# Identification of IPM strategies for Pythium induced root rots in Apiacae vegetable crops

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Minchinton *et al.*

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# Media summary

The Apiaceae vegetables, such as parsley, coriander, parsnip and carrot, are prone to root rots, cankers and cavity spot diseases that can significantly affect yield and marketability of produce. *Pythium*, a group of "water mould" pathogens that are active when soil is saturated with water, are implicated as a major cause of these diseases. This is particularly so for crops sown in autumn and grown over the winter and into spring. The mild, wet conditions in the autumn and the spring are particularly favourable for this group of pathogens. It is not unusual for 90-100% of autumn sown parsnips, for example, to be unmarketable. A three year study examined disease development in parsley and parsnip and evaluated a number of disease management options in parsley, coriander, parsnip and carrot crops, including fungicides, biological and cultural controls and varieties.

This study identified several new pathogens from roots of Apiaceae. In a study of root rots over 3 years of trials in sandy and sandy loam soils in Victoria, *Pythium* was found to be the most common group on diseased roots of autumn sown parsley and parsnip. As parsnip roots matured, other common pathogens invaded. More than 11 different species of *Pythium* were identified, some of which were previously not know to occur on parsley and parsnips in Australia. A species of *Phoma*, not seen before on these crops, was also identified. This suggests that these diseases have a complex of causes, induced by *Pythium*. This information will help better target control treatments.

Two disease management options that provided disease control and improved yields were fungicide treatments and varieties. Metalaxyl, applied to the soil, reduced the severity of root rot in parsley and canker in parsley, resulting in improved yields. This fungicide is active against the "water mould" pathogens such as *Pythium*. However, results were not consistent. Fungicides were not effective in one of two trials in parsley and one of four trials in parsnip. Heavier soil types appeared to be a major constraint. Some parsnip cultivars evaluated in the field proved to be much less susceptible to canker, returning up to 3 times more marketable crop than the standard variety. These varieties may be an option for cultivation when the disease risk is very high.

Three different biological control agents, *Bacillus subtilis*, *Streptomyces lydicus* and *Pythium oligandrum*, did not provide any significant control of root rot in field grown parsley, parsnip or hydroponically grown coriander. There was evidence that *Bacillus subtilis* treatments stimulated the growth of parsnip and coriander. Cultural control treatments of hilling soil over the parsley and parsnip crowns, and blanketing soil with organic mulch, did not reduce root disease, although both hilling and mulch treatments stimulated plant growth in some trials.

Managing diseases in autumn sown crops where disease risk is high, particularly for long growing crops such as parsnip, is particularly challenging. Future research should focus on understanding the importance of the newly discovered pathogen in the diseases complex. Research should also be directed at improving the application and timing of fungicides and biological control agents to ensure that the highest concentration of active material occurs in the root zone at the time when the risk of infection is greatest. Recommendations for control of these *Pythium* induced root rots include rotation with non-Apiacaea crops (e.g. broccoli), selection of fields/beds with relatively good drainage (e.g. avoid the heavier water logged soil), choose varieties that are less prone to disease, and early application of fungicides such as metalaxyl, preferably in the granule form to ensure a good distribution in soil.

# Technical summary

A series of field trials were conducted in Victoria, Tasmania, Queensland, New South Wales (hydroponic systems), and Western Australia, to evaluate a range of control options for *Pythium* related diseases of Apiaceae vegetable crops, including fungicides, biological and cultural controls, and varieties, over the period 2009 to 2011. Apiaceae vegetables included parsley (Victoria, Queensland), parsnip (Victoria, Tasmania, Western Australia), coriander (New South Wales and Queensland) and carrot (Western Australia).

In Victoria, isolation and identification of fungal and oomycete pathogens was conducted on roots that were systematically sampled from untreated control plots during the cropping season in autumn-sown disease management trials in parsley (2010 and 2011) and parsnip (2009, 2010, 2011). Species of *Pythium* were the most predominant of the fungal-like and fungi groups isolated from the roots of parsley and young parsnip crops.

In each year of parsnip trials, species of *Pythium*, and in 2010 and 2011, *Itersonilia perplexans*, were exclusively isolated from asymptomatic and symptomatic young roots at the early stage of crop development. Species of *Fusarium* and *Phoma*, and less frequently *Alternaria*, *Cylindrocarpon* and *Rhizoctonia*, were typically isolated from late infections, indicating a succession of pathogens as the plants matured. Nine species of *Pythium* were identified using ITS region sequence data. Five of these species: the *P. dissotocum* complex, *P. intermedium*, *P. ultimum* var. *ultimum*, *P. sylvaticum* and *P. irregulare*, are typical representatives of the *Pythium* complex in Apiaceae. Four other species, however, *P. tracheiphilum*, and *P. vanterpoolii*, *P. rostratifingens* and *P. camurandrum*, had not been recorded on parsnip roots in Australia and the latter two had not been reported on any crops in Australia. *Phoma exiqua* var. *exigua* was identified for the first time on parsnip roots in Australia. The pathogenicity of the newly discovered species of *Pythium* and *Phoma* to parsnip and their importance in the disease complex on parsnips is yet to be determined.

Cotyledon blight and lesions on the leaves and petioles of parsnip plants attributed to the fungus *Itersonilia perplexans* were common in some parsnip trials in 2010 and 2011. This pathogen has been implicated as a cause of parsnip canker overseas. Despite some reductions in the severity of the disease on parsnip foliage in treatments with organic mulch and foliar applications of tebuconazole (Folicur®), there was no evidence of an associated reduction in disease on parsnip roots.

In parsnip trials, disease symptoms developed gradually during the cropping season but the greatest increments of disease incidence and severity were observed in spring, coinciding with relatively higher rainfall, increasing temperatures and day length, and rapid growth of parsnip roots. Soil properties (sand or loams) and rainfall were associated with higher disease levels and 100% yield loss in crops grown on medium clay soil in the season of relatively high rainfall.

*Pythium* was the most common group isolated from necrotic roots of parsley plants sampled from two trials. Two species, *P. mastophorum* and *P. rostratifingens*, have not previously been recorded on parsley in Australia. The pathogen *Phoma exiqua* var. *exigua* was also identified and has not previously been reported from parsley in Australia. The pathogenicity of one species of *Pythium* to parsley, *P. sulcatum*, was confirmed in a controlled environment experiment. Disease in the two parsley trials was characterised by damping-off of seedlings and the gradual development of root rot in surviving plants through to maturity, resulting in patches of dead and missing plants or a general "thinning" of the crop.

Of the many disease management options evaluated in parsley and parsnip trials in Victoria, only fungicide treatments and new parsnip varieties gave significant reductions in disease and improved yields. However, fungicide treatments were not consistent, being effective in only one of two trials in parsley and one of four trials in parsnip.

In one trial on a sandy soil, the granule formulation of the oomycete specific fungicide metalaxyl (Ridomil®Gold 25SC) reduced the incidence of parsnip roots at harvest with severe (canker and crown rot) and moderate disease (lesions) by an average of 62% and 31%, respectively and improved marketable yields by an average of 50%. The reduction in disease from the metalaxyl was similar for all treatments, irrespective of the number (one, two or four applications) or time of application (10, 1 & 10, 18 & 24 or 1, 10, 18 & 24 weeks after sowing). In the same trial, three applications of an azoxystrobin, difenaconazole mix (Amistar®Top), aimed at controlling oomycetes, *Fusarium* spp. and *Rhizoctonia solani*, reduced the incidence of canker and crown rot in parsnip by 47%, but did not improve marketable yields. It had no effect on disease at a second site in the same season.

Two applications of metalaxyl reduced the incidence of diseased plants by 46% and improved yield by 40%, in a trial in a parsley crop in 2011 but the same treatments had no significant effect on disease in a parsley trial on a different site in 2010.

The relative susceptibility of 11 different varieties of parsnip to root disease (cankers, crown rot and superficial lesions) in a field trial in 2011 varied from 92% roots affected in the standard grower variety to 32% affected in 'Javelin'. The majority of the varieties with the best attributes for marketability were highly susceptible (greater than 80% roots affected). However, growing a variety such as 'Javelin', which does not yield as well as the standard varieties, would be warranted on sites with very high disease pressure. While of the nine cultivars assessed in the Tasmanian trial during 2012, 'Albion', '302-9' and 'Hollow Crown' showed potential as alternative cultivars to the current commercial standards, with good seedling establishment, growth and yield of marketable roots.

There was no evidence of any significant disease reductions from applications of the biological control agent *B. subtilis* (FulzymePlus™) in parsley and parsnip trials, *S. lydicus* (MicroPlus™) in parsnip and the microparasite *P. oligandrum* in parsley in Victoria. There was evidence of improved plant vigour, unrelated to disease control, in one of five trials in parsnip treated with *B. subtilis*. The same biological control agents were evaluated for control of root rots in two trials in organic parsley in Queensland but efficacy was not ascertained because the incidence of root rot in these crops too was low.

Several cultural control treatments applied in parsley and parsnip trials, including hilling soil over the root crowns and organic mulches (composted municipal garden waste) on the soil surface, did not significantly reduce root disease in any trial. However, organic mulches and hilling improved plant vigour in one of two trials in parsnip in 2010. Organic mulches increased laterial root development which is detrimental to marketability of parsnips but may be useful for parsly production.

In a study in New South Wales, *P. sulcatum* was identified as a pathogen of coriander grown in recirculated hydroponic systems in Australia. In a series of four trials conducted on coriander, none of the biocontrol agents tested (*B. subtilis* [FulzymePlus™], *P. oligandrum*) controlled symptoms of root rot sufficiently to give fresh weights similar to healthy plants. Both *B. subtilis* and *P. oligandrum* significantly stimulated plant growth in one trial, but this could not be replicated in other trials.

Autumn sown parsley and parsnip crops are at the highest risk of root disease, being exposed to relatively warm temperatures and autumn and spring rains. The predominance of *Pythium* and the efficacy of the oomycete specific fungicide metalaxyl support the hypothesis that members of this genus are the cause or the inducer of root rot and cankers in the Apiaceae. Effective management of disease complexes over the long growing period of crops, such as parsnip, is particularly challenging. Future research should be focused on a better understanding of the disease complex with regards to the new pathogens identified and also developing much more strategic applications of fungicide and biological control treatments to ensure that the highest concentrations of active materials occur in the root zone at the time when the risk of infection is greatest. Recommendations for control of these *Pythium* induced root rots include rotation with non-Apiacaea crops (e.g. Brassica), selection of fields/beds with relatively good drainage, and early applications of fungicides such as metalaxyl in the granule form to ensure a good distribution in soil.

# Chapter 1

# Review of Pythium Induced Root Rots, Cavity Spots and Cankers of Apiaceae Vegetable Crops

Rudolf de Boer, Joanna Petkowski and Elizabeth Minchinton

## 1 Introduction

The Apiaceae (or Umbelifferae) family of plants includes several important food crops such as carrot (*Daucus carota*), parsnip (*Pastinaca sativa*), parsley (*Petroselinum crispum*), coriander (*Coriandrum sativum*) and celery (*Apium graveolens*). These crops are commonly affected by various types of root rots, which have been the subject of several research projects over the past decade or more. These include cavity spot of carrots (Horticulture Australia Projects VG-036, VG95010, VG98011), root rot of parsley (VG04025, VG06046) and, more recently, canker of parsnip (VG05045).

Species of the fungus-like organism *Pythium* are implicated as a cause of some root diseases, either in their own right or as a complex with other soil-borne pathogens. *Pythiums* are ubiquitous in the vegetable industry and the damage they cause often goes unnoticed. They have been described as the "common cold" of plants (Harvey 2006). They cause damping off and reduce productivity in the early stages of crop production. As soil-borne pathogens, *Pythiums* are relatively fast growing and reproduce quickly. They attack root hairs and lateral roots impairing water and nutrient uptake. They cause cavity spot of carrot, pre- and post-emergence damping off in germinating seedlings, and contribute to symptoms of stress in number of vegetable crops (Porter *et al.* 2007). Preliminary evidence indicates they predispose parsnips to canker (Minchinton *et al.* 2008). *Pythium* is being recognised as a major pathogen of field grown vegetable crops. The main method of control used by growers is calendar applications of fungicides or fumigants which have variable efficacy (Porter *et al.* 2007, VG06092).

*Pythium* is reported to cause losses in field grown crops of baby carrots, carrots, lettuce, baby spinach, parsley and celery (Porter *et al.* 2007, Minchinton *et al.* 2006, Minchinton *et al.* 2008, Karl Riedel pers. comm.). The largest documented losses are in carrots. Carrots are worth $170 M with losses due to *Pythium* estimated to be $8.1 M and cost of controlling the disease $3.25 M. (Davison and McKay 2001, ABS 2004, Porter *et al.* 2007). Parsnips are worth $19 M with losses costing the industry $1.3 M annually (VG05045). *Pythiums* also have a dramatic impact on hydroponics crop yields (Sutton *et al.* 2006, Wulff *et al.* 1998).

This paper reviews *Pythium* root rot diseases of Apiacaeae with particular reference to carrots, parsley and parsnips.

## 2 Cavity Spot of Carrot

Carrots are an important horticultural crop in Australia with a gross value of production of $150 M per annum. Carrots are mainly grown for fresh market, but in Western Australia and Tasmania are also grown for export. Cavity spot is a major disease of mature carrots because the blemishes resulting from this disease make affected carrots unmarketable.

Cavity spot of carrot in Australia was studied extensively Davison and McKay (1998, 1999, 2000, 2001 and 2003). Cavity spot of carrot has been extensively reviewed by Hiltunen and White 2002. Cavity spot is caused by *Pythium* spp., mostly by *Pythium violae* and *Pythium sulcatum*, the latter being the most predominant pathogen in carrots in Australia, and the former being the more predominant causal agent in the main carrot growing areas around the globe. *Pythium viola* causes

cavity spot in carrots grown in irrigated properties along the River Murray in Victoria and South Australia (Davison and McKay 2001). These species are not typical of the more common pythia, having slow growth at normal temperatures, which means in the context of isolation work, plates may be overgrown by other species before they are seen.

In addition to causing cavity spot, *Pythium* spp. cause damping off, leading to low plant numbers and root dieback, resulting in forked or misshapen carrots (White 1986, Liddell *et al.* 1989).

Metalaxyl fungicide was identified as the most effective in controlling cavity spot caused by *P. violae*, but *P. sulcatum* is considered to be naturally tolerant of the fungicide. However, there is evidence of enhanced degradation of metalaxyl with repeated use in some soils (Davison and McKay 1999, Kenny *et al.* 2001). This has forced a renewed focus on other means of controlling this disease. There is evidence that some cultivars have some degree of resistance to the disease (in Hiltunen and White 2002). However, care must be taken in how cultivar resistance is determined. Smith *et al.* (1997) found that the results of experiments with artificial inoculation of different carrots cultivars were inconsistent with field results. Calcium carbonate is known to have significant effects on cavity spot, probably by inducing a soil microflora that is inhibitory to filamentous fungi. Integration of fungicide and/or lime must be considered in an appropriate crop rotation for cavity spot control (Hiltunen and White 2002). Davison and McKay (2003) demonstrated reductions in root damage and cavity spot in carrots grown after a non-Apiaceae crop of broccoli.

In Australia carrot production is estimated at 331,130 tonnes on 7,670 ha (ABS 2004). Carrots and Dutch carrots are direct drilled and grown for 12 to 17 weeks or 9 to 11 weeks, respectively. Carrots are harvested mechanically while Dutch carrots are hand harvested. Victoria is the largest producer growing 34% of the crop followed by Western Australia with 24% of the crop.

# 3 Parsnip Canker

Parsnip canker can cause crop losses of up to 80% in Australia parsnip crops (Minchinton *et al.* 2008). Symptoms of parsnip canker are large black lesions on mature parsnip roots, mainly on the shoulder or the crown. In extreme cases, the canker can cover the entire root. Surveys of parsnip canker at harvest time have found that the incidence of canker peaks in parsnips harvested between September and November on crops sown in February to March. Crop losses peaked at 40% or $1.3M in Victoria during the month of October 2006.

In Australia, parsnip production is estimated at 10,360 t on 415 ha and valued at A$20 million. Victoria produces 82% of the crop which is estimated at 8,535 t on 313 ha and valued at A$15 million (ABS 2001).

Parsnips are direct seeded and grown for 6–8 months. Most Victorian growers have selected their own seed over the years but commercial varieties are still grown both in Victoria and interstate. Locally bred seed produces a whiter rooted parsnip compared with the creamier coloured root of commercial parsnip varieties. The latter are considered less susceptible to canker, but are least preferred by supermarkets. Parsnips are a demanding crop to grow and harvest since their soft root is not amenable to mechanical harvesting. Ongoing issues with parsnip crops affecting marketability include (i) variability in size and shape, (ii) colour, (iii) forking, (iii) powdery mildew and (iv) canker.

Parsnip canker, like cavity spot of carrot, has proved to be an intractable problem to solve and may be due to a complex of pathogens, rather due to a single cause.

## 3.1 A historical background on Parsnip canker

### 3.1.1 Symptoms of parsnip canker

Cankers primarily form on the crown and shoulder of roots, although these lesions can extend along the length of the root (Cerkauskas 2002). Four types of parsnip canker were described by Channon (1965). Overseas, the disease has been attributed to *Itersonilia perplexans*, *Phoma* spp., *M. acerina* and *S. scabies* in the UK (Channon 1965, Fox 2002, Jones 1953). In Canada it was attributed to *Phoma complanata* (Cerkauskas 1985), while in the USA *Itersonilia* was pathogenic (Wilkinson 1952) and in Scotland *Cylindrocarpon destructans* was responsible for canker (Channon and Thomson 1981). It is not considered to be associated with bacteria (Green and Hewlett 1950), but has been associated with the carrot fly larvae *Psila rosae* in the UK (Stone 1954). Fortunately carrot flies are not present in Australia (see 3.2.6).

### 3.1.2 *Itersonilia* spp. as pathogens of parsnip

*Itersonilia perplexans* is thought to be the main cause of parsnip canker in overseas countries, and as a consequence, most research into parsnip canker and its control has been focused on this organism. *Itersonilia* is a basidomycete which can infect roots, leaves, inflorescences and seed (Channon 1969), with an optimal temperature for growth of 20°C. According to the Commonwealth Mycological Institute, *I. perplexans* is endemic to Canada, North America, England, Italy, Australia and New Zealand (Anon 1978). Sowell and Korf (1960) also obtained isolates from the Netherlands.

The type species of *Itersonilia*, *I. perplexans* Derx was first isolated in 1948 (Derx 1948). Not long after, *Itersonilia* sp. was isolated from parsnip crops in the United States, and both cankers and 'typical' leaf spots were induced on parsnips from pure isolates (Wilkinson 1952). Sowell (1953) reported that germinating ballistospores of *Itersonilia* sp. were responsible for both the leaf spot and the canker. Channon (1956) observed similar symptoms in Great Britain and obtained pure cultures from cankers in 1954. Only one of these isolates produced cankers in both unwounded and wounded parsnip tissue. The isolate was subsequently identified as *I. perplexans*, the same species that had been isolated previously (Derx 1948). Similarly, this isolate also produced leaf lesions on young parsnip plants. The leaf lesions were fairly distinctive, with a necrotic centre surrounded by a light-green halo.

A study of 6 isolates from parsnip around the world, as well as 43 other local isolates, determined that all isolates were a single species, *I. perplexans*, and all were proven to be pathogenic on parsnip (Sowell and Korf 1960).

Channon initially isolated *Itersonilia* from cankers in parsnip in Great Britain (Channon 1956) and wrote a series of papers on his studies on parsnip canker (Channon 1963abc, 1964, 1965). He described two kinds of canker in his initial paper, a black canker, caused by *Itersonilia*, *Phoma* or both, and an orange-brown canker, with an unknown cause (Channon 1963a). Over 60 % of black cankers yielded pathogenic *Itersonilia*, but only 16% yielded pathogenic *Phoma*. There was some evidence of a 'consortium' of fungi causing black cankers, since isolates from brown cankers failed to induce similar symptoms, but *Itersonilia* isolates from the same brown cankers produced 'typical' black cankers. These 'brown' cankers appeared to be associated with growth splitting. *Itersonilia* isolates from diseased parsnip roots and leaves were to be pathogenic on parsnips, but similar isolates from chrysanthemums were non-pathogenic on parsnip, and vice-versa. On the basis of profuse chlamydospore production, i.e. the resting stage of the fungus, it was decided that these pathogenic *Itersonilia* isolates were different from the original *I. perplexans* (Derx 1948), and a new species was named that was exclusively pathogenic on parsnip, *I. pastinacae*. Channon also noted that wounding of parsnip roots prior to inoculation with *I. pastinacae* resulted in more rapid and larger cankers (Channon 1963a).

### 3.1.3 Epidemiology

In his second paper, Channon reported the seasonal presence of ballistospores, which appeared on leaves of parsnip, and were the presumptive cause of canker in parsnip roots (Channon 1963b). Ballistospores were present in parsnip crops in late summer and their presence peaked in autumn. Numbers were higher in the morning, and appeared to be associated with dew periods. Drier conditions in subsequent years resulted in fewer *Itersonilia* spores and were associated with less canker incidence in the following season. There was a clear link between rainfalls, numbers of spores collected and canker incidence. It was speculated that abundant spore formation on leaves during the wet season leads to profuse numbers of spores washed down into the soil and subsequent canker. The optimal temperature for growth is 20°C and abundant soil moisture and low temperatures promote the disease whilst hot and dry conditions retard it (Cerkauskas 2002).

### 3.1.5 Presence on seed

The presence of *Itersonilia* in seed was attributed to contamination from infected trash and to infected flowers. Infection of parsnip seedlings from previously pristine fields led to the discovery that *Itersonilia* could be seed-borne (Channon 1967, Smith 1966). A simple bioassay of unsorted seeds stuck to petri dish lids over media demonstrated that *Itersonilia* was present in 20 % of seed lots, with an equal weight of contaminated seed in lots from grower and commercial sources. It was speculated that the presence of *Itersonilia* in seed lots was probably due to exposure to dried plant trash. The level of contamination of the seeds (1–4%) was enough to induce seedling infection. Channon (1969) found *Itersonilia* in flowers, which led to a reduction in seed production and could be a potential problem for emerging seedlings.

### 3.1.6 Persistence in soil

The survival of *Itersonilia* in soil was demonstrated by Smith (1967). When parsnip roots with canker were buried in the soil, *Itersonilia* was still viable after 12 months. When the tops of the roots were excised to simulate harvest damage and stimulate breakdown, viability was cut to 7 months. Soil saprophytes such as *Bacillus subtilis* and *Streptomyces* sp. were introduced to sterile soil inoculated with *Itersonilia* and rapidly lysed both ballistospores and hyphae, but left the more resistant and hardier resting spores (i.e. chlamydospores). This mirrored 'natural soils' and demonstrated the effectiveness of 'hilling' by covering the parsnip crowns progressively with soil and thus encouraging rapid breakdown of the fungus. However, the survivability of *Itersonilia* in soil showed that infected parsnip roots are an obvious source of carry-over in the soil with chlamydospores persisting in a cycle of infection.

### 3.1.7 Host specificity

*Itersonilia perplexans* has been found to be pathogenic on a wide variety of crops and flowers, including parsnip, dill, chrysanthemum, Chinese aster, sunflower, and edible burdock (Channon 1963a, Horita and Yasuoka 2002, Koike 2001, McGovern and Seijo 1999, Seijo *et al.* 2000). It is generally accepted that those isolated from flowers such as chrysanthemum are not pathogenic on parsnip and vice-versa, possibly indicating that *I. perplexans* is a weak pathogen at best (Koike 2001). Alternatively, these differences may be due to different pathotypes. However, isolates from edible burdock were also capable of infecting chrysanthemums, causing petal blight (Horita and Yasuoka 2002).

### 3.1.8 *Itersonilia perplexans* or *Itersonilia pastinacae*?

In his initial studies of parsnip canker, Channon (1963a) isolated an *Itersonilia* strain which was thought to be sufficiently different from the type strain of *I. perplexans*, and was named *I. pastinacae*. However, studies of nutrition requirements, mating and DNA homology determined that *I. pastinacae*, *I. perplexans*, and another species *I. pyrifomans* were *Itersonilia perplexans* (Boekhout 1991, Boekhout *et al.* 1991).

## 3.2 Other causes of parsnip canker

In initial studies of parsnip canker in the UK, other fungi were associated with canker; lending weight to the theory of a 'complex' of fungi was responsible for parsnip canker (Channon 1963c, 1965).

### 3.2.1 Phoma

The initial studies by Channon (1963a) found *Phoma* as well as *Itersonilia*, and both were capable of producing 'black cankers'. Researchers in Canada found that *P. complanata* caused wide spread losses (up to 80 % incidence in field crops) and confirmed that the pathogen was seed-borne (Cerkauskas 1985). There was a strong correlation between the severity and incidence of the foliage phase of *Phoma* and the severity and incidence of the canker phase (Cerkauskas 1987). Unlike *Itersonilia*, *P. complanata* had a narrow pathogenicity range, but like *Itersonilia*, it was capable of over-wintering and surviving in soils for up to 5 months (Cerkauskas 1987).

### 3.2.3 Mycocentrospora

Canker symptoms very similar to those caused by *Itersonilia* yielded another pathogen, *Mycocentrospora acerina*. Cankers associated with this pathogen were also black, but were usually surrounded by a pale brown/red band. Unlike *Itersonilia*, there was no difference in the severity or size of the cankers caused by *M. acerina* on wounded and unwounded parsnip roots. It was pathogenic on a wide variety of crops including parsnip, beetroot, peas, cabbage, cauliflower, tomato and carrot. *M. acerina* was found to be capable of growing and infecting at temperatures below 0°C, which would encourage the advent of canker symptoms under winter conditions (Channon 1965).

### 3.2.4 *Cylindrocarpon*

*C. destructans*, a fungus similar to *Fusarium*, was also isolated from black or dark brown cankers on parsnip. Isolates of this fungus were capable of re-infecting and causing canker symptoms on damaged parsnip roots (Channon and Thomson 1981). The fungus has a wide range of hosts and is considered to be a weak pathogen in most hosts, but a major pathogen of ginseng (Zeizold 1997).

### 3.2.5 *Streptomyces scabies*

*S. scabies*, which causes common scab in potato, was also found to be capable of producing canker in parsnip (Jones 1953).

### 3.2.6 *Psila rosa* (Carrot rust fly)

*Psila rosa* (Carrot rust fly) is a major contributor to the incidence of parsnip canker in the UK, because of the damage it causes to parsnip roots, pre-disposing them to infection (Stone 1954). Controlling carrot fly incidence was found to be consistent with a significant reduction in parsnip canker incidence (Collingwood and Croxall 1954). Control of carrot fly usually involves pre-drilling the soil before seeding and treating with insecticides such as phorate and diazinon (Sivasubramaniam *et al.* 1997). Research is continuing into the location and the amount of insecticide needed to effectively control this pest (Sivasubramaniam *et al.* 1999).

Although carrot rust fly is not in Australia, it is widely distributed around the world. It is in North America, the UK, Europe and Eurasia (Factsheet 17/2001). Its presence has been documented in New Zealand, and thus has the potential to be a major biosecurity issue for carrot and parsnip growers in Australia.

## 3.3 Control of *Itersonilia parsnip* canker

Management of parsnip canker associated with *Itersonilia* has included cultural practices, cultivar resistance and fungicide treatments.

### 3.3.1 Cultural practices

The cultural control of canker is limited in scope, but the following practices have been investigated and promoted.

- Gradual hilling and covering of parsnip shoulders encourages breakdown of *Itersonilia* ballistospores in soil (Channon 1963b, Smith 1967). In the UK, this practice led to a 45 % reduction in canker incidence and a 70 % reduction in the size of the lesions. However, growers in Australia are adamant that this only increases the incidence of *Phoma* canker.
- Sowing and spacing practices were reported to alleviate canker incidence (Channon 1964). There was at least a 3-fold reduction in canker incidence when the crop was late-sown and thinned to 3-cm intervals. However, this was offset by a reduction in root size, with small roots having less canker than larger roots, and there was a balance between a loss in total yield and a reduction in canker incidence and severity that increased marketable roots. While there was a 75 % reduction in canker incidence and a 60 % reduction in lesion size, there was a large drop in marketable yields (50 %), so this method was deemed to be impractical.
- Crop hygiene consisting of removal of all roots and plant trash from beds was suggested by Smith (1967). No hard figures are available for this practice, but it is logical to assume there would be a reduced *Itersonilia* presence in the soil, leading to less canker.
- Crop rotation is imperative as *Itersonilia* can survive on parsnip roots after burial for 12 months. The air-borne stage is not viable after 2 days in soil (Smith 1967).

### 3.2.2 Fungicide treatments

There is very little literature concerning fungicide control of canker caused by *Itersonilia*. Some authors have recommended the application of copper (every 7–10 days) as a foliar spray to eliminate ballistospores on foliage and thus reduce the incidence of canker (Chupp and Sharp 1960). In New Zealand maneb sprayed at fortnightly intervals from February to June, had some efficacy on *Itersonilia* canker (Brandenburg 1965). *Bacillus subtilis* and *Streptomyces* spp. were antagonistic to *Itersonilia in vitro* in Australia (Smith 1967), but biological options do not appear to have been examined in the field. Up to 7 fungicide sprays per crop were required for the control of canker. This frequency of calendar spraying is considered uneconomic by the Australian industry.

Treatment of parsnip seed with hypochlorite or mercuric chloride was insufficient to eliminate the fungus. Hot water treatment eliminated *Itersonilia*, but significantly reduced germination. Thiram only inactivated *Itersonilia* located on seed surfaces (Channon 1969, Smith 1966). The most successful treatment was by steam air at 45.5°C for 30 min which removed it from seed trash without significantly affecting germination (Smith 1966).

Canker caused by *P. complanata* has been successfully controlled by fungicides such as chlorothalonil and mancozeb in Canada (Cerkauskas and McGarvey 1988). The effectiveness of the chemical treatments was dependent on the area in which they were grown. In the UK tebuconazole is registered for canker control (Assured Food Standards 2006).

### 3.3.3 Cultivar resistance

The control of parsnip canker using resistant cultivars is complicated by the fact that more than one organism is responsible for the disease. Parsnip lines have been bred for resistance with varying success against *I. perplexans* (Anon 1966, Channon *et al.* 1970, Davis *et al.* 1989), *P. complanta* (Cerkauskas 1986ab), *Streptomyces scabies* (Green and Hewlett 1954) and *M. acerina* (Channon 1965). Breeding for resistance against *I. perplexans* and *Phoma* also gave rise to resistance against canker caused by *M. acerina* (Channon 1965, Channon *et al.* 1970).

## 3.4 Parsnip Canker in Australia

Etiology, epidemiology and management of this disease have eluded science and industry for 40 years. In recent studies in parsnip crops in Victoria (Minchinton *et al.* 2008), several different potential

pathogens were isolated from cankers on mature parsnips (Table 1). Two fungi attributed with causing parsnip cankers overseas were isolated from cankers in Victoria namely *Itersonilia perplexans*, *Mycocentropsora acerina*. Unidentified *Phoma* spp. and *Cylindrocarpon* spp. were also isolated, *P. complanta* and *C. destructans* have both been implicated as a cause of parsnip canker. Many of the fungi isolated were capable of causing lesions on mature parsnip roots.

In field trials several fungicides were applied to target specific pathogen groups. In one trial, a 75% reduction in the incidence of parsnip canker in plots treated with the oomycete specific fungicide metalaxyl indicated that at this site, *Pythium* spp. were directly or indirectly responsible for the parsnip canker fungicide. Based on field surveys, fungal isolations, pathogenicity tests and field trial it was concluded that parsnip canker is associated with a complex of fungi, with pathogenicity established for *Pythium, Fusarium oxysporum, Itersonilia perplexans*, *Acremonium, Cylindrocarpon, Microdochium, Mycocentrospora acerina*, *Phoma exigua*, and *Rhizoctonia*.

**Table 1 Micro-organisms isolated from parsnip roots with canker and their pathogenicity (Minchinton *et al.* 2008)**

| Organism | Isolates from mature parsnip lesions forming cankers (%) | Pathogenicity on mature parsnips (wounded & unwounded) | Reported causes of parsnip canker in overseas countries |
|---|---|---|---|
| *Acremonium spp.* | 3% | II, IV | |
| *Cylindrocarpon spp* | 11% | I, II | + (*C. destructans*) |
| *Fusarium spp* | 19% | I, II, IV | |
| *Fusarium oxysporum* | | I | |
| *Fusarium solani* | | II | |
| *Itersonilia perplexans* | 22% | I, II, III, IV | + |
| *Microdochium spp.* | 6% | I | |
| *Mycocentrospora acerina* | 5% | I | + |
| *Phoma spp.* | 4% | I | + (*P. complanata*) |
| *Phoma exigua* | | I | |
| *Pithomyces* | 1% | | |
| *Pythium spp.* | 3% | | |
| *Pythium sulcatum* | Isolated from parsnip | II | |
| *Rhizoctonia spp.* | 5% | III | |

Lesion types I, lesions produced on all areas of the parsnip root (crown, middle and lower root), wounded or unwounded; II, lesion on the upper areas only; III, lesions on wounded root only, either along the length or at the crown only; IV, like III, but lesion formed on unwounded crown.

# 4 Root rots of parsley

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.

Crop losses from parsley root rot and post emergence damping off were identified by Minchinton *et al.* (2006, 2007) (VG05045). Growers reported that 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 symptom less.

A number of fungi have been reported to cause root rot or damping off in parsley (Table 2). 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 2002). 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).

The micro-organisms isolated from diseased parsley roots in Australia and their pathogenicity is summarised in Table 3. Based on the results of systematic isolations, pathogenicity testing and the use of selective fungicides in field trials, it was concluded the oomycete pathogens, *Pythium* and *Phytophthora* were the most likely cause of root rot of parsley in winter (Minchinton *et al.* 2006). This was based on root rot symptoms that were consistent with infection by these pathogens and on effective control with metalaxyl. However, a *Fusarium* species was associated with distinctive *Fusarim* like symptoms on root rots of parsley over the summer months (Minchinton *et al.* 2007).

**Table 2 Fungi isolated from parsley roots in overseas trials**

| Parsley Organism – overseas | Symptom | Location | Author |
|---|---|---|---|
| *Phytophthora cryptogea* | Root rot or damping off | California | Davis *et al.* 1994 |
| *P. nicotiana* | + | Hawaii | Uchida & Kodooka 2006 |
| *Pythium paroeccandrum* | + | Northern Ireland | McCraken 1984a |
| *P. masophorum* | + | Germany | Kober & Sauthoff 1999 |
| *P. aphanidermatum* | + | South Africa (hydroponic) | Gull *et al.* 2004 |
| *P. ultimum* | Damping off | USA | Hershman *et al.* 1986 |
| *P. irregulare* | + | + | + |
| *Rhizoctonia solani* | + | + | + |
| *P. debaryanum* | + | + | De Zeeuw 1954 |
| *Alternaria* | + | Belgium & Poland | Nawrocki & Mazur 2004; Nowicki 1997 |
| *Fusarium* | + | + | + |
| *Phoma* | + | + | + |
| *Rhizoctonia* | + | + | + |
| *Sclerotinaia* | + | + | + |
| *Pythium* | + | + | + |
| *Fusarium* | avirulent | USA | Hershman *et al.* 1986 |

**Table 3 Micro-organisms isolated from the roots of parsley in Australian field trials (Minchinton *et al.* 2006, 2007)**

| Organism | Comments | |
|---|---|---|
| | **Pathogenicity tested** | **Isolated** |
| *Pythium spp.* | VIC, not pathogenic<br>NSW, reduced root mass, root browning, collapse of plants, low rates of mortality.<br>QLD, not pathogenic - technique | QLD – root rot in hydroponics<br>QLD – soil<br>NSW – brown root systems<br>VIC – root rot |
| *P. acanthophoron* | | NSW – brown root systems |
| *P. diclinum group* | VIC, not pathogenic, ($2^{nd}$) pathogenic low temp<br>NSW, root browning & collapse<br>QLD, slight to severe root rot all temps ($2^{nd}$) | QLD – root<br>VIC – root |
| *P. intermedium* | VIC, not pathogenic, pathogenic | VIC – root |
| *P irregulare* | VIC, $2^{nd}$ study pathogenic low temp<br>QLD, severe root rot all temps ($2^{nd}$) | QLD – root<br>NSW – roots |
| *P. littorale group* | | QLD – lupin bait soil |
| *P. oligandrum* | VIC, not pathogenic, mycoparasite | VIC – roots<br>NSW – brown root systems |
| *P. paroecandrum* | | NSW – brown root systems |
| *P. sulcatum* | VIC, pathogenic low temp. Stunting, chlorosis, wilt, dull brown soft root rot of neck, *Winter root rot* | VIC – root |
| *P. ultimum* | VIC, not pathogenic, pathogenic ($2^{nd}$) low temp | NSW<br>QLD – root<br>VIC – root |
| *Phytophthora spp.* | NSW, reduced root mass, root browning, collapse of plants, low rates of mortality.<br>QLD, not pathogenic – plants die<br>VIC, not pathogenic | NSW – brown root systems<br>VIC – pear bait soil, water<br>QLD – isolated from crown rot |
| *P cryptogea* | QLD, not pathogenic | QLD – lupin bait & root |
| *P inundata* | VIC, pathogenic, stunting & wilt, taproot | VIC – root |
| *P. megasperma* | VIC, pathogenic, stunting & wilt, taproot | VIC – root |
| *Fusarium spp* | VIC, Red lesions, less feeder roots summer root rot, *Summer root rot* – superficial<br>QLD, weakly pathogenic, non pathogenic ($2^{nd}$), pathogenic slight to severe ($2^{nd}$),<br>NSW, brown & reduced root mass & low rates of mortality | QLD – root, crown, stunting & yellowing<br>NSW– brown root systems<br>VIC – red root lesions |
| *F. oxysporum* | VIC, antagonist to *P. ultimum*<br>QLD, moderate to severe root & crown rot higher temps ($2^{nd}$) | VIC<br>QLD – crown |
| *F. solani* | QLD, collar rot, mild to severe root rot & browning high temps ($2^{nd}$) | QLD – collar |
| *Alternaria petroselini* | QLD, Leaf blight – new report | QLD – leaf |
| *Colletotrichum gloeoporioides* | QLD, slight root browning at 35°C ($2^{nd}$) | QLD – petiole |
| *Cylindrocarpon* | | VIC |
| *Macrophomina phaseolina* | QLD, not pathogenic | QLD – root |
| *Microdochium* | | VIC |
| *Mycocentraspora* | | VIC |
| *Rhizoctonia solani* | NSW, crater-like reddish lesions & collar rot | VIC<br>NSW – crown & collar rot |
| *Septoria* | | VIC – NSW, QLD leaf spot |
| *Sclerotinia sp.* | NSW, watery petiole and crown rot | NSW – watery stem<br>VIC – stem & crown rot |
| *Strentrophomonas maltophilia* | QLD, bacteria, crown and root rot (ID by BioLog?) | QLD – crown & root rot |

# 5 Pythium

Agrios (2005) provides a succinct summary of *Pythium* and diseases caused by this fungal-like pathogen. An example disease cycle for a *Pythium* seedling disease is presented in Fig. 1. *Pythium* is a soil-borne fungal-like organism belonging to the oomycete family, which includes *Pythium, Phytophthora* and the downy mildew causing organisms.

*Pythium* affects seeds, germinating seedlings and the roots of all major grain crops, pastures and horticulture crops in temperature and tropical environments world wide. *Pythium* species are best known for causing 'damping-off' diseases of seedlings resulting in poor germination of seeds and poor emergence of seedlings. Older plants are seldom killed when infected by the damping-off pathogen but develop root and stem lesions and root rots that can retard growth and reduce yields.

*Pythium* species are ubiquitous in all environments. They generally occur in surface waters and soils. They live on dead plant and animal debris as saprophytes or as parasites of fibrous roots of plants.

Pythium survive in the soil as oospores, which are thick walled structures that are resistant to adverse conditions of soil temperature and moisture. Oospores of *P. sulcatum* are known to survive in the soil for at least 21 months in the absence of a host (Davison and McKay 2003). These spores lie dormant in the soil until it rains or when a crop is irrigated.

Germinating seeds and growing roots release chemicals that act as growth stimulants and attractants for hyphae and sporangia enabling the pathogen to grow quickly and infect roots.

*Pythium* produces a white fast growing mycelium. The mycelium gives rise to sporangia, which germinate directly by producing one to several germ tubers, or by producing a short hypha from which a secondary sporangium (vesicle) is formed. Zoospores are produced in the vesicle, which, when released, swarm about for a few minutes, round off to form a cyst and then germinate by producing a germ tube. The germ tuber penetrates the host tissue and starts a new infection. Sometimes the germ tube produces another vesicle from which several secondary zoopores are formed and process this may be repeated.

The mycelium also gives rise to spherical oogonia and club-shaped antheridia which 'mate' to produce an oospore. Oospores are resistant to adverse temperatures and moisture conditions. They germinate in a manner similar to that described for sporangia. The type of germination is determined primarily by temperature: temperatures above 18°C favour germination by germ tubers, whereas temperatures between 10°C and 18°C induce germination by means of zoospores

Spore germ tubes or saprophytic mycelium coming into contact with seeds or seedling tissues infect by direct penetration. Pectoytic, proteolytic and cellulolytic enzymes are released resulting in the complete collapse and disintegration of the cell walls, ultimately causing the characteristic watery rot, collapse of stems resulting in the damping-off symptoms.

If the seedling is infected when it is well developed and has well-thickened and lignified cells, the advance of the pathogen is stopped at the point of infection and only small lesions develop.

Rootlets can be attacked at any growth stage. The pathogen enters the root tips and proliferates, causing rapid collapse and death of the rootlet. Invasion of older roots is usually limited to the cortex. Relatively long and fleshy roots can be invaded resulting in lesions that are several centimetres long.

*Pythium* have come to be known as the 'common cold' of plants (Harvey 2006). Although they are best known for causing dramatic diseases such as damping off and cavity spots, *Pythium* are also implicated in causing significant reductions in productivity in a variety of agricultural crops, even though above ground symptoms may not be apparent. *Pythium* strips off the fine lateral feeder roots

and root hairs reducing nutrient and water uptake and plant growth. Also, plants already weakened by *Pythium* are more vulnerable to infection by other fungal pathogens. Although *Pythium* alone is an important pathogen, its impact can be increased significantly when it forms a disease complex with other root pathogens. For this reason, the impact of *Pythium* is often significantly underestimated.

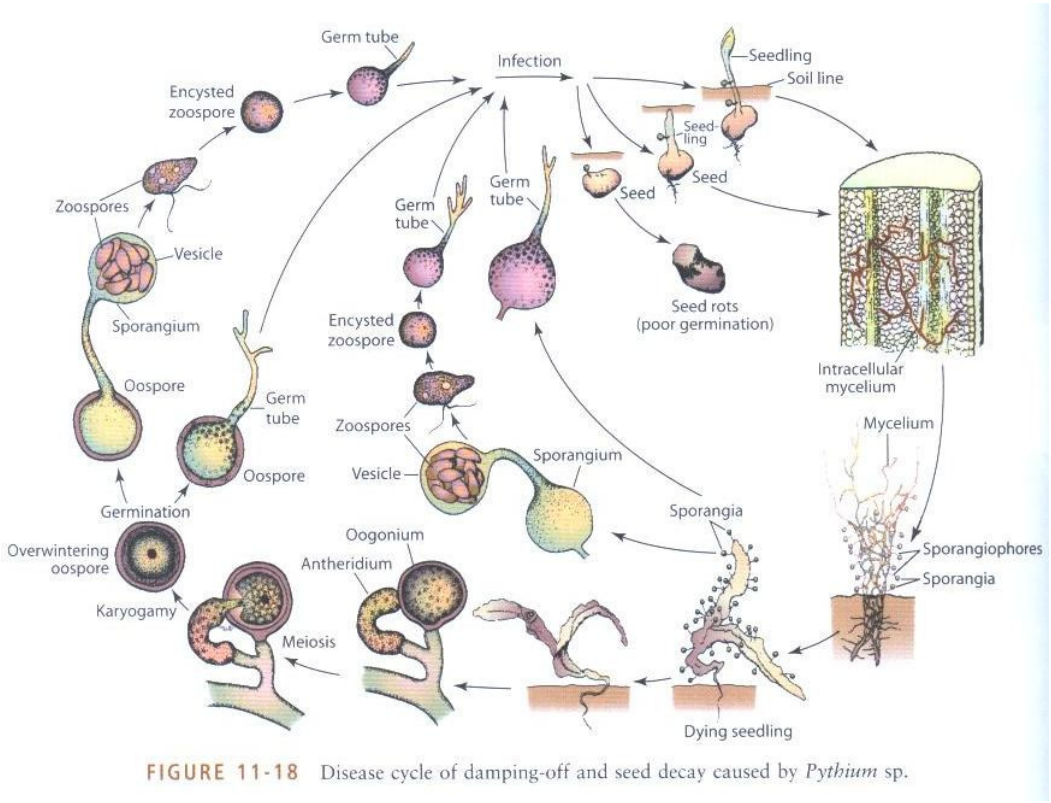


**Figure 1 Life cycle and disease cycle of *Pythium* (Source Agrios 2005)**

## 5.1 Ecology of *Pythium*

Hiltunen and White 2002 suggested a succession of *Pythium* on hosts. For cavity spot of carrots, for example, *P. violae* succeeds *P. sulcatum*, which are then overgrown with faster growing *Pythiums*. Leach 1947 found that *P. ultimum* causing sugar beet pre-emergence damping off was greatest when the ratio of the growth rate of the host to that of the pathogen is lowest. *P. ultimum* grows better at low temperatures, whilst *Rhizoctonia* does not (Hershman *et al.* 1986).

## 5.2 Etiology

*Pythium* spores (zoospores) attach to specific parts of roots or wounded roots. Recent work demonstrated the phenomenon can be attributed to electrical attraction (van West *et al*. 2002). The susceptible root regions had a negative charge, whilst the posterior and anterior flagellae of zoospores had opposite charges (Morris and Gow 1993). Electrotaxis amongst the pythiums tested, however, varied or was weak. The temperature in the root zone can also influence infection. Klaring *et al*. (2001) showed that roots of glass-house grown tomato plants changed from being tolerant to *P. aphanidermatum* to susceptible with a 3°C increase in temperature. Furthermore, oomycetes can exhibit autotaxis or autoaggregation of zoospores, which swim together in groups of hundreds, if not

thousands (van West *et al*. 2003) as well as a non-specific attraction to chemicals such as amino acids (Donaldson and Deacon 1993). This autoaggregation was also found to be Ca-dependent and influenced by pH and acts to enhance zoospore accumulation at plant root surfaces and thus increasing inoculum potential for infection (Reid *et al*. 1995).

The influence of temperature on susceptibility of roots to *Pythium* suggests that a decision support tool can be developed to assist growers in deciding when to action control strategies. The effects of electrotaxis, Ca and pH on the infection process by *P. sulcatum*, which is associated with parsnip canker and cavity spot, are currently unknown, and if demonstrated, would help to better determine the most effective control options.

## 5.3 Epidemiology

Early work on *Pythium* showed that cyclic wetting and drying reduced *Pythium* populations in the field (Stanghellini and Burr 1973). In the UK, studies of *Pythium* populations on cultivated and uncultivated sites suggested an annual pattern of fluctuations with a peak in autumn and winter and a trough in summer (Ali-Shtayeh 1986). Similar observations were reported for *Pythium* root rot of parsley in southern Australia (Minchinton *et al.* 2006, VG04025). UK researchers developed an adequate, but not completely satisfactory model to explain the field variability. Factors that influenced existing large populations in autumn, soil moisture content but surprisingly not soil temperature (Ali-Shtayeh 1986). Conversely, in hydroponic systems, temperature around the root zone determined susceptibility or tolerance to *P. aphanidermatum.* Based on this a hypothetical model was developed, but it did not stand up at high temperatures (Klaring *et al.* 2001). Another theoretical infection model was developed for epidemics of soil-borne plant disease using *Pythium* as an example, which allowed for primary and secondary infection events (Brassett and Gilligan 1988). The model was primarily concerned with concentration of inoculum rather than environmental parameters promoting epidemics. Davis (2007) in California is developing a predictive model for *Pythium* diseases of vegetable crops, but is in the early stages and appears to be aimed at identifying threshold levels of inoculum.

Although there have been a number of theoretical models developed for *Pythium* infections, a comprehensive growth chamber study of all potential parameters such as soil temperature, soil moisture, soil composition, root growth and infection cycles is required for a more practical model.

### 5.3.1 Crop Nutrition and pH

A number of parsnip growers in Victoria plant their crops with reduced or no nutrient inputs at all. Their experience is that parsnip canker appears to be correlated with high nutrient inputs and believe that high growth rates and resulting growth cracks may predispose parsnips to infection by pathogens.

Hiltunen and White (2003) have reviewed the literature on the effects of soil nutrition on cavity spot of carrot (*P. viola* and *P. sulcatum*). In general, there do not appear to be any clear cut or consistent relationships between soil nutrition, plant nutrition or other soil factors (conductivity, moisture holding capacity, organic matter, total and exchangeable calcium and particle size distribution) reported. Early studies suggested a link between disease and low nutrient levels and since then research was focused on studying the effects of soil calcium and potassium levels on cavity spot. There were suggestions that disease was associated with potassium induced calcium deficiency or with excess potassium which could have affected calcium uptake and in one instance high levels of nitrogen were said to have increased cavity spot. However, there are many reports which have not found relationships between cavity spot, nutrients and various soil factors.

Hiltunen and White (2003) reported that for many years lime (calcium carbonate) has been shown to reduce cavity spot of carrots, although the mechanism for suppression by lime is not understood. Furthermore, it is not easy to separate the effects of pH and calcium on cavity spot. There are conflicting reports on the disease incidence in relation to changes of soil pH. Raising soil pH to over 6.9 and 7.4 was shown to reduce cavity spot of carrot (Scaife *et al.* 1983, Perry and Groom 1984, El-

Tarabily *et al*. 1996, Davison and McKay 2000) but disease reduction was also achieved by lowering soil pH below 6.6 (Perry and Harrison 1979). Vivoda *et al*. (1991) did not find any correlation between the incidence of cavity spot and soil pH ranging from 5.7 to 7.7. Soroker *et al*. (1984) and Jacobsohn *et al*. (1984) on the other hand reported the disease from crops grown in calcareous soils (pH 7.8-8.3). Manipulation of soil pH may have not directly affected *Pythium* spp. causing cavity spot as the two most prominent pathogenic species, *P. violae* and *P. sulcatum* grew over pH 5.5-9.0 and pH 5.8-7.8, respectively (Hiltunen and White 2003 and reports therein).

Calcium is a macronutrient, which has several functions within all plants eg. activation and regulation of enzymes and maintenance of membrane and primary cell wall structures. Although it is readily available in most soils, its deficiency in plants, commonly occur from incorrect applications of fertilisers and nutrient solutions. Calcium deficiencies in plants are usually expressed as a yellowing of the upper part of the shoots while lower parts remain dark green. Shoot and root development is limited. Calcium is characterised by low mobility, it can not be translocated from old to new shoots and it is stored in old plant tissues (Pilbeam and Morley 2007). Deficiencies, therefore, often appear in plant organs, which usually store least calcium such as fruit, eg. blossom end rot of tomato, pepper and water melon, internal rust spot of potato and carrot, internal browning of Brussels sprout and tip burn of lettuce (Pilbeam and Morley 2007).

Vegetable growers regularly apply lime to maintain close to neutral soil pH as most of Victorian parsley and parsnip crops are grown on naturally acidic sandy and sandy loam soils. The direct reduction of soil acidity by liming leads to better nutrient availability, e.g. phosphorus, reduced risk of aluminium and manganese toxicities and indirect improvement of soil structure. Adjusting pH to values greater than 7 increases the risk of nutrient deficiencies and toxicities typical for alkaline soils, e.g. phosphorous and iron deficiencies. The benefits of supplying more calcium as calcium carbonate or other forms to improve plant health need to be investigated for parsley and parsnip production systems.

### 5.3.2 Temperature and moisture

Early work on *Pythium* showed that cyclic wetting and drying reduced *Pythium* populations in the field (Stanghellini and Burr 1973). In the UK, studies of *Pythium* populations on cultivated and uncultivated sites suggested an annual pattern of fluctuations with a peak in autumn and winter and a trough in summer (Ali-Shtayeh 1986). Similar observations were reported for *Pythium* root rot of parsley in southern Australia (Minchinton *et al*. 2006, VG04025). Factors that influenced existing large populations in autumn were soil moisture content but surprisingly not soil temperature (Ali-Shtayeh 1986). Conversely, in hydroponic systems, temperature around the root zone determined susceptibility or tolerance to *P. aphanidermatum* (Klaring *et al.* 2001).

### 5.3.3 Alternate Hosts and Crop Rotation

Dormant resting spores of *Pythium* species formed during pathogenic and/or saprophytic colonisation of plant tissues have long been considered to be the primary sources of inoculum for succeeding crops. However, non-pathogen colonisation of other crops and weeds can provide an alternative disease initiating source of inoculum (Staghellini 1974). Significant damage to direct drilled cereals by *Pythium* spp. following knockdown herbicide treatment of the preceding pasture has been observed. This was thought to be due to the colonisation of pasture species roots with a build-up inoculum of *Pythium*. The pasture root systems acted as a 'green bridge' to the cereal crop when there was only a matter of days between pasture knockdown and the drilling of cereal seed (RF de Boer, pers. comm.).

*P. sulcatum*, which causes cavity spot of carrot in Australia (Davison and MacKay 2001) appears to have a relatively restricted host range compared with *P. violae* (main cause of cavity spot in carrots in most other countries) (Hiltunen and While 2002). Apart from carrots, *P. sulcatum* has been isolated from parsley (Plaats-Niterink 1981, Minchinton *et al.* 2006, 2007), from parsnip (Minchinton *et al.* 2008) and in a very low frequency from spinach (McKay and Davison 2000).

A number of reports indicate that in general, there is a tendency for increased incidence of cavity spot with an increased frequency of cropping carrots (Hiltunen and White 2002).

In studies in infested fields in Western Australia, *Pythium sulcatum* was isolated from the roots of carrots and other members of the Apiaceae family (carrot, parsley, parsnip, parsley), but not from vegetables of other plant families (Brassica, beetroot, capsicum, lettuce, onion, spinach, tomato, bean, cucumber, musk melon, barley, maize, oats, rye, wheat) (Davison and McKay 2003). In a rotation experiment, the incidence and severity of carrot seedling tap root infection by *P. sulcatum* was significantly reduced when carrots followed one, two or three broccoli crops. This was apparent in mature carrots as a reduced proportion of carrots marketable as short and unmarketable forked carrots (Davison and McKay 2003). The incidence of cavity spot was significantly reduced when carrots followed one or two crops of broccoli compared with continuously cropped carrots.

Crop rotation is imperative as *Itersonilia* can survive on parsnip roots after burial for 12 months. The air-borne stage is not viable after 2 days in soil (Smith 1967). During hand harvest of parsnips in Australia/Victoria parsnips with symptoms of canker are frequently left behind in the paddock where they can theoretically supply inoculum for future crops. Every effort must be made to remove these roots from the paddock.

## 5.4 Management of *Pythium* Diseases

Management of *Pythium* species has centred on chemical and biological controls, breeding for resistance and crop rotation. Metalaxyl appears to be the fungicide with the most efficacy, especially for carrots, grains and parsley, but being a phenylamide there are resistance management and biological degradation issues (Davison and McKay 2001, Minchinton *et al*. 2006, 2007 (VG06046)). Biological control of *Pythium* was suggested 40 years ago. Populations of the antagonists, *Penicillium* and *Trichoderma*, were observed to be inversely proportional to populations of pythiums in the soil (Watson 1966). A commercial preparation of *Pythium oligandrum* reportedly had biocontrol efficacy, but its optimum temperature is 15°C, and it was not effective under winter conditions in Australia where soil temperatures were below 10°C (Minchinton *et al*. 2006). It could, however, be effective in summer where soil temperatures are above 15°C. Interestingly, *Bacilllis subtilis* inoculated onto tomato plants induced resistance to *Pythium*, especially under saline conditions (Hanafi *et al*. 2007). Identifying a carrot variety less susceptible to cavity spot was most useful and completely changed the industry standard carrot variety in WA (Davison and McKay 2001). Crop rotation with less susceptible hosts has also proved effective for IPM of cavity spot of carrot and *Pythium* root rots of grains in Australia (Davison and McKay 2001, Lawrence and Harvey 2006).

### 5.4.1 Control with fungicides

Fungicides with efficacy against *Pythium* spp. are listed in Table 4.

*5.4.1.1 Metalaxyl*

Metalaxyl appears to be the fungicide with the most efficacy, especially for carrots, grains and parsley, but being a phenylamide there are resistance management and biological degradation issues (Davison and McKay 2001, Minchinton *et al*. 2006, 2007 (VG06046)). Bailey and Coffey (1985) reported that metalaxyl had a half life of 28 d in sandy soils due to biological degradation. Further, metalaxyl rapidly leaches from sandy soil (Sharom and Edgington 1982) and most of the vegetable Apiaceae production in Victoria is on sandy soils. Metalaxyl appears to be the only consistent effective treatment for root rots associated with *Pythium* spp. and clearly an alternative is desperately required for resistant management strategies. Farrar *et al.* (2002) reported the fungicide mefenozam (metalaxyl-M) was loosing its efficacy to control cavity spot of carrot in California.

*5.4.1.2 Other fungicides*

Apart form metalaxyl, few fungicides have efficacy for *Pythium* root rots. Soil fumigation with methyl bromide and chloropicrin has been used to control root rot associated with *Pythium* spp., *Fusarium* spp. and *Rhizoctonia* on spinach (Sumner *et al.* 1976). In field trials Cassini *et a.l* (1971) reported soil pre-treated with methyl bromide, then direct seeded to parsley, resulted in faster emergence and increased yields.

Phosphonic acid and Tachigaren had no efficacy for control of root rots associated with *Pythium* in parsley field trials (Minchinton *et al.* 2007). In field trials metalaxyl, furalaxyl, metalaxyl/mancozeb, copper, thiram and tachigaren failed to control *P. paroecandrum* in Northern Ireland (McCraken 1984a). The lack of efficacy of metalaxyl is surprising, but Minchinton (pers. comm.) had greater efficacy with the granular formulation applied to soil compared to the formulation applied to foliage. Treatment of parsley with the Apron treatment for control of damping off associated with *Pythium* spp. was ineffective at 5 weeks after emergence (Minchinton *et al.* 2007). Seed treatments are considered to be effective for 4 to 6 weeks only, so it is possible insufficient metalaxyl was coated onto the seed. In Poland Nawrocki and Mazur (2004) tried a B-1-4 D-glucosamine polyer, Rovral (iprodine), Sportak (prochloraz and carbendazim) and Zapraw Funaben (carbendazim and tiuram) for root rot control in parsley with the latter having the most consistent success.

