spent doing other productive tasks on the farm or increasing time spent in leisure. However, as has been pointed out in Chapter 5, continual use of metalaxyl may lead to resistance by oomycete root rots. The calculation of the hypothetical case, combining one treatment of metalaxyl with subsequent weekly treatments of phosphonic acid, is therefore, of particular interest. There is little difference in profitability between the top three treatments (Table 7.3, Fig. 7.1). Due to concerns of fungicide resistance in oomycetes, this hypothetical model will enable the prolonged use of metalaxyl as an effective fungicide against oomycetes.

A strong case can therefore be made out for rotating fungicides between metalaxyl and phosphonic acid, or a combination of the two as suggested by the inclusion of the ‘hypothetical’ treatment in the analysis.

Chapter 8

Technology transfer and recommendations

Summary

This chapter reports on the benefits of a project advisory group established to steering 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.

8.1 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 from subcontracting sections of work to industry experts has been of enormous benefit to the project. The herb growers in north Queensland 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. The outcomes of research have been taken up in Victoria and extended to other crops, such as Dutch carrots, with one grower reporting production more than doubled, from 8–10 decks to 25 decks.

8.2 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 VG04025 were:
Craig Arnott – Market Gardener – Arnotts Vegetable Farms – Clyde.
Kevin Clark – Market Gardener – Sims and Clark Pty Ltd – Cranbourne.
Peter Cochrane – Market Gardener – P.J. and J. Cochrane Pty Ltd – Devon Meadows.
Rocky and Tony Lamattina – Market Gardeners – A. D. Lamattina & Sons – Clyde.
Karl Riedel Vegetable Crop Agronomist – E.E. Muir & Sons – Cranbourne.

8.3 Some grower reactions to the field day

Feedback from the Parsley Field Walk held at 3.00 pm on 5 August 2005 at Peter Cochrane's farm, 1435 North Road, Devon Meadows, was attended by 13 growers and industry representatives and is reported here.

CP
Field day was very good, very impressed with the results, easy to follow and see what works and what doesn't. Material given to attendants was good and easy to follow. It is worth extending this project to other states (parsley growing) for instance NSW to help their growers.

KC
Field day was excellent. I thoroughly enjoyed that trial. We covered a number of different treatments some that worked and some that didn't. It will help me save time and money. Time was ok, however, I preferred meetings/field days towards end of the day rather than in the middle.

BB
Very interesting field day and very good. Only comment is that I like to see some follow up work on a couple of fungicides working "backwards" to see what they do to the plant, eg, the way Rovral was worse than control. Friday afternoons are not the best day for Field days.

TL
Field day was very good. Results are clear, easy to distinguish between treatments. Like to see follow up trial to confirm these results. Time, set up everything was good.

GF
The field day was good it gave us a chance to view results at first hand. The booklet was easy to follow and made it very helpful and easy to understand. It also gave us the opportunity to talk to the other growers and industry reps and hear their opinions. It will be interesting to see some final results, if there are any changes in the chemical's performance as the crop gets older?

DEPARTMENT OF PRIMARY INDUSTRIES - VICTORIA

An invitation to learn more about:

Management of Parsley Root Diseases

Workshop on HAL research project VG04025

Research supported by your Vegetable Industry Levy, Horticulture Australia and DPI-Victoria.

Speakers:

Lawrence Kirton Root Zone Solutions WA
What is happening to the nutrients in your soil solution?

Liz Minchington DPI-Knoxfield
A briefing on the scoping study to investigate management of root disease in parsley HAL VG04025

When : 5.30-7:00 pm Tuesday 1st February 2005
Where: Anstel Golf Club – Cranbourne
1000 Frankston Cranbourne Road
Melways Map 133, D5

Contact: Elizabeth Minchinton - 0427 01 2460

Victoria The Place To Be | AUSVEG | HAL Know-how for Horticulture™ | VegCheque extension for the vegetable industries

Veg Cheque
extension for the vegetable industries

An invitation to a Field Walk for Parsley dieback

Research supported by your Vegetable Industry Levy, Horticulture Australia and DPI-Victoria.