Table 4 List of fungicides with efficacy against *Pythium* spp. (CropLife Australia and A World Compendium of the Pesticide Manual 13. edited CDS Tomlin, British Crop Protection Council)

| Activity Group Code | Activity Group | Chemical Family | Active Constituent | Trade Name | Target Pathogen | Situation |
|---|---|---|---|---|---|---|
| A | Methyl Benzimidazole Carbamates | Benzimidazole | Carbendazim | various | Pythium | Seed treatment, root dip |
| C | DMI | Triazole | Difenoconazole | Dividend | Pythium | Seed treatment of wheat |
| D | Phenylamide | Acylalanine | Benalaxyl | Galben | Pythium, Phytophthora, downy mildews | Foliage (resistance) |
| D | Phenylamide | Acylalanine | Furalaxyl | Fongarid | Pythium, Phytophthora, Oomycetes | Soil & seed treatment |
| D | Phenylamide | Acylalanine | Metalaxyl | Ridomil | Pythium, Peronosporales | Soil & seed treatment |
| D | Phenylamide | Acylalanine | Metalaxyl-M | Apron, Ridomil Gold | permit for Pythium | Foliage, soil & seed |
| K | Quinone outside inhibitors QoI (strobilurins) | Methoxy acrylates | Azoxystrobin | Amistar, Dynasty | Pythium, Phytophthora, Rhizoctonia, downy mildews, | Foliage |
| K | QoI (strobilurins) | Methoxy carbamate | Pyraclostrobin | Cabrio | Pythium, Rhizoctonia, Phytophthora | Foliage |
| X | unspecified | Thiadiazole | Etridiazole | Terrazole | Pythium, Phytophthora | Soil |
| X | Phosphonates | Ethyl phosphonate | Fosetyl-Al, | Aliette | Pythium, Phytophthora, downy mildew | Foliage, roots |
| X | phosphonate | Ethyl phosphonate | phosphorous acid | Foli-R-Fos | Phytophthora, downy mildews | Foliage |
| Y | Multi-site activity | Dithiocarbamate | Thiram | Thiram | Pythium, Fusarium | Seed treatment |
| Y | Carbamate | Carbamate | Propamacarb-hydrochloride | Previcur | Pythium, Phytophthora, aphanomyces, downy mildews | Roots and leaves |
| Y | Multi-site activity | Phthalimide | Captan | Captan | Pythium, Phoma, Rhizoctonia | Seed treatment, root dip |

*5.4.1.3 Biological control*

Biological control of *Pythium* was suggested 40 years ago. Populations of the antagonists, *Penicillium* and *Trichoderma*, were observed to be inversely proportional to populations of Pythium spp. in the soil (Watson 1966). A non-pathogenic *Fusarium oxysporum* strain Fo47 in plate trials on cucumber seedlings in pots showed direct inhibition of *P. ultimum* by antibiosis, mycoparasitism and induced plant defence reactions (Benhamou *et al.* 2002). *Zygorrhynchus moelleri* an antagonist of soil-borne fungi when inoculated into compost reduced the disease severity of root rot caused by *P. paroecandrum* on parsley (Brown 1987). A commercial preparation of *P. oligandrum* reportedly had biocontrol efficacy, but its optimum temperature is 15°C, and it was not effective under winter conditions in Australia where soil temperatures were below 10°C (Minchinton *et al*. 2006). It could, however, be effective in summer where soil temperatures are above 15°C. Interestingly, *Bacilllis subtilis* inoculated onto tomato plants induced resistance to *Pythium*, especially under saline conditions (Hanafi *et al*. 2007).

*5.4.1.4 Cultivar resistance*

Identifying a carrot variety less susceptible to cavity spot was most useful and completely changed the industry standard carrot variety in WA (Davison and McKay 2001).

Flat leaf parsley varieties were more susceptible to rots associated with *Pythium* spp. and *Phytophthora* spp. than curly leaf varieties in Victoria (Minchinton *et al.* 2006). Organically grown curly leaf parsley crops cultivated in Biloela Queensland suffered heavy losses from root rots, but these were reduced by changing to another curly leaf variety (Rob Baddman pers. comm.). Overseas Ciccarese *et al.* (2005) reported curly leaf parsley varieties were very susceptible to unspecified wilts, but Nawrocki (1990) maintained all varieties were susceptible to root rot with the incidence worst in autumn and spring. The actual cause of the root rot is often not stated in reports but despite this there does appear to be tolerance in some parsley varieties to unspecified root rots.

In the UK extensive breeding trials were undertaken to select varieties of parsnip tolerant to canker (Channon *et a.l* 1970, Davis *et al.* 1989). Varieties of parsnip with tolerance to canker are 'yellow', but do not have the market acceptability of the 'white' varieties selected by Australian growers, which are very susceptible to parsnip canker (Minchinton *et al.* 2008).

Where breeding programs have been undertaken they have produced resistance to root rots and often changed the industry standard variety. Breeding programs should be encouraged as (i) tolerant varieties would reduce the reliance on fungicides, which often have long residual period in both crop and soil; and (ii) as reducing fungicide applications would reduce the cost of production; and (iii) they enhance the IPM approach to disease management.

*5.4.1.5 Environmental and cultural factors*

Crop hygiene, crop rotation, planting date and crop density, hilling, tillage, residue management, solarisation and roguing are some cultural practice employed to reduce the impact of root disease.

Cultural control of canker is limited in scope, but the following practices have been investigated and promoted.

- Gradual hilling and covering of parsnip shoulders encourages breakdown of *Itersonilia* ballistospores in soil (Channon 1963b, Smith 1967). This practice led to a 45 % reduction in canker incidence and a 70% reduction in the size of the lesions. Growers in Australia are adamant that this only increases the incidence of *Phoma* canker.
- Sowing and spacing practices were reported to alleviate canker incidence (Channon 1964). There was at least a 3-fold reduction in canker incidence when the crop was late-sown and thinned to 3-cm intervals. However, this was offset by the reduction in root size, with small roots having less canker than larger roots, and there was a balance between a loss in total yield, a reduction in canker incidence and severity, but a consequent increase in marketable roots. There was a 75 % reduction in canker incidence, a 60 % reduction in lesion size, but a large drop in marketable yields (50%), so this method was deemed to be impractical.

- Crop hygiene consisting of removal of all roots and plant trash from beds was suggested by Smith (1967). No hard figures are available for this practice, but it is logical to assume there would be a reduced *Itersonilia* presence in the in the soil, leading to less canker. As carrots are machine harvested very little root trash would be expected to remain in the ground.
- Crop rotation is imperative as *Itersonilia* can survive on parsnip roots after burial for 12 months, but the airborne stage is not viable after 2 days in soil (Smith 1967). Research by McKay and Davison (2000) and Kalu *et al.* (1976) showed that *P. sulcatum* of carrot, unlike many other *Pyhtium* spp. had a host range largely restricted to Apiaceae. Maize, tomato, cabbage, broccoli, oats, barley, rye, wheat, musk melon and cucumber were not hosts of *P. sulcatum* and broccoli in the rotation reduced the incidence of cavity spot on carrot (Davison and McKay (2001). The effect of broccoli in reducing cavity spot may be associated with isothiocyanate production having a biofumigant effect on *P. sulcatum* or its inability to colonize the host (Davison and McKay (2001).
- Solarisation was evaluated for cavity spot of carrot but found to be ineffective for P. sulcatium, but possibly had potential for *P. violae* (Davison and McKay (2001). The logistics of the technique are unsuitable for cropping regions where continuous production is the normal practice, such as the Victorian market gardens situated around Melbourne.

*5.4.1.6 Crop rotation*

Crop rotation with less susceptible hosts has also proved effective for IPM of cavity spot of carrot and *Pythium* root rots of grains in Australia (Davison and McKay 2001, Lawrence and Harvey 2006). McCraken 1981 reported rotation with barley was effective to control *P. poroecandrum* in parsley. Control was associated with the liming of the previous barley crop which increased pH prior to sowning. Elsewhere, an unspecified root rot of parsley was highest when grown after potatoes but lowest when grown after grass or beans (Nawrocki 1999). As potatoes are very susceptible to *Rhizoctonia*, this report may well not be associated with *Pythium* root rot.

## 5.5 *Pythium* in hydroponics

*Pythium* spp. have a dramatic impact on hydroponics crop yields (Sutton *et al.* 2006, Wulff *et al.* 1998). Hydroponics is a significant and growing industry and its protected cropping system enables greater control of the environment, creating more options for disease management.

*Pythium* spp. cause major crop losses in hydroponic vegetable production. Some of the crops affected are cucumber, tomato, sweet pepper, eggplant, capsicum, lettuce, parsley and coriander (Sutton *et al.* 2006). Crop losses can be as high as 30% and cost $15,000/ha to control (Porter *et al.* 2007, Tesoriero pers. comm.).

Hydroponic production of coriander and parsley is increasing in the Sydney basin and elsewhere along with a number of other leafy vegetables and herbs (Porter *et al.* 2007). Root browning and rots result in reduced yield losses, downgrade in product quality, and increased labour costs to grade out affected plant material. There have been no formal studies of this particular problem other than preliminary diagnostics that has shown an association with *Pythium* species. Related work has determined similar disease problems, particularly during warmer months, in hydroponic lettuce (Tesoriero *et al.* 2007). The project VG04012 (Improved management for root disease of hydroponic lettuce) has determined the causal species of *Pythium* and *Phytophthora* and has developed biological and cultural control strategies for their management, with temperature management showing much promise. These strategies are important because there is no legal use of pesticides worldwide for control of root diseases in crops growing by the nutrient film technique.

## 5.6 Modelling of *Pythium* root rots

Diseases caused by soil borne diseases are notoriously difficult to model, mainly because we know very little about ecology of the pathogen and critical interactions between pathogen, host and environment. UK researchers developed an adequate, but not completely satisfactory model to explain the field variability. Factors that influenced existing large populations in autumn, soil moisture content but surprisingly not soil temperature (Ali-Shtayeh 1986). Conversely, in hydroponic systems, temperature around the root zone determined susceptibility or tolerance to *P. aphanidermatum.* Based on this a hypothetical model was developed, but it did not stand up at high temperatures (Klaring *et al.* 2001). Another theoretical infection model was developed for epidemics of soil-borne plant disease using *Pythium* as an example, which allowed for primary and secondary infection events (Brassett and Gilligan 1988). The model was primarily concerned with concentration of inoculum rather than environmental parameters promoting epidemics. Davis (2007) in California is developing a predictive model for *Pythium* diseases of vegetable crops, but is in the early stages and appears to be aimed at identifying threshold levels of inoculum.

Although there have been a number of theoretical models developed for *Pythium* infections, a comprehensive growth chamber study of all potential parameters such as soil temperature, soil moisture, soil composition, root growth and infection cycles is required for a more practical model.

## 5.7 Working with *Pythium* and other soil-borne fungi

Soil-borne pathogens are notoriously difficult to work with because of the complexity of the soil environment and the lack of effective tools to detect and quantify the organisms suspected of causing a disease.

### 5.7.1 Timing of sampling and isolation techniques for potential causal organisms of root rots on Apiaceae

*Pythium* and *Phytophthora* spp. were indicated as primary cause of parsley and parsnip root rots in Australia (Minchinton *et al.* 2006, 2008). Various species, which belong to these two genera, have been isolated from symptomatic parsley and parsnip roots, but the accurate identification of a primary causal pathogen is only possible when isolations are conducted from both symptomatic and non-symptomatic root sections (eg. collar, tap and lateral roots) over the entire cropping season. The season in which the crop is grown (summer or winter), the soil physical, chemical and biological properties, soil moisture and temperature can all influence the growth, sporulation, infection and its ability to compete with other micro-organisms in the soil and on the root rhisosphere. For example, the results of serological testing for *Pythium* spp. in the UK were only useful when samples were collected between October to March (Petch 1999). When using using a specific DNA primer to detect *Pythium sulcatum* (cavity spot) in soil (PH Wang, reported by Hiltunen and White 2002), best detection levels were achieved in early spring with a combination of high soil moisture and rising soil temperature. At this time the fungus would be growing actively and saprophytically through soil.

It is generally accepted that *Pythium* spp., once isolated from a lesion onto non-selective media grow faster than *Phytophtora* spp. (Davison and Pascoe, 2005). However, some *Pythium* spp., e.g *Pythium violae* and *Pythium sulcatum*, identified as causal agents of cavity spot on carrot in the UK, were slow growing and needed to be isolated from first formed lesions (Hiltunen and White 2002). Faster growing species such as *Pythium intermedium*, *Pythium irregulare* and *Pythium sylvaticum* were isolated from older lesions on the same host and were also found on non-symptomatic roots (White 1998). While isolating and identifying species of both genera from old lesions, special precautions need to be taken in final conclusion. Some *Phytophthora* spp. are difficult to culture from old lesions as those can be invaded by secondary pathogens. The timing of collection of plant material isolations from both symptomatic and non-symptomatic root sections are therefore critical for identification of species involved in the primary infection.

## 5.7.2 Isolation methods

### *5.7.2.1 Direct plating*

*Pythium* and *Phytophthora* spp. can be isolated by plating either surface-sterilised or non-surface-sterilised plant material sections onto non-selective medium. Sodium hypochlorite solution (1% of active chlorine) is usually used for surface- sterilisation. Some saprophytic species, however, can be destroyed in this process, therefore rinsing in sterile distilled water is often recommended instead. Corn meal agar, V8 or simply WA can be used for isolation of these species. Bacterial growth can be controlled by amending the medium with antibiotics.

### *5.7.2.2 Baiting*

Live trap plant material is being used to bait the organism from soils or from a root sample. The choice of trap material depends on target species, fruit, lupin roots, seedlings or leaf discs are commonly used for that purpose.

### *5.7.2.3 Production of sporangia and zoospore release*

Determination of morphological characters such as the size and shape of sporangia, oogonia and antheridia are necessary to identify *Pythium* and *Phytophthora* species. Some species of both genera form sporangia on the host or agar plates, others require incubation of host material or small agar plugs (cores) in water, pond water or non-sterile soil extract (NSSE). Homothallic species produce gametangia in tissues of invaded host and in culture on agar plates. Heterothallic species require both mating types to produce gametangia.

## 5.7.3 Molecular methods for identification of *Pythium and Phytophthora* spp

Molecular methods of detection for these pathogens would serve two purposes, firstly to help characterise and identify some species, and secondly to be able to quantify soil populations to determine disease risk and conduct ecological studies.

Some species of these genera especially members of *Pythium* genus could not be properly identified using morphological and physiological characteristics due to high variability within these characters or lack of informative morphological structures (Matsumoto *et al.* 1999). A variety of biochemical and molecular methods had been developed for simplification of species identification in both genera (Martin 2000 and therein, Martin and Tooley 2003 and therein). Phylogenetic studies based on sequence analyses of large and small subunit of ribosomal RNA genes, mitochondrial DNA and internal transcribed spacer (ITS) regions of rRNA genes allowed identification of many undescribed *Pythium* and *Phytophthora* species and clarification of their taxonomic position. Several DNA based methods were developed for diagnosis of *Pythium* spp, eg. PCR-RLFP, reverse blot hybridisation, AFLP fingerprinting (Wang and White 1997, Lévesque *et al.* 1998, Garzón *et al.* 2005, Lievens 2005) and for *Phytophthora* spp. (Cooke *et al*. 2000, Martin and Tooley 2003).

### *5.7.3.1 Primers for quantification of Pythium spp. associated with carrot cavity spot (CCS)*

Hiltunen and White (2002) review the development of DNA primers for the detection of *Pythium* spp. PH Wang (reported by Hiltunen and White 2002) has generated a specific primer for the carrot cavity spot pathogen *Pythium sulcatum*. This primer had not been used for detection work in soil at the time of this report. However, Hiltunen and White (2002) considered that for the test to be useful it would be essential to derive a debris-free extract from the soil (White *et al.* 1996b). High soil inoculum levels for *P. violae* have been estimated to be in the order of one propagule per 30 g of soil (Phelps *et al.* 1991*)*, which was considered by Hiltunen and White (2002) to be an unrealistic target for any test. However, they report best detection levels in early spring with the combination of high soil moistures and rising temperatures, which is when the fungus is growing actively and saprophytically through the soil.

# 6 Other root pathogens of the Apiaceae

Table 5 lists other root pathogens of the Apiaceae. Several of them such as white mold (*S. sclerotiorum*) and damping off are ubiquitous to many plant families, where they are associated with similar symptoms. Some diseases are associated with several genera within the Apiaceae such as canker (*I. perplexans*) and *Phoma* crown and root rot (*P. complanata*). Many of the root diseases have only been reported on one genera of the Apiaceae, but it is possible with thorough investigations they may occur on others.

## Table 5 List of root rot pathogens on Apiaceae

| Host | Disease | Symptoms | Pathogen |
|---|---|---|---|
| Parsnip | Bacterial blight | Blight of petioles associated with browning of vascular tissue of crown & root | *Pseudomonas marginalis* |
| Carrot & parsnip | Crown gall | Raised corky lesions on roots, horizontally orientated | *Agrobacterium tumefaciens* |
| Apiaceae | Soft rot | Dirt clings to root, internal soft rot of roots from tip to crown | *Erwinia carotovora, E. chrysanthemi* |
| Carrot, celery, celeriac, | Black rot | Black crown rot, root decay, seedlings, | *Alternaria dauci* |
| Carrot | Phoma rot | Dry brown root rot | *Phoma rostrupii – Leptosphaeria libanotis* |
| Carrot | Cavity spot | Sunken elliptical lesions, ruptured periderm & dark elongate lesions | *Pythium violae, P. sulcatum* |
| Parsnip | Horizontal spot | Sunken elliptical lesions, ruptured periderm & dark elongate lesions | *Pythium violae, P. sulcatum* |
| Carrot, | Cottony rot | Water-soaked, soft lesions, covered with white fluffy mycelial mats & laced with sclerotia | *Sclerotinia sclerotiorum* |
| Celery | Pink rot | Damping off of seedlings, basal stalk rot with pink or brown margins containing sclerotia | *Sclerotinia sclerotiorum* |
| Caraway, chervil, dill, parsley, parsnip | White mold | Water-soaked, soft lesions, covered with white fluffy mycelial mats & laced with sclerotia | *Sclerotinia sclerotiorum* |
| Carrot | Crown rot | Senescence & death of foliage, dry dark brown sunken lesions on crown of root | *Rhizoctonia solani* |
| All Apiaceae | Damping off | Seed decay, pre & post emergence | *Pythium spp., Rhizoctonia* |
| Carrot | Fusarium dry rot | Dry, brown leather lesions on taproot occurring in the field or storage | *Fusarium solani etc* |
| Celery | Fusarium yellows | Stunting & yellowing, vascular tissue in roots and crown is brown | *Fusarium oxysporum* |
| Parsnip, carrot, coriander & parsley | Canker | On roots reddish brown to black cankers, on leaves small brown necrotic lesions surrounded by a green halo, greyish lesions on petiole bases, inflorescences may rot, also seed-borne. | *Itersonilia perplexans* |
| Parsnip | Phoma canker | Leaf spotting, blight and cankers on petioles and roots | *Phoma complanata* |
| Celery, celeriac, carrot, caraway, parsley, parsnip | Phoma crown & root rot | Light brown lesions on roots & crowns, darken & spread to petiole bases causing withering & stunting and in severe cases death of plant | *Phoma apiicola* |
| Carrot & other Apiaceae | Phymatotrichopsis root rot | Wilt & plant death, soil clings to roots, knobbly fungal structures & mycelia cling to the root | *Phymatotrichopsis omnivora* |
| Carrot | Phytophthora root rot | Dark brown to black rubbery lesions on roots leading ot a watery soft rot. | *Phytophthora cactorum, P. cryptogea, P. megasperma, P. porri, Phytophthora sp.* |
| Parsley | Phytophthora root rot | Wilt, stunting & yellowing of foliage, light to dark brown primary roots and lateral roots are sparse or absent. | *Phytophthora cryptogea, P. parasitica* |
| Apiaceae | Pythium root rot | Stunting & chlorosis, dark feeder roots and plant death, especially young plants | *Pythium spp. P. debaryanum. P. irregulare, P. mastophorum, P. paroecandrum, p. ultmum* |
| Carrot | Root dieback | Stubbing & forking of tap root | *Pythium spp. P. irregulare, P. ultimum. P. sylvaticum, P. sulcatum, Rhizoctonia solani* |
| Carrot | Violet root rot | Patches of dying or dead plants, dark purple brown lesions on roots, dense pink to brown mycelial mat around the crown at soil level and above | *Rhizoctonia crocorum* |

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# 8 Appendices

## Appendix 1 Summaries of field trials for root rot control in parsley (Minchinton *et al.* 2006)

| Host | Date | Chemical treatment | Disease (%) | Efficacy | Comments |
|---|---|---|---|---|---|
| Parsley-Cochrane Autumn - Winter | 2005 | Metalaxyl (seed) – Metalxyl (wk 11) | 13% loss<br>87% control | Excellent | Controls both *Pythium* & *Phytophthora* |
| | | Metalaxyl (seed) – phosphonic acid (wk 6, 8, 10, -18) | 2% loss<br>98% control | Excellent | Controls both *Pythium* & *Phytophthora* |
| | | Iprodione (wk 2, 7, 11) – Fludiozonil+cyprodinil (wk 5, 9, 13) | 94 % loss<br>6% control | Disaster | Could be phytotoxic, not a *Fusarium* & *Rhizoctonia* issue |
| | | *Trichoderma* (wk 0, 6, 10, 14, 18) | 86 % loss<br>14% control | None | Biological control ex NZ. |
| | | Calcium cyanamide (wk -2) | 83 % loss<br>17% control | None | Fumigant, oomycetes re-entering from irrigation water? |
| | | Control - unsprayed | 75% loss<br>25% control | | Heaps of disease in this trial. Note the season |
| Parsley – Lamattina Spring - Summer | 2005 | Azoxystrobin+metalaxyl+fludioxonil (seed trt) | 5.5 % loss | None | Not expect seed treatment to last until harvest. No early assessment due to little disease so no data on efficacy of trt for damping off at 6 weeks. No good long term. |
| | | Metalaxyl (seed, wk 8) | 0.9% loss | Excellent | But grower would probably not spray unless >6% loss |
| | | Phosphonica acid (seed, wks 2,4, 6, 8, 10) | 5% loss | None | Not controlling disease. Effective in late stage see above, but not throughout, suspect not working in early stage & losses showing up in late stage. Not controlling Pythium |
| | | Hymexazol (seed, wks 2,4, 6, 8, 10) | 5 % loss | None | Used to control *Phytophthora* and *Aphanomyces* in hydroponics & QLD had some efficacy with root rot of red beet, but needs lots of water for application. Will not be registered in Australia. |
| | | Control - unsprayed | 5 % loss | . | Little disease in this trial. Note the season If losses > 6% grower would spray. |

## Appendix 2 Summaries of field trials for root rot control in parsley and coriander (Minchinton *et al.* 2006, 2007)

| Host | Date | Chemical treatment | Disease (%) | Efficacy | Comments |
|---|---|---|---|---|---|
| Corriander – Lamattina Summer | 2005 | Metalaxyl (seed) | 11% with root rot | Good. | Sig diff to control. Controls both *Pythium* & *Phytophthora*. No observable above ground losses, but symptoms on roots. |
| | | Carbendazim | 22% with root rot | None | Controls a broad range of diseases including Fusarium. |
| | | Boscalid | 19% with root rot | None | Controls a wide range of foliage disease including Rhizoctonia, *Phytophthora, Pythium* etc |
| | | Azoxystrobin+metalaxyl+fludioxonil (seed trt) | 17% l with root rot | Good. | Seed trt expected to last for 4-6 wks, |
| | | Control - untreated | 27 % with root rot | | No observable above ground losses, but symptoms on roots. Symptoms neck rot. Could be early stages of *Pythium*. |
| Parsley – Cochrane Spring - Summer | 2006/7 | Metalaxyl (seed) + phosphonic acid (wk 8 to12) | 56% root rot | None | Symptoms not consistent with oomycetes. *Fusarium* or salinity issue – red roots. |
| | | Polyversum (*P. oligandrum*) (wk -2 to wk 13) | 63 % root rot | None | Imported product. Does not work below 15 °C. May not have been a *Pythium* issue. |
| | | Polyversum (*P. oligandrum*) (wk -2 to wk 8), Phosphonic acid (wk 9 to 13) | 65% root rot | None | Imported product. Does not work below 15 °C. May not have been a *Pythium* issue. |
| | | Control - unsprayed | 63% root rot | | Symptoms not consistent with oomycetes. *Fusarium* or salinity issue – red roots. Fusarium isolated. No above ground symptoms. Note the season. |
| Parsley – Cochrane Autumn - Winter | 2007 | Metalaxyl (seed) + phosphonic acid (wk 8 to13) | 16% root rot | None | Lowest level of root rot & symptoms typical of oomycetes. No significant efficacy a worry. |
| | | Polyversum (*P. oligandrum*) (wk -2 to wk 8, wk 10 & 12) | 39 % root rot | None | Imported product. Does not work below 15 °C. May be to cold. Soil temp ≤ 10° C. |
| | | Polyversum *P. oligandrum* (wk -1 to wk 8) Phosphonic acid (wk 9 to 13) | 36 % root rot | None | Imported product. Does not work below 15 °C. May be to cold. Soil temp ≤ 10° C. |
| | | Control - unsprayed | 27 % root rot | | Symptoms typical of oomycetes. |

## Appendix 3 Summaries of field trials for root rot control in parsnip (Minchinton *et al.* 2008)

| Host | Date | Chemical treatment | Disease (%) | Efficacy | Comments |
|---|---|---|---|---|---|
| Parsnip<br>Kelly Cranbourne<br>Spring -Summer | 2006 | Bavistin (carbendazim) (wk 10, 14, 19) | 0 | None | Targets *Fusarium, Sclerotinia, Rhizoctonia* |
| | | Ridomil G25 (metalaxyl (wk 10, 19) | 0 | None | Targets oomycetes |
| | | Rizolex (tolclocfos-methyl) (wk 10) | 0 | None | Targets soilborne diseases *Rhizoctonis, Sclerotium* |
| | | Control - unsprayed | 0 | None | No symptoms – summer, canker not an issue |
| Parsnip<br>Lamattina Rosebud<br>Spring - Summer | 2006 | Ridomil G25 (metalaxyl) (wk 8, 10, 19) | 0.3 | None | Targets oomycetes. Few symptoms. Canker not an issue in summer. |
| | | Rizolex (tolclocfos-methyl) (wk 10) | 0.72 | None | Targets soilborne diseases *Rhizoctonis, Sclerotium* . Few symptoms. Canker not an issue in summer. |
| | | Control - unsprayed | 1.0 | None | Few symptoms in trial. Note the season. No canker |
| Parsnip<br>Schreurs Devon Meadows<br>Spring - Summer | 2006 | Ridomil G25 (metalaxyl) (wk 2, 11) | 0.5 | None | Targets oomycetes. Few symptoms. Canker not an issue in summer. |
| | | Rizolex (tolclocfos-methyl) (wk 2) | 1.9 | None | Targets soilborne diseases *Rhizoctonis, Sclerotium* . Few symptoms. Canker not an issue in summer. |
| | | Control - unsprayed | 0.9 | None | Few symptoms in trial. Note the season. No canker. |
| Parsnip<br>Schreurs Devon Meadows<br>Autumn - Winter | 2007 | Bavistin (carbendazim)<br>(wk 1, 6, 10, 15) | 14% | None | Targets *Fusarium, Sclerotinia, Rhizoctonia.* Canker not associated with these diseases. |
| | | Ridomil G25 (metalaxyl) (wk 1, 6) | 8.4 % | None | Targets oomycetes. More reps would probably give a significant difference |
| | | Rizolex (tolclocfos-methyl) (wk 1) | 17% | None | Targets soilborne diseases *Rhizoctonis, Sclerotium* . Few symptoms. Canker not associated with these diseases. |
| | | Control - unsprayed | 13 % | None | |
| Parsnip<br>Lamattina Rosebud<br>Autumn - Winter | 2007 | Bavistin (carbendazim)<br>(wk 1, 6, 10, 14) | 12 % | None | Targets *Fusarium, Sclerotinia, Rhizoctonia.* Canker not associated with these diseases. |
| | | Ridomil G25 (metalaxyl) (wk 1, 6) | 3 % | Excellent | Targets oomycetes. Oomycetes an issue |
| | | Rizolex (tolclocfos-methyl) (wk 1) | 12 % | None | Targets soilborne diseases *Rhizoctonis, Sclerotium* . Few symptoms. Canker not associated with these diseases. |
| | | Control - unsprayed | 16 % | | Fumigates so oomycetes return in irrigation water? |
| Parsnip<br>Kelly Cranbourne W<br>Autumn - Winter | 2007 | Bavistin (carbendazim)<br>(wk 1, 5, 10, 14) | 31 % | None | Targets *Fusarium, Sclerotinia, Rhizoctonia.* Canker not associated with these diseases. |
| | | Ridomil G25 (metalaxyl) (wk 1, 56) | 30 % | None | Targets oomycetes. Canker not associated with oomyvetes on this site. |
| | | Rizolex (tolclocfos-methyl) (wk 1) | 31 % | None | Targets soilborne diseases *Rhizoctonis, Sclerotium* . Few symptoms. Canker not associated with these diseases. |
| | | Control - unsprayed | 35 % | | |
| Parsnip<br>Lamattina Rosebud<br>Autumn – Spring | 2007 | MicroPlus (*Streptomyces lydicus*)<br>(wk 1, 5, 9, 14, 18) | 9.1 | None | Biological control. |
| | | Polyversum (*P. oligandrum*)<br>(wk 1, 5, 9, 14, 18) | 9.6 | None | Biological control. Imported product. Does not work below 15 °C. May be to cold. Soil temp ≤ 10° C. |
| | | Control - unsprayed | 10 % | | |

# Chapter 2

# Experiments assessing biological and biorational controls for Pythium root rot of hydroponic coriander

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## Summary

This chapter provides the first report of *P. sulcatum* as a pathogen of coriander grown in recirculated hydroponic systems in Australia. In a series of four trials conducted on coriander, none of the microbial biocontrols or biocontrol agents controlled symptoms of root rot sufficiently to give fresh weights similar to that of *Pythium* free plants. It is possible the concentration *P. sulcatum* may have been too high, despite halving and quartering the concentration in successive trials. Nevertheless Fulzyme®Plus™ (*Bacillus subtilis*) and *P. oligandrum* significantly stimulated plant growth in trial 2, but this could not be replicated. Additionally Bion® + Silicon, Fulzyme®Plus™ + Phoscare®; Fulzyme ®Plus + Bion® + Phoscare® or Phoscare® significantly enhanced plant growth, unrelated to disease control in trials 3 and 4.

## 2.1 Introduction

Hydroponic coriander production has recently expanded across Australia. Compared with soil-grown production, coriander in hydroponics has several distinct advantages in growing coriander in hydroponics. Plants sit in channels on raised benches with their roots suspended in nutrients that are recirculated via a reservoir. This system has the potential to double growth rates while significantly reducing water and fertiliser consumption. Hydroponic production systems have superior ergonomics to soil production, reducing labour costs. Recirculation of nutrients also decreases environmental contamination. Furthermore, hydroponics greatly reduces risks of faecal bacteria contamination of food compared to soil-grown crops using organic manures.

Root rots are a major limitation to the commercial success in production of coriander. They are often caused by particular *Pythium* species. They severely limit yields in both soil and hydroponics (Tesoriero *et al.* 2007) and can lead to complete crop failures. There are no chemicals registered for the control of these root rots in Australia. This is mostly due to the high risk of conventional pesticide residues accumulating in leaves.

Root rots are particularly problematic in recirculated nutrient systems (Gold and Stanghellini, 1985; Menzies *et al.* 1998), particularly where farm and crop hygiene practices are poor or lacking (Jarvis 1992). Further factors of soilless production systems can make them conducive to disease development once plant pathogens enter. They include: elevated nutrient temperatures in summer (Tesoriero and Cresswell 1995; Alhussaen, 2006); stagnant water sitting around roots where there is poor drainage; and accumulation of excess mineral salts (Jarvis 1992). Furthermore, low microbial populations and diversity found in soilless substrates and nutrient solutions are sometimes described as a 'biological vacuum' and one similar in this respect to fumigated soil. This is often associated with a lack of antagonistic microbes that buffer the rhizosphere from plant pathogens (Postma *et al.* 2000). Soilless systems also favour the formation and dispersal of zoospores produced by several *Pythium* species (Stanghellini and Rasmussen 1994).

Microbial biocontrols or biocontrol agents (BCAs) offer an alternative to conventional chemicals and hydroponic production systems provide excellent models to demonstrate their suppression of root rot diseases. BCA products consist of formulated fungal or bacterial inocula. Several products are

commercially available in Australian. However, to date they have only been objectively assessed for Australian hydroponics in leafy lettuce production (Tesoriero *et al.* 2008). In that study, one commercial product containing a strain of the bacterium *Bacillus subtilis* consistently suppressed root disease expression to a level equivalent to uninfected control treatments. It appeared to reduce the colonisation of roots by the pathogen, *Phytophthora cryptogea*. In some trials it stimulated plant growth even in the absence of the pathogen. Mycoparasitic strains of *Pythium oligandrum* have also been used as a BCA for root diseases (He *et al*. 1992). Similarly, certain biorational chemicals such as salicylic acid derivatives and phosphorous acid have been shown to suppress disease development by activating the plant's defences (Doares *et al*. 1995).

The role of silicon as a potential supplement in crop production has in the last twenty years been the focus of a large amount of research in plant biology (Epstein 1999). There is substantial evidence that silicon affects plant development, increasing plant growth and yield in many species and that silicon can modulate plant resistant reactions to multiple pathogens (Epstein 1999, Ma 2004). Cherif *et al.* (1994) demonstrated that silicon application onto cucumber plants resulted in potentiation of chitinases, peroxidases and polyphenol oxidases when plants were later treated with Pythium spp. Recent research has suggested that silicon modulates a type of induced systemic resistance in wheat (Remus-Borel *et al.* 2005), rice (Rodrigues *et al.* 2004) and pea (Dann and Muir 2002), resulting in enhanced production of phytoalexins and pathogenesis-related (PR) proteins.

The purpose of this study is to investigate the potential of certain BCAs and chemicals that stimulate the plants' defences to safely manage *Pythium* root rot diseases in hydroponics.

## 2.2 Materials & Methods

Four trials were established between 2009 and 2010 to determine the relative pathogenicity of *Pythium* isolates and subsequent efficacy of BCAs and biorational chemicals. The experimental facility was a scaled-down version of commercial systems, consisting of 50 independent units, each with a 20L tank, submersible pump and plastic poly-pipe feeding nutrients to the top end of a sloping 3-metre length of PVC channel. Nutrients drained by gravity past suspended plant roots and back into each tank. Trials were designed as replicated blocks with each treatment randomised and consisting of continuously recirculated nutrients supplying 12 plants spaced along each NFT channel. Each treatment was replicated 4-10 times. An overview of the four experiments conducted over the project period is listed in Table 2.1.

**Table 2.1. Pathogenicity and efficacy trials conducted on hydroponic coriander**

| Trial Number | Treatments |
|---|---|
| 1 | Pathogenicity of *Pythium* & *Phytophthora* isolates to coriander |
| 2 | Efficacy of BCAs (*Bacillus subtilis* [Fulzyme®Plus @2mL/L] and *Pythium oligandrum* [isolate 05/590]) and the plant defence activator acibenzolar-S-methyl (Bion®) @2.5µL/L on Pythium root rot (*P. sulcatum* [isolate 03/822]) of coriander. |
| 3 | Efficacy of BCA (*Bacillus subtilis* [Fulzyme®Plus @2mL/L]) plus phosphorous acid Phoscare® @2mL/L, and the plant defence activator acibenzolar-S-methyl (Bion®) @2.5µL/L plus potassium silicate @650µL/L on *Pythium* root rot (*P. sulcatum* [isolate 03/822]) of coriander. |
| 4 | Efficacy of BCA, *Bacillus subtilis* [Fulzyme®PlusTM] +/- phosphorous acid [Phoscare®] @ (i) 2mL/L and (ii) 0.2mL/L on *Pythium* root rot (*P. sulcatum* [isolate 03/822]) of coriander. The lower product concentrations were applied weekly. |

*Pythium* isolates were initially obtained from diseased plant samples collected during surveys of commercial production. Roots were washed and plated to semi-selective agar media (potato carrot agar [PCA] amended with pimaricin [@5ppm] and rifampicin [@10ppm]). Plates were incubated at 25ºC and examined over a 7-day period for mycelial growth. Light microscopy (x100-200) was used to locate growth on agar plates that was then sub-cultured to PCA. Cultural and morphological features on agar media were initially used to identify taxa to genus level. Further morphological and molecular characterisation of selected isolates was used to confirm and distinguish taxa with similar morphologies. The key of Plaats-Niterink (1981) was used to initially identify species of *Pythium.* Molecular characterisation was performed by PCR amplification of the internal transcribed spacer (ITS) region of ribosomal RNA genes using primers ITS1 and ITS4, as described by Levesque and de Cock (2004). Sequences of ITS regions were compared with GenBank databases and similarity analyses were used to place isolates into discrete taxa. *Phytophthora cryptogea* isolates were obtained in a previous study of leafy lettuce growing by hydroponics (Tesoriero *et al.* 2007) and were used in the first experiment to compare relative pathogenicity with *Pythium* isolates.

Seedlings were grown at a commercial nursery in plugs (105/tray) that were transplanted to NFT channels and allowed to establish prior to application of treatments. Seedlings in each plug were thinned to 10 per plug. Samples were taken from 3 plugs in each tray and roots were screened for background or confounding plant pathogens by plating 1 cm lengths to PCA.

Inocula for hydroponic trials were prepared from cultures grown on PCA at 25°C for 10-14 days. Cultures were then homogenised in distilled water and an equivalent of 1-2 plates were added to specified treatment tanks. Serial dilution of the inoculum suspensions and culturing to agar media was used to estimate pathogen concentrations using the most probable number technique (Cochran 1950, Tesoriero 1989). Non-colonised PCA plates where homogenised as negative control treatments. Inoculum rates were halved between experiments 2 and 3 in an effort to reduce disease pressure that may have masked treatment effects in the former trial.

Tanks were topped up with fresh nutrient as required. The plants were grown to maturity (5-6 weeks) and harvested by cutting off leafy tops with scissors. They were then weighed to obtain fresh weights.

Statistical analyses of data were undertaken using Asreml-R and are detailed for each trial in the results section. Where the F-statistic was significant, pair wise differences significant at the 5% level are indicated in the results using the superscript letter-based representation, where non-significant treatments have a common letter.

Roots samples were cultured as described above at the conclusion of trials to determine pathogen colonisation.

## 2.3 Results and Discussion

### 2.3.1 Trial 1

Mean fresh weights of 6 plugs of coriander cv. 'Santo' are presented in Table 2.2. The average coriander fresh weight was significantly lower for *Pythium sulcatum* isolate 03/822 than for the remaining treatments.

**Table 2.2. Mean fresh weights of coriander cv. Santo for pathogen treatments in Trial 1**

| Treatment (isolate #) | Fresh weight (g) |
|---|---|
| Nil | 781.5[b] |
| *Phytophthora cryptogea* (08/174-1) | 795.3 [b] |
| *Phytophthora cryptogea* (08/581) | 792.0 [b] |
| *Pythium coloratum* (07/1122) | 760.9 [b] |
| *Pythium aphanidermatum* (09/89) | 779.3 [b] |
| *Pythium sulcatum* (03/822) | 623.0[a] |
| *Pythium ultimum* (04/710) | 786.3 [b] |
| *Pythium coloratum* (07/1042-3) | 780.3 [b] |
| Average LSD (5%) | 72.09 |
| F-statistic | 4e-04 |

Numbers followed by a different letter differ significantly.

### 2.3.2. Trial 2

Mean fresh weights of 12 plugs of coriander cv. 'Santo' are presented in Table 2.3. *Pythium sulcatum* isolate 03/822 significantly reduced fresh weights compared with nil pathogen treatments. However the analysis showed that there was no significant interaction between the pathogen and biological treatments; that is the biological/chemical controls did not reduce disease expression in the presence of the pathogen. When the data was analysed separately for biological/chemical treatments and pathogen, there was a significant growth stimulation effect by *Bacillus subtilis* (Fulzyme®Plus), and *P. oligandrum* treatment compared with Bion® and Nil (Table 2.4).

**Table 2.3. Mean fresh weights (g) for coriander cv. '*Santo'* in Trial 2**

| Treatment | Fresh weight (g) | Transformed fresh weights ($x^{0.8}$) |
|---|---|---|
| Nil | 867.1 | 1.6966 |
| *P. sulcatum* | 12.0 | 1.1913 |
| Standard error | | 0.0177 |

**Table 2.4. Mean fresh weights (g) for coriander cv. 'Santo' in Trial 2**

| Treatment | Fresh weight (g) | Transformed fresh weights ($x^{0.8}$) |
|---|---|---|
| Nil | 390.0 | 1.3947[a] |
| Bion® | 277.1 | 1.3961[a] |
| Fulzyme®Plus | 628.0 | 1.5097[b] |
| *P. oligandrum* | 463.2 | 1.4753[b] |
| LSD (5%) | | 0.0576 |

Numbers followed by a different letter differ significantly.

### 2.3.3 Trial 3

Mean fresh weights of 12 plugs of coriander cv. 'Santo' are presented in Table 2.5. An analysis was performed in ASReml and a square root transformation was required to stabilise the residuals. Overall, Bion® + Silicon reduced yield in the absence of the pathogen, but increased it with the pathogen. This suggests phytotoxicity at this application rate but also some disease suppression. Future trials could explore lower concentrations of Bion®. In contrast, the *Bacillus subtilis* (Fulzyme®Plus) plus Phosphorous acid (Phoscare®) treatment had no effect on fresh weights in the absence of the pathogen and significantly increased it with the pathogen (Table 2.5). Observations made during the trial were that this treatment suppressed disease symptoms up until a fortnight prior to harvest. Plants appeared to have similar growth rates to those of the uninfected control treatments with white healthy roots. After that time roots began to progressively display typical brown root rot symptoms and older leaves on many plants began to yellow and wilt. A second application of these products may therefore be useful for sustained disease suppression and would be worthy of future experiments.

**Table 2.5. Mean fresh weights (g) for coriander cv. *Santo* in Trial 3**

| Pathogen | Treatment | Fresh weight (g) | Transformed fresh weight (√) |
|---|---|---|---|
| Nil | Nil | 713.1 | 27.23[a] |
| | Bion® + Silicon | 556.5 | 23.58[b] |
| | Fulzyme® Plus + Phoscare® | 772.8 | 27.79[a] |
| | Bion® + Silicon + Fulzyme®Plus + Phoscare® | 480.7 | 21.89[c] |
| *P. sulcatum* | Nil | 73.1 | 8.53[f] |
| | Bion® + Silicon | 121.7 | 10.89[e] |
| | Fulzyme®Plus + Phoscare® | 220.3 | 14.77[d] |
| | Bion® + Silicon + Fulzyme®Plus + Phoscare® | 224.4 | 14.97[d] |
| lsd (5%) | | | 1.46 |

Numbers followed by a different letter differ significantly.

### 2.3.4 Trial 4

Mean fresh weights of 12 plugs of coriander cv. Santo are presented in Table 2.6. None of the microbial or chemical treatments significantly suppressed *Pythium* root rot at either concentration or application time. Disease severity was again very high with no marketable quality coriander produced when *Pythium* was present.

**Table 2.6. Mean fresh weights (g) for coriander cv. Santo in Trial 4**

| Treatment | Mean fresh weight (g) | Standard Error |
|---|---|---|
| Nil | 3138[a] | 101 |
| *Pythium* | 355[c] | 43 |
| Nil + *Bacillus* | 3219[a] | 68 |
| *Pythium* + *Bacillus* | 316 [c] | 41 |
| *Pythium* + *Bacillus* + Phoscare® | 421 [c] | 32 |
| *Pythium* + Phoscare® | 495 [b] | 16 |
| *Pythium* + Bacillus (wk) | 371 [c] | 44 |
| *Pythium* + *Bacillus* + Phoscare® (wk) | 347 [c] | 31 |

Numbers followed by a different letter differ significantly.

## 2.4 Conclusions & Recommendations

*Pythium sulcatum* was confirmed as a significant cause of root rot in coriander growing in recirculated hydroponic systems. This disease has not been previously reported on coriander in Australia. *P. sulcatum* has been recorded as causing cavity spot and root rots of carrots. Isolates of other *Pythium* species were not shown to be pathogenic in a single trial in this study. However, they may still be important pathogens under different production or environmental conditions. Other isolates should be tested in further pathogenicity tests, conducted under varying environmental conditions.

None of the BCAs or biorational chemicals was effective in these preliminary experiments. Their efficacy needs to be assessed at different concentrations and combinations to those tested in this study. In one trial, a single treatment of *Bacillus subtilis* and phosphorous acid significantly suppressed disease symptoms. However, this effect was not sustained and disease severity began to increase toward the end of the trial period. Multiple applications of these products should be assessed in further trials. More isolates of *P. oligandrum* should be screened for their potential to be useful BCAs.

Significantly enhanced plant growth unrelated to disease control was demonstrated with the Fulzyme®Plus treatments in Trials 2 and 3 and with the *P. oligandrum* isolate in Trial 3. This has been previously demonstrated for Fulzyme®Plus (Tesoriero, unpublished) and it is unclear if it is a phyto-hormone response due to the *B. subtilis* isolate or the amino acids in the product formulation. Growth enhancement is valuable to production efficiency and should be considered by growers for further on-farm assessments.

Further trials are required to assess efficacy for different concentrations of biorational chemicals. In particular, lower concentrations of Bion® are required to avoid phytotoxic effects as noted in this study. This product may be best applied as a seed dressing rather than as a post-transplant drench applied through the nutrient system.

The concentrations of *Pythium sulcatum* used as inoculum may have swamped treatment effects in this study. This was despite halving and quartering inoculum concentrations in successive trials. Future experiments should examine inoculum dose-disease severity relationship in order to determine optimal concentrations for resolving treatment effects.

## 2.5 Acknowledgements

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# Chapter 3

# Disease management strategies to control root rot of parsley


## Summary

During Autumn and Winter of 2010 and 2011, in the market garden areas south east of Melbourne, two trials were conducted to identify alternatives to the fungicide metalaxyl (Ridomil®Gold 25G) for control of *Pythium* induced root rot of parsley. Disease at Devon Meadows was characterised by large patches of stunted and dead plants during a period of high rainfall and periods of water logging. The iron treatment was the only one to have improved yield of parsley significantly, by 93%. Metalaxyl increased foliage vigour significantly by 26% and reduced unmarketable parsley by 60% but not significantly at Devon Meadows. At Clyde, root rot caused an overall "thinning" of the plant stand and applications to the soil surface of the granular formulation of metalaxyl reduced the disease incidence (as a measure of plant density) by 46% compared with the untreated control and improved yield by 42%. Other treatments included the biological control agents *Pythium oligandram*, *Bacillus subtilis* (Fulzyme®Plus), foliar applications of the fungicide mix of azoxystobin and difenaconazole (Amistar®Top), the insecticide Diazinon, and the cultural treatments of an organic mulch on the soil surface and hilling of soil over the plant crowns.


## 3.1 Introduction

***Diseases of parsley in Australia caused by species of Pythium***

Post emergence damping-off and root rot are a serious problem for parsley growers (Minchinton *et al.* 2006, 2007). Growers report that the problem is most prevalent during autumn and winter, particularly after several days of heavy rains. Entire bays can be lost to pre and post-emergence damping-off in seedling crops, and to plant wilting and collapse in mature crops. Species of *Pythium* have commonly been isolated from necrotic lateral roots and from crown rots on parsley plants affected with these diseases in Victoria, New South Wales and Queensland (Minchinton *et al.* 2006, 2007).

***Control of Pythium root rots***

Generally, the management of *Pythium* (oomycete) diseases has involved crop rotation and chemical and biological control options. There are no known reports on breeding parsley for resistance to root rots.

Amongst the fungicides available for the control of oomycete pathogens, metalaxyl, a phenylamide, appears to the most effective in controlling root rots of parsley. However, resistance in some oomycete pathogens to this fungicide, and its biodegradation in some soils has been reported (Davison and McKay 2001, Minchinton *et al.* 2006, 2007 (VG06046). Therefore, in some situations, the use of this fungicide may not be sustainable.

Biological control of species of *Pythium* was suggested 40 years ago by Watson (1966). Populations of the antagonists, *Penicillium* spp. and *Trichoderma* spp. were observed to be inversely proportional to populations of *Pythium* species in the soil, which suggested an effect of these fungi on the pathogen. More recently, a commercial preparation of *P. oligandrum* was reported to be efficacious against pathogenic *Pythium* spp. However, this species is active at an optimum temperature of 15°C, and may not be suitable as a biological control agent in the winter months in Australia when soil temperatures are commonly below 10°C (Minchinton *et al.* 2006). This biological control agent could, however, be effective in summer where soil temperatures are above 15°C. Interestingly, *Bacillis subtilis* inoculated onto tomato plants induced resistance to *Pythium* spp. especially under saline conditions (Hanafi *et al.* 2007). In Ireland, McCracken (1984b), had no success in controlling root rot on parsley associated with *P. paroecandrum,* with metalaxyl, furalaxyl, metalaxyl+mancozeb, copper, thiram or

Tachigaren™. However, reduction in disease was achieved by rotating crops with barley, leeks, beetroot or spring onions (McCracken, 1984a).

This study reports on the results of field trials aimed at identifying alternatives to the fungicide metalaxyl for control of *Pythium* induced root rot of parsley.

## 3.2 Materials and Methods

Field trials were conducted in commercial fields in the market garden area east of Cranbourne, Victoria, during the autumn/winter months of 2010 and 2011. Soils were the sandy soils typical of this area. The trials evaluated biological, fungicide, chemical and compost treatments for the control of root rots caused by Oomycetes pathogens, particularly species of *Pythium*.

### 3.2.1 Disease management trials

#### *3.2.1.1 Treatments and their application*

Treatments tested in the two trials are listed in Table 3.1. Liquid treatments were applied with a pressurised Knapsack Sprayer fitted with a single drench nozzle at 30 psi (Silvan Selectra 12v Knapsack). All liquids applied to foliage were sprayed to run-off, unless otherwise stated. The fungicide metalaxyl was applied as a granular formulation (Ridomil®Gold 25G). The product was mixed with fine graded sand, which was broadcast over the soil surface with a perforated vessel to ensure an even distribution over the plots.

Inocula of *P. oligandrum* were prepared from a local isolate (Minchinton *et al.* 2006) grown in a liquid media. Ten, 1 cm diameter plugs from cultures of *P.oligandrum* on petri plates were added to each of 10, 250 mL conical flasks containing 150 mL of V8 broth that were then agitated on a laboratory rotor. Ten days after inoculation, the mycelia from each flask were rinsed with sterile distilled water and vacuum-dried on a Buchner funnel, weighed and homogenised in a domestic blender. Approximately 20g of vacuum dried oospore-mycelium homogenates were resuspended in 10 L of water and applied to soil surface of each of the plots with a watering can.

The fungicide propamocarb hydrochloride (Previcur®Fungicide), used to control damping off caused by species of *Pythium* in ornamental crops, was not applied in the Clyde trial. This fungicide is unregistered for use in parsley and obtaining of an appropriate permit or permission from the growers insurance company allowing its use in research plots, could not be obtained in time for the trial.

**Table 3.1 Fungicide, pesticide, biological and cultural control treatments tested in field trials in commercial parsley crops, Devon Meadows and Clyde, Victoria, 2010 and 2011. (Trial 1, 2010; Trial 2, 2011)**

| Product Trade Name | Active Ingredient | Company | Rate of application | Parsley Trial No. |
|---|---|---|---|---|
| Control | Untreated | na | na | 1,2 |
| Amistar®Top | 200g/L azoxystrobin + 125g/L difenoconazole | Syngenta Crop Protection | 625-725mL/ha | 2 |
| Diazinon® | 800g/L diazinon (APVMA PER9779) | Barmac Pty Ltd | 700ml/ha | 2 |
| Ferric citrate | Ferric citrate | Sigma Aldrich Pty Ltd | 1mM | 1 |
| Fulzyme®Plus | *Bacillus subtilis* | Zadco for Quality Gro Pty. Ltd | 24mL/L | 2 |
| Hilling Mulch | na | na | na | 2 |
| Enviromix™ | Premium Grade MG01 | Enviromix Pty Ltd | 2cm thick | 1,2 |
| *Pythium oligandrum* | *Pythium oligandrum* | In-house | 120mL/5L | 1 |
| Previcur® Fungicide | 600g/L propamocarb | Bayer Crop Science | 45ml/100 $m^2$ | 2 |
| Ridomil®Gold 25G[P] | 25g/kg metalaxyl-M | Syngenta Crop Protection | 120g/100m row | 1,2 |
| Sprayphos®620 | 620g/L phosphorous acid | Spray Gro Liquid Fertilizers | 1.7L/ha | 2 |

[P], APVMA permit

### *3.2.1.2 Devon Meadows 2010*

The trial was located on a commercial property at North Road, Devon Meadows Victoria. The parsley variety Continental was direct seeded at three rows per raised bed on 20 April 2010. Plots were 6m long by 1.68m wide. The trial was a randomised block design with blocks of five treatments replicated six times along a single bed. The crop husbandry was managed by the grower (fertiliser, irrigation and pesticide applications). A schedule of treatments for the trial is listed in Table 3.2. A metalaxyl treatment had been applied to the beds before sowing seed, which would prevent pre- and post-emergence damping off.

**Table 3.2 Schedule of treatments in a disease management trail on parsley *cv.* Continental, Devon Meadows, Victoria, 2010**

| Treatment | Week/date of spray/dap | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 8 | 9 | 10 | 12 | 13 | 15 | 19 | 20 |
| | 10/06/2010 | 15/06/2010 | 24/06/2010 | 9/07/2010 | 16/07/2010 | 28/07/2010 | 24/08/2010 | 30/08/2010 |
| | 50 | 55 | 64 | 79 | 86 | 98 | 125 | 131 |
| Control | - | - | - | - | - | - | - | - |
| Ridomil®Gold 25G[P] | - | - | - | + | - | - | - | - |
| *P. oligandrum* | - | + | - | - | + | - | + | - |
| Mulch Enviromix™ | + | - | - | - | - | - | - | - |
| Ferric citrate | - | - | + | - | - | + | - | + |

[P], APVMA permit; +, treatment applied; -, no treatment application; dap, days after planting.

### *3.2.1.3 Clyde 2011*

The trial was located at Clyde-Fiveways Road, Cranbourne, Victoria. The parsley variety Continental was direct seeded on 1st June 2011 as three rows per bed on raised beds. The trial was a randomised block design with each block of eight treatments replicated seven times. Plots were 5.58m long by

1.5m wide. The trial was located in a "bay" between two irrigation lines. Crop nutrition, irrigation and pesticide applications were maintained by the grower. A schedule of treatments for the trial is listed in Table 3.3. An additional fungicide treatment, Sprayphos®620 (potassium phosphonate), was applied to the Ridomil®Gold 25G treatment plots for control of Phytophthora root rots. This treatment is not effective against species of *Pythium*.

**Table 3.3 Schedule of treatments in a disease management trail on parsley *cv.* Continental, Clyde, Victoria 2011**

| Treatment | Week/date/dap | | | | | |
|---|---|---|---|---|---|---|
| | 1<br>1/06/2011<br>0 | 8<br>22/07/2001<br>52 | 10<br>1/08/2011<br>62 | 12<br>19/08/2011<br>80 | 17<br>16/09/2011<br>108 | 19<br>2/10/2011<br>124 |
| Control | - | - | - | - | - | - |
| Amistar®Top | - | - | - | + | + | + |
| Mulch Enviromix™ | - | + | - | - | - | - |
| Diazinon® | - | - | - | + | + | + |
| Fulzyme®Plus | - | + | - | + | + | - |
| Hilling | - | + | + | - | + | - |
| Ridomil®Gold 25G[P] | + | - | + | - | - | - |
| Sprayphos®620 | - | - | - | - | - | + |

[P], APVMA permit; +, treatment applied; -, no treatment application.

### *3.2.1.4 Assessment of disease, plant growth and yield*

***Devon Meadows 2010***

At Devon Meadows, disease was evident as patches of stunted, wilted and dead plants. On 6th September 2010 (138 days after planting), plants in a 6 m length of row, in each of the 3 rows of each plot, were rated on a scale of 0-2: 0, plants healthy, no stunting or death; 1, foliage obviously stunted but marketable and 2, foliage wilted or dead ("Incidence of foliage loss"). Unmarketable yield for each plot was estimated from the proportion of plants in the 1800 cm row length (3, 6 m lengths/plot) with a rating of 2. Due to the nature of the data a logistic transformation of the data was used and analysis done using ANOVA. Data from replicate 3 was not included in the analysis of disease and yield due to severe water logging in that part of the trial.

The effect of treatments on the marketable yield was calculated as the change in yield relative to the untreated control treatment. Total length of row length rated 2 for vigour was considered unmarketable (plants wilted or dead). Estimates of yield were based on 10 bunches of parsley to a deck (unit of sale), with 0.5 decks harvested per 1 m length of a single row consisting of an average of 75 plants. The average weight (biomass consisting of both foliage and roots) of a healthy plant was 16.74g at the first harvest on 6th September 2010. Due to the nature of the data a logistic transformation of the data was used and analysis done using ANOVA.

***Clyde 2011***

The relative vigour of the foliage in each plot, including plant height and density, was rated on a scale of 0-3 on 16th of September 2011 (108 dap) where 0 was complete loss of foliage, 1 was foliage reduced by two thirds in height and bulk, 2 was foliage reduced by one third and 3 was foliage healthy and no loss.

On the 3rd October 2011 (125 dap), 23 days before harvest, parsley plants were sampled from one location in each row, in a diagonal west, centre and east of each plot, excluding one meter buffer zones on each end of the plot (composite 20 plant sample from each plot). The incidence of plants with root rot (lesions or tap root rots) and plant fresh weight were assessed and recorded. Symptoms on the upper and lower tap root were categorised as mild or severe.

On 26th October 2011 (148 dap), reductions in plant numbers and foliage due to root rot was determined by recording the length of plant row, over the entire length of each plot row (3 rows/plot) in which plants were missing or had rotted tap roots (not secured in the soil). At harvest on 26th October 2011, each Control and Ridomil Gold 25G plot was notionally divided in half and the number of parsley plants in a 30cm length of each plant row, in the centre of each half of the plots, were counted to determine relative differences in plant numbers between the two treatments (total of 1.8 m of plot row counted).

The estimated yield of parsley was calculated only for the Ridomil Gold 25G$^{P}$ + Sprayphos™ and Control treatments as the fungicide treatment was the only one that showed a significant increase in foliage vigour relative to the untreated control. The estimated yield of parsley was based on the number of leaves per bunch (48), the average fresh weight of the bunch, harvested on the 28th October 2011 (212 g) and the average marketable leaves per plant (6) in the control plots (8 plants/bunch).

### 3.2.2 Measuring environmental variables at the trial sites

#### *3.2.2.1 Soil analysis*

Soil samples were collected from the trial site prior to establishing the 2010 field trial (10/08/2009) and analysed for soil nutrient concentrations (Department of Primary Industries, State Chemistry Laboratories, Werribee, Victoria).

#### *3.2.2.2 Weather station*

A Model T™ weather station (Western Electronics Design, Loxton, SA) was installed in the centre of an irrigation line at both trials. The leaf wetness sensor was placed in the crop at a 45 degree angle and its height was adjusted as the crop grew. The station recorded average temperature and relative humidity, the presence or absence of leaf wetness, daylight and total rainfall at 30 minute intervals.

#### *3.2.2.3 Soil moisture, temperature and EC (electrical conductivity)*

Soil moisture, temperature and EC were monitored with Crop Sense Soil Moisture Monitoring Plus EC™ equipment from the T-Systems Australia Pty Ltd, 410 Langbeckers Road, Bundaberg QLD. The equipment was installed within a row of parsley plants in a plot adjacent to the weather station. Soil moisture, EC and temperature were monitored at 2-10cm depth, while soil moisture and temperature were measured at a depth of 3-20 cm.

## 3.3 Results

### 3.3.1 Effects of treatments on disease and yield

#### *3.3.1.1 Devon Meadows 2010*

On the 6th September 2010 root diseases were evident as patches of stunted, wilted and missing plants (Fig. 3.1). Large patches of bare ground due to plant death were evident in some parts of the trial. A map of disease scores (scale 0-2) in the trial plots is presented in Fig. 3.2. The effects of treatments on plant and yield parameters are presented in Table 3.4 and Fig. 3.3. Although there was a trend of a greater percentage of foliage in the Ferric citrate and the metalaxyl treatments compared to the control and the other treatments, these differences were not statistically significant. There was a tendency ($P = 0.087$) of less unmarketable foliage with the ferric citrate and metalaxyl treatments than in the untreated control. There was a trend showing an improvement in yield (t/ha of foliage and decks/ha) for these two treatments relative to the untreated control, although these differences are not statistically significant. The estimated gain in yield from three applications of Ferric citrate was 643.8 decks/ha above that of the Control, although this was not significant (Table 3.4, Fig. 3.2). Applications of *P. oligandrum* reduced yield in comparison to the Control (Fig. 3.3).

**Fig. 3.1.** Trial plots at Devon Meadows 2011 showing patches of stunted plants (right of picture) affected by severe root rot.

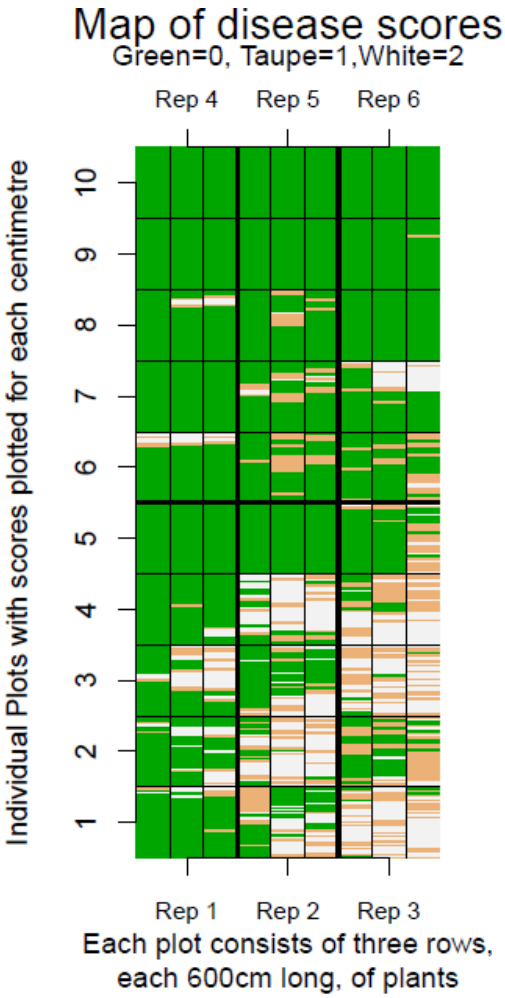


**Fig. 3.2** A map of the Devon Meadows field trial site 2010 showing length of plot row scored for disease severity on a scale of 0-2 at harvest time (September 2010), where 0 was healthy (green), 1 was stunted but marketable (taupe) and 2 was unmarketable (gaps in row and dead plants) (white).

**Table 3.4 Effects of fungicide, nutrient, biological and cultural treatments on disease and yield in a field trial at Devon Meadows, Victoria 2010**

| Treatment | Number of sprays | Incidence of foliage loss[1] (Back transformed) (%) | Proportion of the plot unmarketable[2] (Backtransformed) (%) | Estimated yield gain in foliage relative to Control (t/ha) | Estimated yield gain in foliage relative to Control (decks/ha) |
|---|---|---|---|---|---|
| Ferric citrate | 3 | 6.50 | 0.93 c | 1.365 | 643.8 |
| Ridomil® Gold 25G | 2 | 8.06 | 5.02 bc | 0.908 | 428.3 |
| Mulch Enviromix™ | 1 | 21.15 | 11.78 ab | 0.194 | 91.6 |
| Control (0) | 0 | 25.98 | 12.50 ab | 0.000 | 0.0 |
| *P. oligandrum* | 3 | 27.93 | 19.56 a | -0.714 | -336.7 |
| P value | | ns | 0.087 | | |

[1] Scale 0-2: where 0, no disease; 1, some loss of foliage but marketable; and 2, unmarketable.
[2] Proportion of row length with a vigour score of 2 are deemed unmarketable.
Numbers followed by a different letter differ significantly from each other
ns, not significant.

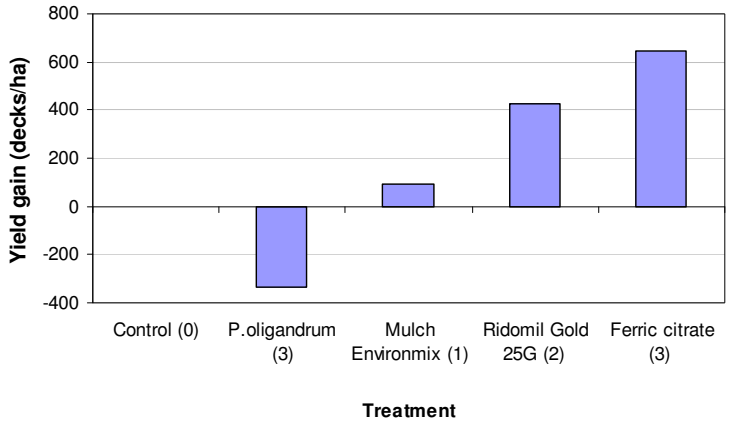


**Fig 3.3** Estimated gain or loss in parsley yield (decks/ha), relative to the untreated control, for nutrient, fungicide, biological and cultural treatments in a trial at Devon Meadows, Victoria 2010. (Number in brackets is the number of treatment applications)

### *3.3.1.2 Clyde, Victoria 2011*

Disease at this site was evident as yellowing and wilting plants and small gaps between plants in the rows as a result of plant death. Tap root rot was evident on yellowing and wilted plants. Large patches of dead plants were not evident as in the Devon Meadows trial in 2010. Plants bordering gaps left by dying and dead plants compensated, being taller and more branched than plants in the denser stands.

Plant density and height ("vigour") was significantly better (P≤0.05) in plots treated with metalaxyl (Ridomil®Gold 25G) than in the untreated control as determined by a score for vigour (16/9/2011) (Table 3.5). Hilling soil over crowns, the compost mulch and the *Bacillus subtilise* treatments did not significantly affect plant vigour compared with the untreated control. Likewise, the foliar treatments Amistar®Top and Diazinon® had no significant effect plant vigour (Table 3.5).

There was no significant effect of any treatment on the incidence of plants with root rot rots (tap root lesions, severe tap root and crown rot), nor on the incidence of plants with severe tap root and crown rot (Table 3.6).

**Table 3.5 Effects of fungicide, pesticide, biological and cultural treatments on plant vigour (foliage density and height) in a field trial at Clyde, Victoria, 2011 (16/9/2011assessment)**

| Treatment | Number of applications | Average vigour of foliage on 16/9/2011 (Scale 0-3) |
|---|---|---|
| Amistar®Top | 3 | 2.0 b |
| Control | 0 | 2.0 b |
| Mulch Environmix™ | 1 | 2.0 b |
| Fulzyme®Plus | 3 | 2.1 b |
| Hilling | 2 | 2.3 b |
| Diazinon® | 2 | 2.3 b |
| Ridomil®Gold 25G | 2 | 2.7 a |
| lsd (5%) | | 0.34 |

Vigour scale 0-3; 0, complete loss of foliage; 3, healthy. Numbers followed by the same letter do no differ significantly from each other.

The incidence of plants sampled from plots with root rots, including mild and severe symptoms of tap root rots (upper and lower tap root) are presented in Table 3.6. Overall, there were no significant effects of fungicide or cultural treatments on the incidence of plants with root rots compared with the untreated control. However, the incidence of plants with upper tap lesions was significantly higher ($P \leq 0.05$) in the Diazinon® treated plots than in untreated control or any other treatment.

**Table 3.6 Effects of fungicide, pesticide, biological and cultural treatments on root rot in a field trial at Clyde, Victoria, 2011 (3rd October 2011 assessment)**

| Treatment | Number of applications | % plants with all root rot diseases | % plants with lesions on upper tap root (mild symptoms) | % plants with lesions on lower tap root (mild symptoms) | % plants with tap root lesions (severe symptoms) | Total weight of healthy plants (g) |
|---|---|---|---|---|---|---|
| Amistar Top | 3 | 52.9 a | 24.3 a | 8.57 | 31.4 | 349 |
| Control | 0 | 60.0 ab | 22.9 a | 7.86 | 20.0 | 477 |
| Mulch Enviromix™ | 1 | 53.6 a | 25.0 a | 7.86 | 22.9 | 453 |
| Fulzyme™ | 3 | 53.6 a | 23.6 a | 13.68 | 23.0 | 497 |
| Hilling | 2 | 64.3 ab | 28.6 a | 6.43 | 24.3 | 423 |
| Diazinon® | 2 | 72.7 b | 43.9 b | 10.00 | 27.9 | 421 |
| Ridomil Gold 25G[P] | 2 | 51.4 a | 26.4 a | 7.14 | 20.7 | 414 |
| lsd (5%) | | 14.71 | 14.61 | ns | ns | ns |

Numbers followed by the same letter do not different significantly from each other.

The proportion of row length of plants lost due to root rot in the metalaxyl treated plots was significantly less ($P<0.05$) than in the control plots (19.3% and 35.7% row length affected) (Table 3.7). The estimated foliage yield (t/ha and decks/ha) was 42% higher in the metalaxyl treatment plants than in the untreated control. The other treatments did not have a significant effect on these variables compared with the untreated control (Table 3.7).

**Table 3.7 Effects of fungicide, biological and cultural control treatments for the control of root rots and for the improvement in yield of foliage on parsley plants Trial No. 2 on 28 October 2011.**

| Treatment | Number of applications | Disease incidence (%)[1] | Estimated yield (t/ha) | Estimated yield (decks/ha) |
|---|---|---|---|---|
| Amistar®Top | 3 | 37.01a | - | - |
| Control | 0 | 35.72a | 17.60 | 8303.6 |
| Mulch Environmix™ | 1 | 34.35a | - | - |
| Fulzyme®Plus | *3* | 34.76a | - | - |
| Hilling | 2 | 34.62a | - | - |
| Diazinon® | 2 | 38.54a | - | - |
| Ridomil®Gold 25G | 2 | 19.28 b | 24.82 | 11,709.82 |
| lsd (5%) | | 7.945 | - | - |

Disease incidence; % length of row lost to root rot. Numbers followed by the same letter do not differ significantly from each other.

## 3.3.2 Environmental data

### *3.3.2.1 Devon Meadows*

***Nutrient analysis***

The nutrient analysis of soil from the Devon Meadows site in 2009 indicated a low level of iron (Appendix 3.6 Table 1). Consequently, iron in the form of ferric citrate was applied as a treatment.

***Weather station***

Air temperature during the course of the trial ranged from 0ºC to 21.5ºC with the coldest temperatures occurring in June and July (see Chapter 4, Appendix 4). There were four major rain events of 2mm or more on 5th June. 31st July, 24th August and 4th September 2010 (refer to Chapter 4).

***Soil moisture, temperature and EC***

During the course of the trial, the EC ranged from 0-2.1 $dSm^{-1}$. Soil temperatures in the 2-10cm profile ranged from 2.5-17.5ºC and soil moisture ranged from 28-34.5%. Soil temperature in the 3 to 30cm soil profile ranged from 2.5-17.5ºC and soil moisture ranged from 24-33%. The lowest soil temperatures occurred in the months of June and July (refer to Chapter 4).

### *3.3.2.2 Clyde 2011*

***Weather station***

Air temperature during the course of the trial ranged from 0.75ºC to 29ºC with the coldest temperatures occurring in June and July (Appendix 4). There were 29 major rain events of 2mm or more of rainfall from 1st June until 20th October 2011 (refer to Chapter 4).

***Soil moisture, temperature and EC***

During the course of this trial, the EC ranged from 0-4 $dSm^{-1}$, with the highest reading being recorded about a week prior to harvest. Soil temperature in the 2-10cm profile ranged from 10.3-17.2ºC and soil moisture ranged from 14-33%. Soil temperature in the 3 to 30cm soil profile ranged from 10.3-17.2ºC and soil moisture ranged from 10.3-36.5%. Soil moisture in both profiles declined towards harvest. The lowest soil temperatures occurred in the months of June and July (Chapter 4).

## 3.4 Discussion

Alternative control treatments for root rot of parsley were not identified in two trials conducted in the sandy soils of the market garden region east of Melbourne. Standard treatments of soil with the fungicide metalaxyl significantly reduced the incidence of diseased plants and improved yields at the Clyde trial but were only marginally effective at Devon Meadows. None of alternative biological control treatments, fungicides and cultural control treatments had any significant effect on disease in the trials.

Disease at Devon Meadows was characterised by large patches of stunted and dead plants during a period of high rainfall and periods of water logging. At Clyde, on the other hand, root rot caused an overall "thinning" of the plant stand, rather than large patches. Disease pressure at the Devon Meadows trial site was particularly high in that season and is exacerbated by the frequent cropping of parsley in the same beds.

In the Clyde 2011 trial, the incidence of plants sampled from the plots with root rot following metalaxyl treatments did not differ significantly from the untreated control, despite a significant effect of the fungicide treatments on plant densities. The plant sample was taken after root rot had already killed off plants in the plots. The sample, taken of the surviving plants was, therefore not representative of the plants affected by disease.

Previous pathogenicity experiments and field work in the Cranbourne area by Minchinton *et al.* (2006, 2007) indicated that *Pythium* species, and to a lesser extent *Phytophthora* species were responsible for parsley root rot in Victoria. These reports showed that metalaxyl treatments could control *Pythium* induced root rots by up to 46%. Six different species of *Pythium* were isolated from plants at the two trial sites but Koch's Postulates have not been conducted to confirm their pathogenicity to parsley.

The fungicide treatment azoxystrobin, in this case mixed with difenaconazole (Amistar®Top), was essentially applied as foliar treatment with some run-off to soil. This treatment may have been more efficacious if applied directly to soil (soil spray and rotary hoed into the surface prior to seeding). It is not registered for this use in Australia at present, but there is currently an APVMA permit for in-furrow applications of azoxystrobin to control *Rhizoctonia* in potatoes in Tasmania.

Iron in the form of Fe citrate was applied at the Devon Meadows trial site to balance a deficiency in iron in the soil. Iron deficiency is not uncommon in sandy soils, which is exacerbated by the use of lime to increase the pH of sandy soils. There was no evidence that improving iron concentration in the soil had any significant effect on the incidence of root rot.

The two biological control agents *Pythium oligandrum*, a mycoparasite, and the bacteria *Bacillus subtillis*, applied to soil and foliage, respectively, did not control disease in these trials. Temperatures may have been too low for the successful establishment of *P. oligandrum* during the Autumn/Winter months, since this organism has an optimum growth temperature of 15°C. Biological control agents are often applied like fungicides but require a more strategic application approach, taking into account their biology and niche in a cropping situation. As a living organism their ability to colonise soil and plant roots will be dictated by their specific requirements of moisture and temperature parameters, and they must also compete with other soil and root microbes.

The organic mulch (Enviromix™) and the hilling treatments were applied as a means of altering soil conditions around the plant crowns and, particularly temperature. Although these treatments did not have a significant affect on disease and yield, the organic mulch was observed to improve lateral root growth. Regular applications of organic mulches may be beneficial in the long term in improving soil carbon levels in these sandy soils. However, such applications would have to be economically feasible.

Growers currently have few alternatives to the fungicide metalaxyl to control root rots of parsley, particularly in the Autumn/Winter months. Disease pressure is high due to the short rotations, cool temperatures and periods of water logging of soil. In addition to chemical controls, integrated management systems for root rots of parsley in the future should include cropping practices that reduce soil conditions conducive to root rots.

## 3.5 References

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## 3.6 Appendix

**Table 1. Nutrient analysis of soil from the trial site at Devon Meadows, 2009**

| Item | Description | Unit | Top Soil | Sub Soil |
|---|---|---|---|---|
| | Depth | | 0-15 | 15-25 |
| Carbonate/Bicarbonate | Bicarbonate | mg/kg | 161.3 | 138.1 |
| | Carbonate | mg/kg | 0 | 0 |
| | Chloride | mg/kg | 200 | 190 |
| Total Carbon/Nitrogen | Carbon | g/100g | 1.8 | 1.4 |
| | Nitrogen | g/100g | 0.15 | 0.1 |
| | Organic matter | g/100g | 3.4 | 2.5 |
| pH and Conductivity | Electrical Conductivity | dS/m | 0.35 | 0.38 |
| | pH(CaCl2) | - | 7.2 | 7.2 |
| | pH(water) | - | 7.7 | 7.7 |
| | Total soluble salts | % | 0.12 | 0.13 |
| Ammonium acetate cations (with prewash) | Calcium | meq/100g | 7.9 | 6.3 |
| | Calcium as % | % | 74 | 74 |
| | Calcium Magnesium ratio | - | 3.8 | 3.7 |
| | Magnesium | meq/100g | 2.1 | 1.7 |
| | Magnesium as % | % | 20 | 20 |
| | Potassium | meq/100g | 0.51 | 0.4 |
| | Potassium as % | % | 5 | 5 |
| | Sodium | meq/100g | 0.17 | 0.14 |
| | Sodium as % | % | 2 | 2 |
| | Sum of four cations | meq/100g | 11 | 8.5 |
| Available Aluminium | Aluminium | mg/kg | <10 | <10 |
| Available Boron | Boron | mg/kg | 2.3 | 1.6 |
| Available Potassium | Potassium | mg/kg | 420 | 350 |
| Available Phosphorus | Phosphorous (Olsen) | mg/kg | 260 | 180 |
| Available Sulfur | Sulfur | mg/kg | 68 | 71 |
| DTPA extractable trace elements | Copper | mg/kg | 5.5 | 3.3 |
| | Iron | mg/kg | 22 | 24 |
| | Manganese | mg/kg | 4 | 3 |
| | Zinc | mg/kg | 9.3 | 6.3 |

# Chapter 4

# Etiology and epidemiology of parsley root rot in Victorian commercial cropping systems


## Summary

The epidemiology of root rot disease on parsley was determined to strategically target control measures. Systematic surveys conducted during the cooler months of 2010 and 2011, showed disease symptoms appeared six weeks into the life of the crop. Development of mild and severe root rot symptoms followed the same trend in both years, with severe symptoms emerging about 6-8 weeks prior to harvest. Mild root rot symptoms did not appear to affect plant biomass and consequently did not reduce yields. Patches of missing plants caused by poor germination and/or damping-off of seedlings at the Devon Meadows site affected yields. Control measures for autumn planted parsley crops need to be targeted therefore before sowing and during the early stage of crop development at 6-8 weeks prior to harvest. *Pythium* spp. were the most frequently isolated pathogens. Six *Pythium* spp. were isolated from necrotic root symptoms and identified using molecular methods. Two of these *Pythium* species, *P. mastophorum* and *P. rostratifingens* have not previously been recorded on parsley roots in Australia. This work also provides the first record of *Phoma exiqua* var. *exigua* on parsley roots in Australia. Pathogenicity of *P. sulcatum*, a commonly occurring species on parsley roots, was confirmed in growth cabinet experiments at 10ºC, 18ºC and 24ºC. *Pythium sulcatum* reduced root biomass at all three temperatures, with losses increasing with increasing temperature. This information could help explain the emergence of severe root rot symptoms in spring and lead to the development of a disease predictive model for *Pythium* root rot of parsley.


## 4.1 Introduction

Root rot is one of the most destructive disease complexes of commercially grown parsley in Victoria. Root disease causes up to 100% yield losses in the winter-grown parsley (Minchinton *et al*. 2007). In a national survey of parsley crops in 2005, the genera of fungi identified from parsley root rots included *Rhizoctonia*, *Fusarium*, *Sclerotinia*, *Mycocentrospora*, *Cylindrocarpon, Microdochium*, *Pythium* and *Phytophthora* (Minchinton *et al.* 2006).

In a study in Victoria (Minchinton *et al*. 2006), species of oomycetes isolated from diseased roots or pear bait bioassays of parsley growing soils were identified using gene sequencing as *Phytophthora megasperma*, *P. inundata*, *Pythium ultimum, P. sulcatum* and the mycoparasite *P. oligandrum*. Their pathogenicity to parsley confirmed following Koch's postulates. Additionally, *Pythium acanthophoron, P. intermedium, P. irregulare, P. paroecandrum, P. sulcatum*, *P. ultimum* and species from the *Pythium diclinum* and *Pythium littorale* groups, as well as unidentified species, were also reported in New South Wales and Queensland (Minchinton *et al.* 2006, 2007).

Overseas, a number of *Pythium* species have been associated with root rot and damping-off in parsley. In Northern Ireland it was associated with *P. paroecandrum* (McCracken 1984a and 1984b), *P. mastophorum* in Germany (Krober and Sauthoff 1999) and *P. aphanidermatum* on hydroponic parsley in South Africa (Gull *et al.* 2004). Damping off of parsley grown in the USA was associated with *P. 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 several fungi and *Pythium* (Nawrocki and Mazur, 2004; Nowicki 1997).

This study reports on the epidemiology of root rot in winter-grown parsley crops, identification of *Pythium* spp. associated with root rot symptoms and explores aspects of the aetiology of *P. sulcatum*, a major pathogen of apiaceae.

## 4.2 Materials and Methods

### 4.2.1 Systematic surveys

Systematic monthly disease surveys were conducted on parsley roots in untreated control plots of two replicated field disease management trials in the market garden area east of Cranbourne, Victoria, (Devon Meadows 2010 and Clyde 2011). Each trial had been direct seeded with parsley cultivar 'Continental' (three rows per bed on raised beds), on 20th April in 2010 and on 1st June in 2011. Both trials were maintained by growers. Refer to Chapter 3 for trial details.

*4.2.1.1 Plant sampling and disease assessment*

***Devon Meadows 2010*** Eight plants were sampled from each of six untreated control plots, on 24th June and 20th July (four plants were randomly taken from a 1m long section of bed at each end of every plot). On the 6th September 2010, 12 plants were randomly sampled from each entire control plot area at harvest time. .

***Clyde 2011*** Four plants were sampled from each of the seven untreated control plots, (two plants were randomly taken from a 1 m long section at each end of every plot) on 22nd June, 22nd July, 19th August and 16th September. Eleven to 17 plants were randomly sampled from each entire plot area on 28th October at harvest time.

***Disease assessment*** Plants from both trials were assessed for severity of root damage. Symptoms were categorised as: (1) healthy roots, (2) mild symptoms including brown lesions on upper and lower tap root, and (3) severe symptoms of tap root and collar rot. Disease incidence was recorded as the percentage of plants in each category of root damage. It needs to be noted that disease assessment may underestimate overall disease incidence and severity in the plots because the most severely affected plants were missing at the time of each sampling.

*4.2.1.2 Yield estimations*

For each harvest sample, individual plants were weighed to calculate the average fresh weight of the whole plant in each root disease category.

### 4.2.2 Pathogen isolations and identification

*4.2.2.1 Isolations and culturing*

Pathogens were isolated from symptomatic parsley roots sampled during monthly surveys at each trial site. Sections of parsley roots, including fine roots, were washed in sterile distilled water, blotted dry and plated onto water agar (WA) and incubated at room temperature. After 2-3 days, hyphal tips of oomycete-like cultures were transferred in 5 mm plugs onto V8 agar or into 250 mL conical flasks with V8 broth. Hyphal tips of fungi were subcultured onto potato dextrose agar (PDA) after 5-7 days. Mycelia of 7 to 10 day old *Pythium*-like isolates growing on V8 agar plates or in V8 broth, and mycelia from 20 day old *Phoma*-like cultures on PDA were scraped from agar plates with a scalpel blade, or vacuum dried on a Buchner funnel, in the case of liquid cultures, and stored in 1.5mL centrifuge tubes at -20°C. Pathogenic genera were identified microscopically. *Pythium*-, and *Phoma*-like isolates were selected for further identification.

*4.2.2.2 DNA extraction and ITS sequencing*

DNA was extracted from eleven *Pythium*-like and two *Phoma*-like isolates using FastDNA®SPIN for Soil Kit (MP Biomedicals, LLC) according to manufacturer's instructions. The ITS region including the 5.8S gene of *Pythium* isolates were amplified using primers UN-UP18S42 (5'-CGTAACAAGGTTTCCGTAGGTGAAC-3') (Bakkeren *et al.* 2000) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al*. 1990) and that of *Phoma* isolates using primers V9G (5'-TTACGTCCCTGCCCTTTGTA-3') (de Hoog and Gerrits van den Ende 1998) and ITS4 (White *et al*. 1990).

DNA was amplified by polymerase chain reactions (PCR) in Applied Biosystems Veriti Thermal Cycler. For both primer pairs, each reaction consisted of 0.2 µM of each primer, 1 mM of each dNTP, 5 µL of 1 x buffer (Invitrogen), 2 mM $MgCl_2$, 1U of Platinum® *Taq*DNA polymerase (Invitrogen) and 2 µL of DNA (of concentration approximately 30 ng/ µL) in a final volume of 50 µL. PCR reaction conditions for *Phoma* were as follows: initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 30 sec, primer annealing at 48°C for 30 sec, and elongation at 72°C for 1.5 min PCR conditions for *Pythium* were as follows: initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 95°C for 30 sec, primer annealing at 50°C for 30 sec, and elongation at 72°C for 1min. The final extension step of 7 min at 72°C was the same for both primers pairs.

PCR products were resolved by electrophoresis on 2% agarose gels stained with ethidium bromide. The products were than purified (QIAquick PCR purification Kit, Qiagen) following the manufacturer's instructions. Sequencing was performed on an AB 3730*xl* automated sequencer (Applied Biosystems) after DNA labelling (sequencing) reaction of PCR products with relevant primers and cleanup was undertaken by Australian Genome Research Facility Ltd. The isolates were identified to the species level by conducting Basic Local Alignment Search Tool (BLAST) searches with the sequence data on GeneBank.

### 4.2.3 Environment data collection

A range of environmental parameters were recorded at each trial site as described in Chapter 3.

### 4.2.4 Pathogenicity of *P. sulcatum* on parsley roots at three temperatures

*4.2.4.1 Plant growth conditions*

Pathogenicity of a local *P. sulcatum* isolate (Minchinton *et al.* 2006) was tested on parsley cv 'Italian Plain Leaf' roots in controlled environments. Parsley was seeded in seed raising mix (Debco™ Debco Pty. Ltd. Tyabb, Victoria), in plastic multicell trays of 144 cells per tray and placed on a glasshouse bench on 25th May 2010. Glasshouse day/night temperatures were set at 25°C and 17°C, respectively. Seedlings were irrigated twice a day for 1 min at 6am and 2pm by overhead sprinklers and fertilised weekly with 5 g/10L solution of Aquasol™ fertiliser. Four week old individual seedlings (4-6 true leaves stage) were transplanted each into 7.5 cm diameter plastic pots, filled with seed raising mix. All 72 pots were fertilised, each with 1 g of slow-release complete fertiliser Nitrophoska ™ (Burnings), and 24 pots were than transferred to each of the three growth cabinets on 23rd June. Temperature in each of the cabinets was set at 10°C, 18°C and 24°C respectively. In each cabinet day/night conditions were set at 12h light/dark with a light intensity of 270 µeinsteins mol $m^{-2}s^{-1}$ and a relative humidity of 70%. Seedlings were watered to saturation every second day.

*4.2.4.2 Preparation of inoculum and inoculation*

A *P. sulcatum* isolate was subcultured from cold storage (collection date, August 2006, Minchinton *et al.* 2006) and grown initially for two days on WA. Mycelial tips were then plated onto V8 agar and incubated at room temperature. In each growth cabinet, six 1 cm diameter agar plugs, cut from the edge of 5 day old *P. sulcatum* cultures, were placed under a thin layer of seedling mix in each of the 12 pots on 13 July (seven weeks after seeding). The remaining 12 pots in each growth cabinet served as uninoculated Controls. All 24 pots in each of the three growth cabinets were flooded (to full water holding capacity of the seedling mix) for 24 hours prior to the inoculation to initiate pathogen zoospore release.

*4.2.4.3 Disease assessment*

Seedling roots were assessed for disease symptoms two weeks after inoculation on 29th July. Incidence of symptoms in each of four categories: (1) healthy, (2) pruning of fine roots, (3) lesion(s) on lateral roots or/and pruning of lateral roots, and (4) lesions and pruning of tap root, was calculated as a percentage of plants with these symptoms. Weights of whole plant and its root were recorded for each inoculated and control plant to calculate yield loss. Sections of infected parsley roots were, rinsed in SDW and plated on WA to fulfil Koch's postulates.

## 4.3 Results

### 4.3.1 Disease development in parsley crops

#### *4.3.1.1 Parsley root rot development at Devon Meadows 2010*

Irregular patches of dead and missing plants were observed at this site early in the life of the crop, due to poor germination and/or seedling damping-off. Symptoms of root rot in the plants sampled from untreated control plots appeared in June 2010 (winter) and by July approximately 50% of the remaining parsley plants sampled from the untreated control plots exhibited symptoms of severe root rot, with incidence changing little up to harvest in September 2010 (Fig. 4.1). The incidence of plants with mild root rot was initially high (40%), declined in July and rose again at harvest. By harvest, over 80% of plants sampled had evidence of root rots.

The average weight of plants with severe root rots was considerably less than those without severe root rots (Table 4.1). These plants had no harvestable foliage. Plants with mild severity of root disease were no different in weight than plants with healthy roots, the former producing harvestable foliage.

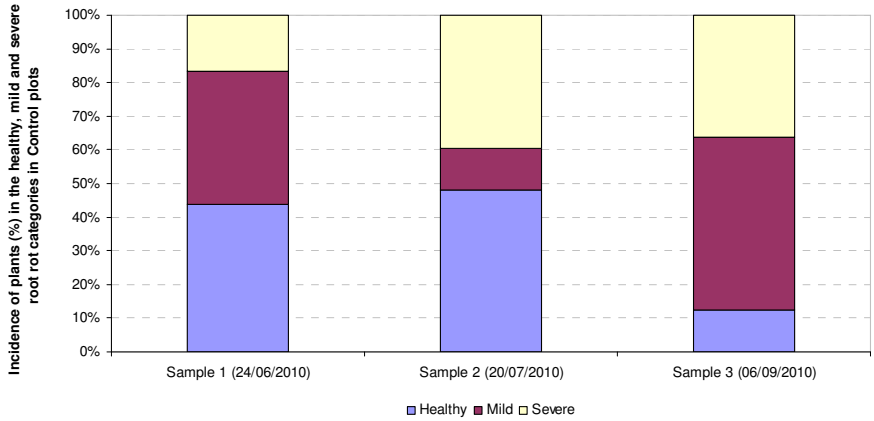


**Fig 4.1** Incidence (%) of parsley plants in the healthy, mild and severe root rot categories untreated control plots of the Devon Meadows trial in winter and spring 2010.

**Table 4.1 Average weights of parsley plants in the three root rot symptom categories from untreated control plots at harvest for the Devon Meadows trial on 6th September 2010.**

| Symptom[1] | No. of plants | Range of weights (g) | Average weight per plant (g) |
|---|---|---|---|
| Healthy: no symptoms | 9 | 7.91-24.98 | 16.74 |
| Mild: Plants with brown lesion on upper or lower tap root or both symptoms | 33 | 2.58-43.52 | 17.4 |
| Severe: Plants with rotten tap root or collar or both symptoms together (other symptoms may also be present) | 28 | 1.26-19.83 | 7.18 |

[1], There were two plants with symptoms other than those listed.

#### *4.3.2.2 Parsley root rot development at Clyde 2011*

In contrast to the trial at Devon Meadows, plots in the Clyde trial exhibited a much more even emergence. Patches of dead and missing plants were not evident in this trial. The incidence of plants sampled from the control treatments during winter and early spring were healthy but by harvest time only 15% of plants had healthy roots (Fig. 4.2). The level of mild symptoms of root rot were initially similar to that of healthy plants but plummeted to approximately 20% in mid September and then

sharply rose to 60% by harvest in early October 2011. Symptoms of severe root rot did not become apparent until late in the life of the crop with 25% of plants affected by harvest time (Fig. 4.2) The proportion of plants with mild root rot severity was relative high (up to 60% of plants affected) over the sampling period. The average weight of plants with severe root rot was less than those with either mild or no symptoms of root rot (Table 4.2). Mild root rots did not reduce the average plant biomass (52.3 g), which was slightly higher than the average plant biomass of a healthy plant (36.3 g).

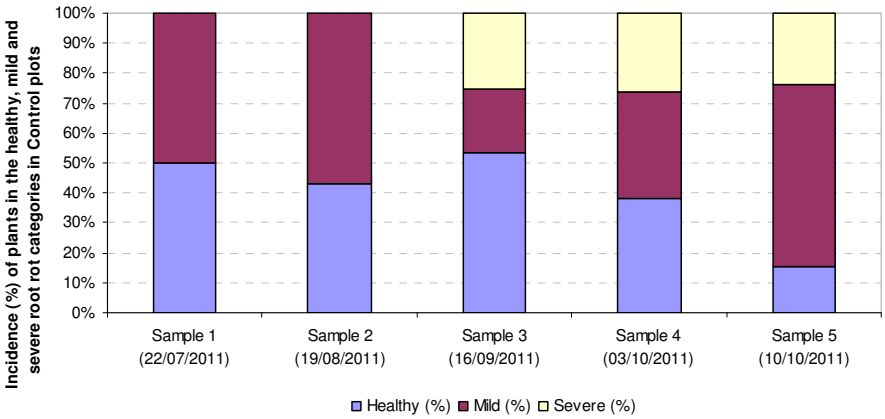


**Fig 4.2** Incidence (%) of parsley plants in the healthy, mild and severe root rot categories untreated control plots of the Clyde trial in winter and spring 2011.

**Table 4.2 Average weights of parsley plants in the three root rot symptom categories from untreated control plots in a field trial at Clyde trial (28 October 2011).**

| Symptom | No. of plants | Range of weights (g) | Average weight per plant (g) |
|---|---|---|---|
| Healthy: no symptoms | 16 | 4.52-144.47 | 36.62 |
| Mild: Plants with brown lesion on upper or lower tap root or both symptoms | 64 | 7.22-177.99 | 52.28 |
| Severe: Plants with rotten tap root or collar or both symptoms together (other symptoms may also be present) | 25 | 2.2-98.03 | 28.61 |

### 4.3.2 Pathogen identification

Six *Pythium* spp. were identified out of eleven sequences of rDNA ITS (ITS1, 5.8S, and ITS2) (Table 4.3), including *P. intermedium*, *P. ultimum* var. *ultimum*, *P. dissotocum* complex, *P. rostratifingens*, *P. sulcatum*, and *P. mastophorum.* Two isolates matched sequences of another *P.* spp., which have not been identified to a species level. Based on molecular phylogeny and taxonomy of the genus *Pythium* (Lévesque and de Cock 2004), species identified in this study belong to five phylogenetic clydes: B, E, F, I, and J. Sequences of the *P. dissotocum* complex are identical to these of *P. lutarium* and *P. coloratum.* These three species are also morphologically similar (Lévesque and de Cock 2004), therefore named here as *P. dissotocum* complex.

Two *Phoma* isolates were identified as *Phoma exigua* var. *exigua.* This species has not been reported from parsley in Australia before. It is known as a pathogen of lettuce, causing Phoma basal rot (Koike *et al*. 2003), and on chicory roots in storage (Dennis and Davis 1978).

**Table 4.3 Identification of *Pythium* and *Phoma* spp. isolated from parsley roots in at Devon Meadows 2010 and Clyde 2011 using sequence data.**

| Species identified | No of isolates |
|---|---|
| ***Pythium*** | |
| *P. dissotocum* complex | 2 |
| *P. intermedium* | 2 |
| *P. ultimum* var. *ultimum* | 2 |
| *P. rostratifingens* | 1 |
| *P. sulcatum* | 1 |
| *P. mastophorum* | 1 |
| *P. sp* | 2 |
| ***Phoma*** | |
| *P. exigua* var. *exigua* | 2 |

### 4.3.3 Environmental data

Generally, the 2010 cropping season was colder than 2011. The average monthly temperature, and average day and night temperature in each month of this season were lower with the exception of June, which was slightly warmer in 2010 than in 2011. The 2011 season was wetter than 2010. Total of 273 mm of rainfall was recorded from June to September on the Clyde trial site and only 193 mm on in the same time on the Devon Meadows site (Fig. 4.3) The Devon Meadows site was additionally frequently irrigated. August was the wettest month of the 2010 with recorded 75 mm of rainfall and it was wetter than August of 2011 with only 57 mm of rainfall.

***Devon Meadows 2010***

The monthly average temperature from June to September 2010 was 9.3ºC, 10.4ºC, 10.3ºC, 12.1ºC, respectively (Fig. 4.4, Appendix 4 Fig. 1 ). There were four rainfall events with ≥ 2mm, but one was approximately 5.5mm and this was associated with severe flooding on the trial site (Appendix 4, Fig. 2).

Soil temperature ranged from 2.25 ºC to 17.3ºC. The changes in soil moisture in the two profiles basically followed the same trend. The percent soil moisture was higher in the 2-10cm profile and ranged from 28-34%, while in the 3-30cm profile it ranged from 23.5-33% (Appendix 4, Fig. 4). Soil EC records collected ranged from 0-2.1 (dS $m^{-1}$). Although soil EC data were recorded by the soil probe, the interpretation of these records in terms of possible relationship between EC and disease is beyond the scope to this project (Appendix 4, Fig. 3).

***Clyde 2011***

The monthly average temperature from July to October 2011 was 10ºC, 9.6ºC, 11.5ºC, 12.6ºC and 14.1ºC, respectively (Fig. 4.4, Appendix 4 Fig. 5). There were about 30 rainfall events with ≥ 2mm during the course of the trial (Appendix 4, Fig. 6). Only seven of these occurred in the first half of crop development with the remainder occurring in the latter half of crop development.

Soil temperature records collected only in September ranged from 10.25 ºC to 17.25 ºC. The soil moisture in the two profiles basically followed the same trend, except on this site the lower profile was wetter than the upper profile and both decreased during the course of the trial (Appendix 4 Fig. 7). The maximum soil moisture in the 2-10cm profile was 32% and in the 3-30cm profile was 35%. Soil EC ranged from 0 – 0.7 (dS $m^{-1}$) but there is no explanation for the spike at 4 dS $m^{-1}$ late in crop development.

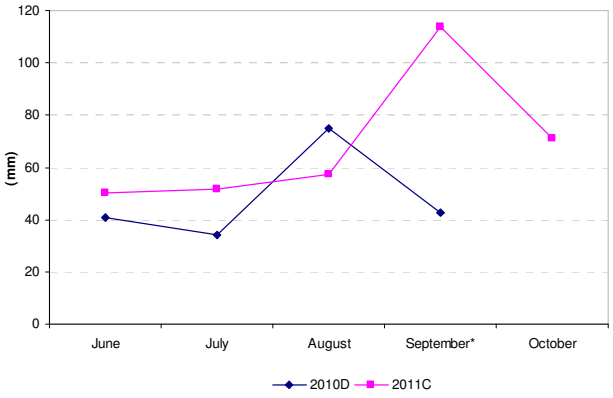


**Fig 4.3** Total monthly rainfalls at two parsley root rot management trial sites, Devon Meadows 2010 (D) and Clyde 2011 (C) recorded on automated ModelT weather stations.

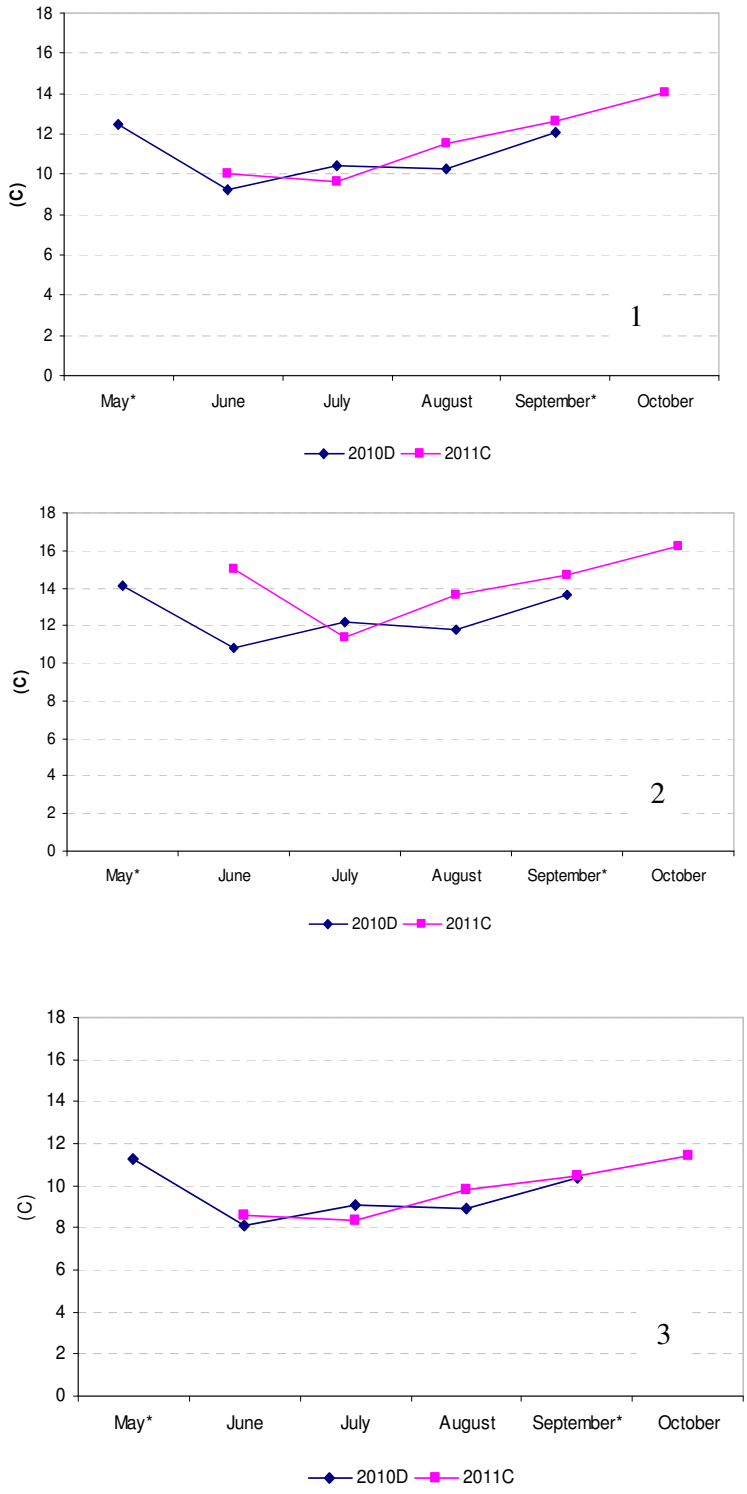


**Fig 4.4** Average monthly temperature (1), average day temperature in each month (2), and average night temperature in each month (3) at two parsley sites, Devon Meadows 2010 (D) and Clyde 2011 (C) recorded on automated ModelT weather stations.

### 4.3.4 Pathogenicity of *P. sulcatum* on parsley roots

The *P. sulcatum* isolate tested was pathogenic to parsely seedlings at 10°C, 18°C and 24°C but caused the most severe lateral and tap rot rot at 18°C. Fine roots were pruned in all inoculated plants in each temperature setting. The incidence of plants with lateral root damage at 10°C, 18°C and 24°C was of 75, 92 and 83% plants affected, respectively. The incidence of plants with lesions on the tap root at 10°C, 18°C and 24°C was 8, 42 and 17% plants affected, respectively. The most severe tap root pruning was recorded on 25% of plants at 18°C. Loss of total and root biomass, relative to the uninfected controls, increased progressively with increasing temperatures (Fig. 4.5). *Pythium. sulcatum* was successfully isolated from diseased roots fulfilling Koch's postulates.

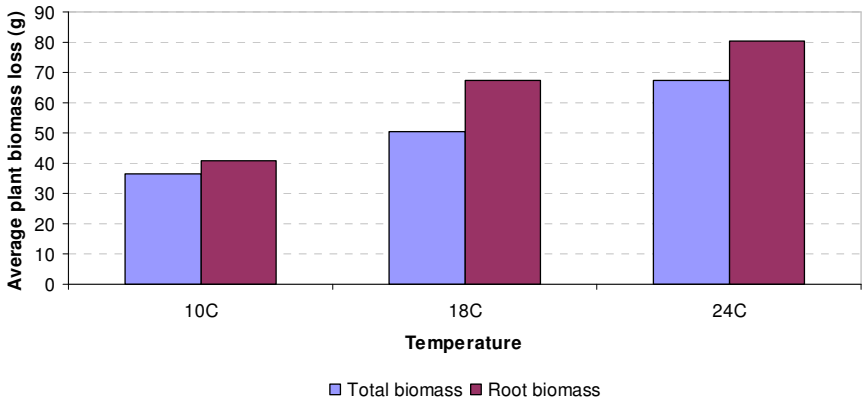


**Fig 4.5** Average total biomass and root biomass losses of parsley plant caused by *P. sulcatum* infections at 10°C, 18°C, and 24°C (Proportion of total and root biomass of uninoculated plant grown at the same temperate).

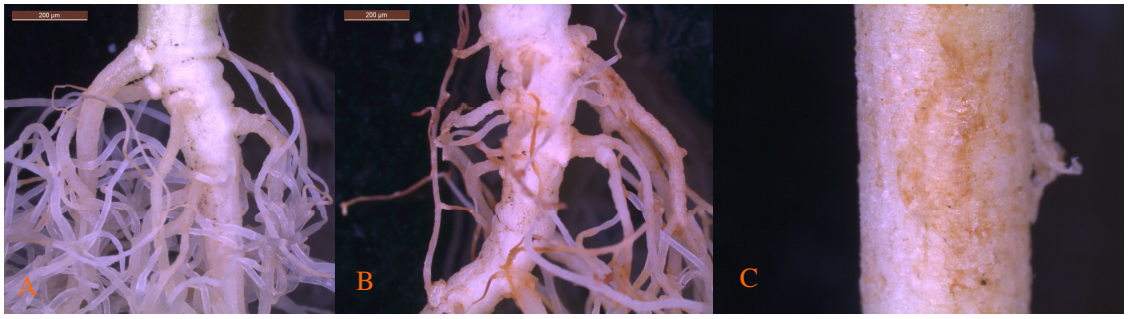


**Fig 4.6** Uninoculated and *P. sulcatum* inoculated roots of parsley seedlings grown in cabinets at A) uninoculated at 18°C, B) inoculated at 18°C, with infections of lateral roots, C) inoculated at 18°C lesion on tap root.

## 4.4 Discussion

### 4.4.1 Disease development in parsley crops

The plots at the Devon Meadows site were characterised by large, irregular patches of dead and missing plants, possibly due to poor germination, water logging, damping-off disease or a combination of these factors. At Clyde, on the other hand, plots exhibited a more even germination and growth. Root disease appeared to be associated with an overall "thinning" of the plant stand, rather than large patches of disease. The plants sampled from the control plots in both trials represent those that had survived, water logging and/or disease and the average disease incidence and severity recorded may be an underestimate of overall disease levels.

The trend in the development of root rot symptoms on surviving parsley was similar for both years during the autumn-winter and into the spring seasons, despite both crops being seeded six weeks apart. In both years, eight to ten weeks into crop development, only 50% of parsley roots were healthy. This indicates that root infections, which do not appear to affect plant foliage occur relatively early during crop development.

Further into the cropping season, approximately eight weeks before harvest, the proportion of healthy roots declined as the proportion of roots with mild symptoms increased and this trend continued until harvest. Interestingly the incidence of roots with severe root rot reached its highest six weeks before harvest in both trials. Severe root rot symptoms developed later in the 2011 crop than in the 2010 crop, which had lower soil temperatures than the former.

It is possible that the initial infection of parsley roots was associated with the warm conditions of autumn as well as rainfall events. Minchinton *et al.* (2006, 2007) observed that parsley root rot occurred in autumn, about a week after heavy rainfall events. In spring the decline in healthy roots and the corresponding rise in mild root rot symptoms may be associated with warmer spring temperatures as well as heavy rainfall events, especially in 2011 season. A major root rot pathogen of parsley, *P. sulcatum*, was shown to be pathogenic over a broad range of temperatures (see 4.4.3) and these temperatures were sufficient for infection by *P. sulcatum* and development of rots on parsley roots.

The information gained from these surveys should enable improvements to timing of targeted registered or permitted fungicide applications to reduce severe root rots, which largely account for weight losses and consequently crop losses, when conditions are conducive for epidemics.

### 4.4.2 Pathogen identification

All except two *Pythium* sp., identified in this study, *P. mastophorum* and *P. rostratifingens* were previously reported from parsley crops in the Cranbourne area (Minchinton *et al.* 2006, 2007). Pathogenicity of *P. sulcatum*, *P. irregulare*, and *P. diclinum* was demonstrated, indicating association of these species with root rot of parsley in Victoria. Isolates identified in the present study as members of *P. dissotocum* complex belong to the same phylogenetic clade B, subclade B2 as *P. diclinum* group previously reported (Minchinton *et al*. 2007). Species concept in the *Pythium* subclyde B2 requires revision (Lévesque and de Cock 2004).

*Pythium mastophorum* was reported from parsley in Germany (Krober and Sauthoff 1999) but not in Australia. *Pythium sulcatum*, *P. ultimum* var. *ultimum* and *P. intermedium* are reported as species commonly associated with root rots of various Apiaceae vegetables, including damping off in parsley in the USA (Hershman *et al.* 1986) and cavity spot in carrots in Europe (Hiltunen and White 2002, Suffert and Guibert 2007, Klemsdal *et al*. 2008). *Pythium sulcatum* has been reported as a predominant species associated with cavity spot of carrots in Australia (Davison and McKay 1998).

*Pythium rostratifingens* is a relatively recently described species (de Cock and Lévesque 2004) and has been isolated mainly from leaf litter, soil under apple trees and corn, but also from vines and

*Brassica, Triticum* and *Medicago* spp. (de Cock and Lévesque 2004, McLoad *et al*. 2009). Its pathogenicity to parsley is yet to be determined.

There are no records of *Phoma exiqua* var. *exigua* on parsley roots in Australia (Australian Plant Pest Database) but this species has been previously isolated from roots of parsnip (Machowicz-Stefaniak *et al*. 2008). The majority of *Phoma exiqua* var. *exigua* records in Australia come from leaf spots on beans (Fabaceae). This species has been also found on parsnip roots during monthly surveys of winter-grown parsnip crops (Refer to Chapter 7).

Identification of *Phoma exigua* var. *exigua* in this study supports a view that this pathogen is potentially part of a parsley root rot complex, together with fungi from *Rhizoctonia* and *Fusarium* genera. *Phoma* isolates were taken from lesions on lateral roots and from brown sunken lesions on tap roots of a relatively advanced (six week after seeding) parsley crop in the mid-July sampling. At this stage of crop development, the incidence of severe root rot symptoms started to increase. This may indicate that *Phoma exigua* var. *exigua* may play a role in parsley root rot.

### 4.4.3 Pathogenicity of *P. sulcatum* on parsley roots

The isolate of *P. sulcatum* caused root rot symptoms at 10°C, 18°C and 24°C. The result of this experiment supports the hypothesis that this species of *Pythium* could potentially cause root disease at soil temperatures as low as 10°C. Although the symptoms caused at this temperature are not as severe as at 18°C and 24°C, the root damage resulting from this infection reduced plant weights.

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Appendix 4

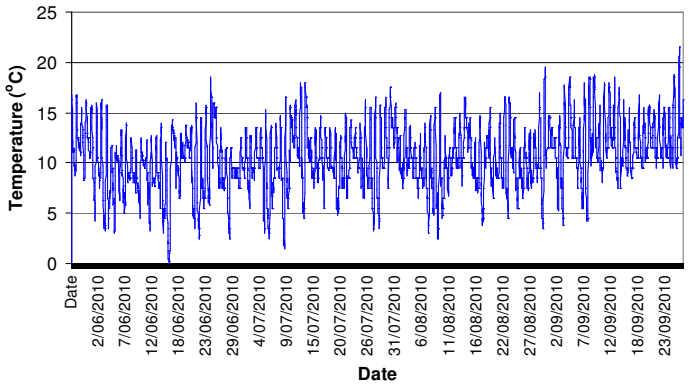


Fig. 1 Air temperature (ºC) for the Devon Meadows trial site collected from 27 May to 27 September 2010

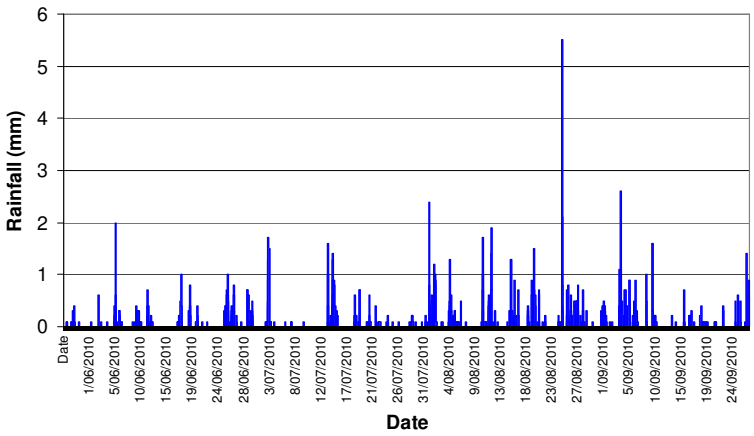


Fig 2. Rainfall (mm) for the Devon Meadows trial site collected from 27 May to 27 September 2010

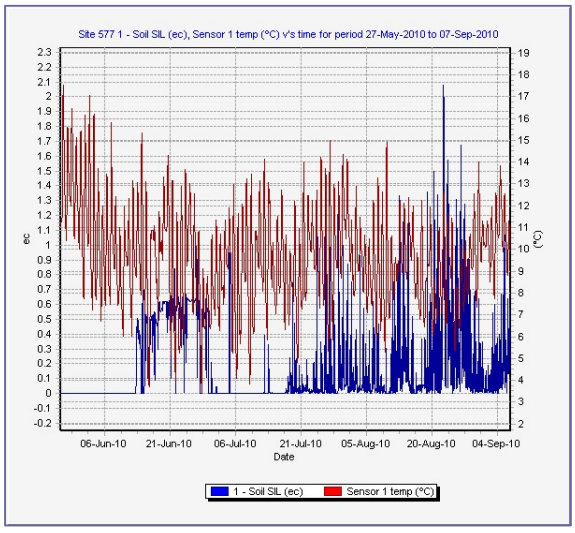


Fig. 3 Soil EC for the Devon Meadows trial site in 2010.

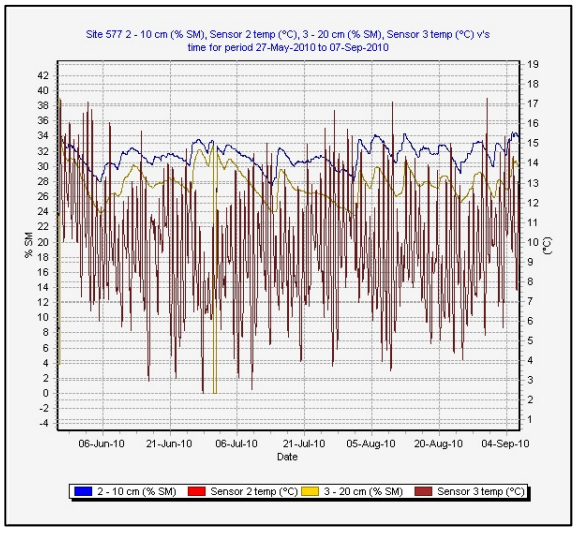


Fig. 4 Soil moisture (%) at 2-10 cm and at 3-20 cm (%) depths and soil temperate (ºC) at the Devon meadows trial site in 2010.

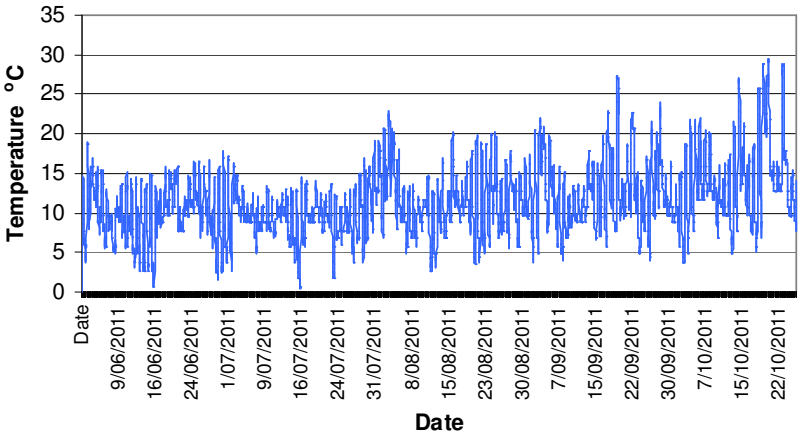


Fig 5. Air temperature (ºC) for the Clyde trial site collected from 1 June to 26 October 2011

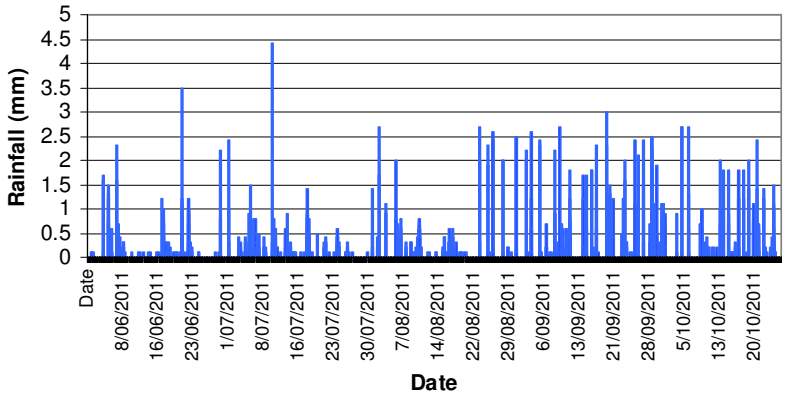


Fig. 6 Rainfall (mm) for the Clyde trial site collected from 1 June to 26 October 2011

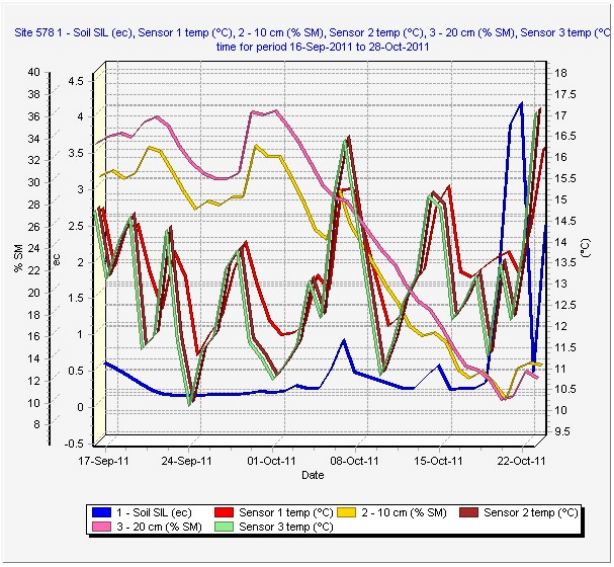


Fig. 7 Daily EC and soil moisture (%SM) and temperature (ºC) at 2 -10cm and 3-30cm data collected from for the Clyde trial site in 2011.