Where: 1435 North Road, Devon Meadows
(at Peter Cochrane's property, behind shed)

When: Friday 5th August 2005 any time between 3 - 5 pm

Speaker: Liz Minchinton DPI Knoxfield

This is an opportunity to look at current trials and discuss recent progress with the research scientist on the scoping study to investigate management of the dieback disease in parsley

Afternoon tea will be provided

For more details contact: Liz Minchinton on 9210-9224
or Slobodan Vujovic on 9210-9297

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DEPARTMENT OF
PRIMARY INDUSTRIES

Primary Industries Research Victoria
Knoxfield Centre

Parsley Root Woes

Dr Elizabeth Minchinton, Len Tesoriero, Heidi Martin, Leif Forsberg, Savitri Nadesan, Dr Fiona Thomson and Slobodan Vujovic

The Project

The joint project between QDPI, NSW DPI and DPI Vic will identify causes of root-rots on parsley in the eastern states and develop management strategies to control the problems and improve parsley production.

Fig 1 Damping off in Victorian parsley

Fig 2 Root rot of Victorian parsley

Background

Parsley production is hampered by root disease problems in Victoria during winter and in Queensland during the wet season.

The Problem

Victorian parsley is affected by:

- Post emergence damping-off (Fig 1)
- Assorted roots rots causing yellowing of foliage, stunted growth and rotting of tap and lateral roots (Fig 2)

Queensland parsley is affected by:

- 'Parsley leaf drop' which causes plant collapse (Fig 3) and rotting of roots (Fig 4).

NSW parsley can be affected by:

- Root knot nematodes which cause sparse crops (Fig 5).

Fig 3 Leaf drop of parsley in Queensland

Fig 4 Root rot of parsley grown in Queensland

Identification of Organisms Associated with Root Rots in Eastern Australia

- Plants with diseased roots were collected from parsley crops in Queensland, NSW and Victoria.
- Fungi consistently isolated from root rots are being inoculated back onto parsley plants to prove they can cause root rot.

Fig 5 Root knot nematode damage in NSW parsley

Fig 6 Planting the parsley trial in Victoria

Field Trial

A field trial in Victoria is testing several management strategies including seed coating with fungicide, systemic fungicides, a fumigant, a biological control agent and nutrient management for disease control (Fig 6).

Manuscript Submission to Australasian Plant Pathology (2005)*

Title: Two new Phytophthora records for Australia

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Phytophthora inundata and *P. hedraiandra* are reported for the first time in Australia. The former was found in soil around carrot and parsley crops in the Cranbourne area of Victoria. The latter is a historical collection from 1996, isolated from soil near horticultural polyhouses in Werribee, Victoria.

Many new *Phytophthora* species have been described in the last 10 years. This is largely due to the use of DNA sequence data in taxonomic studies. To determine which *Phytophthora* species occur in Victoria, we are re-identifying all cultures of *Phytophthora* species in herbarium VPRI (Victorian Department of Primary Industries, Knoxfield) using ribosomal DNA internal transcribed spacer (ITS) sequences. Additionally, many new isolates have also been collected. As a result of this work, two *Phytophthora* species are reported here for the first time in Australia.

Two specimens of *Phytophthora inundata* were pear baited from soil around carrot (VPRI 32407) and parsley (VPRI 32408) crops in the Cranbourne area of Victoria in mid 2005. Initial identification was made by ribosomal DNA internal transcribed spacer sequences. These were identical to the sequence from the type specimen (Brasier *et al*. 2003), and have been deposited in GenBank as accessions xxxxx and xxxxx (TO BE ADDED). The high temperature optimum of this species was confirmed. On carrot agar, growth was 9mm/day at 29ºC, and 1mm/day at 37ºC. Colonies were irregularly to broadly lobed. Oogonia were not seen. Sporangia were non-papillate and internally proliferating.

Phytophthora inundata has been reported from Europe and South America, where it is a pathogen of trees and shrubs in wet or flooded soils (Brasier *et al*. 2003). Hosts include *Salix*, *Olea*, *Prunus* and *Vitis*. It has also been isolated from river water and pond debris. Neither of the two Victorian collections was associated with disease. The associated soils, although sandy, do experience prolonged wet periods.

A single isolate of *P. hedraiandra* was found in the culture collection of herbarium VPRI. This isolate (VPRI 20839) was pear baited from soil next to horticultural polyhouses in Werribee, Victoria, in March 1996. Initial identification was made by a rDNA ITS sequence that was identical to the type specimen (de Cock and Lévesque 2004). The sequence has been lodged in GenBank as accession xxxxx (TO BE ADDED). On V8 agar, morphological characters of the isolate are consistent with those described by de Cock and Lévesque (2004). Sporangia were papillate and caducous, like those of *P. cactorum*. But, unlike *P. cactorum*, the majority of the antheridia were sessile. Oospores were larger (usually 28–30 µm in diameter) than those typically reported for *P. cactorum*. A duplicate of this culture has been deposited in CBS (118732).