# Chapter 5

# Efficacy of three biocontrol agents to control root rot of coriander and parsley in an organic production area in Central Queensland

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## Summary

A field trial was undertaken to evaluate three biocontrol agents for their ability to control root rot of parsley in a commercial organic production. The trial was conducted near Biloela, Central Queensland, from the 9th June 2011 until 21st November 2011. Root disease was very low in all biological treatments and the Control, consequently data analysis was not warranted. No recommendations can be made as to the efficacy of the three biocontrol options under Central Queensland growing conditions. Results showed that yields were above average for all treatments due to good growing conditions and none of the biological treatments affected yield or quality.

## 5.1 Introduction

Six properties in the Biloela area of Central Queensland (an area of subtropical climate with predominantly hot summers and cool dry winters), produce in excess of 400 tonnes of certified organic coriander, dill and parsley for the processing market, annually. These crops are grown for their quality, as defined by essential oil profiles, rather than explicitly for yield. The crops are planted and harvested from late autumn through to late spring. In some years, however, conditions early in the season are cold and wet and later in the season hot and dry with a high water requirement, both conducive to high plant stress. Under these conditions plants develop root diseases, such as root rots. Growers would like to have some biocontrol options available as a management tool for these diseases.

On the properties producing certified organic herbs, soil health and crop vigour is maintained by the use of farm produced compost (mainly a cow manure & lucerne/grass hay base with required trace element corrections), green manures (lablab bean (Lablab purpureus)/forage sorghum/oats) and rotational crops (basil/parsley/maize/coriander). The soil is a high pH, bacterial dominated, brown loam creek soil. Irrigation of organic certified herb production is via trickle and minimal cultivation is practiced to maintain soil microorganisms.

Benefits of composts and green manures have been well documented. Composts have been shown to improve soil physical properties, enhance slow release of nutrients, and promote disease suppression and biological weed control (Ozores-Hampton and Obreza 1998, Stoffella *et al*. 1996). Composts have been shown to suppress damping off caused by Pythium spp., in potting mixes (Diánez *et al.* 2005, Pascual *et al.* 2002, Zhang *et al.* 1996). Long-term use of composts controlled soilborne Fusarium brown rot of Chinese yam in Japan (Hoitink and Fahy 1986) and was attributed to increased populations of Trichoderma spp. (Sekiguchi 1977). Microbial populations of composted soils have been observed to increase, especially that of antagonistic microorganisms such as Trichoderma spp. (Lunsden *et al*. 1986, Pascual *et al.* 2002). Green manures can have a range of effects, including biofumigation, altering the soil temperature, increasing water holding capacity, raising the soil microbial populations and promoting plant growth (Asirifi *et al.* 1994, Perez *et al.* 2008, Stirling and Eden 2008, Smolinska and Horbowicz 1999).

A field trial conducted in 2010, investigated the benefits of biocontrol agents for reducing root rot problems in coriander. The results from this trial were presented to growers at a meeting in March

2011 in Biloela and generated an interest in continuing to evaluate the three biocontrol agents, using parsley as the trial crop, due to its longer growing period.

Coriander crops are typically grown for six to ten weeks, depending on planting date, and harvested once. Parsley crops on the other hand are routinely harvested three times in any one season, but managed so that the last harvest occurs before the beginning of the wet season (before Christmas), to avoid severe plant stresses.

This chapter reports on trials to determine if three commercially available biocontrol formulations (Fulzyme®Plus, Polyversum™ and MicroPlus™) containing antagonistic organisms could (i) control root rot disease and/or (ii) improve yields on organically produced processing coriander and parsley under Central Queensland growing conditions.

## 5.2 Materials and Methods

Two field trials were conducted to evaluate the biological treatments on coriander and parsley crops. Both trials were located on one of the organically certified properties (PK Farming, Latitude 24.49 S, Longitude 150.57 E, on the property of P&K Stringer) and managed as part of a certified organic operation which produces lucerne hay, maize and various herbs (basil, coriander, dill, parsley, lemongrass, oregano and mint) for processing markets. The coriander trial was conducted in 2010 and the parsley trial in 2011. The parsley trial site, prior to the field trial, was flooded in early 2011 and received a covering of silt of approximately 10cm over the whole area with a higher concentration (+2cm) towards the creek end of the bed. Treatments for both field trials were the same and are listed in Table 5.1.

Anticipated plant growth rate of parsley (based on historical data period of 10 years):

- From emergence to Harvest 1 = 10 to 12 weeks of plant growth
- From harvest 1 to Harvest 2 = 6 weeks of plant growth
- From harvest 2 to Harvest 3 = 4 weeks of plant growth.

In a normal season it is anticipated that parsley Harvest 1 will produce approximately 60% of the yield of Harvest 2 and of Harvest 3. Expected commercial yield per 5.78 metres of plot:

- Harvest 1 7.31 kg
- Harvest 2 12.18 kg
- Harvest 3 12.18 kg

**Table 5.1 Treatments in the coriander and parsley field trials.**

| Trade name | Active | Company | Rate |
|---|---|---|---|
| Control | Untreated | na | na |
| Fulzyme ™ | *Bacillus subtilis* | Zadco for Quality Gro Pty. Ltd. | 2L/ha |
| Microplus™ | *Streptomyces lydicus* | Organic Farming Systems | 500g/500L/ha |
| Polyversum™ | *Pythium oligandrum* | Biopreparaty Ltd | 200g/400L |

### 5.2.1 Coriander field trial 2010

#### *5.2.1.1 Layout*

The trial was set up on a single bed (9.6m x 1.2m) which is part of a 1ha production block. The trial was subdivided into 2 blocks (Reps 1&2) with each block containing 4 randomised treatments. Treatment plots were 1.2m in length. The crop was maintained by the grower.

#### *5.2.1.2 Seed and planting*

The trial site was planted with seed from the cultivar, Slowbolt (Terranova Seeds). Planting date was the 21st August 2010.

#### *5.2.1.3 Treatment applications*

Treatments, listed in Table 5.1, were applied as a soil drench to each individual plot using a boom fitted with droppers (Fig. 5.5). Applications commenced on the 30th September 2010 and were applied weekly for four weeks, except application three was missed due to lack of access to the paddock after a 54mm rainfall event. No irrigation was applied to this site post planting.

#### *5.2.1.4 Assessments*

Plots were evaluated on a weekly basis to see if any significant visual differences were observed between treatments, prior to harvest on the 8th October 2010. Harvesting of leaf material was managed exactly as for commercial harvesting and measured as kg per plot. A sample of four roots from each treatment plot and 200g soil sample from each plot were sent to Crop Health Service, DPI Knoxfield, Victoria to conduct fungal isolations from lesions on roots and soil.

### 5.2.2 Parsley field trial 2011

#### *5.2.2.1 Layout*

The trial was set up on a single bed (185m x 1.2m) which is part of a 1ha production block. The trial bed was subdivided into 8 blocks with each block containing 4 randomised treatments. Treatment plots were 5.78m in length.

#### *5.2.2.2 Seed and planting*

The trial site was planted with the seed of cultivar, 'Rialto', from Bejo Seeds. Planting date was the 9th June 2011 (Fig. 5.1), with seed emergence on the 30th June 2011 (Fig. 5.2). Initial growth was very slow due to cold conditions, compare photos from 3rd August 2011 (Fig. 5.3) with growth at the 30th August 2011 (Fig. 5.4).

**Fig 5.1.** Trial site pre emergence

**Fig 5.2.** Checking emergence

**Fig. 5.3.** Slow initial growth on 03/08/2011.

**Fig. 5.4.** Growth on 30/08/2011.

### *5.2.2.3 Treatments and application*

Treatments listed in Table 5.1 were applied mechanically as described earlier to each block (Fig. 5.5):

- Pre Harvest 1 3rd August 2011
  9th September 2011
- Pre Harvest 2 10th October 2011

**Fig. 5.5.** Application of *B.subtilis.*

### *5.2.2.4 Soil and Soil Foodweb analysis*

Soil samples were taken pre plant and post harvest, and analysed for mineral content by Environmental Analysis Laboratory, Southern Cross University, for biological activity by Nutri-Tech Solutions (7 Harvest Rd., Yandina Qld., 4561) and by The Soil Foodweb Institute (1 Crawford Road, Lismore, NSW, 2450). These analyses are part of an ongoing annual monitoring programme at this site.

### *5.2.2.5 Assessments*

*Foliage vigour:* Plots were evaluated on a weekly basis to determine visual differences in growth between treatments, prior to the first harvest, and at the first and second harvests. Foliage vigour at harvest was appraised on a scale of 0-3, where 0, poor growth and 3, tallest growth with best "feel" to foliage.

***Foliage yield:*** Two harvests were undertaken for fresh weight of foliage in this trial (Harvest 1 and Harvest 2). Harvesting of leaf material was managed exactly as for commercial harvesting and measured as kg per plot. Additionally the yield of the adjacent crop was monitored and presented as per trial crops.

***Harvesting:***

Three harvests were undertaken in total:

- Harvest 1: Mechanical leaf harvest of each plot
- Harvest 2: Mechanical leaf harvest of each plot
- Harvest 3: Mechanical root harvest of each plot

Harvesting dates:

- Harvest 1 4th October 2011
- Harvest 2 21st November 2011
- Harvest 3 21st November 2011
-

Harvest measurements:

- Harvest 1 Total yield per plot
- Harvest 2 Total yield per plot
- Harvest 3 Four random samples of root material per plot

Root health: Roots were harvested with a machine used to get rid of patches of nut grass. In each plot, roots were visually appraised for (i) lateral root browning; (ii) crown rot; (iii) lower tap root rot and (iv) cracking of tap root. Additionally, roots of each treatment were combined and four roots were randomly selected from each and sent to the project team at DPI Knoxfield, Victoria for isolation and identification of pathogens. Fungal isolations were made from lesions on roots and from symptomless parsley roots by rinsing plant tissue in sterile distilled water and plating onto water agar (Oxoid). Hyphal tips were transferred onto potato dextrose agar (Oxoid). Fungal genera were identified microscopically.

#### *5.2.2.6 Statistical analysis*

Disease incidence on roots was not analysed as there were only a few plants with symptoms. Means and standard deviations were estimated for foliage weight and vigour for all treatments. Significant differences between treatments were investigated using ANOVA. OLS Regression models were used to estimate differences in outcomes for individual treatment groups relative to Controls. When ANOVA indicated significant differences, means were separated using lsd. All data manipulation and analyses were performed using Stata/SE 12.0, by Masha Fridman, VABC, DPI Victoria.

## 5.3 Results

### 5.3.1 Coriander field trial 2010

Disease pressure was low and no above ground symptoms were observed with weekly visual assessments of coriander plants. There was no difference between treatments for yield or quality, with all yields around 2kg/metre of row.

Crop Health Services, DPI Vic., Knoxfield reported that no *Pythium* species were isolated from necrotic roots of the Microplus™ and Polyversum™ treated plants, but *Pythium* species were isolated from the Fulyzme™ treated plants and untreated Control plants (Table 5.2). *Rhizoctonia, Fusarium* and *Macrophomina* were isolated from all treatments. Baiting of soils detected *Pythium* and *Fusarium* species in all treatments, but no *Phytophthora* species were isolated. The presence of biocontrol agents such as *Trichoderma* spp. was not reported by Crop Health Services as only identification of pathogens was requested.

**Table 5.2 List of fungal and oomycete genera isolated from necrotic coriander roots and baits of surrounding soils**

| Symptom or source | Treatment | Genera identified |
|---|---|---|
| Necrotic roots | Control | *Pythium* spp., *Rhizoctonia, Fusarium, Macrophomina* |
| | Fulzyme ™ | *Pythium* spp., *Rhizoctonia, Fusarium, Macrophomina* |
| | Microplus™ | *Rhizoctonia, Fusarium, Macrophomina* |
| | Polyversum™ | *Rhizoctonia, Fusarium, Macrophomina* |
| Soil baits | Control | *Pythium* spp. *Fusarium* spp. |
| | Fulzyme ™ | *Pythium* spp. *Fusarium* spp. |
| | Microplus™ | *Pythium* spp. *Fusarium* spp. |
| | Polyversum™ | *Pythium* spp. *Fusarium* spp. |

## 5.3.2 Parsley field trial 2011

### *5.3.2.1 Harvest*

The techniques for mechanically harvesting parsley are shown in Figs. 5.7-5.12.

**Fig. 5.7.** Leaf harvest of plot at commercial height

**Fig. 5.8.** Stopping at end of plot to record weight and clear machine

**Fig. 5.9.** 1ha of harvested parsley leaf

**Fig. 5.10.** Leaf remaining after harvest

**Fig. 5.11.** Harvesting parsley roots

**Fig. 5.12.** Harvested roots

### *5.3.2.2 Foliage vigour*

Plants visually evaluated for vigour on a weekly basis, until pre Harvest 1, showed no differences in vigour between treatments, plots and/or blocks. There were also no significant differences in vigour between treatments at Harvest 2 (Table 5.3, Fig. 5.13). There was no correlation between foliage vigour and yield at Harvest 2.

**Table 5.3 Effect of three biocontrol treatments on vigour and yield of parsley at Harvest 1 (4th October 2011) and Harvest 2.1 (21st November 2011).**

| Treatment | Harvest 1 Mean yield ± sd (kg/plot) | Harvest 2 Mean yield ± sd (kg/plot) | Mean vigour at Harvest 2 ± sd (Scale 0-3)[1] |
|---|---|---|---|
| *Control* | 12.9 ± 4.4 | 14.7 ± 2.5 | 2.6 ± (0.4) |
| *B.subtilis* | 13.0 ± 4.4 | 14.2 ± 4.1 | 2.4 ± (0.5) |
| *S. lydicus* | 14.5 ± 4.7 | 15.0 ± 4.5 | 2.5 ± (0.6) |
| *P. oligandrum* | 13.7 ± 3.4 | 14.4 ± 3.2 | 2.4 ± (0.5) |
| P-value | 0.811 (ns) | 0.953 (ns) | 0.824 (ns) |

ns, not significant; sd, standard deviation; [1]Scale of 0-3, where 0, poor growth and 3, tallest growth.

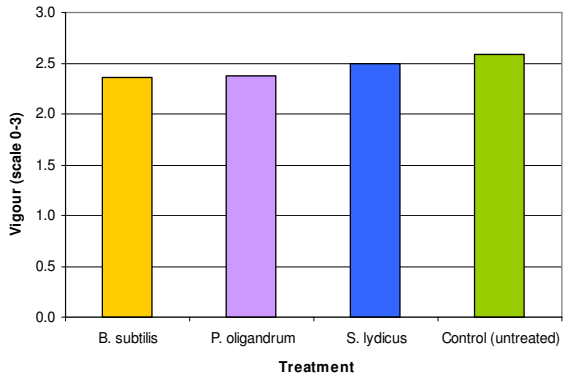


**Fig. 5.13.** Effect of three biocontrol treatments on vigour of parsley at Harvest 2

### *5.3.2.3 Harvest of foliage*

There was no significant difference in the yield (fresh weight) of parsley foliage (kg/plot) between treatments and the untreated Control for the first or second Harvests (Table 5.1, Figs. 5.14 and 5.15). The variation in each treatment (sd) was large and nearly a quarter of the actual mean. Given that there was no significant difference between treatments, fresh weights from all plots for each treatment were combined to examine the overall crop yield per treatment. The total yield (kg/plot) was also calculated for each treatment (Fig. 5.16).

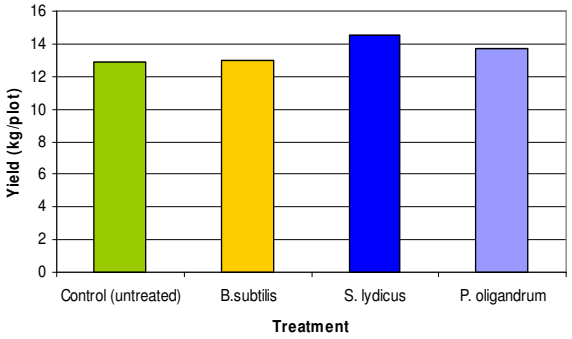


**Fig. 5.14.** Effect of treatments on yield of parsley foliage at the Harvest 1.

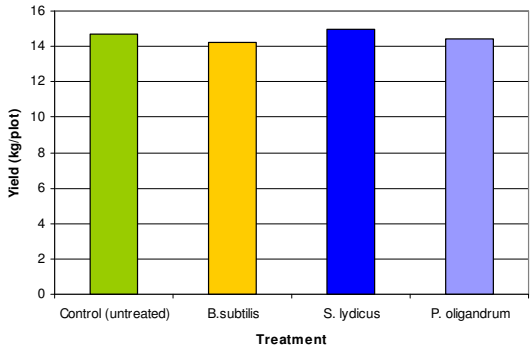


**Fig. 5.15.** Effect of treatments on yield of parsley foliage at the Harvest 2.

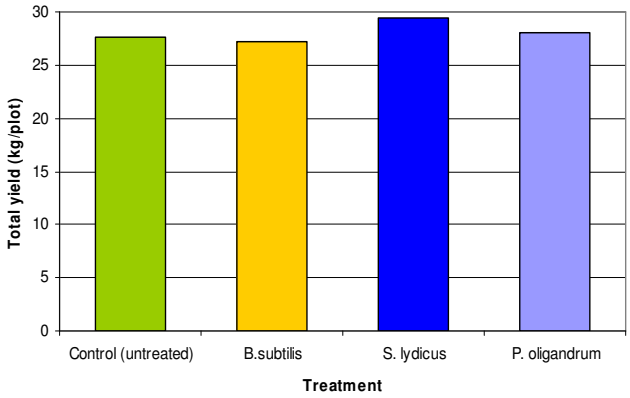


**Fig. 5.16.** Effect of treatments on total crop yield (Harvest 1 and Harvest 2 combined).

It is to be noted that average yields were above the 10 year historical data set (Table 5.4). In the adjacent crop the yields from the first harvest were exceptional. This anomaly was found over the whole production block and above average yield of 20kg per 5.78m was achieved for the 3rd commercial harvest (the trial plot had 2 harvests only), without any loss of quality (as measured by essential oil profile). One explanation offered is that the deposit of extra silty loam from the flooding has influenced yields by increased minerals deposited in silt being available to both plants and microbes.

**Table 5.4 Average yield per trial plot (Expected & Actual)**

| Harvest No. | Harvest date | Expected yield (kg/plot) | Actual yield (kg/plot) |
|---|---|---|---|
| 1 | 4/10/2011 | 7.31 | 13.74 |
| 2 | 21/11/2011 | 12.18 | 14.71 |

### *5.3.2.4 Roots*

Overall root health was excellent with no sign of lateral browning or crown rot (Figs. 5.17- 5.20). Only 6% of roots had a low level of lower tap root rot. Root cracking was found in two samples only. Due to the extremely low incidence of disease symptoms no statistical analysis was warranted.

Isolation of fungi from various symptoms on the tap roots and from the symptomless lateral roots yielded the fungi listed in Table 5.5. Interestingly no *Phytophthora* or *Pythium* species, including, *P. oligandrum,* were isolated from symptoms on tap roots or from symptomless laterals. *Trichoderma* was isolated from all treatments and is considered to be a biocontrol agent. *Epicoccum* is a saprophyte. *Alternaria* can be saprophytic or pathogenic. *Rhizoctonia* spp. were isolated from both the bacterial based biocontrol agents and can be saprophytic or a pathogen. *Microdochium*, *Cladosporium* or *Fusarium* are generally pathogenic, weakly pathogenic or saprophytic.

**Table 5.5 List of fungal genera isolated from symptoms on parsley tap roots and from symptomless lateral roots.**

| Treatment | Symptom | Genera Identified |
|---|---|---|
| *B. subtilis* | Black superficial lesion on the lower tap root | *Trichoderma* |
| | | *Rhizoctonia* |
| | Symptomless laterals | *R. solani* |
| | | *Epicoccum* |
| | Brown lesion on the lower tap root | *Alternaria* |
| | | *unknown* |
| *S. lydicus* | Symptomless laterals | *Epicoccum* |
| | | *R. solani* |
| | | *Trichoderma* |
| | | *Microdochium* |
| | | *unknown* |
| | Cracks on the tap root | *Alternaria* |
| *P. oligandrum* | Symptomless laterals | *Trichoderma* |
| Control | Symptomless laterals | *Trichoderma* |
| | | *Alternaria* |
| | Black lesions on the lower tap root | *Cladosporium or Fusarium* |

### *5.3.2.5 Soil and Foodweb analyses*

Soil mineral analyses results are found in Appendix 5.6, Table 5.6, and reporting on those and the Soil Foodweb analyses taken during the trial can be found in Section 5.4.2

**Fig. 5.17.** Parsley roots from a Control plot.

**Fig. 5.18.** Parsley roots from a *B. subtilis* plot.

**Fig. 5.19.** Parsley roots from a *S. lydicus* plot

**Fig. 5.20.** Parsley roots from a *P. oligandrum* plot.

## 5.4 Discussion

Although coriander had necrotic symptoms on roots, there were no above ground symptoms and no obvious yield loss. This suggests coriander can tolerate a certain level of diseased roots, without loss of yield. It is possible soil conditions were not conducive for the pathogenic fungi to proliferate and cause severe root rots. *Pythium* spp. were not isolated from coriander roots treated with two of the three biological control agents tested (Microplus™ or Polyversum™), suggesting these treatments may be worth pursuing under conditions of higher disease pressure.

In the parsley trial there was little or no root rot present in any of the biocontrol treatments or the untreated Control, therefore treatment comparisons are not possible to control parsley root rot. The biocontrol treatments had no effect on yield, quality or vigour of parsley. However it is possible that larger plots with more replication or a higher frequency of treatment applications may increase the

population of biocontrol organisms and reduce field variability, and under soil conditions conducive for root infections, treatments can be better assessed.

### 5.4.1 Soil tests (parsley trial only) and their relevance

In the parsley trial the grower achieved above average historical yields (with a proven sound yield/quality base) with excellent crop health (roots and foliage). Yet mineral levels are above desirable levels and microbial analysis indicates a soil poor in health. It would be interesting to know what scientific basis these recommendations are made on.

### 5.4.2 Soil Foodweb analyses indicated:

***Pre Planting analysis:***

- High level of active and total bacteria (nitrogen receptacles)
- Negligible active fungi and inadequate levels of total fungi (converters of hard to digest material)
- Poor total fungi/bacteria ratio
- 0% mycorrhizal colonization (colonization is important for release of immobile nutrients and root protection)
- Negligible levels of flagellates and amoebae and barely adequate levels of ciliates (all protozoa which play an important role in mineralising nutrients and making them available for use by plants and other soil microbes)
- High levels of total nematodes identified as Bacterial feeders (per gram) – Acrobeles 0.52, Acrobeloides 2.08, Cephalobus 14.03, Chiloplacus (stubby) 0.52, Fungal feeders – Tylencholaimellus 0.52 and Fungal/Root Feeders - Aphelenchus 3.12. good levels of active and total bacteria

***Post Harvest analysis***

- Good levels of active and total bacteria
- Good levels of fungi, with lower levels of total fungi
- Acceptable total fungi/bacteria ratio
- 0% mycorrhizal colonization
- Negligible levels of flagellates and amoebae, barely adequate levels of ciliates
- High levels of total nematodes identified as Bacterial feeders (per gram) – Acrobeles 0.36, Caenorhabditis 5.43, Panagrolaimus 2.90, Plectus (st) 0.36, Rhabditis 1.81, Fungal/Root Feeders –Aphelenchoides (Foliar nematodes) 2.53, Aphelenchus 0.36, Ditylenchus (Stem and bulb nematode) 0.36 and Root Feeders – Pratlenchus (Lesion nematode) 0.36

### *Trichoderma spp.*

The above analyses failed to identify in the report any *Trichoderma* spp. These species are known to be present in nearly all soils and grow and proliferate best where there are abundant healthy roots (Harman GE, 2012). They are important natural biocontrol agents, now commercially manufactured. They were identified as being present on harvested root material from all four treatments, but were not identified in the Soil Foodweb analyses because this identification was not requested. The persistence of *Trichoderma* spp. in the crop may be contributing to the general lack of disease on the site, as Trichoderma spp. have been observed to increase in composted soils (Lumsden *et al.* 1986, Pascual et al. 2002).

***Nutri-tech soil mineral analyses (Appendix 5.1) highlight:***

- Low Calcium levels
- Excess Magnesium
- Poor Calcium/Magnesium ratio
- Phosphorous, zinc and boron deficiencies
- Good Carbon/Nitrogen ratio
- High post harvest sodium level

Corrective measures include additions of organic carbon, calcium, phosphorous, sulphur, silicon, boron, iron, manganese, copper, zinc and fungal stimulants.

It is interesting to note that Nutri-tech also take a measurement of the paramagnetism of the soil. Paramagnetism has been shown to enhance root development and stimulate the multiplication of beneficial organisms (Dykstra 2000). The effect of paramagnetism is magnified in the presence of compost and organic matter (The Nutri-tech Management Approach). In paramagnetic terms a soil with a µcgs of >300 is described as a "very good soil". Both pre plant and post harvest soil µcgs are >400, with an increase from pre plant to post harvest of 70 µcgs.

Given the excellent yields and sound historical yield base, are paramagnetism theories a key to plant health that needs more serious consideration.

Soil health is of prime importance to growers, but the vast amount of differing information, methodologies and opinions available to them, creates a major difficulty in the understanding of the reactions and interactions which attribute to this soil health, particularly as growers embrace the change from conventional to organic farming. Understandably this forces growers to continue their reliance on, and have difficulty in moving from, experience and "gut feel".

Soil mineral and foodweb analyses as reported above can only be relevant if samples are taken in a manner that can be replicated, and over an extended period of time – snapshots are ineffective.

Looking to a more sustainable future, growers and researchers need to be aware that within this "new" field of farming, because there is little scientific evidence currently available that links biological indicators to productivity (although there is good evidence of links between biological indicators and soil processes) they need to work together, across different disciplines, to determine, given a fixed budget, which measurements will give the most useful information.

Results, such as occurred with this trial, highlight the need for such work to be long term in order to better understand the disease trigger points and the associated contributions of soil inputs and soil health.

Manufactured scenarios, such as inoculation with Pythium species known to be pathogenic to parsley to speed up the process, could be undertaken, but the risk involved in the introduction of such inoculation into a production area is not acceptable.

In conclusion, the crop rotation practices, the maintenance of soil health, the lack of a major rain event, which is known to promote disease, and the avoidance of growing the crop "out of season", may have contributed to low levels of root rot and the excellent yields on the site.

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# 5.6 Appendix

**Table 5.6 Soil analysis at pre-planting for the trial site at Biloela Qld.**

| PK Stringer Parsley Trials Soil Analyses | | | | Pre plant | Post harvest | Desirable |
|---|---|---|---|---|---|---|
| | | | | 10/06/2011 | 21/11/2011 | Range |
| Method | Nutrient | | Units | | | |
| Morgan 1 | Calcium | Ca | mg/kg | 1279 | 2727 | *375* |
| | Magnesium | Mg | mg/kg | 907 | 1475 | *60* |
| | Potassium | K | mg/kg | 151 | 211 | *60* |
| | Phosphorus | P | mg/kg | 4.6 | 18.5 | *10* |
| Colwell | Phosphorus | P | mg/kg | 54 | 122 | *45* |
| Bray2 | Phosphorus | P | mg/kg | 131 | 37 | *48* |
| KCl | Nitrate Nitrogen | N | mg/kg | 25.0 | 43.7 | *10* |
| | Ammonium Nitrogen | N | mg/kg | 9.4 | 8.4 | *15* |
| | Sulfur | S | mg/kg | 8.0 | 18.9 | *8* |
| 1:5 Water | pH | | units | 7.61 | 8.30 | *6.3* |
| | Conductivity | | dS/m | 0.151 | 0.239 | *0.12* |
| Calculation | Organic Matter | | % OM | 4.5 | 2.6 | *>3.5* |
| Ammonium Acetate + Calculations | Calcium | Ca | $cmol^+/Kg$ | 20.22 | 15.23 | *5* |
| | | Ca | kg/ha | 9057 | 6823 | *2240* |
| | | Ca | mg/kg | 4043 | 3046 | *1000* |
| | Magnesium | Mg | $cmol^+/Kg$ | 16.68 | 13.72 | *1.2* |
| | | Mg | kg/ha | 4483 | 3688 | *325* |
| | | Mg | mg/kg | 2001 | 1646 | *145* |
| | Potassium | K | $cmol^+/Kg$ | 1.11 | 0.75 | *0.4* |
| | | K | kg/ha | 973 | 659 | *336* |
| | | K | mg/kg | 434 | 294 | *150* |
| | Sodium | Na | $cmol^+/Kg$ | 0.22 | 1.46 | *0.2* |
| | | Na | kg/ha | 114 | 755 | *113* |
| | | Na | mg/kg | 51 | 337 | *51* |
| KCl | Aluminium | Al | $cmol^+/Kg$ | 0.04 | | *0.5* |
| | | Al | kg/ha | 8 | 16 | |
| | | Al | mg/kg | 3 | 7 | |
| Acidity Titration | Hydrogen | $H^+$ | $cmol^+/Kg$ | 0 | | *0.5* |
| | | $H^+$ | kg/ha | 0 | | *9* |
| | | $H^+$ | mg/kg | 0 | | *5* |
| Calculation | Total Cation Exchange Capacity | | M.E./100g | 38.27 | 31.25 | |
| Base Saturation Calculations | Calcium | Ca | % | 52.8 | 48.9 | *69* |
| | Magnesium | Mg | % | 43.6 | 44 | *16* |
| | Potassium | K | % | 2.9 | 2.4 | *5* |
| | Sodium - ESP | Na | % | 0.6 | 4.7 | *3* |
| | Aluminium | Al | % | 0.1 | 0.0 | *7* |
| | Hydrogen | $H^+$ | % | 0.0 | 0.3 | |
| Calculation | Calcium/ Magnesium Ratio | | ratio | 1.2 | 1.11 | *4.3* |
| DTPA | Zinc | Zn | mg/kg | 1.8 | 0.8 | *4* |
| | Manganese | Mn | mg/kg | 24 | 11.9 | *18* |
| | Iron | Fe | mg/kg | 33 | 19.5 | *18* |
| | Copper | Cu | mg/kg | 2.1 | 1.4 | *1.6* |
| $CaCl_2$ | Boron | B | mg/kg | 0.66 | 0.50 | *1.4* |
| | Silicon | Si | mg/kg | 78 | 59 | *40* |
| LECO IR Analyser | Total Carbon | C | % | 2.59 | 1.51 | *>2* |
| | Total Nitrogen | N | % | 0.17 | 0.10 | *>0.2* |
| Calculation | Carbon/ Nitrogen Ratio | | ratio | 15.4 | 14.70 | *10 to 12* |
| | Basic Texture | t | | Loam | Loam | |
| | Basic Colour | c | | Brownish | Brownish | |
| Calculation | Chloride Estimate | | equiv. ppm | 97 | 153 | |
| Total Acid Extractable | Molybdenum | Mo | mg/kg | 3.46 | 0.66 | *0.5-3.0* |
| | Cobalt | Co | mg/kg | 24.6 | 21.4 | *5.0-50* |
| | Selenium | Se | mg/kg | 1.17 | 0.59 | *0.1-2.0* |
| PCSM | Paramagnetism | | µcgs | 430 | 500 | 200+ |

# Chapter 6

# Disease management strategies to control root rot of parsnip


## Summary

Over the course of three years, seven field trials were conducted to evaluate a range of fungicides, biocontrol agents and cultural controls for parsnip canker associated with *Pythium* spp. and other fungi. The systemic fungicide, Ridomil®Gold 25G (metalaxyl), which is specific for oomycetes, was the only treatment that reduced various symptoms of root rot by 48% to 62% and increased the proportion of marketable parsnips by 34%, thus implicating oomycetes (specifically *Pythium* spp. as no *Phytophthora* spp. were isolated) in canker. Amistar®Top (azoxystrobin) reduced root symptoms by 25% to 47%, but this did not translate to increased yields. Folicur® (tebuconazole) reduced root rots by 27% but did not improve yield. The biofungicides (*Bacillus subtilis* and *Streptomyces lydicus*), Hilling the soil, warming the crop with Fleece™, Stand SKH™ (silicon) and Mulch Enviromix™ did not control canker or improve yield. Foliage symptoms caused by *I. perplexans* were reduced with Folicur® and Mulch Enviromix™; while five treatments stimulated plant growth, Hilling, *Bacillus*, Fleece™, Folicur® and Mulch Enviromix™.


## 6.1 Introduction

***The parsnip industry***

Losses from parsnip canker were estimated to be in the vicinity of A$3 million over a two year period in an industry worth $20 million annually. Victoria produces 80% of Australia's parsnip production. Parsnip canker is worst in crops planted in February-March, which are grown through winter and harvested in October (Minchinton *et al*. 2008). Growers report the pick-out rate in these crops can be as low as 20%. In some plantings up to 100% of the crop has been lost to canker.

***Parsnip canker in Australia***

Although canker is usually attributed to *Itersonilia perplexans,* a basidiomycete, many fungi can also cause canker symptoms on parsnip crowns in Australia (Minchinton *et al.* 2008). In field trials conducted in Victoria, Australia, using fungicides which targeted specific groups of fungi, only Ridomil®Gold 25G (metalaxyl) had efficacy in reducing the incidence of the disease by 70% (significantly) and 43% (although not significant at 5%), suggesting an oomycete was involved in the symptoms. Additionally, pathogenicity tests indicated that oomycete organisms such as *Pythium* spp. may have a role in pre-disposing parsnip roots to canker, (Minchinton *et al.* 2008).

The parsnip industry has not previously managed *Pythium* spp., but does manage leaf spots, including those caused by *I. perplexans*, with several applications of preventative dithiocarbamate fungicides, and powdery mildew, if severe, with DMI fungicides. As parsnips are a 5- to 7-month crop, frequent applications of expensive fungicides, such as metalaxyl, are uneconomical; consequently timing of such applications needs to be targeted to when they will have the most efficacy. Frequent use of metalaxyl, a systemic oomycete specific fungicide, may cause development of pathogens resistant to the fungicide as well as cause its accelerated biological degradation in soil (Davison and McKay 2001, Minchinton *et al*. 2006, 2007, 2008). Previous research by Minchinton *et al*. (2008) also suggests that metalaxyl alone will not control this disease which implies there is a complex of fungi associated with canker.

***Pythium spp.***

*Pythium* spp., are oomycetes, fungus-like organisms, that attack root hairs and lateral roots causing rots, hindering nutrient uptake and causing reduced growth (Harvey 2006). They can cause pre- and post-emergence damping off of seedlings, either alone or in conjunction with other pathogens. They are the causal organism of cavity spot of carrot (Davison and McKay 2001) and are associated with

stress of a number of vegetable crops (Porter *et al.* 2008). Work reported in Chapter 7 indicates they were the first organisms to be isolated from parsnip roots during the cooler weather. Additionally they are very pathogenic on parsley, Apiaceae, during cool conditions (Minchinton *et al.* 2006, 2007).

***Causes of parsnip canker world-wide***

World wide parsnip canker has been attributed to the fungus *Itersonilia perplexans,* a basidiomycete, but also *Phoma camplanata*, *Streptomyces scabies*, *Cylindrocarpon destructans*, *Centrospora acerina* (*Mycocentrospora*), carrot fly larvae, *Psila rosa,* and eelworm, *Anguillulina dipsaci* (Cerkauskas 1985, Channon 1965, Channon and Thomson 1981, Fox 2002, Jones 1953, Stone 1954, Walton 1037). In Canada *P. camplanata* was the cause of major disease problems (Cerkauskas 1987). In the UK the main culprit and the one which has received most research resources is *I. perplexans*. Balistospores are released diurnally with maximum release at dawn and minimum in the afternoon (Channon 1963b). There was a higher incidence of the disease on heavy soils compared to lighter soils, but canker was not related to nutrient deficiency (Green and Hewlett 1950). Brown *et al*. (1964) working in the UK reported that the incidence of canker was correlated with rainfall.

***Parsnip canker management world wide***

Overseas, a reduction of up to 45% in the incidence of canker associated with *I. perplexans* was achieved by hilling soil over parsnip crowns (Brandenburg 1965, Channon 1963ab), but this still left a large incidence of canker in the crop. The practice of hilling, which covered parsnip crowns with soil, was done to prevent *I. perplexans* ballistospores from direct contact with crowns thus reducing the potential for infection and additionally encouraging the breakdown of *I. perplexans* ballistospores released from foliage (Channon 1963b). Chemical or steam air treatment of seed was very successful in removing *Itersonila* from seed coats and trash, respectively (Smith 1966). Crop rotation and crop hygiene have also been suggested by Smith (1967) as the fungus survives in parsnip crop debris, but there is no data on the long term efficacy of the practice. Victorian growers, however, are already practising long rotations. Plastic mulch was trailed in New Zealand where it created warm moist conditions which promoted disease (Brandenberg 1965).

Fungicides considered to control canker in the US were copper sprays applied every 7 to 10 days (Chupp and Sharp 1960); maneb at fortnightly intervals was recommended in New Zealand (Brandenberg 1965), while chlorothalonil and mancozeb had efficacy in Canada (Cerkauskas and McGarvey 1988). More recently in the UK tebuconazole, was registered for canker control on parsnips (Assured Food Standards 2006). Smith (1967) reported that *Bacillus subtilis* and *Streptomyces* spp. were antagonistic to *Itersonilia in vitro* in Australia, but they were not tested in the field.

***Management options for Pythium spp. on parsnips***

Options for management of *Pythium* spp. pre-disposing parsnip roots to canker are needed for the industry. These options could include alternative fungicides such as *Streptomyces lydicus* and *Bacillus subtilis*; cultural practices such as hilling and composts; warming the ground with Fleece and composts/mulches; applications of silicon which promotes disease resistance and reduces cracking of tap roots; and systemic fungicides such as tebuconazole and azoxystrobin which could be alternatives to metalaxyl.

***Biological fungicides***

The biological fungicide, *S. lydicus,* is used to control soil-borne plant root-rotting and damping off fungi, including basidiomyces such as *Armillaria* spp., *Rhizoctonia* spp. and oomycetes such as *Pythium* spp. (Copping 2001). It is a soil actinomycete which colonises plant root tips, producing and excreting anti-fungal metabolites into the surrounding rhizosphere; additionally it is a mycoparasite of spores and hypha of fungal root pathogens. The WYE108 strain is also a plant growth promoting bacterium (Tokala *et al.* 2002). A formulation is available in Australia as Microplus™.

The biological fungicide *Bacillus subtilis* is a spore forming gram-positive bacteria which colonises plant root systems where it competes with and suppresses pathogens (SAR) and thus stimulates plant growth (Tomlin 2003, Ongena *et al*. 2007). It has been used as a seed treatment (Tomlin 2003) and as

a foliar spray to control fungal and bacterial diseases caused by *Alternaria* spp. and *Aspergillus* spp., *Botrytis cinerea, Erysiphe* spp., *Fusarium* spp., *Peronospora* spp., *Rhizoctonia solani*, *Phytophthora* spp. and *Xanthermonas* spp. (Copping 2001, Tomlin 2003). A commercial formulation significantly increased root mass of *Pythium* infected tomato roots of plants grown in a greenhouse (Ingram 2005). Fulzyme™ is a commercial formulation of *B. subtilis* available in Australia.

***Composts and composted mulches***

Composts or composted mulches have been used in agriculture for centuries, but largely fell out of favour with the introduction of inorganic fertilizers. Composts or composted mulch were described by Raviv (2008) as organic matter that has undergone long, thermophilic and aerobic decomposition. Benefits of composts applied to agricultural systems are improved soil structure, weed control, suppression of diseases, release of nutrients, increased microbial activity of soil, retention of moisture and the reduction of erosion (Ozores-Hampton and Obreza 1998, Darby *et al.* 2006). Recent work by Bailey and Lazarovits (2003) suggests that composts formed from waste products could be used to manage a wide range of diseases. Damping off caused by *Pythium* and *Rhizoctonia* spp. was suppressed by sewage sludge, probably by increasing soil microbial activity. Composted bark was shown to be suppressive of *Pythium* spp. causing damping off (Erhart *et al.* 1999) and when pine bark was included in compost it reduced stubble decline of sugarcane caused by *P. arrhenomanes* (Dissanayaka and Hoy 1999). Composted potting mix containing bark was shown to suppress seedling diseases caused by *Pythium* spp. (Diánez *et al.* 2005).

***Fleece***

Fleece™ is used to cover some crops in the United Kingdom to prevent carrot fly larvae attack on basal stems, especially on organically grown produce and to raise the temperature in the crop to encourage growth. It has been trialled on carrots in Tasmania and brought the crop to harvest slightly earlier than would otherwise be expected (Michael Ertler pers. comm.).

***Silicon***

In carrot trials conducted in Tasmania, six applications of Stand SKH™ to foliage significantly reduced splitting and cracks on taproots, improved the pack out by 88% and was cost effective (Hay *et al.* 2009). The many benefits of silicon to agriculture have been summarised by Datnoff *et al.* 2001 and include increased crop growth and yield, improved tolerance to stress and resistance to diseases. Applications of soluble silicon were shown to reduce *Pythium* root rot in hydroponically grown cucumbers (Chérif *et al.* 1992, 1994). The defence reaction is considered to be phenolic based (Datnoff *et al.* 2001).

***Systemic fungicides***

The fungicide Folicur® (tebuconazole), registered for use against parsnip canker in the UK, is a Group 3 DMI fungicide (Crop Life Australia) with systemic, protective and curative action which is rapidly absorbed by the plant and translocated acropetally (Tomlin 2003). It is active against a wide range of diseases including bascidiomycetes, such as rusts (*Puccinia* spp.) and blister blight (*Exobasidium vexans*). Consequently it could have potential to control parsnip canker associated with *Itersonilia perplexans*, also a basidiomycete which produces lesions on leaves, petioles and crowns of parsnips. Theoretically, controlling *I. perplexans* could give an indication of diseases or crop losses associated with this pathogen, as well as with other pathogens.

A new group of fungicides, the strobulurins, do not appear to have been evaluated for efficacy of any diseases on parsnips. Amistar®Top (azoxystrobin) is a fungicide with protective, curative and eradicant properties that has translaminar systemic action. It has efficacy against a wide range of fungi including basidiomycetes, such as *Puccina* spp. and oomycetes such as *Pythium* spp. (Tomlin 2003) and *Albugo candida* (Minchinton *et al.* (2004, 2007, 2011).

Ridomil®Gold 25G (metalaxyl-M) is a phenylamide fungicide with systemic acropetal movement within a plant. It has specific and excellent activity against a wide range of root and foliage oomycete pathogens (Tomlin 2003).

Field trials were conducted to evaluate a number of biological, fungicide, chemical, biocontrol and cultural treatments for their ability to reduce losses from root rot associated with oomycetes, especially *Pythium* species. Additionally, in 2010 the benefit of harvesting parsnips three weeks early was explored.

## 6.2 Materials and Methods

There were seven field trials conducted in the market garden areas east and west of Cranbourne, Victoria, from 2009 to 2011 (Table 6.1).

**Table 6.1. List of field trials and field site locations**

| Trial No. | Year | Location | | Treatments |
|---|---|---|---|---|
| 1 | 2009 | Berwick-Five Ways Rd | Clyde | Various |
| 2 | 2009 | North Road | Devon Meadows | Various |
| 3 | 2010 | Berwick-Five Ways Rd | Clyde | Various |
| 4 | 2010 | North Road | Devon Meadows | Various |
| 5 | 2010 | North Road | Devon Meadows | Silicon |
| 6 | 2010 | North Road | Devon Meadows | Fleece |
| 7 | 2011 | North Road | Devon Meadows | Various |

### 6.2.1 Treatments and their application

All treatments used in the field trials are listed in Table 6.2. Amistar®Top, Fulzyme®Plus, and Microplus™ were applied with a single drench nozzle (Teejet 80 blue), while Folicur® was applied with triple hollow cone nozzles (SPX brown) mounted on a boom. Treatments were applied a Silvan Selectra 12v knapsack (Silvan Pumps and Sprayers (Aus) Pty. Ltd. pressurised at 30psi. All liquids were sprayed until run-off unless otherwise stated. Granular applications of Ridomil®Gold 25G were mixed with sand graded to the same size and manually sprinkled evenly across the bed.

**Table 6.2. List of field trial treatments and their application**

| Trade name | Active | Company | Rate | Parsnip Trial No. |
|---|---|---|---|---|
| Control | Untreated | na | na | 1-7 |
| Amistar Top® | 200g/L azoxystrobin + 125g/L difenoconazole | Syngenta Crop Protection | 625-725mL/ha | 1,2 |
| Folicur® | 440 g/L Tebuconazole | Bayer Crop Sciences | 350mL/ha | 3, 4 |
| Fulzyme®Plus | *Bacillus subtilis* | Zadco for Quality Gro Pty. Ltd. | 170ml/7L/trial area | 3, 4, 7 |
| Hilling | na | na | Tractor rate | 4, 7 |
| Fleece™ | na | Tildener, Bristol, UK | 1 layer/plot | 6 |
| Microplus™ | *Streptomyces lydicus* | Organic Farming Systems | 500g/500L/ha | 1 |
| Mulch Enviromix™ | Premium Grade MG01 | Enviromix Pty. Ltd. | 147.2$m^3$/ha | 3, 4 |
| Mulch NRS™ | na | Natural Recovery Systems | na | 4 |
| Stand SKH™ | Silicon | Agrichem Pty. Ltd. | 6-7L/ha | 5 |
| Natural Wet™ | Surfactant for Fulzyme | Zadco for Quality Gro Pty. Ltd. | 1L/1000L/ha | 3, 4, 7 |
| Ridomil® Gold 25G | 25g/kg metalaxyl-M | Syngenta Crop Protection | 40kg/ha | 1, 2, 3, 7 |

na, not applicable; [1], both mulches were composted.

## 6.2.2 Environmental parameters

### *6.2.2.1 Soil analysis*

Prior to establishing a trial and selecting treatments, soil samples were collected across a bay from the site for Trials Nos. 1 and 2 on 10/08/2009 and sent on the 9th November 2009 to the State Chemistry Laboratories at 621 Sneydes Road, Werribee, Victoria, for analysis.

### *6.2.2.2 Weather station*

A Model T weather station (Western Electronics Design, Loxton, SA) was placed in the middle of an irrigation line in both trials. The station recorded average temperature and relative humidity, the presence or absence of leaf wetness, daylight and total rainfall at 30 min. intervals. The leaf wetness sensor was placed in the crop at a 45 degree angle and its height was adjusted as the crop grew.

### *6.2.2.3 Soil moisture, temperature and EC*

Soil moisture, temperature and EC were monitored with Crop Sense Soil Moisture Monitoring Plus EC equipment from T-Systems Australia Pty Ltd, 410 Langbeckers Road, Bundaberg QLD. The equipment was placed in a row of parsley and soil moisture, EC and temperature were monitored at 2-10cm depth, while only soil moisture and temperature were measured at a depth of 3-20 cm.

In Trials 4 and 6 ambient temperatures were recorded at ground level and soil temperature was recorded at 7cm depth with a Tinytag/Ultra Probe (Hastings Data Loggers) in one plot of the Fleece, Control, Mulch Enviromix and Mulch NRS treatments.

## 6.2.3 Trial No. 1, Clyde 2009

Trial 1 was located on Berwick-Five Ways Road, Clyde, Victoria. The grower's own parsnip seed was direct seeded on 8th April 2009 at two double rows per bed on raised beds. No nutrient deficiencies were detected in the soil sampled from Trial 1 site so no nutrient amendment treatments were applied in the trial (Appendix 6.6). The trial had a randomised block design with eight blocks containing seven plots each, to which the seven treatments were randomly allocated (see Table 6.3). Each plot was 5m long by 1.62m wide and laid out as four by two blocks. An irrigation line was located on the west side of the trial plots. Parsnip seed was spaced 3cm apart and the site had not been sown to parsnips for 15 years. The crop was maintained by the grower and treatments were applied as per the schedule in Table 6.3. This trial was harvested on 21st October 2009.

**Table 6.3. Treatment schedule for parsnip Trial 1, Clyde 2009**

| | Week/date/dap | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 7 | 10 | 12 | 16 | 18 | 21 | 24 |
| Treatment | 8/04/2009 | 18/05/2009 | 11/06/2009 | 23/06/2009 | 22/07/2009 | 5/08/2009 | 27/08/2009 | 17/09/2009 |
| | 0 | 38 | 62 | 74 | 103 | 117 | 129 | 150 |
| Control | - | - | - | - | - | - | - | - |
| Ridomil® Gold 25G 1 & 10 | + | - | + | - | - | - | - | - |
| Ridomil® Gold 25G 10 | - | - | + | - | - | - | - | - |
| Ridomil® Gold 25G 18 & 24 | - | - | - | - | - | + | - | + |
| Ridomil Gold® 25G 1, 10, 18 & 24 | + | - | + | - | - | + | - | + |
| Amistar® Top 10, 18 & 24 | - | - | + | - | - | + | - | + |
| MicroPlus™ | - | + | - | + | + | - | + | + |

+, treatment applied; -, no treatment application, dap, days after planting.

## 6.2.4 Trial 2, Devon Meadows 2009

Trial 2 was located at North Road Devon Meadows, Victoria. The grower's own parsnip seed was direct seeded on 8th April 2009 at four single rows per bed on raised beds. No nutrient deficiencies were detected in the soil sampled from Trial 1 site so no nutrient amendment treatments were applied in the trial (Appendix 6.6). The trial had a randomised block design with six replicates containing six

treatments. Each replicated plot was 6m long by 1.65m wide. The trial was located between two irrigation lines. The crop was maintained by the grower and treatments were applied as per the schedule in Table 6.4. This trial was harvested on 29th October 2009.

**Table 6.4. Treatment schedule for parsnip Trial 2, Devon Meadows 2009**

| Treatment | Week/date/dap | | | |
|---|---|---|---|---|
| | 1 | 10 | 18 | 24 |
| | 8/04/2009 | 11/06/2009 | 5/08/2009 | 17/09/2009 |
| | 0 | 62 | 117 | 150 |
| Control | - | - | - | - |
| Ridomil ®Gold 25G 1 & 10 | + | + | - | - |
| Ridomil ®Gold 25G 10 | - | + | - | - |
| Ridomil ®Gold 25G 10 & 18 | - | + | + | - |
| Ridomil ®Gold 25G 1, 10, 18 & 24 | + | + | + | + |
| Amistar ®Top10, 18 & 24 | - | + | + | + |

+, treatment applied; -, no treatment application; dap, days after planting.

### 6.2.5 Trial No. 3, Clyde 2010

Trial 3 was located on Berwick-Five Ways Road, Clyde, Victoria. The grower's own parsnip seed was direct seeded on 13th May 2010 at two double rows per bed on raised beds. The trial was arranged as two contiguous blocks. One block contained 24 plots arranged as four beds of six plots and the other consisted of 28 plots arranged as seven beds of four plots each. Each bed in the first block contained a full replicate of the six randomly allocated treatments. In the second block, there were four L-shaped replicates of the six treatments plus an extra plot of Mulch Enviromix™, Folicur® and the two Ridomil®Gold 25G treatments. Each plot was 5m long by 1.62m wide. This trial was located between three irrigation lines. Parsnip seed was spaced 3cm apart and the site had not been sown to parsnips for 15 years. The crop was thinned on the 10th June and maintained by the grower. Treatments were applied as per the schedule in Table 6.5 and the crop was harvested on 2nd December 2010.

**Table 6.5. Treatment schedule for parsnip Trial 3, Clyde 2010**

| Treatment | Week/date/dap | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 5 | 6 | 10 | 16 | 17 | 19 | 21 | 23 |
| | 10/06/2010 | 15/06/2010 | 16/07/2010 | 24/08/2010 | 30/08/2010 | 17/09/2010 | 1/10/2010 | 13/10/2010 |
| | 28 | 33 | 64 | 103 | 109 | 127 | 141 | 153 |
| Control | - | - | - | - | - | - | - | - |
| Folicur® | - | - | - | - | + | + | + | + |
| Fulzyme™ | - | + | + | + | - | - | + | - |
| Mulch Enviromix ™ | + | - | - | - | - | - | - | - |
| Ridomil® Gold 25G | - | + | - | - | - | - | - | - |
| Ridomil® Gold 25G + Folicur ® | - | + | - | - | + | + | + | + |

+, treatment applied; -, no treatment application; dap, days after planting.

### 6.2.6 Trial No. 4, Devon Meadows 2010

Trial 4 was located at North Road, Devon Meadows, Victoria. The growers own parsnip seed was direct seeded on 16th April 2010 at four single rows per bed on raised beds. The trial was located between two irrigation lines and had a randomised block design with six blocks each containing six plots to which the treatments were randomly allocated (Table 6.6). Each plot was 6m long by 1.65m wide. The crop was maintained by the grower and treatments were applied as per the schedule in Table

6.6. There was an early harvest on 7th October and a late harvest on the 28th October, the latter being the expected harvest date.

**Table 6.6. Treatment schedule for parsnip Trial 4, Devon Meadows 2010**

| Treatments | Week/date/dap | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 5 | 10 | 14 | 20 | 21 | 23 | 25 | 27 |
| | 20/04/2010 | 13/05/2010 | 15/06/2010 | 16/07/2010 | 24/08/2010 | 30/08/2010 | 17/09/2010 | 1/10/2010 | 13/10/2010 |
| | 5 | 20 | 61 | 92 | 131 | 137 | 155 | 169 | 181 |
| Control | - | - | - | - | - | - | - | - | - |
| Fulzyme™ | - | + | + | + | + | - | - | + | - |
| Folicur® | - | - | - | - | - | + | + | + | + |
| Hilling | - | - | + | + | - | - | - | - | - |
| Mulch Enviromix ™ | + | - | - | - | - | - | - | - | - |
| Mulch NRS™ | + | - | - | - | - | - | - | - | - |

+, treatment applied; -, no treatment application; dap, days after planting.

### 6.2.7 Trial No. 5 Silicon, Devon Meadows 2010

Trial 5 was located at North Road, Devon Meadows, Victoria. The grower's own parsnip seed was air-seeded at 60mm apart on 16th April 2010 at four single rows per bed on raised beds. The trial had a randomised block design consisting of six blocks of two plots each. There were three blocks located on either side of an irrigation line. Each plot was 5.5m long by 1.65m wide. The Stand SKH™ treatment was randomly allocated to one plot in each block. The other plot, the control, was left untreated. The crop was maintained by the grower and Stand SKH™ was applied as per the schedule in Table 6.7. This trial was harvested on 7th and 28th October 2010.

**Table 6.7. Treatment schedule for parsnip Trial 5, Devon Meadows 2010**

| Treatments | Week/date/dap | | | | | |
|---|---|---|---|---|---|---|
| | 16 | 20 | 21 | 23 | 25 | 27 |
| | 28/07/2010 | 24/08/2010 | 30/08/2010 | 17/09/2010 | 1/10/2010 | 13/10/2010 |
| | 104 | 131 | 137 | 155 | 169 | 181 |
| Control | - | - | - | - | - | - |
| Stand SKH™ | + | + | - | - | + | - |

+, treatment applied; -, no treatment application; dap, days after planting.

### 6.2.8 Trial No. 6 Fleece, Devon Meadows 2010

Trial 6 was located at North Road, Devon Meadows, Victoria. The grower's own parsnip seed was direct seeded on 16th April 2010 at four single rows per bed on raised beds. The trial was a randomised block design with six blocks each containing two plots. The Fleece™ treatment was randomly allocated to one plot in each block, the other plot was not treated. There were three blocks located on either side of an irrigation line. Each plot was 8m long by 1.65m wide. Fleece™ (Tildenet, Bristol UK) was supplied courtesy of Mike Erkler, Premium Fresh Tasmania, and was placed over plots on 13th May 2010 and secured by burying the edges in the ground. The height of fleece was adjusted, twice, as the crop grew and removed on 20th August 2010. The crop was maintained by the grower. This trial was harvested on 28th October 2010.

### 6.2.9 Trial No. 7, Devon Meadows 2011

Trial 7 was located at North Road, Devon Meadows, Victoria. The grower's own parsnip seed was direct seeded on 6th April 2011 at three double rows per bed on raised beds. The trial was a randomised block design with 12 blocks each containing four plots, in a bay of six beds, so the first six blocks were in front of the next six blocks and all were situated between irrigation lines. Each plot

was 6m long by 1.65m wide. The four treatments (Table 6.8) were randomly allocated to plots within blocks. The crop was maintained by the grower. This trial was harvested on 24th-25th October 2011.

**Table 6.8. Treatment schedule for parsnip Trial 7, Devon Meadows 2011**

| Treatment | Week/date/dap | | | | |
|---|---|---|---|---|---|
| | 1 | 11 | 13 | 16 | 20 |
| | 8/04/2011 | 15/06/2011 | 1/07/2011 | 22/07/2011 | 19/08/2011 |
| | 2 | 70 | 86 | 107 | 135 |
| Control | - | - | - | - | - |
| Fulzyme™ | - | + | - | + | + |
| Hilling | - | - | + | + | - |
| Ridomil® Gold 25G | + | + | - | - | - |

+, treatment applied; -, no treatment application; dap, days after planting.

### 6.2.10 Assessment

All parsnip trials were sampled by lifting four plants across the row, every half metre from the end of the plot. Sampled plants were assessed for the following symptoms: deep tap root lesions (canker); brown lesions on the upper tap root; brown lesions on the lower tap root; collar rot; skin cracks, forking and healthy (no visible symptoms). At harvest, the number of plants assessed per plot was 64 in Trial 1, 36 in Trial 2 and 20 in Trials 3 to 7. The weight of each harvested parsnip was recorded in Trials 3 to 7.