Phytophthora hedraiandra was described from The Netherlands, where it was associated with leaf spots on *Viburnum* (de Cock and Lévesque, 2004). It has since been isolated from leaf spots on *Rhododendron* in North America, (Schwingle *et al*. 2005). Herbarium records do not indicate the types of plants grown in the vicinity of polyhouses at the site of the Victorian collection.

Simple pathogenicity trials were conducted by inoculating young stems of *Viburnum tinus* with each species of *Phytophthora*. After 2 weeks, *P. hedraiandra* caused lesions 2–3cm long, spreading below the point of inoculation. The fungus was readily re-isolated from the leading edge of the lesion. *Phytophthora inundata* caused no visible symptoms on the plants. This was surprising, as this fungus has been recorded from a wide range of woody plants. Brasier *et al*. (2003) noted *Viburnum* bushes with root necrosis growing near *Salix* with diseased roots containing *P. inundata*, but fungal isolations from the *Viburnum* plants were not undertaken.

Acknowledgements
We thank Arthur de Cock (CBS) for helpful discussions on *P. hedraiandra.*

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***Accepted March, 2006.**
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In press: *Australasian Plant Pathology* **35(3):** xxx–xxx.

8.4 Publication list

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Meetings:
Victoria, Cranbourne, steering committee, 1/2/2005. Notes on 'Management of parsley root diseases'.
Queensland, Biloela 9/2/2005. Notes on 'Management of parsley root diseases'.
Victoria, Cranbourne, final workshop, TBA (May 2006)
Queensland, Biloela, final workshop, TBA (May 2006)
Queensland, Stanthorp, final workshop, TBA (May 2006)

Field day:
Victoria, Devon Meadows 5/8/2005. Notes on 'An invitation to a field walk on parsley dieback'.

8.5 Recommendations

The major findings of the project were that parsley root rot which occurs in Victoria crops during winter can be adequately controlled with Ridomil Gold 25G® (metalaxyl) or Agri-Fos 600® (phosphonic acid). However, the use of metalaxyl may only be a short-term solution due to resistance and degradation in sandy soils. Weekly applications of phosphonic acid may be too expensive.

The cause of the root rot in Victoria was associated with at least one *Pythium* sp and also possibly *Phytophthora* spp., with similar species causing root rot in NSW. Root rot was not associated with *Fusarium* or *Rhizoctonia* species in Victoria, but they have proved to be weak pathogens in warmer areas, such as NSW.

Possible areas of future research:

(i) 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.

(ii) Complete analysis of pathogenicity tests (Koch's postulates).

(iii) Determine predisposing factors to root rot, such as pH, salinity and soil water potential.

- McCracken (1984b) reported that rotating with barley, possibly due to application of lime, appeared to provide some relief from root rot of parsley.
- Salinity is reported to make plants more susceptible to root rot and it is difficult to distinguish root rot symptoms caused by salinity from those caused by fungi (The Connecticut Agricultural Experiment Station, 2006).
- The prevalence of the disease after heavy rains suggests that the duration of high soil water potentials may be predisposing plants to disease (Kraft and Roberts, 1969; Pieczarka and Abawi, 1978).

(iv) Investigate beneficial organisms such as commercial formulations of *Pythium oligandrum* as an alternative to metalaxyl.

(v) Determine if the avirulant *Fusarium oxysporum* isolated in Victoria has potential as a mycoparasite.

8.6 References

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Acknowledgments

This project was facilitated by HAL in partnership with AUSVEG and was funded by the National Vegetable Levy. The Australian Government provides matched funding for all HAL's R&D activities. The researchers gratefully acknowledge the financial support of the Department of Primary Industries, Primary Industries Research Victoria.

The authors thank the members of the advisory group, Craig Arnott, Peter Cochrane, Kevin Clark, Karl Reidel and Rocky and Tony Lamattina for their valuable contribution to this project. The authors thank Peter Cochrane and Rocky and Tony Lamattina for providing field sites and maintaining the field trials. All the growers participating in the surveys in Queensland, New South Wales and Victoria are thanked for their patience and contributions to the project. Peter Carr, Robert Dimsey, Jacky Edwards, Robert Holmes, Fiona Thompson and Dr Keith Leamon are thanked for their useful comments on the final report.