***Severity of root rot diseases***. In Trials 1 and 2, severity of disease on roots was calculated by using the Scale: 0= no symptoms on the root; 1, superficial brown lesions on the upper tap root, lower tap root or forking; 2, skin cracks on the tap root; 3, canker (deep lesions on the tap root); and 4, collar rot. Overall severity was obtained by averaging the severity scores over the total number of plants assessed in the plot. Note some plants could have more than one severity score.

***Vigour of foliage.*** Vigour of foliage was assessed in Trial 4 on 28/7/2010, 20/8/2010 and 1/10/2010 and in Trial 6 on 20/8/2010. Vigour of foliage was assessed on a scale of 0-3, where 0=no growth of foliage; 1, poor growth; 2, moderate growth and 3, highest and most vigour growth in the trial.

***Severity of Itersonilia lesions.*** Severity of *Itersonilia* lesions on petioles and foliage were assessed in Trial 3 on 2/12/2010, in Trial 4 on 7/10/2010 and in Trial 7 on 24/10/2011. Severity of *Itersonilia* petiole and foliage lesions were assessed on a scale of 0-2, where 0, no lesions; 1, a few lesions; 2, lots of lesions.

***Yield.*** The number of plants assessed at harvest varied in Trial 1, where 64 plants were assessed in 53 of the 56 experimental plots. For the remaining three plots (the Control plots in Reps 2, 3 and 8), the number of plants assessed was 140, 143 and 138 respectively. In Trial 2, 36 plants per plot were assessed and in Trials 3 to 7, 20 plants per plot were assessed for symptoms described above and weighed.

In Trials 1 and 3 the estimated number of plants per ha were based on a seed spacing of 30mm per row, four rows of seeds per 1m by 1.65m and a germination rate of 80%. The estimated number of plants per ha is therefore 646,302, but this does not take into account thinning. In Trials 2, 4, 5 and 6, the estimated number of parsnip plants per ha were based on counts of parsnip plants in four rows of three plots of Trial 4 and averaged. On the basis of this data there were estimated to be 204,040 parsnip plants per ha. In Trial 7, which was air-seeded at the rate of one seed per 60mm into three double rows per bed and with an expected germination rate of 80% would be 480,000, but again this

does not take into account thinning, consequently the estimated number of parsnip plants per ha was based on counts of parsnips made in Trial 4.

Yield estimates in Trials 1 and 3 were based on: the proportion of estimated marketable plants x average weight of a Control plant from Trial 4 (196.5g) x estimated number of parsnips per ha (204,040). Yield estimates in Trials 4, 5, 6 and 7 were based on: estimated number of parsnips per ha (204,040) x proportion of plants marketable x average weight of a marketable parsnip. Marketable plants were defined as showing no symptoms of disease on tap roots and having a weight in the range of ≥ 120g to ≤300g, except for Trial 2 where ≥ 90g were considered marketable due to poor growth associated with flooding on the field site.

### 6.2.11 Data analysis

For trials numbered 1, 2, 4, 5 and 6 data were analysed by ANOVA. For Trial 3 REML (Residual Maximum Likelihood) was used to analyse the data. In Trial 7 forking, collar rot, deep tap root lesions were analysed using GLMMs (Generalised Linear Mixed Models) and skin cracks, brown lesions, total number of healthy plants and harvestable plants were analysed by ANOVA.

## 6.3 Results

### 6.3.1 Trial No. 1, Clyde 2009

Strategically timed applications of one, two, three or four sprays of metalaxyl (Ridomil®Gold 25G) significantly reduced (P<0.001) the incidence of parsnips with deep tap root lesion (cankers and crown rot), upper tap root lesions and the overall severity of root rots at harvest compared with the untreated control (Table 6.9). The metalaxyl treatments reduced the incidence of parsnips with canker by an average of 62% and with superficial upper tap rot lesions by an average of 31% of the untreated control (15.9% and 59.7% of parsnips affected with canker and upper tap root lesions, respectively, in the untreated control). The metalaxyl treatments reduced the average root rot severity by 50% of the untreated control (Table 6.9). These treatments did not have a significant effect on the incidence of parsnips with lesions on the lower tap root, which was generally very low (less than 6% of parsnips affected). There was no significant difference in the incidence of parsnips with canker, upper tap root lesions and root rot severity between the different metalaxyl treatments, irrespective of the number and the timing of the applications. The metalaxyl treatments improved the percentage of marketable parsnips by an average of 50% of the untreated control (33% marketable parsnips) (Table 6.9).

Three applications of Amistar®Top (azoxystrobin + difenaconazole) reduced (P<0.001) the incidence of parsnips with canker by 47% and root rot severity by 25% of the untreated control but had no significant affect on the incidence of parsnips with lower tap root lesions or upper tap root lesions (Table 6.9). The treatment did not improve the percentage of marketable parsnips.

**Table 6.9. Effect of treatments to control root rot of parsnip in Trial 1, Clyde 2009**

| Treatment | No. of sprays | Mean incidence of parsnips with deep tap root lesions (Canker) (%) | Mean incidence of parsnips with lower tap root lesions (%) | Mean incidence of parsnips with upper tap root lesions (%) | Average root rot severity (Scale 0=4)[1] | Estimated unmarketable yield (%) | Estimated marketable yield (%) | Estimated marketable yield (t/ha)[2] |
|---|---|---|---|---|---|---|---|---|
| Control | 0 | 15.9 a | 0.5 | 59.7 a | 2.0 a | 77.0 a | 33 a | 13.23 |
| Ridomil® Gold 25G 1 & 10 | 2 | 8.0 bc | 0.2 | 43.6 b | 1.1 c | 54.1 b | 45.9 b | 18.40 |
| Ridomil® Gold 25G 10 | 1 | 6.3 bc | 0.2 | 37.1 b | 1.0 c | 47.5 b | 52.5 b | 21.05 |
| Ridomil® Gold 25G 18 & 24 | 2 | 5.1 bc | 0.2 | 38.3 b | 1.1 c | 47.9 b | 52.1 b | 20.89 |
| Ridomil Gold® 25G 1, 10, 18 & 24 | 4 | 4.5 c | 0.2 | 40.6 b | 1.0 c | 50.2 b | 49.8 b | 19.97 |
| Amistar® Top 10, 18 & 24 | 3 | 8.4 b | 0.59 | 61.1 a | 1.5 b | 72.3 a | 27.7 a | 11.11 |
| MicroPlus™ | 5 | 12.7 a | 0.39 | 63.3 a | 1.6 b | 77.3 a | 22.7 a | 9.10 |
| lsd | | 3.7 | (ns) | 11.6 | 0.3 | 11.8 | 11.8 | - |
| p-value | | <0.001 | 0.947 | <0.001 | <0.001 | <0.001 | <0.001 | |

[1], Scale: 0, no symptoms on the root; 1, superficial brown lesions on the upper tap root, lower tap root or forking; 2, skin cracks on the tap root; 3, canker (deep lesions on the tap root); and 4, collar rot; [2], based on 204,040 parsnips per ha. Numbers followed by a different letter differ significantly; ns, not significantly different. Note, estimated marketable yield does not take into account a weight category of ≥ 120g to ≤ 300g.
Although the MicroPlus™ treatment reduced average severity of root rot by 25% (P<0.001), it did not significantly affect the other disease variables and did not improve the percentage of marketable parsnips compared with the untreated control.

There was a noticeable block effect; this may have been due to blocks 4 and 8 having less disease than the other bocks due to their location or due to an assessor effect. The effect was accounted for by including block in the model that was fitted to the data. No analysis was conducted for brown lesions on the lower tap root as there was a low incidence of these symptoms.

### 6.3.2 Trial No. 2, Devon Meadows 2009

At this site an average of 43% of parsnips harvested from the untreated control plots had symptoms of canker and crown rot (deep tap root lesions) and 49% had superficial lesions on the upper tap rot (Table 6.10). In contrast to the results of the trial at the Clyde site, none of the treatments had a significant effect on the incidence of parsnips with disease, severity of disease or on the percentage of parsnips. There was a tendency of a reduced severity of root rot (P=0.1) with the one and the four applications of Ridomil®Gold 25G compared with the untreated control.

**Table 6.10. Effect of treatments to control root rot of parsnip in Trial 2, Devon Meadows 2009**

| Treatment | No. of sprays | Mean incidence of parsnips with deep tap root lesions (Canker) (%) | Mean incidence of parsnips with lower tap root lesions (%) | Mean incidence of parsnips with upper tap root lesions (%) | Average root rot severity (Scale 0=4)[1] | Estimated unmarketable yield (%) | Estimated marketable yield (%) | Estimated marketable yield (t/ha)[2] |
|---|---|---|---|---|---|---|---|---|
| Control | 0 | 42.6 | 4.2 | 49.1 | 3.5 | 89.8 | 10.2 | 4.090 |
| Ridomil® Gold 25G 1 & 10 | 2 | 42.1 | 5.6 | 53.2 | 3.3 | 89.8 | 10.2 | 4.090 |
| Ridomil® Gold 25G 10 | 1 | 34.7 | 4.2 | 47.7 | 2.9 | 91.2 | 8.8 | 3.528 |
| Ridomil® Gold 25G 10 & 18 | 2 | 42.6 | 4.6 | 38.9 | 3.6 | 89.8 | 10.2 | 4.090 |
| Ridomil® Gold 25G 1, 10, 18 & 24 | 4 | 31.9 | 7.4 | 52.3 | 2.9 | 85.2 | 14.8 | 5.934 |
| Amistar® Top10, 18 & 24 | 3 | 36.1 | 6.5 | 48.6 | 3.2 | 88 | 12 | 4.811 |
| lsd | | ns | ns | ns | 0.5714 | ns | - | - |
| p-value | | 0.354 | 0.871 | 0.252 | 0.096 | 0.621 | | |

[1], Scale: 0= no symptoms on the root; 1 = superficial brown lesions on the upper tap root, lower tap root or forking; 2 = skin cracks on the tap root; 3 = canker (deep lesions on the tap root); and 4 = collar rot. Numbers followed by a different letter differ significantly; ns, not significantly different.

### 6.3.3 Trial No. 3, Clyde 2010

Trial 3 site was severely affected by flooding, which may have contributed the lack of any significant differences between treatments and the untreated Control for the parameters assessed (Table 6.11). Although none of the treatments significantly reduced the incidence of *Itersonilia* lesions on foliage and petioles compared with the Control, the p-value of 10% suggests some treatments differed from each other. Yield of healthy plants was very low and no treatments differed from the Control.

**Table 6.11. Treatment schedule for parsnip Trial 3, Clyde 2010**

| Treatment | No. of sprays | Mean incidence of *Itersonilia* lesions on leaves of parsnips plants (Back transformed) (%) | Mean incidence of healthy tap root on plants of all sizes (predicted) (%) |
|---|---|---|---|
| Control | 0 | 11.1 | 9.8 |
| Folicur® | 4 | 9.2 | 7.7 |
| Fulzyme™ | 4 | 14.5 | 11.4 |
| Mulch Enviromix™ | 1 | 18.7 | 7.0 |
| Ridomil® Gold 25G | 1 | 14.7 | 8.4 |
| Ridomil® Gold 25G + Folicur® | 5 | 5.3 | 5.3 |
| p-value | | 0.100 | 0.567 (ns) |

ns, not significantly different.

### 6.3.4 Trial No. 4, Devon Meadows 2010

Covering the beds with Mulches slightly lowered the average ambient air temperature; the average minimum and average maximum ambient air temperatures by a few degrees compared with the unmulched Control (Table 6.12). The soil temperatures, as measured by the probe, were similar for the average, higher for the minimum and lower for the maximum for Mulch Enviromix™ compared with the Control. The situation was different for Mulch NRS™. In the Mulch NRS™ treatment the average, minimum and maximum soil temperatures were higher compared with the Control.

**Table 6.12. Effect of type of Mulch on ambient and soil temperatures, Devon Meadows 2010**

| Type of temperature | Temperature °C | | | | | |
|---|---|---|---|---|---|---|
| | Control | | Mulch Enviromix™ | | Mulch NRS™ | |
| | Ambient | Probe | Ambient | Probe | Ambient | Probe |
| Average | 12.6 | 11.7 | 12.1 | 11.6 | 12.4 | 12.0 |
| Minimum | 0.8 | 6.4 | 0.6 | 6.9 | -0.2 | 7.2 |
| Maximum | 36.6 | 29.1 | 35.2 | 28.7 | 34.8 | 31.0 |

***Foliage vigour***

There was a significant difference ($p<0.001$) in vigour of foliage over time (Table 6.13). Foliage was more vigorous early in the season when the plants were putting on growth rather than later in the season when tap roots were maturing.

There were significant differences ($p<0.001$) in the overall vigour of foliage between treatments (Table 6.14). Mulch Enviromix™ produced the most vigorous growth, which was 30% more vigorous than the untreated Control. Fulzyme®Plus, Folicur® and Hilling treatments were approximately 19% more vigorous than the untreated Control. Mulch NRS™ was significantly less vigorous than the untreated Control and when first laid, was phytotoxic, burning seedlings. Additionally it contained pieces of plastic, although inert, were an eyesore.

The difference between treatments varied significantly ($p<0.001$) at each assessment date (Fig. 6.1). This means that the vigour of foliage developed differently for each treatment over time. Vigour of foliage on Control plants did not vary over time. Vigour of foliage on plants treated with Hilling and Mulch Enviromix™ was high at the beginning but declined sharply later in the trial, whereas foliage of the Folicur® treatment rose sharply later in the trial.

**Table 6.13. Change in vigour of foliage during the later parts of Trial 4, Devon Meadows 2010**

| Date | Mean vigour of foliage (Scale 0-3)[1] |
|---|---|
| 28/07/2010 | 2.167 a |
| 20/08/2010 | 1.958 b |
| 1/10/2010 | 1.896 b |
| lsd | 0.121 |
| p-value | <0.001 |

Numbers followed by a different letter differ significantly. [1]Scale of 0-3, where 0, no growth of foliage; 1, poor growth; 2, moderate growth and 3, highest and most vigour growth in the trial.

**Table 6.14. Effect of treatment on the overall vigour of foliage in Trial 4, Devon Meadows 2010**

| Treatment | Mean vigour of foliage (Scale 0-3)[1] |
|---|---|
| Control | 1.861 c |
| Fulzyme®Plus | 2.111 b |
| Folicur® | 2.181 b |
| Hilling | 2.222 b |
| Mulch Enviromix™ | 2.667 a |
| Mulch NRS™ | 1.000 d |
| lsd | 0.2475 |
| p-value | <0.001 |

Numbers followed by a different letter differ significantly. [1], Scale of 0-3, where 0=no growth of foliage; 1= poor growth; 2= moderate growth and 3= highest and most vigour growth in the trial.

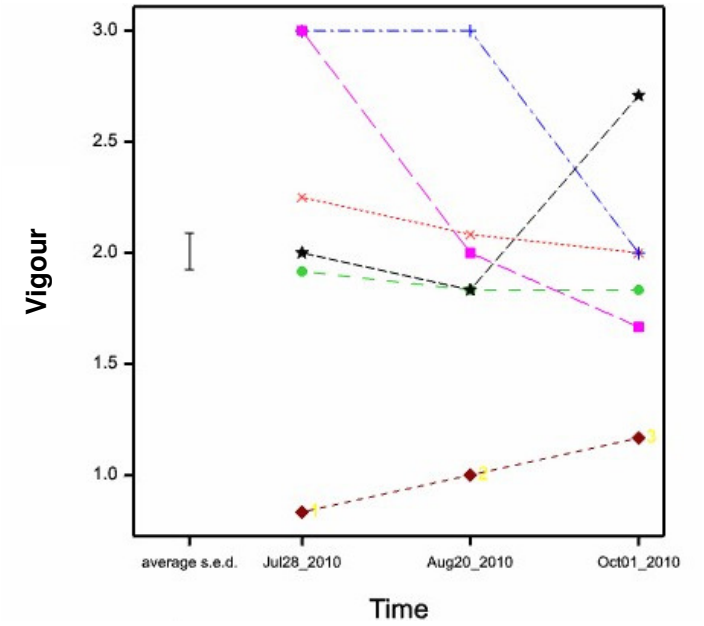


**Fig. 6.1**. Effect of treatments on vigour of foliage over time, Devon Meadows 2010.

***Itersonilia lesions on foliage***

*Itersonilia* is considered one of the causal organisms of parsnip canker and also forms lesions on foliage. *Itersonilia* lesions on foliage at the first assessment date in October were significantly reduced with the Folicur® treatment by 44% and with the Mulch NRS™ treatment compared with the Control (Table 6.15). There were, however, few plants surviving in the latter treatment due to phytotoxicity at planting and the greater spacing of surviving plants would be expected to reduce the spread and consequently the incidence of the disease. Mulch NRS™ should not be considered as a suitable treatment. At the late harvest the mean incidence of *Itersonilia* lesions on foliage followed a similar trend to the early harvest with Folicur® significantly reducing incidence it by 35% and Mulch Enviromix™ significantly reducing it by 24%. Fulzyme®Plus and Hilling had no effect on the incidence of *Itersonilia* lesions on foliage at either the early or late harvest times.

**Table 6.15. Effect of treatments on *Itersonilia* lesions on foliage at the early and late harvests for Trial 4, Devon Meadows 2010**

| Treatment | Number of applications | Severity of Itersonilia on foliage[1] 7/10/2010 (Scale 0-2) | Severity of Itersonilia on foliage[1] 28/10/2010 (Scale 0-2) |
|---|---|---|---|
| Control | 0 | 1.533 ab | 1.185 a |
| Fulzyme®Plus | 5 | 1.558 a | 1.237 a |
| Folicur® | 4 | 1.092 c | 0.775 c |
| Hilling | 2 | 1.467 ab | 1.117 ab |
| Mulch Enviromix™ | 1 | 1.283 bc | 0.898 bc |
| Mulch NRS™ | 1 | 0.467 d | 0.630 c |
| lsd | - | 0.266 | 0.243 |

[1], Foliage is defined as petioles and leaves. Numbers followed by a different letter differ significantly.

***Incidence of diseased roots***

There appears to be no benefit in harvesting parsnips early to avoid disease (p= 0.537), irrespective of treatment. There was no difference between the Early harvest on 7th October 2010 and the Late harvest on 28th October 2010 for the overall incidence of disease (%) on parsnip roots (p=0.241). There was no difference between the Early and Late harvest for incidence of disease (%) on parsnip roots for each treatment (p=0.436). There was no difference between the Early and Late harvest for the average weight (g) of healthy parsnips harvested (p=0.537).

At the Early harvest the Mulch NRS™ had significantly more parsnips with diseases roots compared with the Control (Table 6.16). While the Fulzyme®Plus treatment had 33% less (significant) parsnips with disease roots compared with the Control. At the Late harvest the Mulch NRS™ and the Folicur® treatments had significantly more parsnips with diseases roots compared with the Control (Table 6.16), but none of the other treatments differed from the Control.

**Table 6.16. Effect of treatments to reduce diseased roots at an Early and Late harvest for Trial 4, Devon Meadows 2010**

| Treatment | Number of applications | Mean incidence of diseased roots at the Early harvest[1] (%) | Mean incidence of diseased roots at the Late harvest[2] (%) |
|---|---|---|---|
| Control | 0 | 47.5 bc | 50.8 b |
| Fulzyme®Plus | 5 | 31.7 d | 39.2 b |
| Folicur® | 4 | 55.0 b | 69.2 a |
| Hilling | 2 | 34.2 cd | 39.2 b |
| Mulch Enviromix™ | 1 | 60.8 ab | 50.8 b |
| Mulch NRS™ | 1 | 73.3 a | 75.8 a |
| lsd | - | 15.6 | 18.3 |
| P-value | | <0.001 | <0.001 |

[1], Early harvest was on the 7th October 2010; [2], Late harvest on 28th October 2010. Numbers followed by a different letter differ significantly.

***Yield of parsnip roots***

There were no significant differences in the yield of marketable parsnip roots (120g to 300g category and symptomless) between treatments for the Early and Late harvests (p=0.784). Consequently only the Late harvest data will be considered. None of the treatments at Late harvest significantly improved the marketable yield of parsnips (g) compared with the Control, but Mulch NRS™ significantly reduced marketable yield (Table 6.17). The Mulches noticeable increased lateral root development which is detrimental to marketable parsnips.

**Table 6.17. Effect of treatments on yield of parsnips for Trial No. 4, Devon Meadows 2010**

| Treatment | Number of applications | Mean proportion of marketable parsnips per plots of 20 plants (%) | Mean weight of all marketable parsnips per plot at the Late harvest (g) | Estimated yield of marketable parsnips at the Late harvest (t/ha)[1] |
|---|---|---|---|---|
| Control | 0 | 6 | 1179.0 ab | 12.03 |
| Fulzyme®Plus | 5 | 8 | 1626.0 a | 16.59 |
| Folicur® | 4 | 3.7 | 699.0 bc | 7.13 |
| Hilling | 2 | 7.2 | 1390.0 a | 14.18 |
| Mulch Enviromix™ | 1 | 5.5 | 1035.0 abc | 10.56 |
| Mulch NRS™ | 1 | 2 | 444.0 c | 4.53 |
| lsd | | | 604 | |
| P-value | | | P=0.005 | |

[1], There are estimated to be 204,040 parsnips per ha. Numbers followed by a different letter differ significantly. Late harvest was on 28/10/2010.

## 6.3.5 Trial No. 5, Devon Meadows 2010

The three applications of Stand SKH™ (Silicon) appeared to have little influence on any of the parameters assessed, compared with the untreated Control (Table 6.18, Fig. 6.2). There was no significant difference in the incidence of disease on root (%) between the untreated Control and the Silicon treatment (p=0.889). There was no significant difference in the incidence of deep tap root lesions (canker) between the untreated Control and the Silicon treatment (p=0.688). Additionally there was no significant difference between the incidence of brown lesions (%) between the untreated Control and the Silicon treatment (p=0.122). This trial was located behind the Trial 4 site, so the air temperature, rainfall, soil moisture, soil temperature and EC are the same as for Trial No. 4.

**Table 6.18. Effect of Stand SKH™ (Silicon) on parsnip diseases and yield 2010, Trial 5, Devon Meadows 2010**

| Parameter | Treatment | | p-value |
|---|---|---|---|
| | Control | Stand SKH™ | |
| No. of applications | 0 | 3 | - |
| Incidence of deep tap root lesions (canker ) (%) | 35.8 | 32.5 | p=0.688 |
| Incidence of brown lesions (%) | 18.3 | 29.2 | p=0.122 |
| Incidence of diseased roots (%) | 59.2 | 60 | p=0.889 |
| Proportion of harvested roots with skin cracks (%) | 28 | 30 | - |
| Total No. marketable out of 120 | 30 | 27 | - |
| Proportion marketable out of 120 (%) | 25 | 22.5 | - |
| Total weight of marketable roots (g) | 5664 | 5522 | - |
| Average weight of marketable roots (g) | 188.8 | 204.5 | - |
| Estimated marketable yield (t/ha) | 9.63 | 9.39 | - |

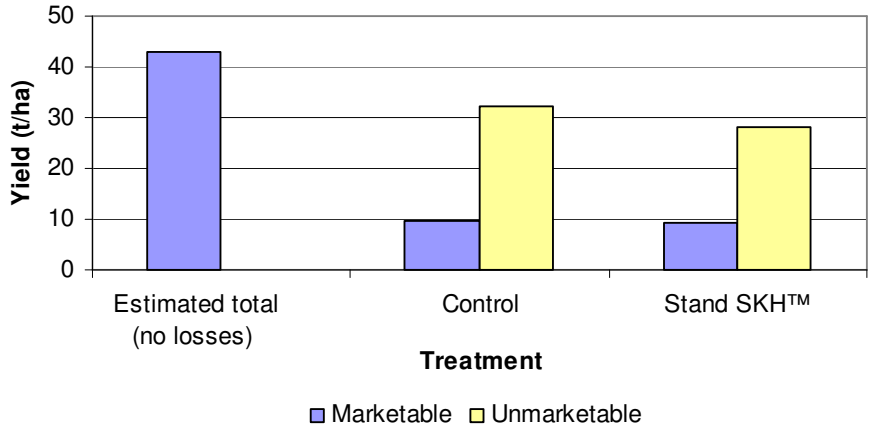


**Fig. 6.2.** The effect of three silicon applications on yield, Trial 5, Devon Meadows 2010.
Estimated total yield assumes all parsnips are healthy and have weight of 210g (median of 120 -300g). Marketable is defined as healthy roots and falling in the weight category of ≥120g to ≤ 300g. Unmarketable includes parsnips with disease or forking and includes all weights.

### 6.3.6 Trial No. 6 Fleece™, Devon Meadows 2010

Covering the crop with Fleece generally raised the minimum temperature and lowered the maximum ambient and soil probe temperatures (Table 6.19). The Fleece™ treatment produced a slightly lower ambient maximum temperature by 4.8ºC compared with the no Fleece™ treatment, but it produced a slightly warmer ambient minimum temperature by 1.3ºC. When the temperatures of the soil probes were compared the Fleece™ treatment increased the minimum temperature by 1.1ºC and reduced the maximum temperature by 1.5ºC compared with the no Fleece™ treatment.

**Table 6.19. Summary of a comparison of soil and ambient temperature between Fleece™ and no Fleece™ in the field trial from 27th May to 29th October 2010**

| Type of temperature | Temperature °C | | | |
|---|---|---|---|---|
| | Control | | Fleece | |
| | Ambient | Soil Probe | Ambient | Soil Probe |
| Average | 12.0 | 11.2 | 11.8 | 11.3 |
| Minimum | 0.8 | 6.4 | 2.1 | 7.5 |
| Maximum | 36.6 | 29.1 | 31.8 | 27.6 |

Despite the Fleece™ reducing the extremes of ambient and soil temperature it had no effect on disease and did not improve marketable yield (Table 6.20, Fig. 6.3). There was no significant difference in the incidence of diseased roots between the Control and Fleece™ (p=0.550). There was no significant difference in the incidence of deep tap root lesions (canker) between the Control and Fleece™ treatments at harvest (p=0.178). Also there was no significant difference in the incidence of brown tap root lesions between the Control and Fleece™ treatments at harvest (p=0.504). Crop losses between the estimated total possible and Control plants were large at 63% (Fig. 6.3).

**Table 6.20. Effect of Fleece™ treatment on parsnip diseases and yield in 2010, Trial 6, Devon Meadows 2010**

| Parameter | Treatment | | p-value |
|---|---|---|---|
| | Control | Fleece | |
| No. of applications | 0 | 1 | - |
| Average vigour (Scale 0-3) | 1.8 | 2.25 | - |
| Incidence of deep tap root lesions (canker ) (%) | 20.8 | 13.3 | p=0.178 |
| Incidence of brown lesions (%) | 28.3 | 33.3 | p=0.504 |
| Incidence of diseased roots (%) | 43.3 | 47.5 | p=0.550 |
| Total No. marketable out of 120 | 49 | 30 | - |
| Proportion marketable out of 120 (%) | 41 | 25 | - |
| Total weight of marketable roots (g) | 9170 | 5772 | - |
| Average weight of marketable roots (g) | 187.4 | 192.4 | - |
| Estimated marketable yield (t/ha) | 15.66 | 9.81 | - |

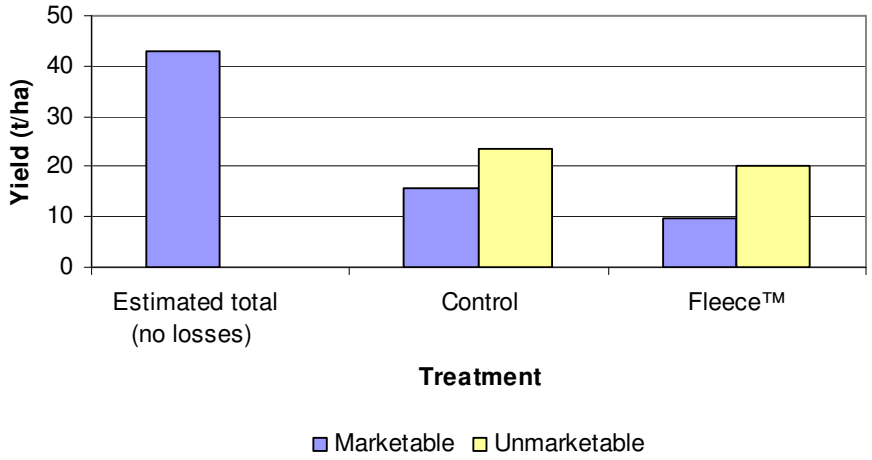


**Fig. 6.3.** Yield of parsnips with and without Fleece in 2010, Trial 6, Devon Meadows 2010.

Estimated total yield assumes all parsnips are healthy and have weight of 210g (median of 120 -300g). Marketable is defined as healthy roots and falling in the weight category of ≥120g to ≤ 300g. Unmarketable includes parsnips with disease or forking and includes all weights.

The Fleece™ was raised twice during the 99 days it was on the crop, which was probably too long as parsnip leaves were compacted (Fig. 6.4). This trial was located behind the Trial 4 site so the air temperature, rainfall, soil moisture, soil temperature and EC are the same as for Trial 4.

**Fig. 6.4.** Fleece™ covering treated plots in the 2010 trial.

### 6.3.7 Trial No. 7, Devon Meadows 2011

***Foliage vigour***
No assessments were undertaken for foliage vigour as there were no observable differences between treatments and the Control.

***Itersonilia lesions on foliage***
In general foliage had few lesions of *Itersonilia* on the rating scale of 0-2, where 0 = no lesions; 1, a few lesions (1-2); and 2, lots of lesions. The Fulzyme™ treatment had slightly less symptoms of *Itersonilia* compared to all the other treatments and the untreated Control (Fig. 6.4).

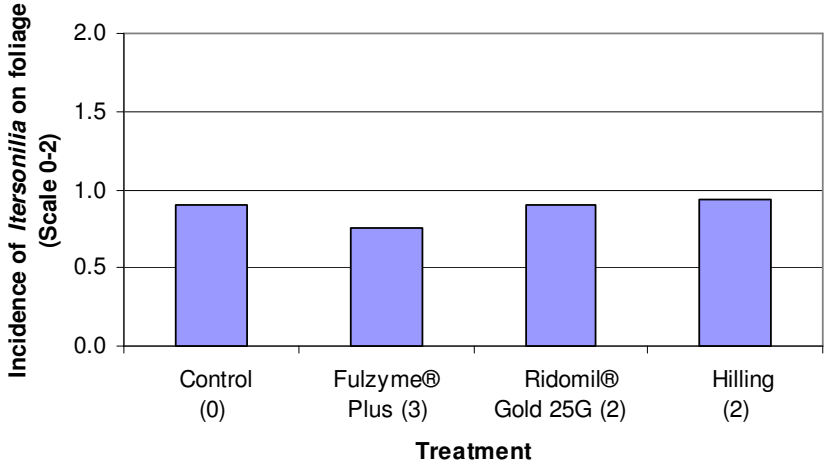


**Fig. 6.4**. Effect of treatment to control *Itersonilia* lesions on foliage in Trial 7, Devon Meadows 2011.
Numbers in brackets are number of sprays applied.
Scale 0-2, where 0, no lesions; 1, a few lesions (1-2) and 2,= lots of lesions.

***Effect of treatment on tap root symptoms***
In this trial Hilling significantly increased the number of parsnips with skin cracks (p-value = 0.075) compared with Ridomil®Gold 25G (Table 6.21). This affected the number of healthy plant taproots for which the Hilling treatment means were significantly lower compared to the other three treatment (p-value = 0.030) (Table 6.22). There is some evidence that Ridomil®Gold 25G is reducing the number of taproots with brown lesions (p-value = 0.131). There appears to be no effect of treatment on the number of plants with forking, collar rot or deep tap root lesions, but their incidence was low (Table 6.21).

**Table 6.21. Effect of treatment on five symptoms on parsnip tap roots, Trial 7, Devon Meadows 2011**

| Treatments | Means back transformed (probabilities) of parsnips with symptoms | | | | | Log Skin cracks[1] |
|---|---|---|---|---|---|---|
| | Forked root | Collar rot | Deep taproot lesions (Canker) | Brown lesions upper taproot | Skin cracks | |
| | (%) | (%) | (%) | (%) | (%) | |
| Control | 3.82 | 4.55 | 6.73 | 21.76 | 15.30 | 2.791 a |
| Fulzyme®Plus | 3.05 | 3.40 | 7.14 | 20.84 | 15.69 | 2.815 a |
| Hilling | 5.01 | 3.79 | 8.37 | 29.36 | 23.97 | 3.218 a |
| Ridomil® Gold 25G | 7.01 | 3.41 | 7.18 | 17.96 | 12.57 | 2.608 b |
| lsd | ns | ns | ns | ns | - | 0.4654 |
| p-value | 0.213 | 0.877 | 0.90 | 0.131 | | 0.075 |

[1], Predicted means for the treatment (ie, not back transformed); -, not applicable. Numbers followed by a different letter differ significantly; ns, not significantly different.

***Effect of treatment on yield***

The analysis of the number of harvestable parsnips provides some evidence that Hilling reduces this number (p-value = 0.111) and has consequently led to the lowest estimated yield (Table 6.22). In this trial there was little difference in the estimated yields with all being slightly less than the Control.

**Table 6.22. Effect of treatments on parsnip yield in Trial 7, Devon Meadows 2011**

| Treatments | Log total number of healthy plants out of 240 harvested[1] | Total number of healthy plants out of 240 harvested (back transformed) (%) | Log harvestable yield (120-300g)[2] | Harvestable yield (back transformed) (120-300g) (%) | Average weight of a parsnip (g) | Estimated yield of parsnips[3] (t/ha) |
|---|---|---|---|---|---|---|
| Control | 4.140 a | 61.83 | 4.370 a | 78.08 | 200.46 | 31.30 |
| Fulzyme®Plus | 4.167 a | 63.51 | 4.360 a | 77.23 | 187.78 | 29.00 |
| Hilling | 3.941 b | 50.46 | 4.260 b | 69.78 | 195.94 | 27.35 |
| Ridomil®Gold 25G | 4.167 a | 63.53 | 4.395 a | 80.03 | 193.85 | 31.03 |
| lsd | 0.171 | - | 0.116 | - | - | - |
| p-value | 0.030 | - | 0.111 | - | - | - |

[1], Based on the harvest of 20 plants from the middle double row of 12 blocks and includes all size categories; [2], harvested as per 1 but defined as healthy roots in the ≥120g to ≤300g category; [3], estimated yield is based on 600,000 parsnips per ha with 80% germination x proportion of parsnips healthy and in the weight category ≥120g to ≤300g out of 240 harvested x average weight of a parsnip.

### 6.3.8 Environmental parameters

Refer to Chapter 7 for a detailed description of environmental parameters and a description of soil types. Generally Trial 2 had higher rainfall (75mm) and a heavier soil (medium clay) compared with Trial 1 which had lower rainfall (61mm) and a lighter soil (sandy loam). Trial 2 had a higher incidence of canker, a higher severity of disease and lower yields compared with Trial 1, for all treatments in common. No comparison is possible with Trials 3 and 4 as Trial 3 was on a low lying area of the farm which flooded after heavy rain.

## 6.4 Discussion

The systemic fungicides Ridomil®Gold 25G, Amistar®Top and Folicur® reduced various symptoms of root rot by 48% to 62%, 25% to 47% and by 27%, respectively. Only Ridomil® Gold 25G increased yield by 34% in one trial and had promising positive effects in another. These results are consistent with oomycetes being involved in parsnip root rots or canker because Ridomil®Gold 25G has efficacy against them. The biofungicides (*Bacillus* and *Streptomyces*), Hilling, Fleece™, Stand SKH™ (silicon) and Mulch Enviromix™ did not control canker or improve yield. Foliage symptoms of *I. perplexans* were reduced with Folicur® (tebuconazole) and Mulch Enviromix™. Hilling, Fulzyme®Plus, Fleece™, Folicur® and Mulch Enviromix™ all stimulated plant growth. Harvesting parsnips three weeks early did not avoid root diseases or increase yields. Growing parsnips on heavy soil should be avoided in Australia, as has been reported in the United Kingdom (Green and Hewlett 1950).

***Ridomil®Gold 25G***

Ridomil®Gold 25G (metalaxyl) was the only treatment to control deep tap root lesions (canker); upper tap root lesions which probably develop into cankers and produce the highest increase in marketable yield, 62%, but in only one of three trials. Interestingly, one, two or four applications had the same efficacy and it was irrelevant whether these applications were targeted to early or late season applications. In the other trials it showed a trend to reduce severity of root diseases and brown lesions on the upper tap root which probably develop into cankers. As metalaxyl is specific for oomycetes and it had efficacy against canker throughout the crop, it suggests oomycetes can play a role in parsnip canker throughout the life of the crop.

The lack of consistent efficacy of metalaxyl between sites and years could be attributable to soil type, sample size and replications. Both this project and Minchinton *et al.* (2008) had difficulty controlling the oomycete component of parsnip canker on clay loam sites. It is possible that metalaxyl is being biologically degraded or bound up in clay and hence unavailable for disease control. Sample size was based on comparison of plants in plots of Trial 1 where significant difference were found between treatments and applied to all subsequent trials. Although replication of treated plots was increased in order to pick up significant differences; the increase from six to 12 plots was still not enough. Trials conduced on parsnips and in particular on soil borne disease should consider employing large plot sizes.

***Amistar®Top***

In both trials where Amistar®Top was sprayed (Trials 1 and 2) it had good efficacy against reducing the average severity of root diseases by up to 25% and in Trial 1 it also had good efficacy and reduced a specific symptoms, deep tap root lesions (canker) by 47%. The inconsistency between trials for canker control may be associated with the poor quality of the crop on the Trial 2 site. Despite good control of canker symptoms and a reduction in severity of root diseases, Amistar®Top did not improve estimated marketable yield. In these two trials if parsnip roots had been weighed and categorized into marketable and unmarketable, as in later trials, it is possible Amistar®Top may have had efficacy to improve yield.

***Folicur® (tebuconazole)***

In Trial 4 Folicur® increased vigour of foliage by 19% and reduced incidence of *I. perplexans* lesions by 35%, suggesting parsnips which carry more of a healthy canopy are better able to support a developing tap root. Unfortunately in our trials this did not translate to healthier tap roots or increased yields, although tebuconazole is registered for parsnip canker control in the UK. This suggests other pathogens could be contributing to canker. It also suggests that parsnips can carry approximately a 20% canopy loss without affecting yield.

***Microplus™***

Microplus™ (*S. lydicu*), had no efficacy against specific disease symptoms. It slightly reduced the average severity of root rot, but other treatments had more efficacy. It had no effect on yields. It is possible that more frequent applications or higher rates may have been beneficial. Although *S. lydicus* has reported efficacy against Basidiomyces such as *Armillaria* spp., *Rhizoctonia* spp. and oomycetes such as *Pythium* spp (Copping 2001), unfortunately parsnip root rot associated with oomycetes (*Pythium* spp.) and other fungal pathogens are unlikely to be added to this list under conditions of our trials. Overseas the efficacy of a commercial formulation of *S. lydicus* to control oomycete pathogens has been has been variable. Up to 9 applications at 7-10 days intervals had no efficacy against *Pythium* root rot of pepper but had significant efficacy against *Phytophthora* blight of fruit (Miller *et al.* 2006). *S. lydicus* may be more suitable for control of foliage diseases rather than diseases of roots.

***Fulzyme®Plus***

Fulzyme®Plus was applied at a much higher rate than recommended by the manufacturer (34 times). In Trial 4 it maintained more vigorous foliage on plants throughout the trial by approximately 25% and reduced disease symptoms on roots at the Early harvest but this did not carry through to the Late harvest or to harvestable yield. The efficacy of *Bacillus* spp, as a biological fungicide, appears variable in the literature for example a commercial formulation of *Bacillis* sp. significantly increased root mass of *Pythium* infected tomato roots of plants grown in a greenhouse but did not control the disease (Ingram 2005). However, when nine applications of a commercial *Bacillus* formulation were mixed with Kocide® and applied at 7-10 day intervals it had no efficacy against *Pythium* induced root rot of field grown pepper (Miller *et al.* 2006). The application of commercial formulations of *Bacillus* may be more suited to glasshouse conditions rather than to field grown crops. Fulzyme®Plus reportedly provided adequate control of *Pythium* induced root rot of hydroponically grown coriander only when populations of the *Pythuim* spp. were low (Len Tesoriero, pers. comm.). This suggests it may have application to maintain low levels of *Pythium* induced root rots in newly established

hydroponic crops. More research is required on timing and rates of application for field grown crops. Fulzyme®Plus had no efficacy to reduce *Itersonilia* lesions on parsnip foliage in Trials 3, 4 or 7.

***Stand SKH™ (Silicon)***

The three applications of silicon to parsnip plants had no effect on any of the disease symptoms, the disorder, skin cracks, or on yields. The lack of any efficacy against skin cracks was unfortunate as they are perceived to be a manor problem for parsnips grown from winter into spring. It is possible that: (i) more frequent applications may be beneficial; (ii) higher levels of replication in trials may pick up differences between plants treated with and without silicon; (iii) silicon levels may already have been high; (iv) the benefits of silicon may be restricted to Cucurbitaceae and not applicable to Apiaceae; or (v) silicon may have benefits for parsnip plants e.g. post harvest, which were not assessed in this trial.

***Fleece™***

Fleece™ reduced the extremes of ambient temperature and soil temperature by a few degrees centigrade and this was associated with a rise in plant vigour, but squashed leaves. It was neither effective in reducing symptoms of disease nor in increasing yields. It was not practicable on a small scale but is used in the United Kingdom to completely cover crops to prevent carrot fly larvae attack of basal stems. In the absence of this pest in Australia, it could possibly be useful in preventing frost damage to high-value crops. Although it was not possible to recycle our Fleece™, when it is machine laid and lifted, re-use is possible, as is done in the UK.

***Hilling***

Hilling did not reduce the incidences of any disease symptoms on parsnip roots in Trials Nos. 4 and 7 and had no effect on skin cracks or yield. Despite the benefits of Hilling, where incidence of canker was reduced by 50%, reported by Channon (1963a) we were unable to duplicate this work, even though our parsnip roots were covered with the same amount of soil as those of Channon (1963a) which was 10.16 cm (4") from the bottom of the trench to the top.

There were, however, some benefits of Hilling. Hilling was beneficial early in the crop life and markedly improved crop vigour but this did not persist until harvest. It did not reduce *I. perplexans* lesions on foliage in Trials Nos. 4 and 7. Assuming *I. perplexans* blastiospores behave in practice as in theory (Channon 1962b), then other pathogens could be responsible for cankers. Alternatively Hilling did not persist long enough to prevent infection from *I. perplexans*. Further applications of Hilling would be difficult to undertake due to the dense canopy of foliage carried by parsnip crops.

***Mulch Enviromix™ Mulch NRS™***

Mulches in our trials were applied to increase soil microbial activity and increase soil temperature in order to decrease diseases associated with *Pythium* spp.. Minchinton *et al*. (2006, 2007, 2008,) reported problems associated with *Pythium* spp. were common in parsnips and parsley production during periods when temperatures were low. The additional nutrients and warmer minimum and cooler maximum temperature, associated with the Mulch Enviromix™, increased foliage vigour and reduce the incidence of *Itersonilia* on foliage at harvest. This mulch had no effect on reducing diseased roots at either the Early or Late harvest and did not improve marketable yields. Mulch NRS™ raised the minimum, maximum and average soil temperatures, but in one trial was not aged sufficiently when laid and was phytotoxic to direct sown seeds. It also contained particles of coloured plastic that were considered an eyesore by the grower.

Both Mulches appeared to increase lateral root development which is detrimental to marketability of parsnips. Mulch applications are probably more suited to crops marketed for their foliage rather than those marketed for their roots. Mulches can be made from a range of organic wastes but those used in our trials were made from municipal green waste, which would have contained some bark. Increased efficacy for control of root diseases may be achieved with mulch containing a higher proportion of bark (Erhart *et al.* 1999) or pine bark (Dissanayaka and Hoy 1999). Additions of a biocontrol agent may increase control, for example compost enriched with *Trichoderma*, reduced onion white rot in Tasmania (Metcalf *et al.* 2004).

Metalaxyl was the most consistent treatment to control tap root lesions (cankers) during the seven field trials conducted over three years. As metalaxyl is specific for oomycetes, especially *Pythium* spp., and as no *Phytophthora* spp. were isolated from parsnip roots (Chapter 7), this implicates *Pythium* spp. as a cause of parsnip canker, probably as a complex with other pathogens.

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# 6.6 Appendix

**Table 1 Soil analysis for Clyde and Deveon Meadows**

| Item | Site | | Trial No. 1 | | Trial No. 2 | |
|---|---|---|---|---|---|---|
| | Description | Unit | Top Soil | Sub Soil | Top Soil | Sub Soil |
| | Depth | | 0-15 | 15-25 | 0-15 | 15-25 |
| Carbonate/Bicarbonate | Bicarbonate | mg/kg | 72.7 | 100.4 | 91.4 | 93.4 |
| | Carbonate | mg/kg | 0 | 0 | 0 | 0 |
| | Chloride | mg/kg | 110 | 150 | 130 | 90 |
| Total Carbon/Nitrogen | Carbon | g/100g | 1.2 | 0.79 | 1.8 | 1.9 |
| | Nitrogen | g/100g | 0.11 | 0.07 | 0.15 | 0.15 |
| | Organic matter | g/100g | 2.2 | 1.5 | 3.2 | 3.5 |
| pH and Conductivity | Electrical Conductivity | dS/m | 0.27 | 0.24 | 0.28 | 0.24 |
| | pH($CaCl_2$) | - | 6.3 | 6.1 | 6.8 | 6.8 |
| | pH(water) | - | 6.6 | 6.4 | 7.3 | 7.3 |
| | Total soluble salts | % | 0.09 | 0.08 | 0.1 | 0.08 |
| Ammonium acetate cations (with prewash) | Calcium | meq/100g | 3.5 | 2.5 | 6.5 | 6.4 |
| | Calcium as % | % | 79 | 79 | 70 | 70 |
| | Calcium Magnesium ratio | - | 4.8 | 4.9 | 3.6 | 3.3 |
| | Magnesium | meq/100g | 0.74 | 0.52 | 1.8 | 1.9 |
| | Magnesium as % | % | 16 | 16 | 20 | 21 |
| | Potassium | meq/100g | 0.15 | 0.11 | 0.79 | 0.69 |
| | Potassium as % | % | 3 | 3 | 8 | 7 |
| | Sodium | meq/100g | 7.20E-02 | 5.00E-02 | 0.16 | 0.19 |
| | Sodium as % | % | 2 | 2 | 2 | 2 |
| | Sum of four cations | meq/100g | 4.5 | 3.2 | 9.3 | 9.2 |
| Available Aluminium | Aluminium | mg/kg | <10 | <10 | <10 | <10 |
| Available Boron | Boron | mg/kg | 0.7 | 0.5 | 1.9 | 1.4 |
| Available Potassium | Potassium | mg/kg | 180 | 180 | 490 | 400 |
| Available Phosphorus | Phosphorous (Olsen) | mg/kg | 83 | 81 | 94 | 58 |
| Available Sulfur | Sulfur | mg/kg | 29 | 33 | 42 | 46 |
| DTPA extractable trace elements | Copper | mg/kg | 6.8 | 6.5 | 2.1 | 1.5 |
| | Iron | mg/kg | 27 | 26 | 78 | 92 |
| | Manganese | mg/kg | 5 | 5 | 4 | 6 |
| | Zinc | mg/kg | 8 | 6.3 | 5.3 | 3.2 |

# Chapter 7

# Evaluating the potential strategies for Pythium control in parsnips in Tasmania

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## Summary
Ridomil 25G®, applied in one or two soil applications had no beneficial effect on parsnip root disease and root quality. Hilling as well as Rovral Aquaflo® and Fulzyme® foliar applications also had no beneficial effect.

## 7.1 Introduction

Pythium is common in soil and may cause seedling damping off or damage to the tip of the tap root resulting in forking of parsnip roots. Trials in Victoria had shown a positive yield response due to reduction of upper root lesions and cankers with Ridomil granule applications (per. comm. Dr Elizabeth Minchinton). Apart from *Itersonilia*, *Pythium* had also been associated with the development of root lesions and cankers.

This study aims to examine the effect of Ridomil granules application in soil as well as other novel strategies for Pythium control and reducing root lesions and cankers within a commercial parsnip crop in Tasmania.

## 7.2 Materials and Methods

### 7.2.1 Treatments

A field trial was conducted within a commercial parsnip crop (cv. Thunder) sown on 06/12/11 at Merseylea, Tasmania. Four strategies that had been identified in previous trials as having potential for *Pythium* and canker disease control in parsnip crops were evaluated (Table 7.1). These strategies included metalaxyl (Ridomil 25G® granules) in one and two applications for Pythium control, the use of hilling to cover root crowns to prevent root lesion and canker development, the use of *Bacillus subtilis* (Fulzyme®) to suppress diseases and iprodione (Rovral Aquaflo®) spray applications for foliar disease control. These were compared against an untreated control. The trial design was randomized complete block with 6 replicates. Plot size was 8 m x 1.2 m. Nemacur®, a granular nematicide, was applied throughout the trial as per commercial standards for nematode control.

**Table 7.1. Treatment applications for the trial at Merseylea, Tasmania**

| No. | Treatment | Treatment application | | | |
|---|---|---|---|---|---|
| | | At sowing (06/12/11) | 50DAS, 7WAS (25/01/12) | 78DAS, 11WAS (22/02/12) | 111DAS, 16WAS (26/03/12) |
| 1 | Untreated control | Nil | - | - | - |
| 2 | Ridomil 1x | Ridomil | - | - | - |
| 3 | Ridomil 2x | Ridomil | Ridomil | - | - |
| 4 | Ridomil 1x + Rovral 2x | Ridomil | - | Rovral | Rovral |
| 5 | Ridomil 1x + Hilling 1x | Ridomil | - | Hilling | - |
| 6 | Ridomil 1x + Fulzyme 3x | Ridomil | - | Fulzyme | Fulzyme |

DAS: days after sowing; WAS: weeks after sowing

At sowing, Ridomil was applied with a precision Gandy applicator on seed rows. At 50 days after sowing (50DAS), Ridomil granules (25 kg/ha) were hand sprinkled evenly over the soil surface, hilling was carried out between plant rows (Fig. 7.1) and Fulzyme was applied with a watering can (120 ml/5 L on each plot). At 78DAS and 111DAS, Rovral Aquaflo (100 ml/100 L) and Fulzyme (2.4 L/100 L) were sprayed until run-off with a backpack pressurized sprayer fitted with a boom.

Soil core samples were obtained from the trial area (10 cm deep) and sent to AgVita Analytical Pty Ltd in Devonport, Tasmania to analyse for soil pH and EC.

At 135 DAS, plant foliage in each plot was assessed for the percentage of leaf area affected by leaf spots due to *Ramularia* infections. At 149 DAS, just before harvest the foliage of plants in the trial plots were slashed and approximately 50 roots were harvested, washed and assessed for disease and marketability (Fig. 7.2).

**Fig. 7.1** Hilling or mounding of plant rows.

**Fig. 7.2** Trial plots showing slashed foliage before harvest.

## 7.3 Results and Discussion

There was excellent seedling establishment in the trial, with no obvious difference between the treatment plots. Soil analysis showed that soil in the trial area has an EC of 0.070 and pH of 6.54 ($H_2O$) and 5.80 ($CaCl_2$). Leaf spots due to *Ramularia* were widespread in April, causing yellowing of foliage (Table 7.2, Fig. 7.3). Rovral foliar applications appeared to increase disease severity. Fulzyme foliar applications appeared to reduce the disease severity slightly compared to treatments that have no foliar applications.

**Fig. 7.3.** Symptoms of *Ramularia* leaf spot

**Table 7.2 Severity of *Ramularia* leaf spots at 135 days after sowing**

| | Ramularia leaf spots | |
|---|---|---|
| Treatment | % Leaf area affected | |
| Untreated control | 21.3 | bc |
| Ridomil 1x | 30.0 | ab |
| Ridomil 2x | 28.8 | abc |
| Ridomil 1x + Rovral 3x | 37.5 | a |
| Ridomil 1x + Hilling 1x | 20.0 | bc |
| Ridomil 1x + Fulzyme 3x | 17.5 | c |
| **P-value** | **0.0251** | |

Means followed by same letter do not significantly differ (P = 0.05, LSD)

In the assessments of harvested parsnip roots at 21 weeks after sowing, there were no significant treatment effects in the marketable and un-marketable parsnip roots (Tables 7.3-7.4). Only approximately 38% of the roots harvested were marketable. Roots that had small and shallow orange lesions were considered to be marketable, as many of these lesions will rub off in commercial washing (Fig. 7.4). Other parsnips were un-marketable due to deep lesions and cankers, root forking and cracks (Fig. 7.4).


**Fig. 7.4.** Quality of parsnip roots

**Table 7.3. Treatment effects on marketable parsnip roots at 21 weeks after sowing**

| Treatment | Total no. of roots assessed | Marketable parsnip roots | | | | |
|---|---|---|---|---|---|---|
| | | % Roots with no blemish | % Roots with shallow small lesions | % Marketable roots | Weight of marketable roots | Mean weight of marketable root |
| | | A | B | A + B | A + B (kg) | (g/root) |
| Untreated control | 53 | 21 | 16 | 37 | 3.91 | 190 |
| Ridomil 1x | 53 | 21 | 16 | 36 | 3.80 | 200 |
| Ridomil 2x | 53 | 27 | 14 | 40 | 3.72 | 170 |
| Ridomil 1x + Rovral 3x | 51 | 23 | 16 | 38 | 3.66 | 190 |
| Ridomil 1x + Hilling 1x | 49 | 21 | 19 | 41 | 3.61 | 180 |
| Ridomil 1x + Fulzyme 3x | 52 | 22 | 16 | 38 | 3.78 | 190 |
| P-value^ | 0.501 | 0.886 | 0.677 | 0.950 | 0.999 | 0.702 |

^ Within each column, means followed by same letter do not significantly differ (P = 0.05, LSD).

Forking of roots was the main cause of un-marketable roots (Table 7.4). No root-knot nematodes could be found in association with the root forking. There was no significant difference in the percentage root forking between the Ridomil soil treatments and untreated control. This indicates that other than *Pythium,* other factors may be causing the root forking. There were also no significant differences in the percentage of deep lesions and cankers between treatments. The lack of differences may be due to the long time lapse since the last treatment application at sowing and 7, 11 and 16 weeks after sowing.

**Table 7.4. Treatment effects on un-marketable parsnip roots at 21 weeks after sowing**

| Treatment | Rejects or un-marketable parsnip roots | | | | |
|---|---|---|---|---|---|
| | % Deep lesions and cankers | % Forking | % Skin cracks | % Other rejects | % Total rejected |
| Untreated control | 17 | 28 | 10 | 8 | 63 |
| Ridomil 1x | 11 | 33 | 10 | 10 | 64 |
| Ridomil 2x | 15 | 29 | 8 | 8 | 60 |
| Ridomil 1x + Rovral 2x | 17 | 27 | 10 | 8 | 62 |
| Ridomil 1x + Hilling 1x | 19 | 23 | 8 | 10 | 59 |
| Ridomil 1x + Fulzyme 3x | 14 | 32 | 9 | 7 | 62 |
| P-value^ | 0.364 | 0.736 | 0.976 | 0.876 | 0.950 |

^ Within each column, means followed by same letter do not significantly differ (P = 0.05, LSD).

## 7.4 Conclusion

Ridomil 25G, applied in one or two soil applications had no effect on parsnip root disease and root quality. Follow-up treatments of hilling and Rovral and Fulzyme foliar applications also had no beneficial effect.

# Chapter 8

# Control of disease in parsnips in Western Australia

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## Summary
Metalaxyl reduced the incidence of forking in a summer parsnip crop in Western Australia. Metalaxyl fungicide reduced forking from the site mean of 30 per cent to 6.7 per cent with a single application of granular product applied 1 day after sowing. Low levels of root and petiole disease were not affected by a range of applied disease control treatments.

## 8.1 Introduction

Recent research in Victoria[1] found that the oomycete active fungicide metalaxyl reduced canker of parsnips. *Pythium* spp. were also commonly isolated from young parsnip roots in these experiments. As part of a national project, the aim of this experiment was to assess the efficacy of potential disease control measures for parsnips in Western Australia.

## 8.2 Materials and methods

The site for this experiment was a commercial vegetable farm at Wanneroo, a northern Perth suburb. The soil type was grey coarse sand (Basendean sand) and the site had a long history of intensive vegetable production including carrots and parsnips. The parsnips were sown on 13 November 2011 with 4 single rows per 1.42 m bed and plants were hand thinned at the 2 true leaf stage. Plots were 5 m long and there were 6 replicates of 10 treatments including 2 untreated controls (Table 8.1). The parsnips grew well and were harvested on the 15$^{th}$ March 2012 coinciding with harvest of the surrounding commercial crop. Parsnips were harvested and washed from a 1.5 m length of each of the middle 2 rows of each plot then weighed to calculate root yield. Twenty roots from each plot were randomly selected for disease and quality assessment.

The incidence and severity of parsnip canker were assessed at harvest with the proportion of marketable parsnips expressed as the sum of parsnip roots without disease and other damage (skin cracks, forking). A root disease rating was calculated using the following 0 – 4 scale for each sampled parsnip where 0 = healthy root; 1 = superficial brown lesions on the upper tap root, lesions on the lower tap root; 2 = elongated lesion on the tap root; 3 = deep lesion or canker on the tap root; and 4 = crown rot. Data were analysed by ANOVA using GENSTAT software.

A sample of root and petiole lesions were plated in the laboratory for isolation of potential pathogens. The lesions were excised from the roots and then surface sterilised in 1.2% sodium hypochlorite before being rinsed in sterile distilled water twice. They were then blotted dry before being plated onto water agar (WA) + Achromycin (A). The petioles lesions (Fig. 8.1) were plated onto potato dextrose agar (PDA) + A and WA + A. No *Itersonilia* spp were isolated.

**Table 8.1. Treatments applied to parsnips sown at Wanneroo, Western Australia on 13 November 2011.**

| Treatment (a.i.) | Product name | Product rate and timing |
|---|---|---|
| 1.Untreated 1 | | |
| 2.Untreated 2 | | |
| 3.Metalaxyl (25g/kg) | Ridomil®Gold 25 G | 40 kg/ha, 1 das |
| 4 Hydrogen Peroxide (35%) | Interox® Ag bath | 25 L/ha drench, 1 das |
| 5.Phosphorus acid (600 g/L) | Agri-Fos®600 | 12 L/ha sprayed monthly[A] |
| 6.Azoxystrobin (500 g/kg) | Amistar WG | 0.3 kg/ha sprayed monthly |
| 7.*Bacillus subtilis* | Fulzyme®Plus | 1.0 L/ha sprayed monthly |
| 8.Hydrated lime | Hydrolime™ | 1 t/ha broadcast 1 das 8 and 12 weeks after sowing |
| 9.Hilling | Cultural practice | |
| 10.Iprodione | Rovral® | 1.0 L/ha sprayed monthly |

[A]Sprayed treatments applied 3 times with mini boom in 384 L water /ha commencing 4 weeks after sowing.

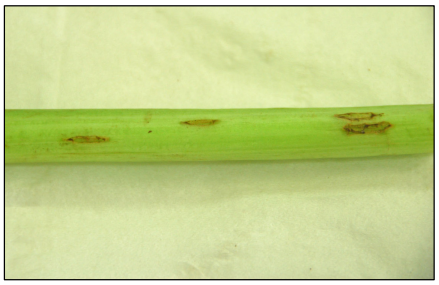

**Fig. 8.1.** Petiole lesions from parsnip experiment

## 8.3 Results

**Fig. 8.2.** Typical root lesions from the experiment

Root forking was the major cause of root rejection and while root disease was present, the incidence and severity, as reflected by the root disease ratings, was low (Table 8.2). Market proportion of roots was highest for the metalaxyl treated plots (Table 8.2) and this was as a result of reduced forking in this treatment.

*Phoma* and *Alternaria* spp were isolated from petiole lesions. Multiple fungi were present in some root lesions. *Fusarium* (53 per cent of lesions) and *Rhizoctonia* spp (58 per cent of lesions) were frequently isolated from root lesions while *Pythium* spp were isolated from 9 per cent of lesions. Typical root lesions symptoms are shown in Fig. 8 2.

**Table 8.2. Results for parsnip crop at Wanneroo, Western Australia, harvested on 15th March 2012**

| Treatment | Total yield (t/ha) | Market (%) | Forked roots (%) | Root disease rating[A] | Petiole disease score[A] |
|---|---|---|---|---|---|
| 1. Untreated 1 | 18.9 | 67.5 | 26.7 | 0.20 | 0.42 |
| 2. Untreated 2 | 17.0 | 44.2 | 49.2 | 0.13 | 0.48 |
| 3. Metalaxyl (25g/kg) | 19.0 | 85.8 | 6.7 | 0.13 | 0.52 |
| 4. Hydrogen Peroxide (35%) | 17.6 | 67.5 | 27.5 | 0.12 | 0.48 |
| 5. Phosphorus acid (600 g/L) | 19.0 | 63.3 | 30.0 | 0.16 | 0.42 |
| 6. Azoxystrobin (500 g/kg) | 17.7 | 53.3 | 34.2 | 0.28 | 0.30 |
| 7. *Bacillus subtilis* | 18.6 | 59.3 | 27.1 | 0.40 | 0.62 |
| 8. Hydrated lime | 18.0 | 60.0 | 38.3 | 0.05 | 0.42 |
| 9. Hilling | 18.6 | 69.8 | 22.7 | 0.18 | 0.34 |
| 10. Iprodione | 18.6 | 59.8 | 34.2 | 0.08 | 0.38 |
| Sig. | ns | *P<0.001* | *P<0.001* | ns | ns |
| LSD *(P=0.05)* | | 24.0 | 14.4 | | |

[A] Average number of petioles per plant with lesions for the 20 plants per plot; das, days after sowing.

## 8.4 Discussion

Forking of parsnips was the major cause of reduced marketability in this experiment. Metalaxyl fungicide reduced forking from the site mean of 30 per cent to 6.7 per cent with a single application of granular product applied 1 day after sowing. The role of *Pythium* spp in causing damping off of seedlings of many plant species as well as root forking in carrots is well documented.

Previous research on parsnip canker (Minchinton *et al*. 2008) found that a range of fungi, including *Itersonilia perplexans*, *Rhizoctonia* spp, *Fusarium* spp and *Pythium* spp, were capable of causing canker-like lesions on parsnip roots. *Rhizoctonia*, *Fusarium* and occasionally *Pythium* spp were isolated from lesions in this experiment. The low incidence and severity of canker symptoms in this experiment hindered the evaluation of control measures.

## Acknowledgments

Thanks to HAL for funding this work via the National Vegetable Levy and the Commonwealth Government. Funding support from the Western Australian Government is also acknowledged. Liz Minchinton of DPI Victoria is thanked for leading the project. Thanks to Figaro and Aaron Natoli of Natoli Produce Farms for hosting the experiment and managing the parsnip crop.

## Reference

Minchinton EJ, Auer DF, Thomson F, Vujovic S (2008) The extent and cause of parsnip canker. HAL Ltd Project VG05045 Final Report. 47pp.

# Chapter 9

# Etiology and epidemiology of parsnip canker in Victorian commercial cropping systems


## Summary

Pathogens associated with parsnip canker were identified and the disease development during the crop life was determined in monthly surveys conducted on winter-grown crops in three consecutive seasons of 2009, 2010 and 2011 in Victoria. *Itersonilia perplexans* and *Pythium* spp., were exclusively isolated from infections on young roots at the early stage of crop development. Two most frequently isolated genera of *Fusarium* and *Phoma* as well as less frequent *Alternaria, Cylindrocarpon* and *Rhizoctonia* were characteristic for late infections. Nine *Pythium* spp. were identified using ITS region sequence data. Five species in the *P. dissotocum* complex, *P. intermedium, P. ultimum* var. *ultimum, P. sylvaticum* and *P. irregulare*, were typical representatives of *Pythium* complex in Apiceae but remaining four specis *P. rostratifingens*, *P. camurandrum*, *P. tracheiphilum* and *P. vanterpoolii* were not recorded on parsnip roots in Australia. The former two species have not been reported in this country. This work also provides the first record of *Phoma exiqua* var. *exigua* on parsnip roots in Australia. Pathogenicities of all typical species to parsnip canker and the newly recorded species are yet to be established. Disease symptoms developed gradually during cropping season but the greatest increments of disease incidence and severity were observed in spring, coinciding with relatively higher rainfall, increasing temperatures and day light, and rapid growth of parsnip roots. Soil properties and rainfall were demonstrated as factors contributing to higher disease levels and 100% yield loss in crop grown on medium clay soil in the season of relatively high rainfall.


## 9.1 Introduction

Parsnip canker can cause crop losses of up to 80% in Australia parsnip crops (Minchinton *et al*. 2008). Surveys of parsnip canker conducted at crop harvest in Victoria in 2006 have found that the incidence of canker peaks in parsnips harvested between September and November on crops sown in February to April (Minchinton *et al.* 2008). Symptoms of parsnip canker are variable in their appearance ranging from superficial orange, grey or brown to large and deep brown or black lesions on mature parsnip roots, mainly on the shoulder or the crown. In extreme cases, the canker can cover the entire root (Channon 1965, Cerkauskas 2002).

In the UK, parsnip canker has been attributed to *Itersonilia perplexans*, *Phoma* spp., *Mycocentrospora acerina, Streptomyces scabies* and *Cylindrocarpon destructans* (Jones 1953, Channon 1956 and 1965, Channon and Thomson 1981, Fox 2002). In Canada, it was attributed to *Phoma complanata* (Cerkauskas 1985), while in the USA *Itersonilia* was pathogenic to parsnip (Wilkinson 1952). It is not considered to be associated with bacteria (Green and Hewlett 1950).

Studies conducted by Minchinton *et al.* (2008) in Victoria concluded that, parsnip canker is a disease complex, which involves potential pathogens from the oomycete genus *Pythium* and several fungal genera such *Fusarium, Rhizoctonia, Acremonium, Cylindrocarpon, Microdochium,* and *Phoma*. Two species of fungi, *I. perplexans*, *M. acerina*, attributed with causing parsnip cankers overseas, unidentified *Phoma* spp. and *Cylindrocarpon* spp. were also isolated from cankers in Victoria. Pathogenicities of *Pythium* sp*, Fusarium oxysporum, Itersonilia perplexans*, *Acremonium, Cylindrocarpon, Microdochium, Mycocentrospora acerina*, *Phoma exigua*, and *Rhizoctonia* were established by this study. Isolates of all pathogens except *Rhizoctonia* and *Itersonilia* caused severe lesions on mature parsnip roots. Little is known on the dynamics of parsnip canker and the stage of crop development. Crop monitoring, including identification of pathogens associated with distinct disease symptoms, from seeding to harvest was recommended by Minchinton *et al.* (2008) to

determine exact cause of the disease and pathogen succession during crop development to appropriately target the disease control methods.

This study reports on the development of parsnip canker during the life of winter-grown parsnip crops and identification of *Pythium* spp. and fungal genera associated with parsnip canker disease complex. Epidemiology of canker resulting from infections of mature parsnip roots by single and combination of two pathogens in controlled environment is also examined.

## 9.2 Materials and Methods

### 9.2.1 Systematic surveys

Systematic monthly disease surveys were conducted on parsnip roots in untreated control plots in five replicated field disease management trials in the market garden area east of Cranbourne, Victoria, (Devon Meadows and Clyde in 2009 and 2010 and Devon Meadows in 2011). Each trial was direct seeded with grower-own parsnip seed at four rows per bed on raised beds in 2009 and 2010 trials, and six rows per raised bed in 2011 trial. All trials were maintained by growers (Table 9.1). Refer to Chapter 6 for further trial details.

**Table 9.1. Site details in Cranbourne market garden area, where monthly surveys were conducted on parsnip roots in untreated control plots of field disease management trials in 2009, 2010 and 2011**.

| Trial | Soil type | Trial dates | | No. of plants sampled | |
|---|---|---|---|---|---|
| | | Set up | Harvest | May to September | Harvest |
| ***2009*** | | | | | |
| Devon Meadows | medium clay | 8 April | 29 October | 4 | 36 |
| Clyde | sandy loam | 8 April | 21 October | 4 | 64 |
| ***2010*** | | | | | |
| Devon Meadows | sandy loam | 16 April | 28 October | 4 | 20 |
| Clyde | sandy loam | 13 May | 2 December | 4 | 20 |
| ***2011*** | | | | | |
| Devon Meadows | sandy loam | 8 April | 24-25 October | 4 | 20 |

*9.2.1.1 Plant sampling and disease assessment*

In the field trials, four plants were sampled from each of untreated control plot, (two plants were randomly taken from 1m long bed section at each end of every plot) in approximately monthly intervals from May to September. Plants were sampled starting from June to October from the Clyde trial in 2010. At harvest 20 plants were sampled from the entire area of each control plot in trials at Devon Meadows in 2010 and 2011 and at Clyde in 2010. In 2009 trials at Devon Meadows and Clyde, 36 and 64 plants, respectively, were samples from the entire area of each control plot.

Parsnip roots were assessed for any root damage that renders them unmarketable. Symptoms included brown superficial lesion(s) on upper and lower tap root, deep lesions (canker) on tap root, collar rot extending to tap root. Incidence of damaged roots was calculated as the percentage of plants with any symptom. Severity of root symptoms was calculated as a sum of scores given to each symptom present for plants assessed in both 2009 trials. The score scale for symptoms ranged from 0 to 4, where 0 was given to a healthy root, 1 for superficial brown lesion(s) on upper and/or lower tap root and/or forking, 2 for skin cracks, 3 for canker – deep lesion on the tap root, and 4 for collar rot extending to tap root.

### 9.2.2 Pathogen isolations and identification

*9.2.2.1 Isolations and culturing*
Pathogens were isolated from non symptomatic and symptomatic parsnip roots sampled during monthly surveys at each trial site. Isolations for oomycetes and fungi were performed as described in 4.2.2 in all three consecutive winter-cropping seasons. Isolations for *Itersonilia* were performed as described by Smith (1966) from samples collected in 2010 and 2011. Sections of disease affected root, leaf base, crown, leaf petiole, leaf blade were attached to a drop of petroleum jelly on the upper lid of 90 mm Petri dish. The lower part of the plate was filled with 10 mL of malt extract agar (MEA). Ballistospores of *Itersonilia* from infected plant sections falling onto MEA germinated and colonised the agar. Pathogen genera of all isolates were identified microscopically. *Pythium*-, and *Phoma*-like isolates were selected for further identification using DNA sequence data.

*9.2.2.2 DNA extraction and ITS sequencing*
DNA was extracted from 35 *Pythium*-like and 13 *Phoma*-like isolates and sequencing of the ITS region of rDNA were performed as described in 4.2.2.2.

### 9.2.3 Environment data collection

A range of instruments consisting of the ModelT weather station, soil moisture, temperature and electrical conductivity (EC) probe (Fig. 9.1), and a personal computer was used to collect environment data. A single weather station and soil moisture, temperature and EC probe was installed in each trial site (Refer to Chapter 3).

**Fig. 9.1.** ModelT weather station and soil moisture, temperature and EC probe installed in parsnip canker management trial at Clyde in 2009.

### 9.2.4 Pathogenicity of *Pythium sulcatum*, *Fusarium oxysporum*, *Cylindrocarpon* sp on mature parsnip roots in single and combined inoculations

Pathogen cultures used in this experiment were originally isolated from parsnip roots by Mr Desmond Auer (Minchinton *et al.* 2008). Agar plugs (10 mm diameter) of *F. oxysporum, Cylindrocarpon* sp and *P. sulcatum* from long term storage in sterile distilled water were placed on WA. Agar plugs were cut from edges of newly grown cultures of fungi and *P. sulcatum* and plated onto PDA and V8 agar, respectively.

Prior to inoculations, mature small parsnip roots courtesy of Mr Mark Milligan, Russell Lamattina Farm, Boneo, Victoria, were misted with 70% ethanol and dried with paper tissues. Two surface sterilised roots were placed into 1 L rectangular plastic take-away container lined with paper towel

moistened with sterile distilled water (SDW). Upper and lower part of one root in each container was damaged using a sterile needle. A single 10 mm diameter plug was cut out from the leading edge of 5-7 day old culture and transferred onto upper and lower section of each, damaged and undamaged parsnip root. Six boxes were prepared for each pathogen consisting of a combination of *P. sulcatum* with either *F. oxysporum* or *Cylindrocarpon* sp. and the uninoculated control. A total of 36 boxes were prepared, sealed with container lids and incubated at 10ºC in the dark. Parsnip roots were visually assessed for symptom development after 6, 10, and 15 weeks of incubation.

## 9.3 Results

### 9.3.1 Disease development in parsnip crops

***9.3.1.1 Development of parsnip canker in three winter-grown crops at Devon Meadows***

In the Devon Meadows trials root damage symptoms were first observed in May 2009 and a month later in 2010 and 2011 trials irrespective of the sowing date (Fig. 9.2). The 2009 site was on medium clay soil, whereas 2010 and 2011 trials were on sandy loam soils. Incidence of root damage at this site was consistently higher at each assessment time than in two sites on sandy loam soils. A month before harvest, all parsnip roots sampled in 2009 were unmarketable, while at harvest in 2010 and 2011 there were 50 and 63 percent of plants with unmarketable roots, respectively.

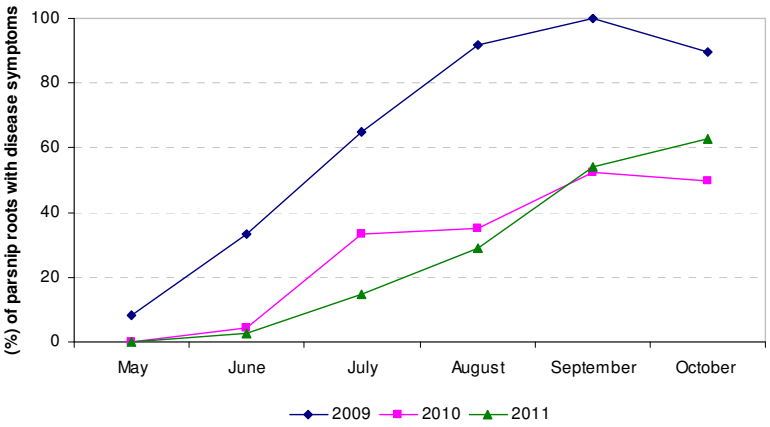


**Fig. 9.2.** Development of disease symptoms on parsnip roots in untreated control plots of the Devon Meadows trials in 2009, 2010 and 2011 winter-cropping seasons.

Generally, the severity score of root damage at 2009 site increased during life of the crop, following the same trend as disease incidence. The greatest increment in disease severity was recorded in spring from August (the average severity score 1.7) to September (the average severity score 3.3) (Fig. 9.3).

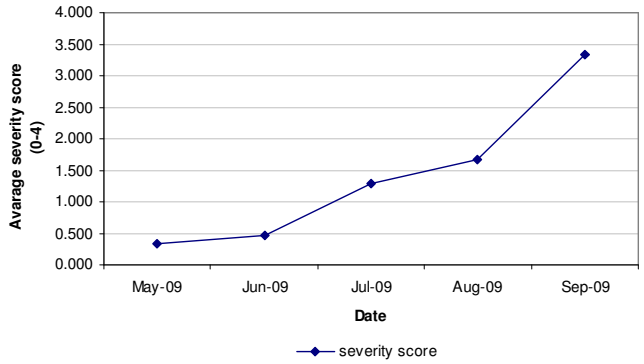


**Fig. 9.3.** Development of parsnip root damage severity in untreated control plots of the Devon Meadows trial in 2009 on the scale of 0-4, where 0 was given to a healthy root, 1 for superficial brown lesion(s) on upper and/or lower tap root and/or forking, 2 for skin cracks, 3 for canker – deep lesion on the tap root, and 4 for collar rot extending to tap root.

***9.3.1.2 Development of parsnip canker in two winter-grown crops at Clyde***

In Clyde, the 2010 trial required reseeding. The first seeds were washed away by heavy rains in late April. The site was often water logged. At the 2010 trial site, there were higher disease levels at each sampling time than in 2009 (Fig. 9.4). At the harvest of the former site, in early December, there were 90 % of plants with unmarketable roots, 13 % more than at the harvest of 2009 site, conducted in late October.

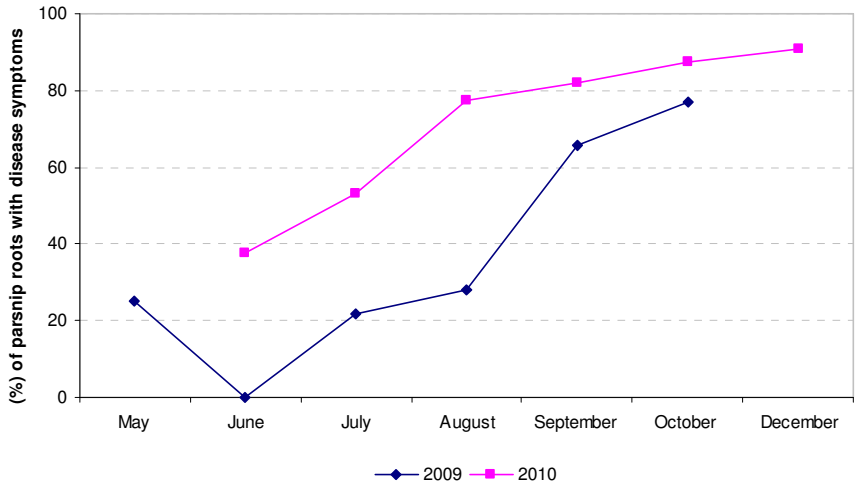


**Fig. 9.4.** Development of disease symptoms on parsnip roots in untreated control plots of the Clyde trials in 2009 and 2010 winter-cropping seasons.

Disease severity at the Clyde site in 2009 was lower than at the Devon Meadows in 2009 and did not exceed the score of 1.3 (Fig. 9.5).

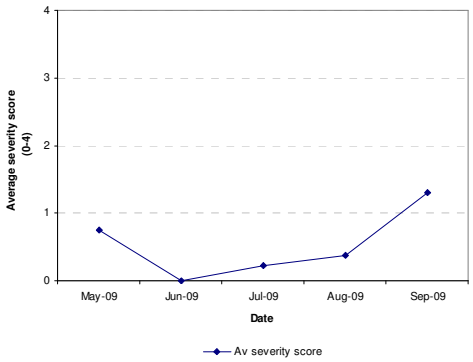


**Fig. 9.5.** Development of parsnip root damage severity in untreated control plots of the Clyde trial in 2009 where 0 was given to a healthy root, 1 for superficial brown lesion(s) on upper and/or lower tap root and/or forking, 2 for skin cracks, 3 for canker – deep lesion on the tap root, and 4 for collar rot extending to tap root.

## 9.3.2 Pathogen identification

***9.3.2.1 Succession of pathogens associated with parsnip canker in two winter-grown crops***

A total of 193 pathogen isolates were collected over three consecutive winter-cropping seasons from monthly surveys of five parsnip canker management trials in Devon Meadows and Clyde (Table 9.2).

In 2009, out of 116 isolates, *Pythium* spp. were the most common on parsnip roots early in the cropping season at both trial sites on medium clay and sandy loam in Devon Meadows and Clyde, respectively (Fig. 9.6 and 9.7). Fungal genera including *Phoma*, *Fusarium*, *Rhizoctonia, Alternaria* become more frequent later in the growing season as soil and air temperature started to increase towards the beginning of spring. Additionally, fungi of the *Cylindrocarpon* and *Botrytis* genera were isolated from roots at the Devon Meadows and Clyde, respectively.

In 2010 growing season, *Pythium* spp. were most common on parsnip seedlings but *Fusarium*, *Alternaria*, *Cylindrocarpon* were isolated mainly from symptomless tap and fine roots of younger plants in early June. Interestingly, *Itersonilia* was isolated from lesions on parsnip cotyledons as early as mid May in all 2010 and 2011 trial sites. In June and July of both seasons, this fungus was isolated from diseased leaf bases, lesions on leaf petioles and blades as well as crowns of parsnip (Fig. 9.8) and it was also isolated from symptomless roots and crowns.

**Table 9.2. Fungal and *Pythium* genera isolated during monthly surveys of parsnip root at five trial sites in three consecutive winter-cropping seasons of 2009, 2010 and 2011 at Cranbourne market garden area.**

| Pathogen genus | Parsnip winter-cropping season | | |
|---|---|---|---|
| | 2009 | 2010 | 2011 |
| *Pythium* | 31 | 7 | 12 |
| *Fusarium* | 17 | 3 | 7 |
| *Phoma* | 18 | 0 | 1 |
| *Alternaria* | 3 | 4 | 2 |
| *Itersonilia* | * | 3 | 21 |
| *Rhizoctonia* | 5 | 1 | 4 |
| Others including sterile and unknown | 42 | 7 | 29 |

*, no isolations for *Itersonilia* were conducted in 2009 cropping season.

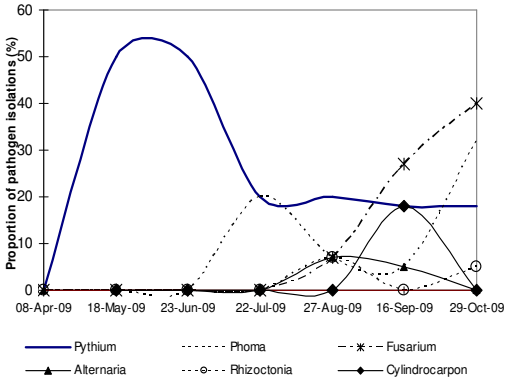


**Fig. 9.6.** Relative proportion (%) of pathogen genera isolated from parsnip roots in monthly surveys of untreated control plots at the medium clay site (Devon Meadows).

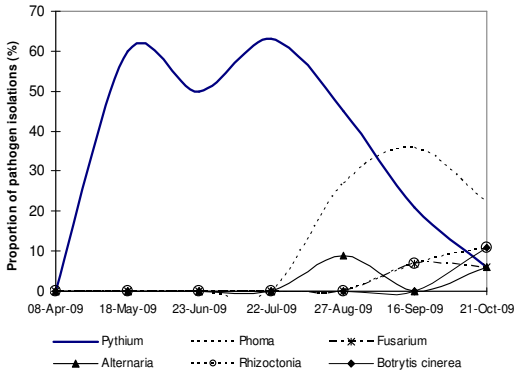


**Fig. 9.7.** Relative proportion (%) of pathogen genera isolated from parsnip roots in monthly surveys of untreated control plots at the sandy loam site (Clyde).

### *9.3.2.2 Identification of Pyhtium and Phoma spp. using ITS region sequence data*

Nine *Pythium* spp. were identified out of 35 sequences of ITS (ITS1, 5.8S, and ITS2) of rDNA (Table 9.3), including *P. intermedium*, *P. ultimum* var. *ultimum*, *P. dissotocum* complex, *P. sylvaticum, P. irregulare, P. rostratifingens*, *P. camurandrum*, *P. tracheiphilum* and *P. vanterpoolii*. Four isolates matched sequences of another *Pythium* sp., which has not been identified to a species level. Based on molecular phylogeny and taxonomy of the genus *Pythium* (Lévesque and de Cock 2004), species identified in this study belong to four phylogenetic clydes: B, E, F, and I. Isolates grouped in the *P. dissotocum* complex have sequences identical to these of *P. lutarium* and *P. coloratum.* These three species are also morphologically similar (Lévesque and de Cock 2004).

All 13 *Phoma* isolates were identified as *Phoma exigua* var. *exigua.* This species was also isolated from parsley (Refer to 4.3.2) and not reported from parsnip in Australia before. Pathogenicity of *Phoma exigua* was verified on mature parsnip roots in studies by Minchinton *et al.* (2008). There is a high probability that this previously tested pathogenic isolate of *Phoma exigua* was *P. exigua* var. *exigua*, since all isolates collected from parsnip in this study belonged to this variety. A more detail description of *Phoma exigua* pathogenicity on other hosts has been provided in 4.3.2.

**Table 9.3. *Pythium* and *Phoma* spp. isolated from parsnip roots at Devon Meadows and Clyde in 2009, 2010 and 2011 and identified using sequence data.**

| Species identified | No of isolates |
|---|---|
| ***Pythium*** | |
| *P. dissotocum* complex | 8 |
| *P. intermedium* | 7 |
| *P. ultimum* var. *ultimum* | 4 |
| *P. sylvaticum* | 3 |
| *P. irregulare* | 2 |
| *P. rostratifingens* | 2 |
| *P. camurandrum* | 2 |
| *P. tracheiphilum* | 2 |
| *P. vanterpoolii* | 1 |
| *P. sp* | 4 |
| ***Phoma*** | |
| *P. exigua* var. *exigua* | 13 |

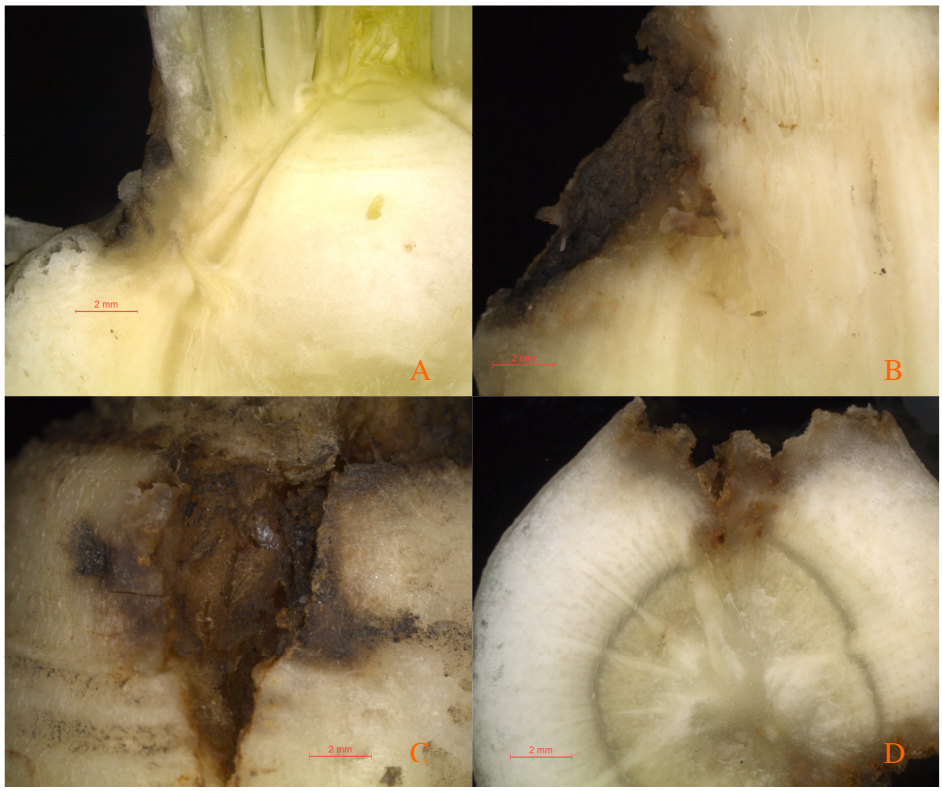


**Fig. 9.8.** Parsnip crown and root with canker A) parsnip root with no apparent disease symptoms and crown with the early stage of the disease initiated at the leaf base, B) canker advancing from crown to root, C) canker of the upper edge of tap root and crown, D) cross section of parsnip root with canker advancing from crown to tap root. *Itersonilia* and *Pythium* spp. are commonly isolated from these symptoms.

### 9.3.3 Environmental data analysis

The 2009 cropping season was the wettest of all three seasons with the highest total monthly rainfall in each month from April to August across all parsnip trial sites in Devon Meadows and Clyde. April and September were the wettest months of this season with the total monthly rainfall of 75.4 and 61.1 mm at Devon Meadows and Clyde. The 2010 was the driest of all three seasons from May to September with the total monthly rainfall of the wettest month August not exceeding 45mm. The 2011 season had the driest April but the wettest September of all three seasons with total monthly rainfall of 16.7 and 114 mm at Devon Meadows (Fig. 9.9).

June, July and August were the coldest months in each cropping season with August of 2010 having the lowest average temperatures of 8.3ºC and 9.4ºC at Devon Meadows and Clyde respectively. Average day and night temperature of this month was 10.1ºC and 6.6ºC and 11.0ºC and 8.1ºC in Devon Meadows and Clyde, respectively. The 2010 season was the coldest of all three seasons. Average temperatures in each month of this season from May to September were lower than in each month of 2009 and 2011 cropping seasons. April, September and October were the warmest months with highest average temperatures of 15.7ºC in April of 2011 and September 12.4ºC and 12.3ºC at both trial sites. October was generally warmer than September of each cropping season. The temperature data for this month, however, does not cover the whole month as all trials were harvested in different times therefore should be interpreted for each site and the season separately (Fig. 9.10).

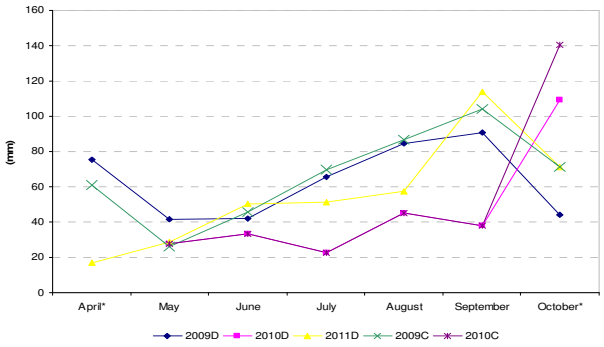


**Fig. 9.9.** Total monthly rainfalls at five parsnip canker management trial sites in three consecutive cropping seasons at Devon Meadows and Clyde based on automatically collected data from ModelT weather stations. Letters following years relate to the trial site, D) Devon Meadows, and C) Clyde.

### 9.3.4 Pathogenicity of selected pathogens on parsnip roots

*Fusarium oxysporum* was most pathogenic on mature parsnip roots. The infections occurred as dark brown sunken lesions, often with visible white-coloured mycelium and were present on 50%, 58% and 92% of inoculated roots after 6, 10 and 15 weeks of incubation (Fig. 9.11). Damaging roots with a needle directly under the *F. oxysporum* bearing agar plug did not affect symptom development. In contrast to *F. oxysporum* infections, more distinct symptoms developed on damaged roots inoculated with *P. sulcatum* and *Cylindrocarpon* sp. Infections were observed on 60% and 33% of damaged roots inoculated with *P. sulcatum* and *Cylindrocarpon* sp respectively, after 15 weeks of incubation.

In double pathogen inoculations with *P. sulcatum* and *F. oxysporum*, symptoms developed on 50% and 83% of undamaged roots, and 17% and 50% of damaged roots after 10 and 15 weeks of incubation respectively. Symptoms occurred on 25% of undamaged and 33% of damaged roots inoculated with both *P. sulcatum* and *Cylindrocarpon* sp, after 15 weeks of incubation, respectively.

## 9.4 Discussion

### 9.4.1 Disease development in parsnip crops in relation to soil and climate

This study demonstrated the development of parsnip root rot symptoms during the life of five parsnip crops grown in the cooler months of 2009, 2010 and 2011 in relation to soil types and weather conditions in the Cranbourne market garden area. Relatively higher disease levels were recorded on parsnip roots early during the crop life (May) at both sites in Devon Meadows and Clyde in 2009 than in the same time of 2010 and 2011. These higher disease levels coincided with the highest rainfall recorded in April (the month of crop establishment and early growth) of all seasons monitored.

The root damage incidence and severity gradually increased in all monitored sites towards the end of each of the three cropping seasons but were highest at the Devon Meadows site in 2009 and at each assessment time than in all other sites surveyed. There was no harvestable yield at this medium clay site but at the sandy loam site in Clyde, 25% of parsnip roots were healthy in the same season. This result suggests that both, rainfall and soil type are important factors contributing to overall disease levels.

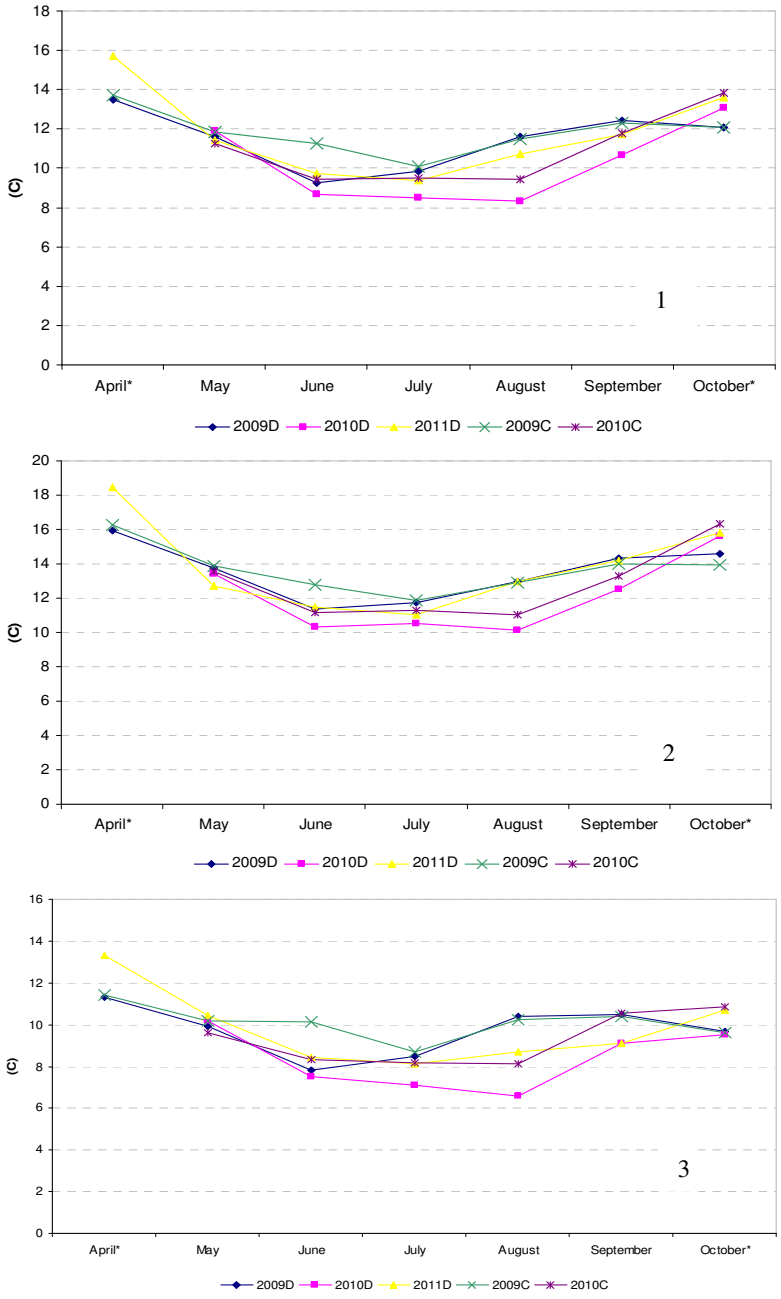


**Fig. 9.10.** Average monthly temperature (1), average day temperature in each month (2), and average night temperature in each month (3) at five parsnip canker management trial sites in three consecutive cropping seasons at Devon Meadows and Clyde based on automatically collected data from ModelT weather stations. Letters following years relate to the trial site, D) Devon Meadows, and C) Clyde.

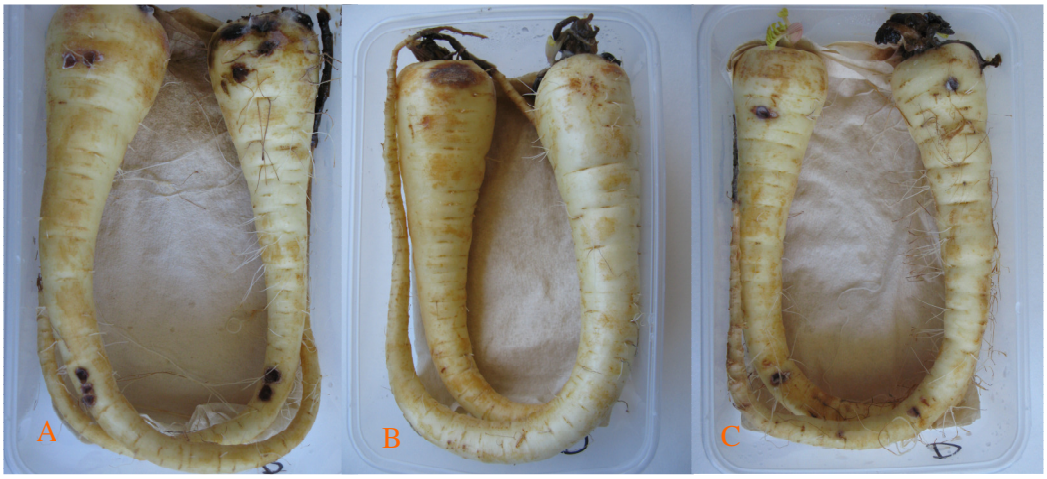


**Fig. 9.11.** Symptoms developed on damaged (right) and undamaged (left) mature parsnip roots incubated at 10°C in the dark for 15 weeks and inoculated with A) *F. oxysporum* B) *P. sulcatum*, C) *P. sulcatum* and *F. oxysporum*.

At the Devon Meadows site, there was relatively less disease in 2010 and 2011 than in 2009 at each assessment time indicating that growing parsnip on sandy loams during cooler and drier season's results in relatively healthier crops and higher yields.

Soils with higher clay content eg. medium clay (median clay content of 50% (Shaw 1999) at the 2009 Devon Meadows site, which have the capacity to hold more water than sandy loams (median clay content of 15% (Shaw 1999) during high rainfall and low temperatures period can be detrimental to crops and conducive to some pathogens eg. typical water moulds such as *Pythium* spp. (Harvey 2006). In contrast low temperatures of the winter months, June, July and August of 2010, in particular, could suppress activity of some pathogens involved in the parsnip canker complex (Refer to 8.4.2).

Greatest increments of disease incidence and severity occurred predominantly in the spring (September) in almost all sites surveyed over three seasons. This coincides with relatively higher rainfall, increasing temperatures and day light and rapid growth of parsnip roots. At this time roots are particularly prone to epidermis fragmentation (skin cracks) and as parsnip does not form a protective layer of wound cork, the inner root tissue is exposed to any soil organism (Sherf and Macnab 1986).

High disease levels at the Clyde site in 2010 are related to later crop establishment than at all other sites in all seasons. Despite lowest rainfall of all three season, sections of this site were frequently waterlogged due to drainage problems. Only 5% of parsnip roots were healthy at harvest.

## 9.4.2 Pathogen identification

***9.4.2.1 Succession of pathogens associated with parsnip canker in winter-grown parsnip crops***

This study identified pathogens associated with parsnip canker, which are responsible for the early and late infections. *Itersonilia perplexans* and *Pythium* spp., were distinct "early invaders" and *Fusarium, Phoma, Alternaria, Cylindrocarpon* and *Rhizoctonia* were commonly associated with late infections.

*Pythium* spp. and *I. perplexans* were isolated from symptomatic and non-symptomatic parsnip root and crown sections during the life of the crop but were almost exclusively isolated early during cropping season (Fig. 9.8). Systematic isolations conducted in the Devon Meadows and Clyde sites in 2009 demonstrated that fungi from *Fusarium, Phoma, Alternaria, Cylindrocarpon* and *Rhizoctonia* genera were relatively more frequent on parsnip roots later in the growing season, from late July to

harvest. *Fusarium* and *Phoma* were the most frequently isolated genera. These results are in line with previous reports on the cause of parsnip canker (Minchinton *et al.* 2008, Fox 2002, Channon 1963abc, 1964, 1965, Wilkinson 1952). Isolates of these fungal genera were also obtained from non-symptomatic root sections early in the 2010 cropping season, indicating that they are present in soils but may not cause initial root damage at this stage of crop development. Low temperatures of the winter months can temporarily suppress pathogenic activity of some fungi and this appears to be the case with parsnip seedlings grown under relatively cool conditions of June, July and August. Young plants may also be less susceptible to infections by these fungi than the mature ones. Results of a pathogenicity test conducted in the present study on mature parsnip roots at relatively low temperature (10°C) support this view for at least one fungus, *Cylindrocarpon* sp.

The frequently isolated fungal genus was *Fusarium*, specifically *F. oxysporum* and *F. solani* from winter parsnip crops. Both species were previously reported to occur on the summer grown crop on sandy soils on the Mornington Peninsula, Victoria (Minchinton *et al.* 2008).

The previous studies on the epidemiology of parsnip canker attributed to *I. perplexans* (Channon 1963b) showed that release of ballistospores increased late in the cropping season and release was rainfall dependent. There were fewer spores in drier conditions and consequently, there was less canker reported in the following cropping season. Sequential parsnip cropping without a winter break together with a relatively warm and wett spring of 2010 and 2011 resulted in infections parsnip seedlings and young plants. These early infections were not evident in the 2009 season, which came at the end of a 15 year drought. Control of *I. perplexans* in seedlings and young plants should include, crop rotations, spatial isolation of crop at different growth stages, management of plant debris and strategically timed fungicide applications, including seed treatments.

More detailed epidemiological studies on the relationship of the most frequently isolated pathogens, *Pythium* spp., *Fusarium*, *Phoma*, and *Itersonilia*, the stage of plant development, when these pathogens cause infections and the effect of temperature on their pathogenicity are required to better understand the dynamics of parsnip canker and to design the most effective disease control methods for parsnip producers.

***9.3.2.2 Identification of Pythium and Phoma spp. using ITS region sequence data***

This study identified nine *Pythium* spp. associated with a variety of parsnip root damage symptoms, including parsnip canker. The most frequently isolated species of *P. dissotocum* complex, *P. intermedium, P. ultimum* var. *ultimum* and less comm. *P. sylvaticum* and *P. irregulare* were previously reported in Australia on other crops from the Apiaceae family such as parsley (Minchinton *et al.* 2006, 2007, Chapter 4) and carrots (Davison and McKay 1998), and on carrots overseas (Hiltunen and White 2002, Suffert and Guibert 2007, Klemsdal *et al.* 2008). Two relatively recently described species, *P. camurandrum* (Bala *et al.* 2010) and *P. rostratifingens* (de Cock and Lévesque 2004), both members of clyde E (Lévesque and de Cock 2004) have not been previously reported in Australia. This is the first report of both species in this country. (Refer to Chapter 4 for description of pathogenicity).

Surprisingly, *P. sulcatum*, which was reported from parsley (Minchinton *et al.* 2006, Chaper 4), and as a predominant species associated with cavity spot of carrots in Australia (Davison and McKay 1998) has not been found on parsnip roots in Victoria. Environmental conditions such as soil properties and crop management practices have been shown to have significant impact on the species diversity in *Pythium* complexes in various crops (Suffert and Guibert 2006, Paulitz and Adams 2003, Dick and Ali-Shtayeh 1986). Both these factors as well as the low temperature during cropping seasons may not support proliferation of this typical pathogenic species to other Apiaceae crops. In contrast to *P. sulcatum*, two other species *Pythium tracheiphilum* and *P. vaterpoolii*, which have not previously been reported on parsnip, were identified. *Pythium tracheiphilum* is a common pathogen of lettuce, which is grown as a rotation crop in both the Devon Meadows and the Clyde site. *Pythium vanterpoolii* has been reported on bent grass in Australia.

Identification of *Phoma exigua* var. *exigua* in this study supports a view that this pathogen and additionally fungi from *Fusarium* genus as well as *I. perplexans*, is involved in the parsnip canker complex. There are no records of *Phoma exiqua* var. *exigua* on parsnip roots in Australia (Australian Plant Pest Database) but this species has been previously isolated from roots of parsnip (Machowicz-Stefaniak *et al*. 2008) overseas. The majority of *Phoma exiqua* var. *exigua* records in Australia come from leaf spots on beans (Fabaceae). *Phoma complanta,* which has been reported as a causal organism of parsnip canker complex in the UK and Canada (Channon 1963a, Cerkauskas 1987) has not been identified in the present study. This fungus is exotic in this country and its absence has been verified using DNA sequence data.

### 9.4.3 Pathogenicity of selected pathogens on parsnip roots

*Fusarium oxysporum* was most pathogenic on mature parsnip roots. The infections occurred as dark brown sunken lesions, often with visible white-coloured mycelium and after 6, 10 and 15 weeks of incubation (Fig. 9.11). This fungus was equally pathogenic in single pathogen inoculations as in double inoculations with *P. sulcatum* in the same test. No synergistic effect of these pathogens activity was observed at the test temperature. *Cylindrocarpon* sp. and *P. sulcatum* were moderately pathogenic on mature parsnip roots. A *Cylindrocarpon* sp. was isolated infrequently from trial sites and *P. sulcatum* not at all. This suggests that they may not play an important role in the parsnip canker complex in Victorian parsnip production systems.

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Appendix 9

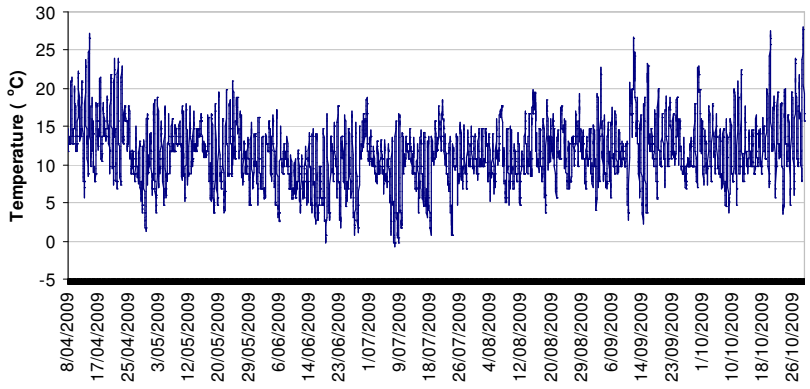


**Fig. 1.** Air temperature (°C) records for the Devon Meadows trial site collected from 8 April to 29 October 2009.

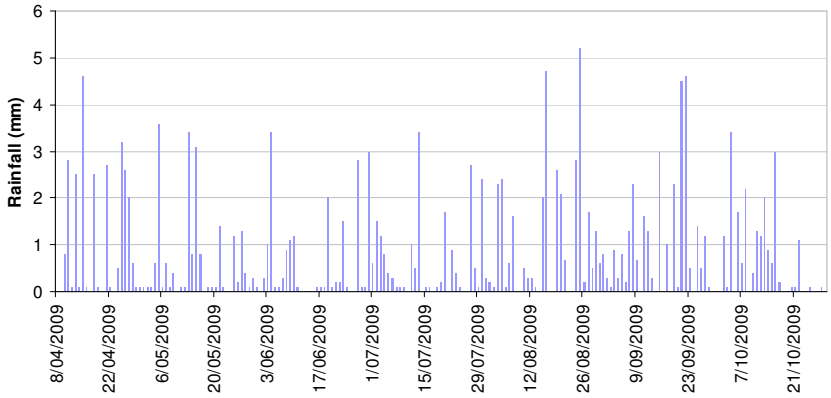


**Fig. 2.** Rainfall (mm) records for the Devon Meadows trial site collected from 8 April to 29 October 2009.

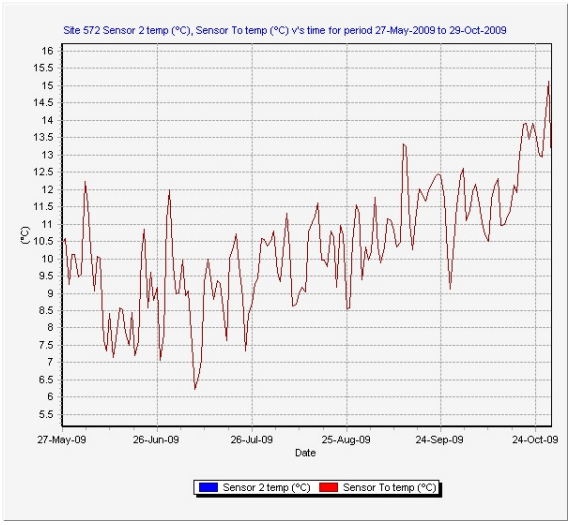


**Fig. 3.** Soil temperature at Devon Meadows 2009.

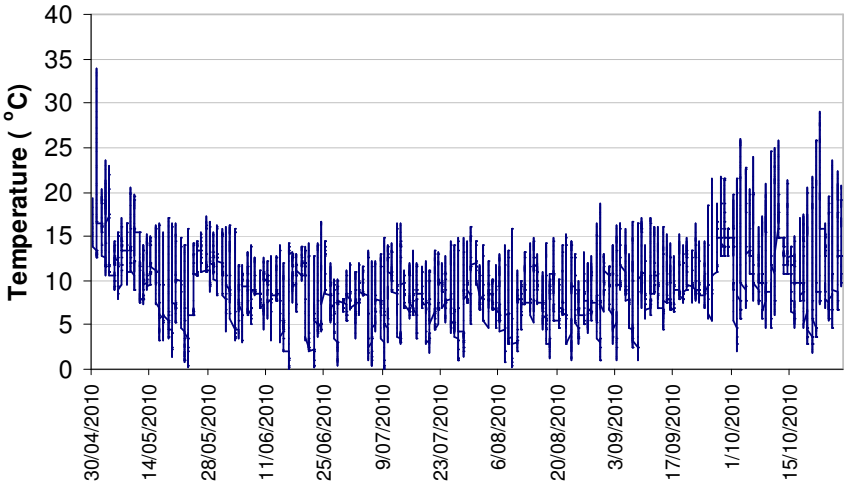


**Fig. 4.** Air temperature (°C) records for the Devon Meadows trial site collected from 30 April to 28 October 2010.

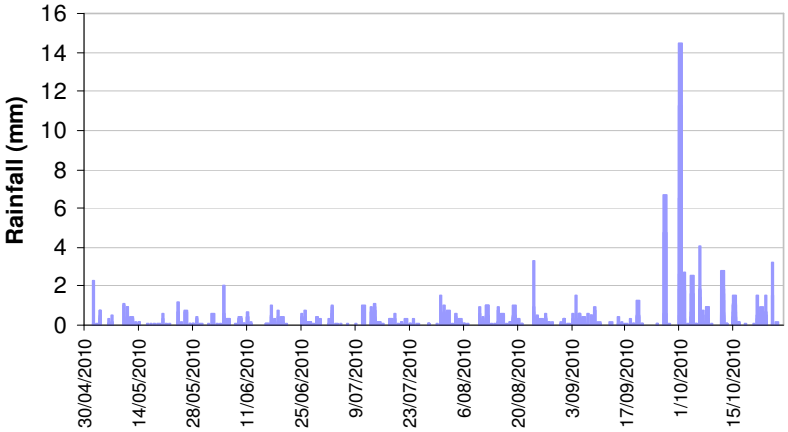


**Fig. 5.** Rainfall (mm) records for the Devon Meadows trial site collected from 30 April to 28 October 2010.

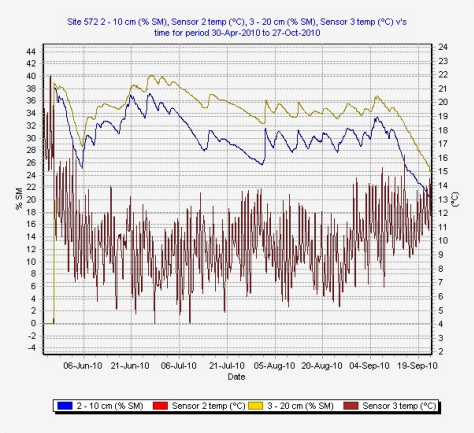

**Fig. 6.** Soil temperature and moisture records for the Devon Meadows trial site 2010.

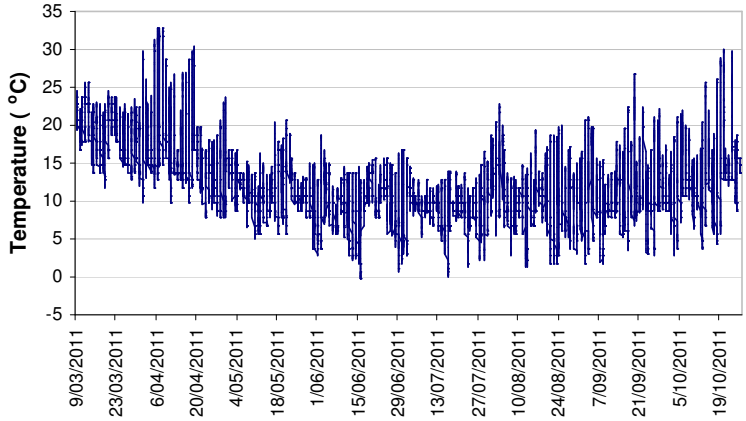


**Fig. 7.** Air temperature (°C) records for the Devon Meadows trial site collected from 8 April to 25 October 2011.

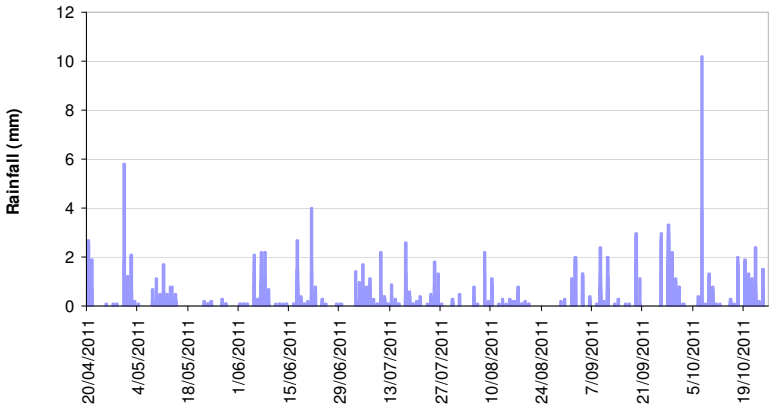


**Fig. 8.** Rainfall (mm) records for the Devon Meadows trial site collected from 16 April to 25 October 2011.

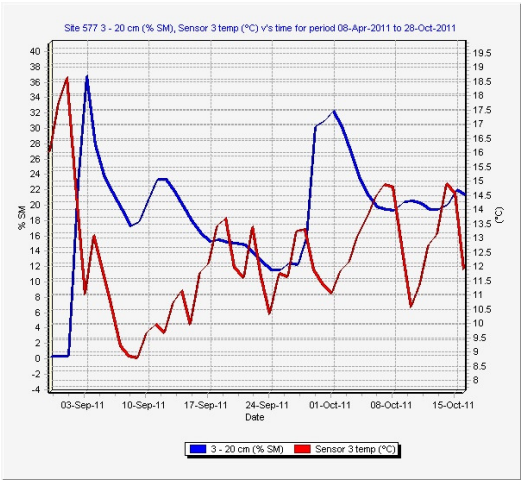


**Fig. 9.** Soil temperature and moisture at Devon Meadows 2011 measured from September to October.

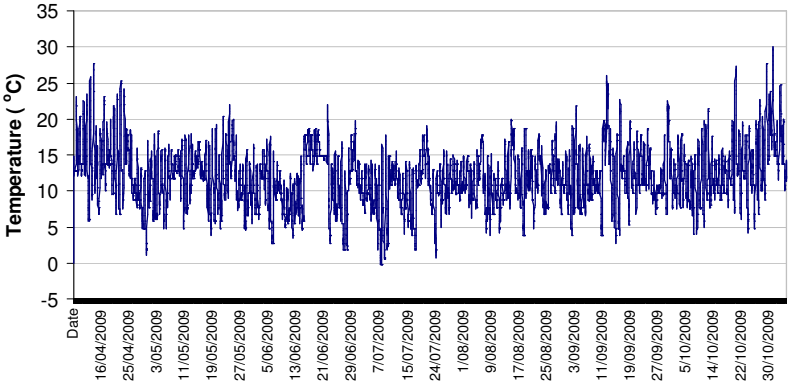


**Fig. 10.** Air temperature (°C) records for the Clyde trial site collected from 8 April to 21 October 2009.

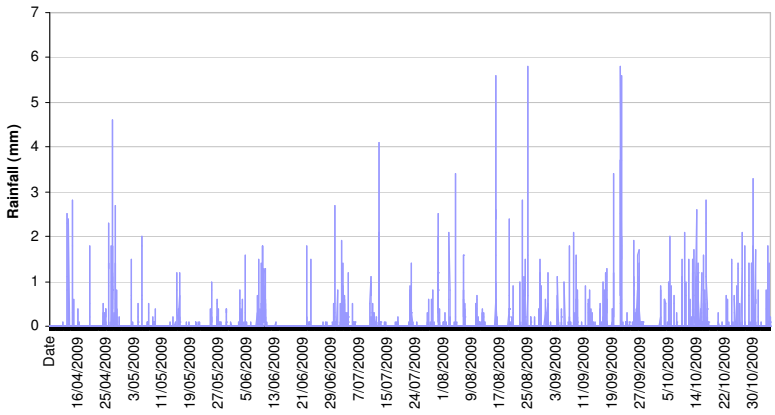


**Fig. 11.** Rainfall (mm) records for the Clyde trial site collected from 8 April to 21 October 2009.

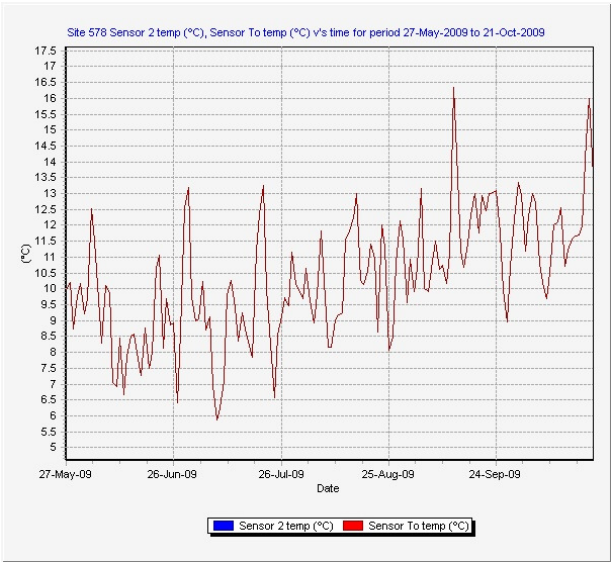


**Fig. 12.** Soil temperature and moisture at Clyde 2009.

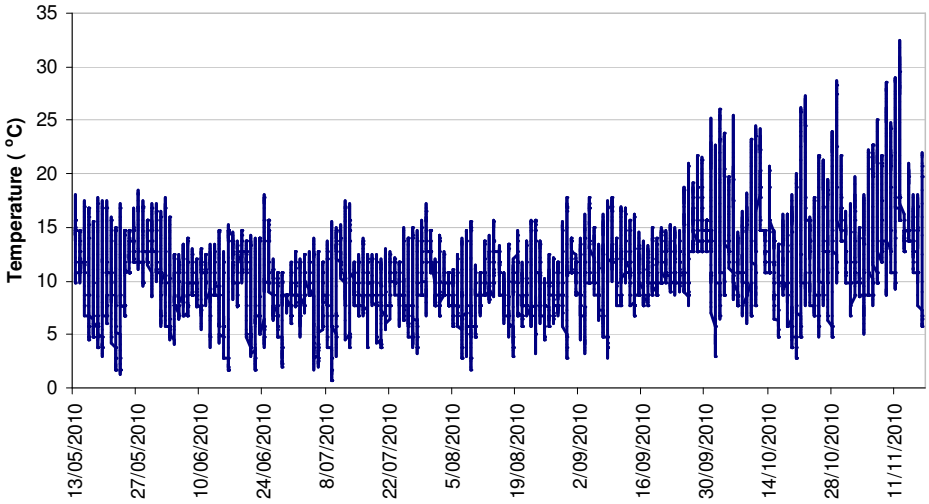


**Fig.13.** Air temperature records for the Clyde trial site collected from 13 May 2010 to 17 November 2010.

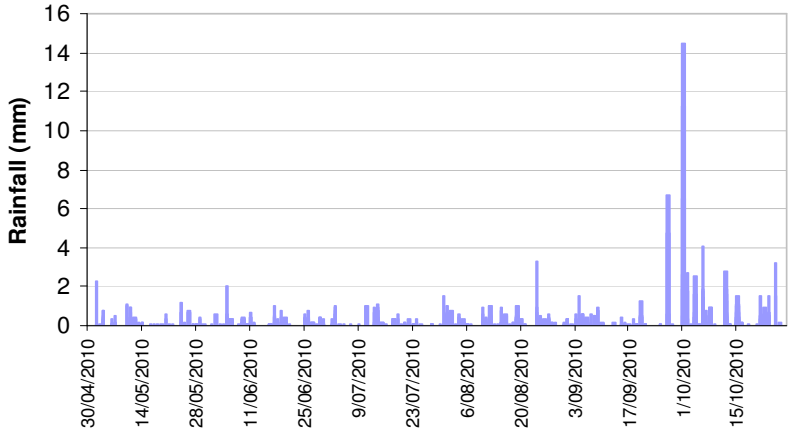


**Fig. 14.** Rainfall (mm) records for the Clyde trial site collected from 30 April to 28 October 2010.

# Chapter 10

# Fungicides for control of cavity spot of carrots

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## Summary
Two consecutive carrot crops (cv Mojo, Syngenta Seeds) were grown on coarse sand at Medina Research Station, Western Australia on a site with a past history of cavity spot disease caused by *Pythium sulcatum*. However the lack of cavity development in the second crop prevented evaluation of the efficacy of a range of biological and chemical disease control agents.

## 10.1 Introduction

Cavity spot, a soil-borne disease of carrots caused by *Pythium sulcatum* in Western Australia (Davison and McKay 1998), reduces the marketablility of carrots. Control options are limited and the development of enhanced biodegradation of soil applied chemicals (eg metalaxyl) in sandy soils in Western Australia limits the effectiveness of fungicide treatment (Davison and McKay1999). The opportunity to assess the efficacy of a range of fungicides and biological control agents against cavity spot arose as part of an Horticulture Australia Limited project (VG08026) led by DPI Victoria.

## 10.2 Materials and methods

### 10.2.1 Site

In 1994, a cavity spot disease nursery site was established at Medina Research Station (latitude 32.13$^{o}$ S) in Western Australia to enable screening of carrot varieties under high disease pressure. The soil was yellow Karrakatta sand of pH 6.5 (in $CaCl_2$) containing 0.4 per cent organic carbon in the surface soil (0-150 mm). In 1994, the site was inoculated with cavity spot infected carrots from a commercial crop which were spread over the site and rotary hoed in. A cavity spot susceptible variety Primo (Vilmorin Seeds, France) was then sown on the site. Following this crop, which developed moderate levels of cavity spot, variety plantings were established on one quarter of the site. The remainder of the site was resown to Primo to maintain a high disease inoculum. Thereafter until 2004, the site was continuously cropped with Primo while the variety plantings (a quarter of site) were rotated around the site and were preceded by at least two bulk crops of Primo to limit variation in disease history. Cavity spot infection persisted in subsequent crops with over 50 percent of roots showing symptoms in all crops. Prior to the 2011 planting, the last carrot crop was harvested from the site in October 2004.

### 10.2.3 Bulk crop

Since 2004, the site had been maintained as bare fallow until a bulk crop (0.24 ha) of the cavity spot susceptible carrot variety Mojo (Syngenta Seeds) was sown on 9$^{th}$ September 2011. The site was irrigated with impact sprinklers and received 0.5 t/ha of double superphosphate and a trace element mix broadcast and incorporated before sowing. Weekly fertilisation commencing 2 weeks after sowing and ceasing 2 weeks prior to harvest totaled 221 kg N/ha, 249 kg K/ha, 10.5 kg Mg/ha and 2.4 kg B/ha. The crop was grid sampled (12 carrots per sample at 1.5 x 9 m) for cavity spot incidence at commercial harvest maturity on 22 December 2011.

### 10.2.4 Experiment 2012

Another crop of carrots cv Mojo was sown on the site on 12th January 2012 and harvested on 4th May 2012Treatments applied are listed in Table 10.1.

Plots were 5 m long by 1.5 m (4 double lines of carrots) wide. There were 6 replicates in a randomized block design. Weekly fertilisation commencing 2 weeks after sowing and ceasing 2 weeks prior to harvest totalled 250 kg N/ha, 258 kg K/ha, 12 kg Mg/ha and 1.5 kg B/ha. At harvest, a 1 m length of the two middle double lines of carrots was hand harvested washed, graded and rated for cavity spot symptoms. Unfortunately cavity spot did not develop in this experiment and symptoms were absent. Data were analysed using GENSTAT statistical software.

**Table 10.1. Treatments applied to carrots cv Mojo sown at Medina Research Station, Western Australia in January 2012.**

| Treatment (a.i.) | Product name | Product rate and timing |
|---|---|---|
| 1.Untreated 1 | | |
| 2.Untreated 2 | | |
| 3.Metalaxyl (25g/kg) | Ridomil®Gold 25 G | 40 kg/ha, 1 das |
| 4.Hydrogen Peroxide (35%) | Interox™ Ag bath | 25 L/ha drench, 1 das |
| 5.Phosphorus acid (600 g/L) | Agri-Fos®600 | 12 L/ha sprayed monthly[A] |
| 6.Azoxystrobin (500 g/kg) | Amistar®WG | 0.3 kg/ha sprayed monthly |
| 7.*Bacillus subtilis* | Fulzyme®Plus | 1.0 L/ha sprayed monthly |
| 8.Hydrated lime | Hydrolime™ | 1 t/ha broadcast 1 das |
| 9.*Streptomyces. lydicus* (+Vitazyme) | MicroPlus® | 0.5 kg/ha (+ 1L/ha), sprayed 1 das then monthly |
| 10.*P.oligandrum* | Polyversum™ | 0.1 kg/ha sprayed, monthly |

[A]Sprayed treatments applied 4 times with mini boom in 384 L water /ha commencing 4 weeks after sowing; das, days after sowing.

## 10.3 Results

### 10.3.1 Bulk crop

The incidence of cavity spot symptoms in the bulk Mojo crop was low with 3 per cent of carrots showing cavity spot lesions at harvest although laboratory isolations failed to recover *Pythium spp* from these lesions.

### 10.3.2 Experiment

Cavity spot did not develop in the experiment at a time of year when cavity spot is normally prevalent. While cavity spot levels were low in the preceding crop, it was expected that some cavity spot would develop. This former cavity spot nursery site had been maintained as bare fallow and had not been planted to carrots for 8 years (last sown April 2004).

There was a small but significant (*P=0.046)* treatment effect on total yield (Table 10.2) such that the metalaxyl treatment had a lower yield than the untreated plots and all other treatments except for the *Pythium oligandrum* treatment. The *Streptomyces lydicus* (+ Vitazyme) treatment had the highest total yield though not significantly higher than the untreated control treatments. Vitazyme is purportedly a biostimulant that improves plant growth and yield. It may be worthy of further investigation.

**Table 10.2. Results for carrot crop sown at Medina Research Station in January and harvested 4 May 2012**

| Treatment | Total yield (t/ha) | Marketable yield (t/ha) | Forked roots (%) | Misshapen roots (%) |
|---|---|---|---|---|
| 1. | 68.7 | 63.8 | 3.5 | 2.6 |
| 2. | 70.9 | 64.9 | 2.3 | 5.2 |
| 3. | 62.3 | 58.2 | 4.0 | 1.9 |
| 4. | 68.4 | 61.7 | 1.7 | 7.8 |
| 5. | 68.0 | 60.2 | 2.2 | 9.1 |
| 6. | 68.4 | 60.5 | 5.7 | 5.6 |
| 7. | 69.6 | 65.5 | 4.5 | 0.8 |
| 8. | 67.3 | 62.2 | 2.6 | 4.2 |
| 9. | 71.8 | 64.8 | 4.0 | 4.4 |
| 10. | 66.7 | 57.9 | 3.9 | 7.9 |
| Sig. | * | ns | ns | ns |
| LSD *(P=0.05)* | 5.08 | | | |

## 10.4 Discussion

The lack of disease development in this experiment prevented any comparison of the applied treatments. Carrots produced in this planting were of excellent quality. Prior to 2004 the site had been used as a cavity spot disease nursery sown to continuous carrots since 1995 with all crops developing high levels of cavity spot caused by *P. sulcatum.* Given that *P. sulcatum* has only been found to be hosted by Apiaceous plants, it is possible that cropping for several years with non-Apiaceous plants would have a similar effect in reducing cavity spot incidence. It would also be of interest to see how quickly cavity spot reappears if intensive carrot cropping recommenced.

Ridomil Gold products have the $R^-$ isomer (= mefenoxam) of metalaxyl as the active ingredient. The $S^+$ isomer which can be phytotoxic (Singh *et al.* 2003) has been removed from the Ridomil Gold products so these are not expected to display phytotoxicity. While a significant total yield reduction was recorded for the metalaxyl (Ridomil Gold 25 G) treatment, the effect was small.

## 10.5 Acknowledgments

Funding for this work from the Federal Government (via HAL Ltd), the National Vegetable Levy and the WA State Government is gratefully acknowledged. Thanks to Liz Minchinton of DPI Victoria for leading the project, the management of the trial by staff of Medina Research Station, especially Gavin d'Adhemar, is also gratefully acknowledged.

## 10.6 References

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Davison EM, McKay AG (1999) Reduced persistence of metalaxyl in soil associated with its failure to control cavity spot of carrots. *Plant Pathology* **48**, 830-835.

Singh M, Mersie W, Brlansky RH (2003) Phytotoxicity of the fungicide metalaxyl and its optical isomers. *Plant Disease* **87**: 1144-1147.

# Chapter 11

# Screening of parsnip cultivars for their susceptibility to cankers and suitability for commercial use in Tasmania

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## Summary

Seven alternative cultivars of parsnips ('Albion', 'Hollow Crown', '300-9', '302-9', 'Lancer', 'Peace' and 'Moonshine') were compared against two commercial standard cultivars ('Melbourne White Skin' and 'Thunder'). 'Albion', '302-9' and 'Hollow Crown' are potential cultivars as alternatives to the current standards, with a combination of high plant density, vigorous growth and acceptable quality and yield of marketable roots.

## 11.1 Introduction

Currently, only two parsnip cultivars are sown commercially in Tasmania, 'Melbourne White Skin' ('White Skin') and 'Thunder'. 'Thunder', a hybrid variety, is more suitable than 'White Skin', especially for later plantings in December, which are harvested in the wet and cold autumn and winter. 'Thunder' is less susceptible to seedling damping off by *Pythium* as well as less susceptible to root lesions and cankers. The limited choice in cultivars available for commercial production has always been a major concern to growers. This study, therefore, aims to screen other cultivars from various seed suppliers, in order to determine their suitability for use in the cold Tasmanian conditions.

## 11.2 Materials and Methods

Only a small quantity of seeds in most cultivars was available for this screening study (Table 11.1). Therefore, with the exception of 'Lancer', all cutlivars were sown in a single plant row 10 metres long, without replications. 'Lancer' was sown in a single row at 20 metres long. This study was conducted within a commercial crop. Seeds were sown on 6/12/11, using a seed tape at the same spacing and depth as the commercial standard (Fig. 11.1). Plants in the trial area were also maintained in the same way as the surrounding commercial crop ('Thunder'). Seedling density and plant vigour were assessed in the field at 50 days after sowing (DAS). At 153 DAS, approximately 52 to 55 roots were harvested, washed and assessed for marketability and yield. Marketable roots were also sorted into different size ranges in order to determine their consistency in root growth.

**Fig. 11.1.** Sowing of parsnip seeds and application of Ridomil®25G (06/12/11)

**Table 11.1 List of parsnip cultivars planted in the Tasmanian field trial**

| Cultivar | Type | Seed Company | Seed Treatment | Seed size (g / 1000 seeds) |
|---|---|---|---|---|
| Melbourne White Skin | Non-hybrid | New Gippsland Seeds | N/a | 3.7 |
| Hollow Crown | Non-hybrid | New Gippsland Seeds | N/a | 4.7 |
| Thunder | Hybrid | Clause Vegetable Seeds | Thiram | 4.2 |
| Commercial Thunder* | Hybrid | Clause Vegetable Seeds | Thiram | 4.2 |
| 300-9 | Hybrid | South Pacific Seeds | Thiram | 6.0 |
| 302-9 | Hybrid | South Pacific Seeds | Thiram | 5.8 |
| Moonshine | Hybrid | South Pacific Seeds | Thiram | 5.3 |
| Peace | Standard | South Pacific Seeds | Thiram | 4.1 |
| Albion F1 | N/a | Johnny's Selected Seeds | N/a | 3.2 |
| Lancer | N/a | Johnny's Selected Seeds | Not treated | 5.2 |

* Commercial 'Thunder' is the same as 'Thunder', except that it was from the same batch of seed used by the grower

## 11.3 Results and Discussion

### 11.3.1 Seedling establishment

In examining seedling establishment at 50 days after sowing (DAS) (Fig. 11.2) 'Albion' with 13 plants/m row had similar plant density as 'White Skin' with 14 plants/m row (Fig. 11.3). 'Hollow Crown' and '302-9' with 9 and 8 plants/m row, respectively, were similar to 'Thunder' with 10 plants/m row. Poor seedling establishment was recorded with 'Peace', '300-9', 'Lancer' and 'Moonshine' seeds, with plant density ranging 4 to 0.3 plants/m row.

**Fig. 11.2.** Plant density and plant biomass at 50 DAS (25/01/12)

'Albion' had the greatest plant biomass at 50 DAS (Fig. 11.4). In comparison to 'Thunder' (100% biomass) and 'Melbourne White Skin' (150%), the biomass of 'Albion' was 250%.

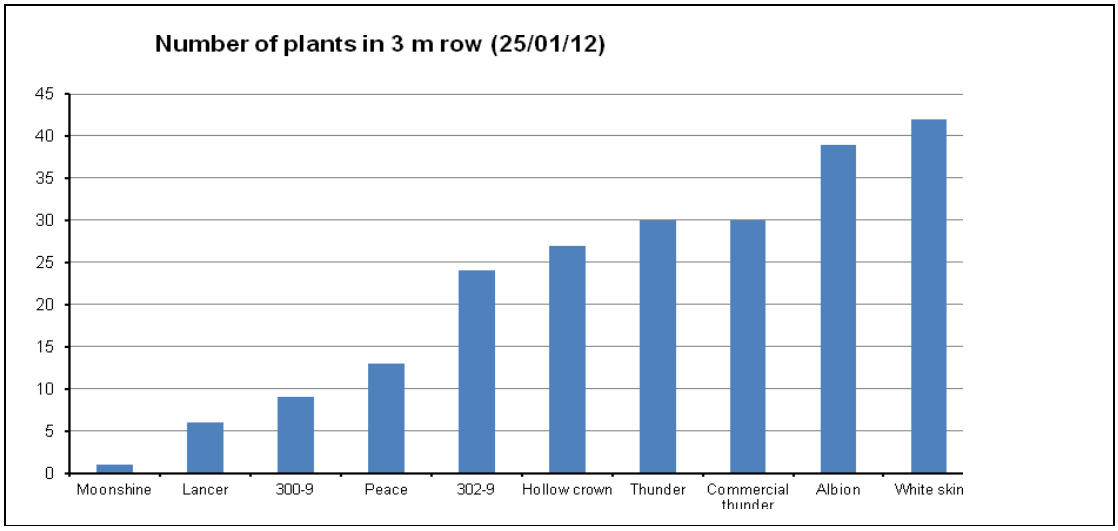


**Fig. 11.3.** Plant establishments of parsnip cultivars at 50 days after sowing.

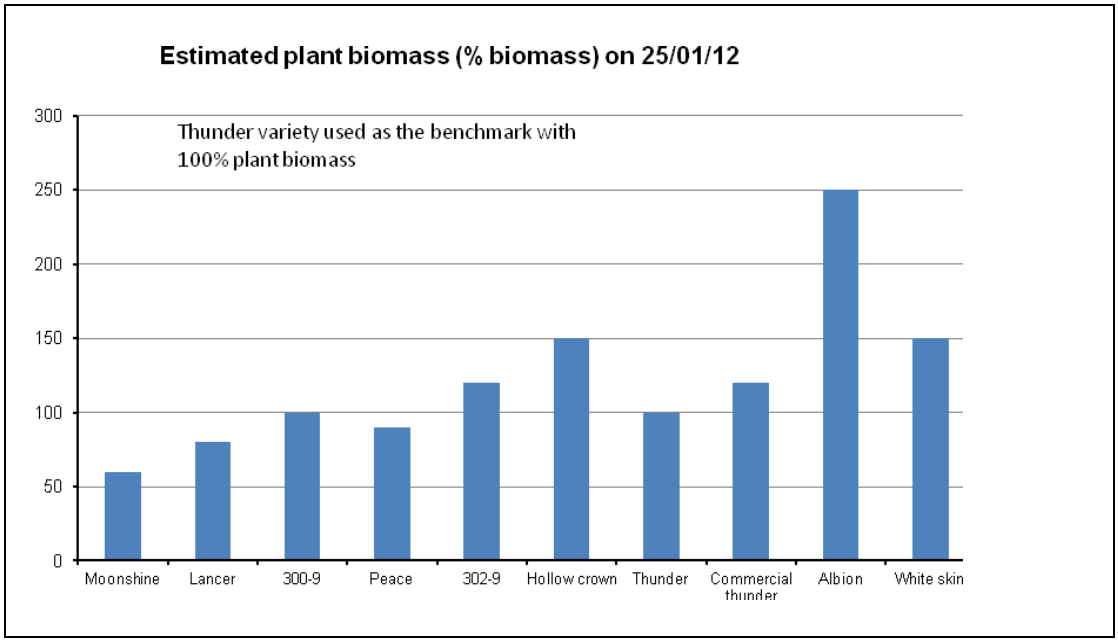


**Fig. 11.4.** Plant biomass of parsnip cultivars at 50 days after sowing.

### 11.3.2 Root weight and marketability

At 153 DAS, the percentages of marketable roots according to the cultivars are shown in Fig. 11.5. Although 'Lancer' and 'Peace' had relatively high percentage of marketable roots, note that their plant densities were very sparse (Fig. 11.3). 'Albion' and '302-9' had higher percentage of marketable roots compared to 'Melbourne White Skin' and 'Thunder'. The average weight of marketable roots of

'Albion' was similar to that of 'Thunder' (Fig. 11.6). While the average weight of marketable roots of '302-9', '300-9' and 'Hollow Crown' were similar to that of 'Melbourne White Skin'.

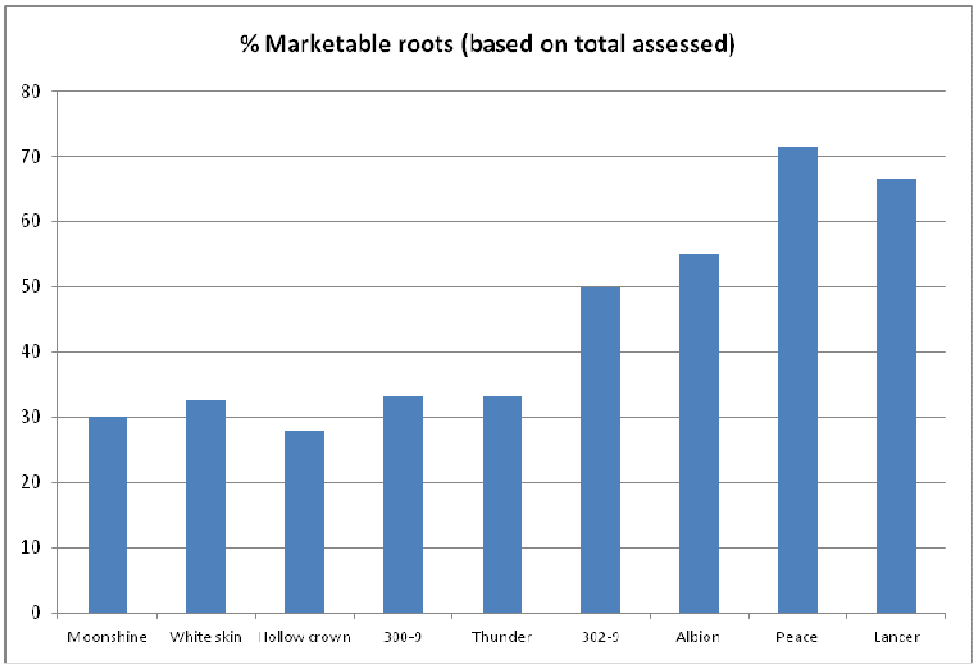


**Fig. 11.5.** Marketable parsnip roots of cultivars at 153 DAS.

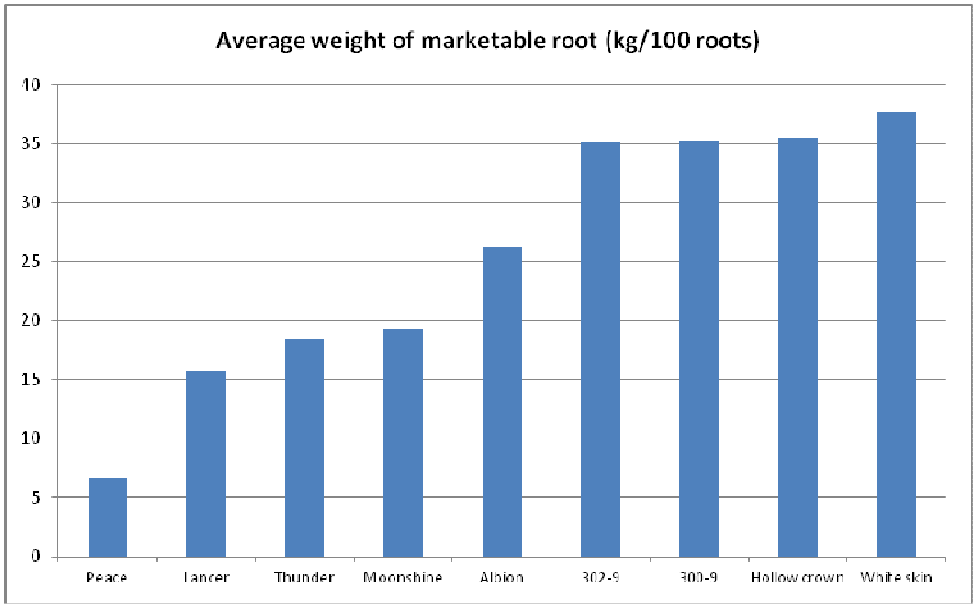


**Fig. 11.6.** Average weight of marketable roots of cultivars at 153 DAS.

### 11.3.3 Root size

In comparing the size range of the marketable roots based on root diameter, ‘Melbourne White Skin’ and ‘Hollow Crown’ had consistently the largest root size (Fig. 11.7). ‘302-9’ had larger size roots than ‘300-9’. ‘Albion’ had variable sizes of marketable roots. ‘Peace' had the smallest root size range. The marketable roots of each cultivar are shown in Fig. 11.8.

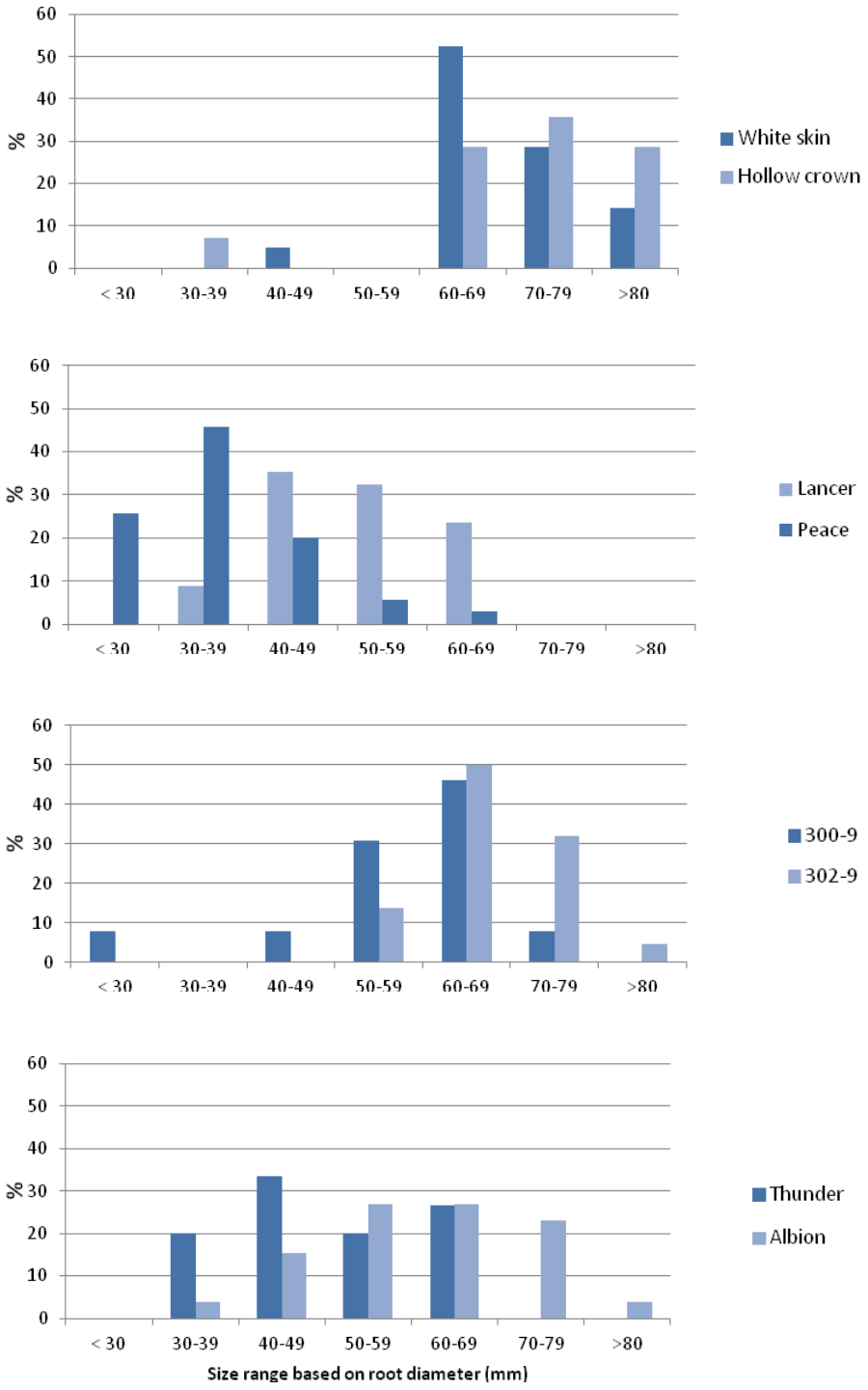


**Fig. 11.7.** Size range of marketable roots according to the cultivars.


**Fig. 11.8.** Marketable roots of each cultivar.

## 11.4 Conclusion

'Albion', '302-9' and 'Hollow Crown' showed potential as alternative cultivars to the current commercial standards, with good seedling establishment, growth and yield of marketable roots.

# Chapter 12

# Parsnip cultivar trial to identify resistance to root rots and Itersonilia in Victoria


## Summary

During the cooler months of 2011 a parsnip cultivar trial was conducted at Cranbourne, Vic, to evaluate 12 parsnip cultivars and one root parsley cultivar for resistance to root rots (cankers) and *Itersonilia* foliage lesions. No cultivars were completely resistant to root rots or to *Itersonilia* symptoms on foliage, most displayed a range of resistance, but some were very susceptible. Incidence of root rot symptoms in 'Javelin' was significantly lower, by 65%, compared with the 'Standard'. 'Javelin' produced the highest marketable yield of 17.2 t/ha, which was 89% higher compared with the 1.95 t/ha marketable yield of the 'Standard'. Additionally, this cultivar had the highest proportion of marketable roots compared with all other cultivars. Its root shape, weight and post harvest root colour however, were not as good as those of the 'Standard'. 'Javelin' had good resistance to foliage symptoms attributed to *Itersonilia.* Incidence of *Itersonilia* lesions on seedlings was 65% and on mature foliage 47% lower than compared with the most susceptible cultivars '300-9' and 'Standard', respectively. Due to the yield advantage of 'Javelin' over other cultivars evaluated, parsnip producers expressed interest in further testing 'Javelin' in coming seasons.


## 12.1 Introduction

Over the years, Victorian growers have bred and selected their own parsnip seed for their own soil type, yield, white-coloured flesh and to conform market specifications. Commercially available cultivars have a cream-coloured flesh which is less acceptable to the market, than the white fleshed cultivars each Victorian parsnip grower has developed. Growers freely admit that the cream fleshed cultivars are more resistant to canker. Canker of parsnip tap roots is a major problem worldwide for parsnip production.

Cankers which form on the crowns and shoulder of tap roots can be caused by a number of pathogens, as well as insect damage. *Itersonilia perplexans* Derx, is considered the main cause of parsnip canker in the UK and USA (Channon 1965, Wilkinson 1952). Other pathogens implicated in parsnip canker in the UK were *Phoma spp. Mycocentrospora acerina* (R. Hartig) Deighton and *Streptomyces scabies* (Thaxt.) Lambert and Loria (Channon 1965, Fox 2002 and Jones 1953). In Scotland, *Cylindrocapon destructans* (Zinssmeister) Scholten was responsible (Channon and Thomson 1981), while in Canada *Phoma complanata* (Tode: Fr.) Desmaz. was pathogenic (Cerkauskas 1985). Additionally, an insect *Psila rosa* Fabricius (carrot rust fly) is a major cause of parsnip canker in the UK as it predisposes tap roots to infection (Jones 1953).

Extensive research has been conducted to breed parsnips for resistance to cankers induced by *I. perplexans* (Anon 1966, Channon *et al.* 1970, Day 1978, Davis *et al.* 1989), *P. complanata* (Cerkauskas 1986ab), *S. scabies* (Green and Hewlett 1954), and *M. acerina* (Channon 1965). Breeding and screening for resistance to parsnip canker is complicated by the fact that this disease has more than one causal organism. Breeding for resistance to canker caused by *Itersonilia* also imparted similar resistance to cankers caused by both *Phoma* and *Mycocentrospora* (Channon 1965, Channon *et al.* 1970). In a separate study, however, conducted on sand and peat soils, the resistance of a cultivar was dependant on soil type (Day 1978). Cultivar trials conducted in the winter of 2007 to identify resistance to parsnip canker in Australia showed that: (i) the seed selection of one grower had consistent resistance to canker on two sites with different soil types; (ii) the cultivar Tusk (Terranova Seeds, Smithfield, NSW, Australia) had good resistance on one site but was significantly more

susceptible on the other site; and (iii) seed from two other growers were consistently susceptible to canker on all soil types tested (Minchinton *et al.* 2008).

Selection of resistant cultivars has been investigated as a way to combat fungal diseases in a variety of crops including parsnips for *Itersonilia* and *Phoma* (Channon *et al.* 1970, Cerkauskas 1986ab), and carrots for *Pythium* (Cooper *et al.* 2006, Davison and McKay 2001, Hiltunen and White 2002). These studies have shown that an effective breeding program for disease resistance can have a profound influence on alleviating the incidence and severity of diseases in vegetables. An analysis of various means of controlling white blister on broccoli showed cultivar resistance was the most economical method of controlling the disease (Minchinton *et al.* 2011).

This chapter reports on a field trial undertaken to identify parsnip cultivars with resistance to root rot or canker symptoms, foliage symptoms of *Itersonilia*, improved yield, desirable foliage and root characteristics, and post harvest root colour.

## 12.2 Materials and Methods

### 12.2.1 The trial design

A cultivar trial was conducted on a commercial vegetable farm producing parsnips on Westernport Highway, west of Cranbourne, Vic. in 2011. In this trial, 12 parsnip cultivars and a root parsley cultivar were screened (Table 12.1). Cultivars were sourced world-wide by Mr Slobodan Vujovic (the then IDO East VGA).

**Table 12.1. List of cultivars tested**

| Cultivar name | Source |
|---|---|
| 300-9 | South Pacific Seeds |
| 302-9 | South Pacific Seeds |
| Albion | Jonny's Selected Seeds |
| Berliner | Theitaliangardner |
| Hollow Crown | New Gippsland Seeds |
| Javelin | West Coast Seeds; Tozer Seeds US; Territorial Seed C. |
| Lancer | Jonny's Selected Seeds |
| Lightning | Clause Vegetable Seeds |
| Moonshine | South Pacific Seeds |
| Melbourne White Skin | New Gippsland Seeds |
| Peace | South Pacific Seeds |
| Standard | Grower's own |
| Thunder | Clause Vegetable Seeds |

The trial was planted on 19th April 2011 and re-sown on 2nd June 2012 due to poor emergence probably associated with planting seed too deep. Seed was planted in single rows, spaced at four rows per bed on raised bed with a hand held planter supplied Courtesy of Westranell Horticultural Solutions Pty Ltd Factory 1, 1820 Rhur St Dandenong, Vic. The trial was a randomised block design with four replicates each containing one of 13 treatments or cultivars. In replicated 4, however, due to poor emergence at the first sowing, Berliner parsley was replaced with the cultivar Lancer. Each replicated plot was 3.36m long by 1.62m wide. The trial site was bordered at either end by a track, on the west side by a track, a shed and an irrigation line and on the east side by the grower's parsnip crop. The crop was maintained by the grower, irrigated, fertilized and thinned as required, but no fungicide sprays were applied.

### 12.2.2 Environmental parameters

***Soil analysis***

Prior to establishing a trial and selecting treatments, soil samples were collected on 10/08/2009, across a near-by bay from the trial site, on the property where the cultivar trial was located and sent on the 9th November 2009 to the State Chemistry Laboratories at 621 Sneydes Road, Werribee, Victoria, for nutrient analysis.

***Weather station***

A Model T weather station (Western Electronics Design, Loxton, SA) was placed in the middle of an irrigation line in both trials. The station recorded average temperature and relative humidity, the presence or absence of leaf wetness, daylight and total rainfall at 30 min. intervals. The leaf wetness sensor was placed in the crop at a 45 degree angle and its height was adjusted as the crop grew.

### 12.2.3 Assessments

***Incidence of Itersonilia on foliage***

The incidence of *Itersonilia* on cotyledon and true leaves was assessed on 19th October 2011 by assessing 20 plants per plot where possible. At harvest on 1st and 2nd December 2011, 12 plants per plots were assessed for foliage and petiole diseases on a scale of 0-2: where 0 = healthy; 1 = some disease; 2 = lots of disease on leaves.

***Foliage characteristics***

At harvest foliage colour and foliage vigour were assessed. Foliage colour was assessed on a scale of 0-3: where 0=no emergence; 1, light green; 2, moderate green; and 3, dark green. Foliage vigour was assessed on a scale 1-3: where 1, shortest; 2, moderate; and 3, tallest.

***Disease at harvest***

At harvest on 1st and 2nd December 2011 cultivars were sampled by lifting four plants across the row, every half metre from the end of the plot to give a total of 12 plants harvested per plot. Sampled plants were assessed for the following symptoms: deep tap root lesions (canker); brown lesions on the upper tap root; brown lesions on the lower tap root; collar rot; skin cracks, forking and healthy (no visible symptoms). Each plant root was weighed.

***Root characteristics***

Root shape Scale 0-2; 0, not acceptable ie forking; 1, poor; 2, marketable ie a good shaped parsnip)

***Post harvest colour***

Up to 10 plants of each cultivar were collected, several from each plot of each block, on 8th December 2011, stored at 4ºC for four days and assessed on 12th and 14th December 2011 for root colour on a scale of 0 to 3, where: 0, most white; 2, intermediate; 3, most creamy.

***Yield***

Estimations of the total yield, yield of healthy parsnip tap roots in the size category ≥120g to ≤300g (marketable) was based on the proportion of healthy plants out of the 12 per plot assessed and the average weight of healthy and marketable plant tap roots. There were estimated to be an average of 119 plants per plot with dimensions of 5.4 $m^2$. There were, however, five plots of 'Lancer' and only three plots of 'Moonshine', 'Albion' and '300-9' due to poor emergence in replicate one, overshadowed for most of the daytime by the neighbouring tall shed.

### 12.2.4 Data analysis

Trial data was analysed by REML (Residual Maximum Likelihood) for incidence of *Itersonilia* lesions on seedlings, average plant weight and disease incidence on parsnip roots at harvest.

## 12.3 Results

During the trial plants in replicate one grew poorly, probably due to their proximity to an adjacent track and additionally due to shade from a near-by shed. Seeds of 'Lighting' were not fungicide coated and this cultivar showed poor germination. Although parsley 'Berliner' grew on this site, the clay loam did not encourage massive root development. It was trialled here in search for a root rot resistant alternative to parsnip and also as a potential new crop. Root parsley is commonly cropped in Europe but it is not popular in Australia. Parsley 'Berliner' and parsnip 'Lighting' have both been omitted from the report due to poor performance and germination of the latter.

### 8.3.1 Incidence of Itersonilia lesions on foliage

There were significant differences in the incidence of *Itersonilia* lesions on cotyledons and true leaves when assessed on 19th October 2011 (Table 12.2). No parsnip cultivars were completely resistant. Parsnip cultivars with less than 30% incidence were 'Peace', 'Moonshine', 'Javelin' and '302-9'. Cultivars '300-9, 'Lancer', 'Standard' and 'Melbourne White Skin' had over 60% of seedlings with symptoms of *Itersonilia* on foliage (Fig. 12.1). Parsley 'Berliner' had the lowest incidence.

At harvest on the 1st and 2nd December 2011 there was a wide range in the severity and incidence of *Itersonilia* lesions on the foliage of the various cultivars (Table 12.2). Both severity and incidence of *Itersonilia* lesions were highest on the 'Standard' and 'Melbourne White Skin', but lowest on 'Javelin' and 'Lancer'.

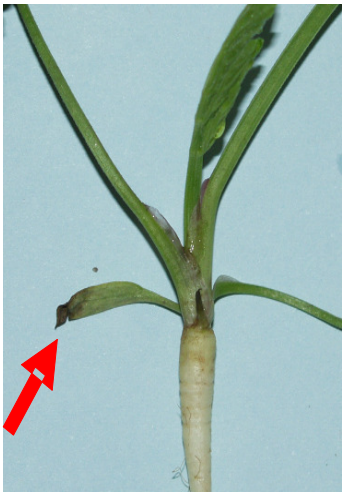

**Fig 12.1.** Symptoms of *Itersonila perplexans* on a seedling.

Interestingly seedlings of 'Lancer' were very susceptible to *Itersonilia*, but mature foliage was not. Perhaps highly infected leaves had defoliated. Conversely, 'Moonshine' had a low incidence on seedlings, but on mature plants it was high. The commonly grown cultivars 'Standard', 'Melbourne White Skin' and 'Hollow Crown' had a moderate incidence on seedlings but the former two developed a very high incidence by harvest, whereas the incidence on 'Hollow Crown' remained moderate. 'Javelin' had a low incidence on seedlings and by harvest only 50% of plants exhibited symptoms.

**Table 12.2. Susceptibility of cultivar foliage, on seedlings and mature plants, to *Itersonilia* lesions**

| Parsnip cultivar | Predicted mean incidence of Itersonilia on seedlings (cotyledons and petioles) (%) | Average severity of *Itersonilia* lesions on foliage at harvest (Scale 0-2) | Incidence of *Itersonilia* lesions on parsnip plant foliage at harvest (%) |
|---|---|---|---|
| 300-9 | 80.59 a | 0.89 | 75.0 |
| Lancer | 77.85 ab | 0.47 | 45.0 |
| Standard | 63.42 b | 1.60 | 95.8 |
| Melbourne White Skin | 62.76 bc | 1.63 | 93.8 |
| Hollow Crown | 47.38 cd | 0.77 | 54.2 |
| Albion | 35.52 de | 0.97 | 58.3 |
| Thunder | 31.66 de | 1.21 | 83.3 |
| 302-9 | 28.64 e | 0.60 | 58.3 |
| Javelin | 28.35 e | 0.55 | 50.0 |
| Moonshine | 27.23 e | 1.19 | 86.1 |
| Peace | 24.58 e | 0.73 | 62.5 |
| Berliner (Hamburg parsley) | 1.24 f | - | - |
| lsd (5%) | 15.9 | - | - |
| p-value | <0.001 | - | - |

Numbers followed by a different letter, differ significantly; -, not applicable. Scale of 0-2: where 0, healthy; 1, little disease; and 2, lots of disease on leaves.

### 12.3.2 Foliage characteristics

Parsnip cultivars exhibited a range of foliage colour from light green to dark green (Fig. 12.2). 'Albion' had the lightest coloured foliage (Fig. 12.2) and had the shortest canopy height or vigour (Fig. 12.3). Cultivars '302-9', 'Lancer' and 'Javelin' had the greenest foliage, while 'Javelin', 'Thunder' and '302-9' had the most vigorous or tallest foliage.

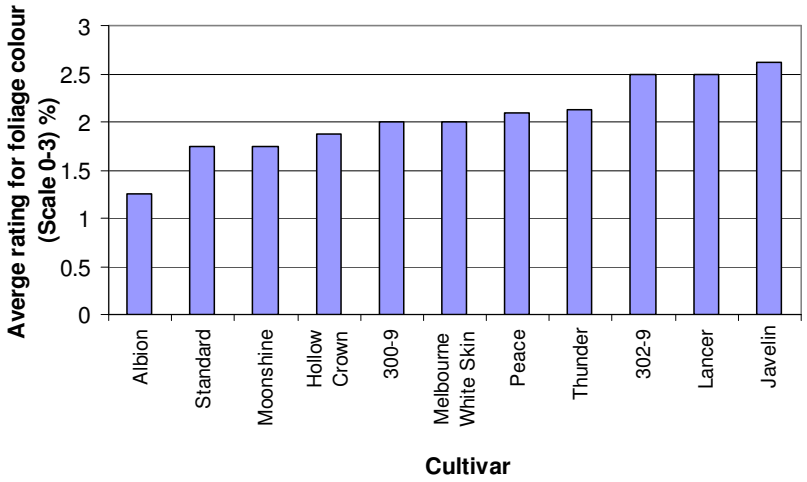


**Fig 12.2.** Assessment of the foliage colour characteristics of parsnip cultivars (Scale 1-3).
(Scale 1-3: 1, light green; 2, moderate green; 3, dark green)

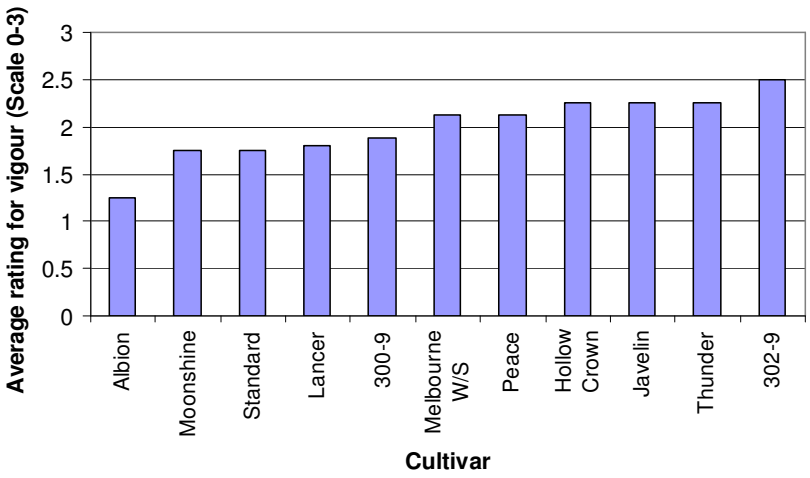


**Fig 12.3.** Assessment of the vigour of characteristics of parsnip cultivars (Scale 1-3).

(Scale 1-3: 1, shortest; 2, moderate; 3, tallest)

### 12.3.3 Disease at harvest

Any disease on parsnip tap roots is detrimental to a marketable harvest, consequently all data relating to symptoms of diseases or disorders (skin cracks and forking) was analysed together, and the data ranked and presented in Table 12.3. A selection of harvested tap roots is shown in Appendix 2 and Fig. 12.4.

Parsnip cultivars exhibited a wide range of susceptibility to root damage. Incidence of tap root damage was significantly ($p<0.001$) lower on 'Javelin' than all other cultivars tested (Table 12.3). The cultivars 'Standard', 'Peace', 'Moonshine', 'Hollow Crown', 'Melbourne White Skin' and 'Lancer' had over 70% of parsnips showing symptoms of root rots or disorders.

Average weight of parsnip roots ranged from 149.2 g for 'Peace' to 278.7 g for '302-9'. The mean weight of '302-9' parsnip tap roots did not differ significantly from 'Standard', 'Melbourne White Skin' and 'Hollow Crown' but was significantly higher compared with all other cultivars. 'Peace', 'Lancer', 'Albion' and '300-9' had the lowest ranked weights. Cultivars with good resistance to tap root disease tended to have lower tap root weights (Table 12.3).

**Table 12.3. Yield of parsnip cultivars and their susceptibility to disease**

| Parsnip cultivar | Mean disease incidence on tap roots (%) | Ranking of mean disease incidence on tap roots | Mean weight of tap root per plant (g) | Ranking of mean weight of tap root per plant | Average root shape (Scale 0-2) | Rank of average root shape |
|---|---|---|---|---|---|---|
| Standard | 91.67 | 11 | 236.6 | 3 | 1.63 | 2 |
| Peace | 89.58 | 10 | 149.9 | 11 | 1.56 | 4 |
| Moonshine | 89.27 | 9 | 225.1 | 5 | 1.28 | 9 |
| Hollow Crown | 83.33 | 8 | 228.2 | 4 | 1.25 | 10 |
| Melbourne White Skin | 81.25 | 7 | 246.8 | 2 | 1.54 | 5 |
| Lancer | 78.47 | 6 | 152.8 | 10 | 1.62 | 3 |
| 302-9 | 64.58 | 5 | 278.7 | 1 | 1.63 | 2 |
| 300-9 | 58.72 | 3 | 167.4 | 8 | 1.44 | 8 |
| Thunder | 58.33 | 4 | 186.1 | 6 | 1.50 | 6 |
| Albion | 53.16 | 2 | 163.4 | 9 | 1.64 | 1 |
| Javelin | 31.82 | 1 | 172.1 | 7 | 1.48 | 7 |
| lsd range | 16-19 | | 78-94 | | | |
| p-value | <0.001 | | 0.035 | | | |

Scale 0-2; 0, unacceptable ie forking and laterals; 1, poor; 2, good shaped tap root.

### 12.3.4 Root characteristics

The cultivars 'Albion', 'Standard', '302-9' and 'Lancer' had the best root characteristics of all cultivars tested, reaching on average 75% of a desirable shaped tap root (Table 12.3). Cultivars 'Hollow Crown' and 'Moonshine' had the worst ranking, showing poorly shaped roots with forking and lateral root development.

### 12.3.5 Post harvest tap root colour

The cultivars 'Standard' and 'Hollow Crown' held their tap root colour the best, up to 6 days after harvest when stored at 4ºC (Table 12.4, Fig. 12.4). The cultivars 'Lancer', '300-9' 'Melbourne White Skin' and 'Moonshine' held their tap root colour at the first assessment, after four days of cool storage; but after six days in cool storage, root colour deteriorated. Cultivar '302-9' and 'Peace' were consistently "creamy" coloured throughout the post harvest trial.

**Table 12.4. Parsnip tap root colour after cold storage for 4 and 6 days post harvest**

| Cultivar | Parsnip tap root colour (scale 1-3[2]) | |
|---|---|---|
| | (4 dah[1]) | (6 dah) |
| Standard | 1 | 1 |
| Hollow Crown | 1 | 1 |
| Lancer | 1 | 2 |
| 300-9 | 1 | 2 |
| Melbourne White Skin | 1 | 2 |
| Albion | 2 | 2 |
| Javelin | 2 | 2 |
| Thunder | 2 | 3 |
| Moonshine | 1 | 3 |
| 302-9 | 3 | 3 |
| Peace | 3 | 3 |

[1], days after harvest; [2], scale 1-3, where 1, whitest; 2, slightly cream coloured; 3, creamiest in colour.


**Fig 12.4.** Parsnip tap roots of various cultivars after 6 days post harvest storage at 4ºC.

Top L to R: 'Lancer', 'Standard', 'Hollow Crown', 'Melbourne White Skin', 'Moonshine', 'Thunder';
Bottom L to R: '300-9', 'Javelin', 'Albion', '302-9', 'Peace'.

### 12.3.6 Summary of parsnip cultivar characteristics

In an attempt to summarise characteristics of parsnip cultivars Fig. 12.5 was constructed from Tables 12.3 and 12.4. It is unfortunate that cultivars with good root shape, tap root weight and colour are often very susceptible to tap root rots, for instance 'Standard' and 'Hollow Crown'. Cultivars with good resistance to tap root rots often had low tap root weights and more creamy flesh compared with susceptible cultivars which had white-coloured flesh. A cultivar which performed well in most categories was '302-9', which had a moderate susceptibility to root rots, but had a good root weight and shape although it was a little creamy in colour.

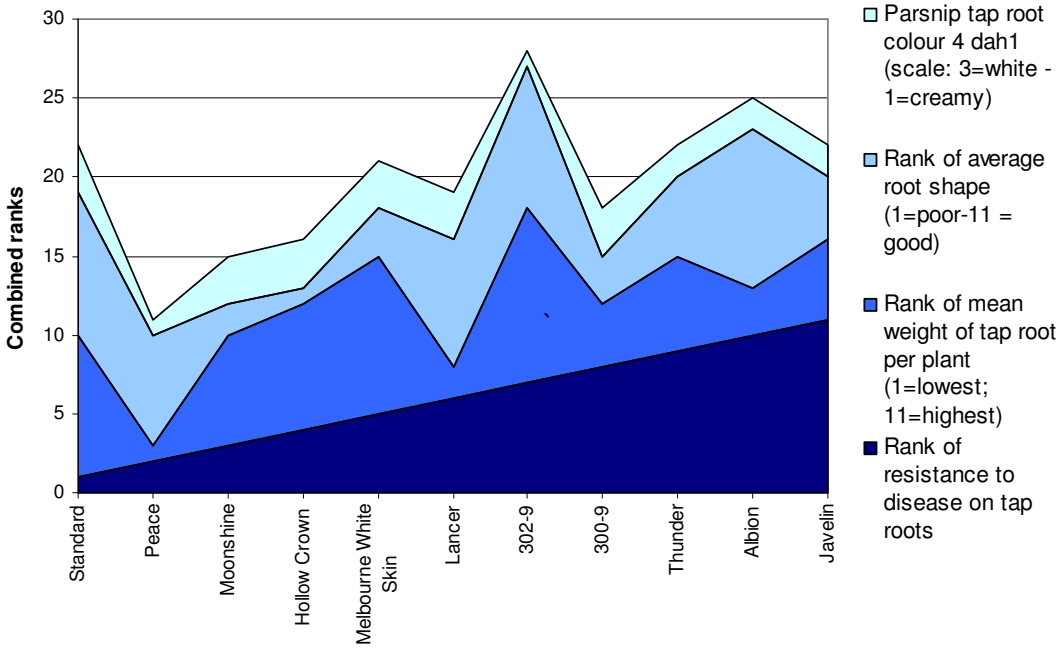


**Fig. 12.5.** Summary of some cultivar characteristics.

### 12.3.7 Yield

The estimated "total" yield of the various cultivars gives an indication of the potential yield of that cultivar (Fig. 12.6). There appears to be three main yield categories of estimated "total" yield, with cultivar '302-9' having the highest estimated "total" yield. 'Melbourne White Skin', 'Standard', 'Moonshine' and 'Hollow Crown' were in the next group. The group with the lowest "total" estimated yields were 'Thunder', '300-9', 'Albion', 'Javelin', 'Lancer' and 'Peace'.

The estimated yield of "healthy" plants was highest for 'Javelin', followed by '302-9', 'Albion', 'Thunder' and '300-9'. Interestingly 'Javelin' had the highest estimated proportion of healthy plants, followed by 'Albion', '300-9' and 'Thunder'. The commonly grown cultivars 'Melbourne White Skin', 'Hollow Crown', 'Standard' and 'Moonshine' had the lowest proportion of healthy plants compared with the estimated "total" for each cultivar.

The estimated "marketable" yield of a cultivar was highest for 'Javelin' followed by 'Albion', 'Thunder' and 'Melbourne White Skin'. Cultivar '302-9' had the highest average root weight and many parsnips were heavier than the marketable weight category. 'Standard' had the lowest rank of all the cultivars for marketable yield, which was probably associated with few (2) parsnips in the healthy and marketable weight categories.

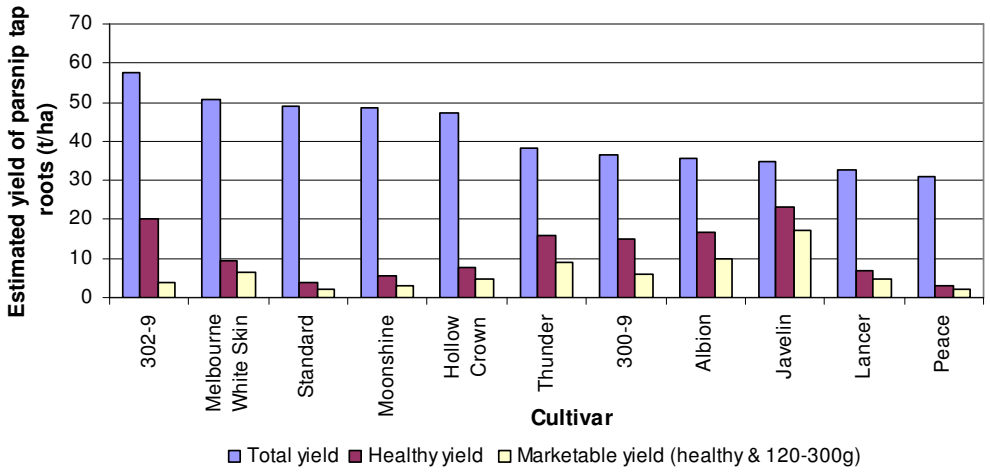


**Fig 12.6.** Estimated total, healthy and marketable root yields of parsnip cultivars December 2011 (t/ha).

### 8.3.8 Environmental parameters

Soil analysis of the site, conducted in 2009, did not detect any nutrient deficiencies on a soil type classed as clay loam (Appendix 1 Table 12.7). The air temperature ranged form 1ºC on 16th June to 34ºC on 18th November 2011 (Appendix 3 Fig. 12. 8). The coldest months were June, July and early August with average monthly temperatures of 9.9ºC, 9.7ºC and 11.4ºC, respectively. The warmest months were November with an average monthly temperature of 17.5ºC and early December. There were 31 events where greater than or equal to 2mm of precipitation was recorded (Appendix 3 Fig. 12.8). During seven of these events, greater than or equal to 4mm of precipitation was recorded; and four of these events, which occurred from October onwards recorded very high rainfall (7.3mm to 13.9mm).

*Itersonilia* lesions on foliage appeared during the coolest temperatures in June and July, with frequent, although not high, rainfall events. They probably persisted on foliage with the high rainfall events which occurred later in the growing season.

## 8.4 Discussion

No cultivars were completely resistance to root rots or to *Itersonilia* symptoms on foliage but, most displayed a degree of resistance, although some were very susceptible. 'Javelin' produced the highest marketable yield of 17.2t/ha, which was 89% higher compared with the yield of 'Standard' cultivar, 1.95t/ha, which was the lowest. As parsnip cultivars can vary in their susceptibility to root rot and produce variable yields on different soil types (Day 1978), yield data from one soil type needs to be viewed with caution. Additionally 'Javelin' had good resistance to symptoms of *Itersonilia* on foliage. The incidence of these symptoms was 65% lower on seedlings and 47% lower on mature foliage compared with the most susceptible cultivars '300-9' and 'Standard', respectively.

Some cultivars appeared to produce very similar results. Discussions with seed company representatives in Australia and overseas have lead to the suspicion that some cultivars may be the same, but sold under a different name. Additionally, 'Standard' often had very similar traits to 'Melbourne White Skin' and 'Hollow Crown', which is not surprising as it was originally selected from these cultivars.

***Disease on tap roots***

Parsnip cultivars varied in their susceptibility to root rot canker and not a single one was completely resistant. The cultivar 'Javelin' had only 32% of parsnips showing root rot symptoms, whereas the grower's own cultivar, 'Standard', had 92% of plants showing symptoms of root rots. Persistent production of this selection, because of its desirable agronomic qualities, such as root shape and weight, will necessitate implementation of better control strategies. Alternatively, perhaps the grower needs to re-select the selection by only selecting seed from plants free of tap root rots.

***Yields***

Newer cultivars, such as 'Javelin', 'Albion' and 'Thunder' had higher proportions of healthy and marketable roots compared with cultivars which have been available for many years such as 'Melbourne White Skin' and 'Hollow Crown'. All these cultivars produced superior yields compared with 'Standard', suggesting that the cultivar grown on this site during winter should be revised. Interestingly, the grower who selected 'Standard' was very interested in 'Javelin' and will trial it next year.

Cultivar '302-9' had the highest total yield and the highest proportion of diseased and oversized unmarketable roots. This result suggests that this cultivar could be harvested earlier than the others. Whilst there was no significant difference in yield between harvesting parsnip Early and Late (refer to Chapter 6), as a rule of thumb, the longer a crop is in the ground; the more time there is for it to be exposed to diseases. Further testing is required to fully evaluate the potential of this cultivar.

The poor health and marketable yields of some cultivars on this site may be associated with soil type. In a study of four parsnip cultivars grown on sandy and peat soils, one cultivar had higher yields and less canker on the peat soil, two cultivars performed better on the sandy soils, whilst one had poor yields and high susceptibility to canker on both soil types (Day 1978). Additionally, Minchinton *et al.* (2008) who conducted parsnip cultivar trials on two sites with different soil types found resistance to canker of some cultivars varied with soil type. It needs to be borne in mind that interpretation of results from a single trial on one soil type may not be directly transferable to another soil type.

***Itersonilia on foliage***

Some parsnip cultivars appear to have some resistance to *Itersonilia* lesions on foliage, but others were very susceptible, none were resistant. The cultivars 'Javelin', '302-9' and 'Albion' showed consistently low incidences of *Itersonilis* symptoms on foliage of seedlings and mature plants. 'Javelin' only had 50% of plants showing *Itersonilia* symptoms at harvest, while 96% of the 'Standard' plants showed *Itersonilia* symptoms. Commonly grown cultivars 'Standard' and 'Melbourne White Skin' were consistently very susceptible to the disease, while the other commercial cultivar 'Hollow Crown' was moderately susceptible.

This trial was memorable for the early appearance of symptoms of *Itersonilia* on seedlings. Brown *et al.* (1964) reported that the incidence of *Itersonilia* on foliage was directly correlated to high rainfall. Whilst rainfall was not especially high during the early stages of the trial it was fairly constant and as the trial progressed precipitation increased, which may account for the early appearance of the disease and its persistence in the crop.

It suggests that growers or their crop consultants should need to monitor weather conditions and seedlings for the disease. If conditions during crop establishment are conducive to infection then remedial action needs to be implemented, probably in the form of prophylactic fungicide sprays. Despite the grower spraying his adjacent parsnip crop with mancozeb, which appeared to control *Itersonila* symptoms on foliage in additions to other foliage disease, he still obtained only a 20% yield, suggesting that:

(i) This fungicide is not controlling root rot symptoms which may be associated with *Itersonilia*, or

(ii) Foliage symptoms of *Itersonilia* are not contributing to root rots and consequently other pathogens may be involved.

***Comparison of foliage and root rot diseases***

Susceptibility to *Itersonilia* symptoms on foliage generally produced a similar or higher incidence of root rots. 'Melbourne White Skin' had a high incidence of *Itersonilia* symptoms on foliage and a high incidence of root rots. 'Albion' had a low incidence of *Itersonilia* symptoms on foliage and moderate susceptibility to root rots, while 'Hollow Crown' went from a moderate incidence of *Itersonilia* on foliage to a high incidence of root rots. The two exceptions were '300-9' and 'Peace'. Cultivar '300-9' displayed highly susceptible foliage but only moderately susceptible roots. 'Peace' had foliage moderately susceptible to *Itersonilia* but tap roots highly susceptible root rots. This suggests that when breeding or selecting for "resistance" to root rots, resistance to *Itersonilia* symptoms on foliage may be a useful trait. Fortunately, when parsnips are breed for resistance to root diseases, resistance is expressed to various forms of causal agents (Channon *et al*. 1970).

***Seed***

Seeds of 'Lighting', which were the only ones not treated with the fungicide Thiram®, had poor emergence. *Itersonilia* is known to be seedborne and the lack of fungicide on the seed may have contributed to poor emergence. Given the high incidence of seedlings with *Itersonilia* symptoms, it is possible that the fungicide treatment is not very persistent, especially as parsnip seed takes about three weeks to emerge. It is possible that a fungicide seed treatment with longer persistence, such as a systemic fungicide, may have more efficacy to control *Itersonilia* at the seedling stage. As parsnips are a five to seven month crop, a fungicide with a long with-holding period would not be expected to cause issues with residues. Treating seed would be expected to be more economical than applying fungicides to field grown crops. An alternative to modifying fungicide seed treatments could be the use of steam-air. Steam-air technology was proposed by Smith (1966) to reduce *Itersonilia* contamination of the surface of parsnip seed. It had efficacy of 100% and did not significantly reduce germination. Despite this evidence, growers are not keen on the use of any kind of heat treatment for seeds. Perhaps the inaccessibility of steam-air treatment is not helpful.

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## 12.5 Appendixes

### 12.5.1 Appendix 1

**Table 12.7 Soil analysis from the trial site.**

| Item | Site Description | Unit | Cultivar trial property Top Soil | Sub Soil |
|---|---|---|---|---|
| | Depth | | 0-15 | 15-25 |
| Carbonate/Bicarbonate | Bicarbonate | mg/kg | 110.6 | 116.6 |
| | Carbonate | mg/kg | 0 | 0 |
| | Chloride | mg/kg | 110 | 88 |
| Total Carbon/Nitrogen | Carbon | g/100g | 1.2 | 1 |
| | Nitrogen | g/100g | 0.1 | 0.081 |
| | Organic matter | g/100g | 2.2 | 1.9 |
| pH and Conductivity | Electrical Conductivity | dS/m | 0.27 | 0.19 |
| | pH($CaCl_2$) | - | 7.1 | 7.2 |
| | pH(water) | - | 7.6 | 7.7 |
| | Total soluble salts | % | 0.09 | 0.06 |
| Ammonium acetate cations (with prewash) | Calcium | meq/100g | 5.2 | 4.8 |
| | Calcium as % | % | 74 | 75 |
| | Calcium Magnesium ratio | - | 4 | 4.2 |
| | Magnesium | meq/100g | 1.3 | 1.1 |
| | Magnesium as % | % | 18 | 18 |
| | Potassium | meq/100g | 0.44 | 0.39 |
| | Potassium as % | % | 6 | 6 |
| | Sodium | meq/100g | 0.1 | 1.00E-01 |
| | Sodium as % | % | 1 | 2 |
| | Sum of four cations | meq/100g | 7.1 | 6.4 |
| Available Aluminium | Aluminium | mg/kg | <10 | <10 |
| Available Boron | Boron | mg/kg | 1.6 | 1.1 |
| Available Potassium | Potassium | mg/kg | 330 | 250 |
| Available Phosphorus | Phosphorous (Olsen) | mg/kg | 130 | 73 |
| Available Sulfur | Sulfur | mg/kg | 27 | 27 |
| DTPA extractable trace elements | Copper | mg/kg | 1.9 | 1.5 |
| | Iron | mg/kg | 50 | 78 |
| | Manganese | mg/kg | 2 | 2 |
| | Zinc | mg/kg | 4.3 | 2.7 |

### 12.5.2 Appendix 2

Fig 12.7 Parsnip cultivars at harvest

‘300-9’

‘302-9’

'Albion'

'Berliner' (Hamburg parsley)

'Hollow Crown'

'Javelin'

'Lancer'

'Melbourne White Skin'

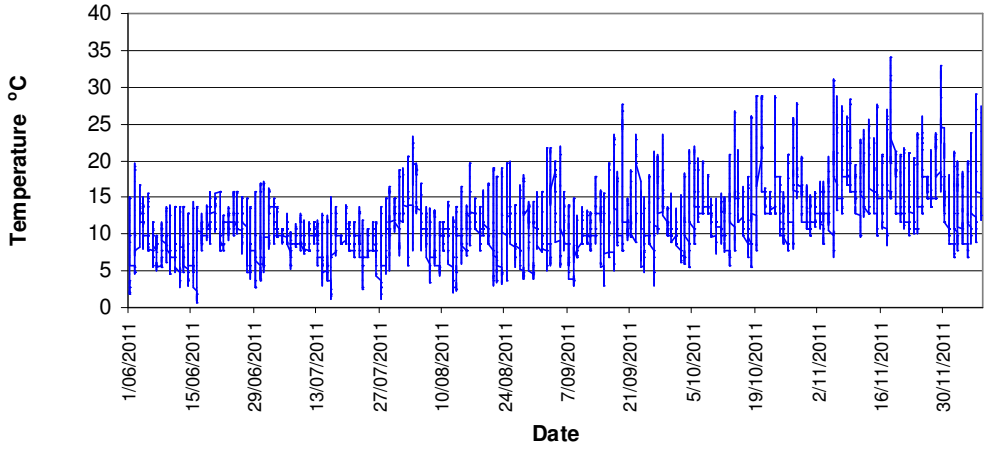
'Moonshine'

'Peace'

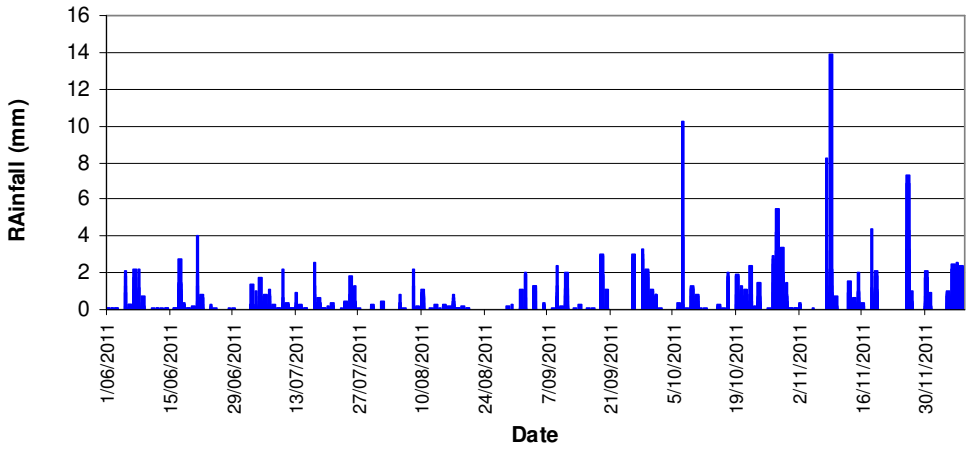
'Standard'

'Thunder'

### 12.5.3 Appendix 3




Air temperature (ºC) for the parsnip cultivar trial, 2011




Rainfall (mm) for the parsnip cultivar trial, 2011

**Fig 12.8** Meteorological data for the cultivar trial site.

# Chapter 13

# Economic analyses of field trials to determine the efficacy of treatments to control root rot on parsley and parsnip

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## Summary

The economic benefits of a range of chemical, non chemical and cultural treatments for controlling root rot diseases were investigated in two parsley and three parsnip trials in sandy soils. In parsley Ridomil®Gold 25G consistently increased the yields and thus contribution to profits by up to 37%, while Ferric Citrate on one site increased farm profit by approximately $5,000/ha, indicating the benefits of identify soil nutrient imbalances with a soil test and then taking corrective action. In parsnips the contribution by treatment to farm profit was variable. In Trial 1 one application of Ridomil®Gold 25G improved the contribution to farm profit by 41% compared with the Control. Fulzyme®Plus and Hilling showed promise on Trial 4 but when tested on a larger scale in Trial 7 with Ridomil®Gold 25G none contributed to farm profit.

## 13.1 Introduction

This chapter reports the economic analysis of field trials 1 and 2 on parsley and field trials 1, 4 and 7 on parsnips. The purpose of the trials was to determine the economic and environmental effects of various treatments for controlling pathogens that cause root rot and affect the plants ability to take up water and nutrients. Details about the trials have been discussed in the above chapters.

## 13.2 Method

The method used was to calculate the increases in the contributions of the various treatments to farm profits above those of the Control. Contribution to profit was calculated as income for the treatments minus variable costs comprising the costs of fungicides and their application for controlling root rot in parsley and parsnip, together with changes in harvesting and packaging costs and labour for inspection of crops. 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. Likewise, overhead or fixed costs for the Control and treatments would be identical.

## 13.3 Results

### 13.3.1 Economic analysis of treatments to minimize the incidence of root rot in parsley

#### *13.3.1.1 Assumptions*

- A deck of parsley comprises 10 bunches and had a farm gate price of $11.00 per deck as determined by the average of prices received in the Melbourne markets during 2010 and 2011.
- The cost of applying fungicides was estimated at $50 per hectare.
- Harvesting and packaging was estimated to cost $3.07 per deck.
- Hilling as a treatment was estimated to cost $60 per hectare.
- Weekly crop inspections cost $5.83 per hectare for labour of $70 per hour.

***13.3.1.2 Costs of treatments and their contribution to farm profit per hectare for controlling root rot in parsley in Trial I and Trial 2***

The costs of chemicals and measures such as the use of mulch and hilling for the various treatments are shown in Table 13.1.

**Table 13.1. Costs of chemicals and other measures for controlling root rot in parsley for Trial 1 and Trial 2**

| Trade name | Rate | Trial No. | Pack size | | Cost excluding GST | Cost per unit L or kg | Cost per hectare |
|---|---|---|---|---|---|---|---|
| | | | | | $ | $ | $ |
| Amistar®Top | 625-725 ml/ha | 2 | 5 | L | 887.10 | 177.42 | 119.76 |
| Diazinon® | 700 ml/ha | 2 | 5 | L | 227.80 | 45.56 | 31.89 |
| Ferric citrate | 190 g/ha | 1 | 250 | g | 71.93 | 287.72 | 54.67 |
| Glucopone™ 600 CS | 772 ml/ha | 1 | 1 | L | 179.92 | 179.92 | 138.90 |
| Fulzyme®Plus | 12.14 L/ha | 2 | 1 | L | 85.00 | 85.00 | 1,032 |
| Hilling | | | | | | | 60.00 |
| Mulch Enviromix™ | 147.2 $m^3$/ha | 1 & 2 | 1 | $m^3$ | 19.50 | | 2,870 |
| Natural Wet™[a] | 500 ml/ha | 2 | 1 | L | 10.00 | 10.00 | 5.00 |
| *Pythium oligandrum* | | 1 | na | na | na | na | |
| Ridomil®Gold 25G | 40 kg/ha | 1 & 2 | 10 | kg | 214.50 | 21.45 | 858.00 |
| Sprayphos®620 | 1.7 L/ha | 2 | 20 | L | 106.80 | 5.34 | 9.08 |

a Surfactant for Fulzyme™

## 13.3.2 Trial 1 costs of treatments and their contribution to farm profit per hectare for controlling root rot in parsley

For Trial 1, the costs per hectare of chemicals and other control measures are shown in Table 13.2 and the contributions of treatments to profit displayed in Table 13.3 with Fig. 13.1.

**Table 13.2. Trial 1 parsley: The number of applications for the various treatments, the cost of the treatments per hectare, cost of application and the total cost of applied treatments**

| Treatment | No. of applications | Cost of chemical per application | Cost of applications | Total cost of chemicals applied per treatment |
|---|---|---|---|---|
| | | $/ha | $/ha | $/ha |
| Control | | | | 0 |
| Ridomil®Gold 25G | 1 | 858 | 50 | 908 |
| *Pythium oligandrum* | 3 | 0 | 50 | 150 |
| Mulch Enviromix™ | 1 | 2,870 | 50 | 2,920 |
| Ferric citrate + wetter | 1 | 194 | 50 | 244 |

[1], *P. oligandrum* was provide in-house so cost of treatment is difficult to calculate.

**Table 13.3. Trial 1 parsley: Contribution to farm profit per hectare for the various treatments**

| Treatment | Total cost of applying chemicals | Yield | Harvesting & packaging @ 3.07 per deck | Crop inspections | Farm gate income @ $11.50 per deck | Contibution to farm profit per hectare |
|---|---|---|---|---|---|---|
| | $/ha | decks/ha | $/ha | $/ha | $/ha | $/ha |
| Control | 0 | 0 | 0 | 0 | 0 | 0 |
| Ridomil®Gold 25G | 908 | 428 | 1,315 | 53 | 4,925 | 2,650 |
| *Pythium oligandrum* | 150 | -337 | -1,034 | 53 | -3,872 | -3,041 |
| Mulch Enviromix™ | 2,920 | 92 | 281 | 53 | 1,053 | -2,201 |
| Ferric citrate + wetter | 244 | 644 | 1,976 | 53 | 7,404 | 5,131 |

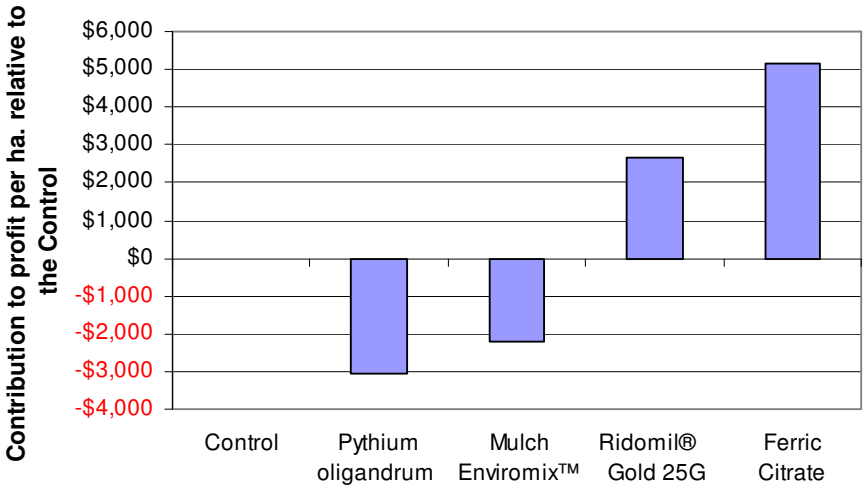


**Fig. 13.1.** Trial 1 parsley: Contribution of various treatments to farm profit compared with the Control

### 13.3.4 Trial 2 costs of treatments and their contribution to farm profit per hectare for controlling root rot in parsley

For Trial 2 parsley, the costs per hectare of chemicals and other control measures are revealed in Table 13.4 and contributions of treatments to profit in Table 13.5 and Fig. 13.2.

**Table 13.4. Trial 2 parsley: The number of applications for the various treatments, the cost of the treatments per hectare, cost of application and the total cost of applied treatments**

| Treatment | No. of applications | Cost of chemical per application | Cost of application | Total cost of chemicals applied per treatment |
|---|---|---|---|---|
| | | $/ha | $/ha | $/ha |
| Control | 0 | | | 0 |
| Amistar®Top | 3 | 120 | 50 | 509 |
| Diazinon® | 3 | 32 | 50 | 246 |
| Ferric citrate + wetter | 3 | 194 | 50 | 731 |
| Hilling | 3 | | | 180 |
| Mulch Enviromix™ | 1 | 2,870 | 50 | 2,920 |
| Ridomil®Gold 25G | 2 | 858 | 50 | 1,816 |
| Sprayphos®620 | 1 | 9.078 | 50 | 59 |

**Table 13.5. Trial 2 parsley: Contribution to farm profit per hectare for the various treatments**

| Treatment | Total cost of applying chemicals | Yield | Harvesting & packaging @ 3.07 per deck | Crop inspections | Farm gate income @ $11.50 per deck | Contibution to farm profit per hectare |
|---|---|---|---|---|---|---|
| | $/ha | decks/ha | $/ha | $/ha | $/ha | $/ha |
| Control | 0 | 8,304 | 25,492 | 0 | 95,491 | 69,999 |
| Amistar®Top | 509 | 8,304 | 25,492 | 111 | 95,491 | 69,379 |
| Diazinon® | 246 | 8,304 | 25,492 | 111 | 95,491 | 69,643 |
| Ferric citrate + wetter | 731 | 8,304 | 25,492 | 111 | 95,491 | 69,158 |
| Hilling | 180 | 8,304 | 25,492 | 111 | 95,491 | 69,709 |
| Mulch Enviromix™ | 2,920 | 8,304 | 25,492 | 111 | 95,491 | 66,968 |
| Ridomil®Gold 25G | 1,816 | 11,710 | 35,949 | 111 | 134,663 | 96,787 |
| Sprayphos®620 | 59 | 8,304 | 25,492 | 111 | 95,491 | 69,829 |

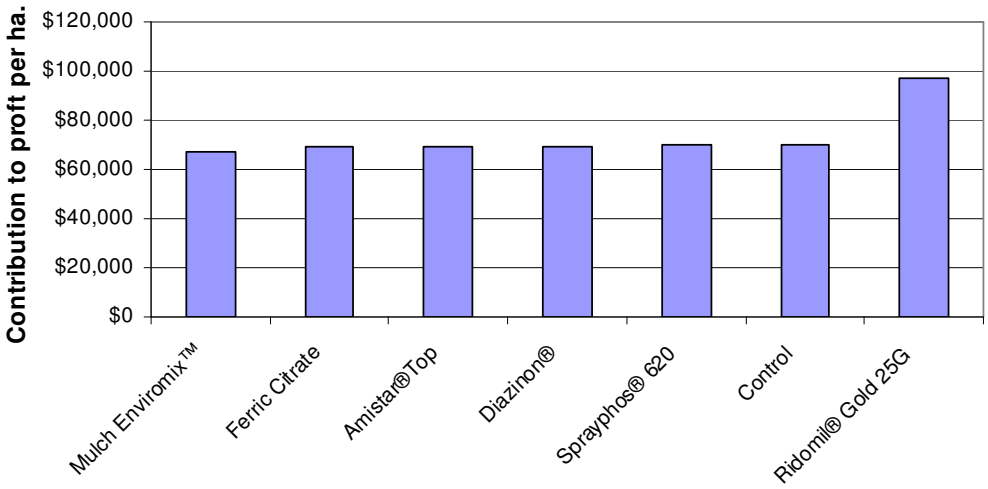


**Fig. 13.2.** Trial 2 parsley: Contribution to profit per hectare for the various treatments

## 13.3.5 Economic analysis of treatments to minimize the incidence of root rot in parsnips

### *13.3.5.1 Assumptions*

- A 10 kg carton of parsnips had a value of $43.75 per carton as determined by the average of prices received in the Melbourne markets during 2009, 2010 and 2011. That corresponds to a return of $4,375 per tonne.
- The cost of applying fungicides was estimated at $50 per hectare.
- Harvesting and packaging for parsnips was quoted by NSW Farm Enterprise gross margins as $200 per tonne.
- Hilling as a treatment was estimated to cost $60 per hectare.
- Weekly crop inspections cost $5.83 per hectare for labour of $70 per hour.

### *13.3.5.2 The cost of chemicals for controlling root rot in parsnips*

The costs per hectare for all treatments for controlling root rot in parsnips are shown in Table 13.6.

**Table 13.6. Costs per hectare for all treatments for controlling root rot in parsnips**

| Trade name | Rate | Trial No. | Pack size | Cost excluding GST | Cost per unit L or kg | Cost per hectare |
|---|---|---|---|---|---|---|
| | | | | $ | $ | $ |
| Amistar®Top | 625-725 ml/ha | 1 & 2 | 5 L | 887.1 | 177.42 | 119.76 |
| Fleece™ | 700 ml/ha | 6 | 5 L | 227.8 | 45.56 | 31.89 |
| Folicur® | 190 g/ha | 3 & 4 | 250 g | 71.93 | 287.72 | 54.67 |
| Fulzyme®Plus | 170 ml/7L | 3, 4 & 7 | 1 L | 85 | 85 | 0.58 |
| Hilling | | 4 & 7 | | | | |
| Microplus™ | 1 L/1000 L/ha | 1 | 500 g | 153.4 | 306.8 | 306.8 |
| Mulch Enviromix™ | 147.2 m3/ha | 3 & 4 | 1 m3 | ? | | |
| Mulch NRS™ | | 4 | | | | |
| Natural Wet | 1 L/1000 L/ha | 3, 4 & 7 | 1 L | 10 | 10 | 0.1 |
| Ridomil®Gold 25G | 40 kg/ha | 1, 2, 3 & 7 | 10 kg | 214.5 | 21.45 | 858 |
| Stand SKH™ | 1.7 L/ha | 5 | 20 L | 106.8 | 5.34 | 9.08 |

### 13.3.6 Trial 1 costs of treatments and their contribution to profit per hectare for controlling root rot in parsnips

For Trial 1 with parsnips, the costs of treatments per hectare for controlling root rot are shown in Table 13.7 and contributions of treatments to profit in Table 13.8 and Fig. 13.3.

**Table 13.7. Trial 1 for parsnips: Costs of treatments per hectare for controlling root rot**

| Treatment | No. of applications | Cost of chemical per application | Cost of application | Total cost of chemicals applied per treatment |
|---|---|---|---|---|
| | | $/ha | $/ha | $/ha |
| Control | | | | 0 |
| Ridomil®Gold 25G 1 & 10 | 2 | 858 | 50 | 1816 |
| Ridomil®Gold 25G 10 | 1 | 858 | 50 | 908 |
| Ridomil®Gold 25G 18 & 24 | 2 | 858 | 50 | 1816 |
| Ridomil®Gold 25G 1, 10, 18 & 24 | 4 | 858 | 50 | 3632 |
| Amistar® Top 10, 18 & 24 | 3 | 858 | 50 | 2724 |
| Microplus™ | 1 | 307 | 50 | 357 |

**Table 13.8.Trial 1 for parsnips: Contribution of treatments to profit per hectare**

| Treatment | Total cost of applying chemicals | Yield | Harvesting & packaging @ 200 per tonne | Crop inspections | Farm gate incomec @ 4375 per tonne | Contibution to farm profit per hectare |
|---|---|---|---|---|---|---|
| | $/ha | tonnes/ha | $/ha | $/ha | $/ha | $/ha |
| Control | 0 | 11.15 | 2,229 | 0 | 48,764 | 46,535 |
| Ridomil®Gold 25G 1 & 10 | 1,816 | 18.29 | 3,658 | 140 | 80,023 | 74,409 |
| Ridomil®Gold 25G 10 | 908 | 18.29 | 3,658 | 140 | 80,023 | 75,317 |
| Ridomil®Gold 25G 18 & 24 | 1,816 | 18.29 | 3,658 | 140 | 80,023 | 74,409 |
| Ridomil®Gold 25G 1, 10, 18 & 24 | 3,632 | 18.29 | 3,658 | 140 | 80,023 | 72,593 |
| Amistar®Top 10, 18 & 24 | 2,724 | 11.15 | 2,229 | 140 | 48,764 | 43,671 |
| Microplus™ | 357 | 11.15 | 2,229 | 140 | 48,764 | 46,038 |

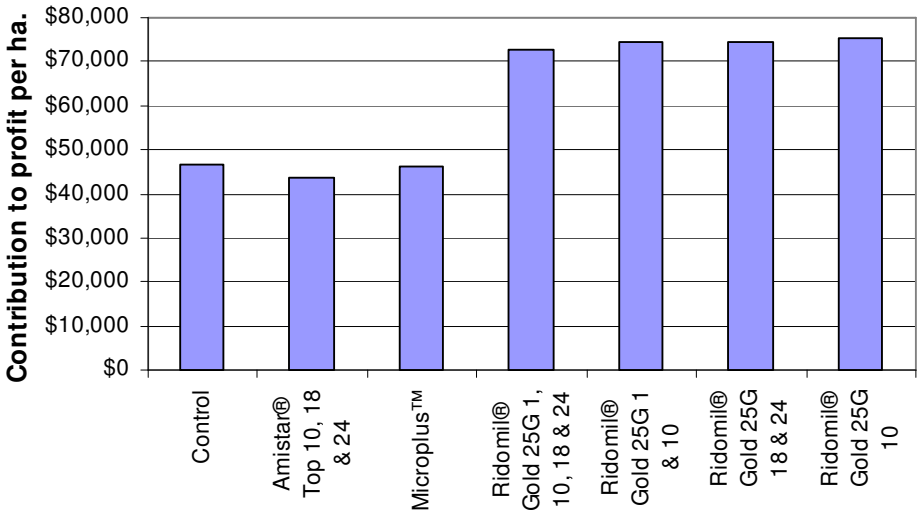


**Fig. 13.3.** Trial 1 parsnips: Contribution of treatments to profit per hectare

### 13.3.7 Trial 4 costs of treatments and their contribution to profit per hectare for controlling root rot in parsnips

For Trial 4 with parsnips, the costs of treatments per hectare for controlling root rot are shownin Table 13.9. Contributions of treatments to profit are displayed in Table 13.10 and Fig. 13.4.

**Table 13.9. Trial 4 parsnips: Cost of treatments per hectare for controlling root rot**

| Treatment | No. of applications | Cost of chemical per application | Cost of application | Total cost of chemicals applied per treatment |
|---|---|---|---|---|
| | | $/ha | $/ha | $/ha |
| Control | 0 | 0 | 0 | 0 |
| Fulzyme®Plus + surfactant | 5 | 1,032 | 50 | 5,411 |
| Folicur® | 5 | 55 | 50 | 523 |
| Hilling | 2 | 60 | 50 | 220 |
| Mulch Enviromix™ | 1 | 2,870 | 50 | 2,920 |
| Mulch NRS™ | 1 | 5 | 50 | 55 |

**Table 13.10. Trial 4 parsnips: Contribution of treatments to profit per hectare**

| Treatment | Total cost of applying chemicals | Yield | Harvesting & packaging @ 200 per tonne | Crop inspections | Farm gate income @ 4357 per tonne | Contibution to farm profit per hectare |
|---|---|---|---|---|---|---|
| | $/ha | t/ha | $/ha | $/ha | $/ha | $/ha |
| Control | 0 | 12.0 | 2,406 | 0 | 52,415 | 50,009 |
| Folicur® | 523 | 7.1 | 1,426 | 157.5 | 31,065 | 29,229 |
| Fulzyme®Plus + surfactant | 5,411 | 16.6 | 3,318 | 157.5 | 72,283 | 68,284 |
| Hilling | 220 | 14.2 | 2,836 | 157.5 | 61,782 | 58,569 |
| Mulch Enviromix™ | 2,920 | 10.6 | 2,112 | 157.5 | 46,010 | 40,820 |
| Mulch NRS™ | 55 | 4.5 | 906 | 157.5 | 19,737 | 18,619 |

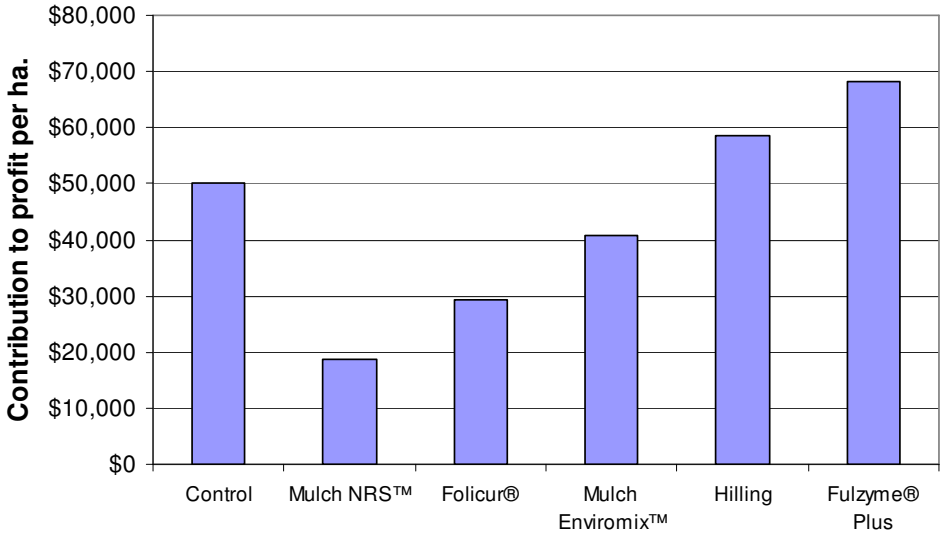


**Fig. 13.4.** Trial 4 parsnips: Graph of treatments contribution to profit per hectare

### 13.3.8 Trial 7 costs of treatments and their contribution to profit per hectare for controlling root rot in parsnips

For Trial 7 on parsnips, Table 13.11 shows the costs of treatments per hectare for controlling root rot. Contributions of treatments to profit are displayed in Table 13.12 and Fig. 4.

**Table 13.11. Trial 7 parsnips: Cost of treatments for controlling root rot**

| Treatment | No. of applications | Cost of chemical per application | Cost of application | Total cost of chemicals applied per treatment |
|---|---|---|---|---|
| | | $/ha | $/ha | $/ha |
| Control | 0 | 0 | 0 | 0 |
| Fulzyme® Plus + surfactant | 3 | 1,037 | 50 | 3,261 |
| Hilling | 2 | 60 | 50 | 220 |
| Ridomil® Gold 25G | 2 | 5 | 50 | 110 |

**Table 13.12. Trial 7 parsnips: Contribution of treatments to farm profit**

| Treatment | Total cost of applying chemicals | Yield | Harvesting & packaging @ 200 per tonne | Crop inspections | Farm gate income @ 4357 per tonne | Contibution to farm profit per hectare |
|---|---|---|---|---|---|---|
| | $/ha | t/ha | $/ha | $/ha | $/ha | $/ha |
| Control | 0 | 30.4 | 6,088 | 0 | 132,627 | 126539 |
| Fulzyme®Plus + surfactant | 3,261 | 30.4 | 6,088 | 117 | 132,627 | 126270 |
| Hilling | 220 | 27.4 | 5,470 | 117 | 119,164 | 113357 |
| Ridomil® Gold 25G | 110 | 30.4 | 6,088 | 117 | 132,627 | 126322 |

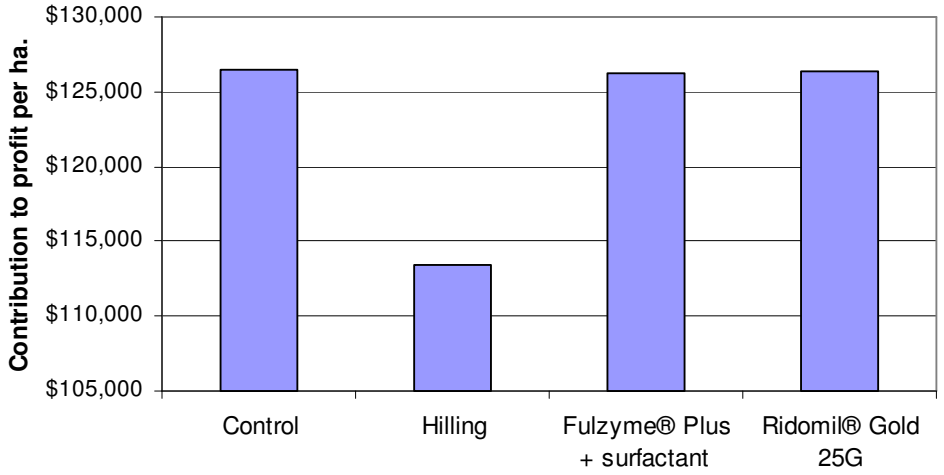


**Fig. 13.5.** Trial 7 parsnips: Contribution of treatments to farm profit

## 13.4 Discussion and conclusions

***Parsley***

The economic benefits of the various treatments for controlling root rot in parsley in Trial 1 reveals that Ferric citrate and Ridomil®Gold 25G increased farm profit by $5,131/ha and $2,650/ha, respectively, above the Control. Ferric citrate was nearly 50% more profitable than Ridomil®Gold 25G. *Pythium oligandrum* and Mulch Environmix™ gave lower profits than the Control. Even though they had greater environmental benefits than treatments that used chemicals, they would not be considered for controlling root rot in parsley. In Trial 2, Ridomil®Gold 25G was the only treatment to improve farm profit, which rose by 38% or $27,000/ha, compared to the Control.

***Parsnip***

Ridomil®Gold 25G, with only one application, gave the highest return in the parsnip trial, which was 41 per cent greater than that of the Control. The economic benefits were fairly similar to other Ridomil®Gold 25G treatments with two and four applications. However, with fewer applications it would be less damaging to the environment. Contributions to farm profit were similar to the Control for Microplus™ with contributions by Amistar®Top being less than the Control.

In parsnip Trial 2, the contribution to profit by Ridomil®Gold 25G, was around 38% greater than that of the Control. The Control was similar to other treatments that used chemicals. Contributions to profit by Fulzyme®Plus and Hilling were less than that of the Control.

Trial 4 for parsnips revealed that Fulzyme®Plus gave the greatest contribution to profit even though its application cost per hectare was $5,411. That high application cost was offset by it having a comparatively higher yield of 16.6 tonnes of parsnips per hectare. However, the initial yield of Fulzyme®Plus did not differ significantly from that of the Control and if yields for both had been average as they did not differ significantly, Fulzyme®Plus would not have produce a greater contribution to profit compared with the Control. The next best treatment with a yield of 14.2 tonnes of parsnips per hectare was Hilling. Hilling has greater environmental benefit than using chemicals. There is therefore a trade off for growers between using a chemical based treatment or one that does not impact the environment. Lower contributions to profit were provided by Mulch NRS, Folicur® and Mulch Enviromix™ than for the Control.

In Trial 7 assessing treatments for the control of root rot in parsnips showed the Control (doing nothing) gave the highest return per ha and the other treatments gave lower returns than that of the Control, although they have environmental benefits. The spacing of seed between Trial 4 and Trial 7 differed, with seeds in trial 4 planted at four rows per bed whilst in Trial 7 they were planted as three double rows per bed and this may have contributed to differences in the contribution to profit. Although Fulzyme®Plus gave the highest contribution to profit in Trial 4 and appeared to be a promising treatment, it failed to perform in Trial 7, due to unknown reasons, it requires further testing.

***Conclusion***

Finally, the trials showed that the treatments with potential environmental benefits did not perform better than treatments that used chemicals for the control of root rot in parsley and parsnips. In Trial 1 with parsley, *Pythium oligandrum* and Mulch Enviromix™ were less effective than the Control.

In Trial 1 with parsnip Microplus™, with *Streptomyces lydicus* as the active ingredient, gave the second lowest economic benefits. Hilling was more economically beneficial than Mulch NRS™ and Mulch Environmix™ in Trial 4 with parsnips, but had a lower contribution to profit than the inconsistent Fulzyme®Plus. In Trial 7 with parsnips Hilling had a very low economic benefit.

# Chapter 14

# Recommendations and technology transfer


## Summary

This report has identified organisms associated with the parsnip canker complex and their succession during the crop development. While parsnip canker was attributed to multiple pathogens, parsley root rot development was largely associated with *Pythium* spp. This chapter summarises the best practice IPM guidelines for control of *Pythium*-induced decline in vegetable production in Victoria, Western Australia and Tasmania. The project elucidated the role of *Pythium* spp. in disease complexes (in field, hydroponics and laboratory studies), identified factors that favour *Pythium* damage (soil moisture, nutrients and temperature in field and growth chamber studies), determined the efficacy of biological, cultural and chemical controls and determined the economics of control options. Recommendations for future research are listed at the end of the chapter.


## 14.1 Discussion of major findings

***Parsley***

Previous work on parsley indicated that losses from damping off were a major problem pre- and post-emergence (Minchinton *et al.* 2006, 2007). This work has shown that mild root rot symptoms were present 6 weeks into crop development while severe symptoms appeared 6-8 weeks before harvest. Parsley plants appeared to tolerate mild symptoms, but not severe symptoms, as biomass loss was only pronounced in the latter. Disease development was consistent between years. *Pythium* species were the first pathogens isolated during the colder months when the crop was relatively young and this may be associated with at least one *Pythium* species. (*P. sulcatum*) having pathogenicity over a wide temperature range. The development of severe root rot symptoms 6-8 weeks prior to harvest, as temperatures warmed up in spring, could be associated with *Pythium* being more pathogenic at higher temperatures, as was evident from the growth cabinet experiments.

This information should enable better targeting of fungicide applications when disease risk is highest. Pre- and post-emergence damping off was identified as a problem for parsley (Minchinton *et al.* 2006, 2007). Applications of a registered or permitted fungicide, such as Ridomil®Gold 25G at 6-8 weeks prior to harvest should prevent development of severe symptoms of root rot. Unfortunately no alternatives to Ridomil®Gold 25G were identified from fungicides, biocontrol agents or cultural practices tested. This is a concern, as metalaxyl, the active ingredient in Ridomil®Gold 25G, is prone to biological degradation in some soil types and some pathogens have developed resistance to this fungicide.

***Parsnip***

*Pythium* were the most common species isolated from parsnip roots early in the cropping season and persisted until harvest; additionally *Itersonilia* was isolated from lesions on cotyledons of seedlings, suggesting both pathogens have the potential to invade the host early in its life cycle. Fortunately *Phoma complanata*, a known pathogen of parsnip overseas, was not identified in our studies. Parsnip canker in Australia appears to be a disease complex as effects of fungicides specifically applied to control *Pythium* spp. and *Itersonilia* were inconsistent over a number of trials. Evaluation of a range of fungicides, biocontrol agents and cultural practices revealed that only Ridomil®Gold 25G controlled the disease, irrespective of application time or frequency. It was also the only treatment to contribute to farm profit, but profits were variable between trials, suggesting a disease complex may be

responsible for canker. As Ridomil®Gold 25G is specific for oomycetes and *Pythium* species were the only oomycete pathogens isolated from parsnips during the three years of trials, this provides circumstantial evidence that *Pythium* spp. have a role to play in parsnip root rot (canker). Parsnip canker appears to be more severe on heavy soils or under conditions of high rainfall. Consequently growers should avoid planting parsnip on heavy soils. Another means to reduce the impact of the disease is to plant cultivars with resistance to canker, but resistance may vary with soil type.

Previous research has demonstrated that *Itersonilia* is pathogenic and can cause canker on parsnips, but controlling *Itersonilia* on foliage of parsnips, with Folicur®, did not lead to a reduction in canker symptoms. The pathogen is known to be seed-borne and seed treatments with either fungicides or steam-air have reduced its incidence on seedlings, suggesting growers should have their own selected lines of parsnip seeds fungicide treated.

### *Pathogens*

Six *Pythium* species were identified from parsley and nine from parsnip roots. Two species from parsley and four parsnips are new records for Australia. Additionally *Phoma exiqua* var. *exigua*, which has a broad host range, was identified on parsley roots and parsnip roots for the first time in Australia. This is an important finding, which may explain the failure of some some fungicide treatments, and will require different strategies for control. Based on the host range records of these newly reported species, *Pythium tracheiphilum* and *Phoma exigua* var. *exigua*, in particular, parsley or parnip growers may consider not growing these crops after lettuce, as lettuce is a host of both these pathogens (Table 14.1).

**Table 14.1. *Pythium* and *Phoma* spp. isolated from parsnip and parsley roots at Devon Meadows and Clyde in 2009, 2010 and 2011and identified using DNA sequence data.**

| Species identified | No of isolates on host | | Common hosts |
|---|---|---|---|
| | Parsnip | Parsley | |
| ***Pythium*** | | | |
| *P. dissotocum* complex[1] | 8 | 2 | Apiaceae (carrot, parsley) |
| *P. intermedium* | 7 | 2 | Apiaceae (carrot, parsley) |
| *P. ultimum* var. *ultimum* | 4 | 2 | Apiaceae (carrot, parsley) |
| *P. sylvaticum* | 3 | 0 | Apiaceae (carrot, parsley) |
| *P. irregulare* | 2 | 0 | Apiaceae (carrot, parsley) |
| *P. rostratifingens*[3] | 2 | 1 | Leaf litter, soil under apple tree, corn, *Brassica, Triticum, Medicago* |
| *P. comurandrum*[3] | 2 | 0 | soil |
| *P. tracheiphilum*[4] | 2 | 0 | lettuce |
| *P. vanterpoolii* | 1 | 0 | grasses |
| *P. sulcatum* | 0 | 1 | Apiaceae (carrot, parsley) |
| *P. mastophorum*[3] | 0 | 1 | Apiaceae (parsley), soil under pine plantations |
| *P.* spp[2] | 4 | 2 | |
| ***Phoma*** | | | |
| *P. exigua* var. *exigua*[3] | 13 | 2 | lettuce, chicory, beans |

[1], Members of clyde B2 (Lévesque and de Cock 2004) including *P. dissotocum, P. coloratum, P. lutarium,* which have identical ITS sequences
[2], *Pythium* spp. not identified to species level
[3], Not reported in Australia on any host
[4], Not reported in Australia on parsnip or parsley

### *Economic analysis*

Economic analysis of the various treatments showed that the one which produced the largest yield contributed the most to farm profit, irrespective of the cost of the treatment.

## 14.2 Best Practice IPM guidelines for control of Pythium-induced decline in Apiaceae vegetable production

- **Check soil nutrient status**
  Conduct soil test before each crop for deficiency of macroelements, which may be contributing to diseases or disorders.

- **Plant broccoli before Apiaceae crops**
  Davison and McKay (2003) reported less cavity spot on carrot if crops followed broccoli as it may have some biofumigation properties. Consequently where possible plant a brassica fumigant crop. Unfortunately radish has no such fumigant activity.

- **Plant parsnip cultivars with resistance to canker (root rot) and *Itersonilia***
  Select those that best suit your soil type. This may necessitate trialling a range of cultivars on your own soil type. Cultivar resistance is the most economical form of disease control.

- **Increase crop rotation**
  Where possible maximise crop rotation periods, away from Apiaceae vegetables, as this will reduce carry-over of inoculum in crop debris.

- **Apply the biocontrol agent *B. subtilis* to control *Pythium* root rot in hydroponic Apiaceae crops**
  When setting up hydroponic systems, applying *B, subtilis* every 2-3 weeks will minimise the build up pathogenic *Pythium* species.

- **Monitor four day weather forecasts for heavy rains**
  Minchinton *et al*. (2006, 2007) noted that parsley drop off and root rot emerged a week after heavy autumn rains. Monitor four day Bureau of Meteorology forecasts for heavy rainfall events and apply a registered or permitted fungicide to prevent disease development.

- **Enhance root health of parsley crops with long-term mulching**
  Continuous mulching of ground, over a five year period improved root health in organic parsley production in Queensland. The mulch is produced on-farm.

- **Avoid planting parsley and parsnips after lettuce**
  *Phoma exiqua* var. *exiqua* and *P. tracheiphilum* infect lettuce as well as parsley and parsnips. Planting lettuce before parsley and parsnip increases the inoculum in soils.

- **Avoid planting parsnips on heavy soils**
  Parsnip crops are more susceptible to root rots (canker) when planted on heavy soils. Clay soils retain more water and are cooler than sandy soils, which favours disease development. Plant parsnips on sandy or sandy loam soils, where possible.

- **Avoid mulching root crops such as parsnip**
  Applications of organic mulch on the soil surface enhanced lateral root development on parsnip roots reducing their quality. Incorporate organic mulches into the soil.

- **Monitor parsley roots for root rot, especially during cooler months**
  Monitor parsley roots for rot symptoms and apply registered fungicides. Apply a registered or permitted fungicide to protect seedlings from damping off on autumn and winter grown crops (Minchinton *et al.* 2006, 2007). Additional fungicide applications

may be required at 6 weeks after emergence and at 6-8 weeks before harvest if symptoms appear.

- **Monitor parsnip roots for root rot, especially during winter**
  Monitor parsnip roots for rots. Applications of a registered or permitted systemic fungicide may have efficacy only on sandy soils.

- **Stimulate plant growth**
  Although not providing disease control, some practices enhanced crop vigour, e.g. hilling of soil over parsnip crowns and applications of the biocontrol agent Fulzyme®Plus (*B. subtilis*) on parsnips.

- **Early control of *Itersonilia perplexans* on foliage**
  More strategic timing of fungicide applications is required to control *I. perplexans* on foliage. Although, the fungicide applied in our trials did not provide control of parsnip canker, they do overseas. Timing of applications is important and fungicides may need to be applied early in the season.

## 14.3 Dissemination of information to industry

Every opportunity was taken to report to industry, through field days, industry publications, workshop meetings and steering committees. The Appendix lists the steering committee meetings, field days, workshops, and industry and technical publications.

The project Steering Committees were an excellent means of ensuring research directions remained consistent with industry needs. The committees also enhanced grower involvement and accelerated industry uptake of R&D outputs. Steering committee meetings were held after workshops. Steering committee members for this project consisted of vegetable growers and representatives from allied support businesses including crop advisers, nurserymen, seed suppliers, chemical manufacturers and chemical resellers. These groups provided an opportunity for researchers to present their work plans and results, while the ensuing discussions gave everyone a chance to participate in the project. The diverse experience and industry networks, both local and overseas, enhanced the project outcomes. The scientists involved were able to ensure the research was relevant for industry, while the industry representatives developed a greater understanding of the scientific rigour and quality assurance behind the research.

The steering committee model has been applied successfully to other vegetable research projects including: 'Benchmarking predictive models, nutrients and irrigation for downy and powdery mildew and white blister' VG07070, Management strategies for white blister (rust) in Brassica vegetables' VG04013, 'A scoping study for race identification, source of epidemic and management of white blister on brassicas' VG02118, the 'Evaluation of a disease forecasting model to manage late blight (*Septoria*) in celery' VG04016, the 'Scoping study to investigate management of root-rot diseases in parsley' VG04025, 'Bunching Vegetables' VG01045, 'Onion White Rot' VG01096, and the Lettuce Aphid Advisory Group under 'Lettuce Best Practice' VG01038.

## 14.4 Areas of future research which would benefit the industry

- Determine the pathogenicity of and epidemiology of the new *Pythium* spp. and *Phoma exigua* var. *exigua* identified on parsley and parsnip and their role in the root rot complexes of vegetables.
- Find a replacement for Ridomil®Gold 25G, in the event metalaxyl loses its efficacy, due to enhanced soil degradation and fungicide resistance.
- Continue evaluating cultural control methods for parsnip canker, as they may be more environmentally friendly and more effective over the long term. Planting style (row configuration and spacing) may influence the practice of hilling; consequently this technique is worth testing with different seed spacings.
- Develop parsley cultivars with resistance to *Pythium* spp. and cultivars of parsnip with resistance to *Pythium* spp. and *Itersonila.*
- Screen a range of biocontrol agents for *Pythium* spp. root rots in hydroponics and their use as preventatives rather than eradicants, as if effective they would be more environmentally friendly.
- Test more strategic timing of applications and application methods of fungicides and biocontrol agents for canker control to improve efficacy of disease control and consequently yield.
- Develop a decision support tool for *Pythium* and *Itersonila* on parsley and parsnip as it could identify strategic timing of fungicide applications.
- Continue evaluation of cultivars to identify those which are best suited to various soil types and have resistance to diseases.

## 14.5 References

Davison E, McKay A (2001). 'Integrated management of *Pythium* diseases of carrots'. Final Report Horticulture Australia Project VG98011 83 pp.

Minchinton EM, Auer D, Martin H, Tesoriero L, Thomson F. Trapnell LN, Forsberg L, Nadesan S, Vujovic S (2006) Scoping study to investigate management of root-rot diseases in parsley. Report of Horticulture Australia Project VG04025, 87 pp.

Minchinton EM, Auer D, Martin H, Thomson F, Vujovic S (2007) Identification and management of parsley root rot. Report of Horticulture Australia Project VG06046, 35 pp.

## 14.6 Communication of information

### Publications

***Technical***

- Minchinton E, Petkowski J, deBoer R (2012) Parsnip cultivar trial identifies resistance to root rots and *Itersonilia*. Abstract accepted 7th Australasian Soilborne Disease Symposium, Freemantle, WA September 17-21 September 2012.
- Minchinton E, Petkowski J, deBoer R, (2012) Evaluating control options for parsley root rot in south eastern Australia. Abstract accepted 7th Australasian Soilborne Disease Symposium, Freemantle, WA September 17-21 September 2012.
- Minchinton EJ, Petkowski JE, deBoer RF, Thomson F (2010) Control of Oomycetes associated with parsnip canker. Abstract. 6th Australasian Soilborne Disease Symposium,

Twin Waters, QLD 9-11 August 2010, p105.

- Petkowski JE, Minchinton EJ, de Boer RF (2012) Strategies for control of parsnip canker in south eastern Australia. Abstract accepted 7th Australasian Soilborne Disease Symposium, Freemantle, WA September 17-21 September 2012.
- Petkowski JE, Minchinton EJ, de Boer RF (2012) *Pythium* spp. Associated with root rot of parsnip and parsley in Victoria, Australia. Abstract accepted 7th Australasian Soilborne Disease Symposium, Freemantle, WA September 17-21 September 2012.
- Petkowski JE, Minchinton EJ, deBoer RF, Thomson F (2010) Fungi and Oomycetes associated with a root rot complex in parsnip crops. Abstract. 6th Australasian Soilborne Disease Symposium, Twin Waters, QLD 9-11 August 2010, p73.
- Petkowski JP, Thomson FM, de Boer RF and Minchinton EJ (2011). Management strategies for root rot of continental parsley. Abstract No. 58. ACCP/APPS Conference Darwin, April 2011. New frontiers in Plant Pathology for Asia and Oceania, 26-29 April 2011, Darwin Convention Centre, Darwin, NT, p 109.
- Petkowski JE, de Boer RF, Norng S, Minchinton EJ *Pythium* species associated with root rot complex in winter-grown parsnip and parsley crops in south eastern Australia, in preparation for APP.
- Minchinton EJ, Petkowski JE, de Boer RF, Thomson F. Efficacy of parsnip cultivars to reduce root rots. In preparation for Plant Disease.
- Minchinton EJ, Petkowski JE, de Boer RF, Thomson F. Disease management strategies to control root rot in parsnips. In preparation for Crop Protection.

***Industry***

- Bradley S (2012) Looking at ways to tackle root rot. Pythium trials are revealing helpful results. *Southern Farmer,* January 2012, p2.
- Minchinton E, DeBoer D, Petkowski J (2009) A new national project on Pythium root rots in Apiaceae vegetables Vic VegeLink - submitted.
- Minchinton E, deBoer R, Petkowski J, Thomson F (2010). National guidelines for *Pythium* control in Apiaceae crops. Vegetable Industry Report 08-09. AUSVEG and HAL, p 38.
- Minchinton E, Petkowski J, deBoer R, Thomson F (2010) Better ways to manage Pythium. Vegetable Industry Annual Report 2009/10. HAL, AUSVEG, p31.
- Minchinton EJ, Petkowski JE, deBoer RF (2009) Update for Vegetable Growers. Victorian Vegetable Grower Notes. 29 Oct 2009.
- Minchitnon E, Petkowski J, deBoer R, Thomson F (2010) IPM strategies for Pythium induced root rots in Apiaceae vegetable crops. *IDO Notes,* May 2010.
- Minchitnon E, Petkowski J, deBoer R, Thomson F (2011) Pythium project update 2011. *In the Field*, February 2011.
- Minchitnon E, Petkowski J, deBoer R, Thomson F (2012) Project: VG08026 Identification of IPM strategies for Pythium induced root rots in Apiacae vegetable crops. Horticultural Australia Annual Report 2011, in press.
- Petkowski J, deBoer R, Minchinton E, Thomson F (2011) Parsnip canker management – research update. Vegetables Victoria (Vegetable Growers Association of Victoria) - submitted.

## Posters

***Technical***

- Petkowski JE, Minchinton EJ, de Boer RF, Thomson F (2010) Fungi and Oomycetes associated with a root rot complex in parsnip crops. *AgriBio Science Conference* 29 November 2010 Department of Primary Industries Conference. Poster.
- Petkowski JE, Minchinton EJ, deBoer RF, Thomson F (2010) Fungi and Oomycetes associated with a root rot complex in parsnip crops. Poster. 6th Australasian Soilborne Disease Symposium, Twin Waters, QLD 9-11 August 2010.
- Minchinton EJ, Petkowski JE, deBoer RF, Thomson F (2010) Control of Oomycetes

associated with parsnip canker. Poster. 6th Australasian Soilborne Disease Symposium, Twin Waters, QLD 9-11 August 2010, p105.

- Petkowski JP, Thomson FM, de Boer RF and Minchinton EJ (2011). Management strategies for root rot of continental parsley. Poster. ACCP/APPS Conference Darwin, April 2011. New frontiers in Plant Pathology for Asia and Oceania, 26-29 April 2011, Darwin Convention Centre, Darwin, NT, p 109.
- Minchinton E, Petkowski J, deBoer R (2012) Parsnip cultivar trial identifies resistance to root rots and *Itersonilia*. Poster in preperartion 7th Australasian Soilborne Disease Symposium, Freemantle, WA September 17-21 September 2012.
- Minchinton E, Petkowski J, deBoer R, (2012) Evaluating control options for parsley root rot in south eastern Australia. Poster in preperartion 7th Australasian Soilborne Disease Symposium, Freemantle, WA September 17-21 September 2012.

***Industry***

- Minchinton L, de Boer D, Petkowski J, Tesoriero L, Galea V (2009). Identification of IPM strategies for Pythium induced root rots in Apiaceae vegetable crops. Poster. HAL booklet, Vegetable Pathology Program.
- Minchinton L, de Boer D, Petkowski J, Thomson F (2009) *Pythium* project. Poster, DPI tent, National Vegetable Expo, 7-8 May 2009.
- Minchinton L, de Boer D, Petkowski J, Tesoriero L (2009) A poster was prepared for the Vegetable Conference at the Melbourne Convention Centre, 5-6/5/2009.

## Conferences

- 6th Australasian Soilborne Disease Symposium, Twin Waters, QLD 9-11 August 2010.
- ACCP/APPS Conference Darwin, April 2011. New frontiers in Plant Pathology for Asia and Oceania, 26-29 April 2011, Darwin Convention Centre, Darwin, NT.
- AgriBio Science Conference 29 November 2010, La Trobe University.
- The National Vegetable Expo at Werribee, Victoria, 7-8/5/2009.
- The National Vegetable Expo at Werribee, Victoria, 6/6/2011.
- AUSVEG Vegetable Conference Melbourne, 5-6/5/2009.
- AUSVEG Vegetable Conference Brisbane, 27-28/6/2010.
- AUSVEG Vegetable Conference Sydney, 15-16/4/2011.
- AUSVEG Vegetable Conference Hobart, 10-12/5/2012.

## Workshops/ Steering committee meetings

Notes were prepared for all meetings and for meetings held in Victorian they were distributed on the day and to four interstate growers, two in WA and two in Tasmania. Additionally field day notes were also distributed to interstate collaborators in WA and Tasmania.

- 19/3/2009. Steering Committee Meeting, DPI Frankston.
- 19/6/2009. Workshop, Amstel Golf Club, Cranbourne.
- 28/9/2009. A conference workshop was held at EMAI NSW for the 17th APPS Conference, Newcastle NSW.
- 18/3/2010. A project report, steering committee meeting and project review were held on at Amstel Golf Club, Cranbourne, Vic. Meeting notes were sent to growers in Tasmania and Western Australia.
- 14/12/2010 Project report and Steering committee meeting, Ranfurlie Golf Club House, Cranbourne.
- 8/11/2010. Report presented to Bathurst growers.
- 11/8/2010. Report presented to Gympie growers at Gympie RSL.
- 12/8/2010. Report presented to Gatton growers at DPI Gatton.
- 20/10/2011. Grower meeting, Settlers Run Golf Course, Cranbourne South, Victoria.
- 3/8/2012. Report to growers, Chisolm TAFE, Cranbourne, Victoria.

## Field days

Field day notes were prepared for all Victorian field days, distributed on the day and to four interstate growers, two in WA and two in Tasmania. Additionally field day notes were also distributed to interstate collaborators in WA and Tasmania.

- A field day was held at Frank and Angelo Lamattina's farm, Berwick-Fiveways Rd, Fiveways, VIC. on 21/10/2009. Field day notes were prepared for the day and sent to parsnip growers in Tasmania and Western Australia.
- A farm tour for the Australian Hydroponic and Greenhouse Association (AHGA) National Conference was held at EMAI NSW on 22/7/2009.
- A field day to view the parsnip variety trial was held at J, D & D. Kelly, 620 Westernport Highway, Cranbourne, VIC 3977, on 8th December 2011.